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Overwhelming role of hydrology-related variables and river types in driving diatom species distribution and community assemblage in streams in Cyprus

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Highlights

- Hydrology plays a major role in shaping diatom assemblages in Cypriot streams.
- Putative “Red List” diatom species for Cyprus are proposed.
- Red List species are more common in intermittent, rather than permanent, streams.
- Several species from the same genus often occur together in a sample.
- Numerous species per genus may enhance resilience of the system.

Abstract

Mediterranean streams are naturally highly-stressed environments mainly due to the wide seasonal and inter-annual fluctuations in water quantity. This natural pressure (which will be exacerbated by climate change) is a significant challenge when establishing efficient assessment methods. We studied environmental parameters (hydromorphology, hydrology, physical and chemical variables) and collected 182 diatom samples from 65 stations in Cyprus (south-western part). Diatoms were found in 171 samples and analyses revealed 290 taxa (273 identified to the species -or intraspecific- level) belonging to 65 genera. Even a tentative application of a Red-List approach underlined the overwhelming importance of hydrology-related variables and river types in determining diatom species distribution and community ecological attributes in the water-stressed Island of Cyprus. Somewhat unexpectedly, both species from threat categories of the diatom Red List for Central Europe (2018) and species one might predict would be included in such categories in a possible future Red List tailored for Cyprus occurred more frequently and were more relevant in assemblages from sites in intermittent streams. We found a majority of motile, medium- to small-sized, diatom species, including a low number of colony-forming species. We found several species known to be effective first colonizers (pioneer species) and, among these, there was a striking preponderance (80%) of *Achnanthidium* species, often with several species co-occurring, particularly at reference sites. A four-factor PERMANOVA found that all type (essentially hydrology-related) variables were significant, and there was also a significant effect of season. Agglomerative hierarchical cluster analysis revealed three end-groups, with groups being separated on ecohydro(geo)logical grounds (lentic/lotic), sediment grain size, discharge and pH. The reference sites were analysed in more detail to identify environmental determinants. 28% of the variation in diatom assemblage composition was explained by the measured variables, with those associated with stream type and hydrology explaining the greatest proportions (12 and 10%, respectively) whilst season accounted for the remainder. All in all, our study emphasised a need for detailed investigations of ecological and distributional (including Red List status) traits of diatom species, and to acknowledge the importance of the hydrological peculiarities of Mediterranean streams, and in particular to account for the dramatic seasonal variability when developing ecological assessment protocols for the region.

Key words: Red List species, phytobenthos, hydrology, intermittent streams, ecological assessment

1. Introduction

The Mediterranean basin is home to approximately 500 million people living in 23 countries, eight of which are members of the European Union (UNEP/MAP, 2012). The large human population coupled with naturally low rainfall (which will be exacerbated by anthropogenic climate change: Giorgi and Lionello, 2008), combine to create a pressure on both quantity and quality of water resources within the region (UNEP / MAP. 2012; Cantonati et al., 2020a). This, in turn, will affect the ability of Mediterranean water bodies to deliver ecosystem services (Terrado et al., 2014) and, thereby, have implications for sustainable growth within the region.

For the countries within the EU, the legislative basis for protection of water resources is provided by the Water Framework Directive (WFD; European Commission, 2000; Dworak et al, 2007; Carvalho et al., 2018). This defines the ecological criteria required for sustainable management of water bodies based on their deviation from a hypothetical “reference state” (defined as “no, or only very minor, anthropogenic alterations to the values of physic-chemical and hydromorphological quality elements”; European Union, 2000). Definition of this reference state has proved to be challenging throughout Europe (Kelly et al., 2012, this volume; Pardo et al., 2012; Bouleau and Pont, 2014) but with some particular issues arising in the Mediterranean region (Feio et al., 2014). However, if objective criteria can be established across the EU for this important benchmark then national implementations of the WFD can be harmonized (Birk et al., 2013; Kelly et al., 2008; Almeida et al. 2014). This, in turn, ensures that the legislation is implemented consistently across the EU (Poikane et al., 2015).

The challenges presented by Mediterranean streams include the high level of natural stress due to seasonal fluctuations in climate, and consequently in the hydrology. While seasonality (dry summers, wet winters) is highly predictable, the marked interannual variability (dry and wet years) is not (e.g., Cantonati et al., 2020a). The extreme manifestation of this, streams that do not flow at all during the summer months, places extreme physiological stress on organisms. This notwithstanding, intermittently flowing streams and rivers are essential to the integrity of river networks as they typically have high biodiversity and support important ecosystem processes. They are a source of valuable ecosystem goods and services, as well as being critical conduits for water, organisms, energy and material even when surface water is not present (Acuña et al., 2014), or allow access to perennial upstream refugia for migratory fish such as the eel (*Anguilla anguilla*) during their (short) flow periods.

The extent to which this stress influences the biota is in part affected by the life cycle of the organisms under consideration. Whilst an organism with a life cycle that extends over several months or years may need special adaptations to cope with this drought period, it is possible that organisms whose life cycle is measured in weeks will cope differently (e.g. with recolonization cycles; Chester and Robson, 2014; Falasco et al., 2018), and, indeed, that the composition of the community will shift over the course of the year in response to these natural changes. Diatoms are one example of a group of organisms with a short life-cycle and which are, furthermore, an integral part of many country's ecological assessment toolkits (Kelly, 2013; Almeida et al., 2014; Poikane et al., 2016). Falasco et al. (2018) concluded that diatom's resistance mechanisms did not play a significant role in recovery patterns after droughts. Artigas et al. (2012) showed that colonization sequences of algae and bacteria in biofilm formation were faster in Mediterranean streams (as compared to Central European ones), and that Mediterranean streams biofilms had larger amount of early-colonizing diatom species (*Ulnaria ulna*, *Karayevia clevei*), as well as higher rates of polysaccharide production in the extracellular matrix.

Calapez et al. (2014) and Piano et al. (2017) found replacement of species sensitive to organic pollution by ones that are tolerant to be the main process in diatom benthic assemblages during droughts. Calapez et al. (2014) also observed that post-drought diatom assemblages showed no significant difference in evenness or abundance (as compared to pre-drought) whilst diatom-quality-index values decreased. Falasco et al. (2018) noted that the extent of droughts in Mediterranean streams has recently intensified and, as mean annual discharge is predicted to further decrease over the coming years (e.g., Giorgi and Lionello, 2008), such effects are likely to be exacerbated. They suggested that endangered (sensu Lange-Bertalot, 1996) diatom species may be less resilient to droughts than generalist and widely-distributed taxa. Falasco et al. (2018) conclude that flow intermittency favours pseudaerial and planktic species whilst it threatens endangered diatom species in Mediterranean streams. Availability of a current velocity of at least 20 cm s^{-1} appears to be the main factor influencing the abundance of endangered species. Elias et al. (2015) found that Mediterranean-stream diatom assemblages, when compared to temperate ones, were characterized by larger and less motile species, and that stalked diatom species decreased after drought.

Almeida et al. (2014) and Kelly et al. (2012) both showed that diatom assemblages in Mediterranean streams did not show strong differences between the stream types defined for the purpose of EU intercalibration (see Feio et al., 2014), with the exception of streams with temporary hydrological regimes. Kelly et al. (2012), furthermore, showed considerable similarity between assemblages from Mediterranean streams and those from other parts of the EU. However, these studies were, necessarily, performed at a coarse resolution, albeit one sufficient to demonstrate broad agreement amongst national approaches. Within smaller geographical areas, however, greater sensitivity in ecological assessment might be possible. First, a key gradient that is significant over a broad geographical area might be less significant in a smaller area, particularly if that area had homogeneous geology. For example, whilst alkalinity has been shown to be a key determinant of diatom assemblage composition in several studies (e.g., Kelly et al., 2008; Cantonati et al., 2012), this is related to bedrock geology and there is scope for considerable hydromorphological and hydrological variability in an area underpinned by a single geological formation.

In Cyprus, for example, most springs and surface waters in the south-western part of the island originate or are influenced by the Troodos ophiolite. The most important rivers and streams (in terms of runoff) come from the Troodos Massif (WDD, 2016). These are mainly basic/ultrabasic rocks from which waters with high alkalinity, calcium, magnesium, and sodium, and often high sulphate and chloride concentrations originate (Neal and Shand, 2002). Thus, virtually all stream waters have relatively high conductivity and alkalinity, and this means that they are less likely to influence the composition of diatom assemblages. This geological peculiarity, however, clearly emerged in the six reference (least-disturbed) diatom assemblages identified by Feio et al. (2014) from seven Mediterranean countries, where group “F” was composed only of sites from Cyprus with ophiolitic geology. Moreover, in an area with pronounced climate-driven hydrological fluctuations, it is possible that there will be natural changes in the diatom assemblages which will be manifested in different values of the metrics used for ecological assessment. The possibility that the reference values from which ecological assessments are derived may vary over the course of a year is not new (e.g., Kelly et al. 2008) but has not been adopted widely for routine assessments.

Whilst sensitive (to organic pollution), characteristic, endangered (Falasco et al., 2018) diatom species are found in Mediterranean-stream sites with high ecological integrity, pollution and contamination of various types typically causes a simplification, generalization and homogenization of diatom assemblages (Tornés et al., 2007), with dominance of a reduced number of pollution-tolerant species and, in some cases, accompanied by an increase in deformed specimens (Tornés et al., 2018). The effects of pollution are aggravated by hydrological stress (Tornés et al., 2018) and metal contamination, which can cause such deformities in Mediterranean streams, is strongly influenced by water-flow regimes due to dilution (Bonet et al., 2013).

In this paper we explore the factors determining diatom assemblages of streams in Cyprus. This is the most easterly of the EU member states in the Mediterranean Basin. As there is a gradient of increasing aridity with distance east in Mediterranean, Cyprus has a distinctive climate characterized by particularly severe summer droughts. Consequently, Cyprus suffers the highest water stress levels in EU and is among the top 20 water-deprived countries worldwide (Giannakopoulos et al., 2010; Sofroniou and Bishop, 2014).

The objectives of this paper are to investigate the factors responsible for shaping diatom assemblages in Cypriot streams, in particular those free from known anthropogenic sources, and to identify those which are major drivers of diatom assemblage composition (including complex attributes such as occurrence of Red List species). A preponderant role of hydrology-related variables and types suggested by the analyses is investigated in detail and highlighted.

2. Methods

2.1. Study area

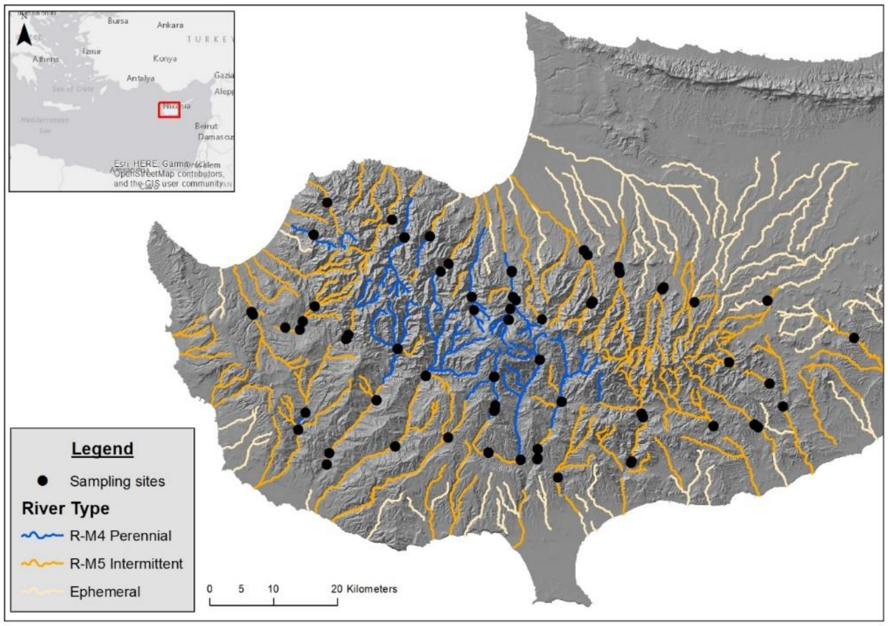

Fig. 1. Location of the sampling sites.

65 sites on 30 rivers in the areas under the effective control of the Republic of Cyprus were sampled on at least one occasion in three main periods: spring, summer and autumn during 2005-2006 and 2010-2011. Some sites were sampled several times (up to 7) in different years (See Supplementary material Table 1). All rivers belonged to one of two main types, as defined for the purposes of the European Commission’s intercalibration exercise (Birk et al. 2013):

a) perennial; comparable in terms of flow regime and overall environmental features to intercalibration type R-M4 (Erba et al., 2009; EC, 2018); or,

b) intermittent, belonging to intercalibration type R-M5 (EC, 2018).

Mean catchment area and altitude of the investigated river sites were respectively 60 km^2 and 405 m a.s.l. The sites' elevation ranged between 88 and 1046 m a.s.l. The predominant catchment geology was ophiolitic formations; some sites were however located on carbonate rocks of the Circum Troodos sedimentary succession although the headwaters of all catchments were within the ophiolitic Troodos rocks. Very few catchments were located entirely on rocks conferring to the waters a high bicarbonate/carbonate content.

Streams were selected to cover the entire quality range present in the region from undisturbed and nearly natural sites (reference sites) to human-impacted sites. 13 river stretches were selected as reference sites from 8 rivers, based on land use, water physico-chemical parameters and hydromorphological alteration criteria specified in Feio (2014). Of 64 sites that were sampled, 13 (= 49 samples) were designated as "reference sites" based on these criteria.

2.2. Diatom analysis

2.2.1. Diatom sampling and preparation of permanent slides

182 diatom samples were collected and treated following the European standard CEN 13946 2003 and CEN 14407 2004 (European Committee for Standardization, 2003; 2004). At least five cobbles were sampled from main flow of the river and an area of approximately 10 cm^2 was scraped from each.

All samples were kept in a cool dry place after collection. Most (149) were preserved with acetic Lugol's iodine and conserved by the Water Development Department in Nicosia, Cyprus, while the rest (33) were preserved using ethanol and conserved in CNR-IRSA laboratories in Brugherio, Italy. 5 of these 33 samples were not well conserved (dry) while 6 samples did not have enough material digested to be processed quantitatively, so indices were computed for a total of 171 samples.

Samples were digested using 30% hydrogen peroxide, and the cleaned material was mounted in Naphrax (refractive index of 1.74) according to European Standard EN 13946: 2003 (CEN, 2003) and labelled accordingly. Each slide was labelled with the station name, station code, date of sampling and date of mounting.

