Hydrobiologia (2022) 849:1821–1837
https://doi.org/10.1007/s10750-022-04829-5

PRIMARY RESEARCH PAPER

Impacts of climate change on stream benthic diatoms—a nation-wide perspective of reference conditions

Elisabet Tornés · Juan Alández-Rodríguez · Alfredo Corrochano · Pepita Nolla-Querol · Maria Carmen Trapote · Sergi Sabater

Received: 7 October 2021 / Revised: 14 February 2022 / Accepted: 19 February 2022 / Published online: 9 March 2022

Abstract Consequences of anthropogenic climate change directly affect freshwater ecosystems and their aquatic biological communities. Diatoms are amongst the most sensitive organisms to hydric stress, making them good indicators of preceding hydrological conditions. We assume that river types with low runoff and associated high temperature and mineralization host the most tolerant diatoms to climate change. We performed a cluster analysis with reference sites throughout Spain, based on their physiographic and hydrological characteristics. We obtained seven end-groups spread in the three existing ecoregions, onto which we estimated the indicator diatom taxa (IndVal). Brackish and aerophilic diatom were indicator taxa in mineralized and low discharge rivers. We simulated the impact of climate change on the river types, to conclude that under the RCP 8.5, the most impacted of all types would be the mineralized rivers. We predict higher homogenization in the diatom assemblages' composition, with higher proportion of planktonic taxa, and a potential increase of terrestrial and aerophilic diatoms, as the best adapted to the harsh conditions imposed by runoff reduction. Formulating clear predictions of climate change effects should rely on planned, long-term monitoring including accurate hydrological and biological data.

Keywords River types · Runoff · Temperature · Mineralized rivers · RCP scenarios · Aerophilic diatoms

Handling Editor: Judit Padisák

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10750-022-04829-5.

E. Tornés (✉) · S. Sabater
Catalan Institute for Water Research (ICRA), Carrer Emili Grahit 101, 17003 Girona, Spain
e-mail: etornes@icra.cat

E. Tornés
University of Girona, Plaça de Sant Domènec 3, 17004 Girona, Spain

J. Alández-Rodríguez
Ministerio para la Transición Ecológica y el Reto Demográfico (MITECO), Dirección General del Agua, Plaza San Juan de la Cruz 10, 28071 Madrid, Spain

A. Corrochano
Gerencia de Planificación y Gestión Hídrica, Tragsatec, Julian Camarillo 6B, 28037 Madrid, Spain

P. Nolla-Querol · M. C. Trapote
DBO5 s.l. - Del. Catalunya, Carrer de Santa Eulàlia 174, entresol B, 08902 L'Hospitalet de Llobregat, Barcelona, Spain

S. Sabater
Institute of Aquatic Ecology, Faculty of Sciences, University of Girona, Campus Montilivi, 17003 Girona, Spain

Introduction

The latest IPCC report (IPCC, 2021) stresses that climate change imprints ramping changes in the global water cycle, reductions in snow and ice cover, and higher frequency of extreme climatic events. Impacts of climate change will be more stressful in some regions than others. In Europe, Northern regions will progress towards wetter conditions, while the Mediterranean region will suffer from more acute drying. Overall, a runoff decrease is expected in southern Europe, which will add to strong human pressures on water resources (Sabater et al., 2018), either directly through flow regulation and water withdrawal for irrigation, or indirectly through effects of landscape change (Jaramillo & Destouni, 2015; Gudmundsson et al., 2021).

Climate change effects on hydrological conditions will directly affect freshwater ecosystems and their biological communities (Dudgeon et al., 2006). While impacts will concern all biological components of the aquatic ecosystem, some are highly sensitive and perform as early warning indicators. This is the case of the diatoms, siliceous microscopic algae making up the largest fraction of algal assemblages in river systems (Pan et al., 1999). Diatoms are highly sensitive organisms to flow reduction and desiccation, this affecting their typology and community composition (Tornés et al., 2021). We have recently observed that under extreme non-flow conditions, only aerophilic diatoms persist, while sensitive species disappear after a few days of non-flow (Tornés et al., 2021). Diatoms may then provide an ideal model group on which to explore the response of biological communities to water flow reduction associated to climate and global change.

We here use diatom assemblages from reference sites in river networks spread throughout several ecoregions in continental Spain. Reference conditions in freshwaters refer to the absence or minimal anthropogenic impact (Wallin et al., 2003). According to the European Water Framework Directive (WFD-European Commission, 2000), reference sites should consistently show this minimal impact in biological, hydromorphological, and physical and chemical characteristics. A central aspect of the WFD is accounting for type-specific ecological assessment and classification of waterbodies, using reference sites as a contrast to impacted sites (Feio et al., 2013). Biological communities, and diatoms amongst them (Tornés et al., 2012), play a central role under this scheme.

We here aim to characterize the distribution of diatom assemblages in reference sites of the main river basins of continental Spain and associate them to the present physiographic conditions of temperature and runoff. We subsequently associate the current assemblages' distribution to predicted climate changes affecting the different ecoregions within Spain. We used an extensive dataset of reference sites distributed throughout continental Spain, and covering a wide range of fluvial typologies, to test the working hypotheses that (a) river types with lower runoff and associated high temperature and high mineralization may harbour the most tolerant diatoms to climate change; and (b) that differences between river types are reduced because of the strong decrease in water runoff caused by climate change, which may lead diatom assemblages towards homogenization.

Material and methods

Study sites

The study was conducted in 346 reference stream sites (Fig. 1) distributed over the Spanish territory and the most relevant watersheds, covering the fluvial typologies recognized in the country (Toro et al., 2009). We used 32 national river types, originally classified using progressive segregation of fluvial network subsets by establishing thresholds for each of the variables. This is an open hierarchical classification based on a GIS tool map. The variables used to determine the river types were altitude (meters a.s.l.), annual thermal amplitude (°C), catchment area (km^2), mean annual discharge (m^3/s), mean annual specific discharge (m^3/s km^2), estimated baseline conductivity (µS/cm), latitude (UTM 30) and longitude (UTM 30), stream order (according to the Strahler classification), mean catchment gradient (%), mean annual air temperature (°C), and the fraction of months with non-flow (%). This last descriptor derives from the SIMPA model (after its Spanish acronym, *Integrated System for Rainfall-Runoff Modelling*, Álvarez et al. 2004).

The area of study has a large spatial heterogeneity regarding its geomorphological and climatic diversity. River sites included in the study range from high, middle mountain, and lowland rivers, headwaters and

