

1 Taxonomic changes and non-native species: An overview of constraints and new
2 challenges for macroinvertebrate-based indices calculation in river ecosystems

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15 **Highlights**

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17 • Biomonitoring tools are required to address new and critical changes to rivers
18 • Taxonomic constraints and non-native species represent new biomonitoring
19 challenges
20 • Existing tools need to be flexible so new scientific developments can be integrated
21 • Mismatches in status classifications may affect management and conservation
22 policies

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26 **Keywords:** Bioassessment, alien species, freshwater ecosystems, ecological indicators,
27 environmental quality, bias

40 **Abstract**

41 Freshwater ecosystems face many threats in the form of reduced water quantity, poor water
42 quality and the loss of biodiversity. As a result, aquatic biomonitoring tools are required to
43 enable the evaluation of these critical changes. Currently, macroinvertebrate-based indices
44 are globally the most widely used biomonitoring tools in fluvial ecosystems. However, very
45 little is known about the potential effects of changes in taxonomic understanding (updating
46 of classification and nomenclature) or the presence of new non-native species for biotic
47 indices calculation. This is especially relevant given that errors, incorrect classification or
48 exclusion of new / updated nomenclature may affect ecological status evaluations and have
49 direct consequences for the management and conservation of freshwater systems. In this
50 discussion paper the main constraints, challenges and implications of these issues are
51 outlined and case studies from a range of European countries are discussed. However,
52 similar challenges affect river and managers globally and will potentially be amplified
53 further in the future. Bioassessment science needs to be open to improvements, and current
54 tools and protocols need to be flexible so that they can be updated and revised rapidly to
55 allow new scientific developments to be integrated. This discussion highlights specific
56 examples and new ideas that may contribute to the future development of aquatic
57 biomonitoring using macroinvertebrates and other faunal and floral groups in riverine
58 ecosystems.

59

60 **1. Introduction**

61 Monitoring freshwater ecosystems is an essential task to fulfil environmental
62 legislation, reflecting attempts to quantify and manage the strong anthropogenic pressures
63 that affect their ecological status. Freshwater biomonitoring is a multidisciplinary field that
64 integrates scientific understanding from different areas of theoretical and applied research,
65 including aquatic ecology, taxonomy, environmental legislation, water resource
66 management and a wide range of stakeholders and end-users (e.g. Nichols et al., 2017). In

67 Europe, after the implementation of the Water Framework Directive 2000/60/CE
68 (European Commission, 2000), the role of biological indicators (usually called
69 bioindicators) has been elevated due to the prominence they are given as indicators of
70 “ecological status” for aquatic ecosystems. Following the implementation of the EU WFD,
71 ecological status is expressed in five classes based on the EQR (Ecological Quality Ratio).
72 This represents the ratio between a measured biological element recorded in the field in
73 relation to the same parameter under ‘reference conditions’ (i.e., without anthropogenic
74 pressures) within the same ecosystem type. Aquatic macroinvertebrates have a long-
75 standing tradition of being used as effective biological indicators of aquatic ecosystems
76 since the early 1900s (Rosenberg and Resh, 1993) and represent the most widely used
77 elements (bioindicators) to characterise and quantify river system conditions (Bonada et
78 al., 2006; Buss et al., 2015). The macroinvertebrate community-based indices currently
79 used in Europe were primarily developed at the end of the Twentieth and beginning of the
80 Twenty-First Century. In response to the EU WFD 2000/60/CE, some European countries,
81 such as France, Italy, and Belgium, replaced their exiting biomonitoring tools with new
82 multi-metric indices and/or new procedures (Buffagni et al., 2006; Buffagni and Erba, 2007;
83 Gabriels et al., 2010; Mondy et al., 2012). However, other countries such as Spain and the
84 UK maintained a connection with pre-existing indices by transforming and improving pre-
85 WFD methods (Munné and Prat, 2009; UKTAG 2014; Bo et al., 2017).

