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

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

3

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

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

14

15

16 **Abstract**

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

35

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

37

38 **Introduction**

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

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

54 Species at risk are non-randomly distributed across phylogeny (Davies 2019). Diatoms are the
55 most species-rich group of algae (Mann 1999). The global diversity of diatoms may be one or more
56 orders of magnitude higher than the current number of species described (Vanormelingen et al. 2008),
57 with as many as ca. 100,000 but at least 30,000 extant diatom species (Mann and Vanormelingen
58 2013). They contribute to global primary production with a proportion as large as 20%, possessing
59 global significance in the carbon and silicon cycles (Mann 1999). They can be found wherever there
60 is a little bit of moisture, and in all aquatic habitats. Most of them have very precise preferences for
61 ecological conditions of paramount relevance in environmental quality assessments, such as pH,
62 mineralization, nutrients etc. The frustules allow species identification, and are usually preserved in
63 sediments, thus providing a precious tool for paleo-environmental reconstructions, and diatom mounts
64 are excellent collection objects, present in many natural history museums. All these characteristics

65 have fostered the use of diatoms in a wide array of disciplines (e.g., Smol and Stoermer 2010). A
66 combination of mechanisms (“melting pot genome” of ca. 10.000 genes with many diatom specific
67 genes but also extensive gene transfer from bacteria to diatoms; active urea cycle, probably used to
68 improve nitrogen storage; fatty acids oxydation in mitochondria; chemical information exchange
69 among cells etc.) may underlie the rapid diversification rates, and may explain why diatoms have
70 come to dominate several contemporary systems in a relatively short period of time (Bowler et al.
71 2008). In oligotrophic habitats, which host diverse and characteristic diatom communities (e.g.,
72 Lange-Bertalot and Metzeltin 1996), a detailed taxonomy, capable of identifying and characterizing
73 typical (and, possibly, new) taxa must be applied. Shoe-horning of taxa in aggregates of species
74 common in meso- and eutrophic waters must be avoided. For diatoms, Mann (1999) advocated a so-
75 called “Waltonian” species concept, a total-evidence approach combining data from morphology,
76 genetics, mating systems, physiology, ecology, and crossing behaviour.

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

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

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

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

105 So far, applications of the diatom Red Lists for Germany (Lange-Bertalot 1996; Hofmann et al.
106 2018) have been relatively rare but included a fair variety of inland-waters' habitats: springs (e.g.,
107 Werum and Lange-Bertalot 2004; Cantonati et al. 2012), mountain mires (e.g., Fránková et al. 2009;
108 Cantonati et al. 2011), glacial streams (e.g., Fell et al. 2018), alpine aquatic habitats (e.g., Falasco and
109 Bona 2011), high-mountain lakes (e.g., Tolotti 2001, including paleolimnology: Cantonati et al.
110 2021a), large peri-alpine lakes (Spitale et al. 2011), Mediterranean streams (e.g., Falasco et al. 2016;
111 Cantonati et al. 2020c).

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

118

119

120 **Methods**

121 **Diatom Red Lists for Germany**

122 The first diatom Red List for Germany was published in 1996 (Lange-Bertalot 1996; hereafter
123 referred to as RL96), and actualized 22 years later by Hofmann et al. (2018; hereafter referred to as
124 RL18). The threat analysis for the latter was grounded on a taxonomically harmonized dataset based
125 on studies from 1985 to 2008, which includes ca. 7600 diatom samples from various German rivers
126 and 3000 samples from German lakes (Hofmann et al. 2013). These data are not evenly distributed
127 across the main inland aquatic habitats occurring in Germany (see Supplementary Information Table
128 1 and Finck et al. 2017) but originate mainly from the stream, river, and lake types considered in the
129 German monitoring network. These, however, include also a fair amount of data from acidification-
130 sensitive silicate streams of the low-elevation mountain ranges of Central Germany, which have been
131 part of the acidification monitoring network since the mid-1980s. Dystrophic aquatic environments,
132 small water bodies, and dilute (soft-water) lakes are largely underrepresented in the dataset. The
133 abundance of the species is recorded as per cent (relative abundance) of the assemblage composition,
134 a quantification method common in diatom studies. Unpublished studies from the years 2008 to 2015
135 that had not been included in the dataset, several individual papers and books (e.g., Reichardt 1997;
136 2006; 2012; Werum and Lange-Bertalot 2004), as well as historical samples from various collections
137 were used to complement habitats not covered by regular/official monitoring.

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

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

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

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

155

156 **RL18 data processing and statistical analyses**

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

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

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

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

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

185 Associations between RL18 diatom taxa and ecological attributes available in Rimet and Bouchez
186 (2012) were tested for significance using *Pearson's Chi-squared* (χ^2) tests with Yates' continuity
187 correction for two-way tables. All numerical analyses were performed using R (R Core Team 2020)
188 and mainly the packages *vcd* (Meyer et al. 2020) and *vcdExtra* (Friendly 2021).

189

190 **Applications of diatom Red Lists**

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

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

199

200

201 **Results**

202 **Diatom Red Lists**

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

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

212

213 **Agreement between RL96 and RL18**

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

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

221 The histogram in Fig. 2 shows the number of taxa (with percentages on each bin) in each of the
222 threat categories of RL96 and RL18. The ‘threat of unknown extent’ (G), and ‘not threatened’ (*)
223 categories increased by 16% and 7%, respectively. On the contrary, there was a marked decrease
224 (22%) of taxa with ‘data insufficient’ (D).

225

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

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

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

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

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

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

254

255 **Association of RL18 with ecological groups**

256 The association plot between RL18 threat categories and RL18 ecological groups (Fig. 4) was highly
257 significant ($\chi^2 = 1385.3$, $df = 48$, $P < 2.2 \times 10^{-16}$). It suggests that: - most diatom extinctions happened
258 in carbonate oligotrophic habitats, - threatened taxa concentrate in dystrophic-oligotrophic
259 environments, - ‘not threatened’ (*) taxa are most frequent in eutrophic and saline ecosystems. These
260 observations are largely confirmed by the highly-significant association plots with ecological
261 attributes according to van Dam et al. (1994): pH (Fig. 5; $\chi^2 = 289.9$, $df = 40$, $P < 2.2 \times 10^{-16}$); nitrogen
262 uptake metabolism (Supplementary Information Fig. 1; $\chi^2 = 204.9$, $df = 24$, $P < 2.2 \times 10^{-16}$); saprobity
263 (Supplementary Information Fig. 2; $\chi^2 = 249.3$, $df = 32$, $P < 2.2 \times 10^{-16}$); trophic status (Fig. 6; $\chi^2 =$
264 377.1 , $df = 48$, $P < 2.2 \times 10^{-16}$). The highly-significant association plot with moisture requirement (M)
265 (Fig. 7; $\chi^2 = 75.7$, $df = 32$, $P < 2.1 \times 10^{-5}$) shows that ‘threatened’ (3) and ‘extremely rare’ (R) taxa are
266 significantly more frequent than expected in moisture categories 4 and 5, respectively, i.e. the
267 categories that include pseud- and euaerial species, whilst ‘not threatened’ (*) taxa are mostly found
268 in submersed assemblages (M = 1).

