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PAPER

Predatory aquatic beetles, suitable trace elements bioindicators

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Predatory aquatic beetles are common colonizers of natural and managed aquatic environments. While as important components of the aquatic food webs they are prone to accumulate trace elements, they have been largely neglected from metal uptake studies. We aim to test the suitability of three dytiscid species, *i.e. Hydroglyphus pusillus*, *Laccophilus minutus* and *Rhantus suturalis*, as trace elements (Al, As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Se and Zn) bioindicators. The work was carried out in a case area representing rice paddies and control sites (reservoirs) from an arid region known for its land degradation (Monegros, NE Spain). Categorical principal component analysis (CATPCA) was tested as a nonlinear approach to identify significant relationships between metals, species and habitat conditions so as to examine the ability of these species to reflect differences in metal uptake. Except Se and As, the average concentrations of all other elements in the beetles were higher in the rice fields than in the control habitats. The CATPCA determined that *H. pusillus* had high capacity to accumulate Fe, Ni and Mn regardless of the habitat type, and hence may not be capable of distinguishing habitat conditions with regards to these metals. On the other hand, *L. minutus* was found less sensitive for Se in non-managed habitats (*i.e.* reservoirs), while *R. suturalis* was good in accumulating Al, Mo and Pb in rice fields. The latter seems to be a promising bioindicator of metal enrichment in rice fields. We conclude that predatory aquatic beetles are good candidates for trace elements bioindication in impacted and non-impacted environments and can be used in environmental monitoring studies. CATPCA proved to be a reliable approach to unveil trends in metal accumulation in aquatic invertebrates according to their habitat status.

1. Introduction

Aquatic insects have been used as bioindicators of pollution, mainly due to their capacity to accumulate contaminants such as trace elements in predictable amounts, *e.g.* following episodic discharges of pollutants into their habitats.[1,2] They can also reflect metal concentrations long after the pollution ceases,[3] which would otherwise be undetected using traditional water/sediment sampling and analysis approaches. The body tissue metal burden of aquatic biota can therefore offer insights into the extent of their exposure to metal contaminants and can also help evaluate the associated ecological risks and status of aquatic ecosystems.

Among aquatic insects, predatory beetles (Fam. Dytiscidae) are widely distributed and have a high capacity to colonize habitats. They are predators, thus prone to bioaccumulate and biomagnify pollutants from lower levels of the food chain. This makes dytiscid beetles potentially useful candidates to assess and monitor pollution in aquatic environments. Most toxicity studies tend to be lab based where aquatic insects are exposed to

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Environmental impact

Due to anthropogenic inputs trace elements are common in rice field ecosystem. Quantifying their accumulation patterns in biota is important to understand their fate, and impacts on the wider ecosystem. As top predators, aquatic beetles are part of a suite of potential trace element bioindicators and can provide information on the existent levels in aquatic ecosystems. This study reports on trace element accumulation in three species of dytiscid beetles and discriminates their bioindicating ability in rice fields and reservoirs through the use of categorical principal component analysis. The use of different species is particularly important as they may provide a more comprehensive picture of metal input in the systems under investigation.

dissolved chemicals for a short period of time.[4] Such studies of metal uptake by biota are not entirely representative of the exposure in natural environments, mainly because of the complexity of the biological/ecological responses.[4] Despite the importance of aquatic predators in contaminants transmission along the food webs, metal bioaccumulation in dytiscid beetles has not received much attention (*e.g.* Barak and Mason[5] and Erman and Gürol[6]).

