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Assessing potential surrogates of macroinvertebrate diversity in North-African Mediterranean aquatic ecosystems

Noura Slimani a,b ,□ , David Sánchez-Fernández c,d , Eric Guilbert a, Moncef Boumaïza b, Simone Guareschi d, Jean Thioulouse e

a UMR7179 CNRS/MNHN, Muséum National d'Histoire Naturelle, CP 50, 57 rue Cuvier, 75005 Paris, France

b Laboratory of Environment Biomonitoring (LEB), Faculty of Sciences of Bizerte, Zarzouna, University of Carthage, Tunisia

c Instituto de Ciencias Ambientales, Universidad de Castilla-La Mancha, Campus Tecnológico de la Fábrica de Armas, 45071 Toledo, Spain

d Department of Ecology and Hydrology, Regional Campus of International Importance "Campus Mare Nostrum" University of Murcia, Espinardo Campus, 30100 Murcia, Spain

e Université de Lyon, 69000 Lyon, Université Lyon 1, CNRS, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, 69622 Villeurbanne Cedex, France

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Corresponding author at: UMR7179 CNRS/MNHN, Muséum National d'Histoire Naturelle, CP 50, 57 rue Cuvier, 75005 Paris, France.

E-mail address: [noura.slimani@edu.mnhn.fr](mailto:noura.slimani@edu.mnhn.fr) (N. Slimani).

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#### ABSTRACT

The need to use surrogates of biodiversity is quite relevant in threatened habitats harboring high values of biodiversity, such as the Mediterranean aquatic ecosystems. In this study, we assess the performance of eight macroinvertebrate groups (Coleoptera, Heteroptera, Odonata, Trichoptera, Plecoptera, Ephemeroptera, Crustacea, and Mollusca) as surrogates of the whole aquatic macroinvertebrate assemblage in 49 localities from Northern Africa (Tunisia). Specifically, we aimed to test i) the congruence of the patterns of species richness and composition among these eight groups (at species level) in order to propose which groups could be accurate as indicators of diversity of the whole community, and ii) if higher-taxon levels (genera or families) are suitable for predicting overall species richness and composition in these ecosystems. In total, we found 72 families, 157 genera and 280 species. Our results show a high congruence between the patterns of species richness and

composition of Ephemeroptera, Coleoptera (even at higher taxonomic levels, especially genus) and the whole community. Thus, we recommend the use of these two groups as surrogates of macroinvertebrate diversity in inland aquatic ecosystem in the study area. They can be used for both i) the rapid and inexpensive monitoring of biodiversity in aquatic ecosystems and ii) conservational studies in order to identify areas with the highest values of freshwater biodiversity in Mediterranean areas. Finally, high values of congruence among taxonomic levels were found suggesting that, in general, higher taxa can be used as biodiversity surrogates for cost-effective practical survey in Mediterranean aquatic ecosystems from Northern Africa.

## 1. Introduction

One of the main concerns for conservation biologists is trying to reduce the high rates of biodiversity loss due to human pressures (Kerr and Currie, 1995). However, most of the species on Earth have not been described so far and major gaps exist in knowledge about their distributions (Brown and Lomolino, 1998). These taxonomic and biogeographical gaps, known as Linnean and Wallacean shortfalls respectively (Lomolino, 2004), place serious limitations on the ability to conserve biodiversity in the face of the ongoing extinction crisis (Hortal et al., 2007). Conservation biologists and environmental managers are indeed striving to find suitable surrogates for mapping and predicting biodiversity as an effective way to overcome this limitations (Humphries et al., 1995; Caro and O'Doherty, 1999), especially in those countries with high biodiversity levels and scarce naturalist tradition. Biodiversity surrogates are groups of organisms with a sound taxonomy that have been well surveyed in a region, and whose patterns of assemblage structure (species richness, endemism, rarity, etc..) or composition, (see Corte et al., 2017) are assumed to be indicative of similar patterns of unsurveyed taxa in the same region (Pearson, 1994). However, the validity of this assumption is rarely evaluated. In this context, researchers have traditionally used plants and/or vertebrates, especially birds, whereas arthropods have received less attention in this kind of conservation studies (Posadas et al., 2001; Cardoso et al., 2011), despite the fact that they represent around 95% of all known animal species (Barnes et al., 2001; Wilson, 2017). An interesting approach to include invertebrate species in the biodiversity assessments could be to consider the number of higher taxonomic groups as a surrogate of the number of local species within the same clade (or other taxonomic groups). The advantage of this approach is that the number of families or genera can be documented more rapidly than the number of species of hyperdiverse groups like invertebrates (Williams and Gaston, 1994; Caro and O'Doherty, 1999; Baldi, 2003; Villaseñor et al., 2004). The need to use surrogates of biodiversity to urgently identify areas of high biodiversity is especially relevant in aquatic ecosystem. These environments are highly diverse, since they constitute only the 0.01% of the world's water, equivalent to only 0.8% of the Earth's surface area, and support at least 100,000 species, i.e., approximately 6% of the estimated 1.8 million described species (Dudgeon et al., 2006; Balian et al., 2008; Heino et al., 2009). Besides, freshwater ecosystems, especially those located in the Mediterranean basin, are subjected to a high