86 During contemporary routine aquatic biomonitoring activities (collecting field
87 samples and processing material in the laboratory), recording multiple non-native
88 invertebrate taxa may be common. The introduction of non-native invasive species is one of
89 biggest threats to aquatic ecosystems globally and represents a growing challenge for
90 environmental regulatory authorities (Havel et al., 2015). Human activities are increasingly
91 affecting the spatial distribution of species both directly and unintentionally (Strayer 2010;
92 Paillex et al., 2009; Lovas-Kiss et al., 2018). Furthermore, Jourdan et al. (2018) recently
93 stressed the relevance of changing climate on European stream communities’ invasibility –

94 referring to the potential increasingly favourable opportunities for non-native and invasive
95 species under many climate change scenarios. Several non-native invasive species have
96 been implicated as being instrumental in modifying native communities (e.g. Simon and
97 Townsend, 2003; Carbonell et al., 2017) with subsequent impacts on freshwater ecosystems
98 (Strayer, 2010; Gallardo et al., 2016; Lovas-Kiss et al., 2018). In most instances, the effects
99 of non-native species on the recipient ecosystem's health have not been fully quantified in
100 the short or medium term as species are not initially identified or recognised as posing a
101 threat, or are not specifically integrated into pre-existing biomonitoring schemes used to
102 assess ecological status (Friberg et al., 2011; Friberg, 2014).

103 To compound this issue, knowledge regarding the correct taxonomy (at least to
104 family and genus level) for field and laboratory identification purposes is crucial to avoid
105 misclassification of both organisms and waterbody conditions. At the same time,
106 improvements in invertebrate taxonomy have been made due to advances in zoological
107 knowledge and scientific advances, which have provided new information regarding the
108 correct classification of some invertebrates (e.g. Arribas et al., 2013; Saito et al., 2018).
109 Changes in taxonomy have occurred over time and are likely to become increasingly
110 common in the future with advances in new molecular tools facilitating the correct
111 classification of cryptic and less studied invertebrate groups and species complexes which
112 may be morphologically almost identical (e.g., Walther et al., 2010; Macadam et al., 2018;
113 Saito et al., 2018).

114 Given the long tradition of employing biotic indices and their widespread
115 application in academic research and use by different stakeholders (e.g. private consultants,
116 water resource managers and regulatory authorities), extensive expertise has been
117 developed, especially in Europe and North America (e.g., Reyjol et al., 2014; Bo et al., 2017;
118 Pawlowski et al., 2018). However, many changes have occurred in European freshwater
119 ecosystems since the WFD was first implemented in 2000. This means that current tools

120 may not accurately reflect some changes that may have become increasingly common in
121 contemporary systems almost 20-years later (see Table 1).

122 Given the limitations identified above, both taxonomic constraints and the spread of
123 non-native species represent significant emerging challenges for the application and
124 reliability of riverine biomonitoring activities. This may have consequences for regulatory
125 environmental agencies, water resource managers and others involved in ecological status
126 evaluations. Mis- or incorrect classification could have direct implications for the
127 management and conservation of freshwaters at national and international scales if they
128 are not addressed or recognised during intercalibration or comparison processes among
129 nation states (e.g., WFD Intercalibration processes; Birk and Hering, 2006). There is
130 therefore an urgent need to address some potentially controversial issues and emerging
131 challenges for existing biomonitoring tools. This discussion paper outlines examples
132 associated with constraints due to the science of taxonomy and the potential and realised
133 effects of non-native invasive species from several European countries. We also discuss the
134 potential options available to address these problems with a view to advancing aquatic
135 biomonitoring activities. The primary purpose of this discussion paper is to focus on how
136 changes in taxonomy and the presence of non-native invertebrate species influence biotic
137 index calculations / metrics and their operation rather than the legislative procedures and
138 policy implementation of biomonitoring management frameworks.

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141 **2. Taxonomic constraints and updates**

142 Many macroinvertebrate-based indices are based on a taxonomic list on which the
143 organisms are grouped and assigned a score based on preferences or tolerances (e.g. a
144 linear scoring system). These lists have typically been approved and validated by an official
145 legislative regulatory authority (government ministry or environmental agency, usually
146 following peer-reviewed publication, e.g. Extence et al. 2013; Chadd et al., 2017) and define

147 the taxa and taxonomic resolution to be considered. For example, the Biological Monitoring
148 Working Party (BMWP) score system was widely used in the UK from 1980 as the official
149 macroinvertebrate based biomonitoring of freshwater lotic ecosystems (Hawkes, 1997)
150 until its refinement in 2014 (UKTAG, 2014). Given its ease of application and reliable results,
151 minor modifications or adaptations have been tested and widely applied in countries
152 throughout Europe, North and South America, Africa and Asia (e.g., Paisley et al., 2014;
153 Aschalew and Moog, 2015). The BMWP score and its derivatives represents a single metric
154 index in which each invertebrate family has been given a score from 1 to 10 based on its
155 known tolerances to organic contamination. The final site score being obtained by summing
156 the individual family scores of the different taxa recorded in the sample. One clear example
157 of its wider application has been the IBMWP index, which has specifically been adapted for
158 use on the Iberian Peninsula (Alba-Tercedor et al., 2002). This has become the most widely
159 used macroinvertebrate biomonitoring method in Spain over the last 25 years (Couto-
160 Mendoza et al., 2015) and the official index used in national legislative based monitoring
161 (MAGRAMA, 2015).