Hydroglyphus pusillus, *Laccophilus minutus* and *Rhantus suturalis* are widely distributed dytiscids, easy to sample and identify; they are present throughout the year and more importantly are tolerant to water pollution.[7] They are commonly found in Mediterranean wetlands, where they inhabit the new habitats created by agriculture, such as rice fields.[8,9] However, these new habitats are not free from environmental disturbance, caused by agriculture production. Monegros, an arid region in NE Spain, has seen the buildup of trace elements in recent years, mainly as byproducts of agricultural intensification and land degradation.[10] Inorganic agrochemicals containing trace metals are routinely applied in rice fields before sowing and during rice growth for pests control and rice production (*e.g.* copper sulfate, Mancozeb and Prochloraz, containing Mn and Zn, respectively). In a previous study in the area, the nickel concentration in the irrigation water (0.112 mg L^{-1}) was found higher than the WHO guide value (0.07 mg L^{-1}).[10] The agricultural land in the area commonly receives large applications of pig manure and chemical fertilizers, which have degraded catchment water quality.[11] In these circumstances, the assessment of the extent of metal accumulation in aquatic wildlife becomes imperative. The objective of the present study was to test the suitability of the aforementioned beetle species as possible trace elements bioindicators, particularly in areas affected by agricultural activities, *i.e.* rice fields, in Monegros. For this we compared trace element concentrations in *H. pusillus*, *L. minutus* and *R. suturalis* collected from rice fields, which are intensively managed and water reservoirs, locally used for storing water for irrigation and livestock farming purposes.

One of the difficulties in environmental research is to convert complex data to information which best uncovers relationships between chemical concentrations, biota species and habitat type. The conventional multivariate approaches such as principal component analysis (PCA) are usually restricted to numerical and linearly related data. Environmental data may, however, not always meet these assumptions. To overcome these limitations, categorical principal component analysis (CATPCA) has been developed as an optimal scaling approach which can model nonlinear relationships between variables with a mixed measurement level, *e.g.* numeric, nominal and ordinal.[12] Despite the potential advantages of CATPCA in environmental studies, its use has been limited, however. Here we hypothesize that CATPCA can reveal reliably the important relationships between trace elements, beetle species and habitats.

2. Methodology

2.1. Study area

The sampling was carried out in a major rice farming area from central Monegros (San Juan del Flumen, 41°46′N, 0°12′W). Monegros (2700 km^2) is one of the most arid regions of Europe.[13] It lies in the central part of Ebro river basin (NE Spain), surrounded by three mountain ranges: the Pyrenees at N, the Iberian chain at SW and the Catalonian coastal ranges at SE. The average annual temperature is 14.5 °C, with extremes from −15 °C to >40 °C. The mountain isolating effect means the average annual rainfall is relatively low, *i.e.* <400 mm.[14] Soils in the area are dominated by Tertiary deposits (lutite and sandstone) with variable level of salinization (conductivity ranges between 1 and 10 mS cm^{-1}).[15] These soils are generally alkaline (pH ranges between 8 and 8.5), with a great abundance of carbonates, and poor in organic matter.[16]

The development of a large-scale irrigation scheme in the 1960s has led to an increase in the land under rice cultivation (45 909 ha in 2008) and crop production.[17,18] These land use changes have caused contamination in the area, as a result of the increased use of agro-chemicals.[19] This is reflected by trace element concentrations in water samples collected from rice paddies and reservoirs in the study area. For example, the concentrations of Ni, Cu, Fe and B in rice paddies (0.023, 0.004, 0.029 and 0.082 mg L^{-1}, respectively) were higher than those from the reservoirs (0.0137, 0.003, 0.021 and 0.036 mg L^{-1}, respectively).[10] Although the levels of other trace elements in the study sites are not available, their concentrations are expected to reflect similar differences between the rice paddies and the reservoirs, at least for certain agri-chemical and manure-borne trace elements. This expectation is supported by the use of metal containing pesticides and fertilizers in rice paddies in the study area.[10]

2.2. Sampling

Sampling strategy was designed to cover habitats with contrasting level of land disturbance/management. It comprised two control areas, *i.e.* permanent reservoirs used for irrigation and livestock farming purposes, and two rice fields (temporary habitats, with high level of land management) (Fig. 1). Reservoirs are of relatively small size (<1/2 ha) and <1.5 m depth while rice fields had <10 cm water depth at the time of sampling. The poor organic matter content of soils from this region and the minimum organic input into the reservoirs means these habitats were unlikely to experience reducing conditions in their bottom sediments.