269 The only significant association (*Pearson* $\chi^2 = 11.19$, $df = 1$, $P = 0.0008$) between RL18 diatom
270 taxa and ecological attributes (Rimet and Bouchez 2012) was between threatened and not-threatened
271 (RL18) and motile and not-motile (Rimet and Bouchez, 2012) taxa (Supplementary Information Table
272 4).

273

274 **The German diatom Red Lists applied to a variety of inland-water ecosystems from different** 275 **geographic areas: strong negative association between threatened taxa and nitrates**

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

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

286 The cumulative percentage of diatom taxa in threat categories (1, 2, 3, G, R, V, D oligotraphentic)
287 of the Red List is negatively associated to nitrate concentration ($R = -0.82$, $t = -6.83$, $df = 23$, $P =$
288 5.8×10^{-7}) (Supplementary Information Fig. 3). A highly-significant linear model (*Residual standard*
289 *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
290 applied on the log-transformed mean nitrate nitrogen concentrations (Supplementary Information
291 Table 5).

292

293

294 **Discussion**

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

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

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

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

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

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

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

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

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

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

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

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

359 light microscope was not found in Germany at all up to the middle of the 20th century. At the end of
360 the last century, while RL96 was still being processed, there were few, undocumented records from
361 the German area south of the Danube. After the turn of the millennium, while *Didymosphenia* blooms
362 were becoming a global problem (Bothwell et al. 2014), massive growths were noted in various
363 tributaries from the Alps (Germany and Austria), rather unexpectedly in view of its apparent absence
364 previously. Similarly, mass developments were observed in recent years in western Germany of
365 *Achnantheidium rivulare*, *A. subhudsonis*, and *A. delmontii*. Six other species are suspected of being
366 neobiota due to their distribution and spreading: *Achnantheidium druartii*, *Capartogramma crucicula*,
367 *Encyonema triangulum*, *Gomphoneis minuta*, *G. transsilvanica*, and *Gomphosphenia oahuensis*.
368 *Achnantheidium*, known to include many pioneer species (Rimet and Bouchez 2012, Cantonati et al.
369 2020c), is the genus with the majority of species amongst the ten taxa discussed as potential
370 neophytes. However, Hofmann et al. (2018) prefer not to classify any of these species as neophytes
371 for the time being, since it cannot be ascertained that their spread resulted from human intervention.
372 Thus, though it is certain that these are opportunistic and relatively tolerant taxa that can spread
373 rapidly under favourable conditions, it still remains to be demonstrated if they also are neophytes in
374 Central Europe.

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

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

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

401

402

403 **Conclusions and way forward**

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

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

- 411 • There is excellent agreement between the two editions of the Red List for Germany, which
412 confirms that an approach already grounded in robust expert knowledge (Lange-Bertalot
413 1996) was supported and validated by a large and taxonomically harmonized database
414 (Hofmann et al. 2018).
- 415 • The association between RL18 threat categories and RL18 ecological groups reveals that: -
416 threatened taxa concentrate in dystrophic-oligotrophic environments, - ‘not threatened’ taxa
417 (= tolerant and opportunistic) are most frequent in eutrophic and saline ecosystems, - most
418 regional extinctions occurred in carbonate oligotrophic habitats.
- 419 • The high frequency of ‘not threatened’ taxa (*) in eutrophic and saline ecosystems reflects
420 two of the current main impacts on freshwaters: eutrophication (pollution) and salinization.
- 421 • The significantly more frequent than expected occurrence of ‘threatened’ and ‘extremely rare’
422 taxa among pseud- and euairal species probably reflects the fact that studies on terrestrial
423 diatoms are still relatively rare when compared to submersed assemblages, which include the
424 majority of the ‘not threatened’ taxa.
- 425 • Nitrates have a highly significant negative association with the cumulative percentage of
426 diatom taxa in threat categories of the Red List. Having included heterogeneous studies, this
427 points to the high potential of diatom-Red-List based approaches with regard to nitrogen
428 management in freshwaters.

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

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

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

440

441

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457

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462

463 **References**

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683 **Tables**

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

688

	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

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691

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

696

	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

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701

702 **Table 3** Main Red List attributes of the 55 most menaced taxa of RL18 (4 taxa in category “0” and 51 taxa in
703 cat. “1”). RL18 (and RL96) threat categories are as in Table 1, RL18 current-distribution categories are as in
704 Table 2, and ecological groups are as in Fig. 4 (or see the Methods section). Long-term development trends are
705 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.
<i>Cyclotella lemanensis</i>	0	oc	ex	*	=	<i>Frustulia krammeri</i>	1	od	er	D	(<)
<i>Cymbella hustedtii</i> var. <i>compacta</i>	0	oc	ex	D	=	<i>Geissleria declivis</i>	1	od	er	1	(<)
<i>Cymbopleura</i> <i>citriiformis</i>	0	o	ex	D	=	<i>Geissleria thingvallae</i>	1	?	er	R	(<)
<i>Encyonema latens</i>	0	oc	ex	D	=	<i>Hippodonta costulatiformis</i>	1	?	er	D	(<)
<i>Achnanthes nodosa</i>	1	od	er	1	(<)	<i>Kobayasiella madumensis</i>	1	od	er	D	(<)
<i>Achnantheidium</i> <i>trinode</i>	1	oc	er	3	(<)	<i>Kobayasiella okadae</i>	1	od	er	1	(<)
<i>Chamaepinnularia</i> <i>soehrensoides</i>	1	od	er	D	(<)	<i>Navicula detenta</i>	1	o	er	1	(<)
<i>Cyclotella schroeteri</i>	1	oc	er	D	(<)	<i>Navicula diabolica</i>	1	?	er	R	(<)
<i>Cyclotella socialis</i>	1	o	er	D	(<)	<i>Nitzschia garrensis</i>	1	od	er	1	(<)
<i>Encyonema hophense</i>	1	oc	er	D	(<)	<i>Pinnularia biceps</i> var. <i>gibberula</i>	1	od	er	D	(<)
<i>Encyonema procerum</i>	1	?	er	D	(<)	<i>Pinnularia inculpata</i>	1	?	er	D	(<)
<i>Encyonopsis tiroliana</i>	1	oc	er	D	(<)	<i>Pinnularia nobilis</i> var. <i>nobilis</i>	1	o	er	D	(<)
<i>Eunotia</i> <i>alkalibiontica</i>	1	oc	er	D	(<)	<i>Pinnularia persudetica</i> var. <i>silvatica</i>	1	od	er	D	(<)
<i>Eunotia bactriana</i>	1	od	er	D	(<)	<i>Pinnularia renatiformis</i>	1	o	er	D	(<)
<i>Eunotia biconstricta</i>	1	od	er	D	(<)	<i>Pinnularia ruttneri</i> var. <i>lauenburgiana</i>	1	?	er	R	(<)
<i>Eunotia braendlei</i>	1	o	er	D	(<)	<i>Pinnularia simonsenii</i>	1	o	er	D	(<)
<i>Eunotia</i> <i>circumborealis</i>	1	od	er	D	(<)	<i>Pinnularia stidolphii</i>	1	o	er	D	(<)
<i>Eunotia cisalpina</i>	1	od	er	D	(<)	<i>Pinnularia subrhombica</i> var. <i>subrhombica</i>	1	od	er	D	(<)
<i>Eunotia denticulata</i>	1	od	er	D	(<)	<i>Pinnularia subrhombica</i> var. <i>angusta</i>	1	od	er	D	(<)
<i>Eunotia diadema</i>	1	od	er	1	(<)	<i>Placoneis navicularis</i>	1	o	er	1	(<)
<i>Eunotia elegans</i>	1	od	er	1	(<)	<i>Platessa rupestris</i>	1	o	er	1	(<)
<i>Eunotia faba</i>	1	od	er	1	(<)	<i>Psammothidium kuelbsii</i>	1	od	er	3	<
<i>Eunotia hexaglyphis</i>	1	od	er	1	(<)	<i>Psammothidium lacus-vulcani</i>	1	od	er	2	<
<i>Eunotia lapponica</i>	1	od	er	D	(<)	<i>Tetracyclus emarginatus</i>	1	od	er	R	(<)