2.2.2. Identification of taxa

Identification and enumeration of diatoms followed European Standard EN 14407 (CEN, 2004). All samples were identified to the species or lower taxonomic levels (i.e. variety) as required for the calculation of indices in OMNIDIA software, with a minimum of 400 valves identified and counted per slide. Broken valves were included in the analysis if at least three quarters of the valve was present. Girdle views were included if several valve characteristics (e.g. length, shape, types of striae, number of striae), could be unambiguously matched to valve views of similar species. Identification and nomenclature followed mainly Krammer and Lange-Bertalot (1986-1991), Krammer and Lange-Bertalot (2004), Krammer (1997a;b; 2000-2003), Lange-Bertalot (1993; 2001), Levkov (2009), Lange-Bertalot et al. (2011), Hofmann et al. (2011), Reichardt (1997, 1999), Werum and Lange-Bertalot (2004), Lange-Bertalot et al. (2003), and Rumrich et al. (2000). For taxa that were only recently described or taxa for which amended taxonomic concepts and / or names were only very-recently published specific literature had to be used (e.g., Novais et al., 2009; Rimet et al., 2010; Van de Vijver et al., 2011b; Romero and Jahn, 2013; Trobajo et al., 2013; Wojtal, 2013). The taxonomic concepts of some species had to be updated using the following recently published papers: Reichardt (2018) for *Nitzschia pseudalpina*, Morales et al. (2020) for *Nitzschia transtagensis* (though we believe that our specimens belong to the taxon discovered in Sardinia and that this population should for now best be kept separated from the newly described *N. transtagensis*). Nomenclature and some taxonomic concepts were updated using Cantonati et al. (2017), AlgaeBase (Guiry and Guiry, 2020), Diatoms of North America (Spaulding et al., 2019), the Freshwater Diatom Flora of Britain and Ireland (Jüttner et al., 2020). Moreover, several new species were identified (e.g., Cantonati et al., 2016; 2018).

2.3. Environmental analysis

For each river site information at different spatial scales was provided. Different land use percentages were estimated from CORINE land cover maps, considering the catchment upstream of each sampling site. Available land use data were summarized into four categories: agriculture (agricultural areas other than pasture: arable land/permanent crops/heterogeneous agricultural areas), urban, pasture (i.e., low impact agricultural areas), and forests. Hydromorphological and habitat information was collected at reach level using the CARAVAGGIO method (Buffagni and Kemp, 2002; Buffagni et al., 2005, 2013) at the same times and places as the biological data. CARAVAGGIO is a modification of the River Habitat Survey (RHS, Raven et al., 1997) optimized for Mediterranean regions.

This method required the operator to recognize channel and bank features in a 500-m length along a river. The 500-m river stretches used were selected to be representative of the water body (sensu WFD).. Water quality was assessed at each site for almost all samples (165 out of 182 samples) by estimating oxygen saturation deficit (%), chloride (mg/L), biological oxygen demand (BOD_5 O_2) (mg/L), ammonium-N (mg/L), nitrate-N (mg/L), nitrite-N (mg/L), chemical oxygen demand (COD) (mg/L), *Escherichia coli* (CFU/100mL), ortho-phosphate (μg/L) and total phosphorus (μg/L). The water quality data were collected by the Water Development Department as part of the Department's routine monitoring.

2.3.1. Calculated environmental indices

The following indices were calculated from the abiotic data:

LUIc – Land Use Index at catchment level. Calculated using the scoring system outlined in Feld (2004). 5 is the score for artificial, 3 for agricultural, 1 for pasture and 0 for natural land uses. The final score is obtained by multiplying the score assigned to each of the different categories of land use to the percentage of the area occupied by that land use.

LUIr – Land Use Index at reach level. The LUIr index (Erba et al., 2015) allows a quantification of land use at the stretch level. For the calculation of the LUIr index, characteristics measured with the CARAVAGGIO method are taken into account.A different score is assigned to the different land uses recorded (32 different categories). The scoring system follows, in broad terms, that developed by Feld (2004).

HMS, HQA, LRD. The habitat modification score (HMS), habitat quality assessment score (HQA), and lentic– lotic river descriptor (LRD) were calculated using CARAVAGGIOsoft (Di Pasquale and Buffagni, 2006). The HMS index (Raven et al., 1998) is used to evaluate the morphological impact at a river stretch and consists of the sum of the scores assigned to features representing types of morphological alteration (e.g., bank modifications, channel modifications,). The index increases with increasing morphological impact. The HQA index evaluates habitat richness and the general quality of a river stretch (Raven et al., 1998; Balestrini et al., 2004). When different habitat features (e.g., flow types and/or different substrates types) are recorded, a high score is assigned to the site. The LRD descriptor (Buffagni et al., 2009, 2010) furnishes information about the lentic–lotic character of the river stretch. Positive values represent rivers with a lentic character (dominance of slow flowing or still water) while negative values represent lotic rivers (dominance of features linked with high turbulence and fast flowing water).

OPD. To describe river sites in terms of water (organic) pollution, the Organic Pollution Descriptor (OPD) (Demartini et al., 2013) was computed. The variables considered here were: Oxygen saturation deficit [%], chloride [mg/L], BOD5 [mg/L O_2], ammonium-N [mg/L], nitrite-N [mg/L], nitrate-N [mg/L], ortho-phosphate-P [µ g/L], total phosphorus [µg/L], COD [mg/L], *Escherichia coli* [CFU/100 mL]. A score is assigned to each chemical variable available in the dataset. The scores obtained from each chemical parameter are then averaged to obtain the final index value.

2.3.2. Hydrological data

Hydrological descriptors were determined from mean daily flow data for all sites where a hydrometric flow gauging station was available at a reasonable distance from a sampling site. Data from flow gauging stations where no diatom data were available were also added in order to cover the entire perennial-temporary continuum gradient (Uys and O'Keeffe, 1997). Due to data limitations, variables were computed using non consistent time ranges between stations (i.e. the data were derived from different time ranges in e.g. station A and station B). Because of a lack of data on abstractions, flow data derived from sites with assumed hydrologic impacts were not corrected by natural flow estimation techniques. The descriptors Mean annual flow, Median number of zero days, Annual Coefficient of Variation (CV), flow predictability and base flow index were calculated using the Indicators of Hydrologic Alteration software (IHAs: Richter et al., 1996); the Richards-Baker Flashiness Index (RB: Baker et al., 2004) was determined in separate calculations. Cluster analysis was subsequently performed with the hydrological descriptors (Statistica 8 software, tree clustering, amalgamation (joining) rule: Ward`s method), applying the parameter combination suggested by Oueslati et al. (2010). The aim of the cluster analysis was to elaborate a preliminary hydrological classification that would be more accurate than a binary split into the R-M4/R-M5 intercalibration types (perennial/temporary rivers, European Commission, 2018). The evaluation of the cluster analyses outcomes with several different combinations of hydrological descriptors led to the hydrological classification presented in Table 1.

Table 1. Hydrological types and relative description based on the WDD hydrological analysis.

Type Code	Type name	Type characteristics
1a	Perennial	less than 4 dry weeks
1b	Perennial Highly Predictable	less than 4 dry weeks, flow predictability around 0.6 (it is around 0.4 for all other perennial sites)
1c	Perennial (Artificial Perennial)	non-natural perennial flow (sewage outfall u/s, ...)
2a	Intermittent	Dry period 1-4 ½ months, R-B index <0.4
2b	Intermittent Flashy	Dry period 1-4 ½ months, R-B index 0.4-0.8
3a	Prolonged Intermittent	Dry period 4 ½ - 8 months, R-B index <0.4
3b	Prolonged Intermittent Flashy	Dry period 4 ½ - 8 months, R-B index 0.4-0.8
4a	Harsh Intermittent	Dry period 8-11 months, R-B index <0.4
4b	Harsh Intermittent Flashy	Dry period 8-11 months, R-B index 0.4-0.8
4c	Harsh Intermittent Highly Flashy	Dry period 8-11 months, R-B index 0.8-1.2
5	Ephemeral/Episodic Hyperflashy	Flow period < 1 month, R-B index >1.2

All sites of the dataset with mean daily flow data were assigned to a hydrologic type based on the type characteristics of Table 1; however, no stations belonging to types 4c and 5 were present. The types were subsequently categorized into four macrotypes: 1. Perennial, 2. Intermittent, 3. Prolonged intermittent, 4 Harsh intermittent. This categorization omits flashiness and flow predictability as criteria and allowed the assignment of hydrologic types to those sites where no hydrometric flow gauging station was available within a reasonable distance. These were classified using the length of the dry period only, based on time series of regular monthly spot flow measurements that were available. This allowed the inclusion of these sites in subsequent analyses.

Table 2. Typological and hydrological variables used to characterise the sites and their biological communities. Variables included in the discriminant function models after testing for multicollinearity between predictors, using the variance-inflation factor (VIF) as described by Fox and Monette (1992): those included in the Type model are marked with an *, while the one in the Hydrological model with **.

Typological and hydrological variables				
Variables from Caravaggio		**Caravaggio indices**	**Other variables**	
Total Channel Width (m) *	Deposition	HQA	Valley Slope (%)	Catchment yield (L/s/km^2) **
Total Water Width (m) *	Bank slope	HMS	Source Distance (Km)*	Mean annual flow (m^3)**
Max Water Depth (m) *	Valley form*	LUIr	Catchment – (Km^2)	Median number of zero days**
Ratio Water Width/Channel Width	Channel Form*	LRD**	Population in Catchments	Presence of Water abstraction
Ratio Water Width/Water Depth	Characteristics of Sez. Q	LUIcatc	Hydrological macrotype	Q.instant (m^3/s)
Substrate type	Erosion - Channel*	LUIbuff	Season	
Erosion - Bank				

Table 3. Chemical-physical and microbiological variables used to characterise the sites and their biological communities.

Chemical-physical and microbiological variables			
Chemical variables		**Physical variables**	**Microbiological variables**
pH	N total (µg/L)	Dissolved Oxygen (mg/L and %)	*E.coli*_total (CFU/100mL)
SO_4 (mg/L)	NH_4-N (µg/L)	Electrical conductivity (µS/cm)	*Enterococcus* (CFU/100mL)
Cl(mg/L)	NO_2-N (µg/L)	Temperature (°C)	
Na(mg/L)	NO_3-N (µg/L)	Turbidity (NTU)	
BOD_5 (mg/L)	P total (µg/L)		
COD (mg/L)	PO_4(µg/L)		

2.4. Data processing and statistical analyses

For all diatom species collected in this study, a threat status (a measure of rarity) was assigned, according to current (Hofmann et al., 2018) and previous (Lange-Bertalot, 1996) Red List data for Central Europe (the only currently available diatom Red Lists). For the species present in both lists, a check was made to confirm whether conservation status was improving or declining. Hofmann et al. (2018) provide further ecological attributes (trophic and mineralization preferences, aerial species) used in this study (Supplementary material Table 2), along with sensitive / tolerant taxa of intermittent Mediterranean streams (sensitive = more abundant in reference sites, and tolerant = more abundant in non-reference sites impacted by pressures, typically organic and nutrient enrichments) as provided by Delgado et al. (2012), and life-form/growth-form, guild and size indications provided by Rimet and Bouchez, (2012).

Some putative Red-List threat-category species in Cyprus could not be found in the Red List for Central Europe (Hofmann et al., 2018), because they are likely to be adapted to typical Mediterranean streams, and they have a Mediterranean / Middle East distribution. Based on information in the literature and our own experience, a number of such “candidate Mediterranean / Cypriot” threat-category Red-List species could be identified and are listed in Table 5 in the Results.

The multiple statistical comparisons to find out more about the distribution of Red List species in non-reference vs reference, perennial (R-M4) vs intermittent (R-M5) intercalibration types, and in the four categories of the hydrological macrotype (Perennial 1 - Intermittent 2 - Prolonged Intermittent 3 - Harsh intermittent 4, see Table 1), detailed in Table 6a & b in the Results, were performed using Mann-Whitney U Tests and Kruskall Wallis Anova with Statistica 7.0 (StatSoft, Inc., Tulsa, OK).

Bray–Curtis dissimilarity measure was selected as a robust indicator of differences among diatom samples (Reynoldson et al., 2001). The dissimilarity matrix was built using relative abundance data,after removing taxa occurring in less than 3 sites and having an abundance less than 5%. 211 taxa remained as the focus of the data analyses.

A four factor Permutational ANOVA (PERMANOVA; Anderson, 2001) with 999 permutations, computed in the *Vegan* package in R version 3.1.0 (Oksanen et al., 2019; R Core Team, 2019), considered hydrological macrotypes (perennial, intermittent, prolonged intermittent and perennial highly predictable), season (winter, spring, and autumn), intercalibration type (“R-M4” and “R-M5”) and year (2005, 2006 , 2009, 2010, 2011, and 2012).

Agglomerative hierarchical cluster analysis (Kauffman and Rousseeuw, 1990) was applied using the *Agnes* function in the R package *Cluster* (Maechler et al., 2005) using an unweighted pair group method with arithmetic averages (UPGMA). The agglomeration coefficient was computed as it provides a measure of the average height of the mergers in a dendrogram. an internal validation approach was used to select the number of clusters to be retained (Handl et al., 2005). Further validation was provided by the metaMDS R function of the *vegan* package (Oksanen et al., 2019) which uses multiple analyses to assess the stress value associated to the number of groups selected. The *Ordispider* function in *Vegan* was used to plot the groups in a non dimensional space (Oksanen et al., 2019).

Potential indicator taxa of the different clusters were identified using the Indicator Value (*IndVal*) (Dufrene and Legendre, 1997) method, using the *duleg* function in the *labdsv* package in R (Roberts, 2016;). This analysis provides a qualitative insight into the composition of the different clusters; consideration of indicator value significance would be inappropriate, due to circularity (i.e. the clusters were observed within the same set of biological observations).

Stepwise ordinations were conducted to identify the environmental variables responsible for the patterns in the biological data using the *vegan* package in R version 3.1.0 (Oksanen et al., 2019; R Core Team, 2019). The forward stepwise ordination regression ran through permutations. Only significant environmental variables were used for the subsequent variance partitioning analysis. Before variance partitioning, all variables were assessed for collinearity to ensure that the statistical outputs were accurate and stable. A variance inflation factor (VIF) >5 was used to determine if variables were collinear ($VIF_x = 1/1-R_x^2$). The procedure was performed in R using the “vif.cca” function (R Core Team, 2019) for each group of environmental variables. The variance partitioning was then conducted separately for three groups of environmental variables: river type; hydrology and season. Variance partitioning was used to determine the relative amount of variance in the diatom assemblage that each group of variables explained. The analysis was conducted using redundancy analysis (RDA: Boccard et al., 2011) in R, using the *vegan* package (Oksanen et al., 2019), to quantify the individual contribution that each variable group had in shaping the diatom assemblage, along with the contributions from interactions amongst the environmental variables.

Based on the output of the variance partitioning, the environmental variables selected were used to build a multiple discriminant function (DF) modelling procedure using the Mass package in R version 3.1.0 (R Core Team, 2019, Venables and Ripley, 2002). Group size was used as a prior probability in predicting group membership probabilities from the DF model (Clarke et al., 2003).

3. Results

3.1. Morphological, physical and chemical characteristics of the Cypriot streams

Supplementary Material Table 1 provides a list of the sampling sites along with their main characteristics: Typological attribution; whether a reference site or not; hydromorphological characteristics (i.e. morphological descriptor calculated from CARAVAGGIO), chemical characterization (OPD) and total number of samples for each site.

Table 4 summarizes the main morphological, physical, and chemical characteristics of all sites, focussing on the determinants most relevant to diatoms. Instant discharge values are relatively low, ranging from 0 to 3, with an average of 0.3 m^3/s, whilst conductivity and pH values are relatively high in good agreement with the basic / ultrabasic ophiolitic rocks of most drainage basins. The main algal nutrients are mostly present in low to moderate concentrations.

3.2. Diatom assemblages of Cypriot streams

A total of 171 samples from 65 stream reaches in Cyprus, each sampled in different seasons, revealed an overall species richness of 290 taxa (273 identified to the species -or intraspecific- level) belonging to 65 genera (Supplementary Material Table 2).

