ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Diatom Red Lists: important tools to assess and preserve biodiversity and habitats in the face of direct impacts and environmental change

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*
Cantonati M., Hofmann G., Spitale D., Werum M., Lange-Bertalot H. (2022). Diatom Red Lists: important tools to assess and preserve biodiversity and habitats in the face of direct impacts and environmental change. BIODIVERSITY AND CONSERVATION, 31(2), 453-477 [10.1007/s10531-021-02339-9].

*Availability:*
This version is available at: https://hdl.handle.net/11585/904129 since: 2024-05-16

*Published:*
DOI: http://doi.org/10.1007/s10531-021-02339-9

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

(Article begins on next page)

12 October 2024

This is the final peer-reviewed accepted manuscript of:

Cantonati M.; Hofmann G.; Spitale D.; Werum M.; Lange-Bertalot H.: *Diatom Red Lists: important tools to assess and preserve biodiversity and habitats in the face of direct impacts and environmental change*

BIODIVERSITY AND CONSERVATION
VOL. 31
ISSN 0960-3115
DOI: 10.1007/s10531-021-02339-9

The final published version is available online at:

https://dx.doi.org/10.1007/s10531-021-02339-9

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

*This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)*

***When citing, please refer to the published version.***

Manuscript

Click here to access/download;Manuscript;Diatom-Red-Lists-R2_text-tables_clean-copy.docx

Click here to view linked References

# Diatom Red Lists: important tools to assess and preserve biodiversity and habitats in the face of direct impacts and environmental change

**Marco Cantonati [1,*], Gabriele Hofmann [2], Daniel Spitale [1], Marcus Werum [3], Horst Lange-Bertalot [4]**

[*] Corresponding author. E-mail address: marco.cantonati@muse.it (M. Cantonati).

https://orcid.org/0000-0003-0179-3842

Open Research statement: Currently available underlying data can be accessed at:

https://www.rote-liste-zentrum.de/de/Download-Pflanzen-1871.html

## Abstract

Freshwater biodiversity is disproportionally endangered and under-prioritized. Algal Red Lists literature is very limited, and there are only two editions of a single diatom-specific Red List (developed for Germany), in spite of the importance of diatoms for biodiversity and global ecosystem functioning. We used and analysed the new diatom Red List, compared to the previous edition, to show that these diatom microalgae threat status data allow, on one hand, a characterization of the ecological integrity and of the diatom diversity of the different types of inland-water ecosystems (also of practical importance to designate the most relevant habitats for conservation purposes), including a clear assessment of the threat status of the habitat, and, on the other hand, they offer ample possibilities to track the effects of stressors and of environmental change. Our results revealed, among other things, that: - threatened taxa concentrate in dystrophic-oligotrophic environments; - 'not threatened', i.e. tolerant and opportunistic, taxa are most frequent in eutrophic and saline ecosystems; - most local diatom extinctions happened in carbonate oligotrophic habitats. In this study, nitrates could be shown to possess a highly significant negative association with the cumulative percentage of diatom taxa in threat categories of the Red List. Having included heterogeneous studies (diverse inland-waters, different geographic areas, from close-to-pristine to impacted, neo- and paleolimnology etc.), we think that this strong negative association is noteworthy and points to the high potential of diatom-Red-List based approaches, especially if these would be tailored for the different biogeographic and climatic ecoregions.

**Keywords** Diatoms · Red Lists · Oligotrophy · Dystrophic environments · Nitrates

## Introduction

International Union for Conservation of Nature (IUCN) Red Lists offer insight into present-day species declines. Foissner (2008) concludes that it is important to include also protists in conservation strategies, and Juráň and Kaštovský (2019) underline that there should be Red Lists also of microalgae because of their role in monitoring and assessment of habitat quality.

Current extinction rates are unprecedentedly high in human history (e.g., Davies 2019), and biodiversity loss in freshwater environments five times higher as compared to terrestrial systems (e.g., Ricciardi and Rasmussen 1999). Moreover, inland-water ecosystems are suffering the impact of multiple stressors acting in combination and the threat multiplier effect of climate change (e.g., Downing 2014; Cantonati et al. 2020a). Fresh water is the main strategic, and nevertheless increasingly scarce, resource but its future, along with that of the species and ecosystems it supports (almost 7% of global biodiversity in spite of being tiny in relative volume) is uncertain (Cantonati et al. 2020a). Freshwater biodiversity is disproportionally endangered and under-prioritized when compared to the marine and terrestrial biota. Yet the Water Framework Directive (WFD; European Union 2000) lacks adequate consideration of wetlands, freshwater ecosystem services, and smaller water bodies (van Rees et al. 2020).

Species at risk are non-randomly distributed across phylogeny (Davies 2019). Diatoms are the most species-rich group of algae (Mann 1999). The global diversity of diatoms may be one or more orders of magnitude higher than the current number of species described (Vanormelingen et al. 2008), with as many as ca. 100,000 but at least 30,000 extant diatom species (Mann and Vanormelingen 2013). They contribute to global primary production with a proportion as large as 20%, possessing global significance in the carbon and silicon cycles (Mann 1999). They can be found wherever there is a little bit of moisture, and in all aquatic habitats. Most of them have very precise preferences for ecological conditions of paramount relevance in environmental quality assessments, such as pH, mineralization, nutrients etc. The frustules allow species identification, and are usually preserved in sediments, thus providing a precious tool for paleo-environmental reconstructions, and diatom mounts are excellent collection objects, present in many natural history museums. All these characteristics

have fostered the use of diatoms in a wide array of disciplines (e.g., Smol and Stoermer 2010). A combination of mechanisms ("melting pot genome" of ca. 10.000 genes with many diatom specific genes but also extensive gene transfer from bacteria to diatoms; active urea cycle, probably used to improve nitrogen storage; fatty acids oxydation in mitochondria; chemical information exchange among cells etc.) may underlie the rapid diversification rates, and may explain why diatoms have come to dominate several contemporary systems in a relatively short period of time (Bowler et al. 2008). In oligotrophic habitats, which host diverse and characteristic diatom communities (e.g., Lange-Bertalot and Metzeltin 1996), a detailed taxonomy, capable of identifying and characterizing typical (and, possibly, new) taxa must be applied. Shoe-horning of taxa in aggregates of species common in meso- and eutrophic waters must be avoided. For diatoms, Mann (1999) advocated a so-called "Waltonian" species concept, a total-evidence approach combining data from morphology, genetics, mating systems, physiology, ecology, and crossing behaviour.

Vanormelingen et al. (2008) pointed out that detailed taxonomic inventories using fine-grained morphological characteristics, molecular markers, and crossing experiments revealed that the geographic distribution of diatoms ranges from global to narrow endemic, and that population divergence apparently takes place over distances of 100s to 1000s of kilometres. According to Juráň and Kaštovský (2019) microorganisms should be viewed in Red Lists primarily as poorly-known, rather than rare.

Oligotrophic aquatic habitats have been neglected also by ecologists and taxonomists, including those working on diatoms (Kociolek and Stoermer 2009), whose attention has been more focussed on aquatic environments affected by human impacts (eutrophication, acidification etc.). The diatom microflora of oligotrophic environments has received renewed attention only since the 1990s. Lange-Bertalot and Metzeltin (1996) found as many as 800 taxa in a small number of samples from three oligotrophic lakes (from carbonate to dystrophic) at a time when the total diatom flora of Central Europe was estimated to comprise 1600 taxa. Oligotrophic habitats are threatened by direct (exploitation for hydropower and drinking water, land-reclamation, nutrient enrichment etc.) and indirect (diffuse airborne pollution, climate change) impacts. Lange-Bertalot (2007) suggests that

there are indeed cosmopolitan diatom species, and that these include, in the first instance, about 250 well-known species adapted to thrive in highly eutrophic to strongly saprobic waters and widely distributed in cultural landscapes e.g. in Europe. Unluckily, strictly oligotrophic freshwaters are becoming rarer and rarer because of direct and indirect (diffuse) human impact.

Diatom Red Lists provide an account of the diatom flora of a geographic area pointing out the distribution and threat status of individual species as determined by environmental factors and impacts. Soininen (2007) identified ion concentration and trophic status as major regional-scale factors and light, current velocity, and substratum composition as key local-scale factors influencing diatom distributions. The main environmental variables affecting composition of diatom assemblages in oligotrophic habitats are pH, conductivity, inorganic N, substrate particle size, and shade (e.g., Cantonati 1998). Among the environmental determinants of diatom distribution, nitrate often stands out as particularly relevant factor (e.g., Bertrand et al. 1999; Tolotti 2001; Gesierich and Kofler 2010; Cantonati et al. 2012).

So far, applications of the diatom Red Lists for Germany (Lange-Bertalot 1996; Hofmann et al. 2018) have been relatively rare but included a fair variety of inland-waters' habitats: springs (e.g., Werum and Lange-Bertalot 2004; Cantonati et al. 2012), mountain mires (e.g., Fránková et al. 2009; Cantonati et al. 2011), glacial streams (e.g., Fell et al. 2018), alpine aquatic habitats (e.g., Falasco and Bona 2011), high-mountain lakes (e.g., Tolotti 2001, including paleolimnology: Cantonati et al. 2021a), large peri-alpine lakes (Spitale et al. 2011), Mediterranean streams (e.g., Falasco et al. 2016; Cantonati et al. 2020c).

We used and analysed the new diatom Red List (Hofmann et al. 2018), compared to the previous edition (Lange-Bertalot 1996), to show that these diatom microalgae threat status data allow, on one hand, a characterization of the ecological integrity and diatom diversity of the different types of inland-water ecosystems (e.g., Cantonati et al. 2020a), including a clear assessment of the threat status of the habitat, and, on the other hand, offer ample possibilities to track the effects of stressors and of environmental change.

## Methods

### Diatom Red Lists for Germany

The first diatom Red List for Germany was published in 1996 (Lange-Bertalot 1996; hereafter referred to as RL96), and actualized 22 years later by Hofmann et al. (2018; hereafter referred to as RL18). The threat analysis for the latter was grounded on a taxonomically harmonized dataset based on studies from 1985 to 2008, which includes ca. 7600 diatom samples from various German rivers and 3000 samples from German lakes (Hofmann et al. 2013). These data are not evenly distributed across the main inland aquatic habitats occurring in Germany (see Supplementary Information Table 1 and Finck et al. 2017) but originate mainly from the stream, river, and lake types considered in the German monitoring network. These, however, include also a fair amount of data from acidification-sensitive silicate streams of the low-elevation mountain ranges of Central Germany, which have been part of the acidification monitoring network since the mid-1980s. Dystrophic aquatic environments, small water bodies, and dilute (soft-water) lakes are largely underrepresented in the dataset. The abundance of the species is recorded as per cent (relative abundance) of the assemblage composition, a quantification method common in diatom studies. Unpublished studies from the years 2008 to 2015 that had not been included in the dataset, several individual papers and books (e.g., Reichardt 1997; 2006; 2012; Werum and Lange-Bertalot 2004), as well as historical samples from various collections were used to complement habitats not covered by regular/official monitoring.

Whilst RL96 was still largely based on empirical assessments by the author based on expertise and literature, RL18 was mainly generated from the above-described dataset and following the standardized method of threat analysis (e.g., Gärdenfors et al. 2001; IUCN 2012). In addition to the current-distribution, the long-term development was the most important criterion to assess population declines and thus the possible endangerment of the diatom taxa. Long-term development trends were achieved by comparing the current distribution situation with the literature and historical diatom samples from the time before the onset of the massive water pollution.

Excel files with the data of the RL18 can be downloaded at: https://www.rote-liste-zentrum.de/de/Download-Pflanzen-1871.html.

RL18 threat categories are as follows: 0 = extinct or lost, 1 = threatened with extinction, 2 = strongly threatened, 3 = threatened, G = threat of unknown extent, R = extremely rare, V = declining, D = data insufficient, * = not threatened. RL18 current-distribution categories are as follows: ex = extinct or lost, er = extremely rare, vr = very rare, r = rare, mf = moderately frequent, f = frequent, vf = very frequent, ? = unknown, na = not assessed.

RL18 ecological attributes of the taxa are as follows: ae = aerial, o = oligotraphentic, oc = oligotraphentic carbonate, od = oligotraphentic distrophic, eu = eutraphentic to tolerant, hal = halophilic, ? = unknown.

**RL18 data processing and statistical analyses**

Diatom taxa of RL18 were automatically matched, using code in R (R Core Team 2020), to ecological attributes in the lists of van Dam et al. (1994; providing information on trophic status, moisture, pH etc.) and Rimet and Bouchez (2012; providing information on size, growth forms/ecological guilds, pioneer species etc.). The matches were then checked and expanded manually accounting for synonyms. Tables 3 and Supplementary Information Table 3 provide details for the most threatened (cat. 0 + 1) taxa and the putative neophytes, respectively. As concerns the most menaced species' and neophytes' tables (Table 3, Supplementary Information Table 3), matches with van Dam et al. (1994) were too few to be used in a meaningful way, and matches with Rimet and Bouchez (2012) were available in fair amount (50%) for the neophytes only.

To test for agreement between RL96 and RL18, using only taxa with congruent taxonomy in the two Red Lists, we used Cohen's kappa (k) (Cohen 1960, 1968), a commonly used measure of agreement that compares the observed agreement to the congruency expected by chance if two observers' panels rate independently (k = 1 means perfect agreement). The original (unweighted k) only counts strict agreement (the same category is assigned by both panels). A weighted version of k (Cohen 1968) allows for partial agreement. For example, exact agreements can be given full weight,

while a one-category difference might be given a weight of 1/2. This typically makes sense only when the categories are ordered. Cohen's kappa values were used to construct a contingency table. Based on this, an Observer Agreement Chart between RL96 and RL18 was plotted (Fig. 1).

To measure the association between the RL18 threat categories and the current-distribution situation of the diatom taxa we used Cramer's V (Friendly and Meyer 2015), varying from 0 (no association) to 1 (perfect association).

To measure the association between the RL18 threat categories and the ecological groups available in RL18, and between RL18 threat categories and selected ecological attributes reported by van Dam et al. (1994) and Rimet and Bouchez (2012), we used association plots. In the association plot, each cell is shown by a rectangle, constructed in a way that each cell is proportional to the respective raw residual. The rectangles are positioned relative to a baseline representing independence shown by a dotted line. Cells with observed > expected frequency rise above the line and are coloured blue, cells with observed < expected frequency fall below it and are shaded red.