Three most abundant predatory aquatic beetles in the area were collected from all study sites: *H. pusillus* (Fabricius, 1792), a small species (body length 1.9–2.2 mm), *L. minutus* (McLeay, 1825), medium sized (4.3–4.8 mm), and *R. suturalis* (Linnaeus, 1758), somewhat bigger (10.5–11.9 mm).[9] Species identification was carried out following the keys for aquatic insects of Nilsson and Holmen.[20] The sampling was conducted in February 2008 using a D-frame net by sweep netting along the shore in the decomposing vegetation. Overwintering adults are common in February, when they tend to cluster under floating or bottom vegetation/debris.[21] Their potential migration is also reduced during winter. The three species exhibit life cycles of about one year, similar to most dytiscids from temperate regions.[22] Although the precise age of the adult beetles is difficult to determine and it was unknown at the time of their capture, previous field observations on their adult class distribution in the

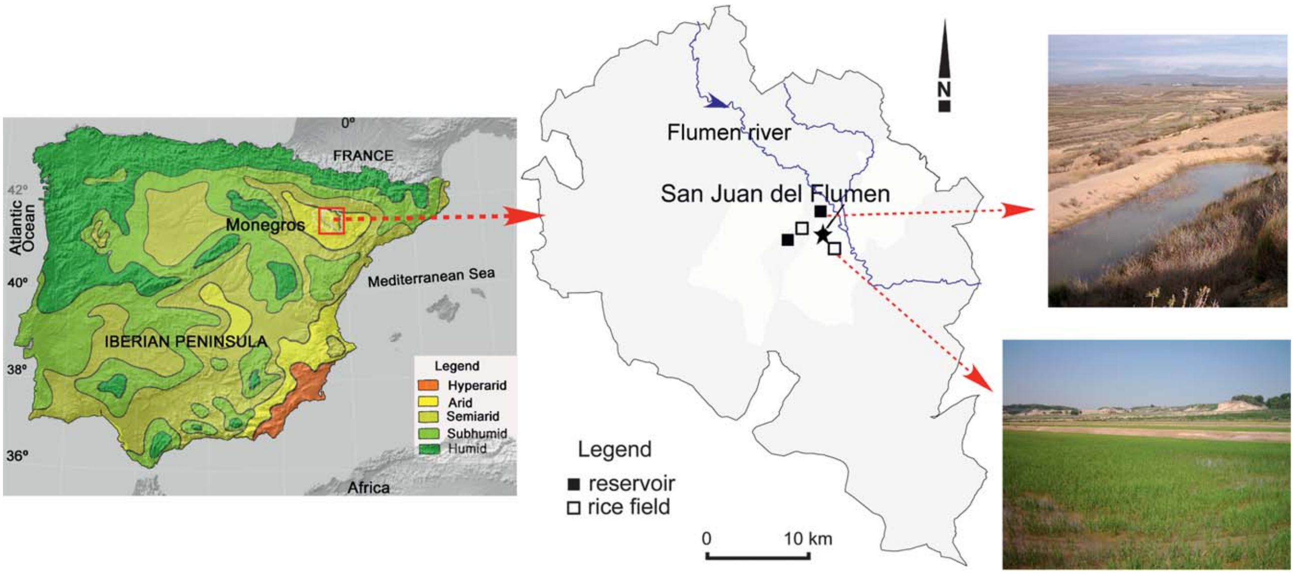

Fig. 1 Monegros area with location of the sampling sites.

area led us to assume that in February the beetles were near the end of their life cycle.[9] Therefore the body metal content is assumed to represent concentration at similar life stages.

Altogether 778 individuals of the three species were collected from two rice paddy areas and two reservoirs, separated in the landscape at an inter-site distance of 3–5 km. From each sampling point, 15 beetles were taken randomly, at habitat depths <10 cm. The samples were collected with the minimum amount of water, vegetation or sediment possible and kept in sterile polyethylene bags until laboratory processing.

2.3. Samples digestion and analytical procedure

The beetles were washed with Milli-Q water to remove potential residues and kept in covered Petri dishes to prevent cross-contamination. The samples were dried at 60 °C for 48 h and ground using a laboratory ball mill and following a clean protocol. The ground samples were acid digested using Aristar HNO_3 and H_2O_2.[23]

Prepared solutions were analyzed for trace elements (Al, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd and Pb) by inductively coupled plasma mass spectroscopy (ICP-MS). The ICP-MS analysis was highly sensitive for all trace elements in terms of detection limits and reproducibility.