human pressure (e.g., overexploitation; water pollution; flow modification; degradation of habitat; and invasion by exotic species: Dudgeon et al., 2006; Janssen et al., 2015), and climate change (Heino et al., 2009).

A number of studies have focused on the assessment of different taxonomic groups as biodiversity surrogates. Some of them have criticized the use of flagship species and other surrogate concepts in conservation, especially when the chosen areas are not consistently associated with high local biodiversity over space and time (Roberge and Angelstam, 2004). In an exhaustive review on freshwater ecosystems, Heino (2010) found that indicator groups and, more generally, cross-taxon congruence do not appear to be particularly relevant for conservation in the freshwater realm. Guareschi et al. (2015) found a limited concordance between assemblage patterns of macroinvertebrates and water birds in Iberian wetlands. However, other studies point out to high congruence among groups, suggesting that water beetles in Mediterranean semi-arid regions (Sánchez-Fernández et al., 2006) or snails in wetlands of Northeastern China (Guan et al., 2018) could be useful as effective indicators of freshwater biodiversity. In this study, we assess for the first time in Africa the performance of eight macroinvertebrate groups as potential surrogates of aquatic (macroinvertebrate) biodiversity. Specifically, we aimed to test i) the congruence of the patterns of species richness and composition among these eight groups (at species level) in order to identify which groups can be used more accurately as biodiversity indicators in inland aquatic ecosystem in Northern Tunisia, and ii) if higher-taxon richness are suitable for predicting overall species richness and composition in these ecosystems.

## 2. Methods

### 2.1. Study area

The study was performed in northern Tunisia (Fig. 1), a Mediterranean region located in the Maghreb (North Africa), encompassing four watersheds (Medjerda; Northwestern; Ichkeul organized within two large sub-basins: Sejnane and Joumine; and northeastern watershed. Tunisia is located in a contact zone marking the transition from the temperate humid Mediterranean climate to the dry Saharan climate (Zielhofer and Faust, 2008). The northern area of Tunisia is characterized by a typical Mediterranean climate, ranging from humid (Mogods-Kroumirie region) to sub-humid (Bizerte region). A total of forty-nine non-impacted sites were selected to include a quantitative representation of the main aquatic habitats (see Arrignon, 1976), in the study area (Fig. 1 and Table S1 in Supplementary Material): lotic freshwater (20 sites), lentic freshwater (7 sites), lotic saline (17 sites) and lentic saline waters (5 sites).

### 3. Biological data

For each one of the 49 selected sampling sites, faunistic compositions of eight taxonomic groups of macroinvertebrates (Crustacea, Mollusca and 6 groups of insects: Coleoptera, Heteroptera, Odonata, Trichoptera, Plecoptera, Ephemeroptera) were obtained from standardized fieldwork. All localities were sampled in 2013, conducting the same sampling effort in each one of them. In each site, macroinvertebrates were sampled using both Surber nets (300  $\mu$ m) and kick

net (filet Troubleau) during twenty-five minutes across the entire habitat heterogeneity. Samples were preserved in 70% ethanol and taken to the laboratory for identification at species level. The applied methodology is common in studies on freshwater macroinvertebrates (e.g., Picazo et al., 2012).

#### 4. Statistical analysis

##### 4.1. Richness patterns

Spearman rank correlations were used to evaluate the relationship among the species richness patterns of the different groups of macroinvertebrates and the Total Species Richness (TSR). To avoid giving higher weight in the correlation of the groups with a greater number of species, for each taxonomic group we also calculated the Remaining Richness value (RR), defined as the total number of species at a site (of all eight groups considered) minus the number of species belonging to the considered indicator group. Finally, we also conducted Spearman correlations to explore whether the higher taxon richness (family, genus) is correlated with the species richness (and with the RR values).