162 However, even since the last refinement of the IBMWP faunal list (MAGRAMA,
163 2013), some taxonomic changes have occurred and still need to be integrated into the index.
164 An examination of the current taxonomic family list highlights the presence of the gastropod
165 family Ancyliidae (with a score of 6). New taxonomic developments have resulted in
166 Ancyliidae no longer being recognised and species which were part of the family are
167 currently included taxonomically in the family Planorbidae (Bouchet and Rocroi, 2005;
168 Oscoz et al., 2011; Bank, 2013); which obtains an IBMWP score of 3. Given the IBMWP's
169 additive character and sensitivity to low abundance taxa (Guareschi et al., 2017), this could
170 result in elevated final index values and potentially ecological status in some cases. In this
171 instance, advances in taxonomy have moved faster than updates to environmental
172 legislation. This issue is not unique to Spanish waterbodies since Ancyliidae at the family
173 level is also present on other taxonomic lists, for example, the multimetric STAR_ICM Index

174 (ISPRA, 2014 and see Table 1a). This index has been used in Europe as the Intercalibration
175 Common Metric Index, and is the official index currently used in Italy and Cyprus to assess
176 river ecological statuses to fulfil EU WFD legislation (details in Buffagni et al. 2006, Feio et
177 al., 2014, ISPRA 2014). The STAR_ICM index is comprised of 6 metrics: ASPT (Average Score
178 Per Taxon), logarithm of the selected families of Ephemeroptera, Plecoptera, Trichoptera
179 and Diptera ($\log(\text{sel_EPTD}+1)$), total number of taxa, number of EPT taxa, 1 minus the
180 relative abundance of Gastropoda, Oligochaeta and Diptera (1-GOLD) and the Shannon
181 index.

182 The most common Palearctic species of the former Ancyliidae family is *Ancylus*
183 *fluvialitis* Müller, 1774, a rheophilic species with ecological and biological traits that are
184 markedly different to most limnophilic Planorbidae, especially in relation to current
185 velocity and dissolved oxygen preferences (Oscoz et al., 2004). Keeping these taxa separate
186 (*Ancylus* sp. separate from Planorbidae) would appear to be a sensible choice for riverine
187 biomonitoring purposes and one option would be to replace Ancyliidae on official lists but
188 to include the genus taxonomic designation - *Ancylus*. This change has already been applied
189 to the Whalley, Hawkes, Paisley and Trigg Index (WHPT), one of the indices currently used
190 in the UK (UKTAG, 2014) which considers the *Ancylus* group separately from other members
191 of the family Planorbidae.

192 Another example is illustrated by the caddisfly species *Pseudoneureclipsis*
193 *lusitanicus* Malicky, 1980 that has been recorded in Portugal, Spain and France (González
194 and Martínez, 2011). It was formerly considered part of the family Polycentropodidae but
195 is currently assigned to the family Dipseudopsidae (Tachet et al., 2001) which is not
196 reported or recognised on the official Spanish IBMWP lists. Similarly, *Acroloxus* Beck, 1838,
197 now belongs to the family Acroloxidae (Gastropoda) and Pediciidae (Diptera, Tipuloidea)
198 are not included as scoring taxa on the IBMWP taxonomic list but, are considered in other
199 European macroinvertebrate indices (e.g., STAR_ICM index and WHPT, Table 1a).

200 Consideration of the taxonomic level utilised in biomonitoring tools is an interesting
201 topic worthy of attention and discussion. The use of a higher taxonomic level for
202 invertebrates (e.g. family) is widely employed for most biomonitoring indices and is
203 considered a good compromise between classification effort and obtaining appropriate
204 biological information (e.g., Gayraud et al., 2003; Monk et al.,2012). A greater taxonomic
205 resolution (genus or species level) may provide additional information but may be
206 extremely time consuming and incur a greater economic cost. For instance, the IBMWP taxa
207 list is composed primarily of taxa at the family level, with a few exceptions for higher
208 taxonomic levels: Acariformes, Oligochaeta and Ostracoda. The other exception concerns
209 the only genus currently included on the IBMWP list: *Ferrissia* Walker, 1903. The regulatory
210 authority stopped considering Ferrissidae as a separate family in its own right (now
211 incorporated within Planorbidae), but uses the genus: *Ferrissia* (MAGRAMA, 2013) with a
212 score of 6. The use of *Ferrissia* as the only genus currently considered is odd given that little
213 is known scientifically regarding its tolerances, preferences and spatial distribution (Oscoz
214 et al., 2011). Furthermore, the taxonomy of the Palearctic *Ferrissia* taxa is currently under
215 debate, and no consensus has been reached on the presence or identity of any true
216 autochthonous Palearctic species (Vecchioni et al., 2017). Moreover, the cryptic invasion by
217 the North American gastropod, *Ferrissia fragilis* (Tryon, 1863), has been highlighted in
218 Southern Europe ecosystems (Marrone et al., 2011) and in other countries with surprising
219 conservation implications (e.g., invasive species considered endangered freshwater
220 limpets, Saito et al., 2018).

