Multivariate analysis applied to agglomerated macrobenthic data from an unpolluted estuary

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ABSTRACT

We agglomerated species into higher taxonomic aggregations and functional groups to analyse environmental gradients in an unpolluted estuary. We then applied non-metric Multidimensional Scaling and Redundancy Analysis (RDA) for ordination of the agglomerated data matrices. The correlation between the ordinations produced by both methods was generally high. However, the performance of the RDA models depended on the data matrix used to fit the model. As a result, salinity and total nitrogen were only found significant when aggregated data matrices were used rather than species data matrix. We used the results to select a RDA model that explained a higher percentage of variance in the species data set than the parsimonious model. We conclude that the use of aggregated matrices may be considered complementary to the use of species data to obtain a broader insight into the distribution of macrobenthic assemblages in relation to environmental gradients.

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1. Introduction

The distribution of macrobenthic assemblages in relation to environmental drivers has been often described at the species level (Rodrigues et al., 2006; Ysebaert et al., 1998; Zajac and Whitlatch, 1982). Agglomeration or aggregation of species at low resolution levels has also been used to describe environmental gradients. According to the concept of taxonomic sufficiency, species data are aggregated into higher taxonomic levels deemed to be sufficient for the purposes of a study. The term 'taxonomic sufficiency' (coined by Ellis, 1985) has been used to identify the effects of pollution on marine communities (e.g. Ferraro and Cole, 1990). The family level has been proposed as an appropriate level of description for pollution studies (Gómez Gesteira et al., 2003), although higher levels may also be used (Warwick, 1988). However, it has been argued that the likelihood of detecting a stressor at higher taxonomic levels will depend on the severity of the stressor (Ferraro and Cole, 1990), and therefore the taxonomic resolution used in a study must take into consideration the environmental problem in question. Similar conclusions were reached in long term monitoring studies that propose periodical analysis at species level rather than at lower taxonomic resolution, such as the family level (Musco et al., 2011).

Agglomeration of species into guilds (usually feeding guilds) has also been considered (Gaudêncio and Cabral, 2007; García-Arberas and Rallo, 2002). The use of functional groups (or functional traits) subdivides the aggregated data at a finer functional level. For instance, the carnivore guild may be further subdivided in accordance with the motile or sessile characteristic of the species, reflecting different ecological niches that are not indicated by the 'carnivore' grouping alone. Similarly, although species in the same feeding guild commonly compete for the same food resource, the interaction is not necessarily reflected in a taxonomic approach (Rosenberg, 2001). Improvements in computational methods enable more complex analyses that allow species, biological traits and environmental matrices to be considered simultaneously (Dolédec et al., 1996; Legendre et al., 1997). Analysis and visualization of patterns based on species agglomeration matrices in relation to environmental factors may be accomplished by common ordination techniques such as Non-metric Dimensional Scaling (NMDS, Terlizzi et al., 2008) and Redundancy Analysis (RDA, Boström et al., 2006).

A few studies have shown that the family level provides an adequate description of macrobenthic assemblages along natural

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gradients (De Biasi et al., 2003; Dethier and Schoch, 2006; Wlodarska-Kowalczuk and Kedra, 2007, and references therein). The family level is a better descriptor than higher taxonomic levels or trophic groups for explaining the distribution of the assemblages (Dethier and Schoch, 2006), although a lower level of data resolution may also be useful (Wlodarska-Kowalczuk and Kedra, 2007).

Aggregation of species has been underexplored as a means of describing natural gradients (Wlodarska-Kowalczuk and Kedra, 2007). The aim of this study was to assess the usefulness of agglomerated data sets in explaining the distribution of macrobenthic assemblages along a natural gradient in an unpolluted estuary. As estuaries may display horizontal, vertical or cross-sectional spatial gradients, among others (McLusky, 1993), the study was conducted in an area of an estuary between the zones most affected by marine and freshwater influences. We measured a number of abiotic variables (redox potential, total nitrogen content of the sediments, salinity and others) with the aim of explaining the distribution of the macrobenthic assemblages. We compared the results of applying multivariate analyses to the species dataset and the results of applying the same analyses to agglomerated data grouped at low level of resolution (order and class taxonomic level and gross feeding guilds), which are rather overlooked in comparison with finer aggregation types such as the family taxonomic level (De Biasi et al., 2003) or subdivisions of feeding guilds (Fauchald and Jumars, 1979). The underlying null hypothesis of the study was that there was no relation between multivariate patterns in agglomerated data sets and the species data set.

2. Material and methods

2.1. Study site

The River Minho is the longest river in the Northwest Iberian Peninsula. The annual average discharge of the River Minho is 13,560 h m^3, with a monthly flow average ranging from 100 m^3 s^{-1} in August to 1000 m^3 s^{-1} in February (deCastro et al., 2006). The estuarine part of the river (Fig. 1) lies between Portugal and Galicia (Spain). The Minho estuary has mesotidal features and is partially mixed, although it tends to be a salt wedge estuary when high floods occur (Sousa et al., 2005). Numerous small tributaries drain into the estuary (Fig. 1).

A number of studies have highlighted the low level of anthropogenic pressure on the Minho estuary (Monteiro et al., 2007;

Fig. 1. Map of the Minho estuary showing the location of the sampling sites.

Moreira et al., 2006). Other authors have recommended using this estuary as a pristine reference site for comparison with other metal polluted estuaries (Reis et al., 2009). However, Sousa et al. (2008a) have identified up to 11 well-established alien species of invertebrates and fish in the Minho estuary. Among these, the invasive bivalve *Corbicula fluminea* (Müller, 1774) has achieved a key position in the benthic assemblages because of its high abundance, biomass and production (Sousa et al., 2008b).

2.2. Sampling and laboratory procedures

Sampling was conducted on both banks of the estuary during a summer spring tide, between 23 and 26 August 2010. We sampled ten sites and assigned them codes in alphabetical order from AM, BM… to KM, excluding IM (Fig. 1). We conducted intertidal sampling during low spring tides immediately above the water edge. These sampling surveys included sites along the main axis of the estuary, in salt marshes and on an estuarine island (Boega Island, site JM). The length of the sampling area was approximately 13 km, extending from the mouth of the estuary to a few kilometres upstream of the village of Vilanova da Cerveira (Fig. 1).