The most 50 frequent and abundant species of the main genera were as follows: *Achnanthidium* (*A. jackii*, *A. minutissimum* s.s., *A. minutissimum* sp. gr., *A. polonicum*, *A. pyrenaicum*, *A. straubianum*, *A. saprophilum*, *A. tepidaricola*), *Gomphonema* (*G. pumilum* var. *rigidum*, *G. rosenstockianum*, *G. tergestinum*, *G. lateripunctatum*), *Halamphora* (*H. veneta*, *H. submontana*, *H*. sp. aff. *oligotraphenta*; less important: *H. paraveneta*, *H. normannii*), *Nitzschia* (important *Nitzschia* spp. in groups*: N. soratensis / N. inconspicua / N. frustulum*, *N. pseudalpina*, *N. fonticola / N. costei*, *N. dissipata / N. dissipata* var. *media*, *N. palea*, *N. communis / N. pusilla*, *N. amphibia*, *N. capitellata*, *N. linearis* + *Grunowia denticula* & *Tryblionella apiculata* and *T. hungarica*), *Navicula* (*N. veneta*, *N. tripunctata*, *N. cryptotenella / N. cryptotenelloides*, *N. caterva / N. reichardtiana*, *N. antonii*, *N. capitatoradiata*), *Encyonopsis* (*E. minuta*, *E. subminuta*, *E. microcephala*, *E. fonticola*), *Cymbella* (*C. vulgata*, *C. kolbei*, *C. affinis*), *Planothidium* (*P. frequentissimum* -including *P. victorii*-, *P. lanceolatum*), *Ulnaria* (*U. monodii* most important species, followed by *U. acuscypriacus*; *U. vitrea* not very frequent but locally abundant), *Reimeria* (only *R. uniseriata* is frequent and abundant), *Diatoma* (*D. moniliformis*, *D. polonica*), *Rhoicosphenia* (only *R. abbreviata*), *Diploneis* (*D. separanda* is by far the most frequent and abundant species), *Craticula* (*C. subminuscula* most frequent and abundant). Taxa that were sporadic but which could be abundant when they were found were: *Crenotia rumrichorum*, *Brachysira neglectissima*, *Odontidium mesodon*, *Cavinula cocconeiformis*.

Table 4. Main morphological, physical, and chemical characteristics of all 65 sites studied (HQA: Habitat Quality Assesment; HMS: Habitat Modification Score. LUIcara: Land Usex Index derived from CARAVAGGIO survey; OPD: Organic Pollution Descriptor).

	Average	**Min**	**Max**	**Median**
HQA	49,38	35,00	68,00	50,00
HMS	13,42	0,00	49,00	10,00
LUIcara	2,59	0,00	13,47	0,88
OPD	0,82	0,13	1,00	0,83
Instant Discharge (m^3/s)	0,31	0,00	3,00	0,15
pH	8,30	5,77	9,02	8,35
EC field_ Us (µS/cm)	726	202	3230	611
DO%_field (%)	97	40	191	98
Temp (°C)	14,0	6,3	24,3	13,4
Turb_field (NTU)	3,63	0,05	60,57	1,90
$\mathbf{SO_4^{2-}}$ (mg/L N)	123,9	3,5	1493,5	55,0
$\mathbf{Cl^-}$ (mg/L N)	63,1	12,9	368,0	43,3
$\mathbf{Na^+}$ (mg/L N)	49,31	7,84	220,00	36,00
$\mathbf{BOD_5}$ (mg/L O_2)	1,4	0,5	19,0	0,5
COD (mg/L O_2)	4,6	0,5	51,0	3,0
coli_total (/100mL)	2741	1	24190	1325
N_total (µg/L N)	1419	100	10350	450
$\mathbf{NO_3^-}$**-N** (µg/L N)	1051	2	9671	407
P_total (µg/L P)	41	1	3650	3

Least-impacted sites hosted: - some *Delicata* species (including comparatively large-celled species); - a number of species of *Epithemia* (capable of N fixation via cyanobacterial endosymbionts); - *Mastogloia* and *Surirella* species (but only with low frequency, and, especially, very low abundance); - *Frustulia spicula* subsp. *judaica*; - some *Pinnularia* spp. with very low frequency and abundance; and, - occasional cells of large Alpine species (*Cymbella helvetica*, *Surirella helvetica*).

The species found also included some new-to-science (*Ulnaria acuscypriacus* Lange-Bertalot et Cantonati in Cantonati et al., 2018), and poorly-known, re-discovered species [*Ulnaria monodii* (Guermeur) Cantonati et Lange-Bertalot in Cantonati et al., 2018]. *Navicula veronensis* Lange-Bertalot et Cantonati was described from a spring in the surroundings of Verona (Italy) but it was relatively frequent in the Cyprus streams studied, and these data allowed the ecological characterization of the species (Cantonati et al., 2016). Several others are still in the process of being described (see manuscript names "MN" in the Supplementary Material Table 2, MC unpublished material).

Red-List threat-category data were available for 240 of the 273 taxa identified at least to species level (Hofmann et al., 2018). More than a quarter (27.5% = 66 taxa) of the species for which Red List information was available were found to belong to one of the threat categories (1, 2, 3, G, R, V, D oligotraphentic). These are listed in Table 5 with threat categories in decreasing order of severity, and are also available in Supplementary Material Table 2, which allows a comparison with the threat status published in the previous Red List for Central Europe (Lange-Bertalot 1996), along with 18 "candidate Mediterranean / Cypriot" Red List species. No species with threat category 1 ("threatened with extinction") were present in the dataset.

Table 5. Red List, and putative "Mediterranean / Cypriot", Red List species in threat categories (2 = strongly threatened, 3 = threatened, G = threat of unknown extent, R = extremely rare, V = on the way to be threatened, D = data insufficient). RL 2018 = Red List of Central Europe (and 'Ecology': ae = aerial, o = oligotraphentic, oc = oligotraphentic carbonate, od = oligotraphentic distrophic, eu = eutraphentic to tolerant, hal = halophilic, ? = unknown) according to Hofmann et al. (2018). % mean = average relative abundance (%) and N.O. = Number of Occurrences in this dataset. Size class 1 to 5 as reported in Rimet and Bouchez (2012).

Species belonging to threat categories of the Red List for Central Europe	RL 2018	Ecology	% mean	N.O.	Size class	**Species belonging to threat categories of the Red List for Central Europe**	RL 2018	Ecology	% mean	N.O.	Size class
Brachysira vitrea	2	oc		3	2	*Diploneis elliptica*	V	o		4	5
Cymbella tumidula	2	?	2	30	3	*Diploneis krammeri*	V	oc		1	5
Cymbellonitzschia diluviana	2		3	1		*Encyonopsis cesatii*	V	o	1	19	4
Eunotia intermedia	2	od		2	2	*Eucocconeis laevis*	V	o		1	3
Nitzschia alpinobacillum	2	oc		3		*Gomphonema lateripunctatum*	V	oc	2	15	4
Sellaphora stroemii	2	oc	2	11	1	*Gomphonema sarcophagus*	V	?	0	4	3
Cymbella helvetica	3	oc	0	4	5	*Grunowia denticula*	V	?	12	19	2
Cymbella vulgata	3	?	1	61	3	*Halamphora normanii*	V	ae	2	13	5
Diploneis petersenii	3	o	0	5	2	*Hannaea arcus*	V	o		2	4
Eunotia arcubus	3	oc		1	5	*Navicula veronensis*	V		0	10	
Fragilaria amphicephaloides	3	oc		1		*Nitzschia acidoclinata*	V	?	1	4	2
Gomphonema auritum	3	o	0	6		*Nitzschia dissipata* var. *media*	V	?	2	19	4
Gomphonema vibrio	3	oc	1	1		*Psammothidium grischunum*	V	?		1	2
Navicula subalpina	3	oc	2	6	3	*Achnanthidium deflexum*	D	?	7	20	1
Achnanthidium lineare	G	eu	6	10	1	*Amphora lange-bertalotii*	D	o	3	15	

Species belonging to threat categories of the Red List for Central Europe	RL 2018	Ecology	% mean	N.O.	Size class
Cavinula cocconeiformis	G	o	11	6	3
Cymbopleura frequens	G	o	0	18	4
Delicata delicatula var. *angusta*	G	oc	2	1	
Encyonopsis falaisensis	G	o	0	1	2
Encyonopsis krammeri	G	oc	4	14	1
Encyonopsis lanceola	G	o	0	3	
Encyonopsis subminuta	G	o	2	30	1
Epithemia goeppertiana	G	oc	0	18	5
Eunotia soleirolii	G	od		2	
Fragilaria austriaca	G	oc		1	2
Gomphonema pseudotenellum	G	o	5	16	1
Mastogloia grevillei	G	oc		1	
Mastogloia lacustris	G	oc	0	6	4
Navicula cariocincta	G	?	1	8	3
Navicula oblonga	G	?		3	
Navicula wygaschii	G	oc	0	9	
Neidiomorpha binodiformis	G	oc		1	
Nitzschia lacuum	G	?	1	6	1
Nitzschia oligotraphenta	G	oc	0	2	
Pinnularia irrorata	G	?		8	
Rhopalodia parallela	G	oc		7	

Species belonging to threat categories of the Red List for Central Europe	RL 2018	Ecology	% mean	N.O.	Size class
Brachysira neglectissima	D	oc	16	5	
Crenotia rumrichorum	D	?	20	4	
Cymbella hantzschiana	D	oc	0	3	
Cymbella subcistula	D	o	0	4	5
Diploneis calcilacustris	D	?		1	
Diploneis separanda	D	oc	5	36	2
Humidophila contenta	D	ae	0	4	2
Surirella terricola	D	ae	0	5	3
Ulnaria lanceolata	D	?	6	17	
Ulnaria vitrea	D	?	13	13	
Candidate "Mediterranean / Cypriot" Red List species					
Achnanthidium tepidaricola			35	3	1
Caloneis sp. aff. *pseudoclevei* sp. nov.			0	13	
Craticula mediterranea sp. nov. MN			1	3	
Cymbella kolbei			1	55	5
Cymbella vulgata var. *plitvicensis*		?	1	23	
Delicata judaica			2	3	
Delicata verena			1	9	
Delicata verena var. *sandrae*			2	7	
Encyonema alpiniforme			0	14	

Species belonging to threat categories of the Red List for Central Europe	RL 2018	Ecology	% mean	N.O.	Size class
Surirella helvetica	G	o		2	5
Cymbella kolbei var. *angusta*	R	oc	0	7	
Frustulia spicula subsp. *judaica*	R	ae	1	5	4
Pinnularia kneuckeri	R	ae	0	5	

Species belonging to threat categories of the Red List for Central Europe	RL 2018	Ecology	% mean	N.O.	Size class
Encyonopsis fonticola			1	15	
Gomphonema rosenstockianum			6	77	4
Halamphora sp. aff. o*ligotraphenta* sp. nov.			2	24	
Halamphora sp. aff. *subcapitata* small sp. nov.			1	4	
Mastogloia cyprica sp. nov. MN		hal	0	1	
Navicula cyprica sp. nov.			0	2	
"*Nitzschia* aff. e*broicensis*" (*N. transtagensis*?)				1	
Nitzschia pseudalpina			5	69	
Ulnaria ungeriana			1	11	

Only 14 species were classified as “aerial” (Supplementary Material Table 2): *Fallacia insociabilis*, *Frustulia spicula* subsp. *judaica*, *Halamphora normanii*, *Hantzschia abundans*, *H. amphioxys*, *Humidophila contenta*, *Luticola mutica*, *L. nivalis*, *L. ventriconfusa*, *Pinnularia borealis*, *P. kneuckeri*, *Simonsenia delognei*, *Surirella terricola*, *Tryblionella debilis*. 17 species were classified as halophilic (e.g., *Craticula buderi*, *Entomoneis paludosa* var. *subsalina*, *Fallacia pygmaea*, *Nitzschia dubia*, *N. frustulum*, *Surirella ovalis*). Analyses, similar to those carried out for Red List species (see following section), were performed also to find out more about the distribution of aerial species in non-reference vs reference, perennial (R-M4) vs intermittent (R-M5) intercalibration types, and in the four categories of the hydrological macrotype (not shown) but were mostly not significant and could only show a higher number of aerial species in non-reference sites as compared to reference ($U = 2507$, $Z = 2.319560$, $p = 0.020$, $Z_{\text{adjust.}} = 3.029223$, $p = 0.002$). 70 species were classified as ‘eutraphentic to tolerant’ (Supplementary Material Table 2, Table 9).

We found 29 of the 42 sensitive and 31 of the 55 tolerant diatom taxa listed in Delgado et al. (2012) for temporary Mediterranean island streams (Supplementary Material Table 2).

Size-class values (Rimet and Bouchez, 2012) were available for 209 taxa (Supplementary Material Table 2): 32 taxa (16%) were in size class 1 (biovolume between 0-99 μm^3), 54 taxa (27%) in size class 2 (100-299 μm^3), 42 taxa (21%) in size class 3 (300-599 μm^3), 42 taxa (21%) in size class 4 (600-1499 μm^3), 32 taxa (16%) in size class 5 (>1500 μm^3).

Information on motility, pioneer character and life/growth form of taxa (Rimet and Bouchez, 2012) was available for 209 taxa (Supplementary Material Table 2), with 184 taxa (88%) classified as motile, and 10 taxa (4.8%) known as typical pioneer species (e.g., *Achnanthidium druartii*, *A. jackii*, *A. minutissimum*, *A. polonicum*, *A. tepidaricola*, *Amphora pediculus*). 19 taxa (9%) were classified as adnate, 69 taxa (33%) as pedunculate (stalk or pad attached to substrate), 28 taxa (13%) as ‘pad’ (attached to substrate), 41 taxa (20%) as ‘stalk’ (attached to substrate). 34 taxa (16%) were classified as colonial whilst 175 taxa (84%) as non-colonial. Six different types of colonies were found, in decreasing order: Ribbon colony 16, Mucous tubule colony 6, Zig-zag colony 4, Arbuscular colony 3, Filament colony 2, Rosette colony 2. Information on guild type (Rimet and Bouchez, 2012) was available for 209 taxa (Supplementary Material Table 2), with the four guilds represented as follows in decreasing order: Motile 108, Low profile guild 48, High profile guild 47, Planktonic 6.