Associations between RL18 diatom taxa and ecological attributes available in Rimet and Bouchez (2012) were tested for significance using *Pearson's Chi-squared* ($\chi^2$) tests with Yates' continuity correction for two-way tables. All numerical analyses were performed using R (R Core Team 2020) and mainly the packages *vcd* (Meyer et al. 2020) and *vcdExtra* (Friendly 2021).

**Applications of diatom Red Lists**

We assessed all studies we were aware of (own, published and unpublished, and those available in the literature) in which RL96 or RL18 were applied on a variety of current (and past) inland-water ecosystems from different geographic areas. In particular, we focussed on the relationship between Red List threat categories and nitrate concentrations, and, therefore, only studies providing these data were retained.

The relationship between the cumulative percentage of diatom taxa in threat categories of the Red List and mean nitrate nitrogen concentrations was investigated with a Pearson's product-moment correlation, and applying a linear model on the log-transformed nitrate concentrations.

## Results

### Diatom Red Lists

An ‘Article title, Abstract, Keywords’ query ( diatom* AND "red list*" ) carried out in Scopus on May 21st 2021 yielded only 29 relevant document results, of which about 50% were authored/co-authored by one of the authors of the present paper.

RL96 contained a total of 1,435 taxa whilst RL18 includes 715 more (2,103). This 50% increase is mainly the result of intensified investigations and monitoring programs since 1996 which significantly expanded the database. After 1996, numerous species were detected for the first time from German waters, e.g. from the previously poorly-investigated waters of the northern German lowlands. Also newly described species and those which were the result of differentiation/splitting made a significant contribution: 110 out of the 715 taxa (= 15%) added to the new edition (Hofmann et al. 2018).

### Agreement between RL96 and RL18

The association table (Table 1) shows the extent of overlap in the Red List categories between taxa with congruent taxonomy in both lists. The highest congruency (0.91) was found for the ‘extremely rare’ category, meaning that 91% of the taxa were listed R in RL96 as well as RL18. The lowest congruency between the same category was related to category 2 with only 36% of taxa in this category in RL96 persisting in this category in RL18.

The excellent consistency between the two Red Lists is confirmed by Cohen’s kappa (Fig. 1; Supplementary Information Table 2).

The histogram in Fig. 2 shows the number of taxa (with percentages on each bin) in each of the threat categories of RL96 and RL18. The ‘threat of unknown extent’ (G), and ‘not threatened’ (*) categories increased by 16% and 7%, respectively. On the contrary, there was a marked decrease (22%) of taxa with ‘data insufficient’ (D).

**The new diatom Red List (RL18)**

The association table based on Cramer's V values (Table 1) between the RL18 threat categories and the current-distribution status of the diatom taxa shows a very strong and consistent association. The overall Cramer's V value of 0.674 is statistically highly significant ($P$ <0.0001).

The histogram in Fig. 3 shows the current distribution status of the diatom taxa of the Red List (RL18). A striking observation is that rare taxa are about three times more numerous than common ones.

Table 3 shows the 55 most menaced taxa of RL18 (4 taxa in category "0" = extinct or lost, and 51 taxa in cat. "1" = threatened with extinction). The effort of expanding and updating Red List knowledge allowed to assign three taxa for which Red List status was unknown (D) in 1996 to category 0, and 28 of these taxa to category 1. Consistently with their threat category, the four taxa in the 'extinct or lost' category, and the remaining fifty-one considered nearly extinct are 'extremely rare'. For the 51 non-extinct taxa, the long-term development is predicted to be "decline of unknown extent" for most taxa, with only two estimated to be affected by "moderate decline". As many as 31 of the most endangered taxa are restricted to oligo-dystrophic environments, 10 to oligotrophic habitats, and 8 to oligotrophic calcareous ecosystems (for 6 taxa the preferred environment is unknown). Consistently, the two most well-represented genera among the 51 taxa 'threatened with extinction' are *Eunotia* with 18 taxa and *Pinnularia* with 10 taxa.

Following Hofmann et al. (2018), Supplementary Information Table 3 lists the ten species that have been suggested in the literature to be neophytes for central Europe. *Achnanthidium* is the genus with most species (4) in this group. The most common threat category is 'not threatened' (4) followed by 'data insufficient' (3). Most (6) of the ten species are classified as eutraphentic, with two halophilic species, and two with insufficiently known ecology. As concerns the current distribution, three species are 'moderately frequent', three are 'rare', one is 'very rare', and three are 'extremely rare'. All species (3) for which a long-term development indication could be assigned have 'clear increase'. Life forms/ecological guilds were available for 50% of these species. As concerns size class, three

species are large and two are small. The large majority (4) of species for which data were available are motile.

**Association of RL18 with ecological groups**

The association plot between RL18 threat categories and RL18 ecological groups (Fig. 4) was highly significant ($\chi^2$ = 1385.3, *df* = 48, $P < 2.2\text{x}10^{-16}$). It suggests that: - most diatom extinctions happened in carbonate oligotrophic habitats, - threatened taxa concentrate in dystrophic-oligotrophic environments, - 'not threatened' (*) taxa are most frequent in eutrophic and saline ecosystems. These observations are largely confirmed by the highly-significant association plots with ecological attributes according to van Dam et al. (1994): pH (Fig. 5; $\chi^2$ = 289.9, *df* = 40, $P < 2.2\text{x}10^{-16}$); nitrogen uptake metabolism (Supplementary Information Fig. 1; $\chi^2$ = 204.9, *df* = 24, $P < 2.2\text{x}10^{-16}$); saprobity (Supplementary Information Fig. 2; $\chi^2$ = 249.3, *df* = 32, $P < 2.2\text{x}10^{-16}$); trophic status (Fig. 6; $\chi^2$ = 377.1, *df* = 48, $P < 2.2\text{x}10^{-16}$). The highly-significant association plot with moisture requirement (M) (Fig. 7; $\chi^2$ = 75.7, *df* = 32, $P < 2.1\text{x}10^{-5}$) shows that 'threatened' (3) and 'extremely rare' (R) taxa are significantly more frequent than expected in moisture categories 4 and 5, respectively, i.e. the categories that include pseud- and euaerial species, whilst 'not threatened' (*) taxa are mostly found in submersed assemblages (M = 1).

The only significant association (*Pearson* $\chi^2$ = 11.19, *df* = 1, *P* = 0.0008) between RL18 diatom taxa and ecological attributes (Rimet and Bouchez 2012) was between threatened and not-threatened (RL18) and motile and not-motile (Rimet and Bouchez, 2012) taxa (Supplementary Information Table 4).

**The German diatom Red Lists applied to a variety of inland-water ecosystems from different geographic areas: strong negative association between threatened taxa and nitrates**

In Table 4 we summarize the studies in which RL96 or RL18 were applied to a variety of current (and some former) inland-water ecosystems from different geographic areas. Even considering all personal published and unpublished datasets and those available in the literature, only 25 studies provided both

Red List data and nitrate concentrations. The habitat types include springs (different types including limestone precipitating springs), high-mountain lakes, streams, mires, and a large peri-Alpine lake. The substrata from which diatoms were sampled are benthic, mainly lithic materials but also bryophytes, surface sediments, etc. The study sites are located mainly in the Alps but also in the northern Apennines, central Germany, Cyprus, Bosnia and Herzegovina, France, and Belgium. The main reported impacts are nitrates from agricultural runoff, pastures and cattle watering, alteration of the morphology and water diversion, airborne pollution.

The cumulative percentage of diatom taxa in threat categories (1, 2, 3, G, R, V, D oligotraphentic) of the Red List is negatively associated to nitrate concentration ($R$ = -0.82, $t$ = -6.83, $df$ = 23, $P$= $5.8 \times 10^{-7}$) (Supplementary Information Fig. 3). A highly-significant linear model (*Residual standard error* = 8.25, $df$ = 23, *Multiple* $R^2$ = 0.67, *Adjusted* $R^2$ = 0.65, $F_{[1,23]}$ = 46.6, $P = 5.8 \times 10^{-7}$) could be applied on the log-transformed mean nitrate nitrogen concentrations (Supplementary Information Table 5).

## Discussion

There is nowadays specialists' agreement that Red Lists for microorganisms, microalgae, and heterotrophic protists are important, and that they should be used primarily to guide protection of their habitats, and only secondarily as a means to achieve protection of particular taxa (Juráň and Kaštovský 2019). Some authors (Geissler 1988) even claimed that Red Lists of algae point to the urgent necessity of maintaining high ecological integrity in as many inland waters as possible.

Using the two diatom Red Lists and studies in which they were used, we gathered examples and evidence supporting the high potential of Red Lists for a sound representation and characterization of the ecological integrity and diversity of the different types of inland-waters' ecosystems. This has also important applied implications, since IUCN Red Lists of e.g. birds and plants are widely used to designate the most relevant habitats and conservation areas, and to identify regions where resident

species are most vulnerable (e.g., Paini and Mokany 2016), whereas this remains extremely limited in the case of diatom Red Lists.

However, literature on algal Red Lists is scarce and there are basically only two editions of a single diatom-specific Red List (Lange-Bertalot 1996; Hofmann et al. 2018), in spite of the paramount importance of this group of protists for biodiversity and global ecosystem functioning. Only in Estonia (Lilleleht et al. 2008), Hungary (Neméth 2005), Poland (Siemińska et al. 2006), and Slovakia (Hindák and Hindáková 2001) are diatoms included, more or less extensively, in algal red lists.

Here we have shown that this approach is useful, and should be extended to other geographic areas. It is also an excellent way to preserve expert knowledge, and make it available in an objectively-ordered way to future scholars (compare Juráň and Kaštovský 2019).

There is excellent agreement between the two editions of the Red List for Germany (Table 1, Fig. 3), showing statistical support and validation from a large and taxonomically harmonized database for an initially expert-based classification. Nevertheless, a marked decrease (22%) of taxa with ‘data insufficient’ (D) highlights the benefits of a data-driven approach. The accuracy of RL18 is also underpinned by the very strong, partly-inherent association between the RL18 threat categories and the current-distribution situation of the diatom taxa.

Considering the latter, it appears clearly that rare taxa outnumber common ones. This is consistent with previous observations, in particular with Lange-Bertalot (1997) who noted that most diatoms are bound to specific habitats but that there is a relatively small group of cosmopolitan diatom species that is widely distributed in cultural landscapes, e.g. in Europe.

Our focus on the 55 most menaced taxa of RL18 (Table 3) pinpoints clear relationships between groups of the most endangered taxa and the different types of inland-waters’ ecosystems. This was reported as being particularly evident in springs and groundwater-dependent ecosystems, in particular when these are considered from an ecohydrogeological perspective (Cantonati et al. 2020d).

Species at risk are non-randomly distributed across space (Davies 2019). The association between RL18 threat categories and RL18 ecological groups reveals that: - threatened taxa concentrate in dystrophic-oligotrophic environments, - ‘not threatened’ (= tolerant and opportunistic) taxa are most

frequent in eutrophic and saline ecosystems, - most regional extinctions occurred in carbonate oligotrophic habitats. Carbonate inland waters are common also at low and medium elevations, where they are often affected by multiple human impacts, such as water abstraction and morphological alteration, nitrates and other pollutants from intensive agriculture etc. (e.g., Angeli et al. 2010; Cantonati et al. 2020a). Low-conductivity, naturally acidic (organic acids), oligotrophic (including nitrogen), dystrophic environments were singled out for their rich and peculiar diatom microflora by previous works (e.g., Cantonati et al. 2009; 2011; 2020b). Conversely, the fragility of oligotrophic and dystrophic habitats explains the fact that most oligo- and oligo-mesotraphentic diatom taxa are in threat categories of the Red List for central Europe (Lange-Bertalot 1997). The high frequency of 'not threatened' (*) taxa in eutrophic and saline ecosystems reflects two of the current main impacts on freshwaters: eutrophication (pollution) and salinization (e.g., Downing 2014; Kaushal et al. 2018).

The significantly more frequent than expected occurrence of 'threatened' (3) and 'extremely rare' (R) taxa among aerial species probably reflects the fact that studies on terrestrial diatoms are still relatively few (e.g., Foets et al. 2021) compared to submersed assemblages, where the majority of the 'not threatened' (*) taxa are found (e.g., Cantonati et al. 2017).

Several papers (e.g., van Rees et al. 2020) list invasive alien species among the main anthropogenic threats faced by freshwater ecosystems. Supplementary Information Table 3 lists the ten species that have been suggested in the literature to be neophytes for central Europe according to Hofmann et al. (2018). However, extreme caution is necessary in considering a diatom species as non-native and invasive. For instance, Hofmann et al. (2018) discovered that the putative tropical invasive species *Diadesmis confervacea*, was present in various historical Kützing samples from a habitat in north-west Germany, where the species persisted unnoticed for many years (Cantonati et al. 2017). A classification of this species as a neophyte has therefore to be viewed with reservation, also because the reasons for its immigration, whether natural or anthropogenic, cannot be assessed. Only one of the ten potential neobiota listed in Supplementary Information Table 3 doesn't have North America as the region of origin but neighbouring northern regions of Eurasia: *Didymosphenia geminata*. This species which forms conspicuous nuisance growths, and by its large cells is also very noticeable under the

light microscope was not found in Germany at all up to the middle of the 20th century. At the end of the last century, while RL96 was still being processed, there were few, undocumented records from the German area south of the Danube. After the turn of the millennium, while *Didymosphenia* blooms were becoming a global problem (Bothwell et al. 2014), massive growths were noted in various tributaries from the Alps (Germany and Austria), rather unexpectedly in view of its apparent absence previously. Similarly, mass developments were observed in recent years in western Germany of *Achnanthidium rivulare*, *A. subhudsonis*, and *A. delmontii*. Six other species are suspected of being neobiota due to their distribution and spreading: *Achnanthidium druartii*, *Capartogramma crucicula*, *Encyonema triangulum*, *Gomphoneis minuta*, *G. transsilvanica*, and *Gomphosphenia oahuensis*. *Achnanthidium*, known to include many pioneer species (Rimet and Bouchez 2012, Cantonati et al. 2020c), is the genus with the majority of species amongst the ten taxa discussed as potential neophytes. However, Hofmann et al. (2018) prefer not to classify any of these species as neophytes for the time being, since it cannot be ascertained that their spread resulted from human intervention. Thus, though it is certain that these are opportunistic and relatively tolerant taxa that can spread rapidly under favourable conditions, it still remains to be demonstrated if they also are neophytes in Central Europe.