<i>Eunotia pseudopectinalis</i>	1	od	er	1	(<)	<i>Tetracyclus glans</i>	1	od	er	3	(<)
<i>Eunotia renata</i>	1	od	er	D	(<)						
<i>Eunotia rhynchocephala</i>	1	od	er	1	(<)						
<i>Eunotia serra</i>	1	od	er	1	(<)						
<i>Eunotia silvahercynia</i>	1	od	er	1	(<)						
<i>Eunotia superpaludosa</i>	1	od	er	D	(<)						

706

707

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

Habitat type (substrata sampled)	Location	N	[N-NO ₃ ⁻] µg L ⁻¹				Impacts	Reference
			%RL	Mean	Med.	TNT		
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 Rocciavè Nature Park, Piedmont, northwestern Italy	14	55	271	284	103	long-distance airborne airborne 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)
Helocene 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)

718 **Figures**

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

724

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

727

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

730

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

735

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

740

741 **Fig. 6** Association plot between Red List categories and trophic status (T; van Dam et al., 1994). Please see the
742 Methods section for correct interpretation of the association plot. 1 = oligotraphentic, 2 = oligo-mesotraphentic,
743 3 = mesotraphentic, 4 = meso-eutrathentic, 5 = eutrathentic, 6 = hypereutrathentic, 7 = oligo- to eutrathentic
744 (hypereutrathentic). Threat categories are as in Table 1 (or see the Methods section)

745

746 **Fig. 7** Association plot between Red List categories and moisture (M; van Dam et al., 1994). Please see the
747 Methods section for correct interpretation of the association plot. 1 = never, or only very rarely, occurring
748 outside water bodies; 2 = mainly occurring in water bodies, sometimes on wet places; 3 = mainly occurring in
749 water bodies, also rather regularly on wet and moist places; 4 = mainly occurring on wet and moist or
750 temporarily dry places; 5 = nearly exclusively occurring outside water bodies. Threat categories are as in Table
751 1 (or see the Methods section)

752

753

754 **Supplementary Information (SI)**

755 **Tables**

756 **Supplementary Information Table 1** Main Red-List inland-water habitats of Germany. nG = national long-
757 term threat (1 = threatened with complete destruction, 2 = strongly endangered, 3 = endangered, ★ = currently
758 no recognizable threat, # = risk classification does not make sense), TE = current development trend (↓ =
759 decrease, → = constant / stable, ↑ = increase, # = classification does not make sense), SE = rarity (X = very
760 rare), RLD = Red-List status (1 = threat of complete destruction, 1-2 = endangered up to threatened with
761 complete destruction, 2-3 = endangered to strongly threatened, 3-V = acute pre-warning list, ★ = currently no
762 risk of loss, # = risk classification does not make sense, V = pre-warning list), RE = restorability [N = cannot be
763 restored, K = hardly regenerable (>150 years), S = difficult to regenerate (15-150 years), B = conditionally
764 regenerable (approx. up to 15 years), X = no classification useful] (selected and modified from Finck et al.,
765 2017)

766

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

770

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

775

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

778

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

782

783 **Figures**

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

791

792 **Supplementary Information Fig. 2** Association plot between Red List categories and saprobity (S; van Dam
793 et al., 1994). Please see the Methods section for correct interpretation of the association plot. 1 = oligosaprobic,
794 2 = beta-mesosaprobic, 3 = alpha-mesosaprobic, 4 = alpha-meso-/polysaprobic, 5 = polysaprobic. Threat
795 categories are as in Table 1 (or see the Methods section)