3.3. Exploring the distribution of Red-List and candidate Red-List diatom species

Whilst species that are on the Central European Red List showed no preference for reference over non-reference sites, more taxa from the putative Mediterranean / Cypriot Red List were found in non-reference sites than in reference sites, though the overall relative abundance of these two groups showed no significant difference (Table 6a). Similarly, there were no differences in either number of taxa or relative abundance of Central European Red List taxa between the two intercalibration types, R-M4 and R-M5, but the latter (intermittent streams) did have a greater overall relative abundance of putative Mediterranean / Cypriot Red List taxa than the former (Table 6a). A more refined analysis of the influence of hydrology on these Red List taxa showed significant trends for both Central European and Mediterranean / Cypriot Red List taxa to prefer intermittent streams (Table 6b, Fig. 2)

Table 6 Results of the statistical comparisons (Mann-Whitney U Tests [6a] e Kruskall-Wallis Anova [6b] performed to detail the distribution of Red List. Close-to-significant probabilities are italics, significant ($p < 0.05$) probabilities are bold, and highly significant ($p < 0.01$) probabilities are bold and highlighted in light grey.

a

Comparison:	***U***	***Z***	***p***	$Z_{adjust.}$	***p***
- species belonging to all threat categories of the diatom Red List for Central Europe:					
* Number of taxa:					
** non-reference vs reference	3225	-0.029	0.977	-0.029	0.977
** R-M4 vs R-M5	3225	-1.465	0.143	-1.475	0.14
* Σ relative abundances (%):					
** non-reference vs reference	2974	-0.83	0.406	-0.831	0.406
** R-M4 vs R-M5	3109	-1.811	*0.07*	-1.812	*0.07*
- candidate "Mediterranean / Cypriot" Red List species:					
* Number of taxa:					
** non-reference vs reference	2638	1.903	*0.057*	1.972	**0.049**
** R-M4 vs R-M5	3108	-1.814	*0.07*	-1.88	*0.06*
* Σ relative abundances (%):					
** non-reference vs reference	3048.5	-0.592	0.554	-0.595	0.552
** R-M4 vs R-M5	3020	-2.074	**0.038**	-2.084	**0.037**

b

Comparison:	***H***	***p***
- species belonging to all threat categories of the diatom Red List for Central Europe:		
* Number of taxa:		
** Hydrological macrotype (1,2,3,4)	8.939	**0.03**
* Σ relative abundances (%):		
** Hydrological macrotype (1,2,3,4)	10.77	**0.013**
- candidate "Mediterranean / Cypriot" Red List species:		
* presence / absence:		
** Hydrological macrotype (1,2,3,4)	19.178	**< 0.001**
* Σ relative abundances (%):		
** Hydrological macrotype (1,2,3,4)	16.75	**< 0.001**

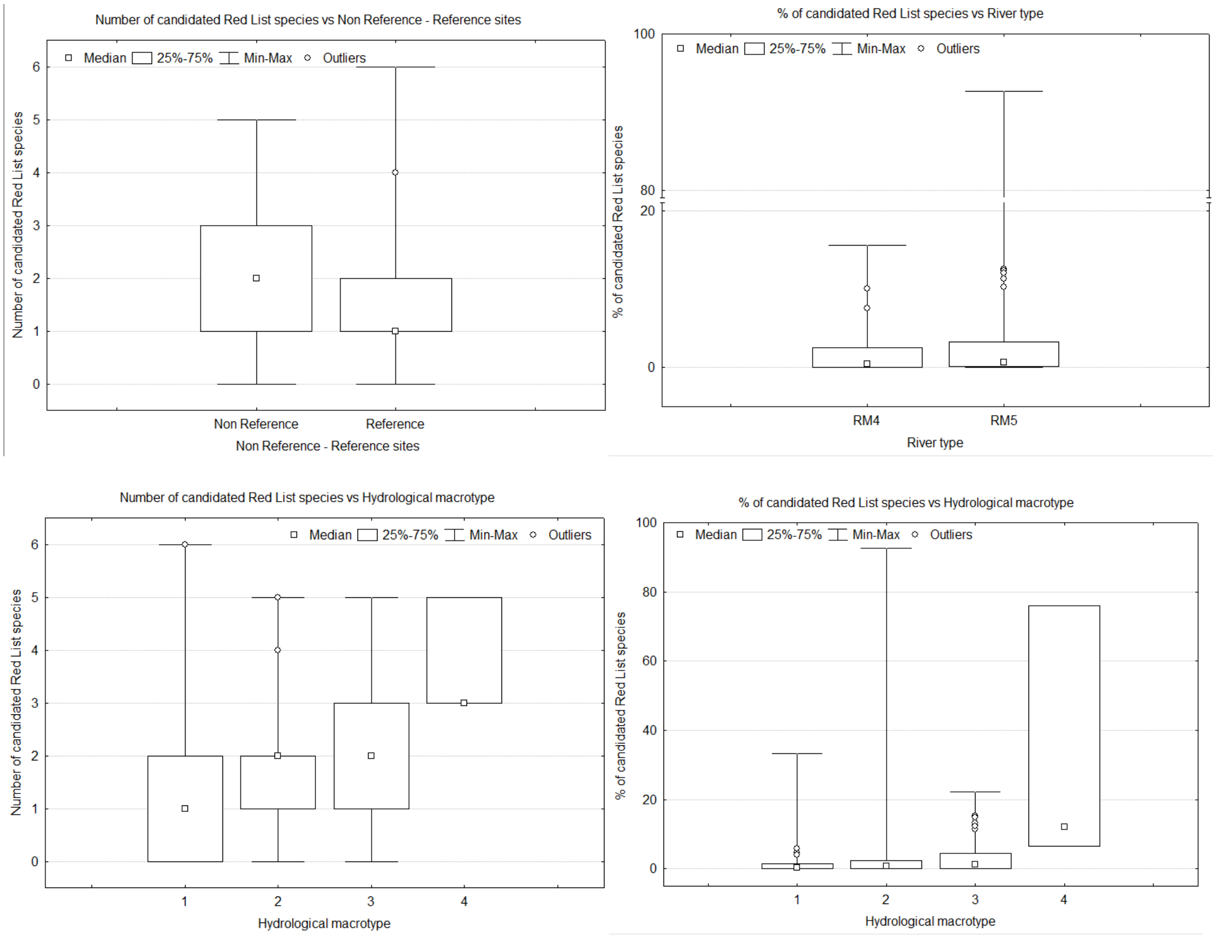

Fig. 2. Selected diagrams showing the results of statistically significant analyses (Table 6) confirming higher numbers / cumulative relative abundance (%) for candidate "Mediterranean / Cypriot" Red List species in non-reference, intercalibration type intermittent (R-M5), and hydrological macrotype 4 ("harshly intermittent") streams.

3.4. Environmental determinants of the diatom assemblages in reference streams

~~Twelve~~ Thirteen sites fulfilled all the reference criteria and were used for to determine the abiotic, non-pressure, and pressure-related factors which shaped diatom assemblages in Cyprus (Table 7). The final dataset of reference samples consisted of 43 samples, from which a total of 211 species were identified. 61 of these were present in less than 5% of samples and so were removed from the dataset (McCune and Grace, 2002)., leaving 150 species in the final data matrix. The 61 species removed had an abundance less than 1%.

Table 7. Main morphological, physical, and chemical characteristics of the reference sites (Abbreviations as in table 4).

Monitoring code	**r1-3-5-05**	**r1-3-6-53**	**r1-4-3-22**	**r1-4-3-35**	**r2-2-5-02**	**r2-3-8-48**	**r2-4-6-65**	**r2-7-2-75**	**r2-8-3-10**	**r3-1-2-30**	**r3-1-2-30_u/s**	**r3-3-1-60**	**r3-3-1-63**
R-M4_5	RM4	RM4	RM5	RM5	RM5	RM4	RM5	RM5	RM5	RM5	RM5	RM4	RM4
Hydrological macrotype	1a	2a	3a	3a		1b	3a	2a	2a	3a	3a	1b	1b
Reference (Y/N)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
HQA	4	3	4	4	3	3	4	4	4	4	4	4	4
HMS	1	1	1	1	1	1	2	2	1	1	1	1	1
LUIcara	1	1	1	1	1	1	1	0	1	1	1	1	1
OPD Mean	0,88	0,89	0,94	0,89	0,90	0,93	0,90	0,89	1,00	1,00	0,95	0,90	0,91
Instant Discharge (m^3/s)	0.2825	N/A	0.06	0.18067	0.02	0.022	0.016	0.38625	0.48056	0.3175	0.1	0.2748	N/A
pH	8,34	8,04	8,50	8,32	8,42	8,28	8,56	8,10	7,98	8,11	8,22	8,83	N/A
EC field_ Us (µS/cm)	423	629	556	532	690	677	752	501	519	444	448	496	512
DO%_field (%)	107	86	105	114	95	112	83	89	92	88	99	90	65
Temp (°C)	13,7	17,7	16,5	13,6	19,4	14,1	18,3	15,9	15,3	14,6	15,6	12,5	11,3
Turb_field (NTU)	0,97	N/A	2,25	1,24	0,27	3,54	0,05	0,76	1,70	1,10	0,57	1,57	N/A
$\mathbf{SO_4^{2-}}$ (mg/L N)	50,0	86,0	39,7	75,0	59,6	48,9	60,5	54,1	54,5	N/A	29,9	10,7	N/A
$\mathbf{Cl^-}$ (mg/L N)	41,6	47,2	44,2	102,2	52,4	65,2	66,0	53,0	34,4	N/A	27,1	23,9	38,0
$\mathbf{Na^+}$ (mg/L N)	30,2	50,6	35,7	65,0	37,0	59,2	54,9	37,8	28,3	N/A	24,8	12,1	N/A
$\mathbf{BOD_5}$ (mg/L O_2)	1,00	1,65	0,50	1,17	3,00	1,17	1,50	1,00	0,83	0,50	0,50	1,00	0,50

Monitoring code	**r1-3-5-05**	**r1-3-6-53**	**r1-4-3-22**	**r1-4-3-35**	**r2-2-5-02**	**r2-3-8-48**	**r2-4-6-65**	**r2-7-2-75**	**r2-8-3-10**	**r3-1-2-30**	**r3-1-2-30_u/s**	**r3-3-1-60**	**r3-3-1-63**
COD (mg/L O_2)	2,88	2,68	10,00	3,00	3,50	2,00	3,50	3,75	3,00	3,00	10,00	3,10	0,50
coli_total (/100mL)	348,3	26,1	N/A	301,3	0,5	100,0	0,5	331,0	495,3	236,0	276,0	80,7	N/A
N_total (µg/L N)	725	338	100	250	100	100	100	450	475	525	100	250	N/A
NO_3^--N (µg/L N)	56	24	2	256	2	100	2	57	30	93	2	150	119
P_total (µg/L P)	6	3	1	5	1	3	1	3	12	3	1	4	10

Reference sites were also characterized by the co-occurrence of several species from the same pollution-sensitive genera such as *Achnanthidium* and *Gomphonema* (Fig. 3a,c). In the case of *Achnanthidium*, the number of taxa recorded was significantly higher ($P < 0.001$) in reference compared to non-reference sites whilst the total percentage of valves did not show a significant difference (Fig. 3a,b). For *Gomphonema*, on the other hand, both number of taxa and the percent of valves were higher in reference sites (Fig. 3c,d). The opposite tendency was seen for genera which are typically associated with enriched sites: species of both *Navicula* and *Nitzschia* were more numerous, and their overall percentage greater, in non-reference sites (Fig. 3e,f,g,h).

A four-factor PERMANOVA analysis, comparing taxa composition based on year, season, hydrological macrotype and intercalibration type showed no significant effect was detected for year, whilst season was significant (partial R^2=0.09, P=0.003). Both type variables were significant: hydrological macrotype (partial R^2=0.15, P=0.001) and intercalibration type (partial R^2=0.09, P=0.001) (Table 8). No significant interactions were observed between factors.

Table 8. Results of the Permanova performed on reference sites, using 999 permutations, with: i) Inter-calibration type (ICT); ii) hydrological macrotype (HMn); iii) year and iv) season. Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	**Sums of squares**	**Mean squares**	**F.Model**	**R^2**	**Pr(>F)**
Season	2	1.053	0.526	2.466	0.092	0.003**
ICT	1	1.012	1.012	4.739	0.089	0.001***
HMn	3	1.685	0.562	2.632	0.148	0.001***
Year	1	0.354	0.354	1.656	0.031	0.094
Season:ICT	2	0.343	0.171	0.803	0.030	0.711
Season:HMn	5	1.065	0.213	0.998	0.094	0.473
Season:Year	1	0.225	0.225	1.053	0.020	0.401
ICT:Year	1	0.163	0.163	0.764	0.014	0.674
HMn:Year	3	0.666	0.222	1.040	0.058	0.425
Season:HMn:Year	2	0.347	0.173	0.812	0.030	0.722
Residuals	21	4.483	0.214		0.39339	
Total	42	11.395			1	

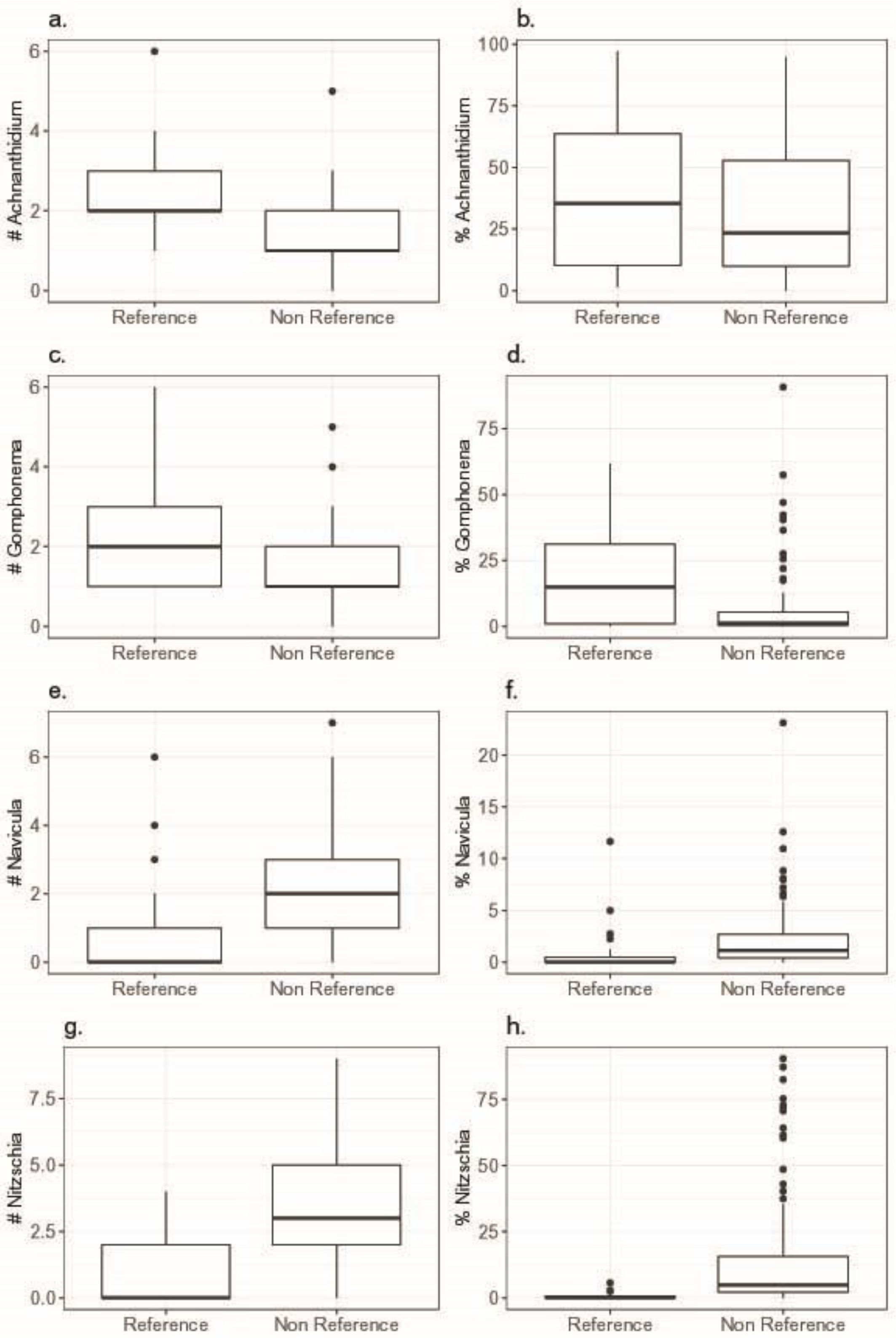

Fig. 3. Differences in the number of species and percent of *Achnanthidium*, *Gomphonema, Navicula* and *Nitzschia* recorded at reference and non-reference sites in Cypriot streams.