##### 4.2. Community composition patterns

We used non-metric multidimensional scaling (NMDS, 'metaMDS' function in vegan) to summarize composition patterns in the aquatic macroinvertebrates dataset. We repeated the NMDS using different taxonomic levels for the entire community (family, genus and species) and within each one of the eight invertebrate groups. The analyses were performed on a biological matrix based on presence-absence data and using the Jaccard index. As some taxonomic groups do not occur in all

Fig. 1. Study area. Red dots indicate sampling localities in North Tunisia.

sites, a dummy taxon was added (when necessary), to allow the comparison among groups. A Procrustean analysis was applied to evaluate the degree and significance of community concordance among the NMDS ordinations of the different taxonomic levels and different taxonomic groups. Procrustean rotation analysis is regarded as a robust method for concordance analysis (Peres-Neto and Jackson, 2001) being frequently used to study aquatic communities (e.g., Virtanen et al., 2009; Guareschi et al., 2015; Valente-Neto et al., 2018). Three dimensional NMDS ordinations were compared with the function 'protest' (vegan package) where the Procrustean rotation analysis was accompanied by a permutation test ( $n = 9999$ ). The statistic obtained is a Procrustes correlation  $r$  derived from the symmetric Procrustes residual  $m^2(r = \sqrt{1 - m^2})$ . Congruence analyses were performed in three parts: i) comparing each order at family-genus and species level with the entire community at species levels; ii) comparing inside each order the concordance among its different taxonomic levels (only where the Order is present); and iii) using the entire community and testing the concordance among taxonomic levels. With the first two parts we aim to understand which group may be surrogates of others composition patterns, while the last step provides useful information about taxonomic resolution useful in aquatic biodiversity assessment. All statistical analyses were performed with R software (R Core Team, 2014), using ade4 and vegan package (<http://pbil.univ-lyon1.fr/ade4/>, Thioulouse and Dray, 2007; Oksanen, 2011).

#### 5. Results

## 5.1. Richness patterns

A total of 72 families, 157 genera and 280 species were found in the 49 localities studied (Table 1). The group with highest richness was Coleoptera (117 species) followed by Mollusca (40) and Heteroptera (36). Coleoptera and Heteroptera were the most widespread groups in the study area, being present in all sampling sites (Table 2). Ephemeroptera, Mollusca, Odonata and Crustacea were absent from one type of habitat (lentic or lotic saline waters). Trichoptera species were found in 10 sites, including two types of habitat (both lotic and lentic freshwaters), while Plecoptera species were confined to freshwater lotic ecosystems appearing only in two sites (Table 2).

The richness patterns of all groups except Plecoptera were significantly correlated with the pattern of total richness (TSR) with Coleoptera showing the highest values, followed by Ephemeroptera (Table 3). In the same way, the species richness patterns of all groups but Plecoptera and Trichoptera were significantly correlated with their respective RR values, with Ephemeroptera showing the highest values (Table 3).

Among groups, the strongest correlation was found between Ephemeroptera and Mollusca, followed by Mollusca and Odonata (Table 3). The groups with highest number of significant correlations were Ephemeroptera (with all groups) and Trichoptera (with 4). However, Plecoptera were just correlated with one group, and Coleoptera with two groups ( $p < 0.05$ ; see Table 3). On the other hand, the richness of families and genera considering the whole community were significantly correlated with TSR ( $r = 0.845$  and  $0.977$ ;  $p < 0.001$ , respectively). In the same way, the richness of families and genera of any taxonomic group, were significantly correlated with TSR, their respective species richness and RR values, showing in general high Spearman correlation coefficients, especially with their respective species richness values (see Table 4).