We used a standard field probe (WTW 340i) to measure temperature, salinity, oxygen, redox potential in the interstitial water, at a depth of approximate 10 cm in the sediment. We sampled the top 3 cm of the sediment (making composite sample from three replicate samples per site) to determine the sediment grain size by the dry sieving method. We defined the grain size fractions as follows: gravel (>2 mm), coarse and medium sand (2−0.250 mm), fine sand (0.250−0.063 mm) and finest grains (<0.063 mm), following the method of Rodrigues et al. (2006) and Silva et al. (2006), although these authors considered coarse and medium sand content separately. At each sampling site, we placed samples of the top 3 cm of the sediment in plastic containers and kept them in a cooler at 4 °C for subsequent determination of total organic carbon and total nitrogen (on dried samples) in an elemental analyser (LECO CN2000).

We inserted a corer of inner diameter 95 mm to a depth of 25 cm in the sediment (7 replicates = 0.05 m^2) to sample infaunal organisms. We sieved all samples through a 1 mm mesh and preserved the material retained on the mesh in 70% ethanol. We used a dissecting microscope to sort, count and identify samples of benthic fauna to the lowest possible taxonomic level.

2.3. Taxa aggregation and categories

We agglomerated the taxa into four aggregation types, each constituted by different categories, as explained below. We first aggregated the taxa into two different taxonomic levels: order and class; the number of categories for both aggregation types depended on the fauna found in each sampling site. We also aggregated the taxa into four categories of trophic guilds: suspension feeders, deposit-feeders (including surface and sub-surface-deposit feeders), carnivores (or predators) and omnivores. This trophic aggregation, with the exception of the omnivore category, follows that used by Chardy and Clavier (1988). We categorized feeding guilds in accordance with Ysebaert et al. (1998), Cummins et al. (1989) and Mancinelli et al. (2005). The feeding guilds are hereinafter referred to as guilds. For the final aggregation, we combined class and guilds in the same data matrix, to produce a mixed data matrix. This approach is based on that used by Pagola-Carte and Saiz-Salinas (2001), although in the present study each taxon occurs twice, as a category of both class and guilds. For example, the species *Hediste diversicolor* occurs in the polychaete category (class) and the omnivore category (guild). We labelled taxa that did not fit any of the categories within a specific aggregation type as *other* (e.g. we
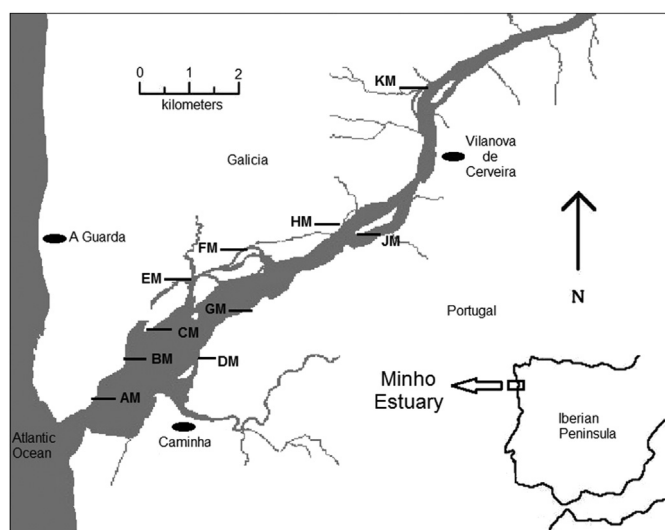
defined insects as *other*). We also considered the species data set (including some undetermined taxa) in the analysis.

2.4. Statistical analysis

We used multivariate analysis to examine the spatial distribution and composition of the assemblages within the estuary. We used the Vegan package (Oksanen et al., 2011), which is run in the free R environment for statistical computing (R Development Core Team, 2011), for most of the analyses. We calculated a dissimilarity matrix, based on the Euclidean distance of the Hellinger transformed raw data, to overcome acute differences in the numbers of individuals between sampling stations (Legendre and Gallagher, 2001). We then applied non-metric Multidimensional Scaling (NMDS) to site centroids (7 replicates). NMDS is an ordination technique that displays the distance between the considered objects in accordance with a previously computed dissimilarity matrix (e.g. Legendre and Legendre, 1998). We identified groups of sites by cluster analysis (Legendre and Legendre, 1998), in accordance with their similarity in species composition. We formed three groups of sites in each cluster analysis by choosing an arbitrary threshold dissimilarity distance (Clarke and Warwick, 1994). We used complete linkage agglomerative clustering because it is considered an appropriate approach for detecting discontinuities in data (Borcard et al., 2011).

We used non-parametric permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001, 2005) to test for differences between the assemblages in groups identified by cluster analysis, with sites nested within groups of sites. We mainly used the PERMANOVA test to assess the convergence of the results obtained with different data sets, not to test for differences on the groups identified a posteriori by cluster analysis, because in such cases circularity makes invalid any inference (Clarke and Warwick, 1994). We used the Hellinger distance in the analysis. PERMANOVA enables post-hoc analysis based on uncorrected permutations for multiple testing. The Monte Carlo asymptotic *P*-value (Anderson, 2005) is provided as the *P*-value (P) for the pairwise comparisons.

We applied the indicator value index (IndVal; Dufrêne and Legendre, 1997) to identify the components of the fauna that characterized the groups of sites by comparing their abundance and occurrence. In relation to the type of sites under analysis, the specificity and fidelity of the fauna yield an IndVal value that is higher for the most prominent members of the groups; the significance of the differences in these values is determined by a permutation test (Dufrêne and Legendre, 1997; Borcard et al., 2011). In IndVal, specificity (designated as Aij, see below) is calculated by considering the mean number of individual of species i (or any category in this case) across sites of group j divided by the sum of the mean number of individuals of species i over all groups. Fidelity (designated as Bij, see below) is equal to the number of sites in cluster j, where species i occurs, divided by the total number of sites in the cluster. Finally, IndVal is calculated as the product between specificity and fidelity: IndVal = Aij * Bij.