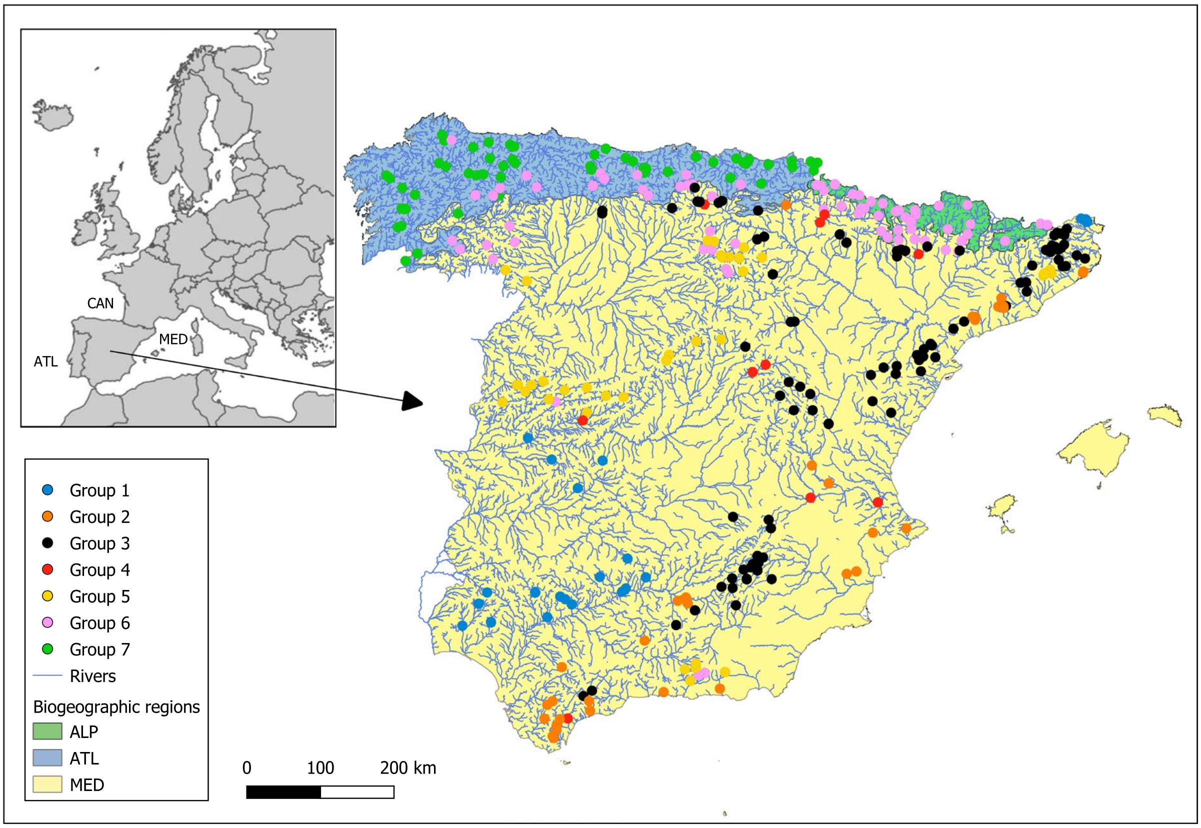

Fig. 1 Study area and location of reference stream sites in the continental Spain. Coloured areas represent the three biogeographic areas present (Alpine *ALP*, Atlantic *ATL*, and Mediterranean *MED*), following the classification of the European Environment Agency (Codelist for bio-geographical regions, Europe 2011; www.eea.europa.eu). Sampling sites are labelled following the new classification of river types (Fig. 2) obtained in this study

large rivers, floodplain rivers, and coastal streams. These rivers and their basins include the Mediterranean, Atlantic, and Alpine ecoregions in Spain. Those under Mediterranean climate show a strong seasonality and interannual rainfall variability (Gasith & Resh, 1999) and are submitted to low-flow periods during late spring and summer, and high-flow periods in late autumn and winter. Headwaters and middle-order streams in the Mediterranean ecoregion often become intermittent or have intermittent reaches during long periods of the hydrological cycle (Colls et al., 2019). Rivers in the Cantabrian-Atlantic ecoregion are short in length and receive abundant rainfall, even in summer, due to the dominance of northern winds, and a narrow yearly temperature range (Amigo et al., 2017). The climate in the Cantabrian-Atlantic region is oceanic, with cool winters and warm summers, and with a mean annual rainfall of 1000–1200 mm distributed throughout the year. Althouh these systems do not dry up, the lowest precipitation occurs during summer (Gartzia de Bikuna et al., 2015). The Alpine ecoregion mostly has mountain rivers, with steep gradients along most of the channel length. These systems experience minimum temperatures below 0 ºC, annual rainfall of above 1000 mm, and heavy snowfall in winter (Wohl, 2010; Sabater et al., 2022). Although dry periods are characteristic from the Mediterranean ecoregion, drying events increasingly occur in the humid and temperate regions. A general decrease in snow cover, along with an increase in the annual temperature and air pressure has been observed in the Alpine ecoregion, leading to a significant decreasing trend in the annual runoff (Falasco et al., 2018).

We used diatom data from two nation-wide surveys (TRAGSATEC, 2020), complemented with others obtained in regional surveys (CHE, 2017; Tornés et al., 2021). Overall, most of the sites were sampled more than once, spanning from 2014 to 2020, and providing a total of 603 samples (Supplementary Table S1).

Environmental characterization

The environmental variables of the sites were extracted from the characterization of Spanish river types (Toro et al., 2009). The ones selected were altitude (meters a.s.l.), catchment area (Km^2), mean annual specific discharge (m^3/s/km^2), estimated baseline conductivity (µS/cm), mean catchment gradient (%), months of non-flow (%), and mean annual air temperature (°C) (Table 1). We did not use annual thermal amplitude (°C), mean annual discharge (m^3/s), latitude (UTM 30), longitude (UTM 30), and stream order (Strahler), due to their redundancy with the other variables.

Actual hydrological data in the reference sites were sought using the QGIS software (version 3.16.0; QGIS Development Team, 2020). We constructed a layer with the gauging stations of the region, using data available from different water authorities (www.miteco.gob.es; www.aca.gencat.cat; www.juntadeandalucia.es). We then used the Distance Matrix algorithm from QGIS Network Analysis Toolbox (QNEAT3) plugin, to find out the nearest gauging station to each reference site, using the fluvial systems as the network layer. QNEAT3 computes the network route-based cost of Origin–Destination relations between the points of two layers and calculates the shortest paths between all the points. We considered appropriate gauging stations that ones located upstream of a site and at a distance less than 10 km, with natural flow regime, and where dams and/or reservoirs, or tributaries between the gauging station and the sampling site, did not occur. Results from the QGIS QNEAT3 plugin indicated that only few sites fulfilled those criteria. Even cases where the gauging station existed, data were not available for the period of the diatom sampling. Then, from the 346 reference stream sites selected for the analysis, less than 50 accomplished the fixed criteria, representing less than 100 samples (from a total of 603), not evenly distributed across the river types.

Overall, hydrological data for each river site could not be used in the subsequent analyses and forced us to define theoretical values after those of Toro et al. (2009). For that, we used the extensive dataset of reference sites in the continental Spain, and applied the predictions of the Representative Concentration Pathways (RCP) 4.5 and 8.5 to the present characteristics of runoff, temperature, and water conductivity of the different river types. With these, we defined new values under the two RCP scenarios based on the IPCC 2013 (CEDEX, 2017). A conductivity-temperature relationship was applied after Hayashi (2004) to estimate future values of water conductivity in the different types.

Diatom sampling and laboratory analyses

Diatom samplings were conducted mostly during summer, although some of the sites were sampled in spring and autumn. Sampling periods, therefore, covered low-flow (summer) and high-flow (spring, autumn) periods. Sampling followed standard procedures (Kelly et al., 1998; MAGRAMA, 2013). In short, diatoms were sampled from riffle sections and obtained from several colonized cobbles. From each cobble, the total algal materials were scraped with a brush into 20 mL of distilled water. A composite sample was created from the samples of the five cobbles from each stream, and each composite sample was preserved in 4% formaldehyde or ethanol until analysis. Materials were later cleaned for the organic material using boiling hydrogen peroxide, and cleaned frustules were mounted on permanent slides using Naphrax (r.i. 1.74; Brunel Microscopes Ltd., Chippenham,Wiltshire, UK). Up to 400 valves were counted on each slide by performing random transects under light microscopy using differential interference contrast optics at a magnification of ×1000.

Diatom assemblages were examined for their taxonomical composition. Diatoms were identified using reference floras (Krammer & Lange-Bertalot, 1991a, b, 1997a, b; Cantonati et al., 2017), and complemented through the monographs of "Diatoms of Europe" and "Bibliotheca Diatomologica". As diatom data derived from different datasets, they were checked for their homogeneity in the identification. Then, last accepted names were validated for each taxon, and same taxa with different names (synonymies) were combined in a single entry. Teratological forms were summed to the normal forms as they were not considered in all surveys.

Table 1 Mean annual values, coefficient of variation (CV, %), and range of the environmental variables defining the Spanish national river types