221 Some exceptions regarding the use of genus level data can be found within the
222 biomonitoring tools used across Europe. For example, Buffagni and Erba (2007) stressed
223 the importance of Operational Units (genus and subgenus) to the Order Ephemeroptera for
224 surveillance and investigative monitoring surveys. This has subsequently been integrated
225 into Italian monitoring legislation. Similarly, the Belgian MMIF index, and the I2M2 Index
226 used in France, requires some invertebrate orders to be identified to the genus level

227 (Gabriels et al. 2010; Mondy et al. 2012). However, in the latter, as well as in the STAR_ICM,
228 taxa belonging to Planorbidae are always recorded at the family level.

229 Specific research at the genetic level and in relation to experimental tolerances of
230 *Ferrissia* and its Iberian, and wider European populations, is therefore recommended
231 considering that information regarding the presence of native European or western
232 Mediterranean species is pending. Given current knowledge, a score of 6 for a genus with
233 doubts raised regarding its origin and taxonomy requires reflection. However, a traditional
234 taxonomic approach (although this is also problematic) would still consider it at the family
235 level (Planorbidae - 3 points). Should no new findings regarding the autochthonous *Ferrissia*
236 be forthcoming, questions regarding whether the genus should be given a score on any
237 European taxonomic list may need to be addressed.

238 These effects and constraints on multiple national taxonomic lists and family level
239 metrics are common and given that legislation should be responsive to scientific advances,
240 periodic updating and greater flexibility is recommended. Modifications made to taxonomic
241 lists should be confirmed on official documents validated by the national regulatory
242 authority, after careful scientific-technician evaluation of potential consequences, to
243 standardise scoring systems and avoiding inhomogeneity when interpreting data and
244 results.

245

246 **3. The role of alien species in river biomonitoring: how should they be considered?**

247 Although there is a growing body of literature on non-native species, relatively little
248 is known about their effect on routine biomonitoring results or about which metrics could
249 be particularly affected. Some notable exceptions include recent research undertaken in
250 Central Europe and the UK, which has demonstrated how the presence of non-native
251 invasive species may affect the metric scores and even the potential classification of a
252 freshwater body's ecological status (e.g., McNeil et al., 2013, Mathers et al. 2016).

253 Non-native freshwater invertebrates represent a global pressure, exemplified by
254 Mollusca and Crustacea fauna (Fenoglio et al., 2016). The geographical range of non-native
255 invasive bivalves, such as *Corbicula fluminea* (Müller, 1774), are expanding in many
256 European countries (e.g., Zamora-Marín et al., 2018) but are not typically integrated into
257 existing biomonitoring schemes despite being recognised as a problem in Belgium for
258 interpreting biomonitoring outputs (Gabriels et al., 2005). Other species, such as *Dreissena*
259 *polymorpha* (Pallas 1771) (zebra mussel), which are widespread in many waterbodies, may
260 benefit from future climate change in some European areas, but less in others (Gallardo and
261 Aldridge, 2013) with potentially diverse effects on wider communities and ecosystem
262 functioning (Ward and Ricciardi, 2007).

263 The North American signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), belongs
264 to the family Astacidae, a non-tolerant family with relatively high score on both the WHPT
265 and IBMWP lists (scoring 8-10). In this instance, the presence of a non-native taxon (if
266 considered at the family level, see Table1b) could increase the final index value, with
267 potential consequences for the ecological status classification. In the UK, the WHPT index
268 explicitly includes non-native species information when considering Astacidae taxa but
269 utilises the same tolerance values (UKTAG 2014). However, Mathers et al., (2016) found
270 that sites subject to invasion by signal crayfish may experience elevated biotic index scores
271 because of their predation of leeches and snails (typically lower scoring taxa). This means
272 that some sites could theoretically obtain higher index scores as a result of the presence and
273 activities of a non-native species and not because of specific improvements in river
274 ecosystem quality.

