

How do low-abundance *taxa* affect river biomonitoring? Exploring the response of different macroinvertebrate-based indices

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ABSTRACT

The contribution of rare *taxa* to aquatic bioassessments remains a subject of debate, and generates contrasting positions among researchers. Very little is known about the effect of low-abundance *taxa* (LAT) for calculating both single and multimetric macroinvertebrate-based indices, as well as the ecological status classification. In this study, we aimed to: i) identify the aquatic macroinvertebrates that need special attention during index applications given their low abundance; ii) analyse the effect of excluding LAT on single (IBMWP and IASPT) and multimetric (STAR_ICMi) biological indices; and iii) investigate the influence of LAT on river ecological status assessments. To this end, two different river basins in SE Spain and N Italy with contrasting climatic conditions and river types were selected. Our results showed that almost all the *taxa* at the family level can act as low-abundance *taxa*. In particular, the LAT belonged mainly to Diptera, Trichoptera, Coleoptera, Gastropoda and Hemiptera. The IndVal analysis stressed Tabanidae, Cordulegasteridae and Hydroptilidae as the most characteristic low-abundance families in the Spanish data set, while Dryopidae and Athericidae were identified mostly in N Italy. Excluding LAT affected the studied index values and the resulting bioassessment classification, except for the IASPT index. Loss of the entire LAT pool reduced the ecological status for 78% of the samples for the IBMWP index. Changing took place in 41% of the samples when considering the STAR_ICM index. Relevant changes were detected even when considering loss of 50% of the LAT, especially with the IBMWP index. Similar values and patterns were obtained in each considered quality class and river type. Our results provide useful information about controversial *taxa* and stress the significance of LAT in river biomonitoring. Excluding LAT is discouraged, although different responses according to the considered index were detected. The IBMWP index always obtained lower values (coupled mainly with an underestimation of ecological classes), while STAR_ICMi and, especially the IASPT index, were less affected by excluding LAT. Paying special attention to all the protocol application stages is recommended, with emphasis placed when using the IBMWP index.

Key word: Bioassessment; rare *taxa*; ecological classes; IBMWP; IASPT; STAR_ICMi.

INTRODUCTION

River assessment is extensively based on aquatic macroinvertebrates and numerous biomonitoring indices have been developed for this purpose (Doledéc and Statzner, 2010; Birk *et al.*, 2012; Bo *et al.*, 2016). These indices are generally based on rapid biological assessment protocols (Barbour *et al.*, 1999), followed to obtain a practical and rigorous assessment of river ecological conditions. However, macroinvertebrate sampling activities may reflect just one part of the community, and documenting some *taxa* (*e.g.*, with either low abundances or small distribution ranges) is often time-consuming and cost-intensive in terms of fieldwork, laboratory work, data processing and analyses (Nijboer and Schmidt-Kloiber, 2004).

These *taxa* are generally called “rare” (Gaston 1994; Nijboer and Verdonshot, 2004) and their contribution to aquatic biomonitoring has received particular attention in multivariate analyses for bioassessment (Cao *et al.*, 2001; Marchant 2002) and predictive methods, especially in

Anglo-Saxon countries (*e.g.*, the RIVPACS approach; Clarke and Murphy, 2006; Van Sickle *et al.*, 2007). Nevertheless, this debate is ongoing with researchers offering contrasting positions and findings (Cao and Williams, 1999; Marchant *et al.*, 1999; Poos and Jackson, 2012). Very little is known about the effect of rare *taxa* for calculating single or multimetric macroinvertebrate-based indices or about the effect on ecological status assessments, especially in South Europe.

Contrary to *taxa* with small distribution ranges, low-abundance *taxa* (herein referred to as LAT) might be present in numerous samples, sites and habitats, but have the general peculiarity of presenting low number of individuals during sampling activities. Although excluding this kind of *taxa* may be cost-effective (especially in the field) when applying rapid biological assessment protocols, LAT may be common contributors to the assemblage structure in undisturbed streams (Robinson *et al.*, 2000), and might even represent the largest overall richness component (Cao *et al.*, 1998).

The low abundance of a macroinvertebrate *taxon*, or

even its absence, from a sample can be attributed to both structural (*i.e.*, an inhospitable environment that does not allow a species to establish) and stochastic (*i.e.*, incomplete characterisation of a macroinvertebrate community) causes (Gray, 2005). In this study, we focused specially on this second aspect as community characterisation can be strictly related with difficulties during fieldwork or laboratory procedures, such as operator efficiency (Metzeling *et al.*, 2003), sorting errors (Haase *et al.*, 2010), or subsampling methods (Nichols and Norris, 2006). As a result, some methods and procedures may be biased towards large, abundant and widely distributed *taxa* (Gillies *et al.*, 2009; Haase *et al.*, 2010). LAT have fewer chances of being sampled, so their distribution range can be underestimated (Nijboer and Verdonschot, 2004). The imperfect detection of these *taxa* can be a common problem that may affect numerous biological metrics; for instance, total taxon richness and EPT richness, which are two of the main metrics used in biomonitoring (Birk *et al.*, 2012).

In order to improve knowledge on this topic and to provide applied information and recommendations for environmental agencies, technicians and researchers, we aimed to: i) identify the aquatic macroinvertebrates that need special attention during biomonitoring (in different river types) given their potential low abundance; ii) analyse the effect of excluding LAT from the single (IBMWP and IASPT) and multimetric (STAR_ICMi) indices; and iii) test their influence on river ecological status assessments.

When addressing the first objective, we stressed those *taxa* that need special attention while applying protocols by discussing their ecological preferences and features. When dealing with the other two, we investigated whether the exclusion of these *taxa* could be considered crucial for determining not only the index final values, but also ecological classes, to provide useful information about effi-

cient future sampling strategies or line guides. In this way, the comparison of different index outcomes and behaviours is possible and useful as study metrics and indices are among the most widely used tools in river bioassessments (Armitage *et al.*, 1983; Munné and Prat, 2009; Laini *et al.*, 2014; Buss *et al.*, 2015). Finally, controversial issues and possible future implications are discussed.