Cluster	Type	Altitude (m a.s.l.)			Catchment area (Km^2)			Specific discharge (m^3/s/km^2)			Conductivity (µS/cm)		
group	code	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range
G1	R-T01	348	31.2	200–550	255	172.5	15–1100	0.0051	58.8	0.002–0.012	196	95.4	< 595
G1	R-T06	153	50.6	20–280	464	596.5	20–1700	0.0058	29.3	0.003–0.008	218	56	< 365
G1	R-T08	470	31.1	260–710	237	431.8	15–860	0.0056	50	0.002–0.011	156	79	< 410
G1	R-T19	129	87.3	–	754	84.4	–	0.0077	20.8	–	229	24.6	–
G2	R-T02	68	88.8	0–180	231	156.1	25–1380	0.0040	25	0.003–0.005	715	32.2	> 370
G2	R-T07	229	74.6	20–600	443	153.2	20–1820	0.0055	72.7	0.001–0.014	543	39.5	> 190
G2	R-T10	231	65.1	–	33	104	–	0.0029	41.4	–	715	12.2	–
G2	R-T13	250	79.1	–	613.4	101	–	0.0009	66.7	–	448.5	23.9	–
G2	R-T18	93	125.2	0–350	130	1337	10–210	0.0060	61.7	0.002–0.014	454	49.2	> 80
G2	R-T20	245	84.9	15–690	99	111.4	10–320	0.0164	29.3	0.011–0.027	559.3	24.6	> 305
G3	R-T04	808	10.9	680–950	435	138.5	40–1620	0.0029	65.5	0.001–0.006	575	36.1	> 195
G3	R-T05	736	14.4	580–930	763	177.6	50–2400	0.0020	45	0.001–0.004	656.6	30.3	> 285
G3	R-T09	425	51.3	70–790	499	394.2	25–1880	0.0036	69.4	0.001–0.009	545	31.1	> 325
G3	R-T12	855	29.5	450–1280	275	208.6	15–1090	0.0055	56.4	0.002–0.011	566	36.9	> 300
G4	R-T14	114	84.5	5–320	4878	57	550–9100	0.0054	68.5	0.002–0.014	586	11.2	> 505
G4	R-T15	522	48.4	140–940	3532	106.5	660–11,050	0.0123	44.7	0.005–0.022	257	54.2	< 450
G4	R-T16	554	34.8	260–840	6445	67.6	2.090–15,700	0.0040	40	0.001–0.007	571	14.5	> 435
G4	R-T17	286	78.9	5–710	34.132	57.4	7.000–81,200	0.0046	45.7	0.002–0.010	390	32.1	> 120
G5	R-T03	772	16.4	600–1000	162	145	20–650	0.0052	44.2	0.003–0.010	136	87.3	< 380
G5	R-T11	961	28.8	390–1380	118	132.1	10–470	0.0098	45.9	0.004–0.018	130	82.4	< 310
G5	R-T24	613	37.5	280–1000	74	134.1	10–270	0.0223	37.2	0.008–0.035	37	111.4	< 105
G6	R-T25	937	20.4	600–1240	123	154.4	10–550	0.0244	38.9	0.009–0.038	145	68.1	< 345
G6	R-T26	707	38	420–1180	419	162.6	10–1730	0.0210	44.3	0.011–0.038	360	32.5	> 220
G6	R-T27	1289	19.8	890–1800	64	134.4	10–280	0.0326	43.3	0.014–0.058	217	53.7	> 15
G7	R-T21	421	40.8	115–690	35	78.3	10–95	0.0263	27.4	0.016–0.039	119	93.1	< 305
G7	R-T22	284	69.4	20–670	39	77	10–100	0.0269	23.8	0.017–0.038	349	31.3	> 250
G7	R-T23	269	45	100–490	34	67	10–75	0.0355	23.1	0.022–0.049	342	42.1	> 150
G7	R-T28	248	67.9	15–550	2.677	126.2	450–12,800	0.0270	19.6	0.020–0.036	121	34.2	< 205
G7	R-T29	70	95.5	10–170	669	39.1	400–1160	0.0312	17	0.021–0.039	342	24	> 210
G7	R-T30	92	85.2	0–230	31	78.5	10–85	0.0252	25.8	0.016–0.036	181	97.9	> 20

Table 1 (continued)

Cluster group	Type code	Altitude (m a.s.l.)			Catchment area (Km^2)			Specific discharge (m^3/s/km^2)			Conductivity (µS/cm)		
		Mean	CV	Range	Mean	CV	Range	Mean	CV	Range	Mean	CV	Range
G7	R-T31	393	61.1	25–850	238	52	95–450	0.0291	22.3	0.019–0.041	118	73.3	< 215
G7	R-T32	163	85.3	15–430	228	49.5	85–450	0.0306	21.2	0.020–0.043	350	28.3	> 215

Cluster group	Type code	Catchment gradient (%)			Months of non-flow (%)			Air temperature (ºC)		
		Mean	CV	Range	Mean	CV	Range	Mean	CV	Range
G1	R-T01	2	59.2	0.7–4.2	43	62.9	–	15.6	6.3	14–17
G1	R-T06	3	35.8	1.2–4.2	41	68.8	–	17.4	3.8	16–18
G1	R-T08	4	47.9	1.7–7.3	46	48.4	–	15.4	7.5	14–17
G1	R-T19	3	19.4	–	36	60.9	–	17.6	3.1	–
G2	R-T02	1	50.5	0.4–2.4	22	120.8	–	17.9	2	17–18
G2	R-T07	5	52.3	1.7–10.3	31	84	–	17.1	6.3	15–18
G2	R-T10	6	46.7	–	5	187.2	–	15.2	5.9	–
G2	R-T13	4	28.9	–	23	97.4		17	7.3	–
G2	R-T18	6	63.1	0.8–12.3	14	143.5	–	16.8	5.8	15–18
G2	R-T20	8	30.4	5.0–12.3	29	75	–	16.9	3.2	16–18
G3	R-T04	2	69	0.4–3.6	5	204.2	–	11.3	6.9	10–12
G3	R-T05	1	44.4	0.6–2.7	7	238.6	–	13.8	4.4	13–15
G3	R-T09	5	45.8	1.9–9.1	13	161.4	–	14.8	9	13–17
G3	R-T12	5	57.6	1.6–10.1	7	199.2	–	11.7	12.6	9–14
G4	R-T14	5	18.6	3.9–7.4	1	450	–	17.5	3.2	17–18
G4	R-T15	6	38.7	2.6–10.2	1	378.8	–	12.7	13.5	10–15
G4	R-T16	4	41.9	1.5–6.1	0	3423.7	–	13.8	11.7	11–16
G4	R-T17	3	31.4	2.0–5.0	2	449.8	–	15.3	12.7	12–18
G5	R-T03	2	77.7	0.7–3.4	34	73.5	–	12.1	8.2	10–14
G5	R-T11	7	48.3	2.6–13.3	29	64.9	–	11.1	16.1	9–14
G5	R-T24	9	50	1.9–16.9	31	38.9	–	12.4	13	9–14
G6	R-T25	8	42.7	3.0–14.7	11	123.9	–	9.7	11.4	8–11
G6	R-T26	10	38.3	4.0–16.6	4	218.2	–	10.7	18.9	7–13
G6	R-T27	13	28.1	7.6–18.7	9	129.5	–	8.4	14.3	6–10
G7	R-T21	6	63.9	1.8–13.7	1	435.5	–	12	9.9	10–14
G7	R-T22	9	33.7	4.4–14.9	5	167.2	–	12	14	9–14

Table 1 (continued)

Cluster group	Type code	Catchment gradient (%)			Months of non-flow (%)			Air temperature (ºC)		
		Mean	CV	Range	Mean	CV	Range	Mean	CV	Range
G7	R-T23	10	14.6	7.9–12.4	4	130.7	–	12.7	5.8	11–14
G7	R-T28	7	52	2.3–13.4	0	4875	–	12.3	9.9	10–14
G7	R-T29	11	28.8	2.3–10.2	0	307	–	12.8	13.7	12–14
G7	R-T30	6	41.1	2.3–10.2	1	509.6	–	13.3	6.4	12–14
G7	R-T31	7	57.8	2.2–14.8	0	2096.7	–	11.6	13.5	9–14
G7	R-T32	10	24.8	7.4–15.4	0	358.7	–	12.3	14.5	7–14

The corresponding end-group of cluster analysis (Fig. 2) for each type is indicated and used for their arrangement. Codes of the river types correspond to those described in Table 3

Data analyses

Sites ordination

We departed from the original classification of national river types of Toro et al. (2009) and then proceeded to group them according to their physiography and hydrology. Classification of the sites was conducted by means of hierarchical agglomerative clustering. Pearson's correlation was performed to define co-linear variables ($R^2 > 0.8$) which were then not included in the cluster analysis. As such, catchment gradient was removed because of its high correlation with specific discharge ($R^2 = 0.83$). The cluster analysis was performed using group average as cluster mode, on previously calculated Euclidean distances for the available environmental variables. The statistical significance of between-group differences was tested using a Permutational MANOVA (PERMANOVA). The PERMANOVA was based on the Euclidean distances scores. The PERMANOVA operates on a resemblance matrix and is similar to traditional parametric multivariate analysis of variance (MANOVA) but does not require a multivariate normal distribution. It provides a pseudo-F statistic by expectations of mean squares, and a *P*-value based on permutations of the data (Anderson, 2001).