275 In another instance, the Ponto Caspian killer shrimp *Dikerogammarus villosus*
276 (Sowinsky, 1894), which was recorded in Italy more than 10 years ago (Casellato et al.,
277 2006), belongs to the family Gammaridae (occurring on many European taxonomic lists)
278 and would be positively considered in the STAR_ICM index calculation if specific taxonomic
279 information for this species was absent (see Table1b). Similarly, the alien euryhaline corixid

280 *Trichocorixa verticalis verticalis* (Fieber, 1851), recorded in Spain and Portugal downstream
281 to river estuary mouths and wetlands (Guareschi et al., 2013), belongs to the same family
282 (Corixidae) as the native species within the genus *Sigara* Fabricius, 1775, among others.
283 These examples, illustrate how additional taxonomic resolution (e.g., genus level
284 resolution) would provide greater information and if combined with taxonomic updates to
285 national lists avoid the effects of colonisation and invasion being overlooked. Analogous
286 problems may appear with other cryptic taxa, such as some Oligochaeta where multiple
287 families may appear morphologically analogous (e.g., non-native genus *Sparganophilus*
288 Benham, 1892 and numerous common Lumbricidae taxa, see Rota et al., 2016). The
289 development of specific tools such as DNA metabarcoding could help mitigate, at least
290 partially, some of the issues of reliably identifying species for morphologically similar and
291 cryptic groups (e.g. Pawlowski et al., 2018).

292 The case of the New Zealand mud snail *Potamopyrgus antipodarum* (J.E. Gray, 1843)
293 highlights multiple issues associated with taxonomic changes and the effects of non-native
294 species on aquatic ecosystems. New molecular studies by Wilke et al. (2013) supported the
295 designation of the species belonging to the family Tateidae (former subfamily of
296 Hydrobiidae, see Batzer and Boix, 2016), but this family is not considered in most European
297 indices. In addition, juvenile life stages of Hydrobiidae (scored family) and Tateidae, such as
298 the native species *Mercuria similis* (Draparnaud, 1805) and non-native *Potamopyrgus*
299 *antipodarum*, could lead to misclassification due to their morphological similarities (Table
300 1b).

301 When considering the EQR (Ecological Quality Ratio) and focussing on taxonomic
302 metrics, the presence of non-native invasive species could be considered a shift from the
303 site's reference conditions, or at least a pressure on specific water bodies (ADAS, 2008).
304 However, thus far no official metric exists to characterise the effects of emerging stressors
305 such as non-native taxa in a European WFD context (Hering et al., 2010) or globally.
306 Arbačiauskas et al. (2008) proposed assessing the biocontamination of benthic

307 macroinvertebrate communities using a site-specific biocontamination index derived from
308 two metrics: an abundance contamination index and a richness contamination index at the
309 ordinal rank. Their research stressed the relevance of biocontamination affecting ecological
310 status assessments using BMWP type methods in Central and Eastern Europe.

311 Most official biotic indices currently ignore the presence of non-native invasive
312 species or integrate them within the family level designations of native fauna, sometimes
313 without acknowledgement. Non-native species (when detected) are usually reported in the
314 “observations space” of the official field card used by qualified operators when undertaking
315 routine biomonitoring activities. Thanks to this procedure (sometimes not easy for cryptic
316 species), biomonitoring reports could act as an important quantitative resource for
317 research into biodiversity threats, biological invasion(s) and biogeography. This common
318 procedure may be informative but is insufficient given that it has no practical effect on the
319 final index value (e.g. IBMWP Index) and any potential shift in status or functioning is not
320 considered at the ecosystem evaluation stage. In other instances, the taxonomic list used to
321 calculate metrics such as, *Average Score Per Taxon (ASPT)* and Total Family Richness for the
322 multimetric STAR_ICM Index considers some non-native families such as Corbiculidae and
323 Dreissenidae, despite no BMWP scores currently being available (ISPRA, 2014).