A number of quality control measures were implemented to assure the integrity of the results, which included reagent blanks, duplicate samples and standard reference materials (ERM-CE 278). The analysis was highly precise with the coefficient of variability between replicates <5% and the relative standard deviation between measurements of the same sample <2%. The mean percentage recovery for the elements considered was within the acceptable range. The certified and obtained values of Mn, Cu, Zn, As, Cd and Pb were in agreement.

All reagents were ultra-pure quality (Aristar grade). Stock standard solutions were Merck Certificate AA standards. Ultra-pure (Milli-Q) water was used in all samples, standard solutions and dilutions, as appropriate.

2.4. Data analysis

Categorical principal component analysis (CATPCA) was performed to reveal whether the considered species are suitable trace element bioindicators in one habitat *vs.* the other. Compared to traditional linear methods (*e.g.* PCA), CATPCA is a multivariate analysis applicable to nonlinearly related and multiple-scaled data, *e.g.* nominal, categorical/ordinal and numeric variables. In this analysis categorical/nominal variables with ordered and unordered (discrete) categories are optimized/transformed by assigning optimal scale values (numeric values) to their categories through a process called optimal quantification. The category quantifications are transformed in such a way that the total variance extracted in the components is maximized.[12] These values are essential for variance and Pearson correlation calculations between the quantified variables and the principal components, which give the component loadings. To determine what kind of transformation fits better to CATPCA solution (results), different types of quantification (scaling levels of variables, *e.g.* ordinal, spline ordinal, numeric and multiple nominal) available in the SPSS package were screened in turn. Comparison of the solutions for CATPCA obtained with these different scaling levels was based on the total percentage of variance-accounted-for (total PVAF) in the transformed variables and Cronbach's α for each model. Cronbach's α is a measure for the internal consistency of categorical principal components. Additionally, we compared the total variance explained by the first two dimensions of CATPCA with the variance explained by classical PCA.

2.5. CATPCA stability

Because CATPCA is an explorative approach, there is a risk of fitting structures that are sample-specific. It is therefore important to conduct stability tests on the solution provided by CATPCA.[12] The robustness of the CATPCA results of our data, *i.e.* the constancy of assignment of the variables to the main components, was checked by the bootstrap procedure applied to the component/dimension loadings.[24] This is a resampling procedure which is useful to determine the sampling error in estimating the component loadings. It implies repeating CATPCA on different samples randomly drawn from the original dataset to construct 90% confidence ellipses of the component loadings. 1000 bootstrap samples with replacement were created randomly in order to determine the probability of obtaining a sample with the degree of variation identical to that observed in the original estimates. 90% confidence regions of CATPCA component loadings were created by bootstrap, giving therefore a general idea about the significance/stability of CATPCA solution. If the results provided by CATPCA are stable, we expect narrow confidence ellipses.

The analyses were performed in PASW Statistics 18 (former SPSS) for Windows. Bootstrap procedure was computed with macro files Categories CATPCA Bootstrap for PASW (available online at http://www.spss.com/devcentral/).

3. Results and discussion

3.1. Trace element contents in predatory aquatic beetles

The average metal concentrations in the studied species are listed in Table 1. It appears that *H. pusillus* accumulated relatively more Al, Mn, Fe, Ni and Se, but less Cd (below detection limit) as compared to the other species. *Laccophilus minutus* had the highest concentrations of As and *R. suturalis* accumulated comparatively more Cu, Zn and Mo. The variation in metal concentrations between species may well reflect differences in patterns of uptake and excretion, as well as differences in prey choice and life history.[25]

The concentrations of Al, Cu, Ni, Pb and Cd in the three species were similar to levels reported for other predatory beetles (Fam. Gyrinidae) from Canada, and are relatively low to produce negative effects at higher levels of the food chain, *e.g.* aquatic birds.[26] Likewise, Se, Fe and Mn concentrations were lower than toxic levels that would affect other top aquatic predators such as fishes.[27,28] On the other hand, Mo body contents (Table 1) were higher than its average concentrations reported in other aquatic insects, such as ephemeropterans: Heptageniidae (1.40 μg g^{-1}) and Ephemerellidae (1.18 μg g^{-1}).[29] This suggests that the predatory beetles may be more efficient in accumulating Mo from the environment. However, this inference should be considered preliminary as this study did not deliberately target this question.