The environmental variability of the resulted end-groups of the cluster analysis was explored by means of a Principal Components Analysis (PCA). Before performing the PCA, variables were normalized by subtracting the mean and dividing it by the standard deviation.

Diatom analyses

Principal Coordinates Analysis (PCO) was used to ordinate the taxonomical composition of diatom assemblages in the 603 stream samples available. PCO can be based on any (symmetric) resemblance matrix like non-metrical multi-dimensional scaling, and it is a projection of the points onto axes that minimize residual variation in the space of the resemblance measure chosen (Anderson et al., 2008). The PCO was performed on previously calculated Bray–Curtis similarities for the taxonomical composition of diatom assemblages. We used the complete diatom taxa list to avoid arbitrary decisions associated to the removal of rare taxa; downweighing the

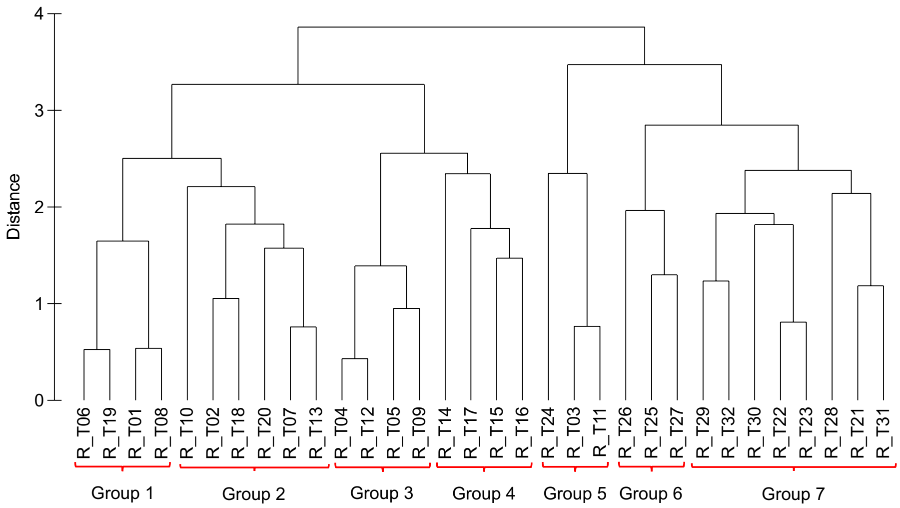

Fig. 2 Group average clustering dendrogram of river types based on their Euclidean distances using data from Table 1. The resulted seven end-groups are identified. Codes correspond to those of Table 3. Table 2 and Fig. 3 show the environmental variables characterizing each end-group and their distribution in an ordination space, respectively

contributions of rare taxa is an inherent property of the computation of Bray–Curtis similarities (Capone & Kushlan, 1991; Hansen & Ramm, 1994). Afterwards, and independently of the environmental variables, the PCO was linked to the cluster by indicating membership of each sample to a determined cluster end-group using symbol factors.

We finally used the indicator value method (IndVal; Dufrêne & Legendre, 1997) to identify the indicator species of the resulted groups of types in cluster dendrogram. IndVal is a simple and useful method to identify indicator species and species assemblages in groups of samples (Dufrêne & Legendre, 1997). The relevance of this method lies in the way it combines information on the specificity and the fidelity of occurrence of a species in a group. It produces indicator values for each species in each group expressed as the product of the specificity and fidelity. Therefore, indicator species are defined as the most characteristic species in each group. Taxa which were mostly observed in only one type of stream were nominated as type-specific. The statistical significance of the species indicator values is evaluated using a randomization procedure. The indicator value of a species i is the largest value of IndVal_{ij} observed amongst all groups j. The indicator value is at its maximum when all individuals of a species are found in a single group of sites (high specificity) and when the species occurs in all sites of that group (high fidelity) (Dufrêne & Legendre, 1997).

Cluster analysis, PCA, PCO, and PERMANOVA (999 permutations) were performed with PRIMER-E 6 v.6.1.11 and PERMANOVA + v.1.0.1 (PRIMER-E Ltd., Plymouth, UK). Analyses were carried out with square-root-transformed diatom data further converted into a resemblance matrix using Bray–Curtis similarity. Environmental data (except those expressed as ranked variables) were logarithmically transformed $(x+1)$ before analyses to reduce skewed distributions. IndVal analysis was conducted using R package "labdsv" (version 4.0.3; R Core Team, 2020).

Results

The stream sites we used in this study derive from a larger group of sites previously validated as references by the corresponding water authorities (CHE, 2017; ACA, 2020; TRAGSATEC, 2020). The selected sites accomplished the reference conditions defined by the WFD REFCON guidelines (European Commission, 2003). Setting the high ecological status required for the reference conditions, in terms of biological, hydromorphological, and physical and chemical quality elements, may be difficult in the Mediterranean region, due to the long history of human presence and the strong human exploitation of water resources (Feio et al., 2013). This is a general problem in European Mediterranean rivers, where almost all river types have experienced hydromorphological changes of anthropological origin (Feio et al., 2013; Almeida et al., 2014). Our reference sites adjusted to minimally disturbed conditions (i.e. absence of

Table 2 Mean values, coefficient of variation, and ranges of environmental variables characterizing the different cluster groups of Spanish national river types, with the number of river types per group indicated in brackets

	Group 1 (n = 4)	Group 2 (n = 6)	Group 3 (n = 4)	Group 4 (n = 4)	Group 5 (n = 3)	Group 6 (n = 3)	Group 7 (n = 8)
Altitude (m a.s.l.)							
Mean	275	186	706	369	782	978	243
CV	51.3	40.5	23.8	48.8	18.2	24.5	49.6
Range	129–470	68–250	425–855	114–554	613–961	707–1289	70–421
Catchment área (Km^2)							
Mean	428	258	493	12,247	118	202	494
CV	48.8	79.6	35.7	103.5	30.4	76.9	172.0
Range	237–754	33–613.4	275–763	3532–34,132	74–162	64–419	31–2677
Specific discharge ($m^3/s/km^2$)							
Mean	0.0061	0.0060	0.0035	0.0066	0.0124	0.0260	0.0290
CV	16.3	83.5	36.8	50.8	58.1	18.7	10.9
Range	0.0051–0.0077	0.0009–0.0164	0.0020–0.0055	0.0040–0.0123	0.0052–0.0223	0.0210–0.0326	0.0252–0.0355
Conductivity (µS/cm)							
Mean	200	572	586	451	101	241	240
CV	14.0	19.0	7.2	30.2	44.9	37.1	44.6
Range	156–229	448.5–715	545–656.6	257–586	37–136	145–360	118–350
Months of non-flow (%)							
Mean	42	21	8	1	31	8	1
CV	8.8	43.0	37.5	70.7	6.6	36.8	135.8
Range	36–46	5–31	5–13	0–2	29–34	4–11	0–5
Air temperature (ºC)							
Mean	16.5	16.8	12.9	14.8	11.9	9.6	12.4
CV	6.1	4.8	11.2	12.1	4.7	9.8	4.1
Range	15.4–17.6	15.2–17.9	11.3–14.8	12.7–17.5	11.1–12.4	8.4–10.7	11.6–13.3

significant human disturbance), or least disturbed conditions (i.e. sites with the lowest signs of human disturbance in areas with extensive human disturbance), following the categories defined by Stoddard et al. (2006). Reference conditions could be attributed to each of the river types considered in the classification of Toro et al. (2009). However, reference sites could not be nominated in large areas of some river basins (Fig. 1). In particular, the northern and southern parts of the Central Plateau, and the Ebro Depression, have large areas onto which prevail disturbed conditions (Sabater et al., 2022). The absence of reference sites was the most notorious in the main axes of rivers flowing through these areas.

The cluster analysis lead to the definition of 7 end-group river types (Fig. 2). The groups were significantly different (PERMANOVA; pseudo-$F_{6,25} = 13.548$, $P = 0.001$). The physiographic and hydrological characteristics of each of the 7 end-groups are detailed in Table 2. Cluster group 1 (4 national river types) consisted of siliceous lowland rivers. A second cluster group (6 national river types) included mineralized rivers of low altitude. Cluster group 3 (4 national river types) accounted for mineralized rivers having low specific discharge. The fourth cluster group (4 national river types) encompassed both Mediterranean and Mediterranean-continental axes. Cluster group 5 (3 national river types) included rivers with the smallest catchment area and the lowest mineralization. Groups 1 to 5 were situated in the Mediterranean biogeographic region (Fig. 1). The sixth group (3 national river types) consisted of mountain rivers, with the highest altitude and high specific discharge. This group was mostly located in the Alpine and the Atlantic regions. Finally, cluster group 7 (8 national river types) included the Cantabrian-Atlantic rivers, with streams short in length and high specific discharge. Group 7 was only found in the Atlantic biogeographic region. A Principal Components Analysis (PCA) based on the available environmental variables of the groups of types in the cluster dendrogram (Fig. 3), stressed the prevailing environmental characteristics for the different cluster groups.