796

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

800

801

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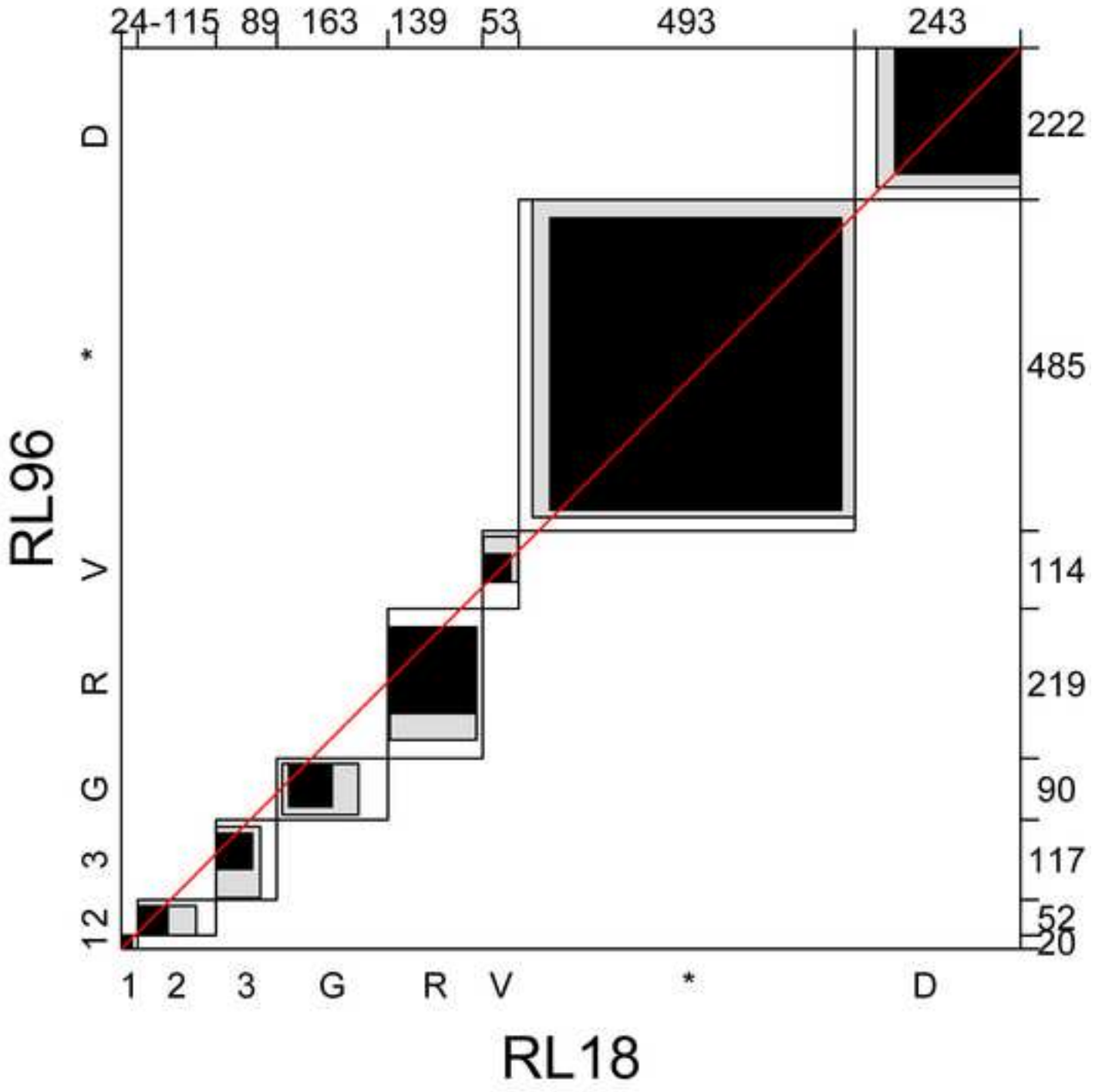