In this study we observed a markedly negative association between the cumulative percentage of diatom taxa in threat categories of the Red List and nitrate concentrations. Nitrates were shown to be important environmental determinants of diatom assemblages in different types of ecosystems, such as springs (e.g., Cantonati 1998), high-mountain lakes (e.g., Tolotti 2001), limestone-precipitating springs (e.g., Denys and Oosterlynck 2015), large peri-Alpine lakes (e.g., Spitale et al. 2011) and streams (e.g., Smucker et al. 2020). Werum (2001) observed a significant reduction of the proportion of the RL96 species in threat categories when springs were affected by acid deposition with higher nitrate concentrations or in springs with intermittent discharge, while this did not occur in springs of slightly higher trophic status. Werum and Lange-Bertalot (2004) noted that the relative portion of endangered Red List species was more sensitive to the impact caused by spring-capturing than the Shannon-Wiener entropy index.

Diatom communities always react to multiple stressors that can act in combination. Nevertheless, the negative relation of nitrates to the cumulative percentage of diatom taxa in threat categories of the Red List persisted throughout a wide range of habitats, geographic regions differing in human impact, and neo- as well as paleolimnological records, making it particularly noteworthy.

Denys (2000) observed that, if some sensitive habitat, e.g. dystrophic mires, is not represented in a geographic area of more limited extent, this should be taken into due account when applying a specific diatom Red List for the wider region. On a similar note, Denys (2000) pointed out the relevance of developing specific lists for regions in which naturally-eutrophic waters are particularly common. Cantonati et al. (2020c), during a tentative application of a Red-List approach to streams in the water-stressed island of Cyprus, found, somewhat unexpectedly, that both species from threat categories of RL18 and putative threat-category species of a possible future Red List tailored for Cyprus occurred more frequently and were more relevant in assemblages from sites in intermittent streams. Since temporary streams are more characteristic than permanent ones in the Mediterranean climatic setting, this points to the necessity of developing diatom red lists tailored for the different biogeographic and climatic ecoregions.

## Conclusions and way forward

The main conclusions that can be drawn from our study are:

- Literature on algal Red Lists is very limited, and there are basically only two editions of a single diatom-specific Red List (developed for Germany), in spite of the paramount importance of this group of protists for biodiversity and global ecosystem functioning.
- We have gathered evidence to show that this approach is useful, and should be extended to many more geographic areas. It is also an efficient way to preserve expert knowledge, and make it available in an objectively ordered way to future scholars.

- There is excellent agreement between the two editions of the Red List for Germany, which confirms that an approach already grounded in robust expert knowledge (Lange-Bertalot 1996) was supported and validated by a large and taxonomically harmonized database (Hofmann et al. 2018).
- The association between RL18 threat categories and RL18 ecological groups reveals that: - threatened taxa concentrate in dystrophic-oligotrophic environments, - 'not threatened' taxa (= tolerant and opportunistic) are most frequent in eutrophic and saline ecosystems, - most regional extinctions occurred in carbonate oligotrophic habitats.
- The high frequency of 'not threatened' taxa (*) in eutrophic and saline ecosystems reflects two of the current main impacts on freshwaters: eutrophication (pollution) and salinization.
- The significantly more frequent than expected occurrence of 'threatened' and 'extremely rare' taxa among pseud- and euaerial species probably reflects the fact that studies on terrestrial diatoms are still relatively rare when compared to submersed assemblages, which include the majority of the 'not threatened' taxa.
- Nitrates have a highly significant negative association with the cumulative percentage of diatom taxa in threat categories of the Red List. Having included heterogeneous studies, this points to the high potential of diatom-Red-List based approaches with regard to nitrogen management in freshwaters.

The main suggestions emerging from our study for the future development of freshwater diatom Red Lists are:

- In future applications of diatom-based Red Lists, if affordable, approaches applying Red Lists to large numbers of individual samples, rather than to databases reflecting the overall composition of the species pool, should be preferred.
- We suggest to ground future Red Lists on collations of databases adequately representing all types of inland-water habitats occurring in the region of interest and not only those considered in monitoring networks. Any future diatom Red List approaches should be primarily data-driven, and additionally validated by expert knowledge.

- The applications of the diatom Red List so far point out the importance to develop diatom Red Lists tailored for the different biogeographic and climatic ecoregions.

**Acknowledgements** The authors (other than HL-B) are most glad to use the occasion of the publication of this paper to celebrate Prof. Horst Lange-Bertalot, distinguished diatomist and pioneer of diatom Red Lists, on the occasion of his 85th birthday.

We thank the Editors and Reviewers for useful suggestions.

**Author contributions** MC: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing - original draft, Writing - review & editing. GH: Conceptualization, Data curation, Funding acquisition, Methodology, Writing - review & editing. DS: Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. MW: Data curation, Methodology, Resources, Writing - review & editing. HLB: Validation, Writing - review & editing.

**Funding** The work was financed entirely by the authors.

## Compliance with ethical standards

**Conflict of interest** The authors have no conflict of interest to declare that are relevant to the content of this article.

**Ethical issues** None.

## References

Angeli N, Cantonati M, Spitale D, Lange-Bertalot H (2010) A comparison between diatom assemblages in two groups of carbonate, low-altitude springs with different levels of anthropogenic disturbances. Fottea 10(1):115–128. https ://doi.org/10.5507/fot.2010.006

Bertrand J, Renon, JP, Monnier O (1999) Les diatomées des sources du rebord karstique de la Beauce de la région orléanaise. Symbioses 1:3–14

Bothwell ML, Taylor BW, Kilroy C (2014) The Didymo story: the role of low dissolved phosphorus in the formation of *Didymosphenia geminata* blooms, Diatom Res 29:229–236. https ://doi.org/10.1080/0269249X.2014.889041

Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A, Maheswari U, Martens C, Maumus F, Otillar RP, Rayko E, Salamov A, Vandepoele K, Beszteri B, Gruber A, Heijde M, Katinka M, Mock T, Valentin K, Verret F, Berges JA, Brownlee C, Cadoret JP, Chiovitti A, Choi CJ, Coesel S, De Martino A, Detter JC, Durkin C, Falciatore A, Fournet J, Haruta M, Huysman MJ, Jenkins BD, Jiroutova K, Jorgensen RE, Joubert Y, Kaplan A, Kroger N, Kroth PG, La Roche J, Lindquist E, Lommer M, Martin-Jezequel V, Lopez PJ, Lucas S, Mangogna M, McGinnis K, Medlin LK, Montsant A, Oudot-Le Secq MP, Napoli C, Obornik M, Parker MS, Petit JL, Porcel BM, Poulsen N, Robison M, Rychlewski L, Rynearson TA, Schmutz J, Shapiro H, Siaut M, Stanley M, Sussman MR, Taylor AR, Vardi A, von Dassow P, Vyverman W, Willis A, Wyrwicz LS, Rokhsar DS, Weissenbach J, Armbrust EV, Green BR, Van de Peer Y, Grigoriev IV (2008) The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. Nature 456:239–44

Cantonati M (1998) Diatom communities of springs in the Southern Alps. Diatom Res 13:201–220. https ://doi.org/10.1080/0269249X.1998.9705449

Cantonati M (2004) Le diatomee di tre sorgenti del Parco Naturale delle Prealpi Giulie (Italia nord-orientale). Gortania – Atti Mus Friul di Storia Nat 25:95–108

Cantonati M, Angeli N, Bertuzzi E, Spitale D, Lange-Bertalot H (2012b) Diatoms in springs of the Alps: spring types, environmental determinants, and substratum. Freshw Sci 31:499–524. https://doi.org/10.1899/11-065.1

Cantonati M, Kelly MG, Demartini D, Angeli N, Dörflinger G, Papatheodoulou A, Armanini D (2020c) Overwhelming role of hydrology-related variables and river types in driving diatom

species distribution and community assemblage in streams in Cyprus. Ecol Ind 117, 106690. https://doi.org/10.1016/j.ecolind.2020.106690

Cantonati M, Kelly MG, 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 (2010) Diatom biodiversity of springs in the Berchtesgaden National Park (northern Alps, Germany), with the ecological and morphological characterization of two species new to science. Diatom Res 25:251–280. https://doi.org/10.1080/0269249X.2010.9705849

Cantonati M, Lange-Bertalot H (2011) Diatom monitors of close-to-pristine, very-low alkalinity habitats: three new *Eunotia* species from springs in Nature Parks of the south-eastern Alps. J Limnol 70:209–221. https://doi.org/10.4081/jlimnol.2011.209

Cantonati M, Lange-Bertalot H, Decet F, Gabrieli J (2011) Diatoms in very-shallow pools of the site of community importance Danta di Cadore Mires (south-eastern Alps), and the potential contribution of these habitats to diatom biodiversity conservation. Nova Hedwigia 93:475–507. https://doi.org/10.1127/0029-5035/2011/0093-0475

Cantonati M, Poikane S, Pringle CM, Stevens LE, Turak E, Heino J, Richardson JS, Bolpagni R, Borrini A, Cid N, Čtvrtlíková M, Galassi DMP, Hájek M, Hawes I, Levkov Z, Naselli-Flores L, Saber AA, Di Cicco M, Fiasca B, Hamilton PB, 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. https://doi.org/10.3390/w12010260

Cantonati M, Scola S, Angeli N, Guella G, Frassanito R (2009) Environmental controls of epilithic diatom depth-distribution in an oligotrophic lake characterised by marked water-level fluctuations. Eur J Phycol 44:15–29. https://doi.org/10.1080/09670260802079335

Cantonati M, Segadelli S, Spitale S, Gabrieli J, Gerecke R, Angeli N, De Nardo MT, Ogata K, Wehr JD (2020b) Geological and hydrochemical prerequisites of unexpectedly high biodiversity in spring ecosystems at the landscape level. Sci Total Environ 740:140157. https://doi.org/10.1016/j.scitotenv.2020.140157

Cantonati M, Spitale D (2009) The role of environmental variables in structuring epiphytic and epilithic diatom assemblages in springs and streams of the Dolomiti Bellunesi National Park (south-eastern Alps). Fundam Appl Limnol 174:117–133. https://doi.org/10.1127/1863-9135/2009/0174-0117

Cantonati M, Stevens LE, Segadelli S, Springer AE, Goldscheider N, Celico F, Filippini M, Ogata K, Gargini A (2020d) Ecohydrogeology: The interdisciplinary convergence needed to improve the study and stewardship of springs and other groundwater-dependent habitats, biota, and ecosystems. Ecol Ind 110. https://doi.org/10.1016/j.ecolind.2019.105803

Cantonati M., Van de Vijver B., Lange-Bertalot H (2009) *Microfissurata* gen. nov. (Bacillariophyta), a new diatom genus from dystrophic and intermittently-wet terrestrial habitats. J Phycol 45:732–741. https://doi.org/10.1111/j.1529-8817.2009.00683.x

Cantonati M, Zorza R, Bertoli M, Pastorino P, Salvi G, Platania G, Prearo M, Pizzul E (2021a) Recent and subfossil diatom assemblages as indicators of environmental change (including fish introduction) in a high-mountain lake. Ecol Ind 125:107603. https://doi.org/10.1016/j.ecolind.2021.107603

Cohen J (1960) A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20:37–46

Cohen J (1968) Weighted kappa: Nominal scale agreement with provision for scaled disagreement or partial credit. Psychological Bull 70:213–220

Davies TJ (2019) The macroecology and macroevolution of plant species at risk. New Phytologist 222:708–713. https://doi.org/10.1111/nph.15612

Denys L (2000) Historical distribution of 'Red List Diatoms' (Bacillariophyceae) in Flanders (Belgium). Systematics Geogr Plants 70 (2):409–420. https://doi.org/10.2307/3668653

Denys L, Oosterlynck P (2015) Diatom assemblages of non-living substrates in petrifying *Cratoneurion* springs from lower Belgium. Fottea, 15 (2), 123–138. https://doi.org/10.5507/fot.2015.014

Downing JA (2014) Limnology and oceanography: two estranged twins reuniting by global change. Inland Waters 4, 215–232. https://doi.org/10.5268/iw-4.2.753.

European Union (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23rd October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities, L327/1. Brussels, European Commission.

Falasco E, Bona F (2011) Diatom community biodiversity in an Alpine protected area: a study in the Maritime Alps Natural Park. J Limnol 70(2):157–167.

Falasco E, Piano E, Bona F (2016) Diatom flora in Mediterranean streams: flow intermittency threatens endangered species. Biodivers Conserv 25 (14):2965–2986. https://doi.org/10.1007/s10531-016-1213-8

Fell SC, Carrivick JL, Kelly MG, Füreder L, Brown LE (2018) Declining glacier cover threatens the biodiversity of alpine river diatom assemblages. Glob Chang Biol 24(12):5828-5840. https://doi.org/10.1111/gcb.14454

Finck P, Heinze S, Raths U, Riecken U, Ssymank A (2017) Rote Liste der gefährdeten Biotoptypen Deutschlands. Dritte fortgeschriebene Fassung 2017. Natursch Biol Vielf 156:637 pp.

Foets J, Stanek-Tarkowska J, Teuling AJ, Van de Vijver B, Wetzel CE, Pfister L (2021) Autecology of terrestrial diatoms under anthropic disturbance and across climate zones. Ecol Ind 122:107248. https://doi.org/10.1016/j.ecolind.2020.107248

Foissner W (2008) Protist diversity and distribution: some basic considerations. Biodivers Conserv 17:235–242

Fránková M, Bojková J, Poulíčková A, Hájek M (2009) The structure and species richness of the diatom assemblages of the Western Carpathian spring fens along the gradient of mineral richness. Fottea 9:355–368. https://doi.org/10.5507/fot.2009.035

Friendly M 2021 Package ‘vcdExtra’, extension and addition. R package version 0.7-5.

Friendly M, Meyer D (2015) Discrete data analysis with R: visualization and modelling techniques for categorical and count data (Vol. 120). CRC Press.