We performed the ordination of the diatom assemblages by means of a Principal Coordinates Analysis (PCO) and labelled them according to the different groups of sites (Fig. 4). Even though the comparison of the two analyses has inherent limitations, the ordination of the diatom assemblages is largely coherent with that of the cluster groups. The subsequent IndVal analysis revealed that all cluster end-groups had significant indicator diatom taxa (Supplementary Table S2), and nearly all type groups had species with IndVals higher than 25% (Dufrêne & Legendre, 1997). Higher IndVals mostly occurred in groups 1, 4, and 6. *Planothidium frequentissimum* (Lange-Bertalot) *Lange-Bertalot* var. *frequentissimum* showed a high IndVal (48%) in the siliceous lowland rivers (Group 1). *Sellaphora nigri* (De Not.) C.E. Wetzel et Ector comb. nov. emend., *Melosira varians* Agardh, *Nitzschia palea* (Kützing) W. Smith, *Cocconeis euglypta* Ehrenberg, and *Navicula cryptocephala* Kützing showed an indication between 20 and 35%. The species with the highest indicator value in Group 2 were *Halamphora tenerrima* (Aleem & Hustedt) Levkov, *Nitzschia inconspicua* Grunow, *Halamphora coffeaeformis* (Agardh) Levkov, and *Brachysira aponina* Kützing, characteristic in mineralized lowland rivers. The highest IndVals for mineralized mid-altitude rivers with low specific discharge (Group 3) corresponded to *Encyonopsis microcephala* (Grunow) Krammer and *Encyonopsis minuta* Krammer & Reichardt. Aerophilic taxa such as *Sellaphora stroemii* (Hustedt) Kobayasi in Mayama Idei Osada & Nagumo, *Luticola mutica* (Kützing) D.G. Mann, *Humidophila contenta* (Grunow) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová, and *Halamphora montana* (Krasske) Levkov emerged as indicator species in Group 3. These taxa had low IndVals (≤ 11%) but showed a high specificity to this group. *Diatoma vulgaris* Bory var. *vulgaris*, *Navicula cryptotenella* Lange-Bertalot, *Cymbella affinis* Kützing, *Diatoma moniliformis* Kützing, *Achnanthidium druartii* Rimet & Couté in Rimet & al., *Pantocsekiella costei* (Druart et F. Straub) K.T. Kiss et Ács, and *Nitzschia dissipata* subsp. *dissipata* (Kützing) Grunow var. *dissipata* were indicator species in Mediterranean and Mediterranean-continental main axes (Group 4), with an indication between 21 and 38%. The best indicators for Group 5 (poorly mineralized rivers) were *Gomphonema rhombicum* M. Schmidt, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot, and *Encyonema minutum* (Hilse in Rabh.) D.G. Mann in Round Crawford & Mann. The species with the highest indicator value in Mountain rivers (Group 6) were *Gomphonema angustivalva* E. Reichardt, *Achnanthidium lineare* W. Smith, *Diatoma*

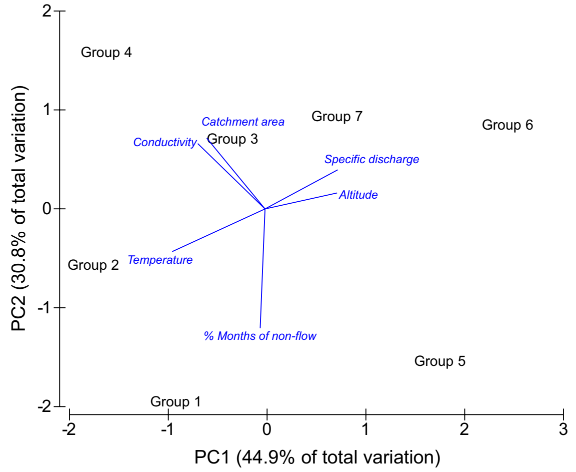

Fig. 3 Principal components analysis (PCA) based on available environmental variables of the resulted groups of types in cluster dendrogram. The vector length and direction reflect the importance of each variable's contribution to each of the two axes

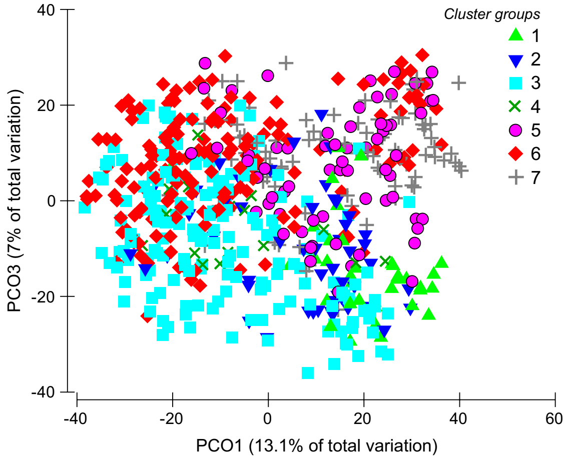

Fig. 4 Principal coordinates analysis (PCO) performed with the diatom assemblages of the 603 samples available. Independent to this ordination, the membership of each sample to one of the cluster end-groups of Fig. 2 was indicated with symbol factors

ehrenbergii Kützing, *Achnanthidium pyrenaicum* (Hustedt) Kobayasi, and *Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot. The highest IndVals for Cantabrian-Atlantic rivers (Group 7) were for *Navicula angusta* Grunow, *Surirella roba* Leclercq, and *Achnanthidium subatomus* (Hustedt) Lange-Bertalot. Some terrestrial (*Humidophila laevissima* (P.T.Cleve) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová) and aerophilic (*Eunotia minor* (Kützing) Grunow in Van Heurck, and *Nitzschia hantzschiana* Rabenhorst var. *hantzschiana*) taxa also appeared as indicators for this group, having a low IndVals (6, 15, and 5%, respectively) but high specificity.

Discussion

There is general agreement that future climate change will cause higher variability in water regimes and further reduction in water flow over large areas of the planet (Messager et al., 2021). Low water flow periods may become longer (Döll & Zhang 2010), and rivers will experience lentification, i.e. a progressive conversion towards slower flowing condition (Sabater, 2008). In many cases, streams may be driven towards desiccation for long periods (Stubbington et al., 2017) and even become terrestrialized. Under lower-than-normal dilution conditions, concentrations of nutrients and other dissolved materials increase (Petrovic et al., 2011), water warms up, and the mobility of materials and organisms becomes compromised (Liu et al., 2013; Cañedo-Argüelles et al., 2015; Death et al., 2015; Dong et al., 2016). These are conditions for significant changes in biological communities. In the case of diatom assemblages, climate change might favour taxa less sensitive to warmer and lentic conditions while affect those preferring colder and fast-moving waters (Tornés et al., 2021).