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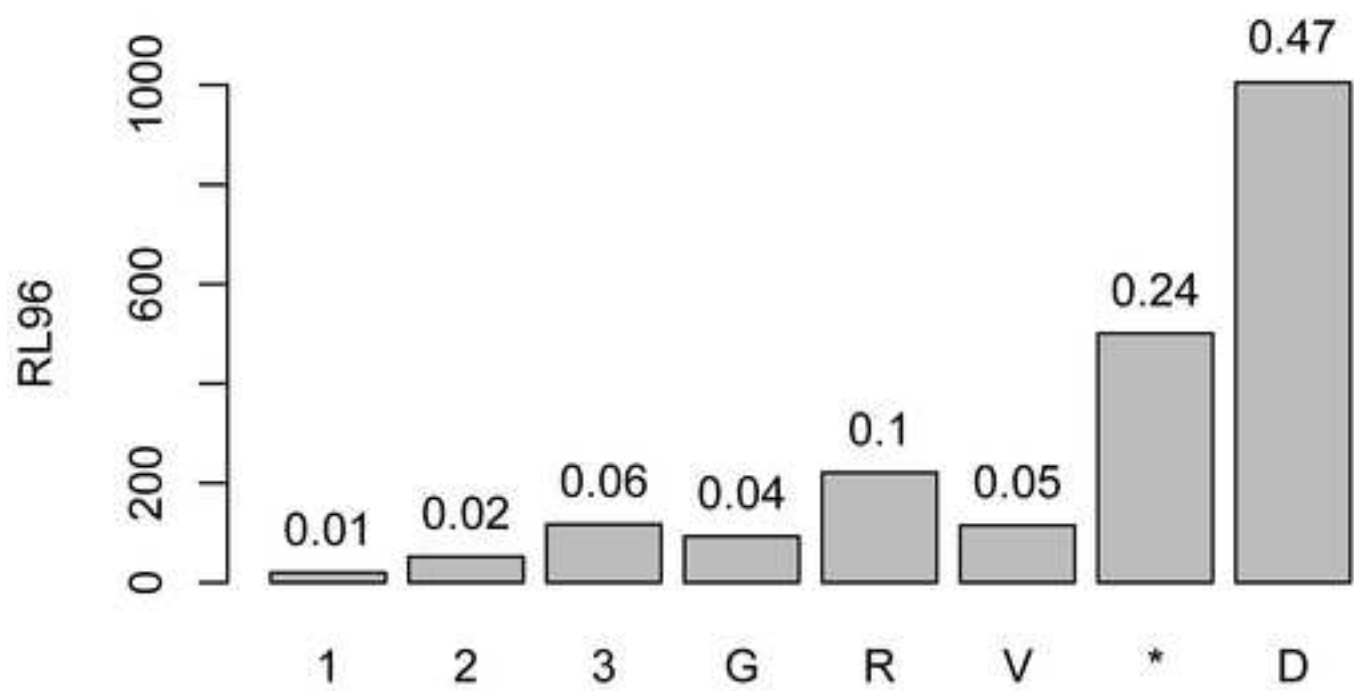
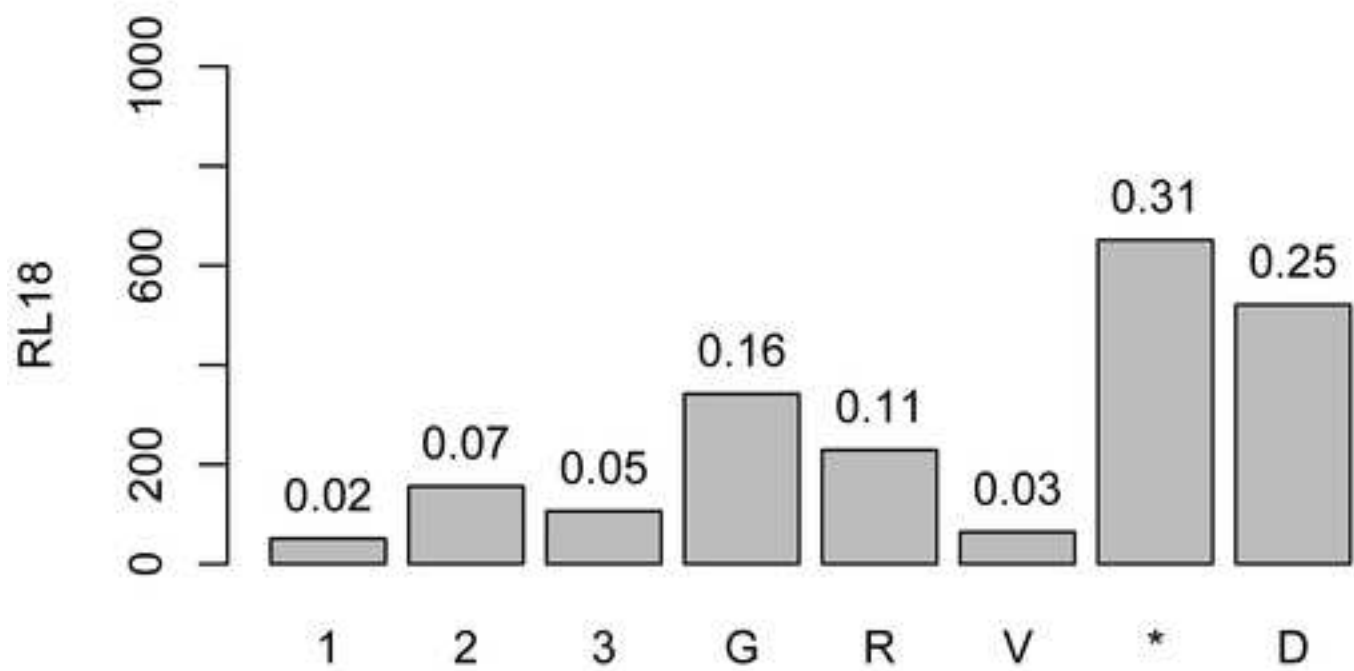
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