METHODS

Study area and sampling sites

The present study was carried out by analysing different biogeographical and climatic regions (S Spain and N Italy). We focused on the Segura Basin (SE Spain, Fig. 1a) with 30 sites located in the Murcia, Andalusia and Castilla-la Mancha regions, which were sampled mainly during three seasons in 2000-2003 for 81 sample data (Supplementary Tab. 1). The Segura Basin is an environmentally diverse basin, considered a good candidate to be utilised as a Mediterranean pilot basin (Bruno *et al.*, 2014). Agricultural impacts and dam regulation have been recognised as representing the most important pressures on aquatic ecosystems in this catchment (Kroll *et al.*, 2013; Sánchez-Montoya *et al.*, 2009; Bruno *et al.*, 2014). We considered types, reference values and class boundary values according to those proposed by Sánchez-Montoya *et al.* (2007, 2010) and developed in the Spanish study area. These biological data and types were developed as part of the GUADALMED II Project for Mediterranean Spanish rivers (Prat, 2004). To obtain a representative data set, the sites of four of the five river types in Mediterranean rivers were considered (including temporary and perennial, and different lithologies; Tab. 1).

We also studied the effect of LAT on biomonitoring in the Po Basin (N Italy, Fig. 1b). It is the widest river basin

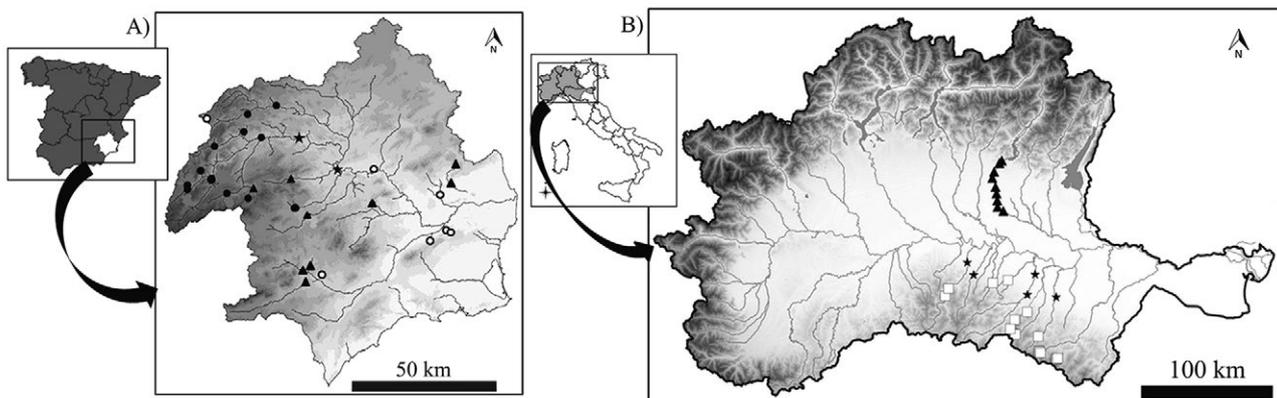


Fig. 1. Study area and location of sampling sites. A) Segura Basin (SE Spain; white circles, T1 sites; black triangles, T2 sites; black circles, T4 sites; black stars, T5 sites). B) Po Basin (N Italy, black triangles, 06GL sites; white squares, 10SS sites; black stars, 06SS sites).

in Italy. We considered 29 representative sites (Lombardia and Emilia-Romagna regions) that were sampled mainly twice (86 sample data; Supplementary Tab. 1). The macroinvertebrate data originate from the fieldwork activities performed from 2009 to 2015. Similar to the Segura Basin, agriculture and flow alterations can be considered the main pressures in the area (Laini *et al.*, 2011; Guareschi *et al.*, 2014). We considered types, reference values and class boundary values according to those published in the Italian ministerial decree DM 260/2010. The data set encompasses three river types from lowland to Apennine streams (Tab. 1, see details on Italian hydro-regions in Buffagni *et al.*, 2008).

According to the Köppen-Geiger climate map of Europe (Peel *et al.*, 2007), the study area in SE Spain is classified mostly as arid (code: BSk), while Italian regions are temperate (code: Cfa).

Macroinvertebrate indices: IBMWP, IASPT and STAR_ICMi

We defined low-abundance *taxa* as all the *taxa* whose abundance was ≤ 3 individuals *per* sample, which corresponds to the first abundance class in the IBMWP index, and as ≤ 3 *per* 0.5 m² for the quantitative STAR_ICMi. Similar criteria have been considered in other studies (Bradley and Ormerod, 2002; Gillies *et al.*, 2009). The response of three different macroinvertebrate-based indices, IBMWP (Iberian Biological Monitoring Working Party), IASPT (IBMWP value/number of families) and STAR_ICMi (Intercalibration Multimetric Index), to LAT exclusion was tested. The first two indices were tested with the Spanish data set, while the Italian data set was used with the last one (see the details below).