We used canonical ordination to determine the spatial structure of the species assemblages in response to an environmental explanatory matrix. We chose redundancy Analysis (RDA) because it enables transformation of the species data matrix (Legendre and Gallagher, 2001; Borcard et al., 2011) and posterior projection on a Euclidean space (Legendre and Gallagher, 2001). We constructed RDA triplots by using site scores based on the weighted sum of species (wa) as a counterbalance or midpoint between the descriptors used in the analysis and environmental constraints (following Oksanen, 2011). The so-called species–environment correlation is also provided (Oksanen, 2011). We used Akaike's Information Criteria (AIC) to measure the goodness of fit of the RDA models (Oksanen et al., 2011). We assessed linear dependencies between environmental variables by computing variance inflation factors (Borcard et al., 2011; Oksanen et al., 2011), so that the values of none of the variables was higher than 10, as recommended by Borcard et al. (2011). We used permutation tests to assess the significance of the environmental variables and RDA axes of the constrained ordination. We used forward selection (Blanchet et al., 2008) to determine which environmental variables significantly explained the distribution of the assemblages.

We used Procrustean superimposition (Gower, 1971) to compare constrained and unconstrained ordinations between different species aggregations. Procrustes rotation minimizes the sum of squared residuals between matrices that are compared. Therefore, the optimal superimposition between matrices is used as a metric of association (Gower, 1971). The statistical significance of the differences highlighted by Procrustean analysis can be assessed by a permutational procedure (PROTEST; Jackson, 1995). We calculated the corresponding values on the first two dimensions of the ordinations to maintain the same dimensionality between the comparisons (Peres-Neto and Jackson, 2001). We used the PROTEST approach because it has been shown to be more powerful than the Mantel test for this purpose (Peres-Neto and Jackson, 2001).

We assessed and compared the dispersion within each aggregation type to identify any intrinsic differences in the multivariate dispersion among aggregation types. Anderson (2001) pointed out that the mean distance to the group centroid could be tested in multivariate data in the same way as by a Levene's test for homogeneity of variances in univariate analysis. The comparison used here was based on the mean distance for each replicate (7 per site) in relation to the general centroid within an aggregation type prior to Hellinger transformation. Pairwise comparisons can be conducted after an overall significance test through the calculation of the Tukey's Honest Significant Differences (Oksanen, 2011) between aggregations.

Values are shown as means ± standard deviations (±sd).

3. Results

The environmental data obtained at each sampling site are shown in Table 1. The porewater temperature ranged from

Table 1
Mean values (±sd) of the environmental variables measured at each site.

Variable	Units	AM	BM	CM	DM	EM	FM	GM	HM	JM	KM
Temp	°C	22.2 ± 2.2	24.0 ± 0.8	23.2 ± 0.5	21.5 ± 0.3	22.3 ± 0.3	22.3 ± 0.3	22.4 ± 1.0	24.0 ± 0.8	24.2 ± 0.6	21.9 ± 0.5
Sal	–	17.8 ± 0.8	7.1 ± 0.0	0.8 ± 0.0	10.0 ± 0.0	2.7 ± 0.1	0.7 ± 0.0	6.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
O$_2$	mg l^{-1}	4.4 ± 0.1	4.3 ± 0.7	4.3 ± 0.0	3.6 ± 0.1	4.1 ± 0.1	3.9 ± 0.1	1.5 ± 0.2	2.7 ± 0.1	4.2 ± 0.0	4.6 ± 0.1
Redox	mV	183 ± 1.4	181 ± 0.7	182 ± 0.0	177 ± 4.2	190 ± 2.8	182 ± 0.7	177 ± 1.0	144 ± 2.8	186 ± 0.0	159 ± 0.7
pH	–	5.2 ± 0.03	5.8 ± 0.08	6.4 ± 0.06	6.3 ± 0.03	6.1 ± 0.03	6.2 ± 0.01	5.2 ± 0.00	5.8 ± 0.08	6.1 ± 0.06	5.3 ± 0.05
Sand	%	0.98	0.51	0.30	0.67	0.59	0.58	0.22	0.39	0.52	0.76
Mud	%	0.00	0.02	0.04	0.01	0.02	0.03	0.07	0.01	0.02	0.04
TOC	%	0.10	0.12	0.22	0.10	0.16	0.48	0.73	0.10	0.30	0.73
TN	%	0.04	0.07	0.09	0.06	0.09	0.10	0.10	0.06	0.07	0.09

Table 2

Mean (±sd) density per replicate (0.007 m^{-2}) of the species (or unidentified taxa) considered for analysis at each site. Codes for each species, class (Mal, Malacostraca; Oli, Oligochaeta; Pol, Polychaeta; Biv, Bivalvia; Gas, Gastropoda) and feeding guild (DF, Deposit-feeder; SF, Suspension-feeder; O, Omnivorous; P, Predators) are shown. The categories of the order aggregation type are provided in full. Species are shown in decreasing total abundances accounted over the whole set of sampling sites (last column).