324 The development of new metrics or modifying existing regulatory methods is
325 beyond the scope of this discussion. However, updates and information from relevant
326 environmental authorities regarding non-native invasive taxa (e.g., a periodically updated
327 list of non-native taxa at a national level potentially with notes on taxonomy, observed
328 tolerances and other faunal associations) would help to avoid overlooking these issues
329 when analysing and interpreting data. Moreover, some flexibility in existing methods and
330 adaptations should be considered. For additive scoring systems such as IBMWP (and
331 numerous other BMWP derived approaches), applying a negative score to each non-native
332 taxon or a generic negative score if non-native taxa are observed in the sample may be an
333 option worthy of further research. Another possibility that may require further research is

334 an adaptive attribution of the family level scores: if non-native species are present then a
335 revised score could be use (ideally integrating both native and non-native species tolerance
336 and relevant abiotic / biotic information). However, if no non-native species occur the
337 original score should be used in an unmodified form. In both instances this requires a good
338 species level knowledge of non-native species present in a given country / river basin. In
339 addition, regular updating of lists of non-native aquatic species and new records of recently
340 invaded sites may be crucial for effective management. The same constraints that affect
341 additive scores occur in other commonly used multimetric indices that incorporate an
342 average score / ASPT approach as a core metric (e.g. Cyprus, Italy, Portugal, UK; Feio et al.
343 2014; UKTAG 2014; Laini et al., 2018). The ASPT and WHPT ASPT Index (total BMWP or
344 WHPT score / number of families scored) is a direct derivative of the additive scoring system
345 BMWP (Hawkes, 1997). It would be possible to test the effect of a zero score(s) for non-
346 native families on the final metric. In this way, the effect of non-native taxa could be
347 integrated (e.g., the ASPT or WHPT ASPT value would be lower as the denominator value
348 would increase).

349 Similar limitations affect macrophyte-based indices like the IBMR (Macrophyte
350 Biological Index for Rivers, Haury et al. 2006) developed in France, but adapted and used in
351 Spain and Italy. The presence of non-native taxa does not affect the final scores in most
352 instances, except for three taxa: *Azolla filiculoides* Lam, *Elodea canadensis* Michx and *Elodea*
353 *nuttalii* (Planchon) St John, which have been included in the French and Italian scoring
354 systems with their tolerance values. In the case of macrophytes, congeneric species (native
355 and non-native) or cryptic species represent an ongoing challenge to scoring systems (e.g.
356 Ceschin et al., 2016). Fish-based methods for rivers and lakes have a longer tradition of
357 dealing with non-native taxa (Birk et al., 2012) and negative values have been proposed in
358 some biomonitoring systems such as the NISECI Index (Macchio et al., 2017) used in Italy,
359 or the German FIBS (Diekmann et al., 2005), where the occurrence of non-native or hybrid
360 species are penalised in the index final score.

361 However, non-native species are not all equal (in terms of ecologic effects or
362 impacts) and should not necessarily all be treated with the same negative score. Depending
363 on their success in receipt systems, some may have a strong effect on ecosystems by
364 becoming “invasive”, whereas others do not represent any clear pattern of effects or may
365 simply occur sporadically (e.g., depending on the waterbody or geographic areas, see
366 examples of *Menetus dilatatus* (Gould, 1841) or *Potamopyrgus antipodarum*, Múrria et al.,
367 2008). Could we use some (or all) non-native species to evaluate river ecological status or
368 derive other biotic indexes? Could a river supporting and inhabited by only non-native
369 species be evaluated? Information regarding non-native species’ tolerance to anthropogenic
370 pressures or pollution remains scarce for many taxa. It should be investigated, and even
371 incorporated into biomonitoring research, by considering that some non-native species
372 may have similar tolerances to indigenous native species. This would provide ecosystem
373 information when comparable native taxa are missing (see Lagrue et al., 2014) and non-
374 native taxa could also be assigned an indicator value in their own right for some stressors
375 or conditions, but it may bring into question the EQR and reference conditions (especially
376 in an European WFD context). Another option would be to develop and test metrics
377 specifically to assess the introduction/invasion of non-native taxa (e.g. Arbačiauskas et al.,
378 2008). These new tools should be integrated into the toolbox available to environmental
379 managers and should deal with specific intercalibration procedures if they are intended to
380 complement ecological status evaluation.

381 The issue of community dominance appears more complicated, in lowland or
382 moderate altitude rivers, where some non-native species may represent the most common
383 taxa in terms of abundance (no. of individuals) or biomass, making it more difficult to
384 correctly apply current biomonitoring indices. For instance, Arndt et al. (2009) showed that
385 the dominance of non-native species may affect the reliability and interpretations of the GSI
386 (German Saprobic Index) results given reduced native macroinvertebrate abundance.
387 However, quantifying biological invasion and potential dominance by specific taxa is still

388 not integrated into the final score of biomonitoring indices; remaining an open topic of
389 discussion in bioassessment science and ecological research (e.g., Arbačiauskas et al. 2008;
390 Catford et al., 2012).