Gärdenfors U, Hilton-Taylor C, Mace G, Rodríguez JP (2001) The application of IUCN Red List Criteria at Regional levels. Conserv Biol 15(5):1206–1212

Geissler U (1988) Some changes in the flora and vegetation of algae in freshwater environments. Helgoländer Meeresuntersuchungen 42 (3-4):637–643. https://doi.org/10.1007/BF02365632

Gesierich D, Kofler W (2010) Epilithic diatoms from rheocrene springs in the eastern Alps (Vorarlberg, Austria). Diatom Res 25:43–66

Hindák F, Hindáková A (2001) Červený zoznam siníc/cyanobaktérií a rias Slovenska. In: Baláž D, Marhold K, Urban P (eds) Červený zoznam rastlín a živočíchov Slovenska. Štátná ochrana prírody Slovenskej republiky and Centrum ochrany prírody a krajiny, Bánska Bystrica, pp 14–22

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

Hofmann G, Werum M, Lange-Bertalot H (2013) Diatomeen im Süßwasser-Benthos von Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis. Über 700 der häufigsten Arten und ihre Ökologie. 2. korrigierte Auflage. – Ruggell (A.R.G. Gantner):908 pp.

IUCN (2012) Guidelines for Application of IUCN Red List Criteria at Regional and National Levels: Version 4.0. Gland, Switzerland and Cambridge, UK: IUCN. iii + 41pp. ISBN: 978-2-8317-1247-5

Juráň J, Kaštovský J (2019) The procedure of compiling the Red List of microscopic algae of the Czech Republic. Biodivers Conserv DOI: 10.1007/s10531-019-01792-x

Kamberović J (2020) Springs Ecosystem of the Konjuh Mountain. Tuzla. B&H. OFF – SET Štamparija. 176 pp.

Kamberović J, Plenković-Moraj A, Kralj Borojević K, Gligora Udovič M, Žutinić P, Hafner D, Cantonati M (2019) Algal assemblages in springs of different lithologies (ophiolithes vs. limestone) of the Konjuh Mountain (Bosnia and Herzegovina). Acta Botanica Croatica 78(1):66–81. https://doi.org/10.2478/botcro-2019-0004

Kaushal SS, Likens GE, Pace ML, Utz R, Haq S, Gorman J, Grese M (2018) Freshwater salinization syndrome on a continental scale. Proceedings of the National Academy of Sciences of the United States of America https://doi.org/10.1073/pnas.1711234115

Kociolek JP, Stoermer EF (2009) Oligotrophy: the forgotten end of an ecological spectrum. Proceedings of the 20th International Diatom Symposium (Dubrovnik, Croatia). Acta Botanica Croatica 68:465–472

Lange-Bertalot H, Metzeltin D (1996) Indicators of Oligotrophy. In: H. Lange-Bertalot (Ed.), Iconogr Diatomol 2. Koeltz, Koenigstein: 1–390

Lange-Bertalot H (1996) Rote liste der limnischen Kieselalgen (Bacillariophyceae) Deutschlands. Schriftenreihe für Vegetationskunde 28:633–677

Lange-Bertalot H (1997) A first ecological evaluation of the diatom flora in Central Europe. Lauterbornia 31:117–123

Lilleleht V, Ernits P, Hang V et al. (2008). Red data book of Estonia. Commission for nature conservation of the Estonian academy of sciences. http://www.zbi.ee/punan e/engli sh/index .html. Accessed 28 Jan 2018

Mann DG (1999) The species concept in diatoms. Phycological. Reviews 18. Phycologia 38:437–495

Mann DG, Vanormelingen P (2013) An Inordinate Fondness? The Number, Distributions, and Origins of Diatom Species. J Eukaryot Microbiol 60:414–420. https://doi.org/10.1111/jeu.12047

Medlin LK, Kooistra WHCF, Gersonde R, Sims PA, Wellbrock U (1997) Is the origin of the diatoms related to the end-Permian mass extinction? Nova Hedwigia 65:1–11

Meyer D, Zeileis A, Hornik K, Gerber F, Friendly M, Meyer MD (2020) Package ‘vcd’. Visualizing Categorical Data. R package version, 1-4-8.

Mogna M, Cantonati M, Andreucci F, Angeli N, Berta G, Miserere L (2015) Diatom communities and vegetation of springs in the south-western Alps. Acta Botanica Croatica 74:265–285. https://doi.org/10.1515/botcro-2015-0024

Neméth J (2005) Red list of algae in Hungary. Acta Bot Hung 47:379–417. https ://doi.org/10.1556/ ABot.47.2005.3-4.7

Paini DR, Mokany K (2016) Birds of a feather: using species assemblages to assess vulnerability to extinction. Ecol Appl 26(2):415–423

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ (accessed 07 March 2020).

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

Reichardt E (2006) Bemerkenswerte Diatomeenfunde aus Bayern. V. Neue und seltene Arten aus der Schwarzachklamm. Berichte der Bayerischen Botanischen Gesellschaft 76:41–51

Reichardt E (2012) Der Artenkomplex um *Gomphonema occultum* E. Reichardt & Lange-Bertalot (Bacillariophyceae): Variabilität und drei neue Arten. Nova Hedwigia, Beiheft 141:155–168

Ricciardi A, Rasmussen JB (1999) Extinction Rates of North American Freshwater Fauna. Cons Biol 13:1220-1222. http://dx.doi.org/10.1046/j.1523-1739.1999.98380.x

Rimet F, Bouchez A (2012) Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. Knowledge Manag Aquat Ecosyst 406:01–12

Siemińska J, Bąk M, Dziedzic J, Gąbka M, Gregorowicz P et al. (2006) Red list of the algae in Poland – Czerwona lista glonów w Polsce. In: Z. Mirek et al. (Eds) Red list of plants and fungi in Poland – Czerwona lista roślin i grzybów Polski. Polish Academy of Sciences, Kraków, 35–52

Sims PA, Mann DG, Medlin LK (2006) Evolution of the diatoms: insights from fossil, biological and molecular data. Phycologia 45:361–402

Smol JP, Stoermer EF (2010) The Diatoms: Applications for the Environmental and Earth Sciences, Second Edition. Cambridge University Press, 667 pp.

Smucker NJ, Pilgrim EM, Nietch CT, Darling JA, Johnson BR (2020) DNA metabarcoding effectively quantifies diatom responses to nutrients in streams. Ecol Appl 30(8):e02205. 10.1002/eap.2205

Soininen J (2007) Environmental and spatial control of freshwater diatoms – a review. Diatom Res 22:473–490

Spitale D, Scalfi A, Lange-Bertalot H, Cantonati M (2011) Using different epilithic-diatom assemblage metrics for an ecological characterization of the shores of Lake Garda. J Limnol 70:197–208. https://doi.org/10.4081/jlimnol.2011.197

Taxböck L, Karger DN, Kessler M, Spitale D, Cantonati M (2020) Diatom species richness in Swiss springs increases with habitat complexity and elevation. Water 12:449. https://doi.org/10.3390/w12020449

Taxböck L, Linder HP, Cantonati M (2017) To what extent are Swiss springs refugial habitats for sensitive and endangered diatom taxa? Water 9(12):967. https://doi.org/10.3390/w9120967

Taxböck L, Preisig HR (2007) The diatom communities in Swiss springs: a first approach. Proceedings of the 1st Central European Diatom Meeting, Kusber. Pages 163–168 in W.-H. Kusber and R. Jahn (editors). Botanic Garden and Botanical Museum Berlin-Dahlem. Freie Universität Berlin, Berlin, Germany.

Tolotti M (2001) Littoral diatom communities in high mountain lakes of the Adamello–Brenta Regional Park (Trentino, Italy) and their relation to acidification. 327–352 in R. Jahn, J. P. Kociolek, A. Witkowski, and P. Compère (editors). Lange–Bertalot Festschrift. A.R. G. Gantner Verlag K. G., Ruggell, Liechtenstein.

van Dam H, Mertens A, Sinkeldam J (1994) A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands J Aquat Ecol 28(1):117–133. https://doi.org/10.1007/bf02334251

van Rees CB, Waylen KA, Schmidt-Kloiber A, Thackeray SJ, Kalinkat G, Martens K, Domisch S, Lillebø AI, Hermoso V, Grossart H, Schinegger R, Decleer K, Adriaens T, Denys L, Jarić I, Janse JH, Monaghan MT, De Wever A, Geijzendorffer I, Adamescu MC, Jähnig SC (2020) Safeguarding Freshwater Life Beyond 2020: Recommendations for the New Global Biodiversity Framework from the European Experience. Cons Lett 2020;e12771. https://doi.org/10.1111/conl.12771

Vanormelingen P, Verleyen E, Vyverman W (2008) The diversity and distribution of diatoms: from cosmopolitanism to narrow endemism. Biodiversity and Conservation 17: 393–405.

Werum M (2001) Die Kieselalgengesellschaften in Quellen. Abhängigkeit von Geologie und anthropogener Beeinflussung in Hessen (Bundesrepublik Deutschland) – Hessisches Landesamt für Umwelt und Geologie, pp. 273. ISBN 3-89026-331-3.

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

# Tables

**Table 1** Agreement between RL96 and RL18. Computations were done only on taxa with congruent taxonomy. 1 = perfect association, 0 = no association. RL18 threat categories are as follows: 0 = presumed extinct, 1 = threatened with extinction, 2 = strongly threatened, 3 = threatened, G = threat of unknown extent, R = extremely rare, V = declining, D = data insufficient, * = not threatened

| | **1** | **2** | **3** | **G** | **R** | **V** | ***** | **D** |
|---|---|---|---|---|---|---|---|---|
| **1** | 0.62 | 0.03 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| **2** | 0.04 | 0.36 | 0.01 | 0.04 | 0.00 | 0.02 | 0.00 | 0.00 |
| **3** | 0.12 | 0.37 | 0.58 | 0.06 | 0.02 | 0.00 | 0.01 | 0.02 |
| **G** | 0.00 | 0.06 | 0.13 | 0.39 | 0.00 | 0.00 | 0.01 | 0.02 |
| **R** | 0.17 | 0.16 | 0.06 | 0.24 | 0.91 | 0.00 | 0.03 | 0.06 |
| **V** | 0.00 | 0.03 | 0.15 | 0.14 | 0.01 | 0.75 | 0.05 | 0.03 |
| ***** | 0.00 | 0.01 | 0.07 | 0.07 | 0.01 | 0.21 | 0.87 | 0.11 |
| **D** | 0.04 | 0.00 | 0.00 | 0.05 | 0.06 | 0.02 | 0.04 | 0.76 |

**Table 2** Association (Cramer's V) between the RL18 threat categories and the current-distribution situation of the diatom taxa. RL18 threat categories are as in Table 1 (or see the Methods section). RL18 current-distribution categories are as follows: ex = extinct or lost, er = extremely rare, vr = very rare, r = rare, mf = moderately frequent, f = frequent, vf = very frequent, ? = unknown

| | **ex** | **er** | **vr** | **r** | **mf** | **f** | **vf** | **?** |
|---|---|---|---|---|---|---|---|---|
| **0** | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **1** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **2** | 0 | 0.3 | 0.7 | 0 | 0 | 0 | 0 | 0 |
| **3** | 0 | 0 | 0.1 | 0.9 | 0 | 0 | 0 | 0 |
| **G** | 0 | 0 | 0.8 | 0.2 | 0 | 0 | 0 | 0 |
| **R** | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| **V** | 0 | 0 | 0 | 0.7 | 0.3 | 0 | 0 | 0 |
| ***** | 0 | 0 | 0 | 0.3 | 0.4 | 0.2 | 0.05 | 0 |
| **D** | 0 | 0 | 0.1 | 0.1 | 0 | 0 | 0 | 0.86 |

**Table 3** Main Red List attributes of the 55 most menaced taxa of RL18 (4 taxa in category "0" and 51 taxa in cat. "1"). RL18 (and RL96) threat categories are as in Table 1, RL18 current-distribution categories are as in Table 2, and ecological groups are as in Fig. 4 (or see the Methods section). Long-term development trends are as follows: (<) decline of unknown extent, < moderate decline

| Taxon (name) | threat category | ecol. group | distribution | RL96 | long-term dev. | | threat category | ecol. group | distribution | RL96 | long-term dev. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cyclotella lemanensis* | 0 | oc | ex | * | = | *Frustulia krammeri* | 1 | od | er | D | (<) |
| *Cymbella hustedtii* var. *compacta* | 0 | oc | ex | D | = | *Geissleria declivis* | 1 | od | er | 1 | (<) |
| *Cymbopleura citriformis* | 0 | o | ex | D | = | *Geissleria thingvallae* | 1 | ? | er | R | (<) |
| *Encyonema latens* | 0 | oc | ex | D | = | *Hippodonta costulatiformis* | 1 | ? | er | D | (<) |
| *Achnanthes nodosa* | 1 | od | er | 1 | (<) | *Kobayasiella madumensis* | 1 | od | er | D | (<) |
| *Achnanthidium trinode* | 1 | oc | er | 3 | (<) | *Kobayasiella okadae* | 1 | od | er | 1 | (<) |
| *Chamaepinnularia soehrensioides* | 1 | od | er | D | (<) | *Navicula detenta* | 1 | o | er | 1 | (<) |
| *Cyclotella schroeteri* | 1 | oc | er | D | (<) | *Navicula diabolica* | 1 | ? | er | R | (<) |
| *Cyclotella socialis* | 1 | o | er | D | (<) | *Nitzschia garrensis* | 1 | od | er | 1 | (<) |
| *Encyonema hophense* | 1 | oc | er | D | (<) | *Pinnularia biceps* var. *gibberula* | 1 | od | er | D | (<) |
| *Encyonema procerum* | 1 | ? | er | D | (<) | *Pinnularia inculpata* | 1 | ? | er | D | (<) |
| *Encyonopsis tiroliana* | 1 | oc | er | D | (<) | *Pinnularia nobilis* var. *nobilis* | 1 | o | er | D | (<) |
| *Eunotia alkalibiontica* | 1 | oc | er | D | (<) | *Pinnularia persudetica* var. *silvatica* | 1 | od | er | D | (<) |
| *Eunotia bactriana* | 1 | od | er | D | (<) | *Pinnularia renatiformis* | 1 | o | er | D | (<) |
| *Eunotia biconstricta* | 1 | od | er | D | (<) | *Pinnularia ruttneri* var. *lauenburgiana* | 1 | ? | er | R | (<) |
| *Eunotia braendlei* | 1 | o | er | D | (<) | *Pinnularia simonsenii* | 1 | o | er | D | (<) |
| *Eunotia circumborealis* | 1 | od | er | D | (<) | *Pinnularia stidolphii* | 1 | o | er | D | (<) |
| *Eunotia cisalpina* | 1 | od | er | D | (<) | *Pinnularia subrhombica* var. *subrhombica* | 1 | od | er | D | (<) |
| *Eunotia denticulata* | 1 | od | er | D | (<) | *Pinnularia subrhombica* var. *angusta* | 1 | od | er | D | (<) |
| *Eunotia diadema* | 1 | od | er | 1 | (<) | *Placoneis navicularis* | 1 | o | er | 1 | (<) |
| *Eunotia elegans* | 1 | od | er | 1 | (<) | *Platessa rupestris* | 1 | o | er | 1 | (<) |
| *Eunotia faba* | 1 | od | er | 1 | (<) | *Psammothidium kuelbsii* | 1 | od | er | 3 | < |
| *Eunotia hexaglyphis* | 1 | od | er | 1 | (<) | *Psammothidium lacus-vulcani* | 1 | od | er | 2 | < |
| *Eunotia lapponica* | 1 | od | er | D | (<) | *Tetracyclus emarginatus* | 1 | od | er | R | (<) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Eunotia pseudopectinalis* | 1 | od | er | 1 | (<) | *Tetracyclus glans* | 1 | od | er | 3 | (<) |
| *Eunotia renata* | 1 | od | er | D | (<) | | | | | | |
| *Eunotia rhynchocephala* | 1 | od | er | 1 | (<) | | | | | | |
| *Eunotia serra* | 1 | od | er | 1 | (<) | | | | | | |
| *Eunotia silvahercynia* | 1 | od | er | 1 | (<) | | | | | | |
| *Eunotia superpaludosa* | 1 | od | er | D | (<) | | | | | | |