As listed in Table 1 the most abundant trace elements in the aquatic beetles were Fe and Zn. The pattern of metal distribution in the three species followed the order:

Fe > Zn > Cu > Al > Mn > Mo > Se > Ni > As > Pb > Co > Cd in *H. pusillus*; *Fe > Zn > Cu > Al > Mn > Mo > Se* > As > Ni > Pb > Co > Cd in *L. minutus*, and *Fe > Zn > Cu > Al > Mn > Mo > Se* > As > Pb > Ni > Cd > Co in *R. suturalis*.

Fe, Zn, Cu, Al, Mn, Mo and Se (in italics) had a common pattern of accumulation in all species. Similar trend is also found in other predatory aquatic insects.[30] However, the accumulative behavior signatures for Ni, As, Pb, Co and Cd showed different patterns between species, which could indicate a species-specific metal bioaccumulation behaviour, *e.g.* either as a reflection of the order they accumulate in the insect body, or species requirement, exclusion or excretion which can be different for various elements/species. Variation in metal accumulation within each species may be a reflection of energy requirements associated with excreting and/or detoxifying the ingested metals. If energy burden is significant this may be translated into effects on species biology (*e.g.* growth and survival) and ecology.

3.2. Variation in metals bioaccumulation between habitats

Except As and Se, the rest of trace elements showed higher concentrations in the rice field exemplars than in those from the control sites (Table 2). This is hardly surprising as the rice fields are expected to have a comparatively larger pool of trace elements than the reservoirs since the former receives additional inputs *via* agro-chemical use. Although we did not measure total or soluble amounts of trace elements in the habitats, it is not unreasonable to assume that the concentrations of As and Se in the rice fields can be expected to be at least similar to those in the reservoirs (control) bottom sediments. This then raises a question why the beetles should accumulate similar level of As or slightly greater amount of Se from the control reservoirs compared to the rice paddies. The more reducing environment of rice paddies is

Table 1 Inter-species variation in metal concentrations (μg g^{-1} dry weight). Figures represent mean ± SE, based on data pooled across the two habitat types (rice paddies and reservoirs)

	H. pusillus	*L. minutus*	*R. suturalis*
Al	30.83 ± 2.31	24.29 ± 9.57	24.80 ± 6.80
Mn	22.15 ± 2.24	14.86 ± 3.75	13.06 ± 1.86
Fe	202.64 ± 89.59	145.35 ± 24.67	123.43 ± 16.57
Co	0.05 ± 0.02	0.05 ± 0.02	0.03 ± 0.008
Ni	3.08 ± 2.98	0.10 ± 0.06	0.14 ± 0.04
Cu	34.56 ± 7.39	35.39 ± 2.04	39.35 ± 1.87
Zn	61.43 ± 5.98	63.54 ± 3.45	64.02 ± 2.02
As	0.32 ± 0.04	0.41 ± 0.10	0.32 ± 0.05
Se	1.42 ± 0.92	0.93 ± 0.04	0.97 ± 0.15
Mo	2.66 ± 0.28	1.79 ± 0.97	4.44 ± 3.69
Cd	n.d.[a]	0.03 ± 0.02	0.04 ± 0.03
Pb	0.08 ± 0.06	0.07 ± 0.06	0.15 ± 0.07

[a] n.d. = below detection limit.

Table 2 Inter-site variation of trace elements in Monegros' predatory aquatic beetles from different types of aquatic habitats, averaged across the species. Results are presented ±SE

	Habitat type	
Element/μg g^{-1}	Rice fields	Control
Al	32.81 ± 6.13	20.50 ± 4.41
Mn	18.33 ± 2.02	13.56 ± 2.73
Fe	186.44 ± 38.47	117.86 ± 12.64
Co	0.05 ± 0.02	0.04 ± 0.00
Ni	1.66 ± 1.47	0.08 ± 0.02
Cu	37.62 ± 3.66	36.44 ± 1.65
Zn	64.10 ± 3.42	62.64 ± 1.67
As	0.32 ± 0.05	0.37 ± 0.06
Se	0.85 ± 0.19	1.22 ± 0.28
Mo	5.03 ± 3.53	1.67 ± 0.61
Cd	0.04 ± 0.04	0.02 ± 0.02
Pb	0.16 ± 0.08	0.06 ± 0.04