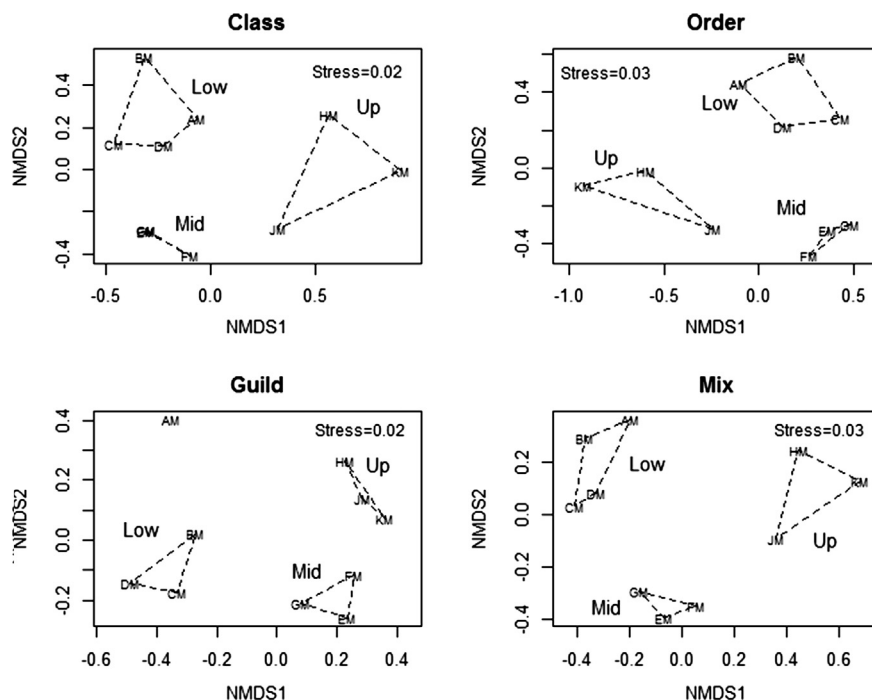| Species	Code	Class	Order	Guild	AM	BM	CM	DM	EM	FM	GM	HM	JM	KM	Totals
Corophium multisetosum	Com	Mal	Amphipoda	DF			2.29 ± 1.8	0.86 ± 0.9	79.86 ± 36.89	68.57 ± 35.93	33 ± 7.66	0.86 ± 0.69	5.57 ± 7.04		1337
Limnodrilus hoffmeisteri	Lim	Oli	Haplotaxida	DF								18.14 ± 7.88	1.43 ± 2.94	22.86 ± 22.56	297
Hediste diversicolor	Hed	Pol	Phyllodocida	O	1 ± 0.82	7 ± 2.71	7.43 ± 1.9	6.14 ± 2.19	0.86 ± 0.69	0.71 ± 0.75	2.71 ± 1.98	1.14 ± 1.07	0.71 ± 0.49		194
Streblospio shrubsolii	Str	Pol	Spionida	DF		9 ± 3.83	9.14 ± 9.15	3.14 ± 4.02	4.71 ± 2.29		0.29 ± 0.76				184
Corbicula fluminea	Cof	Biv	Veneroida	SF					0.86 ± 0.69	3.43 ± 1.4		5.71 ± 2.63	7.43 ± 2.7	6.86 ± 1.46	170
Potamopyrgus antipodarum	Pot	Gas	Littorinimorpha	DF						1.71 ± 1.6		0.14 ± 0.38	19.29 ± 14.11	2.57 ± 1.51	166
Cyathura carinata	Cya	Mal	Isopoda	P		0.29 ± 0.49	2 ± 0.82	1.57 ± 1.72	8.29 ± 2.93	3.71 ± 3.09	0.57 ± 0.79		0.57 ± 0.53		119
Chironomida und.	Qui	Oth	Oth	DF						0.57 ± 0.98		0.14 ± 0.38	0.43 ± 0.79	13.14 ± 6.33	100
Psammoryctides barbatus	Psa	Oli	Haplotaxida	DF								0.29 ± 0.76		12.71 ± 11.18	91
Insecta und.	Ins	Oth	Oth	Ot	0.14 ± 0.38			0.14 ± 0.38						3.43 ± 5.62	26
Oligochaeta und.	Oli	Oli	Oth	DF										2.71 ± 3.45	19
Neomysis integer	Neo	Mal	Mysida	O		0		0.14 ± 0.38	1.14 ± 2.19			0.49 ± 1.13		0.86 ± 1.21	18
Tubifex tubifex	Tub	Oli	Haplotaxida	DF										2.14 ± 4.49	15
Saduriella losadai	Sad	Mal	Isopoda	Ot						1 ± 1	0.14 ± 0.38		0.86 ± 0.69		14
Scrobicularia plana	Scr	Biv	Veneroida	DF		0.71 ± 0.76		0.43 ± 0.53							8
Gyraulus laevis	Gyr	Gas	Hygrophila	DF										1 ± 0.82	7
Cerastoderma edule	Cer	Biv	Veneroida	SF	0.29 ± 0.49	0.57 ± 0.53									6
Hydrobia ulvae	Hyd	Gas	Littorinimorpha	DF				0.71 ± 0.76	0.14 ± 0.38						6
Crangon crangon	Cra	Mal	Decapoda	P			0.43 ± 0.79								3
Gammarus chevreuxi	Gam	Mal	Amphipoda	O						0.43 ± 1.13					3
Nematoda und.	Nem	Oth	Oth	Ot										0.43 ± 0.53	3
Arenicola marina	Are	Pol	Capitellida	DF		0.29 ± 0.49									2
Heterotanais oerstedii	Het	Mal	Tanaidacea	DF				0.14 ± 0.38		0.14 ± 0.38					2
Lekanesphaera levii	Lek	Mal	Isopoda	DF						0.29 ± 0.76					2
Sphaeroma serratum	Sph	Mal	Isopoda	DF	0.29 ± 0.49										2
Spiophanes bombyx	Spio	Pol	Spionida	DF				0.29 ± 0.49							2
Capitella capitata	Cap	Pol	Capitellida	DF	0.14 ± 0.38										1
Scolelepis fuliginosa	Sco	Pol	Spionida	DF	0.14 ± 0.38										1
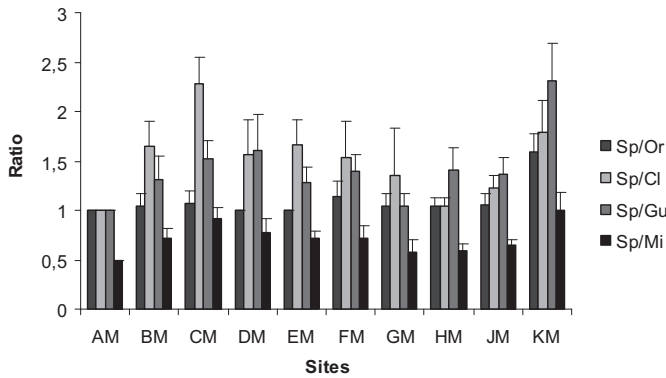
Fig. 2. Mean values of the ratio (±sd) between number of species and number of the following: orders (Sp/Or), classes (Sp/Cl), guilds (Sp/Gu), and mixed categories (Sp/Mi) per site.

21.5 ± 0.3 °C to 24.2 ± 0.6, reflecting summer values. Salinity was maximal close to the mouth of the estuary (site AM) and decreased upriver until reaching zero values at the uppermost sampling stations. The south bank tended to higher salinity values relative to the longitudinal axis (see CM compared with DM and EM compared with GM sites). Concentrations of dissolved oxygen were similar at all sampling sites (approximately 4 mg l^{-1}), except for the minimum values of 2.7 ± 0.1 and 1.5 ± 0.2 mg l^{-1} recorded at sampling sites HM and GM, respectively. The redox potential was positive at all sites (minimum 144 ± 2.8 in site HM), although the values tended to be higher in the lower part of the estuary. The pH tended to be low, and the maximum value (6.4 ± 0.06) was measured in sampling site CM. Coarse and medium sand predominated in most of the sampling sites (7 of 10 sites). The organic matter contents (estimated as total organic carbon) were generally higher in the uppermost sampling sites, while total nitrogen concentration, measured as a proxy for the quality of the organic matter in the sediment, were highest in the mid-estuary sites (FM, GM).