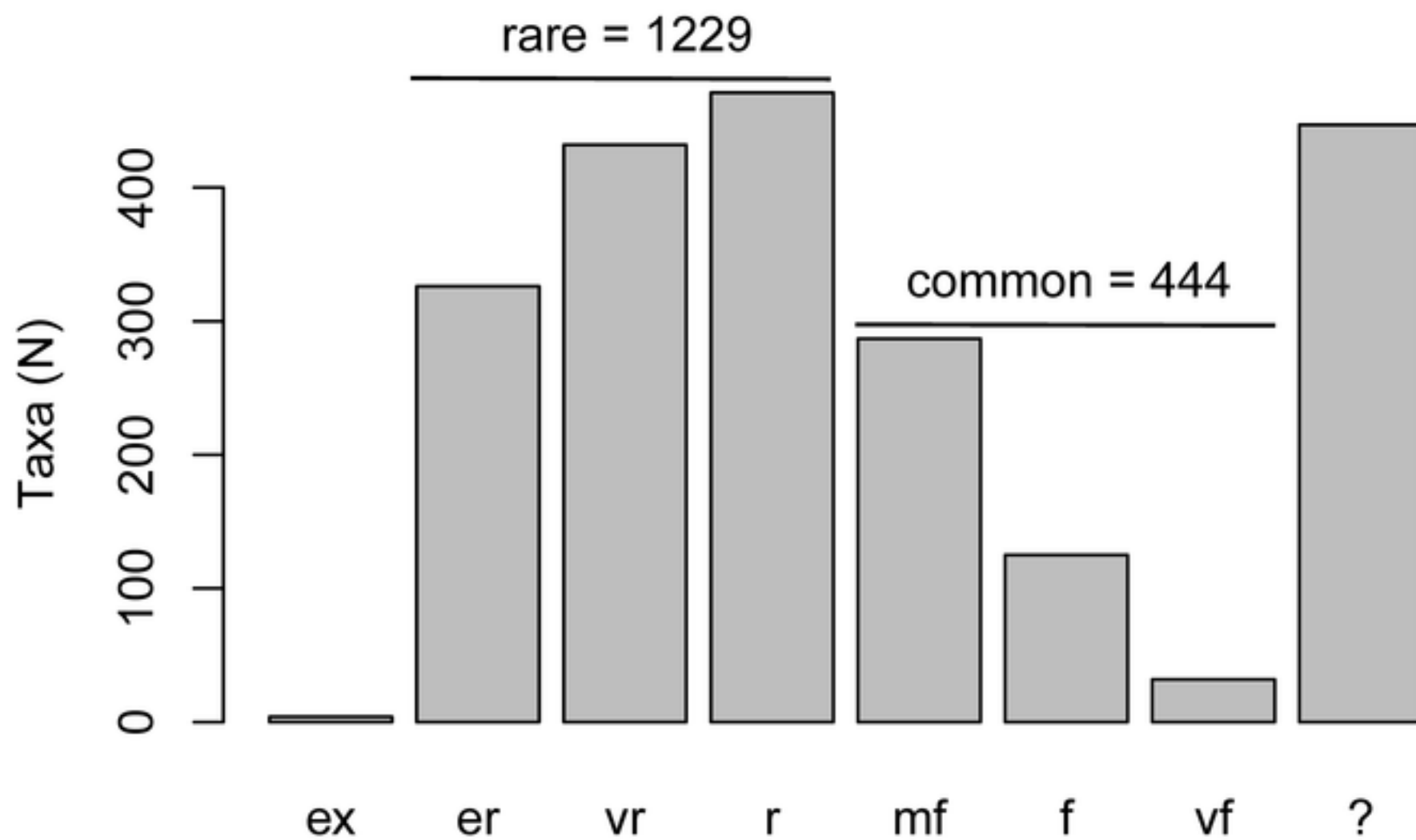
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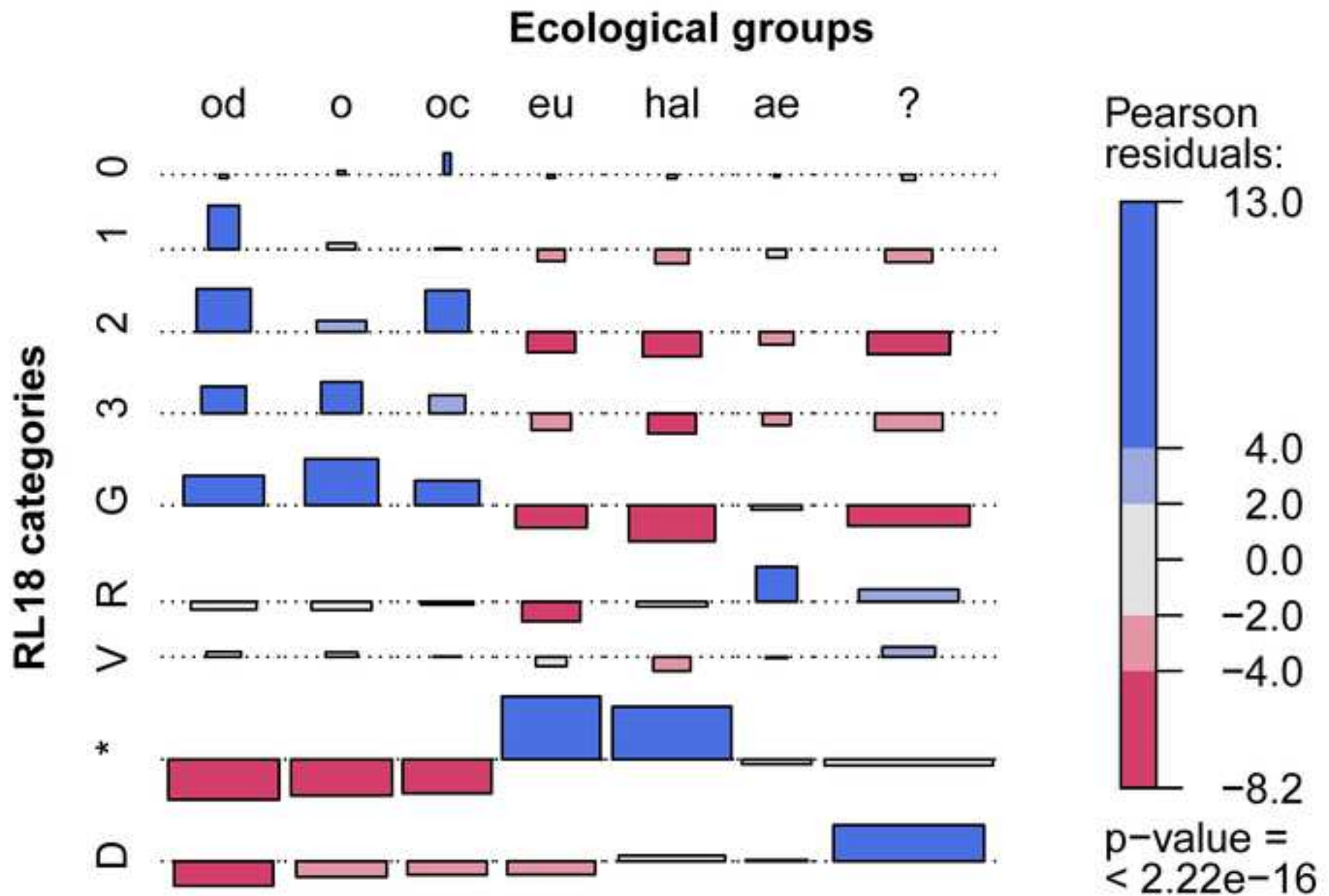