The taxonomic resolution needed to calculate these indices is the family level and five ‘Ecological status’ levels

have been established according to European legislation (WFD 2000/60/CE, European Commission 2000) for IBMWP and STAR_ICMi. IBMWP is the most widely used index in Spanish Mediterranean rivers (Alba-Tercedor *et al.*, 2002; Munné and Prat, 2009). It is currently the official index proposed in Spanish rivers (MAGRAMA, 2015) and is an adaptation of the British BMWP scoring system for the Iberian Peninsula, where each family presents a score from 0 to 10 according to their known tolerance to pollution. The IBMWP index is a single metric index (Munné and Prat, 2009) and the sample value is obtained by summing these family scores. This index is considered a simple one (Couto-Mendoza *et al.*, 2015) with a multi-habitat semi-quantitative kick procedure in the field (Jáimez-Cuéllar *et al.*, 2002). IASPT represents a sensitive *taxa* index that is easily calculated for each sample as the IBMWP value divided by the number of scoring families detected. It was calculated only for the Spanish data set as this index is currently recognised as a biomonitoring tool in Spain and is commonly used in this area (*e.g.*, Sánchez-Montoya *et al.*, 2010). Unlike the other indices, STAR_ICMi is a multimetric index and is the official index used in Italy for assessing water course quality statuses according to European legislation (Buffagni *et al.*, 2006, 2008). It requires a quantitative sampling activity referee on a surface (0.5 m² or 1.0 m² depending on the Italian hydro-regions) and has also been used in Europe as an Intercalibration Common Metric Index (Buffagni *et al.*, 2006). It is composed of six metrics: ASPT (Average Score Per Taxon), logarithm of the abundances of the selected families of Ephemeroptera, Plecoptera, Trichoptera and Diptera abundances ($\log(\text{sel_EPTD}+1)$), total number of *taxa*, number of EPT *taxa*, 1 minus the relative abundance of Gastropoda, Oligochaeta and Diptera (1-GOLD) and the Shannon index. After calculating the metrics, they were normalised with the reference community values and weighed (see Buffagni *et al.*, 2006, 2007; and the Italian ministerial decree DM 260/2010 for further details).

Statistical analyses

First of all, the percentage of LAT and the contribution of each taxonomic group (mainly Order) to the total number of LAT were calculated. To refine the analysis and to deal with the first goal, the Indicator Value analysis (Ind-Val) was carried out to select specific rare families per river type (Dufrene and Legendre, 1997; De Cáceres *et al.*, 2010). Such affinity was calculated according to the frequency of each *taxon* in the previously identified groups by taking into account only the sub-communities that composed of LAT. The significance of the Indicator Value (IV) was tested by a Monte Carlo test (999 runs), and the alpha level was set at 0.05.

In order to test the effect of excluding LAT on river biomonitoring indices, they were removed from each

Tab. 1. Code and description for each river type in the study area. The details for each site are available in Supplementary Tab. 1.

Spanish dataset	
Code	Description
T1	Temporary streams
T2	Evaporite calcareous at medium altitude
T4	Calcareous headwaters at medium and high altitude
T5	Large watercourse
Italian dataset	
Code	Description
06GL	Floodplain watercourse originated from lakes
06SS	Floodplain streams
10SS	Northern Apennine streams

sample after considering 100% and 50% of LAT (see details below). Then each index was recalculated and all the samples were reclassified in the corresponding ecological classes. The first case corresponded to excluding the entire pool of LAT (*e.g.*, relevant problems in the sorting or picking phase, operators have little experience). We also tested a more conservative exclusion threshold, which corresponded to a 50% loss of LAT. To do this, the excluded LAT were obtained by considering 100 randomisations of LAT constant loss one by one, and by stopping at the value that equalled a 50% loss of the total LAT pool. At this point, we once again recalculated each index and all the samples were reclassified in the corresponding ecological classes.

The differences between the index values obtained before and after totally excluding LAT were statistically tested by a non-parametric Mann-Whitney test. The results were reported for both the Spanish and the Italian data sets, and even after splitting each data set according to its ecological classes and river type. The behaviour of each index was also studied graphically by focusing on the trend obtained after performing the LAT loss simulations.

To determine whether possible changes in ecological status were equally distributed among the ecological quality classes, we classified the sample data into three classes: High, Good and Less than Good (codes: H, G, <

G). The boundary between Good and Moderate is crucial according to European legislation (WFD 2000/60/CE) because it sets the targets for restoration plans in measuring programmes of water bodies which fail the environmental objectives of achieving a good ecological status. All the statistical analyses were performed with the statistical computing R software (R Development Core Team, 2013) with packages “Vegan”, “ade4” and “indicpecies”.

RESULTS

Low-abundance *taxa* characterisation

Ninety-five macroinvertebrate *taxa* (92 families, plus Hydrachnidia, Ostracoda, Oligochaeta) were found in the Spanish basin (mean \pm SD = 30 \pm 11; SD, standard deviation), with a mean value of 11 *taxa* (\pm 5) as LAT (details in Tab. 2). Ninety-one of the 95 *taxa* were identified as rare at least once. The *taxa* with low abundances belonged mainly to Diptera (20%), Trichoptera and Coleoptera (both 14%) in this area (Fig. 2).

The IndVal analysis stressed just one indicator family in river types T1 and T2 as Baetidae and Tabanidae, respectively. Larger groups of indicator families were obtained for types T4 and T5. Cordulegasteridae and Gyrinidae showed the highest IV for T4, with Hydroptil-

Tab. 2. Mean number (\pm SD) of the low-abundance *taxa* (LAT) for each river type in each data set. The total number of *taxa* (TT) and the total number of LAT are also displayed.

	T1	T2	T4	T5	Total	06GL	10SS	06SS	Total
LAT	8 \pm 5	10 \pm 3	15 \pm 3	10 \pm 5	91	8 \pm 3	10 \pm 4	8 \pm 3	90
TT	73	73	83	55	95	68	66	42	92