In total, 70 samples from the ten sampling sites yielded 2798 individuals belonging to 28 different taxa (Table 2). The most frequent taxonomic grouping at the order level was Isopoda and at the class taxonomic level, Malacostraca. The most common feeding type was deposit feeders. The ratio of the number of species to the number of higher taxa, guilds and mix categories per site is shown in Fig. 2. The ratio was maximal for guilds in site KM (2.3 ± 0.4) but the grand mean of the ratio for all sites was higher for data aggregated in class (1.5 ± 0.3). The ratio between the number of species and the number of classes was higher in the low and mid estuary, whereas the ratio tended to be higher for guilds in the upper estuary. Because the ratio of the number of species to the number of categories in the mix aggregation included each species twice, its value was below or equal to 1 for sites along the estuary (grand mean for all sites 0.7 ± 0.2).

The ordination of the sites by NMDS yielded similar results for each aggregation type, with a low stress value (maximum 0.03), indicating an excellent fit (Clarke and Warwick, 1994). Cluster analysis identified three groups of sites within each aggregation, with the exception of the ordination of guilds, for which four groups were formed because of the isolated representation of site AM (Fig. 3). The stress value for the NMDS based on species (data not shown) was also low (0.03), and the same three clusters of sites as those produced by order, class and mixed aggregation types were identified. Basically, the clusters of sites reflected their location along the longitudinal axis of the estuary within the study site (lower, mid and upper location, Figs. 1 and 3).

The results of the PERMANOVA test applied to these groups of sites (except site AM for the low cluster of sites) were consistent with those obtained by NMDS and cluster analysis. The test found significant differences in macrofaunal composition between the three clusters of sites regardless of the aggregation type used for the analysis. The lowest *F* value corresponded to the species data set ($F_{2,6} = 8.317$, $P = 0.001$). The pairwise comparisons between groups were also consistent among the aggregation types; all three groups of sites were found to differ significantly in all cases, and the

Fig. 3. Non-metric Multidimensional Scaling plot displaying the similarity between sites on the basis of the presence of different aggregation types. The groups of sites identified by cluster analysis are also shown (lower, mid and upper estuary).

highest probability was 0.023 ($t = 2.511$) for comparison of the lower and upper clusters of sites within the "order" aggregation.

We used the IndVal procedure to identify the faunal categories of the different aggregation types that characterized each of the groups of sites (Table 3). The IndVal procedure revealed different faunal compositions for each cluster of sites, in the same way as identified by NMDS and PERMANOVA. The upper group was best characterized in any aggregation type by a larger number of taxa. Conversely, the middle group was mainly characterized by taxa of the class Malacostraca. Interestingly, the orders Amphipoda and Isopoda were found to be characteristic, but only one amphipod species (*Corophium multisetosum* Stock, 1952) was clearly attributable to the middle group of sites (Tables 2 and 3). The output for the mixed aggregation was identical (by definition) to the joint results obtained for the class and guild aggregations. The statistical significance (P) in the IndVal analysis was generally higher for the species data set (Table 3).

We preceded the search for the best parsimonious RDA model by excluding the mostly highly correlated environmental variables (see variance inflation factor in Section 2.4). We considered a full model, including the set of environmental variables shown in Table 1, except temperature. Temperature is a more appropriate variable for seasonal studies (e.g. Silva et al., 2006) or temporal gradients (McLusky, 1993). The variance inflation factors (vif) applied to the constrained variables in a full RDA model (based on sites centroid for the species data set) yielded the highest vif value (146) for sediment mud content. We recomputed the full model without mud as an environmental variable. The remaining environmental variables showed weak linear dependencies. The highest vif value (7.29) in this case corresponded to TN. The resulting full RDA model was significant ($P < 0.05$) and explained 94% of the observed variance (Table 4).

We calculated the parsimonious models centroids of each aggregation type for each site; the resulting triplots are shown in Fig. 4. Most of the parsimonious RDA ordinations revealed one significant environmental variable, usually redox potential (Fig. 4; Table 4). This was the case for ordinations based on mixed ($P = 0.0280$) and class ($P = 0.025$) aggregations and species data set ($P = 0.032$) (Fig. 5a; Table 4). The ordination based on order aggregation also identified redox potential and TN content as significant factors ($P = 0.020$ and $P = 0.045$, respectively). By contrast, the RDA ordination based on guilds identified salinity as a significant factor ($P = 0.008$; Fig. 4), although this environmental variable was not significant in the remaining ordinations. The model based on the order aggregation explained the highest percentage of variance (48%) and also showed the highest species–environment correlation (0.86), while the ordination based on guilds yielded the best fit (AIC = −19.85; Table 4). The grouping of sites identified by cluster analysis was still recognizable in the RDA ordination, as shown in Fig. 5a for the species data set. The clustering of sites in RDA by the different aggregation types was similar to that found by MDS (Figs. 3 and 4). The correlations between the categories within each aggregation type in the RDA triplots were similar to those yielded by IndVald analysis (Table 3). Finally, the RDA model selected for the species data set (Fig. 5b) included the set of environmental variables identified by the parsimonious models as exerting significant effects. The model summarized the general spatial distribution of sites in relation to the environmental variables. Thus, the redox potential tended to be higher for the lower and mid group of sites, salinity was higher for the lower group and TN was higher for the mid group (Figs. 4 and 5). The selected model for species data set included the latter environmental variables. This model improved the quantity of variance explained (53%) and the goodness-of-fit (AIC = −5.14) relative to the parsimonious model for the species data set (Table 4), although it included a non-

Table 3
Indicator values (IndVald) for the significant categories submitted to the IndVal test ($P < 0.05$) within each aggregation type used in the analysis. Codes for the categories of each aggregation type are displayed in Table 2.