likely to reduce selenium to insoluble forms resulting in its lower bioavailability.[31] Arsenic, on the other hand, presents a more complex biogeochemistry. Its reduction from As(IV) to As(III) while may not reduce its bioavailability, the further expected transformation to methylated forms means it may not be bioavailable.[32] Re-adsorption and co-precipitation of As, however, are known to decrease its solubility/bioavailability under long-term and moderately reducing conditions such as in rice paddies.[31] While it is acknowledged that the geochemistry of trace elements in rice paddy environment is rather complex and highly fluctuating, mainly due to organic carbon and water regime triggered redox conditions, it is evident from our findings that generally the species are capable of distinguishing between habitats in terms of their response to the levels of exposure to trace elements, and hence can potentially be used as bioindicators.

3.3. Exploring the association of trace elements with species and habitat type by CATPCA

Metal bioaccumulation in aquatic insects can show strong variation among different species and between habitats. To analyze habitat–species relationships on the basis of metal concentrations, and thus assess their suitability as metal bioindicators, a categorical principal component analysis (CATPCA) was carried out on three sets of variables, *i.e.* body tissue metal concentrations, habitat type and the beetle species.

3.4. Setting CATPCA optimal solution

In CATPCA, the correlations between variables depend on the scaling level chosen for each variable.[33] In order to assess the appropriateness of scaling level for CATPCA solution an initial step was therefore to compare the solutions obtained with different scaling levels of variables, *i.e.* the total percentage of variance-accounted-for (total PVAF) and Cronbach's α (Table 3).

In terms of PVAF and Cronbach's α, the spline (smooth function to obtain nonlinear transformations) ordinal solution/scaling level was relatively more accurate than ordinal and numeric ones (Table 3). Total Cronbach's $\alpha > 0.95$ indicates a high reliability of our data structure as well as high inter-variable correlations on the two main dimensions extracted by CATPCA. Multiple nominal scaling level allowed plotting of categories of the categorical variables in a two dimensional space. The multiple nominal scaling level (non-monotonic transformations) for the categorical variables (species and sites) and spline ordinal level (monotonic transformations) for metal variables seemed therefore to be the most suitable to reveal nonlinear relationships between the variables involved (Table 3).

Table 3 Comparison of solutions (percentage of variance-accounted-for, PVAF; and eigenvalue, EV) for 1st and 2nd dimensions (Dim.) obtained with different scaling levels applied to metal variables while keeping species and sampling sites as multiple nominal in CATPCA

	Scaling level		
	Ordinal	Spline[a] ordinal	Numeric
PVAF Dim. 1	37.99%	40.56%	31.23%
PVAF Dim. 2	32.56%	32.26%	27.37%
PVAF total	*66.34%*	*68.12%*	*54.9%*
EV Dim. 1	5.32	5.68	4.37
EV Dim. 2	4.56	4.52	3.83
EV total	*9.29*	*9.54*	*7.69*
Cronbach's α Dim. 1	0.87	0.89	0.83
Cronbach's α Dim. 2	0.84	0.84	0.80
Cronbach's α total	*0.96*	*0.96*	*0.94*

[a] Most suitable function for CATPCA solution.

Two main components (dimensions) extracted by CATPCA on the optimal/chosen scaling captured 68.12% of total variance in the dataset, with a Cronbach's $\alpha > 0.8$ for each, indicating a high explanatory power of this analysis. This was more efficient than a classical PCA, which was able to extract only 57.63% of total variance in its two principal components. Besides, one of the major advantages of CATPCA is that it allows the graphical representation of the relationships between the categories of variables, *i.e* of species and sites.

3.5. CATPCA stability

Because CATPCA is an exploratory approach and may be influenced by the sample characteristics we evaluated its level of stability (degree of sensitivity to changes in the data) by nonparametric bootstrap procedure.[24] This computed 90% confidence limits (displayed as ellipses) for the component/dimension loadings from CATPCA after 1000 bootstrap resampling of the data (Fig. 2).