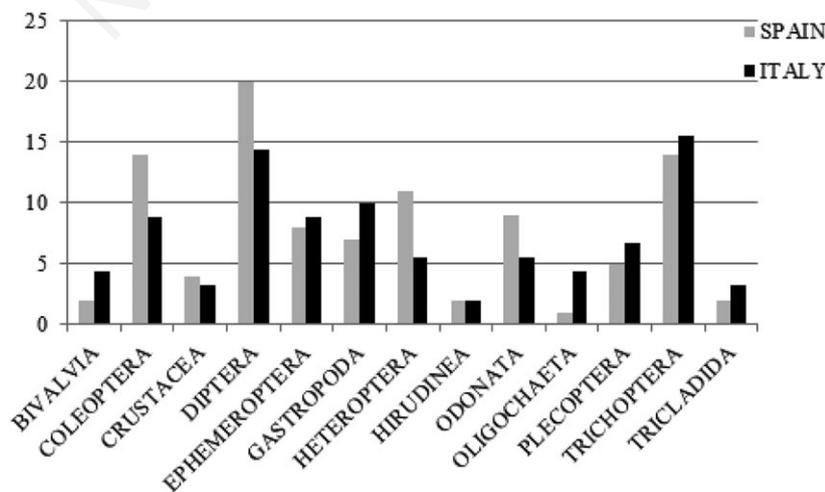


Fig. 2. Distribution of LAT (in %) among the main taxonomic groups (mainly Order). Spanish results are labelled in grey and Italian ones in the darker tone.

idae, Ceratopogonidae and Philopotamidae in the T5 ecosystems (Tab. 3).

Regarding the Italian dataset, 92 *taxa* (91 families, plus Hydrachnidia) were detected (21 ± 5 *per sample*) with a mean value of 9 ± 3 LAT *per sample*. Ninety *taxa* were identified as rare at least once (Tab. 2), where the highest percentage of low-abundance *taxa* belonged to Tri-

choptera (16%), followed by Diptera, Gastropoda and Coleoptera (Fig. 2).

The IndVal analysis showed only two families as being characteristic LAT in the 06GL river type (Corbiculidae and Aphelocheiridae), whereas a larger number of *taxa* were found in the other types (Tab. 4). Dryopidae, Leuctridae and Oligoneuriidae showed the highest IV for

Tab. 3. The IndVal results for the Spanish data set of LAT (Segura Basin) at the family level.

Order	Family	T	IV	P value
Ephemeroptera	Baetidae	T1	0.19	0.050*
Diptera	Tabanidae	T2	0.37	0.005**
Odonata	Cordulegasteridae	T4	0.32	0.005**
Coleoptera	Gyrinidae	T4	0.31	0.020*
Megaloptera	Sialidae	T4	0.29	0.020*
Diptera	Psychodidae	T4	0.26	0.025*
Trichoptera	Polycentropodidae	T4	0.25	0.040*
Odonata	Aeshnidae	T4	0.24	0.035*
Ephemeroptera	Leptophlebiidae	T4	0.24	0.010**
Plecoptera	Perlodidae	T4	0.21	0.040*
Trichoptera	Hydroptilidae	T5	0.42	0.005**
Diptera	Ceratopogonidae	T5	0.34	0.020*
Trichoptera	Philopotamidae	T5	0.32	0.020*
Odonata	Calopterygidae	T5	0.31	0.005**
Gastropoda	Planorbidae	T5	0.31	0.005**
Coleoptera	Dytiscidae	T5	0.29	0.010**
Gastropoda	Ancylidae	T5	0.22	0.050*

T, river type; IV, indicator value; ** $P < 0.01$; * $P < 0.05$.

Tab. 4. The IndVal results for the Italian basin of LAT (Po Basin) at the family level.

Order	Family	T	IV	P value
Bivalvia	Corbiculidae	06GL	0.26	0.035*
Hemiptera	Aphelocheiridae	06GL	0.24	0.050*
Coleoptera	Dryopidae	6SS	0.58	0.005
Plecoptera	Leuctridae	6SS	0.30	0.020*
Ephemeroptera	Oligoneuriidae	6SS	0.29	0.005**
Ephemeroptera	Ephemerellidae	6SS	0.27	0.040*
Trichoptera	Polycentropodidae	6SS	0.25	0.040*
Amphipoda	Gammaridae	6SS	0.20	0.050*
Diptera	Athericidae	10SS	0.40	0.005**
Diptera	Empididae	10SS	0.31	0.035*
Coleoptera	Dytiscidae	10SS	0.27	0.005**
Plecoptera	Perlidae	10SS	0.23	0.040*
Trichoptera	Limnephilidae	10SS	0.23	0.010**
Trichoptera	Sericostomatidae	10SS	0.17	0.040*
Coleoptera	Scirtidae	10SS	0.13	0.025*
Hemiptera	Gerridae	10SS	0.13	0.045*
Trichoptera	Glossosomatidae	10SS	0.11	0.040*

T, river type; IV, indicator value; ** $P < 0.01$; * $P < 0.05$.

floodplain rivers (06SS), while Athericidae and Empididae were identified in Apennine streams (10SS, Tab. 4).

Effect of excluding low-abundance *taxa* on single metric indices: IBMWP and IASPT

Excluding the entire pool of LAT (100%) resulted in significant differences compared with the IBMWP results ($W=3321$; $P<0.0001$). The original IBMWP mean value was 148; on the contrary when the index was recalculated, we obtained a mean value that equalled 93. The ecological quality classification of 29 of the 30 sampling sites changed at least once, and the ecological status of 78% of the samples changed to a minor class (see Tab. 5). Moreover, 82% of the values first classified as High shifted to a minor status (Good). Similar results were obtained (74-75%) when the starting point was Good or Less than Good. Substantial changes were noted for each river type in the analysis (from 33% to 100%), which was especially true for the T2 streams that exhibited huge changes in quality assessment when the starting points were High or Less than Good classes.

The 50% randomised LAT loss also led to relevant changes in the IBMWP values, but the percentage of class changes lowered compared to the total LAT loss (Tab. 5). The ecological status of about half the overall samples (48%) changed (24 of the 30 sites changed at least once). Once again, percentages were similar among different classes (43-53%). When focusing on each river type separately, the patterns were basically the same (compared with the total LAT loss), with changes falling between 33% and 100% of cases depending on ecological class. When LAT were not considered, all the river types presented similar patterns, and their IBMWP values clearly and constantly dropped (Fig. 3).