There is a clear prediction of strong runoff reductions in river ecosystems of the Iberian Peninsula under the worst climate change scenarios (IPCC, 2021). These models contemplate a 20% of runoff reduction, accompanied with an increase in temperature and conductivity for most river types. We present the associated variations in runoff, air temperature, and conductivity under the two climate change scenarios. While the predictions for the RCP 4.5 in the period 2070–2100 are moderate (Table 3), those under the RCP 8.5 scenario show significant changes to the main physiographic characteristics of many river types. Runoff (19 to 36% decrease), air temperature (increase of 4–5.3 C), and water conductivity (increase between 10 and 13%) changes will be striking at the 2070–2100 horizon. The outputs from the RCP 8.5 simulation led to the worst-case situation in

Table 3 Variations in runoff, air temperature, and conductivity predicted under the Representative Concentration Pathways (RCP) 4.5 and 8.5 for the impact period 2070–2100, for each river type in the region based on the IPCC 2013 (CEDEX, 2017)

Cluster group	Type code	River type	RCP 4.5			RCP 8.5		
			Δ Runoff (%)	Δ Temperature (°C)	Δ Conductivity (%)	Δ Runoff (%)	Δ Temperature (°C)	Δ Conductivity (%)
G1	R-T01	Siliceous plain rivers of the Tagus and Guadiana	– 16	3.9	10	– 28	5.3	13
G1	R-T06	Siliceous rivers of the foothills of Sierra Morena	– 19	3.5	9	– 31	4.7	12
G1	R-T08	Siliceous low-mountain Mediterranean rivers	– 15	3.6	9	– 26	4.8	12
G1	R-T19	Tinto and Odiel rivers	– 18	3.3	8	– 29	4.4	11
			– 17	***4***	***9***	***– 28***	***5***	***12***
G2	R-T02	Rivers of the Guadalquivir Valley	– 19	3.7	9	– 32	5.0	13
G2	R-T07	Low-altitude mineralized Mediterranean rivers	– 20	3.3	8	– 31	4.4	11
G2	R-T10	Mediterranean rivers with karst influence	– 8	3.4	9	– 19	4.5	11
G2	R-T13	Highly mineralized Mediterranean rivers	– 18	3.5	9	– 33	4.7	12
G2	R-T18	Coastal Mediterranean rivers	– 16	3.4	8	– 29	4.5	11
G2	R-T20	Wet Andalusian mountain range rivers	– 20	3.3	8	– 31	4.4	11
			– 17	***3***	***9***	***– 29***	***5***	***11***
G3	R-T04	Mineralized rivers of the Northen Plateau	– 14	3.8	10	– 25	5.1	13
G3	R-T05	Rivers of Castilla-La Mancha	– 19	3.7	9	– 33	5.0	12
G3	R-T09	Mineralized low-mountain Mediterranean rivers	– 17	3.5	9	– 30	4.7	12
G3	R-T12	Mediterranean calcareous mountain rivers	– 16	3.6	9	– 29	4.8	12
			– 16	***4***	***9***	***– 29***	***5***	***12***
G4	R-T14	Low-altitude mineralized Mediterranean axis	– 20	3.3	8	– 31	4.4	11
G4	R-T15	Mediterranean-continental low-mineralized axis	– 13	3.8	10	– 26	5.2	13
G4	R-T16	Mediterranean-continental mineralized axis	– 18	3.7	9	– 31	5.0	12
G4	R-T17	Major axes in Mediterranean environments	– 21	3.4	9	– 36	4.6	12
			– 18	***4***	***9***	***– 31***	***5***	***12***
G5	R-T03	Siliceous peneplain rivers of the Northen Plateau	– 14	3.8	10	– 25	5.1	13
G5	R-T11	Mediterranean siliceous mountain rivers	– 14	3.6	9	– 25	4.9	12

Table 3 (continued)

Cluster group	Type code	River type	RCP 4.5			RCP 8.5		
			Δ Runoff (%)	Δ Temperature (°C)	Δ Conductivity (%)	Δ Runoff (%)	Δ Temperature (°C)	Δ Conductivity (%)
G5	R-T24	Gredos-Béjar Canyon	– 14	3.9	10	– 25	5.3	13
			– 14	***4***	***9***	***– 25***	***5***	***13***
G6	R-T25	Siliceous wet mountain rivers	– 11	3.2	8	– 22	4.3	11
G6	R-T26	Calcareous wet mountain rivers	– 11	3.5	9	– 23	4.7	12
G6	R-T27	High-mountain rivers	– 13	3.5	9	– 24	4.7	12
			– 12	***3***	***8***	***– 23***	***5***	***11***
G7	R-T21	Siliceous Cantabrian-Atlantic rivers	– 10	3.0	8	– 20	4.0	10
G7	R-T22	Calcareous Cantabrian-Atlantic rivers	– 10	3.0	8	– 25	4.0	10
G7	R-T23	Rivers of the Basque Country and the Pyrenees	– 10	3.0	8	– 26	4.0	10
G7	R-T28	Main Cantabrian-Atlantic siliceous river networks	– 10	3.0	8	– 20	4.0	10
G7	R-T29	Main Cantabrian-Atlantic calcareous river networks	– 10	3.0	8	– 23	4.0	10
G7	R-T30	Coastal Cantabrian-Atlantic rivers	– 10	3.0	8	– 23	4.0	10
G7	R-T31	Small Cantabrian-Atlantic siliceous networks	– 10	3.0	8	– 20	4.0	10
G7	R-T32	Small Cantabrian-Atlantic calcareous networks	– 10	3.0	8	– 25	4.0	10
			– 10	***3***	***8***	***– 23***	***4***	***10***

The corresponding end-group of cluster analysis (Fig. 2) for each type is indicated and used for their arrangement. Mean values for each cluster end-group are given in italics and boldface type

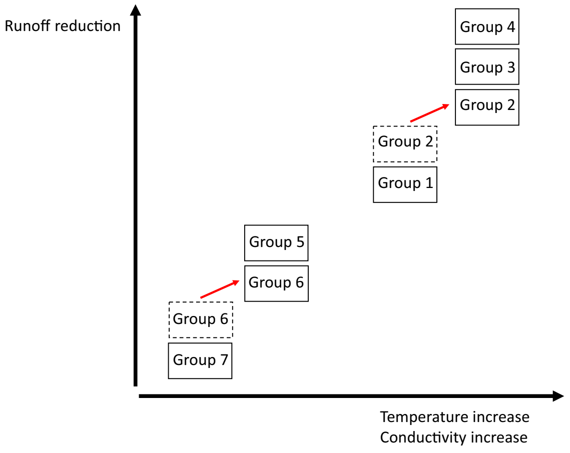

Fig. 5 Simulated displacement of end-group types of reference sites, based on the projected physiographic values of the national river types of Table 3 for the impact period 2070–2100. Displacements are indicated with dotted-lined boxes and red arrows

the southern part of the Peninsula, with runoff reductions reaching nearly 40% in the river types of the Southeast. The hydrological alterations under this scenario may lead to longer low-flow periods and drastic changes in water chemistry, particularly in the mineralized river types of Groups 2, 3, and 4.

Since hydrological data were not available for most of the sites, and therefore, they could not be used for modelling, the future effects of runoff reduction had to rely on the autecology of the diatom taxa. That is, we used the current environmental attribution of diatom taxa to define which changes would occur in the diatom assemblages in the event of the environmental changes predicted under the RCP 8.5. Then, using the projected physiographic values of the national river types in Table 3, we propose a new configuration of end-group types, which contemplates a displacement in the inter-group classification (Fig. 5).

This simulation (Fig. 5) shows that differences between the diatom assemblages in the Northern and Southern Spain, and between siliceous and calcareous regions (roughly corresponding to the Western and Eastern parts, respectively; Sabater et al., 2022), will become more pronounced. In this new arrangement, Groups 2, 3, and 4 become more alike to each other than presently are. Mediterranean and Mediterranean-continental axes (Group 4), and mineralized rivers (Groups 2 and 3), account for the highest impact (Table 3), and will become similar under future climate change conditions. In this future, scenario terrestrial and aerophilic diatoms will be the best adapted taxa to the harsh conditions imposed by runoff reduction. Also, brackish diatoms will be more frequent due to the increase in water mineralization. As such, the presence of aerophilic taxa such as *S. stroemii*, *L. mutica*, *H. contenta*, and *H. montana* (Delgado et al., 2013; Novais et al., 2014; Falasco et al., 2016; Tornés et al., 2021) and brackish taxa such as *H. tenerrima*, *N. inconspicua*, *H. coffeaeformis*, and *B. aponina* (Ros et al., 2009; Heudre et al., 2020), might become more prevalent. On the other hand, sensitive freshwater species to warmer and lentic conditions such as *A. pyrenaicum*, *G. angustivalva,* and *D. vulgaris* may become less common (Centis et al., 2010; Cantonati et al., 2012; Falasco et al., 2020).

In the new arrangement derived from the RCP 8.5 scenario, mountain rivers (Group 6) may become closer to small and poorly mineralized mid-altitude rivers (Group 5), then becoming more distinct than the present Cantabrian-Atlantic rivers (Group 7). The river types in the Atlantic biogeographic region (Group 7) and those of Group 1 which assemble poorly mineralized lowland streams in the Mediterranean region, will likely become apart from all other groups. Such a singularity is under the assumption that these river types will be the least affected under the RCP 8.5 conditions. However, even in the Cantabrian-Atlantic rivers, presently the wettest parts of the Iberian Peninsula, terrestrial, and aerophilic diatoms are already present, with a high group specificity. This may indicate a potential risk if the lentification of these systems is progressing, which needs to be confirmed by future investigations. In these systems, lentic (planktic) species might be also abundant, replacing large-sized taxa in presently lotic areas (B-Béres et al., 2019).