Species Code	Group	IndVal	P	Order Code	Order	Group	IndVal	P	Class Code	Group	IndVal	P	Guild Code	Group	IndVal	P	Mix Code	Group	IndVal	P
Str	Lower	0.741	0.002	Spionida	Lower	0.741	<0.001	Pol	Lower	0.741	<0.001	O	Lower	0.690	<0.001	Pol	Lower	0.741	0.001
Hed	Lower	0.717	0.002	Phyllodocida	Lower	0.717	<0.001	Mal	Mid	0.635	<0.001	DF	Mid	0.385	<0.001	O	Lower	0.690	0.001
Scr	Lower	0.333	0.002	Amphipoda	Mid	0.749	<0.001	Oli	Upper	0.810	<0.001	SF	Upper	0.751	<0.001	Mal	Mid	0.635	0.001
Cer	Lower	0.190	0.059	Isopoda	Mid	0.350	0.046	Biv	Upper	0.646	<0.001					DF	Mid	0.385	0.001
Hyd	Lower	0.173	0.059	Haplotaxida	Upper	0.810	<0.001	Gas	Upper	0.536	<0.001					Oli	Upper	0.810	0.001
Com	Mid	0.748	0.002	Veneroida	Upper	0.646	<0.001									SF	Upper	0.751	0.001
Cof	Upper	0.812	0.002	Littorinimorpha	Upper	0.493	<0.001									Biv	Upper	0.646	0.001
Lim	Upper	0.667	0.002	Hygrophila	Upper	0.238	0.02									Gas	Upper	0.536	0.001
Pot	Upper	0.583	0.002																
Qui	Upper	0.442	0.002																
Psa	Upper	0.381	0.002																
Oli	Upper	0.286	0.009																
Gyr	Upper	0.238	0.041																
Ins	Upper	0.234	0.041																

Table 4
Summary of the RDA models for each aggregation type. The significant environmental variables are shown, jointly with the explained variance, the correlation between the biotic and environmental variables (I or II first axes), the goodness-of-fit of the models (AIC), the F and significance (P) of each model. DF = degrees of freedom (model, residuals). TN = Total nitrogen; Sal = Salinity.

Category tested	Type of model	Environmental variables	Explained variance (%)	Biotic—env correlation	AIC value	DF (mod, res)	F value	Significance P
Species	Full	All	94	I-0.99/II-0.99	−18.03	7, 2	4.651	0.002
Species	Parsimonious	Redox	23	I-0.84	−4.1	1, 8	2.332	0.028
Class	Parsimonious	Redox	31	I-0.83	−8.32	1, 8	3.522	0.025
Order	Parsimonious	Redox + TN	48	I-0.86/II-0.77	−7.33	2, 7	3.219	0.003
Guild	Parsimonious	Sal	34	I-0.77	−19.85	1, 8	4.078	0.012
Mix	Parsimonious	Redox	25	I-0.81	−11.66	1, 8	2.708	0.028
Species	Chosen	Redox + Sal + TN	53	I-0.88/II-0.84	−5.14	3, 6	2.278	0.009

significant third constrained axis (variance explained = 3.5%; $F_{1,6} = 0.697$; $P = 0.640$).

The degree of multivariate correlation between the ordinations obtained by both MDS and RDA ordination methods are shown in Table 5. The MDS ordinations were more consistent than the RDA ordinations. The lowest value corresponded to the comparison between class and guilds aggregation types ($m^2 = 0.786$), although the degree of correlation was statistically significant ($P = 0.002$), as in the entire set of comparisons ($P = 0.001$ in the remaining cases). Examination of the correlation between the RDA ordinations revealed the weakest correlation between the class and guilds aggregations ($m^2 = 0.734$, $P = 0.005$) and between species and guilds aggregations ($m^2 = 0.756$, $P = 0.002$). Interestingly, the correlation between species and class matrices was higher ($m^2 = 0.991$, $P = 0.001$) than that between species and order aggregations ($m^2 = 0.967$, $P = 0.001$) among the RDA ordinations.

The test of multivariate homogeneity of group dispersions detected significant differences in the overall variability across aggregation types ($F_{4,345} = 40.715$, $P < 0.001$; Fig. 6). Pairwise comparisons identified significant differences between aggregation types, except between species and order ($P_{adj} = 0.964$) and between order and class aggregations ($P_{adj} = 0.085$). The average distance to the aggregation centroid was minimal for the guilds (0.449) and maximal for the species data set (0.782).

4. Discussion

We have shown that multivariate analysis of agglomerated data grouped at a low level of resolution (at the order and class taxonomic level and at gross feeding guilds categories) provide similar results and high degree of correlation and consistency with those obtained using the species data set. The mixed approach (after merging the class and guild categories in a single matrix) also proved to be useful for describing the distribution patterns of intertidal macrobenthic assemblages in the Minho estuary. The use of agglomerated data in RDA models also identified as significant a number of environmental variables that would be disregarded by considering only the species data. Thus, although agglomerated data may used to describe patterns of macrobenthic assemblages, they were also found to be complementary to the species data set for selecting better RDA models.