Unlike the IBMWP results, the IASPT recalculations (when all the LAT were excluded) gave values that did not statistically differ from the original ones ($W=1382$, $P=0.1906$). Graphically, the IASPT behaviour of LAT loss

appeared constant and displayed a horizontal trend, with the only exception being river type T1, where more diversified responses were obtained (Fig. 4).

Effect of excluding low-abundance *taxa* on a multimetric index: STAR_ICMi

The exclusion of all the LAT (100%) led to significant differences when we compared the STAR_ICMi results ($W=3781$; $P<0.0001$). The original STAR_ICMi mean value was 0.74, which became 0.62 when recalculated. The ecological quality classification of 19 of the 29 sampling sites underwent at least one class change. The ecological status of 41% of all the samples changed to a minor class. Similar values and patterns were obtained through different ecological classes (36-41%), except when the starting point was High class (100% change, but a limited number of data were available; Tab. 6).

The 50% randomised loss of LAT brought about changes in the STAR_ICMi values (Tab. 6). The ecological status of 33% of the overall samples changed (17 of 29 sites underwent at least one change). Once again, the percentages were similar among the different classes (23-36%), except when the original starting point was the High class. When focusing on each river type separately, and compared to the total LAT loss, the percentage of class changes lowered, except for river type 06SS (Tab. 6). When we graphically analysed the behaviour of STAR_ICMi, the response patterns were not as clear as they were for the other indices. A decreasing tendency seemed the commonest behaviour in the three studied river types when LAT were excluded (Fig. 5).

DISCUSSION

Low-abundance *taxa*

Despite the climatic and environmental differences in the two study areas, LAT belonged mainly to the same or-

Tab. 5. Number of changes of ecological quality classes for the 100% or 50% LAT loss. The results are displayed after considering the overall number of samples (Total), divided by river type (T1, T2, T4, T5) and ecological class (the classification represents the starting point class before excluding LAT) in the Spanish data set. Number of sampling data *per* river type is also displayed.

	Spain (Segura Basin)									
	Total		T1 (n=16)		T2 (n=25)		T4 (n=34)		T5 (n=6)	
	100%	50%	100%	50%	100%	50%	100%	50%	100%	50%
Changes	78%	48%	12 (75%)	9 (56%)	21 (84%)	13 (52%)	27 (79%)	16 (47%)	3 (50%)	1 (17%)
No changes	22%	52%	4 (25%)	7 (44%)	4 (16%)	12 (48%)	7 (21%)	18 (53%)	3 (50%)	5 (83%)
Classes										
High	82%	53%	2 (67%)	2 (67%)	5 (100%)	5 (100%)	22 (81%)	12 (44%)	2 (67%)	1 (33%)
Good	74%	43%	7 (88%)	5 (63%)	13 (76%)	6 (35%)	5 (71%)	4 (57%)	1 (33%)	0
<Good	75%	50%	3 (60%)	2 (40%)	3 (100%)	2 (67%)	-	-	-	-

ders in both data sets (*e.g.*, Diptera, Trichoptera and Coleoptera) and were basically those stressed by Nijboer and Schmidt-Kloiber (2004) for Dutch streams. When focusing on the Spanish data set, and according to the crite-

riion of $IV > 25$ as a key value to judge an indicator *taxon* as being adequate (Bonada *et al.*, 2008, following Dufrière and Legendre, 1997), Baetidae was not a good indicator for the T1 streams. On the contrary in the T2 streams, Ta-