**Table 4** Different inland-waters habitats ranked by decreasing percentage of threatened RL96 (shaded background) and RL18 diatom taxa. %RL = % diatom taxa belonging to the sum of all threatened Red List categories, N = N. of sampling stations, TNT = total number of taxa found, org. pol. = organic pollution, str. = streams. ABNP = Adamello-Brenta Natural Park (Trentino, Italy), CRENODAT Project (Autonomous Province of Trento = Trentino, Italy), DBNP = Dolomiti Bellunesi National Park (Veneto Region, Italy), CESSPA Project (Province of Verona, Veneto Region, Italy), BeNP = Berchtesgaden National Park (Bavaria, Germany), GNP = Gesäuse National Park (Styria, Austria), JPNP = Julian Prealps Natural Park (Friuli Venezia Giulia Autonomous Region, Italy). lm = lithic material, b = bryophytes, ss = surface sediment, fga = filamentous green-algae, ll = leaf litter, sa = sand

| | | | | **[N-$NO_3^-$]** µg $L^{-1}$ | | | | |
|---|---|---|---|---|---|---|---|---|
| **Habitat type (substrata sampled)** | **Location** | **N** | **%RL** | **Mean** | **Med.** | **TNT** | **Impacts** | **Reference** |
| Mire pools (b, ss) | Danta di Cadore, south-eastern Alps | 5 | **72** | **5** | **6** | 86 | trails, roads | Cantonati et al. (2011) |
| Springs of different ecomorphological types on various geological substrata (lm, b, ss) | Emilia-Romagna Region, Northern Apennines, Italy | 16 | **60** | **186** | **196** | 285 | Agriculture and industrial activities in the lowlands, water abstraction | Cantonati et al. (2020a) |
| High-mountain lakes on holocrystalline rocks (lm, b, ss) | ABNP, south-eastern Alps | 16 | **56** | **192** | **196** | 204 | used as reservoirs for hydropower | Tolotti (2001) |
| Shallow (max depth = 6.4 m) high mountain lake (neolimnology: lm, paleolimnology: sediment core) | Balma Lake, Orsiera Rocciavrè Nature Park, Piedmont, northwestern Italy | 14 | **55** | **271** | **284** | 103 | long-distance airborne aiborne pollutants, grazing & cattle watering, fishing | Cantonati et al. (2021a) |
| Mountain carbonate springs (lm, b, ss) | BeNP, north-eastern Alps | 9 | **54** | **303** | **248** | 104 | forestry, hunting, cattle breeding | Cantonati and Lange-Bertalot (2010) |
| Springs (all main lithologies) (lm, b) | Trentino, CRENODAT Project, s.-e. Alps | 110 | **53** | **735** | **559** | 370 | water abstraction, pastures, roads, agricultural runoff | Cantonati et al. (2012) |
| Low-elevation siliceous-metamorphic mountain springs (lm, b, ss) | BFNP 2018 | 15 | **53** | **760** | **780** | 120 | in the recent past: acidification, forestry | Cantonati et al. unpublished |
| High-mountain lake on holocrystalline rocks (lm) | Trentino, Lake Cornisello | 8 | **69** | **170** | **173** | 96 | | MC & M. Segnana unpublished |
| Carbonate and holocrystalline mountain springs (lm, b, ss) | ABNP, south-eastern Alps | 30 | **48** | **516** | **468** | 254 | pastures | Cantonati (1998) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mountain carbonate springs (lm, b, ss) | BeNP 2018 | 15 | **47** | **754** | **761** | 162 | forestry, hunting, cattle breeding | Cantonati et al. unpublished |
| Siliceous metamorphic springs (lm, b, ss) | South-western Alps springs | 48 | **46** | **302** | **230** | 223 | cattle watering | Mogna et al. (2015) |
| Swiss springs: 7 spring types over a wide altitudinal range & wide range of anthropogenic alterations (lm, ss, b, fga, ll) | springs in central and eastern Switzerland as well as the Jura Mountains | 74 | **45** | **878** | **512** | 504 | eutrophication, morphology alteration | Taxböck et al. (2017, 2020) |
| Carbonate springs and streams | DBNP, south-eastern Alps | 21 | **41** | **497** | **429** | 131 | org. pol. (str.) | Cantonati and Spitale (2009) |
| Low-altitude carbonate springs (lm, b, ss) | JPNP, south-eastern Alps | 3 | **40** | **509** | | 60 | org. pol. | Cantonati (2004) |
| Lake Tovel (epilithon euphotic z.) | ABNP, south-eastern Alps | 50 | **40** | **350** | **340** | | | Cantonati et al. (2009) |
| Springs (all main lithologies) (lm) | Vicinities of Frankfurt/M. (Südhessen) | 96 | **37** | **1580** | | 416 | water abstraction, airborne pollution, roads, agricultural runoff | Werum and Lange-Bertalot (2004) |
| Helocrene springs (spring fens) | Western Carpathians | 13 | **33** | **587** | | 188 | | Fránková et al. (2009) |
| Lake Garda (lm) | South-eastern Alps | 24 | **33** | **420** | **440** | 75 | ($NO_3^-$), shore-morphology alteration | Spitale et al. (2011) |
| Pre-Alpine carbonate springs (lm) | Areas of Basel and Zürich, CH | 17 | **30** | **2851** | **2210** | 118 | | Taxböck and Preisig (2007) |
| Mountain springs (lm) | Springs in Vorarlberg, Austria, Alps | 27 | **28** | **2300** | **1600** | 197 | water abstraction, pastures, deforestation | Gesierich and Kofler (2010) |
| Mediterranean island streams (lm) | Cyprus | 65 | **27** | **985** | **380** | 290 | water scarcity and abstraction | Cantonati et al. (2020b) |
| springs on ophiolites and limestone) | Konjuh Mountain, Bosnia and Herzegovina | 20 | **27** | **1309** | **1230** | 187 | alterations of the morphology, water abstraction, deforestation, trampling by cattle | Kamberović et al. (2019), Kamberović et al. (2020) |
| Cratoneurion limestone-precipitating springs (sa, lm) | lower Belgium | 13 | **25** | **7550** | **8070** | 310 | nitrate-enriched GW | Denys and Oosterlynck (2015) |
| Carbonate springs (lm) | Verona Province, pre-Alpine | 25 | **24** | **1971** | **1242** | 138 | $NO_3^-$, capturing | Angeli et al. (2010) |
| Carbonate springs (lm) | Beauce region, Orléanais, N France | 14 | **23** | **14266** | **8465** | 135 | $NO_3^-$, | Bertrand et al. (1999) |

# Figures

**Fig. 1** Observer Agreement Chart between RL96 and RL18 (only taxa with congruent taxonomic concept in the two Red Lists). In this chart black squares show observed agreement, and are positioned within larger rectangles (the large rectangle shows the maximum possible agreement). When there is perfect agreement, the rectangles are all squares, completely filled by the shaded squares. Threat categories are as in Table 1 (or see the Methods section)

**Fig. 2** Number of taxa (with percentages on each bin) in each of the threat categories of RL96 and RL18. Threat categories are as in Table 1 (or see the Methods section)

**Fig. 3** Current distribution situation of the diatom taxa of the Red List (RL18). RL18 current-distribution categories are as in Table 2 (or see the Methods section)

**Fig. 4** Association plot between RL18 threat categories and RL18 ecological groups. Please see the Methods section for correct interpretation of the association plot. ae = aerial, o = oligotraphentic, oc = oligotraphentic carbonate, od = oligotraphentic distrophic, eu = eutraphentic to tolerant, hal = halophilic, ? = unknown. Threat categories are as in Table 1 (or see the Methods section)

**Fig. 5** Association plot between Red List categories and pH (van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. Please see the Methods section for correct interpretation of the association plot. 1 = acidobiontic, 2 = acidophilous, 3 = circumneutral, 4 = alkaliphilous, 5 = alkalibiontic, 6 = indifferent. Threat categories are as in Table 1 (or see the Methods section)

**Fig. 6** Association plot between Red List categories and trophic status (T; van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = oligotraphentic, 2 = oligo-mesotraphentic, 3 = mesotraphentic, 4 = meso-eutraphentic, 5 = eutraphentic, 6 = hypereutraphentic, 7 = oligo- to eutraphentic (hypereutraphentic). Threat categories are as in Table 1 (or see the Methods section)

**Fig. 7** Association plot between Red List categories and moisture (M; van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = never, or only very rarely, occurring outside water bodies; 2 = mainly occurring in water bodies, sometimes on wet places; 3 = mainly occurring in water bodies, also rather regularly on wet and moist places; 4 = mainly occurring on wet and moist or temporarily dry places; 5 = nearly exclusively occurring outside water bodies. Threat categories are as in Table 1 (or see the Methods section)

## Supplementary Information (SI)

## Tables

**Supplementary Information Table 1** Main Red-List inland-water habitats of Germany. nG = national long-term threat (1 = threatened with complete destruction, 2 = strongly endangered, 3 = endangered, ★ = currently no recognizable threat, **#** = risk classification does not make sense), TE = current development trend (🡻 = decrease, 🡺 = constant / stable, 🡹 = increase, **#** = classification does not make sense), SE = rarity (X = very rare), RLD = Red-List status (1 = threat of complete destruction, 1-2 = endangered up to threatened with complete destruction, 2-3 = endangered to strongly threatened, 3-V = acute pre-warning list , ★ = currently no risk of loss, **#** = risk classification does not make sense, V = pre-warning list), RE = restorability [N = cannot be restored, K = hardly regenerable (>150 years), S = difficult to regenerate (15-150 years), B = conditionally regenerable (approx. up to 15 years), X = no classification useful] (selected and modified from Finck et al., 2017)

**Supplementary Information Table 2** Agreement between RL96 and RL18. Computations were done only on taxa with congruent taxonomy. 1 = perfect association, 0 = no association. K = Cohen's Kappa; ASE: approximate standard error; CI = confidence interval

**Supplementary Information Table 3** Taxa listed as potential neophytes in Hofmann et al. (2018). RL18 attributes are as in Table 3 (or see the Methods section). Size class, and growth forms / ecological guilds are those available in Rimet and Bouchez (2012). Long-term development indications: > clear increase, ? data insufficient

**Supplementary Information Table 4** Association between motile and not-motile (Rimet and Bouchez, 2012) and threatened (1,2,3, G,R, V,) an not-threatened (*) RL18 diatom taxa

**Supplementary Information Table 5** Parameters of the linear model for the relation of the logarithm of nitrate-nitrogen averages and the cumulative percentage of diatom species in Red List threat categories (dependent variable)

## Figures

**Supplementary Information Fig. 1** Association plot between Red List categories and nitrogen uptake metabolism (N; van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; 2 = nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; 3 = facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen; 4 = obligately nitrogen-heterotrophic taxa, needing continuously elevated concentrations of organically bound nitrogen. Threat categories are as in Table 1 (or see the Methods section)

**Supplementary Information Fig. 2** Association plot between Red List categories and saprobity (S; van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = oligosaprobic, 2 = beta-mesosaprobic, 3 = alpha-mesosaprobic, 4 = alpha-meso-/polysaprobic, 5 = polysaprobic. Threat categories are as in Table 1 (or see the Methods section)

**Supplementary Information Fig. 3** Linear model applied to the association between the cumulative percentages of threat categories and nitrate concentrations in diverse inland-water habitats (both neo- and paleolimnological data)

# Authors and Affiliations

**Marco Cantonati [1,*], Gabriele Hofmann [2], Daniel Spitale [1], Marcus Werum [3], Horst Lange-Bertalot [4]**

Gabriele Hofmann

GM.Hofmann@t-online.de

Daniel Spitale

spitale.daniel@gmail.com

Marcus Werum

werum@aglhh.de

Horst Lange-Bertalot

lange-bertalot@web.de

[1] MUSE – Museo delle Scienze, Limnology & Phycology Section, Corso del Lavoro e della Scienza 3, I-38123 Trento, Italy

[2] Hirtenstraße 19, 61479 Glashütten, Germany

[3] Karl-Bieber-Höhe 23, 60437 Frankfurt am Main, Germany

[4] Goethe Universität Frankfurt, Biologicum, Max-von-Laue Straβe 13, 60438 Frankfurt, Germany