Overall, our predictions may be summarized by saying that climate change may likely act by reducing the differences between river types, and causing that diatom assemblages could simplify their composition towards higher homogenization. Biotic homogenization has been described as one of the most likely effects promoted by climate change (e.g. Tornés & Ruhí, 2013; Petsch, 2016; Falasco et al., 2021), with likely implications for loss of resistance and resilience of biological communities, and alteration of related ecosystem functions.

Finally, the limitation of hydrological data on the reference sites leads to a general concern. The scarcity of hydrological data in our study highlights a global problem, especially in the case of non-perennial rivers and streams (Messager et al., 2021). Hydrological characterization of reference conditions is limited in many river networks, due to their poor instrumentation in many tributaries. In fact, most gauging stations are installed on large, perennial rivers worldwide (Zimmer et al., 2020), while headwaters are underrepresented. A temporal bias is added to this spatial constraint, since in some monitoring schemes, gauging stations may change location or data frequency over time. Advancing on effects of climate change on water flow and biological communities in reference sites requires shifting from the current scarcity of primary hydrological data to a planned, long-term monitoring including hydrological and biological data (Zimmer et al., 2020; Messager et al., 2021). These are essential requisites to produce reliable predictions on the effects of climate change in riverine biota.

Funding This research was funded by the project “Technical service for monitoring reference stations and obtaining the baseline to assess alterations due to climate change and atmospheric pollution. Current situation (21.803-0985/0421)” of the Spanish Ministry of Ecological Transition—DG Water.

Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

ACA, 2020. Estudi general de la demarcació, anàlisi d’impactes i pressions de l’activitat humana i anàlisi econòmica de l’ús de l’aigua a les masses d’aigua del districte de conca fluvial de Catalunya. Document IMPRESS 2019. Generalitat de Catalunya, Departament de Territori i Sostenibilitat. http://aca.gencat.cat/ca/plans-i-programes/pla-de-gestio/3r-cicle-de-planificacio-2022-2027/document-impress/index.html.

Almeida, S. F. P., C. Elias, J. Ferreira, E. Tornés, C. Puccinelli, F. Delmas, G. Dörflinger, G. Urbanič, S. Marcheggiani, J. Rosebery, L. Mancini & S. Sabater, 2014. Water quality assessment of rivers using diatom metrics across Mediterranean Europe: a methods intercalibration exercise. Science of the Total Environment 476–477: 768–776.

Álvarez, J., A. Sánchez & L. Quintas, 2004. SIMPA, a GRASS based tool for hydrological studies. In Proceedings of the FOSS/GRASS Users Conference, Bangkok.

Anderson, M. J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences 58: 626–639.

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER. Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK.

Amigo, J., M. A. Rodríguez-Guitián, J. J. P. Honrado & P. Alves, 2017. The lowlands and midlands of Northwestern Atlantic Iberia. In Loidi, J. (ed), The vegetation of the Iberian Peninsula, plant and vegetation, 12. Springer, Cham: 191–250.

B-Béres, V., B. Tóthmérész, I. Bácsi, G. Borics, A. Abonyi, K. Tapolczai, F. Rimet, A. Bouchez, G. Várbíró & P. Török, 2019. Autumn drought drives functional diversity of benthic diatom assemblages of continental intermittent streams. Advances in Water Resources 126: 129–136.

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

Cantonati, M., M. G. Kelly & H. Lange-Bertalot, 2017. Freshwater benthic diatoms of Central Europe: over 800 common species used in ecological assessments. English edition with updated taxonomy and added species, Koeltz Botanical Books, Schmitten-Oberreifenberg:

Cañedo-Argüelles, M., K. S. Boersma, M. T. Bogan, J. D. Olden, I. Phillipsen, T. A. Schriever & D. A. Lytle, 2015. Dispersal strength determines meta-community structure in a dendritic riverine network. Journal of Biogeography 42: 778–790.

Capone, T. A. & J. A. Kushlan, 1991. Fish community structure in dry-season stream pools. Ecology 72: 983–992.

CEDEX, 2017. Evaluación del impacto del cambio climático en los recursos hídricos y sequías en España. Informe Técnico para el Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, Secretaría de Estado de Medio Ambiente, Oficina Española de Cambio Climático. Tomo Único, clave CEDEX 42-407-1-001. Centro de Publicaciones, Secretaría General, Técnica del Ministerio de Fomento. http://www.cedex.es/CEDEX/LANG_CASTELLANO/ORGANISMO/CENTYLAB/CEH/Documentos_Descargas/EvaluacionimpactoCCsequiasEspana2017.htm.

Centis, B., M. Tolotti & N. Salmaso, 2010. Structure of the diatom community of the River Adige (North-Eastern Italy) along a hydrological gradient. Hydrobiologia 639: 37–42.

CHE, 2017. Análisis de presiones e impactos y evaluación del riesgo de incumplir los objetivos medioambientales de la Directiva Marco del Agua en aguas superficiales de la cuenca del Ebro. Documento IMPRESS 2015. Confederación Hidrográfica del Ebro, Área de Calidad de las Aguas. http://www.chebro.es/contenido.visualizar.do?idContenido=22071&idMenu=4041.

Colls, M., X. Timoner, C. Font, S. Sabater & V. Acuña, 2019. Effects of duration, frequency, and severity of the non-flow period on stream biofilm metabolism. Ecosystems 22: 1393–1405.

Death, R. G., I. C. Fuller & M. G. Macklin, 2015. Resetting the river template: the potential for climate-related extreme floods to transform river geomorphology and ecology. Freshwater Biology 60: 2477–2496.

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

Döll, P. & J. Zhang, 2010. Impact of climate change on freshwater ecosystems: a global-scale analysis of ecologically relevant river flow alterations. Hydrology and Earth System Sciences 14: 783–799.

Dong, X. Y., B. Li, F. Z. He, Y. Gu, M. Q. Sun, H. M. Zhang, L. Tan, W. Xiao, S. R. Liu & Q. H. Cai, 2016. Flow directionality, mountain barriers and functional traits determine diatom metacommunity structuring of high mountain streams. Scientific Reports 6: 24711.

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

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z.-I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A.-H. Prieur-Richard, D. Soto, M. L. J. Stiassny & C. A. Sullivan, 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81: 163–182.

European Commission, 2000. 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. Official Journal of the European Communities L327: 1–73.

European Commission, 2003. Common implementation strategy for the Water Framework Directive (2000/60/EC), Working Group REFCON, guidance document no. 10, rivers and lakes – tipology, reference conditions and classification systems.

Falasco, E., E. Piano & F. Bona, 2016. Diatom flora in Mediterranean streams: flow intermittency threatens endangered species. Biodiversity and Conservation 25: 2965–2986.

Falasco, E., E. Piano, A. Doretto, S. Fenoglio & F. Bona, 2018. Lentification in Alpine rivers: patterns of diatom assemblages and functional traits. Aquatic Sciences 80: 36.

Falasco, E., D. Alberto, S. Fenoglio, E. Piano, & F. Bona, 2020. Supraseasonal drought in an Alpine river: effects on benthic primary production and diatom community. https://doi.org/10.4081/jlimnol.2020.1933.

Falasco, E., F. Bona, A. M. Risso & E. Piano, 2021. Hydrological intermittency drives diversity decline and functional homogenization in benthic diatom communities. Science of the Total Environment 762: 143090.

Feio, M. J., F. C. Aguiar, S. F. Almeida, J. Ferreira, M. T. Ferreira, C. Elias, S. R. Serra, A. Buffagni, J. Cambra, C. Chauvin, F. Delmas, G. Dörflinger, S. Erba, N. Flor, M. Ferréol, M. Germ, L. Mancini, P. Manolaki, S. Marcheggiani, M. R. Minciardi, A. Munné, E. Papastergiadou, N. Prat, C. Puccinelli, J. Rosebery, S. Sabater, S. Ciadamidaro, E. Tornés, I. Tziortzis, G. Urbanič & C. Vieira, 2013. Least disturbed condition for European Mediterranean rivers. Science of the Total Environment 476–477: 745–756.