391 It is worth highlighting that, despite not being specifically designed for non-native
392 taxa, some metrics like Evenness, the Shannon Index and 1 minus the relative abundance of
393 Gastropoda, Oligochaeta and Diptera (called "1-GOLD"), which are abundance-based
394 metrics sensitive to high densities of individuals, can reflect the dominance of some taxa in
395 the final metric value. Thus the 1-GOLD metric would decrease if there was a high
396 dominance associated with Gastropoda, Oligochaeta and Diptera families. Unfortunately,
397 the taxonomic resolution at the family level would not allow the identification of some non-
398 native taxa belonging to other groups (e.g., the case of some Crustacean taxa). However, in
399 other instances the opposite scenario may also occur and, paradoxically, this metric would
400 give high values (close to 1) for the low abundances of Gastropoda, Oligochaeta and Diptera,
401 but a very high abundance for taxa from other families, with the consequent risk of "hidden"
402 dominant invasive taxa (in abundance terms) possibly raising the final metric value.

403

404 **4. Conclusions**

405 Aquatic ecosystems face ongoing global challenges due to global environmental
406 change, new non-native/invaser taxa, biodiversity loss and hydrological regime
407 modification, and these pressures will affect the results of aquatic biomonitoring.
408 Bioassessment science needs to be open to improvements, and current tools should be
409 flexible so that new scientific advances can be integrated (from not only molecular /genetic
410 perspectives, but also associated with taxonomic, biogeographic, hydro-morphologic and
411 non-native species management advances). For the indices based on the BMWP score /
412 ASPT type metrics, there are specific adjustments that could lead to improved
413 characterisation of waterbody status following wider testing of large datasets. Taxonomic
414 lists of single and multimetric biotic indices should not be considered fixed but should be

415 periodically reviewed (e.g., regularly adapted in the regulatory context of European WFD
416 survey networks) to update and consider possible taxonomic modifications associated with
417 new non-native taxa/invasers. At the European scale, updating and refining taxonomic lists
418 should ideally be accompanied by updating reference condition values and thresholds
419 among ecological classes to allow direct comparison with historical data series. The latter
420 wouldn't be an easy task, but considering that European intercalibration relies on at least
421 partially outdated data (e.g., Birk and Hering, 2006) and that significant changes have
422 occurred within freshwaters over the last 20 years (e.g. new aquatic invaders, taxonomic
423 changes, climatic and hydrological pressures) revised and validated updates would refine
424 and improve bioassessment accuracy of river ecosystems.

425 Solving these common constraints may bring positive consequences to functional
426 diversity assessments (e.g., updated information on non-native species' functional traits or
427 tolerances would be useful), which could complement bioassessment alongside other WFD-
428 compliant tools (Reyjol et al. 2014). It seems crucial to address the challenges outlined
429 above because mismatches in ecological status classifications may directly affect
430 management and conservation policies and the future conservation status of freshwater
431 ecosystems. Both challenges, in addition to other global freshwater challenges, may allow
432 us to reflect on the potential to improve the family level approach that often hides or ignores
433 taxonomic issues, especially where non-native and native taxa occur in the same family.
434 Similarly, the potential advantages of multimetric indices over single metric indices should
435 also be considered; this topic has already been subject of debate in some instances (e.g.
436 Couto-Mendoza et al., 2015). To avoid criticisms associated with scoring systems limited to
437 faunal tolerances in relation to a single parameter (a common criticism of the BMWP
438 approach which focuses on organic contamination), a multimetric approach would facilitate
439 the assessment of multiple stressors (e.g., potentially including the presence and impacts of
440 new invaders). However, the "core metrics" that compose any multimetric tool should be
441 complementary and assessed each in turn to understand which directly responds to specific

442 conditions. The focus just on the final multimetric score may overlook or ignore information
443 that may be apparent when considering the individual components. For example, Meier et
444 al., (2006) proposed the use of 3 different modules to characterising biotic response to: i)
445 organic pollution, ii) general degradation, and iii) acidification in German rivers. These are
446 derived independently (with specific biotic metrics) and subsequently integrated in final
447 evaluation stage to provide a reliable multimetric.

448 Given the intrinsic multidisciplinary character of biomonitoring, discussion and
449 possible adjustments need to be shared with all “freshwater science” stakeholders,
450 including researchers and practitioners in universities, research centres, government
451 agencies, environmental managers and private consultancies, which deal and work with
452 these issues on a daily basis. Finally, the next generation of genetic sequencing approaches
453 (e.g., DNA metabarcoding) appear to be on the brink of revolutionising ecology and there
454 are strong opportunities to complement and improve aquatic bioassessment methods at
455 least for presence/absence data of most macronvertebrate groups (e.g., Elbrecht & Leese,
456 2017; Pawlowski et al., 2018). However, these new tools should also provide a bridge
457 between the past and the present by allowing the comprehensive use of long-term data
458 series.