By inspecting the bootstrap component loadings of the quantified variables (*i.e.* the correlations between variables quantified by CATPCA and the two extracted components/dimensions; Fig. 2), we observed that almost all variables had high loadings (>0.6) on their associated components, which means that they make an important contribution to that particular component. As displayed in Fig. 2, the confidence ellipses of most of the variables component loadings were fairly small, indicative of their relatively good stability. Cd, however, did not contribute much to the CATPCA solution, as shown by

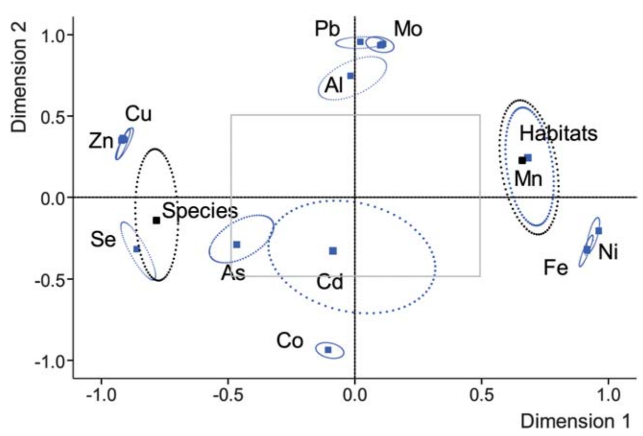

Fig. 2 Bootstrap component loadings for quantified variables and their 90% confidence ellipses from CATPCA. Black (species and sites) and blue (body metal content) symbols represent centroids and the bootstrap clouds. Correlations of variables with dimensions >0.5 are outside the inset square.

its low loading (0.33) on the first component and a relatively large confidence ellipse.

The multiple nominal variables, *i.e.* species and habitats, received two quantifications for each dimension. We chose to represent graphically only their highest loadings on any of the components. Overall, the analyses showed that CATPCA is reliable for this type of data and it could be confidently used to examine the relationships between body metal content, species and habitats.

3.5.1. Species relationship with metal body content and habitats. The results of CATPCA presented in Fig. 3 show the capacity of the species in accumulating trace elements in habitats with different disturbance levels. The first two resulted dimensions shared together metal variation between species and sites within 68.12% of the total variance. In graphical terms, variables with the multiple nominal scaling level, *i.e.* species and sites, are represented by category points, while metals are indicated by vectors. The first dimension, explaining nearly 41% of total variance (eigenvalue = 5.68, Cronbach's $\alpha = 0.89$), clearly associated *H. pusillus* with Ni, Fe and Mn on its negative side and Cu and Zn on the positive side (Fig. 3). It shows *H. pusillus* as a good bioaccumulator of Ni, Fe and Mn, regardless of the site management level. The opposite projection of Cu and Zn to the previous group implies *H. pusillus* likely excludes these elements. Brown[34] reported that the major pathway for uptake and bioaccumulation of metals in aquatic invertebrates is through food, from which they are often better assimilated.[35] Some predatory aquatic insects are reported to take up and accumulate trace elements such as Ni, a metal easily transferred along the aquatic food chain, almost exclusively from their prey.[4] Another possibility is that Ni could bind to iron oxides, which can precipitate on the chitinous exoskeleton of some burrowing aquatic insects.[36] Such conditions would be propitious for the sorption of other elements such as Ni, Mn, Pb and As on the Fe oxide incrustations of the body surface,[36] especially in *H. pusillus*, the species with the largest area-to-volume ratio. Although no association with Pb and As was observed for this species in our study, possibly due to their limited bioavailability, this mechanism, however, cannot be totally excluded. The physiological mechanisms of metals uptake–release in dytiscid beetles are still poorly known and their efficiency in avoiding metal uptake or having efficient detoxification mechanism needs to be further investigated.