## 5.2. Community composition: patterns and congruence

The ordination space of the first three axes of the NMDS including all groups presented a final stress value of 0.15 (for both family, genus and species level). Focusing on each order separately, Heteroptera and Coleoptera families showed the highest levels of concordance with the entire macroinvertebrate community at species level ( $r = 0.55$ ;  $p < 0.001$ ; Table 5). On the other hand, Plecoptera showed the lowest level of concordance with no significant p-value. In the case of Coleoptera and Heteroptera the same results were always obtained with or without using dummy taxa. At genus level Coleoptera and Heteroptera presented the highest level of concordance with the entire community at species level ( $r = 0.69$  and  $r = 0.61$  respectively) while Coleoptera and Ephemeroptera obtained the best concordance at species level ( $r = 0.88$  and  $r = 0.56$ , respectively; see Table 5). Focusing inside each order, the analyses were performed using only the sites where the order appears and it was not possible to proceed with Plecoptera data (just two sites with presence). In these cases Crustacea at family level presented the highest community concordance with their respective species composition ( $r = 0.90$ ;  $p < 0,001$ ) followed by Heteroptera ( $r = 0.80$ ;  $p < 0,001$ ; Table 5). Overall, every order at genus level acts like a good surrogate of its community composition at species levels (minor value were obtained for Mollusca  $r = 0.67$ ). Finally, according

to protest analysis, the community variation was, in all cases, significantly concordant across different taxonomic levels. However, the concordance between genus and species community assemblages was higher ( $r = 0.95$ ;  $p < 0.001$ ) than the concordance between family and species community ( $r = 0.69$ ;  $p < 0.001$ ).

## 6. Discussion

This is the first study addressing the potential use of biodiversity surrogates in North-African inland aquatic ecosystems. One of the main strengths of this study is the wide taxonomic spectrum considered. Unlike other studies (e.g. Heino et al., 2003; Bilton et al., 2006; Guareschi et al., 2015), we consider here the main macroinvertebrate groups (excepting Diptera) in Mediterranean freshwater ecosystems, both in terms of species richness and abundance (see Tierno de Figueroa et al., 2013).

The high correlation values found between the species richness of the whole community and the species richness of most of the considered taxonomic groups, especially Coleoptera ( $r = 0.847$ ) and Ephemeroptera ( $r = 0.730$ ) suggest that at least these groups can be used as effective surrogates of the whole macroinvertebrate community, being congruent with the results found in Sánchez-Fernández et al. (2006) and Bilton et al. (2006). Coleoptera and, even if in a less way Ephemeroptera, have been already stressed as surrogate of aquatic macroinvertebrate richness in freshwater ecosystems belonging to the Spanish National Parks (Guareschi et al., 2012).

Nevertheless, examining the correlation between species richness patterns is only one of the possible ways to evaluate biodiversity indicators (Kati et al., 2004), and these results only partially match with those obtained with the community composition assessment. Previous studies suggest that strong concordance between multiple organism groups should be indicated by  $r$ -values  $> 0.7$  (e.g., Heino, 2010). In this case, just Coleoptera at species level showed a  $r$ -value higher than 0.7, being Coleoptera, Heteroptera and Ephemeroptera (at any

Table 1

Number of families, genera and species of the eight taxonomic groups recorded in the study area.

...

Families Genera Species

...

...

Coleoptera (Col)	12	51	117
Heteroptera (Het)	13	19	36
Ephemeroptera (Eph)	8	19	24
Plecoptera (Ple)	6	10	16
Odonata (Odo)	7	14	19
Trichoptera (Tri)	6	9	18
Mollusca (Mol)	13	27	40
Crustacea (Cru)	7	8	10
Total	72	157	280

...

taxonomic resolution) the taxonomic groups whose community composition showed the highest levels of concordance with the entire community at species level.

Thus, it seems that Coleoptera at species level stands out in both

richness and (even if in less minor) composition assessments. The use of Coleoptera as surrogate shows some important advantages, as it encompasses the gradient of environmental heterogeneity in semi-arid Mediterranean regions, i.e., they are present in all localities irrespective of the type of habitat, showing similar results in each type of habitat separately (see Table S1 in Supplementary Material). However, the correlation values when excluding beetles species from the complete pool (RR), although significant, falls from 0.847 to 0.399 (Table 3), suggesting that the high species richness of this group is biasing the obtained results with the whole community. In the same way, the results on congruence of community composition were conducted only considering the whole community and not separately with RR due to methodological problems to compare values when some species groups are missing. Besides, excepting Ephemeroptera, the congruence among richness patterns of the different groups was weak in most of the cases. This result was similar to those found by Heino et al. (2003) across 110 headwater streams in Finland. Patterns of Coleoptera species richness just correlated significantly with patterns of species richness of two groups (Heteroptera and Ephemeroptera). These issues call in question in some degree the performance of water beetles as unique surrogate of biodiversity in Mediterranean freshwater ecosystems (see Heino, 2010), and suggest its use in combination with others like Ephemeroptera and Heteroptera as a combined effective strategy. On the other hand, the correlation values between family and genus levels with TSR were high, suggesting that, in general, the higher taxa (both genera and families) could be used as biodiversity surrogates for cost-effective practical survey. Besides, we observed that assemblage composition patterns among taxonomic levels are concordant and significant, with relevant values of significance (minor value of  $r = 0.69$ ). In this context, the community composition even at family level presented a significant level of concordance with the community composition at species level. The  $r$ -values obtained between groups at family level and the whole community at species level (0.23-0.55) may be considered quite similar to those obtained by Dolph et al. (2011) studying concordance between fish and macroinvertebrate