Agglomerative hierarchical cluster analysis of the reference samples revealed three end-groups, consisting of 13, 12, and 18 samples (Fig. 4). The median Bray-Curtis dissimilarity was 0.72 (mean value: 0.70) with an inter-quartile range from 0.61 to 0.83. Visual assessment of the dendrogram and breaks in slope of the agglomeration function indicated the selection of three groups as an optimal clustering and an *adonis* analysis confirmed that there were significant differences among diatom assemblages in these groups (*adonis*, F = 7.69, $R^2 = 0.27$, p = 0.001). The final stress value in the 3-dimensional NMDS ordination was 13.5. End-groups 1 and 2 from the cluster analysis had low scores on axis 2, but were separated along axis 1. End group 3, by contrast, was differentiated from groups 1 and 2 by a higher score on axis 2 (Fig. 5).

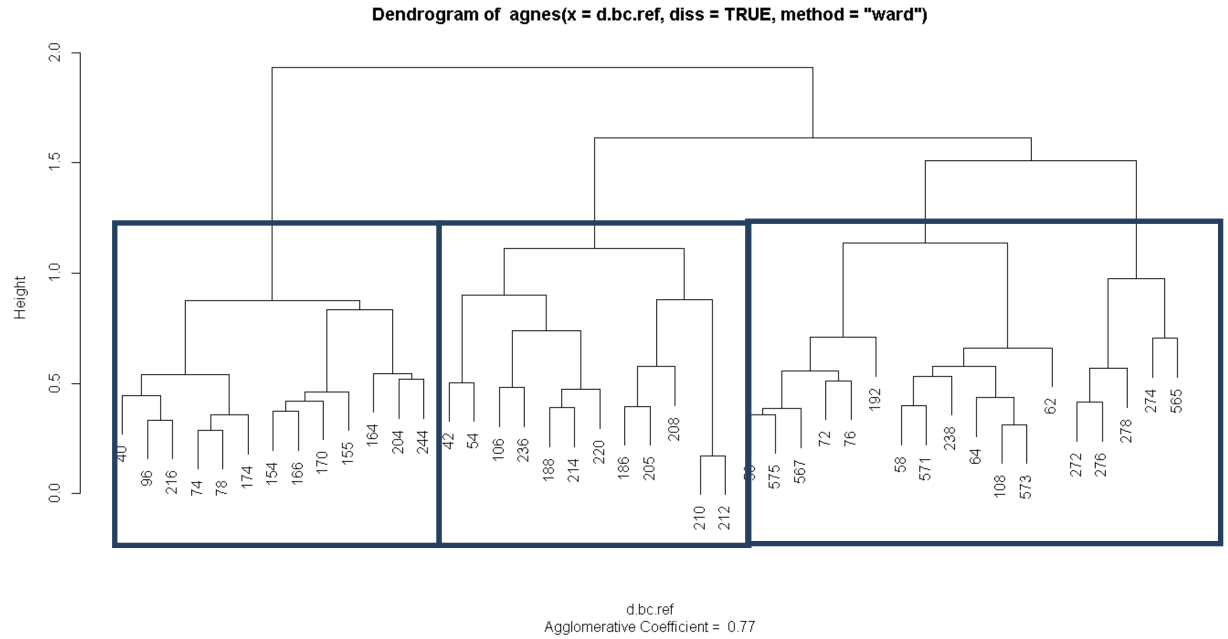

Fig. 4. Dendrogram showing the agglomerative structure of the diatom matrix (Reference sites).

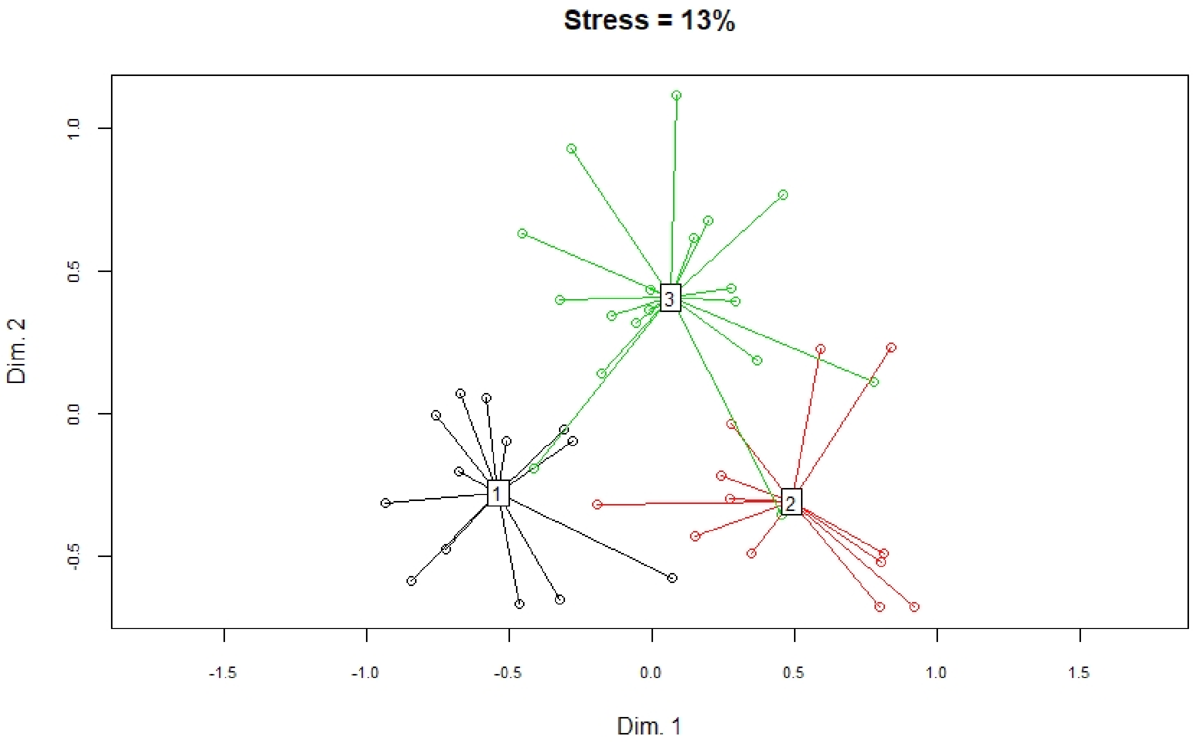

Fig. 5. Nonmetric multidimensional scaling (NMDS) ordination of the diatom data from the reference sites showing that the stress values and samples were clustered by the group identified with the agglomerative hierarchical clustering using the function *ordispider* of the R package *vegan*.

Achnanthidium minutissimum, *A. minutissimum* spp. gr., *Cocconeis euglypta*, *Gomphonema pumilum* var. *rigidum*. were common in all reference sites. Group 1 was further characterized by: *Amphora micra, Reimeria uniseriata, Navicula cryptotenelloides, Navicula tripunctata, Rhoicosphenia abbreviata*. Group 2 was further dominated by *Ulnaria monodii, Gomphonema rosenstockianum, Diatoma moniliformis, A. straubianum, A. saprophilum, A. polonicum*. Further common species in Group 3 were *Ulnaria monodii, G. tergestinum*, *Cymbella vulgata*, *Diatoma moniliformis, Gomphonema rosenstockianum* (Table 9).

IndVal analysis confirmed the presence of significant indicators for each group, and these are listed in Table 10.

Table 9. Characteristics of the diatom indicator species identified by IndVal (in bold) and of the species most abundant in the three main groups obtained with the agglomerative clustering for reference sites. Size class 1 to 5 as reported in Rimet and Bouchez (2012).

	Ecology Hofmann et al., 2018	% MEAN	Number of Occurrences	Sensitive Delgado et al., 2012	Tolerant Delgado et al., 2012	Size class	Motile	Pioneer	Adnate	Pedunculate (stalk or pad)	Pad (attached to substrate)	Stalk (attached to substrate)	Colonial	High profile guild	Low profile guild	Motile guild
Achnanthidium minutissimum	?	28	128	X		1	X	X		X		X			X	
***Achnanthidium minutissimum* spp. gr.**		13	44			1	X	X		X		X			X	
Achnanthidium polonicum		12	32			1	X	X		X		X			X	
Achnanthidium saprophilum	eu	11	13			1	X	X		X		X			X	
Achnanthidium straubianum	?	4	20		X	1	X	1		X		X			X	
Adlafia bryophila	?	1	5			2	X									X
Amphora micra		7	77													
Cocconeis euglypta	?	27	137		X	5	X		X						X	
Cymbella vulgata	?	1	61	X		3	X			X		X			X	
Diatoma moniliformis	eu	4	73	X		3				X	X		1	X		
Gomphonema pumilum var. *rigidum*	?	8	99	X		2	X			X		X		X		
Gomphonema rosenstockianum		6	77	X		4	X			X		X		X		
Gomphonema tergestinum	?	6	55			4	X			X		X		X		
Navicula antonii	eu	1	30			3	X									X
Navicula cryptotenelloides	?	1	62	X		2	X									X
Navicula simulata	eu	0	19			4	X									X
Navicula tripunctata	eu	1	74		X	4	X									X
Reimeria uniseriata	?	1	81	X		3	X		X	X		X			X	
Rhoicosphenia abbreviata	eu	2	55		X	3				X		X	X		X	
Ulnaria monodii		9	126			5				X	X		X	X		

Table 10. IndVal results for key indicator species for the different biological groups identified with the agglomerative analysis of the diatom matrix for reference sites. Significant (p) codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘. ‘A’ is the specificity of the species as an indicator of the site group whilst ‘B’ is the fidelity, or sensitivity of the species as an indicator of the group.

Group 1

Species name	**A**	**B**	**Stat**	**P value**
Navicula tripunctata	1	0.462	0.679	***
Reimeria uniseriata	0.847	0.538	0.675	***
Rhoicosphenia abbreviata	0.987	0.385	0.616	**
Adlafia bryophila	1	0.231	0.48	*
Navicula simulata	1	0.231	0.48	*

Group 2

Species name	**A**	**B**	**Stat**	**P value**
Achnanthidium polonicum	0.842	0.833	0.838	***
Achnanthidium minutissimum spp. gr.	0.761	0.75	0.756	***
Achnanthidium straubianum	0.858	0.5	0.655	**
Achnanthidium saprophilum	0.823	0.417	0.586	*
Navicula antonii	0.783	0.333	0.511	*

Group 3 #sps. 1

Species name	**A**	**B**	**Stat**	**P value**
Cymbella vulgata	1	0.222	0.471	*

The groups identified with the agglomerative hierarchical cluster analysis were also significantly different in terms of a number of key environmental variables: lentic-lotic character of the streams (LRDall, Kruskal-Wallis Test=9.6, P=0.008, Fig. 6A), granulometry and substrate type found at the stream’s sites (Substrate, Kruskal–Wallis Test=11.5, P=0.008, Fig. 6B); pH (Kruskal–Wallis Test=12.6, P=0.002, Fig. 6C) and instant discharge (m^3/s, Kruskal–Wallis Test=13.5, P=0.001, Fig. 6D). No significant effect was detected with respect to Valley Slope (%), distance from the source (km), and catchment size (km^2).

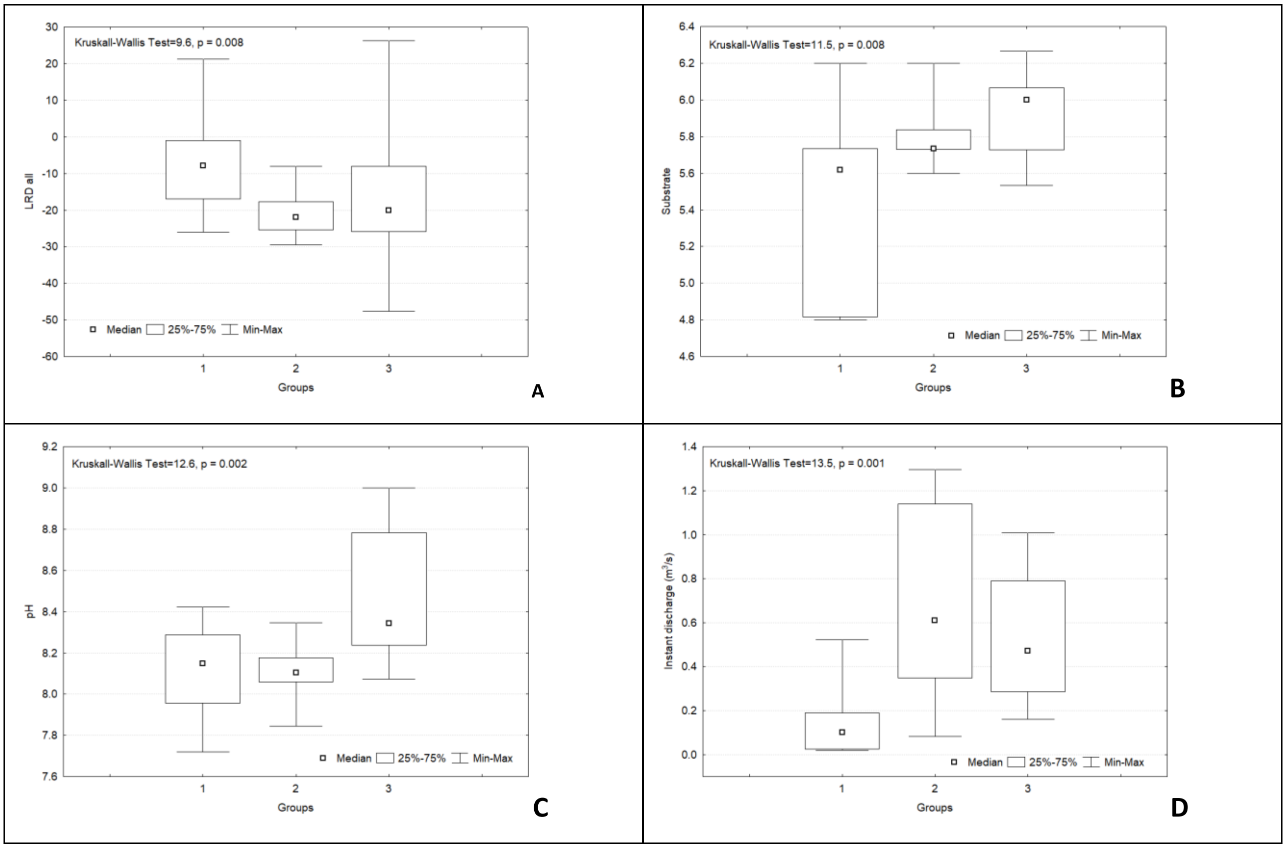

Fig. 6. Box plot and Kruskal–Wallis Test for a selection of environmental variables for the groups identified with the agglomerative hierarchical cluster analysis (reference sites)

Factors responsible for driving the composition of these three groups were then investigated by discriminant analysis following preliminary stepwise ordinations to select potential drivers from the categories stream type and hydrology. A series of individual stepwise ordination regressions enabled a set of Type and Hydrological variables to be selected: i) variables related to river type were included in the first RDA: total channel width (m), maximum water depth (m), valley form and source distance (Km); ii) variables related to river hydrology were included in the second RDA: LRD, catchment yield (L/s/km^2), mean annual flow (m^3) and median number of zero days. Both models were significant when tested using an ANOVA with 999 permutations ($p < 0.001$: Table 11) as were the single variables included in the analysis, with the exception of median number of zero days in the hydrological model ($p = 0.076$). Source distance and channel form were the most important type variables for shaping the diatom assemblage, whilst catchment yield and mean annual flow were the key hydrological ones.