Gartzia de Bikuna, B., E. Lopez, J. M. Leonardo, J. Arrate, A. Martinez & A. Manzanos, 2015. Development of a multimetric benthic macroinvertebrate index for assessing the ecological condition of Basque streams (north of Spain). Fundamental and Applied Limnology 187: 21–32.

Gasith, A. & V. H. Resh, 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology and Systematics 30: 51–81.

Gudmundsson, L., J. Boulange, H. X. Do, S. N. Gosling, M. G. Grillakis, A. G. Koutroulis, M. Leonard, J. Liu, H. Müller Schmied, L. Papadimitriou, Y. Pokhrel, S. I. Seneviratne, Y. Satoh, W. Thiery, S. Westra, X. Zhang & F. Zhao, 2021. Globally observed trends in mean and extreme river flow attributed to climate change. Science 371: 1159–1162.

Hansen, M. J. & C. W. Ramm, 1994. Persistence and stability of fish community structure in a southwest New York stream. American Midland Naturalist 132: 52–67.

Hayashi, M., 2004. Temperature-electrical conductivity relation of water for environmental monitoring and geophysical data inversion. Environmental Monitoring and Assessment 96: 119–128.

Heudre, D., C. E. Wetzel, B. Van de Vijver, L. Moreau & L. Ector, 2020. Brackish diatom species (Bacillariophyta) from rivers of Rhin-Meuse basin in France. Botany Letters 168: 56–84.

IPCC, 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, New York. In Press.

Jaramillo, F. & G. Destouni, 2015. Local flow regulation and irrigation raise global human water consumption and footprint. Science 350: 1248–1251.

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

Krammer K. & H. Lange-Bertalot, 1991a. Bacillariophyceae 3. Teil: Centrales, Fragilariaceae, Eunotiaceae. In Ettl H., J. Gerloff, H. Heynig & D. Mollenhauer, (eds) Süsswasserflora von Mitteleuropa, 2. Gustav Fischer Verlag, Stuttgart, Germany.

Krammer, K. & H. Lange-Bertalot, 1991b. Bacillariophyceae 4. Teil: Achnanthaceae, Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema. In Ettl H., J. Gerloff, H. Heynig & D. Mollenhauer (eds), Süsswasserflora von Mitteleuropa, 2. Gustav Fischer Verlag, Stuttgart, Germany.

Krammer K. & H. Lange-Bertalot, 1997a. Bacillariophyceae 1. Teil: Naviculaceae. In Ettl H., J. Gerloff, H. Heynig, D. Mollenhauer (eds) Süsswasserflora von Mitteleuropa, 2. Gustav Fischer Verlag, Jena, Germany.

Krammer, K. & H. Lange-Bertalot, 1997b. Bacillariophyceae 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In

Ettl H., J. Gerloff, H. Heynig & D. Mollenhauer (eds), Süsswasserflora von Mitteleuropa, 2. Gustav Fischer Verlag, Jena, Germany.

Liu, J., J. Soininen, B. P. Han & S. A. Declerck, 2013. Effects of connectivity, dispersal directionality and functional traits on the metacommunity structure of river benthic diatoms. Journal of Biogeography 40: 2238–2248.

MAGRAMA, 2013. Protocolo de muestreo y laboratorio de flora acuática (organismos fitobentónicos) en ríos. Código ML-R-D-2013. Centro de Publicaciones, Secretaría General Técnica del Ministerio de Agricultura, Alimentación y Medio Ambiente. https://www.miteco.gob.es/es/agua/temas/estado-y-calidad-de-las-aguas/ML-R-D-2013-Muestreo%20y%20laboratorio_Flora%20acu%C3%A1tica%20Fitobentos_R%C3%ADos%2024_05_2013_tcm30-175285.pdf

Messager, M. L., B. Lehner, C. Cockburn, N. Lamouroux, H. Pella, T. Snelder, K. Tockner, T. Trautmann, C. Watt & T. Datry, 2021. Global prevalence of non-perennial rivers and streams. Nature 594: 391–397.

Novais, M. H., M. M. Morais, J. Rosado, L. S. Dias, L. Hoffmann & L. Ector, 2014. Diatoms of temporary and permanent watercourses in Southern Europe (Portugal). River Research and Applications 30: 1216–1232.

Pan, Y. D., R. J. Stevenson, B. H. Hill, P. R. Kaufmann & A. T. Herlihy, 1999. Spatial patterns and ecological determinants of benthic algal assemblages in Mid-Atlantic streams, USA. Journal of Phycology 35: 460–468.

Petrovic, M., A. Ginebreda, V. Acuña, R. J. Batalla, A. Elosegi, H. Guasch, M. López de Alda, R. Marcé, I. Muñoz, A. Navarro-Ortega, E. Navarro, D. Vericat, S. Sabater & D. Barceló, 2011. Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers. Trends in Analytical Chemistry 30: 1269–1278.

Petsch, D. K., 2016. Causes and consequences of biotic homogenization in freshwater ecosystems. International Review of Hydrobiology 101: 113–122.

QGIS Development Team, 2020. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org.

R Core Team, 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Ros, M. D., J. P. Marín-Murcia & M. Aboal, 2009. Biodiversity of diatom assemblages in a Mediterranean semiarid stream: implications for conservation. Marine and Freshwater Research 60: 14–24.

Sabater, S., 2008. Alterations of the global water cycle and their effects on river structure, function and services. Freshwater Reviews 1: 75–88.

Sabater, S., F. Bregoli, V. Acuña, D. Barceló, A. Elosegi, A. Ginebreda, R. Marcé, I. Muñoz, L. Sabater-Liesa & V. Ferreira, 2018. Effects of human-driven water stress on river ecosystems: a meta-analysis. Scientific Reports 8: 11462.

Sabater S., A. Elosegi, M. J. Feio, R. Gómez, M. A. S. Graça, I. Muñoz, I. Pardo & A. M. Romaní, 2022. In Tockner, K., Ch. Zarfl & C. T. Robinson (eds), The Iberian Rivers. In: Rivers of Europe. Academic Press, 2nd Edition: 181–224.

Stoddard, J. L., D. P. Larsen, C. P. Hawkins, R. K. Johnson & R. H. Norris, 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16: 1267–1276.

Stubbington, R., J. England, P. J. Wood & C. E. Sefton, 2017. Temporary streams in temperate zones: recognizing, monitoring and restoring transitional aquatic-terrestrial ecosystems. Wiley Interdisciplinary Reviews Water 4: e1223.

Tornés, E. & A. Ruhí, 2013. Flow intermittency decreases nestedness and specialisation of diatom communities in Mediterranean rivers. Freshwater Biology 58: 2555–2566.

Tornés, E., M. Leira & S. Sabater, 2012. Is the biological classification of benthic diatom communities concordant with ecotypes? Hydrobiologia 695: 43–55.

Tornés, E., M. Colls, V. Acuña & S. Sabater, 2021. Duration of water flow interruption drives the structure and functional diversity of stream benthic diatoms. Science of the Total Environment 770: 144675.

Toro, M., S. Robles, I. Tejero, E. Cristóbal, S. Velasco, J. R. Sánchez & Pujante A., 2009. Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España. Ministerio de Medio Ambiente, y Medio Rural y Marino, Madrid.

TRAGSATEC, 2020. Estudio, validación y selección final de estaciones de referencia en ríos. Informe REFCON. Grupo TRAGSA. Ministerio para la Transición Ecológica y el Reto Demográfico, Madrid.

Wallin M, Wiederholm T, Johnson RK (2003). Guidance on establishing reference conditions and ecological status class boundaries for inland surface waters. Common Implementation Strategy Working Group 2.3 – REFCOND, 2.

Wohl, E. E., 2010. Mountain Rivers Revisited, Water Resources Monograph, 19, American Geophysical Union, Washington, DC:

Zimmer, M. A., K. E. Kaiser, J. R. Blaszczak, S. C. Zipper, J. C. Hammond, K. M. Fritz, K. H. Costigan, J. Hosen, S. E. Godsey, G. H. Allen, S. Kampf, R. M. Burrows, C. A. Krabbenhoft, W. Dodds, R. Hale, J. D. Olden, M. Shanafield, A. G. DelVecchia, A. S. Ward, M. C. Mims, T. Datry, M. T. Bogan, K. S. Boersma, M. H. Busch, C. N. Jones, A. J. Burgin & D. C. Allen, 2020. Zero or not? Causes and consequences of zero-flow stream gage readings. Wiley Interdisciplinary Reviews Water 7: e1436.