459

460

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794 **Table**

795

796 **Table 1.** Summary of the main taxonomic constraints (groups with taxonomic revisions,
797 Table 1a) and non-native taxa that may affect the performance of macroinvertebrate-based
798 indexes (1b). Examples and references are also provided (for further details please see the
799 main text).

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801

Table 1. Summary of the main taxonomic constraints (groups with taxonomic revisions, Table 1a) and non-native taxa that may affect the performance of macroinvertebrate-based indexes (1b). Examples and references are also provided (for further details please see the main text).

a. Taxonomic constraints

Order	Taxa	Constraints	Example	References
Diptera	Pediciidae	Lack of consensus regarding status of family	Not considered in IBMWP but included in STAR_ICM and WHPT	Tachet et al. 2010; MAGRAMA, 2013; ISPRA, 2014; UKTAG, 2014
Mollusca	Ancylidae	Currently within the family Planorbidae	Indices not updated to incorporate change (e.g. IBMWP, STAR_ICM)	Oscoz et al., 2011; Bank, 2013, MAGRAMA, 2013, ISPRA, 2014
Mollusca	Acroloxidae	Taxonomically recognised family	Not considered in IBMWP but included in STAR_ICM and WHPT	Oscoz et al., 2011; MAGRAMA, 2013; ISPRA, 2014; UKTAG, 2014
Mollusca	<i>Ferrissia</i>	Lack of consensus regarding autochthonous Palaearctic taxa	Considered at the genus level in one index (IBMWP)	Mondy et al., 2012; MAGRAMA, 2013; Vecchioni et al., 2017
Trichoptera	Dipseudopsidae	Formerly considered part of the family Polycentropodidae	<i>Pseudoneureclipsis lusitanicus</i> and family Dipseudopsidae not considered in existing indices	Tachet et al., 2001; González and Martínez, 2011; MAGRAMA, 2013

b. Non-native taxa

Order	Taxa	Constraints	Example	References
Crustacea	Cambaridae	Non-native taxa frequently dominant in terms of biomass where they occur	Not considered in most indices (e.g. IBMWP) but included in STAR_ICM	MAGRAMA, 2013; ISPRA, 2014
Crustacea	Astacidae	Native and non-native species occur within the same family	<i>Pacifastacus leniusculus</i> and <i>Austropotamobius pallipes</i> complex	Tachet et al., 2010
Crustacea	Gammaridae	Native and non-native species occur within the same family	<i>Dikerogammarus</i> sp. and <i>Echinogammarus</i> sp.	Tachet et al., 2010; Casellato et al., 2006
Hemiptera	Corixidae	Native and non-native species occur within the same family	Native <i>Sigara</i> sp. and non-native <i>Trichocorixa verticalis</i>	Guareschi et al., 2013
Haplotaxida	Sparganophilidae	Cryptic and less studied invertebrate Order / Families	Classification (native and non-native) may be difficult for non-expert operators	Rota et al., 2016
Mollusca	Corbiculidae	Non-native taxa usually dominant in terms of biomass and / or densities where they occur	Not always considered in existing indices (e.g. IBMWP). When it is, its presence may increase richness metrics (e.g. STAR_ICM). May cause problems with interpreting outputs (MMIF index)	Gabriels et al., 2005; MAGRAMA, 2013; ISPRA, 2014
Mollusca	Dreissenidae	Non-native taxa usually dominant in terms of biomass and / or densities where they occur	Not always considered in existing indices (e.g. IBMWP). When it occurs, its presence may increase richness metrics (e.g. STAR_ICM)	Ward and Ricciardi, 2007; MAGRAMA, 2013; ISPRA, 2014
Mollusca	Hydrobiidae	Former subfamily Tateidae raised to taxonomic family and removed from Hydrobiidae	<i>Potamopyrgus antipodarum</i> (Tateidae) and <i>Mercuria similis</i> (Hydrobiidae) can be confused by non-expert operators	Wilke et al., 2013; Batzer and Boix, 2016
Mollusca	Planorbidae	Native and non-native species occur within the same family	North American <i>Menetus dilatatus</i> and numerous <i>Planorbarius</i> species	Kołodziejczyk and Lewandowski (2015)