Table 11. Output of an ANOVA performed on reference sites, using 999 permutations, for the Type and Hydrological RDA models, including details on the relative contribution of each selected variables. Significant (p) codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘.

	Variables	Df	Variance	F	Pr(>F)
Type	**Model**	4	0.138	2.715	***
	Source Distance	1	0.039	3.079	**
	Erosion - Bank	1	0.030	2.393	*
	Valley form	1	0.027	2.119	*
	Channel Form	1	0.040	2.393	***
	Residual	38	0.482		
Hydrological	**Model**	3	0.111	2.847	***
	Catchment yield	1	0.050	3.877	***
	Mean annual flow	1	0.031	2.380	*
	Median number of zero days	1	0.023	1.791	.
	Residual	39	0.508		

Overall, 28% of the total variance in the diatom dataset was explained by these abiotic variables, with type (10%) and hydrology (12%) explaining the largest proportions (Fig. 7). Although season explained a smaller proportion of the total variation, group 2 was composed entirely of samples collected during the winter. The level of interaction between the three classes of variables was relatively low (no more than 1% of the total variation).

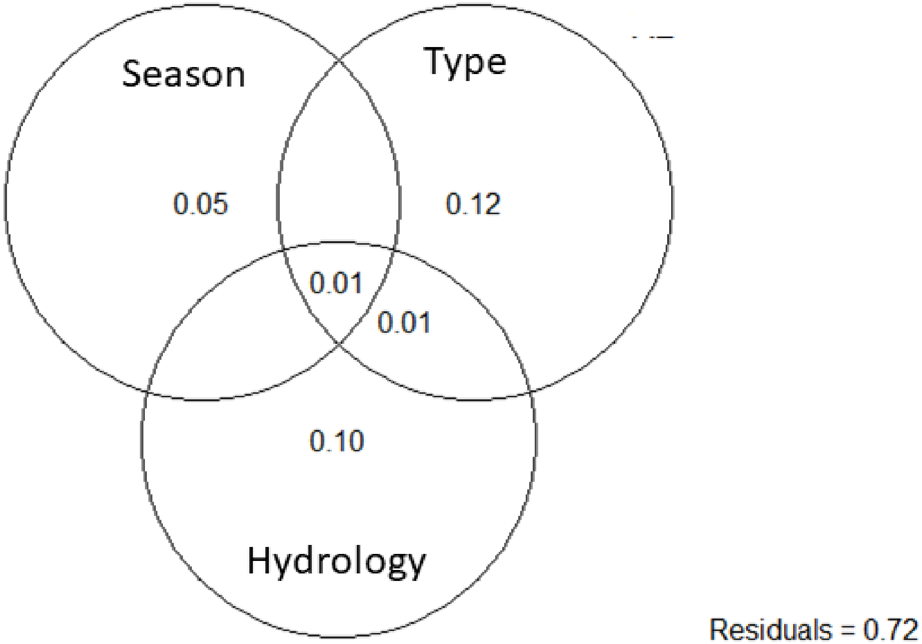

Fig. 7. Outcome of the variance partitioning (reference sites) for the diatom assemblage considering type, hydrological and season as drivers.

4. Discussion

This is the first paper describing the diatom assemblages of Cypriot streams, providing a robust ecological, as well as taxonomic, characterization of the assemblages, as a benchmark for future work. Our results highlight the overwhelming importance of hydro(geo)logy in shaping the diatom assemblages of stream sites (the least-impacted ones, in particular) on the island of Cyprus. Almost all explained variance was due to hydrological factors and to stream type, which, again, is mainly identified on hydrogeological grounds. Hydromorphological impacts, including water-level and discharge fluctuations in lakes, rivers, and streams are increasingly recognized as one of the main and insufficiently addressed impacts in the EU (Poikane et al., 2019).

4.1 The importance of hydrology

Even a tentative analysis of our diatom dataset using Red-List criteria underlined the overwhelming importance of hydrology-related variables and river types in determining diatom species distribution and community ecological attributes in the water-stressed Island of Cyprus. Species that fulfil the descriptions of Red List threat categories, and which might be included in a future Cypriot / eastern

Mediterranean Red List, occurred more frequently and were more relevant in assemblages from intermittent (RM5) as compared to perennial (RM4) streams (Table 6a, Fig. 2), and in the harshly intermittent hydrological macrotype (Table 6b, Fig. 2). A similar trend was seen when the threat categories of the diatom Red List for Central Europe (2018) were used, although differences were only significant between stream hydrotypes (Table 6b).

This contradicts the idea of higher diversity and percentages of endangered Red List species in groundwater-fed, permanent (stable) streams. Falasco et al. (2016), for example, reported that Mediterranean streams with more stable discharge (as compared to intermittent ones) had higher numbers of endangered species, and even proposed a current-velocity threshold (0.2 m/s) to maintain abundant occurrence of endangered species. However, to identify Red List species in threat categories they used the only diatom Red List available at that time (Diatom Red List for Germany: Lange-Bertalot, 1996). It is reasonable to assume that sensitive Red-List species with a relatively wide distribution (i.e. occurring both in Germany and in Mediterranean streams) may be adapted to streams retaining some discharge and current velocity across the seasons. On the contrary, characteristic species of Mediterranean streams are likely to be adapted to the marked, often extreme, seasonal and inter-annual discharge fluctuations of these environments.

Many diatom taxa recorded in this study could be found in the recently published Red List for Central Europe (Hofmann et al., 2018). The amount of diatom diversity at the landscape level found in these 65 stream sites (290 taxa, 273 identified to the species -or intraspecific- level, belonging to 65 genera), and the percentage of species belonging to threat categories of the Central European Red List (27.5%) is about half that found in high-ecological integrity environments of the Alps. As an indirect comparison, 15 springs (unique and highly diverse habitats) of a mainland Mediterranean region(Emilia-Romagna: northern Apennines and Po floodplain: Cantonati et al., 2020b) had an overall species richness of 285 taxa (272 identified to the species -or intraspecific- level) belonging to 63 genera, with a high proportion (60%) of the species for which Central European Red List information was available found to belong to one of the threat categories. This indicates that streams, not all of which are in pristine condition, still retain the potential to host diatom assemblages which are relatively rich in threatened Red List species (both widely-distributed Central European Red List species and putative Mediterranean Red List species, see below).

However, several taxa found during our Cyprus study were not included in the most recent Red List available (Hofmann et al., 2018), and, most important, these taxa include sensitive species which are likely to be highly characteristic of the Mediterranean and/or Middle East region (e.g., *Delicata judaica*, *D. verena*, *U. ungeriana*). This reinforces the need to apply Red Lists outside the biogeographical areas where they have been developed with caution. A much refined and effective approach would be the development of a diatom Red List for Cyprus based on revised and harmonized project databases, ideally with an associated iconography. This could also serve as an excellent basis on which to anchor new knowledge that will be produced by the application of molecular approaches to typical Mediterranean and local taxa.

Among the diatom species we found, there is a preponderance of motile, medium- to small-sized, species. A low number of species has the capability to form colonies, with ribbon and mucous-tubule colony type being the most common.

We found several species known to be effective first colonizers (pioneer species) and, among these, there is a striking preponderance (80%) of *Achnanthidium* species. *Achnanthidium tepidaricola* deserves a special mention as it was described from the wet stonewall situated in the Evolutionary Greenhouse in the National Botanic Garden of Belgium where it formed an almost pure monoculture (Van de Vijver et al., 2011a). The stones used to build the wet stonewall were imported from the southern Anatolian peninsula (Bart Van de Vijver, personal com.). In this study in Cyprus, *A. tepidaricola* was found in three samples only, being very abundant only at one site. However, in a follow up project in Cyprus in 2019, *A. tepidaricola* was found 16 times with a maximum abundance of 391 valves out of 400 (MC unpublished data). It is possible, therefore, that *A. tepidaricola* is a species indigenous to the eastern Mediterranean that has been largely overlooked until now.

The frequent and abundant occurrence of *Achnanthidium* pioneer species in our dataset could be an adaptation to extreme flow reduction and the consequent need to quickly recolonize stream stretches. This could explain the striking co-occurrence of several, apparently similar, *Achnanthidium* species. As an application of the redundant species hypothesis (e.g., Lawton and Brown, 1994; Ehrlich and Walker, 1998) to benthic diatom communities in Mediterranean streams, we hypothesize that the abundant occurrence of species within genera (Fig. 3) might serve to improve the resilience capability of ecosystems.

The number of aerial species found in our study was lower than expected for Mediterranean streams with strongly fluctuating hydrological regimes. This may in part be explained by the fact the autecology of several species is still not known in detail, and some pseudaerial and euaerial species can thus occur unnoticed. For instance, *Eunotia arcubus* was noted in our study in Cyprus as a Red List species of oligotrophic carbonate streams (Table 5) but studies on springs of the Alps (Cantonati et al., 2012) show it to be also typical of unstable, low-discharge carbonate environments. *E. arcubus*, along with the well-known and widespread aerial species *Humidophila contenta* (e.g., Cantonati et al., 2017), can be found among the tolerant species in the lists of species sensitive and tolerant to organic pollution provided by Delgado et al. (2012) for insular Mediterranean temporary streams, suggesting that there still is some confusion on the major environmental determinant for these species. It is possible that occurrence of multiple aerial species in a single sample might be more significant than that of any single species (Kelly, 2019).

Hydrology was a dominant factor also in the outcomes of the other analyses carried out. The four-factor PERMANOVA analysis found that all type (essentially hydrology-related) variables were significant. No significant effect was detected for the year, whilst season was significant. However, our follow up projects on Cyprus streams' diatoms (MC unpublished observations) showed that the (unpredictable) inter-annual differences can be even more dramatic than the (predictable) seasonal changes (e.g., Cantonati et al., 2020a). The most important differences between years and season are, again, primarily related to hydrological regimes. Agglomerative hierarchical cluster analysis revealed three end-groups, with groups being separated on ecohydro(geo)logical (in the sense of Cantonati et al., 2020c) grounds: - Group 1 was lentic, with fine sediments, and dominated by motile species; - Group 2, composed of samples collected during winter, had the highest discharge, coarse lithic substrata and was dominated by low-profile, pedunculate or adnate, pioneer species; - Group 3 had average discharge and substrate grain size and high pH, with oligotraphentic, sensitive, Red-List species (Table 9-10, Supplementary Material Table 2).

The stepwise ordinations to select potential drivers of these three groups from the categories stream type and hydrology by discriminant analysis confirmed the statistical significance of almost all selected variables. It should be note that also the river-type variables (channel width, water depth, valley form, and source distance) were evidently hydro(geo)logy-related. Overall, 28% of the total variance in the diatom dataset was explained by the selected abiotic variables, with (hydrology-related) type (10%) and hydrology (12%) explaining the largest proportions.

4.2 Opportunities for improved ecological assessment

These results all suggest an opportunity for more refined ecological assessment of Mediterranean streams using phytobenthos. In contrast to the relatively conservative approaches both to use of metrics and definition of stream types used at present (Almeida et al., 2014). We see potential for using information about hydrological regime both define stream types in a more meaningful manner. At the same time, this could start a move away from the current reliance on metrics such as the Indice de Polluosensibilité Specifique (IPS: Coste, in CEMAGREF, 1982) and Indice Biologique Diatomées (Coste et al., 2009) that assume that chemical pressures predominate. Given the urgency of the climate crisis, the new generation of metrics for the Mediterranean region needs to also be sensitive to hydrological stresses. However, rather than focus on individual stresses per se this study suggests opportunities for using fundamental ecosystem properties that should, in theory, be responsive to a wide range of pressures.

The first opportunity suggested by our data is the refinement of “expected” values, which form the denominator in calculations of Ecological Quality Ratio. Currently, these are typically average values for a metric for all sites in least disturbed condition for a particular stream “type” (Feio et al., 2014). This typology used for WFD assessments in the Mediterranean region is, however, not particularly sensitive discriminating only between permanent and intermittent streams (Almeida et al., 2014). Our study suggests a number of additional variables that could be used to refine this typology, one of which is season. There are precedents (Kelly et al., 2008) and, also, a strong theoretical case in the Mediterranean Basin, given the strong seasonal hydrological patterns, combined with the short life cycles of diatoms. One problem with using hydrology to refine the typology is that changes may be expected due to global warming. It is important, under such circumstances, to use a fixed point in time as a benchmark against which future changes may be measured (Kelly et al., 2019).

The second opportunity is for the development of a set of metrics that can complement IPS for stream assessments in Cyprus and elsewhere in the Mediterranean Basin. The first of these would be the use of Red List species which, we have already shown, are capable of discriminating between reference and non-reference sites (Table 6a). Whilst the focus of most ecological assessment has been the definition of “good ecological status”, as this should be consistent with sustainable use of aquatic resources (Kelly, 2013), detailed knowledge of Red List species specific to a region could provide criteria for objective discrimination of high ecological status (in a similar way as done to designate tier 1 sites, representing “natural, or undisturbed”, in the Biological Condition Gradient; see e.g. Paul et al. 2020).

Another possibility is to make more use of diversity within genera. Whilst traditional measures of diversity are problematic when applied just to diatoms (Denicola & Kelly, 2014) and are rarely insightful (e.g. Blanco et al., 2012), the greater diversity of *Achnanthidium* and *Gomphonema* at reference sites (Fig. 3a,c) suggests that the time might be ripe for revisiting this. We hypothesise that the co-existence of several species from a single genus imparts resilience to the biofilm community and, therefore, high diversity within the reference assemblage could complement metrics such as the IPS in future assessments.   Furthermore, the opposite trend shown by genera such as *Navicula* and *Nitzschia* (Fig. 3e,g), generally associated with more enriched conditions, is both a reminder that resilience is a two-tailed phenomena, with the scope for several taxa to exploit niches within a degraded or enriched community, and a clue to the poor performance of diversity metrics in earlier studies. As the diversity associated with the expected community decreases, so increased diversity of genera which are adapted to these conditions dampens any signal that traditional diversity metrics impart. Within the wide suite of methods currently used for assessments for the WFD (Birk et al., 2012), those using freshwater phytobenthos are relatively unusual in the absence of diversity metrics and the time is ripe for a re-examination of the role of this measure.

4.3 Conclusion

This study has demonstrated the major role played by hydrology in determining diatom assemblages in a eastern Mediterranean streams and highlighted the need for greater recognition of this in ecological assessment protocols.   As for the terrestrial flora of Cyprus (Christofides, 2017), diatom assemblages show a degree of endemism, with several new species being recorded (Cantonati et al., 2016, 2018) described, with others recognised but yet to be formally described. This combination of harsh climate and geographical isolation means that assessment concepts developed elsewhere in Europe should be transferred to Cyprus with caution.   Whilst it has been possible to adapt the IPS to produce assessments of ecological status that are harmonised with those from elsewhere in the Mediterranean Basin (Almeida et al., 2014), there is potential for developing a new suite of metrics that will better reflect ecological conditions here and ensure responsible management of the Cypriot environment.