Table 2

Number of sites with the presence of each group in the different types of habitat.

...

Lotic-freshwater

(n = 20)

...

...

Lentic-freshwater

(n = 7)

...

...

Lotic-saline

(n = 17)

...

...

Lentic-saline

(n = 5)

...

...

All sites



```

...
...
Coleoptera 20 17 7 5 49
Heteroptera 20 17 7 5 49
Ephemeroptera 20 15 7 0 42
Plecoptera 2 0 0 0 2
Odonata 17 7 0 1 25
Trichoptera 8 2 0 0 10
Mollusca 15 1 5 0 21
Crustacea 12 5 0 1 18
...

```

Table 3

Results of pairwise Spearman correlation coefficients for the species richness of the eight groups of taxa studied (49 sampling sites). TSR: Total Species Richness; RR: Remaining Richness. See codes of the groups in Table 2. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

```

...
Col Het Eph Ple Odo Tri Mol Cru TSR RR
...
...
Col - 0.352* 0.402** 0.030 0.229 0.031 0.159 0.251 0.847*** 0.399**
Het - 0.464*** 0.319* 0.083 0.160 0.119 0.255 0.539*** 0.407**
Eph - 0.316* 0.400** 0.493*** 0.525*** 0.317* 0.730*** 0.610***
Ple - 0.150 0.471*** 0.247 0.062 0.286 0.
Odo - 0.369** 0.519*** 0.207 0.477*** 0.394**
Tri - 0.487*** 0.166 0.344* 0.
Mol - 0.204 0.524*** 0.365**
Cru - 0.366** 0.297*
...

```

Table 4

Spearman correlation coefficient within each group at different taxonomic levels (families, genera and species), with the Total Species Richness (TSR) and the remaining richness of each group (RR) (49 sampling sites). \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

```

...
S_Col TSR RR_Col
...
...
F_Col 0.842*** 0.733*** 0.368**
G_Col 0.984*** 0.793*** 0.334*
...
...
S_Het TSR RR_Het
...
...
F_Het 0.763*** 0.393** 0.302*
G_Het 0.926*** 0.481*** 0.365**
...
...
S_Eph TSR RR_Eph
...
...
F_Eph 0.892*** 0.550*** 0.430**

```

```

G_Eph 0.990*** 0.705*** 0.584***
\,\,
...
S_Ple TSR RR_Ple
\,\,
...
F_Ple 1.000*** 0.286* 0.
G_Ple 1.000*** 0.286* 0.
\,\,
...
S_Odo TSR RR_Odo
\,\,
...
F_Odo 0.986*** 0.478*** 0.399**
G_Odo 0.999*** 0.474*** 0.392**
\,\,
...
S_Tri TSR RR_Tri
\,\,
...
F_Tri 0.993*** 0.340* 0.
G_Tri 0.996*** 0.348* 0.282*
\,\,
...
S_Mol TSR RR_Mol
\,\,
...
F_Mol 0.986*** 0.521*** 0.372**
G_Mol 0.994*** 0.534*** 0.378**
\,\,
...
S_Cru TSR RR_Cru
\,\,
...
F_Cru 1.000*** 0.366** 0.297*
G_Cru 1.000*** 0.366** 0.297*
\,\,

```

Table 5

Concordance among assemblages of each group at different taxonomic levels and A) the whole dataset at species level and; B) the species of each specific

taxonomic group (only in sites where species of each group were detected).