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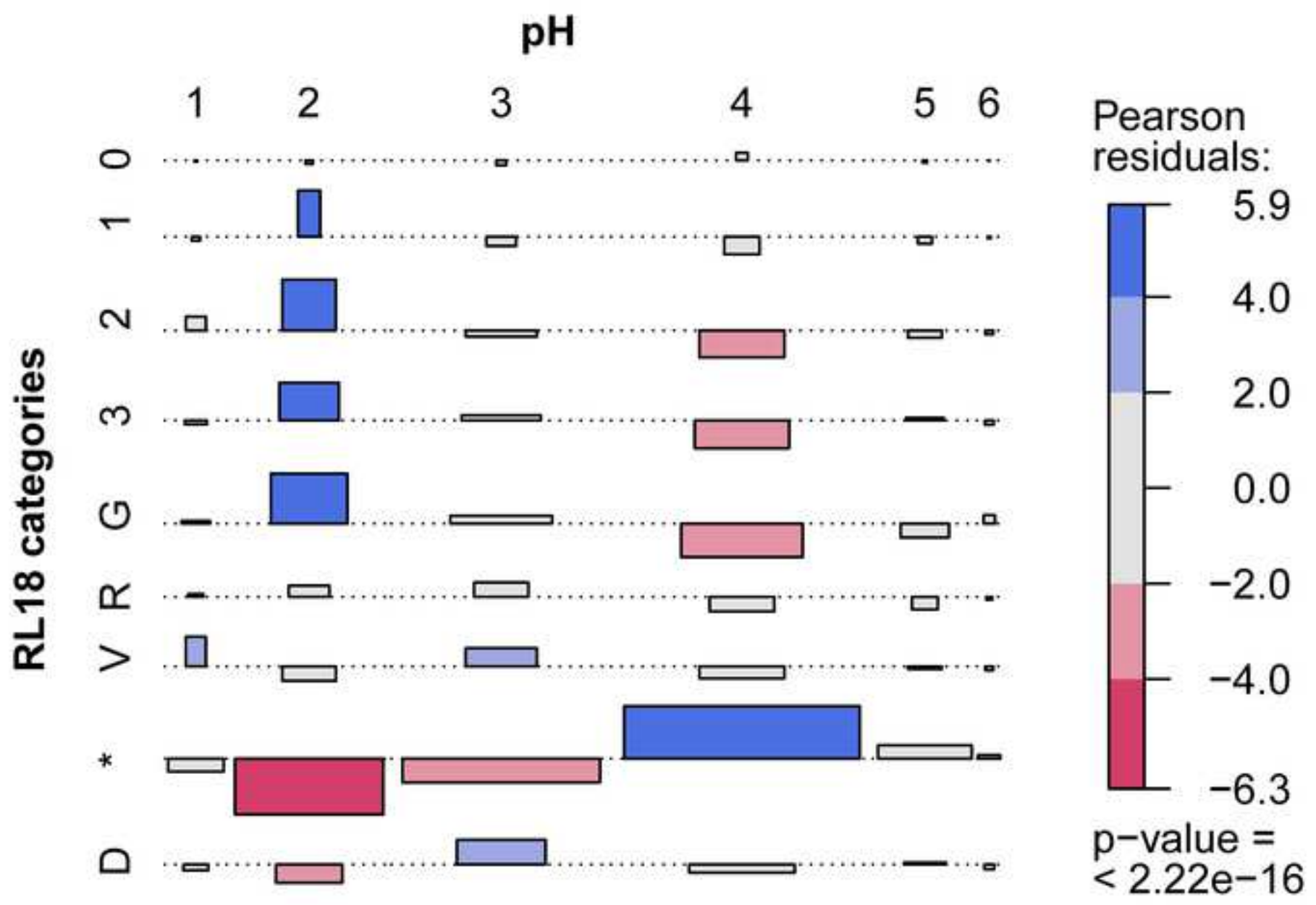
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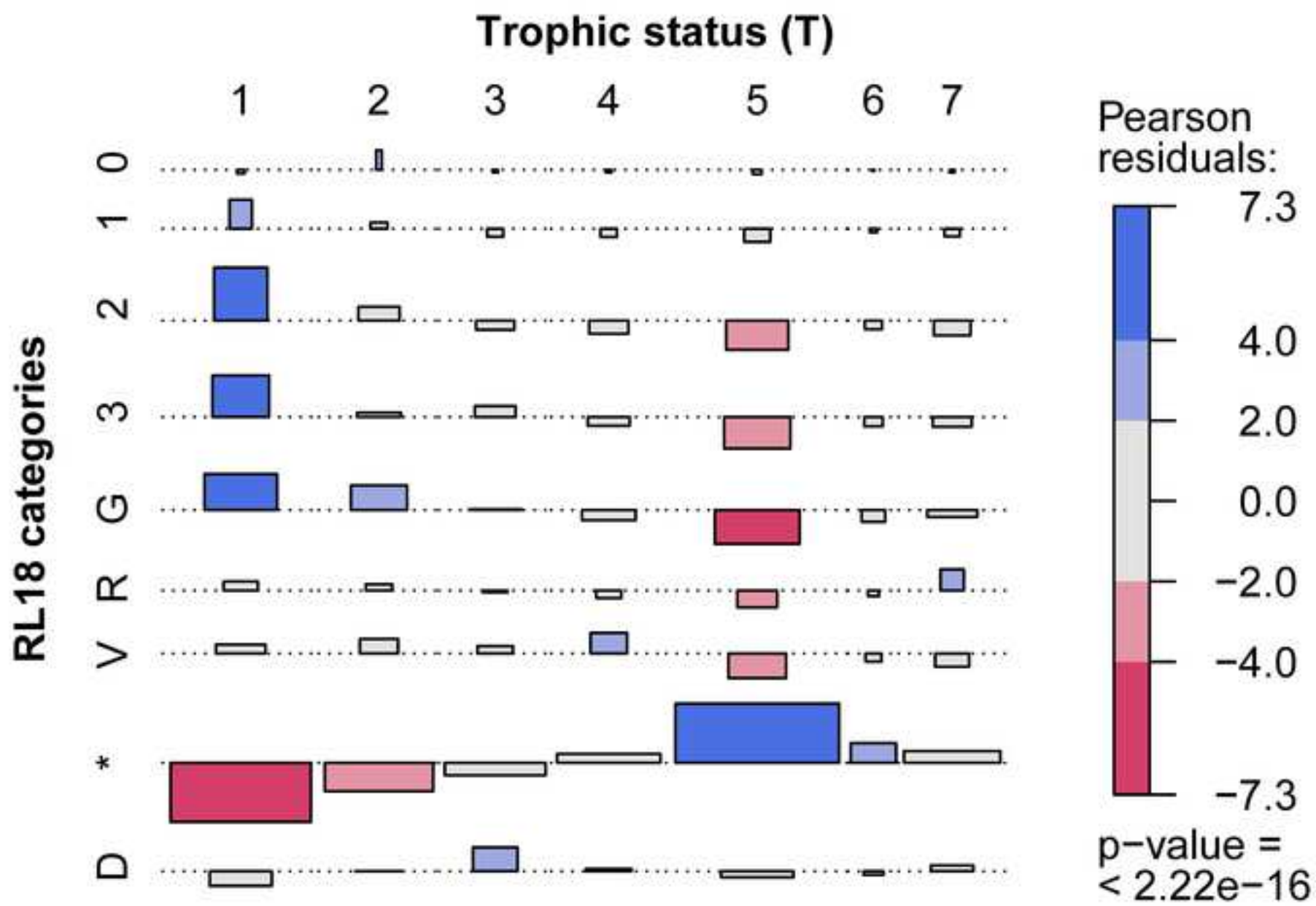