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References

Acuña, V., Datry, T., Marshall, J., Barceló, D., Dahm, C.N., Ginebreda, A., McGregor, G., Sabater, S., Tockner, K., Palmer,M.A., 2014.Why should we care about temporary waterways? Science 343, 1080–1081.

Almeida S.F., Elias C., Ferreira J., Tornés E., Puccinelli C., Delmas F., Dörflinger G., Urbanič G., Marcheggiani S., Rosebery J., Mancini L., Sabater S. 2014. Water quality assessment of rivers using diatom metrics across Mediterranean Europe: a methods intercalibration exercise. Science of the Total Environment 476-477:768-76. doi: 10.1016/j.scitoten.v.2013.11.144

Anderson, M. J. (2001). A new method for a non-parametric multivariate analysis of variance. Austral Ecology, 26, 32– 46

ARCADIS, 2006. Diatom Survey and Water Quality Assessment in the Arda Basin According to the EU-WFD, PROJECT PHARE BG 2003/005-630.05. Technical Assistance for Water Quality Management of Arda River.

Artigas, J., Fund, K., Kirchen, S., Morin, S., Obst, U., Romanı´, A. M., Sabater, S., and Schwartz, T. 2012. Patterns of biofilm formation in two streams from different bioclimatic regions: analysis of microbial community structure and metabolism. Hydrobiologia 695, 83–96. doi:10.1007/S10750-012-1111-3

Baker, D.B., Richards, R.P., Loftus, T.T. and Kramer, J.W. (2004). A new flashiness index: Characteristics and applications to midwestern rivers and streams. JAWRA Journal of the American Water Resources Association, 40(2), pp.503–522.

Balestrini, R., Cazzola, M., Buffagni, A., 2004. Characterising hydromorphological features of selected Italian rivers: a comparative application of environmental indices. Hydrobiologia 516, 365–379.

Birk, S., Bonne, W., Borja, Á., Brucet, S., Courrat, A., Poikane, S., Solimini, A., Van De Bund, W., Zampoukas, N., Hering, D., 2012. Three hundred ways to assess Europe’s surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. Ecological Indicators 18, 31–41. doi:10.1016/j.ecolind.2011.10.009

Birk, S., Willby, N.J., Kelly, M.G.**,** Bonne, W., Borja, A., Poikane, S. & van de Bund, W. (2013). Intercalibrating classifications of ecological status: Europe’s quest for common management objectives for aquatic ecosystems. *Science of* the Total Environment 454-455: 490-499.

Blanco, S., Cejudo-Figueiras, C., Tudesque, L., Bécares, E., Hoffmann, L., & Ector, L., 2012. Are diatom diversity indices reliable monitoring metrics? Hydrobiologia 695, 199-206.. https://doi.org/10.1007/s10750-012-1113-1

Bonet B., Corcoll N., Acuňa V., Sigg L., Behra R., Guasch H. 2013. Seasonal changes in antioxidant enzyme activities of freshwater biofilms in a metal polluted Mediterranean stream. Science of The Total Environment, 444: 60-72. https://doi.org/10.1016/j.scitotenv.2012.11.036.

Boccard et al., 2011 D. Boccard, F. Gillet, P. Legendre Numerical Ecology with R. Springer (2011), p. 306

Bouleau. G. & Pont, D. (2014). Did you say reference conditions? Ecological and socio-economic perspectives on the European Water Framework Directive. Environmental Science and Policy doi: 10.1016/j.envsci.2014.10.012.

Buffagni A., Kemp J. L. (2002): “Looking beyond the shores of the United Kingdom: addenda for the application of River Habitat Survey in South European rivers”. J. Limnol. 61 (2): 199-214.

Buffagni, A. Ciampittiello M., ERBA S. 2005. Il River Habitat Survey Sud Europeo: Principi e schede di applicazione. Notiziario dei Metodi Analitici Ist. Ric. Acque. Dicembre 2005

Buffagni, A., Armanini, D.G., Erba, S., 2009. Does the lentic–lotic character of rivers affect invertebrate metrics used in the assessment of ecological quality? J. Limnol. 68 (1), 92–105.

Buffagni, A., Erba, S., Armanini, D.G., 2010. The lentic–lotic character of Mediterranean rivers and its importance to aquatic invertebrate communities. Aquat. Sci. 72, 45–60.Buffagni, A., D. Demartini & L. Terranova, 2013. Manuale di applicazione del metodo CARAVAGGIO - Guida al rilevamento e alla descrizione degli habitat fluviali. 1/i. Monografie dell'Istituto di Ricerca Sulle Acque del C.N.R., Roma (301 pp, ISBN: 9788897655008) [available on internet at https://www.life-inhabit.it/it/download/tutti-file/doc_download/123-manuale-caravaggio] (In Italian).

Buffagni, A., Demartini, D., Terranova, L., 2013. Manuale di applicazione del metodo CARAVAGGIO – Guida al rilevamento e alla descrizione degli habitat fluviali. Monografie dell'Istituto di Ricerca Sulle Acque del C.N.R., Roma 1/i, pp. 293.

Calapez, A. R., Elias, C. L., Almeida, S. F. P., and Feio, M. J. 2014. Extreme drought effects and recovery patterns in the benthic communities of temperate streams. Limnetica 33, 281–296.

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

Cantonati M., Angeli N., Spitale D. & Lange-Bertalot H. 2016. A new *Navicula* (Bacillariophyta) species from low-elevation carbonate springs affected by anthropogenic disturbance. Fottea 16: 255–269. DOI: 10.5507/fot.2016.013 With supplementary materials (Plate & Table).

Cantonati M., Kelly M.G. & Lange-Bertalot H., 2017. Freshwater benthic diatoms of Central Europe: over 800 common species used in ecological assessment. Koeltz Botanical Books, Schmitten-Oberreifenberg, Germany, 942 pp.

Cantonati M., Lange-Bertalot H., Kelly M. & Angeli N. 2018. Taxonomic and ecological characterization of two *Ulnaria* species (Bacillariophyta) from streams in Cyprus. Phytotaxa 346(1): 078-092. https://doi.org/10.11646/phytotaxa.346.1.4

Cantonati M., Poikane S., Pringle C.M., Stevens L.E., Turak E., Heino J., Richardson J.S., Bolpagni R., Borrini A., Cid N., Čtvrtlíková M., Galassi D.M.P., Hájek M., Hawes I., Levkov Z., Naselli-Flores L., Saber A.A., Di Cicco M., Fiasca B., Hamilton P.B., Kubečka J., Segadelli S., Znachor P. 2020a. Characteristics, Main Impacts, and Stewardship of Natural and Artificial Freshwater Environments: Consequences for Biodiversity Conservation. Water 12: 260. DOI: 10.3390/w12010260

Cantonati M., Stevens L.E., Segadelli S., Springer A.E., Goldscheider N., Celico F., Filippini M., Ogata K. & Gargini A. 2020b. Ecohydrogeology: The interdisciplinary convergence needed to improve the study and stewardship of springs and other groundwater-dependent habitats, biota, and ecosystems. Ecological Indicators 110. DOI: 10.1016/j.ecolind.2019.105803

Cantonati M., Segadelli S., Spitale S., Gabrieli J., Gerecke G., Angeli N., De Nardo M.T., Ogata K. & Wehr J.D. 2020c. Geological and hydrochemical prerequisites of unexpectedly high biodiversity in spring ecosystems at the landscape level. The Science of the Total Environment, 140157. DOI: 10.1016/j.scitotenv.2020.140157.

Carvalho, L, Mackay, E.B., Cardoso, A.C., Baattrup-Pedersen, A., Birk, S., Blackstock, K.L., Borics, G., Borja, A., Feld, C.K., Ferreira, M.T., Globevnik, L., Grizzetti, B., Hendry, S., Hering, D., Kelly, M., Langaas, S., Meissner, K., Panagopoulos, Y., Penning, E., Rouillard, J., Sabater, S., Schmedtje, U., Spears, B.M., van de Bund, W. & Solheim, A.L. (2019). Protecting and restoring Europe’s waters: an analysis of the future development needs of the Water Framework Directive. Science of the Total Environment 658: 1228-1238.

CEMAGREF, 1982. - Etude des méthodes biologiques d’appréciationquantitative de la qualité des eaux. Rapport Q. E. Lyon, Agencede l’Eau Rhône-Méditerranée-Corse-CEMAGREF, Lyon218 p.

CEN (Comité Européen de Normalisation). 2003. Water qual-ity—Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers. EN 13946:2003.Comité Européen de Normalisation, Geneva, Switzerland.

CEN (Comité Européen de Normalisation). 2004. Water quality—Guidance standard for the identification, enumerationand interpretation of benthic diatom samples from runningwaters. EN 14407:2004. Comité Européen de Normalisation,Geneva, Switzerland

Chester, E. T., and Robson, B. J. 2014. Do recolonization processes in intermittent streams have sustained effects on benthic algal density and assemblage composition? Marine and Freshwater Research 65, 784–790. doi:10.1071/MF13239

Christofides, Y. (2017). *Illustrated Flora of Cyprus*. Yannis Christofides, Cyprus.

Clarke, R. T., Wright, J. F., Furse, M. T. 2003. RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. Ecological Modelling, 160, 219–233.

Coste, M., Boutry, S., Tison-Rosebery, J., Delmas, F., 2009. Improvements of the Biological Diatom Index: description and efficiency of the new version (BDI-2006). Ecological Indicators 9, 621-650.

Delgado C., Pardo I., García L. 2012. Diatom communities as indicators of ecological status in Mediterranean temporary streams (Balearic Islands, Spain). Ecological Indicators 15 (2012) 131–139. doi:10.1016/j.ecolind.2011.09.037

Demartini D., Armanini, D.G., Tziortzis, I., Dörflinger, G., Buffagni, A. 2016. Developing ecological assessment systems for rivers in Cyprus along a gradient of hydrological stability. Notiziario dei Metodi Analitici, 2, 83-89.

Denicola, D. M., Kelly, M., 2014. Role of periphyton in ecological assessment of lakes. Freshwater Science, 33: 33: 619-638. https://doi.org/10.1086/676117

Di Pasquale, D., Buffagni, A., 2006. Il software CARAVAGGIOsoft: uno strumento per l'archiviazione e la gestione di dati di idromorfologia e habitat fluviale. Notiziario dei Metodi Analitici IRSA 12, 20–38.

Dufrene, M., & Legendre, P. (1997). Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs, 67, 345–366.

Dwoorak, T., Kampa, E., de Roo, C., Alvarez, C., Back, S., Benito, P. (2007). Simplification of European Water Policies. European Parliament.

Elias C.L., Calapez A.R., Almeida S.F.P., Feio M.J. 2015. From perennial to temporary streams: an extreme drought as a driving force of freshwater communities' traits. Mar Freshwater Res 66:469–480. doi:10.1071/MF13312

Erba, S., Furse, M.T., Balestrini, R., Christodoulides, A., Ofenböck, T., van de Bund,W., Wasson, J.-G., Buffagni, A., 2009. The validation of common European class boundaries for river benthic macroinvertebrates to facilitate the intercalibration process of the Water Framework Directive. Hydrobiologia 633, 17

Erba, S., Pace, G., Demartini, D., Di Pasquale, D., Dörflinger, G., Buffagni, A., 2015. Land use atthe reach scale as a major determinant for benthic invertebrate community in Medi-terranean rivers of Cyprus. Ecol. Indic. 48:477–491.http://dx.doi.org/10.1016/j.ecolind.2014.09.010.

Ehrlich, P., Walker B. 1998. Rivets and redundancy. BioScience 48: 387. https://doi.org/10.2307/1313377

European Commission, 2000. European Commission Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. Off. J. Eur. Communities, 2000 (2000)

European Commission. (2018). Commission Decision (EU) 2018/229 of 12 February 2018 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise and repealing Commission Decision 2013/480/EU. [online]. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2018:047:TOC.

Falasco, E., Piano, E., and Bona, F. 2016. Diatom flora in Mediterranean streams: flow intermittency threatens endangered species. Biodiversity and Conservation 25, 2965–2986. doi:10.1007/S10531-016-1213-8

Falasco E., Piano E., Doretto A., Fenoglio S., Bona F. 2018. Resilience of benthic diatom communities in Mediterranean streams: role of endangered species. Marine and Freshwater Research 70(2): 212-224 https://doi.org/10.1071/MF17282

Feld, C.K., 2004. Identification and measure of hydromorphological degradation in Central European lowland streams. Hydrobiologia 516, 69–94.

Feio, M.J., Aguiar, F.C., Almeida, S.F., Ferreira, J., Ferreira, M.T., Elias, C., Serra, S.R., Buffagni, A., Cambra, J., Chauvin, C., Delmas, F., Dörflinger, G., Erba, S., Flor, N., Ferréol, M., Germ, M., Mancini, L., Manolaki, P., Marcheggiani, S., Minciardi, M.R., Munné, A., Papastergiadou, E., Prat, N., Puccinelli, C., Rosebery, J., Sabater, S., Ciadamidaro, S., Tornés, E., Tziortzis, I., Urbanič, G. & Vieira, C. (2014). Least disturbed condition for European Mediterranean rivers. Science of the Total Environment DOI:10.1016/j.scitotenv.2013.05.056.

Fox, J., and G. Monette. 1992. Generalized collinearitydiagnostics. Journal of the American Statistical Association87:178–183.

Giannakopoulos, P., Hadjinicolaou, E., Kostopoulos, K., Varotsos, V., Zerefos, C. (2010). Precipitation and temperature regime over Cyprus as a result of global climate change. Adv. Geosci. 23: 17-24.

Giorgi, F., & Lionello, P. 2008. Climate change projections for the Mediterranean region. Global Planet Change, 63, 90-104. doi:10.1016/j.gloplacha.2007.09.005

Guiry, M.D. & Guiry, G.M. 2020. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org; searched on 07 February 2020.

Handl, J., Knowles, K., & Kell, D. (2005). Computational cluster validation in post-genomic data analysis. Bioinformatics, 2115, 3201–3212.

Hofmann, G., M. Werum und H. Lange-Bertalot. 2011. Diatomeen im Süßwasser-Benthos von Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis. Über 700 der häufigsten Arten und ihre Ökologie. A.R.G. Gantner Verlag K.G.

Hofmann G., Lange-Bertalot H., Werum M., Klee R. 2018. Rote Liste der limnischen Kieselalgen. Naturschutz und Biologische Vielfalt 70(7): 601-708.

Jüttner I., Bennion H., Carter C., Cox E.J., Ector L., Flower R., Jones V., Kelly M.G., Mann D.G., Sayer C., Turner, J. A., Williams D.M. 2020 Freshwater Diatom Flora of Britain and Ireland. Amgueddfa Cymru - National Museum Wales. Available online at https://naturalhistory.museumwales.ac.uk/diatoms. [Accessed: 7 February 2020].

Kelly, M.G. (2013). Data rich, information poor? Phytobenthos assessment and the Water Framework Directive. *European Journal of Phycology* **48**: 437-450.