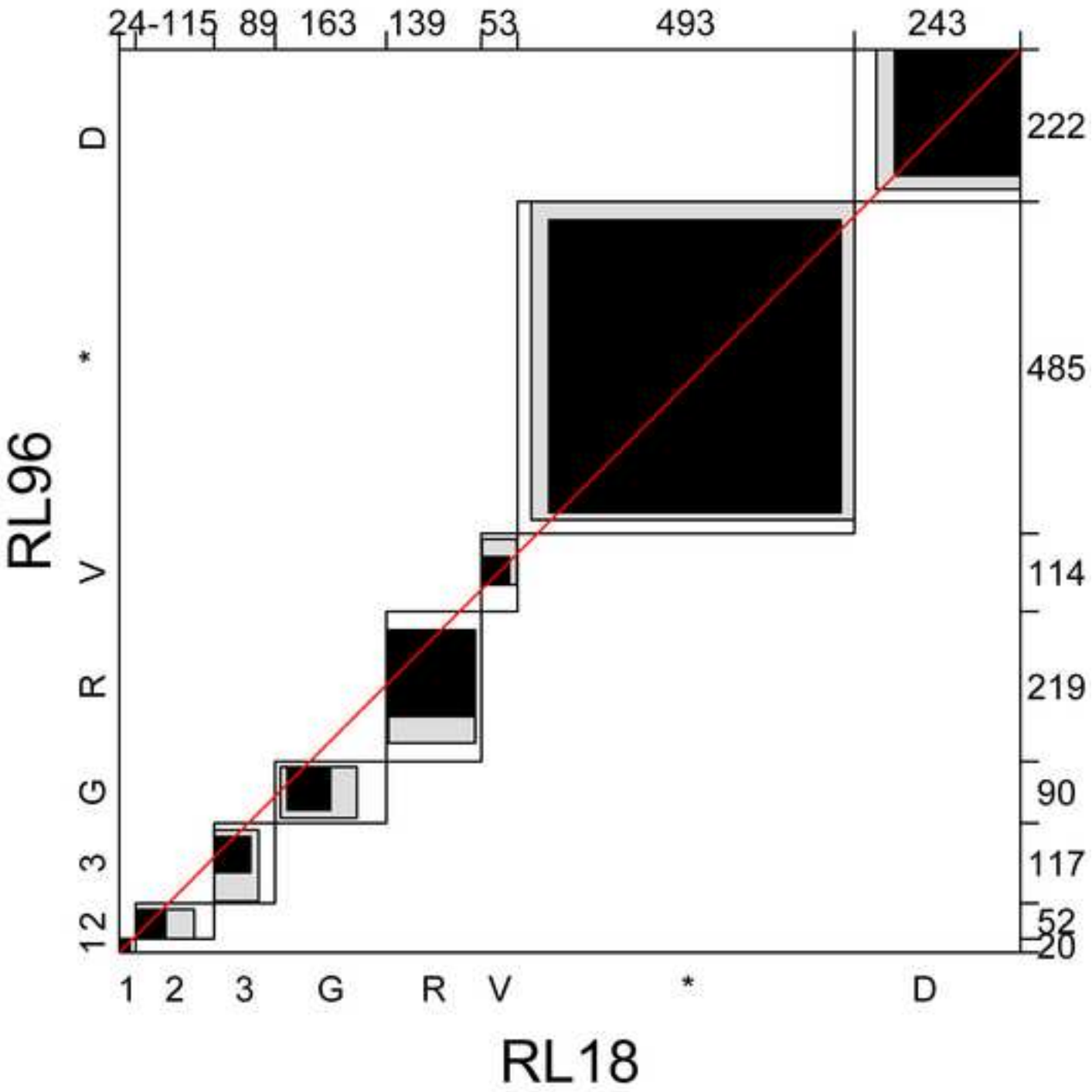
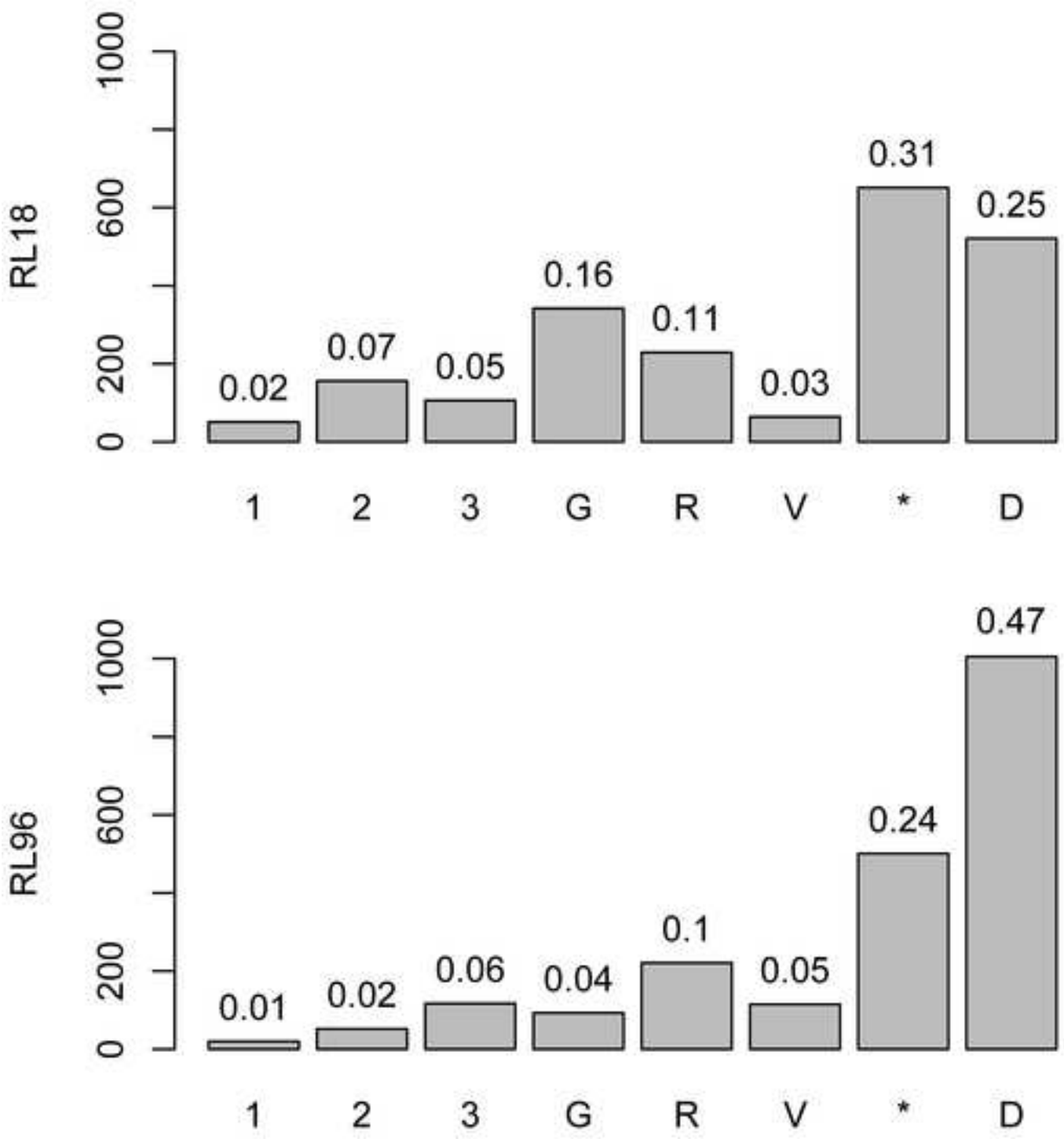
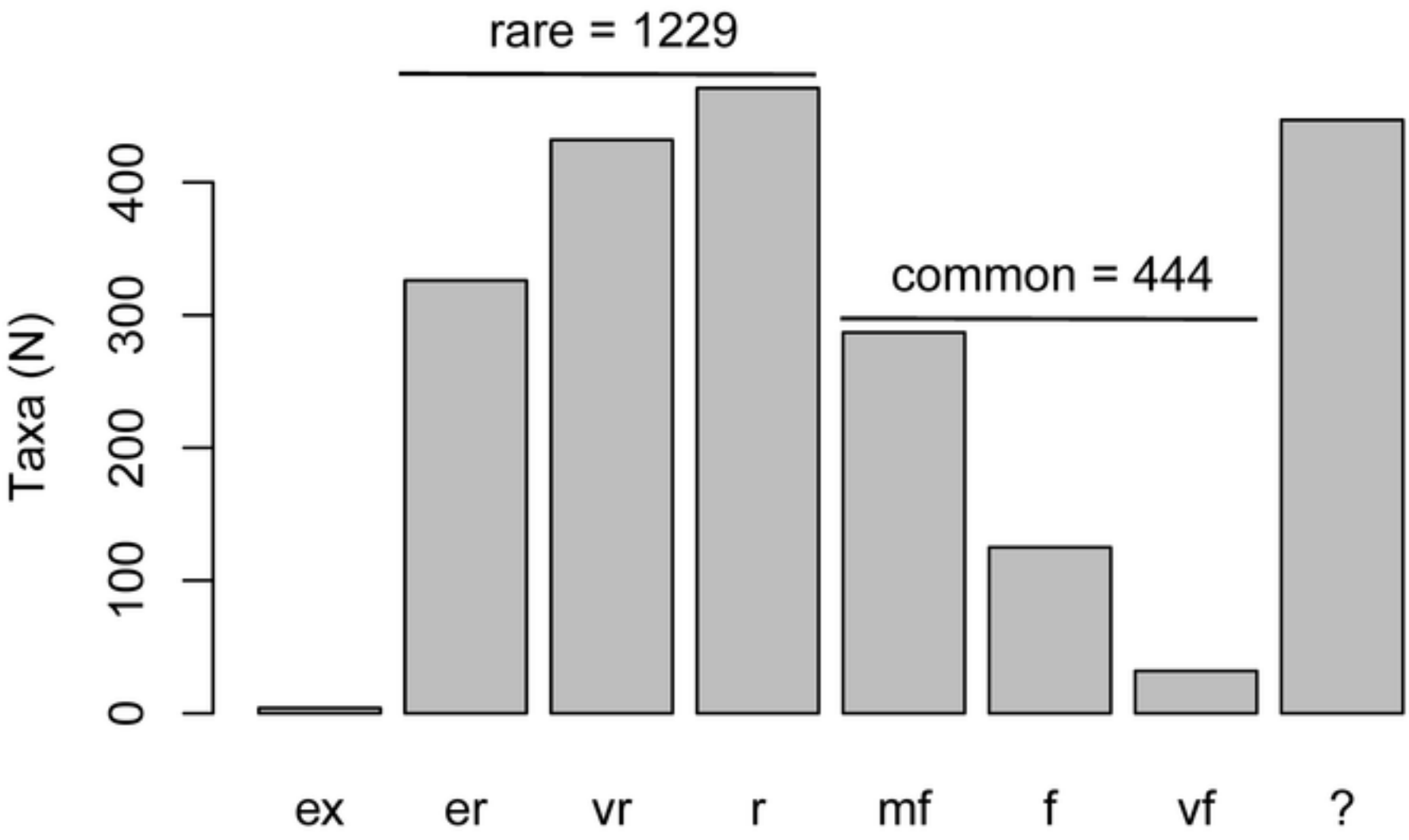