There is clear evidence that the results of the analysis of agglomerations of macrobenthic species were consistent with previous findings for these species in the Minho estuary. The observed distribution of the intertidal macrofauna is comparable to that described by Sousa et al. (2008a) for subtidal macrobenthic assemblages in similar sampling sites in the Minho estuary. These authors also found that salinity was an important environmental driver explaining the spatial distribution of the assemblages, and
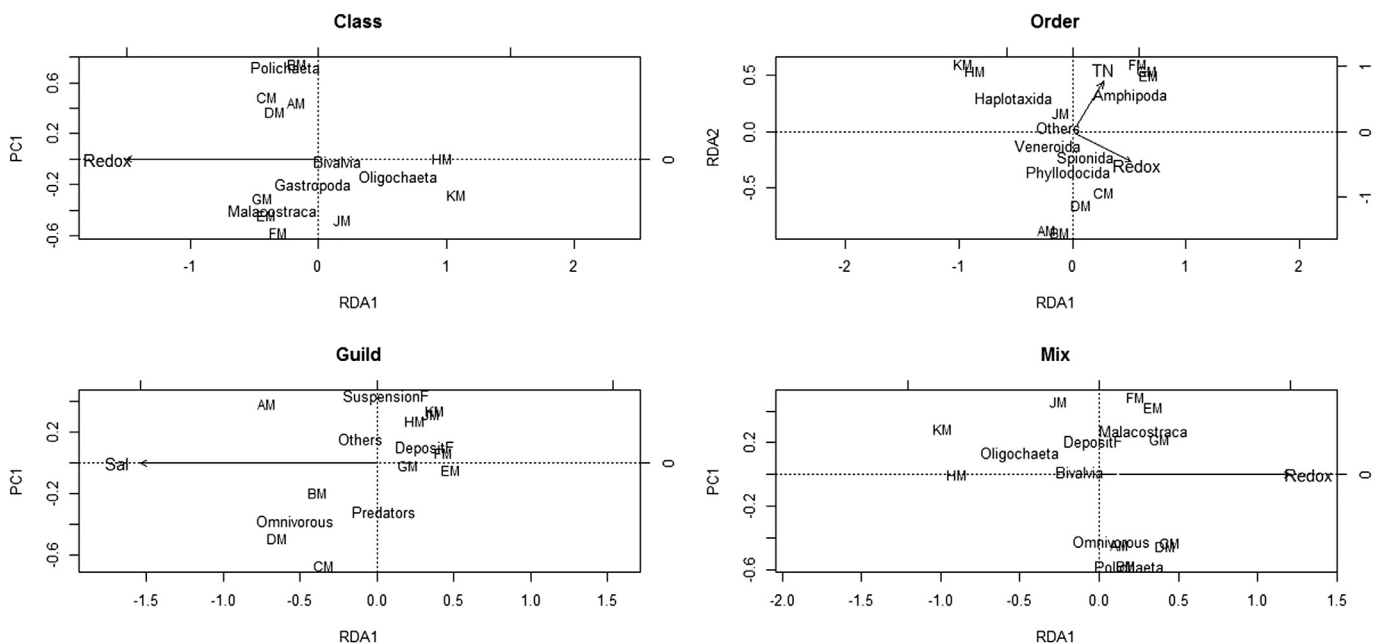
Fig. 4. Triplot graphs of the RDA parsimonious model. Note the different environmental variables found to be significant in relation to the aggregation type considered.
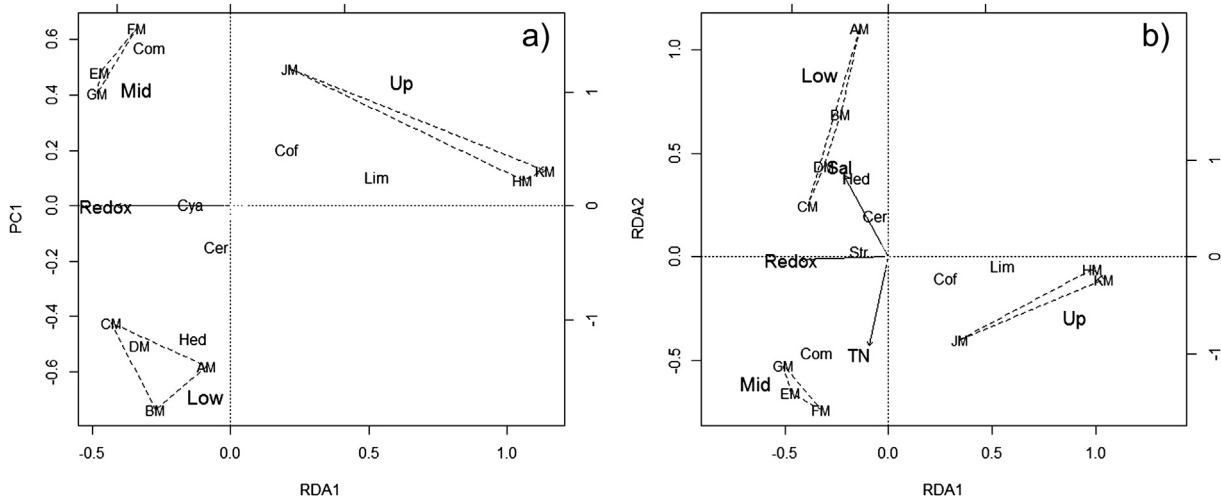
Fig. 5. Triplot graphs of RDA models for species with groups of sites identified by cluster analysis (lower, mid and upper): a) parsimonious model b) selected model. Species codes are shown in Table 2.

that grain size and organic matter content were important environmental constraints within clusters of sites. Mazé et al. (1993) characterized the distribution of the macrobenthic assemblages as a sharp transition from marine to freshwater communities in the Minho estuary. The present results are also consistent with those of the latter study, although these authors had not yet described *C. fluminea* as part of the macrobenthic assemblages at the upper sampling sites, where species of the class Malacostraca predominated. The current presence of *C. fluminea* in the upper reaches has clarified the transition from marine to freshwater assemblages in the Minho estuary (Sousa et al., 2008a).

The macrobenthic assemblages were grouped into three clusters in relation to their distribution on the longitudinal axis of the Minho estuary (except for the guilds, which did not include site AM in the lower group of sites). Redox potential was the most significant environmental factor, and it differentiated the lower and mid cluster of sites from the upper group (Figs. 4 and 5; Table 4). Redox potential values are associated with the organic matter content, grain size and chemical forms of nutrients in estuarine sediments (e.g. Mucha and Costa, 1999). Salinity was also identified as a longitudinal environmental descriptor, and it differentiated the lower group from the mid and upper clusters of sites (Fig. 4). The distribution of macrobenthic assemblages in estuaries is largely explained by the salinity gradient (Ysebaert et al., 1998). Moreover, the joint influence of salinity and redox potential as important environmental variables in relation to the distribution of macrobenthic assemblages has been reported previously (Rodrigues et al., 2011). Total nitrogen was associated with the mid-estuary group of sites, which probably indicates some difference in the nutrient quality of the sediment (Rice and Rhoads, 1989). Interestingly, these important environmental factors were found by considering different species aggregation matrices as response variables

(Table 4). Muniz and Pires-Vanin (2005) and Olsgard et al. (1997) also found different results for the environmental variables in different aggregation types, although they did not consider these differences as major findings.