Kelly, M.G. (2019). Life out of water. https://microscopesandmonsters.wordpress.com/2019/02/11/life-out-of-water/

Kelly, M.G., Bennett, C., Coste, M., Delgado, C., Delmas, F., Denys, L., Ector. L., Fauville, C., Ferreol, M., Golub, M., Jarlman, A., Kahlert, M., Lucey, J., Ní Chatháin, B., Pardo, I., Pfister, P., Picinska-Faltynowicz, J., Schranz, C., Schaumburg, J., Tison, J., van Dam, H. & Vilbaste, S. (2008). A comparison of national approaches to setting ecological status boundaries in phytobenthos assessment for the

European Water Framework Directive: results of an intercalibration exercise. *Hydrobiologia* 695 109-124.

Kelly, M. G., Chiriac, G., Soare-Minea, A., Hamchevici, C., & Juggins, S. (2019). Use of phytobenthos to evaluate ecological status in lowland Romanian lakes. Limnologica. https://doi.org/10.1016/j.limno.2019.125682

Kelly, M.G., Gómez-Rodríguez, C., Kahlert, M., Almeida, S.F.P., Bennett, C., Bottin, M., Delmas. F., Descy, J.-P., Dörflinger,G., Kennedy, B., Marvan, P., Opatrilova,L., Pardo, I., Pfister, P., Rosebery, J., Schneider, S. & Vilbaste, S. (2012). Establishing expectations for pan-European diatom based ecological status assessments. *Ecological Indicators* 20: 177-186.

Kelly, M.G., Juggins, S., Guthrie, R., Pritchard, S., Jamieson, B.J., Rippey, B, Hirst, H & Yallop, M.L. (2008). Assessment of ecological status in UK rivers using diatoms. *Freshwater Biology* 53: 403-422.

Kelly, M.G., Phillips, G., Juggins, S., Willby, N.J., 2020. Re-evaulating expectations for river phytobenthos assessments and understanding the relationship with macrophytes. *Ecological Indicators* (this volume).

Krammer, K & H. Lange-Bertalot. 2004. Sűβwasserflora von Mitteleuropea. In: Ettl, H., Gärtner, G., Heynig, H. & Mollenhauer, D. (eds): Bacillariophyceae. Achnanthaceae, **2/4**, 468 pp. G. Fischer, Stuttgart. New York.

Krammer, K. & H. Lange-Bertalot. 1986-1991. Sűβwasserflora von Mitteleuropa. In: Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (eds): Bacillariophyceae. Naviculaceae, **2/1**, 876 pp.; Bacillariaceae, Epithemiaceae, Surirellaceae, **2/2**, 596 pp.; Centrales, Fragilariaceae, Eunotiaceae, **2/3**, 576 pp.; Achnanthaceae, **2/4**, 437 pp. – G. Fischer, Stuttgart. New York.

Krammer, K. 2000-2003. Diatoms of Europe. The genus *Pinnularia*. **1**, 703 pp.; *Cymbella*, **3**, 584 pp.; *Cymbopleura*, *Delicata*, *Navicymbula*, *Gomphocymbellopsis*, *Afrocymbella*, **4**, 530 pp. Edited by H. Lange Bertalot, A.R.G. Gantner Verlag K.G.

Krammer, K., 1997a. Die cymbelloiden Diatomeen. Teil 1. Allgemeines und *Encyonema* Part. – Bibliotheca Diatomologica 36: 1−382.

Krammer, K., 1997b. Die cymbelloiden Diatomeen. Teil 2. *Encyonema* part., *Encyonopsis* and *Cymbellopsis*. – Bibliotheca Diatomologica 37: 1−469.

Kaufman, L., & Rousseeuw, P. J. (1990). Finding groups in data: an introduction to cluster analysis. New York: Wiley.

Lange-Bertalot H., Cavacini P., Tagliaventi N. & Alfinito S., 2003. Diatoms of Sardinia. Rare and 76 new species in rock pools and other ephemeral waters. *Iconographia diatomologica* 12: 1–438. Krammer, K. (1997a): Die cymbelloiden Diatomeen. Teil 1. Allgemeines und *Encyonema* Part. Bibliotheca Diatomologica **36**: 1-382.

Lange-Bertalot, H. 1993. 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süßwasserflora von Mitteleuropa Vol. 2/1-4. - Bibliotheca Diatomologica **27**: 1-454. J. Cramer. Berlin, Stuttgart.

Lange-Bertalot, H. 1996. Rote Liste der limnischen Kieselalgen (Bacillariophyceae) Deutschlands. - Schriften-Reihe für Vegetationskunde **28**: 633-677.

Lange-Bertalot, H. 2001. *Navicula* sensu stricto, 10 genera separated from *Navicula* sensu lato, *Frustulia*. In Lange-Bertalot, H. (Ed.) Diatoms of Europe, 2: 1-526. A.R.G. Gantner Verlag , K.G., Ruggell.

Lange-Bertalot, H. 2001-2011. Diatoms of Europe. Volls 1-6. A.R.G. Gantner Verlag K.G., Ruggell.

Lange-Bertalot, H., Witkowski, A. & M. Bąk. 2011. *Eunotia* and some related genera. In Lange-Bertalot, H. (Ed.) Diatoms of Europe, 6: 1-780. A.R.G. Gantner Verlag, K.G., Ruggell.

Lawton, J.H., Brown, V.K. 1994. Redundancy in Ecosystems. In: Schulze E.D., Mooney H.A. (eds) Biodiversity and Ecosystem Function, pp 255-270. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-58001-7_12

Levkov, Z. 2009. *Amphora* sensu lato. In.: H. Lange-Bertalot (ed.), *Diatoms of Europe: Diatoms of the European Inland Waters and Comparable Habitats*. Vol. 5 pp. 5-916.: A.R.G. Gantner Verlag K.G.

Maechler, M., Rousseeuw, P., Struyf, A., & Hubert, M. (2005). Cluster analysis basics and extensions. http://cran.r-project.org/ web/packages/cluster/.

Mann, D.G., Thomas, S.J. & Evans, K.M. 2008. Revision of the diatom genus *Sellaphora*: a first account of the larger species in the British Isles. *Fottea*, **8**(1), 15-78.

MCCUNE, B., AND J. GRACE. 2002. Analysis of ecological communities. MJM Press, Gleneden Beach, Oregon.

Morales E.A., Wetzel C.E., Novais M.H., Morais M.M. & Ector L. 2020. *Nitzschia transtagensis* sp. nov. (Bacillariophyceae) from a spring in Southern Portugal. Botany Letters, 167(1): 32-41. DOI: 10.1080/23818107.2019.1688676

Neal, C. and Shand, P. (2002). Spring and surface water quality of the Cyprus ophiolites. Hydrology and Earth System Sciences 6 (5),797-817.

Novais, M.H., Blanco, S., Hlubikova, D., Falasco, E., Gomá, J., Delgado, C., Ivanov, P., Acs, E., Morais, M., Hoffmann, L. & Ector, L. 2009. Morphological examination and biogeography of the *Gomphonema rosenstockianum* and *G. tergestinum* species complex (Bacillariophyceae). *Fottea* 9: 257-274.

Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'hara R.B., Simpson G.L., Solymos P., Stevens M.H.H., Wagner H. (2013) Vegan: Community Ecology Package. R package version 2.0-7. http://CRAN.R-project.org/package=veganhttp://CRAN.R-project.org/package=vegan

Oueslati, O., De Girolamo, A.M., Abouabdillah, A. and Lo Porto, A. (2010). Attempts to flow regime classification and characterisation in Mediterranean streams. In EGU General Assembly Conference Abstracts. EGU General Assembly Conference Abstracts. p. 13806.

Pardo, I., Gómez-Rodríguez, C., Wasson, J.-G., Owen, R., van de Bund, W., Kelly, M., Bennett, C., Birk, S., Buffagni, A., Erba, S., Mengin, N., Murray-Bligh, J., Ofenböeck, G. (2012). The European reference condition concept: A scientific and technical approach to identify minimally-impacted river ecosystems. *Science of the Total Environment* 420: 33-42.

Paul M.J., Jessup B., Brown L.R., Carter J.L., Cantonati M., Charles D.F., Gerritsen J., Herbst D., Stancheva R., Howard J., Isham B., Lowe R., Mazor R., Mendez P.K., Ode P., O'Dowd A., Olson J., Pan Y., Rehn A.C., Spaulding S., Sutula M., Theroux S. 2020. Characterizing benthic macroinvertebrate and algal Biological Condition Gradient models for California wadeable streams, USA. Ecological Indicators. Accepted.

Piano, E., Falasco, E. & Bona, F. 2017. How does water scarcity afect spatial and temporal patterns of diatom community assemblages in Mediterranean streams? Freshwater Biology 62, 1276–1287.

Poikane, S., Birk, S., Böhmer, J., Carvalho, L., de Hoyos, C., Gassner, H., Hellsten, S., Kelly, M., Solheim, A.L., Olin, M., Pall, K., Phillips, G., Portielje, R., Ritterbusch, D., Sandin, L., Schartau, A.-K., Solimini, A.G., van den Berg, M., Wolfram, G. & van de Bund, W. (2015). A hitchhiker's guide to European lake ecological assessment and intercalibration. *Ecological Indicators* 52: 533-544.Poikane S., Kelly M.G. & Cantonati M. 2016. Benthic algal assessment of ecological status in European lakes and rivers: challenges and opportunities. Science of the Total Environment 568: 603–613. DOI: 10.1016/j.scitotenv.2016.02.027

Poikane S., Zohary T., Cantonati M. 2019. Assessing the ecological effects of hydromorphological pressures on European lakes. Inland Waters. DOI: 10.1080/20442041.2019.1654800

Raven, P.J., Fox, P., Everard, M., Holmes, N.T.H., Dawson, F.D., 1997. River Habitat Survey: a new system for classifying rivers according to their habitat quality. In: Boon, P.J., Howell, D.L. (Eds.), Freshwater Quality: Defining the Indefinable?. The Stationery Office, Edinburgh pp. 215–234.

Raven, P.J., Holmes, T.H., Dawson, F.H., Fox, P.J.A., Everard, M., Fozzard, I.R., Rouen, K. J., 1998. River Habitat Survey, the physical character of rivers and streams in the UK and Isle of Man. River Habitat Survey Report, 2. The Environment Agency, Bristol pp. 86.

Reynoldson, T. B., Rosenberg, D. M., & Resh, V. H. (2001). Comparison of models predicting invertebrate assemblages for biomonitoring in the Fraser River catchment, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences, 58, 1395–1410.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reichardt, E. 1997. Taxonomische Revision des Artenkomplexes um *Gomphonema pumilum* (Bacillariophyceae). 1997. Nova Hedwigia 65 (1-4): 99- 129.

Reichardt, E. 1999. Zur Revision der Gattung *Gomphonema*. Die Arten um *G. affine*/*insigne*, *G. angustatum*/*micropus*, *G. acuminatum* sowie gomphonemoide Diatomeen aus dem Oberoligozän in Böhmen. In: Lange-Bertalot (Ed.). Iconographia Diatomologica 8. Koeltz Scientific Books: 1-203.

Reichardt, E. 2018. Die Diatomeen im Gebiet der Stadt Treuchtlingen. Selbstverlag der Bayerischen Botanischen Gesellschaft, München. 2 vols., 1184 pp. ISBN 978-3-00-060715-8

Richter, B.D., Baumgartner, J.V., Powell, J. and Braun, D.P. (1996). A Method for Assessing Hydrologic Alteration within Ecosystems. Conservation Biology, 10(4), pp.1163–1174.

Rimet, F., & Bouchez, A. 2012. Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. Knowledge and Management of Aquat Ecosystems, 406: 01–12.

Rimet, F., Couté, A., Piuz, A., Berthon, V. and Druart, J.C. 2010. *Achnanthidium druartii* sp. nov. (Achnanthales, Bacillariophyta), a new species invading European rivers. *Vie et Milieu - Life and Environment* 60(3):185-195.

Roberts, D.W. 2016. Labdsv: Ordination and Multivariate Analysis for Ecology. R package version 1.8-0.

Romero, O. and Jahn, R. 2013. Typification of *Cocconeis lineata* and *Cocconeis euglypta* (Bacillariophyta). *Diatom Research* 28(2): 175-184.

Rott, E., Pfister, P., Van Dam, H., Pipp, E., Pall, K., Binder, N., Ortler, K., 1999.Indikationlisten für Aufwuchsalgen in österreichischen Fliessgewässern. Teil 2:Trophieindikation sowie geochemische

Präferenzen, taxonomische und24S. Blanco, E. Bécares/Chemosphere 79 (2010) 18–25 toxikologische Anmerkungen. Wasserwirtschaftskataster Herausgegeben vomBundesministerium für Land- und Forstwirtschaft, Wasserwirtschaftskataster,Wien.

Rumrich U., Lange-Bertalot H. & Rumrich M., 2000. Diatomeen der Anden. *Iconographia diatomologica* 9: 1–649.

Sofroniou, A. and Bishop, S. 2014. Water scarcity in Cyprus: A review and call for integrate Policy. Water 6: 2898-2928.

Spaulding, S.A., Bishop, I.W., Edlund, M.B., Lee, S., Furey, P., Jovanovska, E. and Potapova, M. 2019. Diatoms of North America. https://diatoms.org/

Terrado, M., Acuna, V., Ennaanay, D., Tallis, H. & Sabater, S. (2014). Impact of climate extremes on hydrological ecosystem services in a heavily humanized Mediterranean basin. Ecological Indicators 37: 199-209.

Tornés, E., Cambra, J., Gomà, J., Leira, M., Ortiz, R., Sabater, S., 2007. Indicator taxa of benthic diatom communities: a case study in Mediterranean streams. Ann. Limnol. - Int. J. Lim. 43: 1-11. https://doi.org/10.1051/limn/2007023

Tornés, E., Mor J.-R., Mandaric L., Sabater S. 2018. Diatom responses to sewage inputs and hydrological alteration in Mediterranean streams. Environmental Pollution, 238: 369-378. https://doi.org/10.1016/j.envpol.2018.03.037.

Trobajo, R., Rovira, L., Ector, L., Wetzel, C.E., Kelly, M. & Mann, D.G. 2013. Morphology and identity of some ecologically important small *Nitzschia* species. Diatom Research, 28 (1): 37-59.

UNEP/MAP (2012) State of the Mediterranean Marine and Coastal Environment. United Nations Environment Programme / Mediterranean Action Plan – Barcelona Convention, Athens.

Uys, M.C. and O'Keeffe, J.H. (1997). Simple Words and Fuzzy Zones: Early Directions for Temporary River Research in South Africa. Environmental management, 21(4), pp.517–531.

Van de Vijver B., Jarlman A., Lange-Bertalot H., Mertens A., de Haan M., Ector L. 2011a. Four new European Achnanthidium species (Bacillariophyceae). Algological Studies Volume 136-137: 193 – 210.

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

Venables, W.N. & Ripley, B. D., 2002. Modern Applied Statistics with S. Fourth edition, Springer, pp- 301-330.

WDD- Water Development Department (2016). River Basin Management Plan of Cyprus for the Implementation of the Directive 2000/60/EC (Period 2016-2021). LDK Consultants, ECOS. Republic of Cyprus. Nicosia.

Werum, M., and H. Lange-Bertalot. 2004. Diatoms in springs from Central Europe and elsewhere under the influence of hydrogeology and anthropogenic impacts. Iconographia Diatomologica 13. A. R. G. Gantner Verlag K. G., Ruggell, pp. 480.

Wojtal AZ. 2013. Species composition and distribution of diatom assemblages in spring waters from various geological formations in southern Poland. Bibl Diatomol. 59: 1–436.