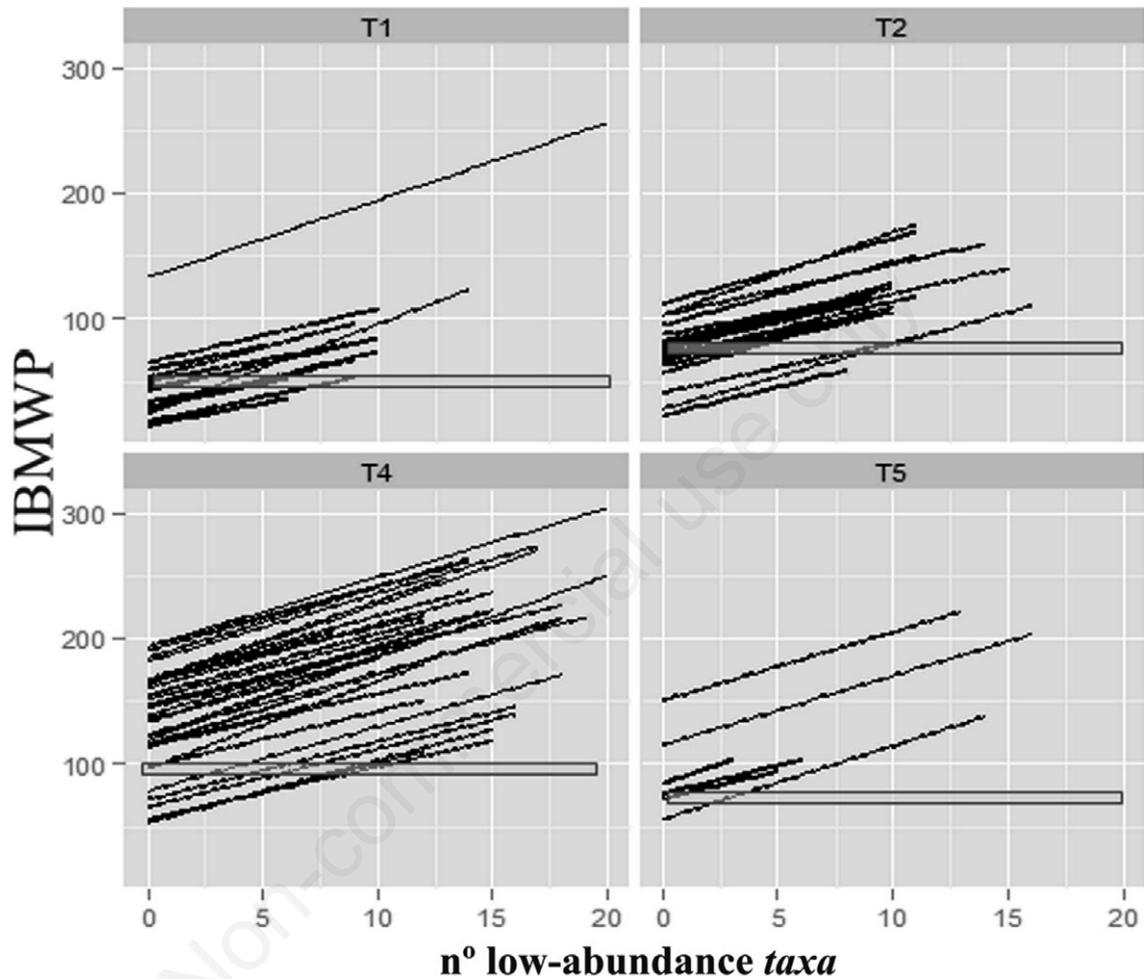


Fig. 3. IBMWP index behaviour per river type (T1, T2, T4, T5) after randomisations of constant LAT loss. The boundary value between the Good and Moderate conditions is displayed. The index values are displayed on the y-axis and the number of considered LAT is found on the x-axis.

Tab. 6. Number of changes of ecological quality classes for the 100% or 50% LAT loss. The results are displayed after considering the overall number of samples (Total), divided by river type (06GL, 10SS, 06SS) and ecological class (the classification represents the starting point class before excluding LAT) in the Italian data set. Number of sampling data *per* river type is also displayed.

	Total	Total	Italy (Po Basin)							
			100%	50%	06GL (n=55)		10SS (n=24)		06SS (n=7)	
					100%	50%	100%	50%	100%	50%
Changes	41%	33%	21 (38%)	17 (31%)	10 (42%)	7 (29%)	4 (57%)	4 (57%)		
No changes	59%	67%	34 (62%)	38 (69%)	14 (58%)	17 (71%)	3 (43%)	3 (43%)		
Classes										
High	100%	100%	-	-	-	-	3 (100%)	3 (100%)		
Good	41%	36%	9 (38%)	8 (33%)	8 (50%)	7 (44%)	1 (25%)	1 (25%)		
<Good	36%	23%	12 (39%)	9 (29%)	2 (25%)	0 (0%)	-	-		

banidae needed special attention during sample and sorting activities, as did a large group of *taxa* dominated by Cordulegasteridae and Hydroptilidae for the T4 and T5 streams, respectively. Cordulegasteridae larvae (genus *Cordulegaster*), Tabanidae and Athericidae (see below) are generally predators of other aquatic invertebrates (Tachet *et al.*, 2010). Members of these feeding groups have already been stressed as being relatively less abundant than prey species (Spencer, 2000). Hydroptilidae larvae (Trichoptera) usually prefer specific microhabitats with submerged vegetation where they anchor their cocoons which, coupled with their limited size and lack of movements (under dry and wet conditions) (Tachet *et al.*, 2010), may make them particularly difficult to detect.

None of the *taxa* stressed by the analysis was characterised by extreme IBMWP scores, and higher values were depicted by some Odonata (*e.g.*, Cordulegasteridae) or Trichoptera (*e.g.*, Philopotamidae). Most of the indicator *taxa*

presented intermediate values, basically with scores of around 3, 4, or even 6 points, as confirmed by the IASPT response to LAT loss (Fig. 4). The mean *taxa* scores did not change, but stabilised with values between 4 and 6. In this situation, LAT presented a comparable IBMWP score with the commonest *taxa*. These results (Fig. 4) agree with the research of Nijboer and Schmidt-Kloiber (2004), who found that the mean scores for saprobic valences were similar for both *taxa* types (low and high abundances). If LAT had been *taxa* with an extreme IBMWP score (1 or 10), the IASPT responses to their loss would have shifted towards a marked change in their slopes.

According to the Italian results, it was difficult to suggest specific LAT for the 06GL rivers if we considered that the IndVal stressed *taxa* with IV came close to 25. On the contrary, Dryopidae (Coleoptera) and Athericidae (Diptera) were underlined as characteristic LAT in 06SS and 10SS, respectively. Special attention needs to be paid