The use of taxonomic sufficiency at a family level has been recommended for studies involving pollution monitoring (Gómez Gesteira et al., 2003) or identification of natural spatial patterns (Dethier and Schoch, 2006). In some cases, the effect of pollution is portrayed at lower taxonomic resolution, at the level of order (Ferraro and Cole, 1990) or even phylum (Warwick, 1988). In both of the latter studies, the authors proposed that an environmental perturbation is reflected at higher taxonomic levels as the severity of the perturbation increases. This may be the case in the unpolluted Minho estuary, in which a strong natural gradient within a distance of a few kilometres (Fig. 1) allows identification of the
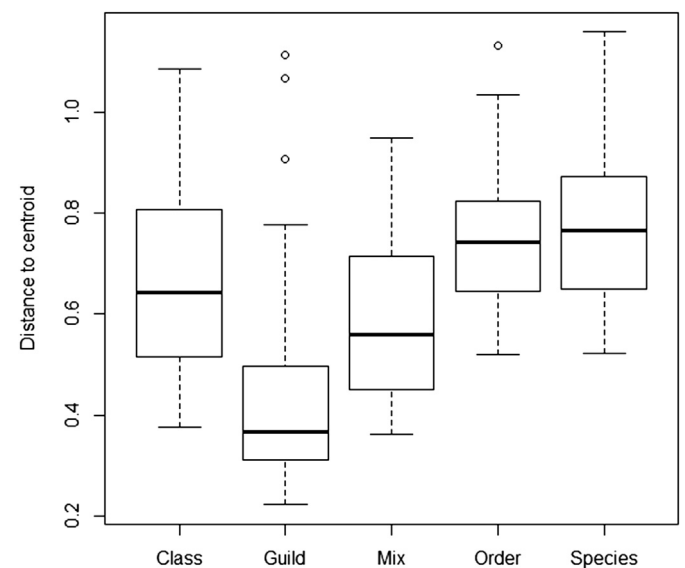
Table 5
Degree of correlation between the ordinations obtained by MDS and the RDA parsimonious models. All the correlations between ordinations were statistically significant ($P < 0.05$).

MDS	Species	Order	Class	Guild	RDA	Species	Order	Class	Guild
Order	0.974				Order	0.967			
Class	0.945	0.978			Class	0.991	0.945		
Guild	0.869	0.815	0.786		Guild	0.756	0.783	0.734	
Mix	0.977	0.976	0.971	0.899	Mix	0.986	0.940	0.993	0.784

Fig. 6. Boxplot of the multivariate dispersion of the different aggregation types used in relation to their general centroid. Horizontal lines within each box show the median plus the 25th and 75th percentiles at the box ends. Vertical dashed lines show either the maximum and minimum value or 1.5 times the interquartile range; individual points are outliers.

distribution of estuarine assemblages from the marine to the freshwater edges of the estuary, even with low resolution agglomerated data. Similarly, the nearby poikilohaline Mondego estuary characterized by a strong seasonal saline fluctuation was adequately described with regard to the distribution of macrobenthic assemblages from the lower to the upper estuary at the taxonomic resolution of order (Chainho et al., 2007). By contrast, the order level was considered a poor habitat descriptor in a relative small Mediterranean estuary with a sharp community transition (De Biasi et al., 2003). It would be interesting to test experimentally whether the degree of correlation of the species data set with aggregation types of lower resolution (such as class or phylum, characterized by a few categories) is higher at higher levels of perturbation. This may include coarse aggregations other than taxonomic levels, such as guilds, which showed an acceptable degree of correlation to the ordination based on species (Table 5).

The mix aggregation approach appears valid for analysing the distribution and composition of macrobenthic assemblages. In the present case, the class and guilds matrices were merged to produce a matrix of 11 categories, in contrast to the 30-variable-matrix resulting from the combination of 5 (classes) and 6 (guilds) categories. Each individual is represented twice in the mixed matrix, although under different categories. However, the categories may overlap, as was the case for the class Malacostraca and deposit-feeders (Fig. 4, Table 3). This may be complementary rather than redundant information, given that both types of aggregation are a priori unrelated because, unlike taxonomic aggregations, functional groups characterize ecosystem processes (Odum, 1969; Rosenberg, 2001). However, this may not always occur, e.g. in the class Bivalvia, which is largely dominated by suspension-feeding habits or when feeding groups overlap to a large extent with polychaete families (Fauchald and Jumars, 1979). It would be particularly interesting to assess the usefulness of the mixed aggregation when individual types of aggregation are found to be poor surrogates. Low ratios of the number of species to aggregated data sets (as in Fig. 2) usually indicate an adequate correlation between similarity matrices (Wlodarska-Kowalczuk and Kedra, 2007). As shown by the latter authors, higher ratios are sometimes associated with poorer correlations of similarity between the matrices based on species and matrices constructed on aggregated data. Because each species is considered twice in the mixed approach, this seems a good strategy for lowering the ratio between the species data set and data aggregated at lower resolution levels, such as the family level, which usually provides satisfactory correlations (Wlodarska-Kowalczuk and Kedra, 2007 and references therein). This hypothesis needs to be specifically tested in further studies. Regarding the merging of data sets, the mixed ordinations (Figs. 3 and 4) appear to be driven by the subset of data belonging to the most dispersed aggregation, (class aggregation, Fig. 6).

The RDA ordinations probably identified different environmental variables as being important (Fig. 4; Table 4) because of the different multivariate dispersion within each aggregation type (Fig. 6). The multivariate dispersion of the data sets decreased at lower levels of resolution (Fig. 6), as reported by Terlizzi et al. (2008) and Musco et al. (2011) for the class taxonomic level. Given that response variables are constrained by linear combinations of the environmental variables, so that their dispersion in RDA analysis is maximized (Legendre and Legendre, 1998), the different nature of the aggregated data used may explain why different environmental variables are identified as being significant. For instance, the agglomeration of species in guilds allowed identification of salinity as significant because the spread of this aggregation type better matched the dispersion of salinity.

5. Conclusions

Ordination of the sampling sites by constrained and unconstrained multivariate methods provided similar results for the different levels of species aggregations. The degree of correlation in relation to the species ordination was generally high in all cases, but it was weaker for guilds, especially in the RDA ordination. The use of species data sets aggregated into groups of lower taxonomic resolution or functional groups may replace macrobenthic estuarine species data sets in environmental studies. Moreover, the simultaneous use of various aggregation matrices to explain the distribution of estuarine macrobenthic assemblages appears to provide complementary information and broader ecological insight than the use of species data alone to describe the assemblages. Therefore, agglomeration of species into coarse taxonomic or functional groups may be used in combination with (rather than instead of) species data sets, for a more comprehensive analysis of the distribution of macrobenthic assemblages in relation to environmental gradients.

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