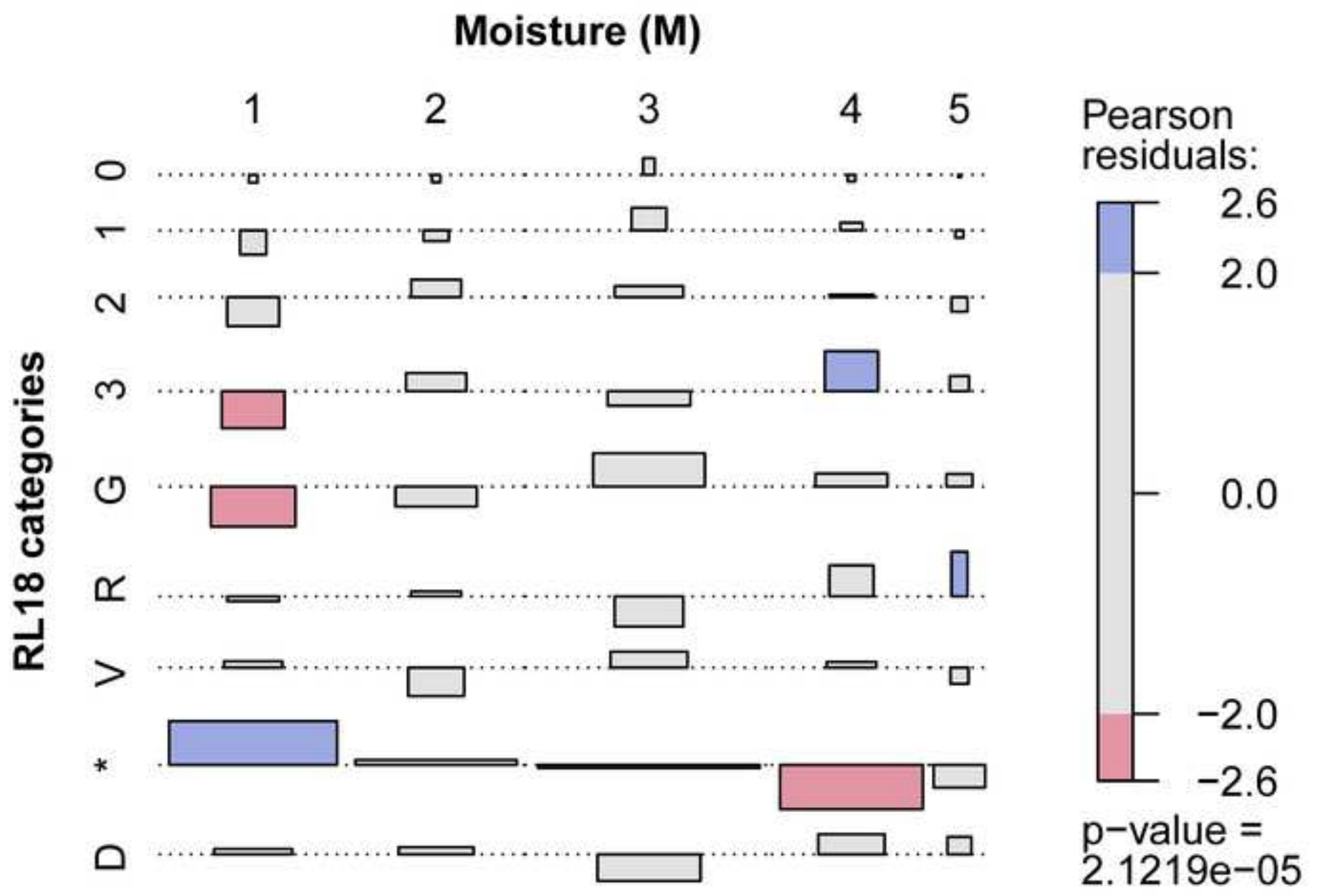












Supplementary Information Table 1 Main Red-List inland-water habitats of Germany. nG = national long-term threat (1 = threatened with complete destruction, 2 = strongly endangered, 3 = endangered, ★ = currently no recognizable threat, # = risk classification does not make sense), TE = current development 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

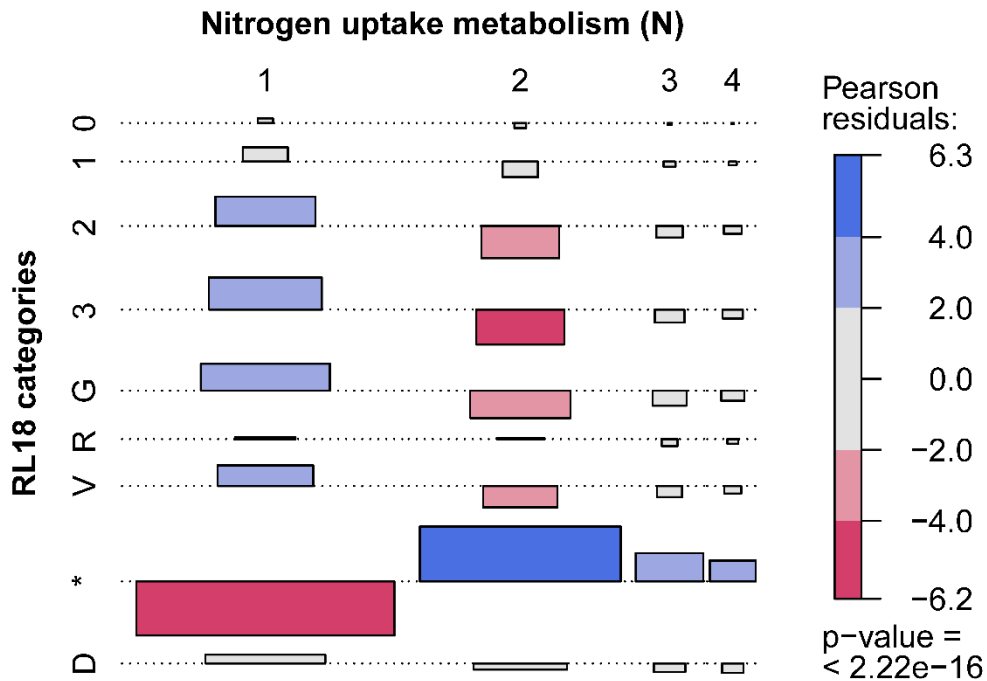
Supplementary Information Table 4 Association between motile and not-motile (Rimet and Bouchez, 2012) and threatened (1,2,3, G,R, V,) an not-threatened (*) RL18 diatom taxa

<i>N (%)</i>	threatened	not-threatened
not-motile	12 (16.4)	148 (37.5)
motile	61 (83.6)	247 (62.5)

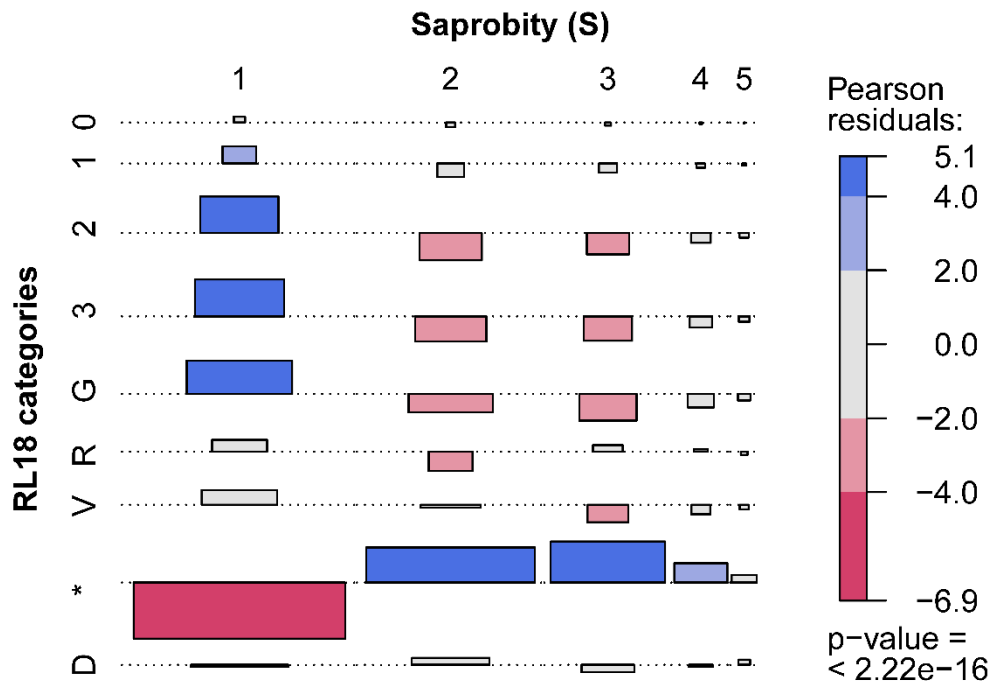
Supplementary Information Table 5 Parameters of the linear model for the relation of the logarithm of nitrate-nitrogen averages and the cumulative percentage of diatom species in Red List threat categories (dependent variable)

	Min	Q1	Median	Q3	Max
Residuals:	-12.7	-7.1	1.5	5.7	16.3
Coefficients:	Estimate	Std. Error	<i>t</i> value	<i>p</i> (> <i>t</i>)	
Intercept	92.3	7.5	12.4	1.2x10 ⁻¹¹	
logmean	-7.7	1.1	-6.8	5.8x10 ⁻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)