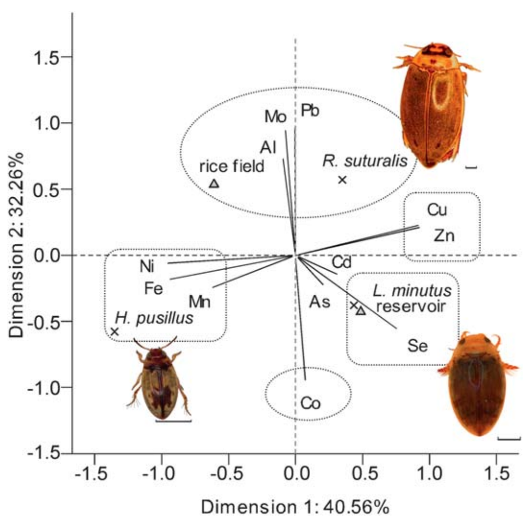

Fig. 3 Clustering of trace elements, species and habitat type in their projection on dimensions 1 and 2 of Categorical principal component analysis (CATPCA). Variables clusters with high loadings on the first dimension are encircled and ones with high loadings on the second dimension are enclosed in polygons. Symbol key: △, site type; X, species. Scale bar for species is 1 mm.

Laccophilus minutus, on the other hand, clustered together with Se and control sites, *i.e.* reservoirs of low agricultural disturbance, and projected independently on the positive side of first CATPCA dimension (Fig. 3). Selenium, a naturally occurring anion in soils/sediments, is highly soluble under the alkaline oxidizing conditions prevalent in arid climate environments such as the shallow reservoirs,[37] from where it can be readily assimilated by predatory aquatic insects.[38] In fact, diet has been identified as the main entry route for Se into aquatic animals,[39] the bio-transformation of Se into organo-Se compounds by its incorporation into the prey increasing its availability to predators.[40] Although *L. minutus* generally accumulated less Se than the other dytiscids (Table 1), it is the higher proportion of Se accumulated by this beetle, compared to other elements, which determines its association with control sites (Fig. 3). It seems therefore from our results that this species is a good bioindicator candidate for selenium in the less managed habitat.

The second dimension of CATPCA (32.26% of total variance; eigenvalue = 4.52, Cronbach's $\alpha = 0.84$) shows Al, Mo and Pb projecting together with *R. suturalis*, close to rice fields position in the ordination space, while Co was negatively related to this first cluster (Fig. 3). It is evidence of the capacity of this species to uptake Al, Mo and Pb in rice paddy environments, and being limited in bioaccumulating Co. The uptake of higher proportions of Al, Mo and Pb, of different solubilities, would rather be an indication of the bioaccumulation behaviour of these insects in the natural environment, as has been suggested by other studies.[23,41,42] Thus, they may reflect the contamination state of their habitat. Long-term application of agro-chemicals such as inorganic phosphate fertilizers and possibly organic manure in arable lands from arid regions is known to contribute to the buildup of Pb and other metals.[43,44] Likewise, the emissions from gasoline powered equipment/machinery can make important Pb contribution to rice fields.[45] The intensification of agriculture on the relatively poorly developed soils (arid and saline) following the introduction of irrigation in Monegros in the 1960s meant large amounts of inorganic fertilizers, pesticides and organic wastes were used in the area to increase agricultural production.[46] Because of the long-term application of these soil amendments and agro-chemicals, the enhanced accumulation of trace elements such as Pb and Mo in living organisms was expected as indicated by predatory aquatic beetles in this study.

4. Conclusions

The study provides a baseline dataset of trace element levels in predatory aquatic beetles from sites with different levels of land-use disturbance. Categorical principal component analysis (CATPCA) proved to be a reliable method for uncovering associations between species, metal uptake and habitat type when using metal concentration variables as spline ordinal scaled, and species and sites variables as multiple nominal scaled.

The results demonstrate that predatory aquatic beetles are capable of reflecting trace elements bioaccumulation in habitats with different disturbance levels and thus can be used in environmental monitoring/contamination studies. Although the three species are related predators (Fam. Dytiscidae), their innate ability to take up/excrete metals differs. According to our results, *R. suturalis* had a high capacity to bioaccumulate Al, Mo and Pb in habitats with high management impact, *i.e.* rice paddies, while *L. minutus* was prone to accumulate Se in the least managed sites, *i.e.* reservoirs. *Rhantus suturalis* can therefore be suitable as a bioindicator of trace element pollution. Surprisingly, despite *H. pusillus* showing the highest metal uptake it proved to be less efficient in discriminating the effects of habitat management/type.

Our results help strengthen the knowledge of the interaction between freshwater insects and trace elements. The nature or the small size of these insects should thus not be an argument against using dytiscid beetles as metal bioindicators in the natural/anthropic environments.

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