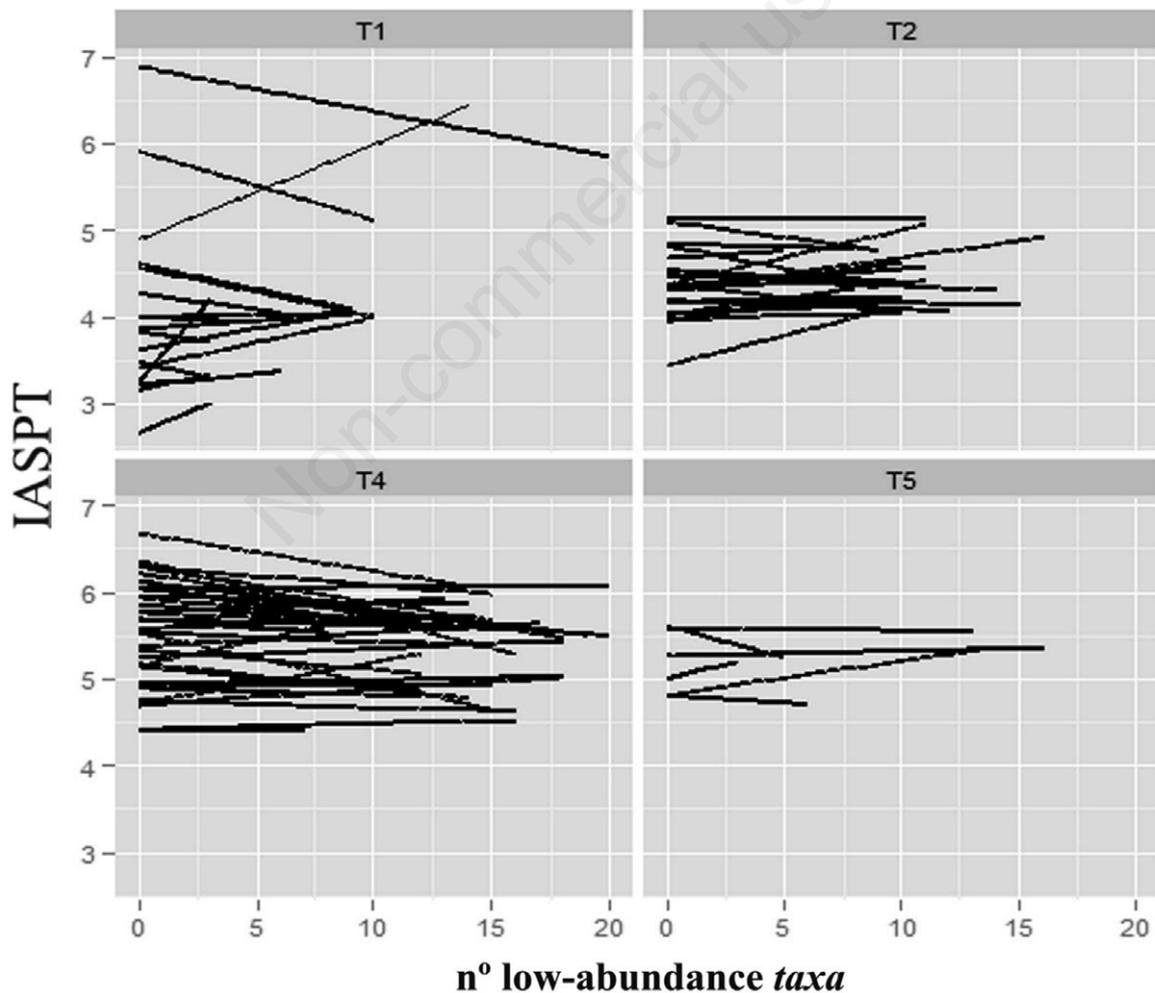


Fig. 4. IASPT index behaviour per river type (T1, T2, T4, T5) after randomisations of constant LAT loss. The index values are displayed on the y-axis and the number of considered LAT is found on the x-axis.

to these *taxa* in all the sampling phases and for all laboratory activities. Dryopidae larvae are generally semi-aquatic or riparian (Jäch and Balke, 2008) and are, therefore, rarer to detect during sampling activity as only adults are strictly aquatic. Furthermore, Dryopidae have already been stressed as infrequent in low mountainous streams in other European countries (Slovakia: Zatovičová *et al.*, 2004). Regarding specific LAT features, other studies have generally found that these *taxa* are associated with standing or slowly flowing waters (Nijboer and Schmidt-Kloiber, 2004).

Our results in both data sets only partially confirmed this finding by considering that a heterogeneous group of LAT was found. This group was composed of some lotic preference *taxa*, but also some highly diverse *taxa* (Coleoptera, Diptera), and even some strictly lentic ones (Odonata, Gastropoda, and Hemiptera). However, benthic

sampling methodologies like those utilised (D-net and Surber, following official legislation) could also be the reason for the low abundance found in some groups (*e.g.*, swimmers or surface skaters). Most of these *taxa* (*e.g.*, Coleoptera or Hemiptera) live on the water surface or among vegetation, and can easily escape sampling nets. Nevertheless, the LAT identified herein cannot be considered rare or endemic in terms of small range areas, especially given taxonomic resolution (family level).

Performance of indices and implication for bioassessment

Excluding LAT resulted in underestimating ecological status (values lower than reality) for STAR_ICM and IBMWP indices. This effect became considerably stronger after applying the IBMWP index, with relevant