24-115
89
163
139
53
493
243
222
485
114
219
90
117
52
20
D
*
V
R
G
3
12
1
2
3
G
R
V
*
D
RL96
RL18

Figure Click here to access/download;Figure;Fig2.tiff

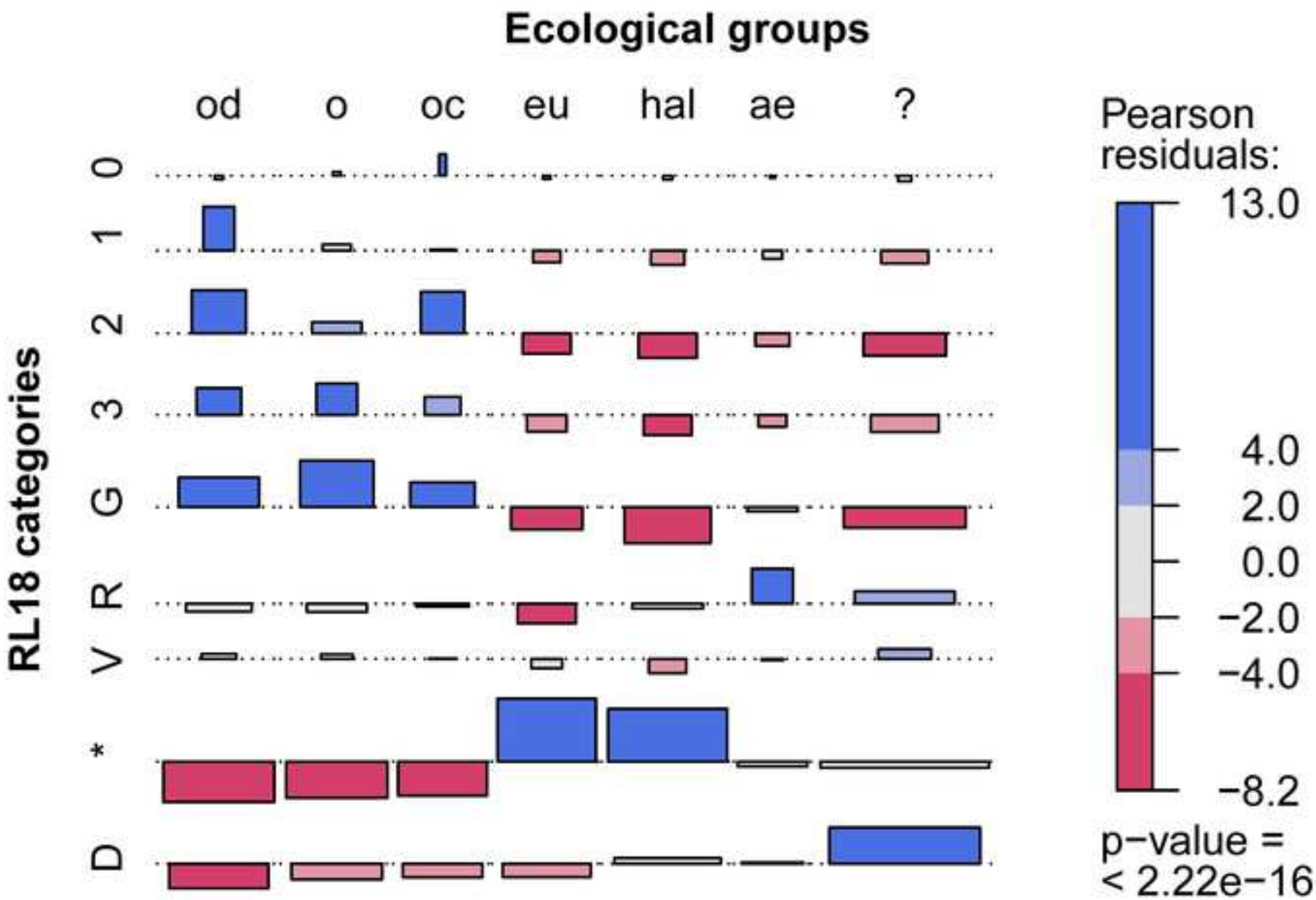


Click here to access/download;Figure;Fig3.tiff



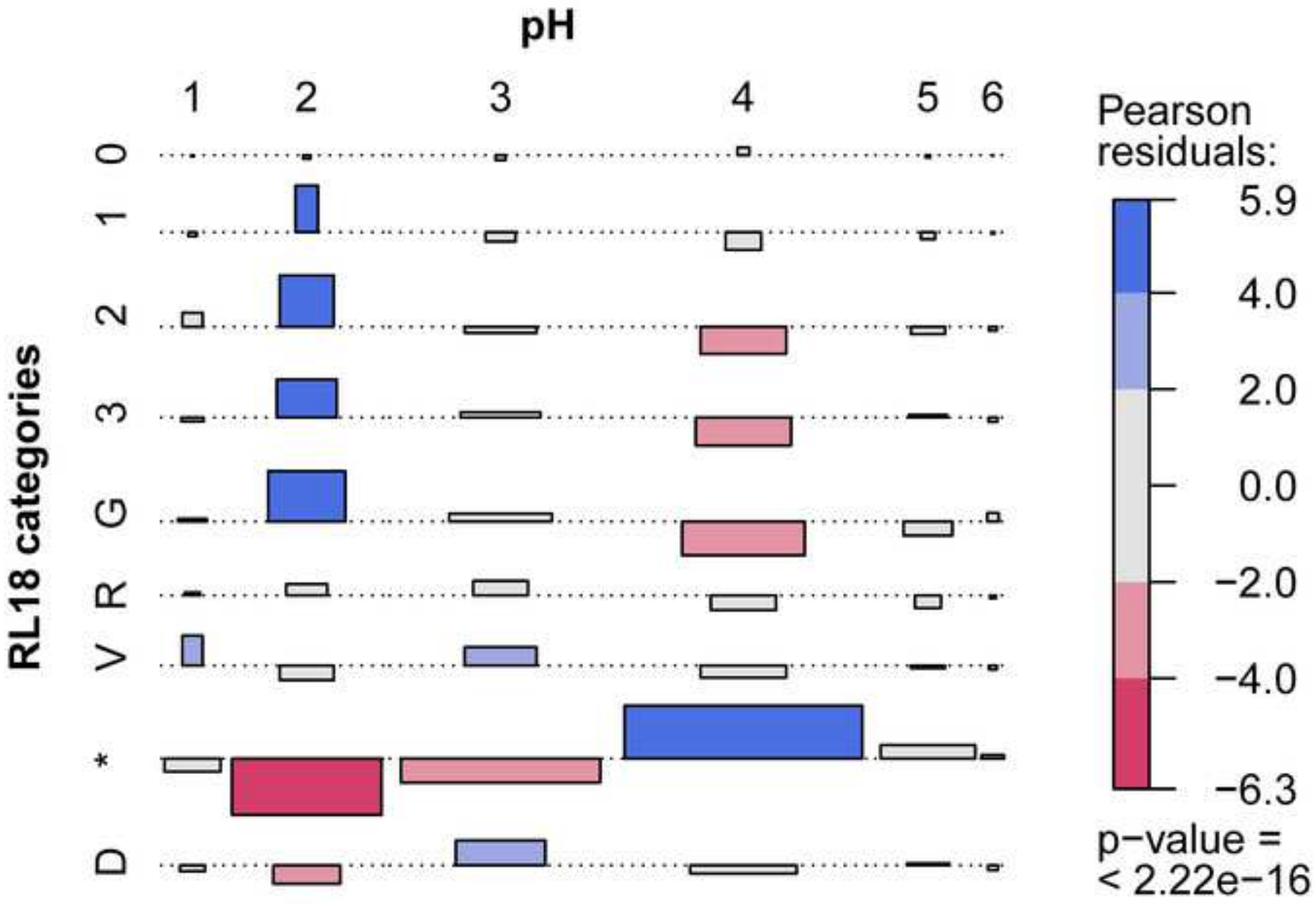
Figure

Click here to access/download;Figure;Fig4.tiff



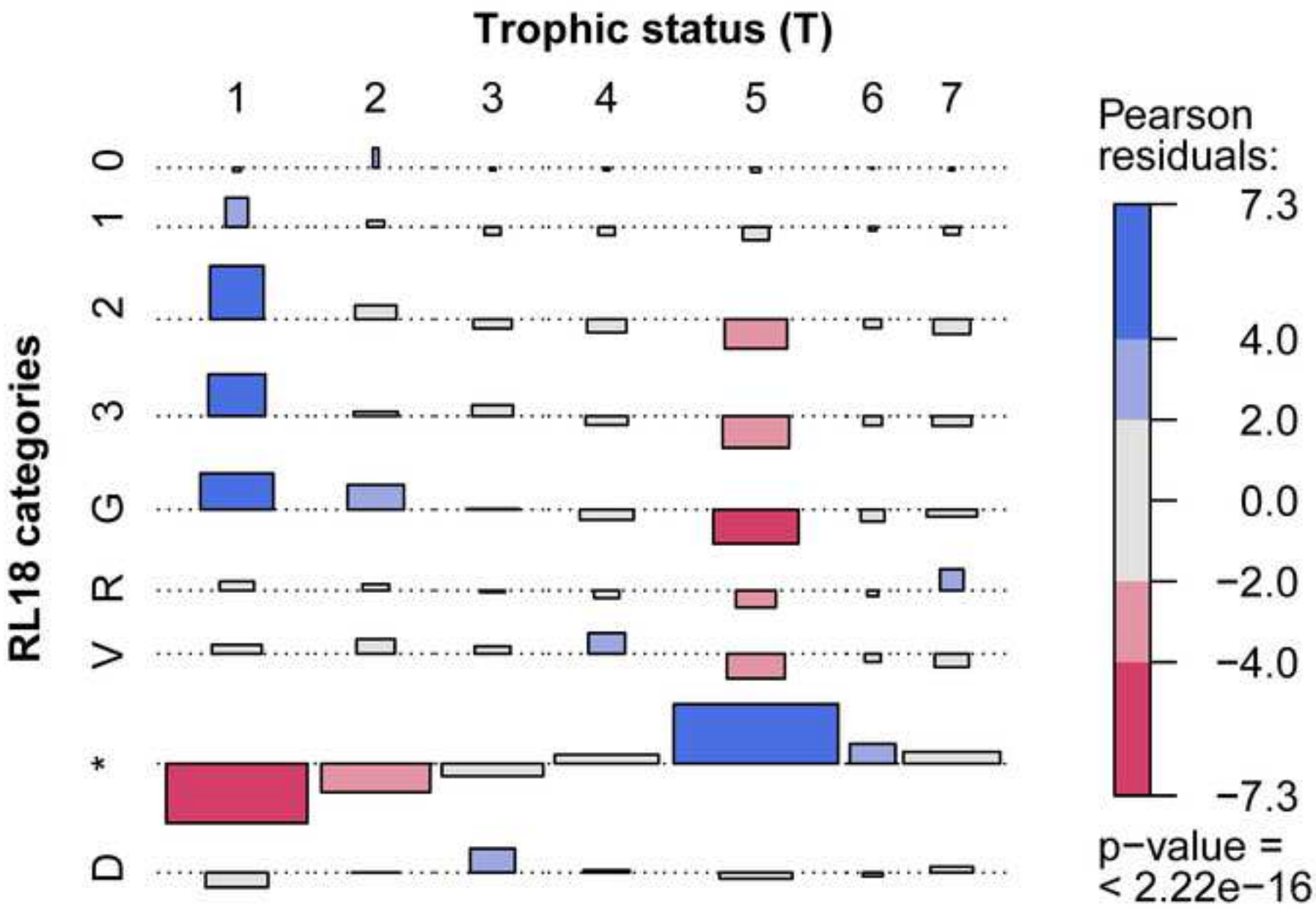
Figure

Click here to access/download;Figure;Fig5.tif



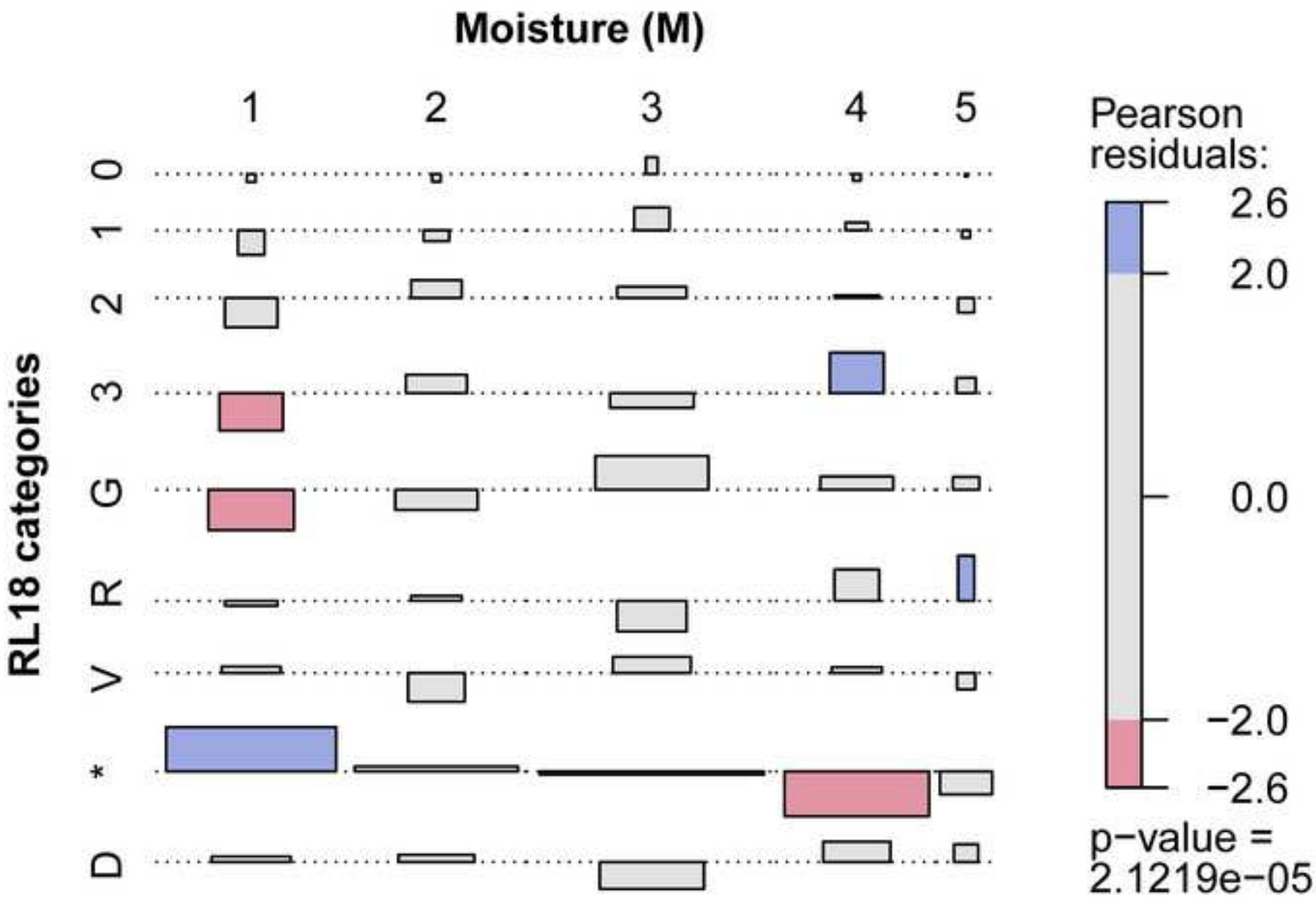
Figure

Click here to access/download;Figure;Fig6.tiff



Figure

Click here to access/download;Figure;Fig7.tiff



**Supplementary Information Table 1** Main Red-List inland-water habitats of Germany. nG = national long-term threat (1 = threatened with complete destruction, 2 = strongly endangered, 3 = endangered, ★ = currently no recognizable threat, **#** = risk classification does not make sense), TE = current development trend (🡻 = decrease, 🡺 = constant / stable, 🡹 = increase, **#** = classification does not make sense), SE = rarity (X = very rare), RLD = Red-List status (1 = threat of complete destruction, 1-2 = endangered up to threatened with complete destruction, 2-3 = endangered to strongly threatened, 3-V = acute pre-warning list , ★ = currently no risk of loss, **#** = risk classification does not make sense, V = pre-warning list), RE = restorability [N = cannot be restored, K = hardly regenerable (>150 years), S = difficult to regenerate (15-150 years), B = conditionally regenerable (approx. up to 15 years), X = no classification useful] (selected and modified from Finck et al., 2017)