Concordance based on Procrustes. It is shown the Procrustean correlation (r) and

the associated m<sup>2</sup> statistic. Significances of the Procrustean statistic were cal-

culated from randomisation tests (9999 permutations). F = family level;

G = genus level; S = species level. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

AB

\,\,

m<sup>2</sup> rm<sup>2</sup> r

\,\,

\,\,

F\_Col 0.700 0.547\*\*\* 0.731 0.518\*\*\*

G\_Col 0.531 0.685\*\*\* 0.442 0.747\*\*\*

S\_Col 0.222 0.882\*\*\* --

F_Het	0.693	0.554***	0.362	0.799***
G_Het	0.627	0.611***	0.235	0.874***
S_Het	0.746	0.504***	--	
F_Eph	0.755	0.495***	0.732	0.517***
G_Eph	0.715	0.534***	0.375	0.791***
S_Eph	0.683	0.564***	--	
F_Ple	0.949	0.227	NA	NA
G_Ple	0.923	0.277	NA	NA
S_Ple	0.952	0.220	--	
F_Odo	0.795	0.452***	0.672	0.573***
G_Odo	0.836	0.405***	0.177	0.907***
S_Odo	0.841	0.399***	--	
F_Tri	0.851	0.386***	0.691	0.
G_Tri	0.887	0.336**	0.446	0.744**
S_Tri	0.859	0.375**	--	
F_Mol	0.790	0.458***	0.675	0.569***
G_Mol	0.772	0.477***	0.555	0.667***
S_Mol	0.777	0.472***	--	
F_Cru	0.902	0.314*	0.194	0.898***
G_Cru	0.900	0.317*	0.183	0.904***
S_Cru	0.893	0.327**	--	

communities in North American rivers and Guareschi et al. (2015) studying concordance among waterbirds and macroinvertebrates in Mediterranean wetlands. However, the highest level obtained between assemblages at genus and species levels, seems to stress genus level as the ideal compromise between classification effort and gathered information. The identification of individuals at genus level, generally possible at their last larvae stage or with adults, can be conducted using exclusively morphological characters (see e.g. Tachet et al., 2010). Nevertheless, among adult insects, only the majority of Coleoptera and Heteroptera are strictly aquatic and they generally represent the most frequently recorded taxonomic groups in lentic systems (e.g., Nicolet et al., 2004; Guareschi et al., 2015). Valente-Neto et al. (2018) also recommend EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) at genus level as the best indicator group for monitoring the effects of riparian vegetation loss on aquatic biodiversity in Neotropical streams. Thus, considering all results together, we propose the use of Ephemeroptera in combination with Coleoptera both at genus level as the best surrogate of biodiversity macroinvertebrates in inland aquatic ecosystems of Tunisia. Besides, it is important to note that any potential surrogate of biodiversity must cover a reasonably wide geographic range and occur in a broad range of habitat types (Caro and O'Doherty, 1999), and this is the case of both Ephemeroptera and Coleoptera (Tachet et al., 2010). In sum, we recommend the use of higher taxa as surrogates for the rapid assessment of aquatic biodiversity only when accurate information on species level (rare, endemic or endangered species) is not available (most of the cases working with invertebrates, especially in Africa).

This proposal of biodiversity surrogates can be used i) for the rapid and inexpensive monitoring of biodiversity in aquatic ecosystems of Mediterranean areas and ii) for conservational studies in order to identify areas with the highest values of biodiversity in which the aquatic environment has been or will be threatened (Dudgeon et al., 2006; Vörösmarty et al., 2010; Chadwick, 2010; Tierno de Figueroa

et al., 2013). Indeed, the five first sites ordered by the criteria of complementarity using the data of Coleoptera and Ephemeroptera at genus level, encompassed 172 species (62% of the total), which is interesting for conservational purposes and could be useful for future biomonitoring programmes and biodiversity research. Our findings provide a relevant contribution to the task of searching areas of high aquatic biodiversity in poorly sampled areas such as North Africa, as well as to explore congruencies among taxonomic groups in inland aquatic ecosystems from this area. However further studies in other areas of Tunisia and other North African states are recommended to improve and update the distribution mainly on water beetles and mayflies (also from the whole macroinvertebrate community in some localities) to complement and validate our main results. Thus, different stakeholders (e.g. conservationists, environmental consultants, natural resource managers, universities) may directly benefit from these findings establishing the first practical information on biodiversity surrogate selection in these aquatic systems.

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#### Appendix A. Supplementary data

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