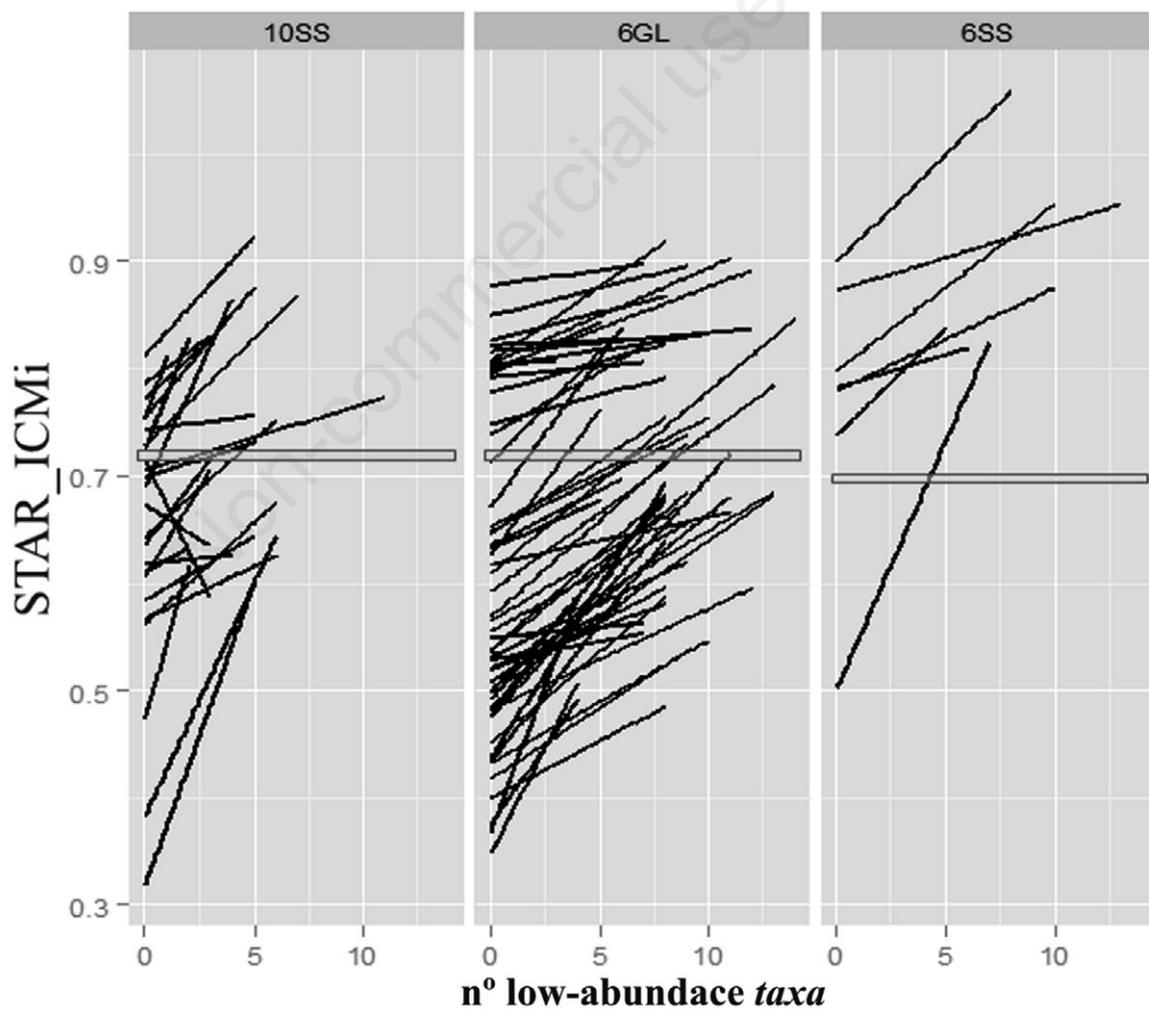


Fig. 5. STAR_ICM index behaviour per river type (10SS, 06GL, 06SS) after randomisations of constant LAT loss. The boundary value between the Good and Moderate conditions is displayed. The index values are displayed on the y-axis and the number of considered LAT is found on the x-axis.

changes in the ecological evaluations. This is strictly related with the index formula, which exclusively represents a sum of family scores and is strongly affected by a reduction in the total recognised taxa.

Different outcomes were detected when focusing on the IASPT index. This index did not appear sensitive when LAT were excluded from three of the four Mediterranean river types, and displayed a contrasting response in temporary rivers (T1). Applying biomonitoring procedures to temporary aquatic ecosystems is currently one of the main bioassessment challenges and requires specific tools (Nikolaidis *et al.*, 2013; Datry *et al.*, 2014; Prat *et al.*, 2014). The general observed lack of sensitivity of this index can be considered an interesting attribute (*i.e.*, no specific knowledge or experience required), but this approach may be questionable, or even dangerous (Metzeling *et al.*, 2003; Haase *et al.*, 2006). We should also consider that the IASPT index has been reported to be less sensitive to stressors than the IBMWP index in Mediterranean rivers (Sánchez-Montoya *et al.*, 2010).

The Italian index STAR_ICM gave an intermediate response compared to the other indices. Following LAT loss, the results were generally underestimated, but more slightly than for the IBMWP index. Once again, this is probably related with the index formula. As it was a multimetric index, and not just a mathematic sum, it could be less affected by the reduction in the total taxa considered herein. Richness metrics formed part of the index (*e.g.*, total of families, total EPT *taxa*), but the metrics with the heaviest weight in the definition of the index was the Average Score *Per Taxon* (ASPT) value. Knowing the specific behaviour of the Spanish ASPT (investigated herein) could help explain the attenuated STAR tendencies.

Unlike our results, Nijboer and Schmidt-Kloiber (2004) reported that excluding LAT overestimated the ecological quality class in Dutch lowland streams with the AQEM software (Hering *et al.*, 2004). These contrasting findings, which are especially strong between IBMWP and AQEM, may be due to the different geographic contexts and river types considered (*e.g.*, Northern vs Southern Europe), but could also be due to specific differences between protocols (*e.g.*, taxonomic resolution, sampling area). Despite the AQEM method and STAR_ICMi presenting similar procedures (Hering *et al.*, 2006), contrasting results were obtained as LAT exclusion did not generally lead to overestimates in the Italian data set. Nevertheless, in both cases (underestimation and overestimation), these mismatches could have serious consequences for environmental agencies and water managers during environmental management (*e.g.*, restoration where it is not necessary), which can provide equivocal pictures of river ecosystem health.

CONCLUSIONS

Our study is one of the first attempts to test the effects of excluding LAT in several widely used European indices. Previous studies have tested macroinvertebrates at the genus or species level (Cao *et al.*, 1998; Resh *et al.*, 2005), and have mainly examined the effects of LAT on richness metrics. Our results demonstrated that even at the family level and in two different study areas, LAT represent a large part of overall richness. Their exclusion strongly lowered the number of *taxa* in the samples regardless river typology, with effects on bioassessment depending on the index employed. In this context, the implementation of quality control mechanisms into macroinvertebrate assessment procedures seems recommendable.

Finally, by considering the increasing impacts and challenges of alien species on aquatic ecosystems (Havel *et al.*, 2015; Fenoglio *et al.*, 2016), alien *taxa* must be specially considered because they can be generally characterised by initial low abundances in their first invasion phases. Indeed, in our study, Corbiculidae (*e.g.*, *Corbicula fluminalis*) are reported as LAT in some Italian rivers. This fact may lead to some mismatches or variations in the final index scores, and similar problems have already been stressed in other European countries (Gabriel *et al.*, 2005). Specific reflexions and future adjustments to studied systems in both countries should be made (*e.g.*, definition of specific tolerance classes or periodically reviewing the *taxa* list by considering possible taxonomic modifications and future invaders) to improve ecological assessment tools in freshwater ecosystems.

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