| Code | Biotope type | nG | TE | SE | RLD | RE |
|---|---|---|---|---|---|---|
| 22.01 | Seepages and wetland springs (helocrenes) | 2 | 🡻 | | 1-2 | K |
| 22.02 | Pool springs (Limnocrenes) | 2 | 🡺 | | 2-3 | K |
| 22.03 | Flowing springs (Rheocrenes) | 2 | 🡻 | | 1-2 | B |
| 22.04 | Saline or brine springs | 1 | 🡺 | X | 1 | N |
| 22.05 | Tapped (captured) springs | ★ | # | | ★ | X |
| 23.01 | RUNNING WATERS | 2 | 🡻 | | 1-2 | K |
| 23.01.01 | Natural and near-natural rhithral | 2 | 🡻 | | 1-2 | K |
| 23.01.02 | Natural and near-natural potamal | 1 | 🡺 | | 1-2 | K |
| 23.02 | Running waters with moderate anthropogenic impact | 2 | 🡺 | | 2-3 | S |
| 23.03 | Running waters with strong anthropogenic impact | ★ | # | | ★ | X |
| 23.04 | Running waters with very strong anthropogenic impact | ★ | # | | ★ | X |
| 23.05.01 | Ditches with a flowing-water character all year round | 3 | 🡺 | | 3-V | B |
| 23.05.02 | Technical -gutter and half-round ditches | ★ | # | | ★ | X |
| 23.05.03 | Piped ditch | # | # | | # | X |
| 23.06 | Deltas in inland waters | 3 | 🡺 | | 3-V | K |
| 23.07.01 | Waterfall | 2 | 🡺 | | 2-3 | B |
| 23.07.02 | Oxbow lake | 1 | 🡺 | | 1-2 | S |
| 23.07.03 | Lake runoff | 3 | 🡺 | | 3-V | S |
| 23.07.04 | Dammed river stretch | ★ | # | | X | X |
| 23.07.05 | Saline stream | 1 | 🡺 | X | N | N |
| 23.08 | Intermittently wet habitats below the mean water level in running waters | 2 | 🡻 | | 1-2 | S |
| 23.09 | Natural and near-natural temporary running waters | 2 | 🡻 | | 1-2 | S |
| 24.01 | Dystrophic standing waters / Mire pools | 2 | 🡻 | | 1-2 | K |
| 24.02 | Oligotrophic standing waters | 2 | 🡻 | | 1-2 | S |
| 24.03 | Mesotrophic standing waters | 2 | 🡻 | | 1-2 | B |
| 24.04 | Eutrophic standing waters | 3 | 🡺 | | 3-V | B |
| 24.05 | Poly-hypertrophic standing waters | ★ | # | | ★ | X |
| 24.06 | Saline inland waters | 2 | 🡻 | X | 1 | N |
| 24.07 | Standing waters of anthropogenic origin | | | | | X |
| 24.07.04 | Ditches with very slow flowing to standing water | 3 | 🡻 | | 2-3 | X |
| 35.01 | Forest-free, oligo- to mesotrophic fens and swamps | 1 | 🡻 | | 1! | K |
| 35.02 | Grassland in wet to (intermittently) moist locations | | | | | |
| 36.01 | Raised bogs (intact to a large extent) | 1 | 🡻 | | 1! | N |
| 36.02 | Transitional and intermediate mires | 2 | 🡻 | | 1-2 | N |
| 36.03 | Mire degeneration stages | 3 | 🡺 | | 3-V | X |
| 36.05 | Mire regeneration areas | ★ | 🡹 | | ★ | X |
| 38.01 | Club-rush reed | 2 | 🡻 | | 1-2 | S |

| | | | | | | |
|---|---|---|---|---|---|---|
| 38.02.01 | Reeds | 2 | ↓ | | 1-2 | S |
| 60.01 | Springs of the subalpine to alpine elevation belt | V | → | | V | K |
| 60.01.01 | Wetland (helocrenic) spring (seepage) of the subalpine to alpine elevation belt | V | → | | V | K |
| 60.01.02 | Pool (limnocrenic) spring of the subalpine to alpine elevation belt | ★ | → | | ★ | K |
| 60.01.03 | Flowing (rheocrenic) springs & gushets of the subalpine to alpine elevation belt | 3 | → | | 3-V | K |
| 60.02 | Running waters of the subalpine to alpine elevation belt | 3 | → | | 3-V | K |
| 60.02.01 | Glacial stream | 3 | ↓ | X | 2 | K |
| 60.02.02 | Upper part of the running-water system (rhithral) of the subalpine to alpine elevation belt | 3 | → | | 3-V | K |
| 60.03 | Standing waters of the subalpine to alpine elevation belt | 3 | ↓ | | 2-3 | K |
| 60.03.01 | Lake of the subalpine to alpine elevation belt | 3 | → | | 3-V | K |
| 60.03.02 | Tarn of the subalpine to alpine elevation belt | 3 | → | | 3-V | K |
| 60.03.03 | Pools of the subalpine to alpine elevation belt | 3 | ↓ | | 2-3 | K |
| 65.01 | Raised and transitional mires of the subalpine to alpine elevation belt | 3 | → | | 3-V | N |
| 65.02 | Fen or swamp of the subalpine to alpine elevation belt | 3 | → | | 3-V | N |

**Supplementary Information Table 2** Agreement between RL96 and RL18. Computations were done only on taxa with congruent taxonomy. 1 = perfect association, 0 = no association. K = Cohen's Kappa; ASE: approximate standard error; CI = confidence interval

| | K | ASE | z | P | lower CI | upper CI |
|---|---|---|---|---|---|---|
| **Unweighted** | 0.645 | 0.015 | 43.6 | <0.0001 | 0.616 | 0.674 |
| **Weighted** | 0.744 | 0.014 | 55.0 | <0.0001 | 0.718 | 0.771 |

**Supplementary Information Table 3** Taxa listed as potential neophytes in Hofmann et al. (2018). RL18 attributes are as in Table 3 (or see the Methods section). Size class, and growth forms / ecological guilds are those available in Rimet and Bouchez (2012). Long-term development indications: > clear increase, ? data insufficient

| | RL18 | | | | Rimet and Bouchez (2012) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **threat cat.** | **ecol. group** | **distribution** | **long-term d.** | **size class** | **motile** | **pioneer** | **pedunculate** | **stalk** | **colonial** | **mucous** | **arbuscular** | **high-profile** | **low-profile** | **motile guild** |
| *Achnanthidium delmontii* | * | eu | mf | > | | | | | | | | | | | |
| *Achnanthidium druartii* | D | eu | r | ? | | | | | | | | | | | |
| *Achnanthidium rivulare* | * | ? | mf | > | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Achnanthidium subhudsonis* | * | eu | mf | > | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Capartogramma crucicula* | R | hal | er | ? | | | | | | | | | | | |
| *Didymosphenia geminata* | * | ? | r | ? | 5 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| *Encyonema triangulum* | R | eu | er | ? | 5 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| *Gomphoneis minuta* | D | eu | vr | ? | 5 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| *Gomphoneis transsilvanica* | D | eu | r | ? | | | | | | | | | | | |
| *Gomphosphenia oahuensis* | R | hal | er | ? | | | | | | | | | | | |

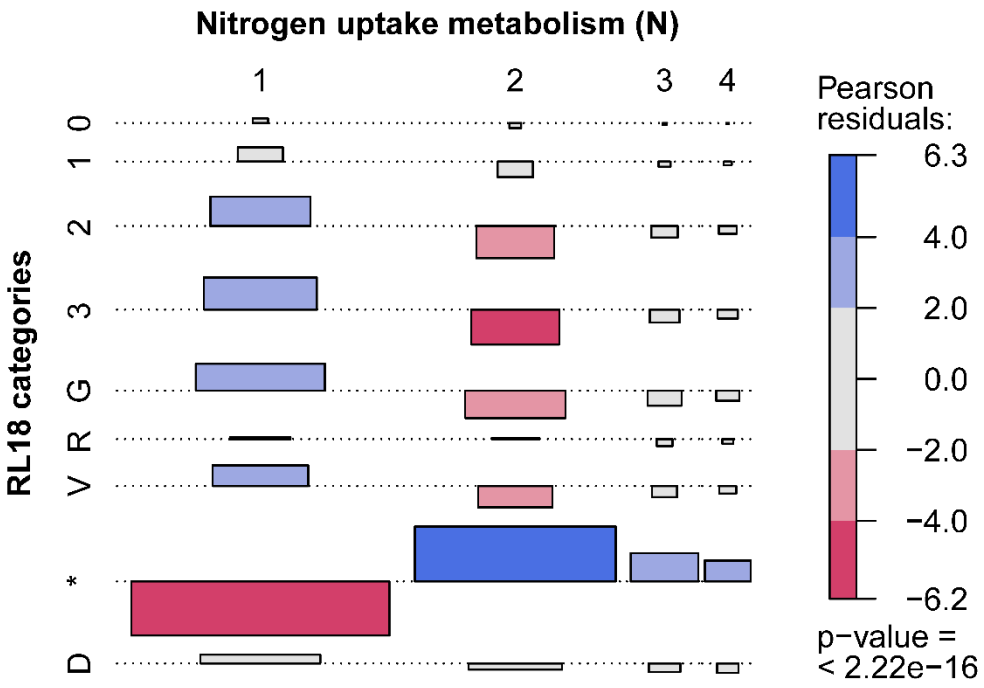
**Supplementary Information Table 4** Association between motile and not-motile (Rimet and Bouchez, 2012) and threatened (1,2,3, G,R, V,) an not-threatened (*) RL18 diatom taxa

| ***N (%)*** | **threatened** | **not-threatened** |
|---|---|---|
| **not-motile** | 12 (16.4) | 148 (37.5) |
| **motile** | 61 (83.6) | 247 (62.5) |

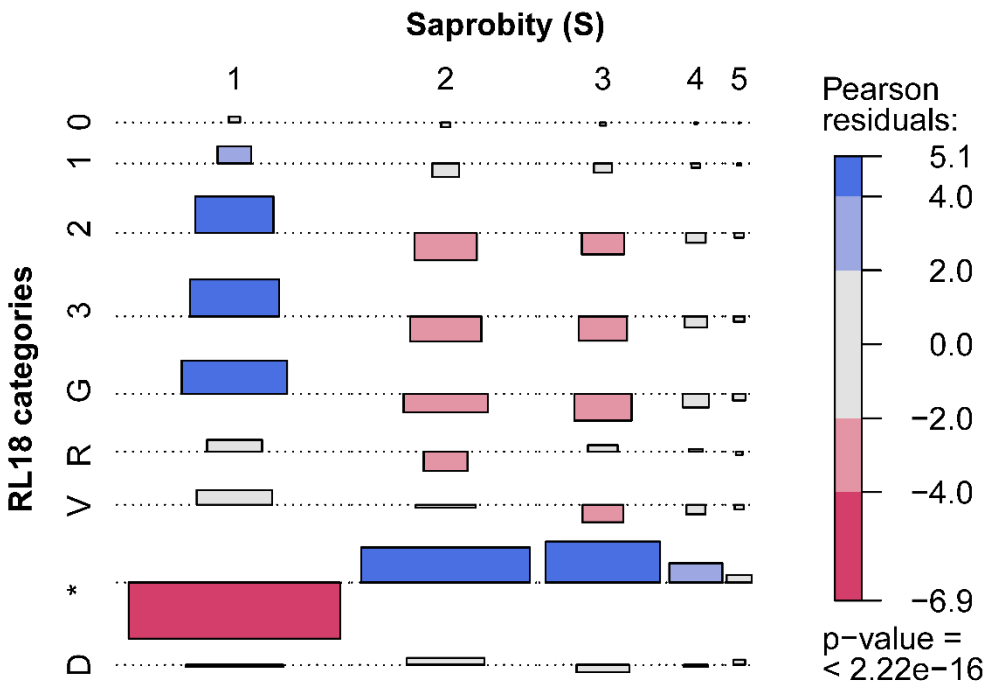
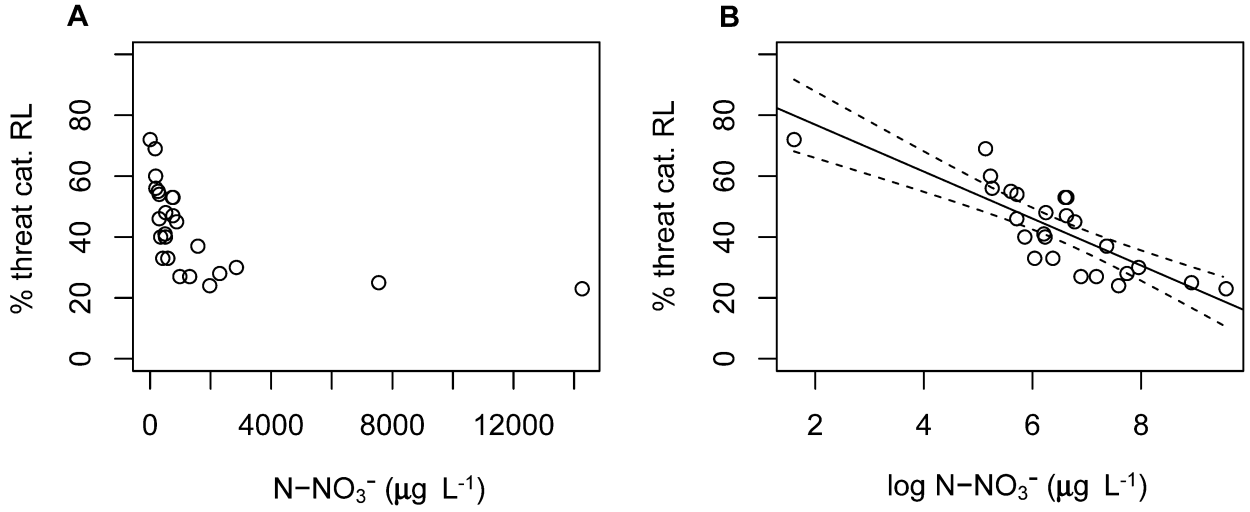
**Supplementary Information Table 5** Parameters of the linear model for the relation of the logarithm of nitrate-nitrogen averages and the cumulative percentage of diatom species in Red List threat categories (dependent variable)

| | **Min** | **Q1** | **Median** | **Q3** | **Max** |
|---|---|---|---|---|---|
| **Residuals:** | -12.7 | -7.1 | 1.5 | 5.7 | 16.3 |
| | | | | | |
| **Coefficients:** | **Estimate** | **Std. Error** | ***t* value** | ***p* (>\|*t*\|)** | |
| **Intercept** | 92.3 | 7.5 | 12.4 | $1.2 \times 10^{-11}$ | |
| **logmean** | -7.7 | 1.1 | -6.8 | $5.8 \times 10^{-07}$ | |

**Supplementary Information Fig. 1** Association plot between Red List categories and nitrogen uptake metabolism (N; van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; 2 = nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; 3 = facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen; 4 = obligately nitrogen-heterotrophic taxa, needing continuously elevated concentrations of organically bound nitrogen. Threat categories are as in Table 1 (or see the Methods section)



**Supplementary Information Fig. 2** Association plot between Red List categories and saprobity (S; van Dam et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = oligosaprobic, 2 = beta-mesosaprobic, 3 = alpha-mesosaprobic, 4 = alpha-meso-/polysaprobic, 5 = polysaprobic. Threat categories are as in Table 1 (or see the Methods section)



**Supplementary Information Fig. 3** Linear model applied to the association between the cumulative percentages of threat categories and nitrate concentrations in diverse inland-water habitats (both neo- and paleolimnological data)