The performance of trait-based indices in an estuarine environment

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22 **Abstract**

23 The performance of several indices of benthic functioning, based on the traits of estuarine macro-
24 invertebrates, was tested in the lower Mondego estuary (Portugal), whose two arms exhibit
25 different disturbance levels related to hydromorphology. The results showed that some indices
26 responded clearly to this type of disturbance and others not so well. We argue that the community-
27 weighted mean (CWM) trait values in combination with the newly developed SR-FRED index
28 provided the best overall picture of how the benthic communities might have been affected by
29 hydromorphological disturbance. This study also showed that certain indices should be used with
30 caution when dealing with communities with few and dominant species, such as in estuarine
31 environments.

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1. Introduction

Traditionally, species-environment relationships have often been studied using taxonomic-based indices (e.g. richness, diversity and abundance of species) (Tilman et al. 2001, Vandewalle et al. 2010). These indices may accurately describe spatial and temporal differences in the composition and structure between species communities. However, they do not capture the causal mechanisms underlying species-environment relationships (Statzner & Bêche 2010, Mouillot et al. 2013a, Stuart-Smith et al. 2013, Verberk et al. 2013). Trait-based indices are based upon the richness, diversity and abundance of species 'traits' (morphological, physiological and life-history characteristics of species), and offer a useful alternative approach, since a species' ability to deal with environmental disturbance is at least partly prompted by its traits (e.g. Dolédec et al. 1996, Townsend et al. 1997, Statzner & Bêche 2010, Mouillot et al. 2013a). The concept behind this approach is based upon Southwood's 'habitat templet theory' (1977), which states that the habitat provides the template upon which evolution forges species traits. When disturbance increases, only species with specific combinations of traits suitable for survival pass through the environmental filter.

Since the 1990s, the number of studies using trait-based indices to investigate the effects of environmental disturbance on different species communities has been steadily increasing (Statzner & Bêche 2010, Vandewalle et al. 2010, Verberk et al. 2013). Many of these studies have shown that species traits are, to some extent, predictably affected by disturbance (e.g. Statzner & Bêche 2010, Vandewalle et al. 2010, Stuart-Smith et al. 2013). The functional structure of communities (the traits displayed by the species in a community) have often been described quantitatively by calculating two trait-based indices: (a) the dominant trait-categories in a community, which can be measured by calculating the community-weighted mean trait values (CWM) and/or (b) functional diversity (FD) (Petchey & Gaston 2006, De Bello et al. 2010, Vandewalle et al. 2010). FD has been defined as the extent of trait differences among species in a community (Petchey & Gaston 2006), and can be further partitioned into three components: 1) functional richness, i.e. the number of species traits in a community; 2) functional evenness, i.e. the distribution of traits in a community weighted by the relative abundance of species; and 3) functional divergence, i.e. the degree of dissimilarity among traits weighted by the relative abundance of species (Mason et al. 2005, Villéger et al. 2008). Each component provides independent information on the trait structure, and a separate index is required to quantify each component (Mouchet et al. 2010, Mason et al. 2013). To date, there are about a dozen trait-based indices, most of which measure one component of FD, while only a few integrate more

72 components. Existing trait-based indices and the methods to calculate them are constantly being

73 upgraded and new indices, or forms of computing them, have been developed (e.g. Villéger et al.

74 2008, Laliberté & Legendre 2010, Mouchet et al. 2010, Schleuter et al. 2010, Mason et al. 2013,

75 Mouillot et al. 2013a). Most of these studies used theoretical models as surrogates for biological

76 communities along a hypothetical stress gradient, and their general conclusion is that many of the

77 tested FD indices are complementary, each one illustrating its own unique information of

78 community functioning. Mouchet et al. (2010) and Mason et al. (2013) recommended that any

79 study examining changes in assembly processes along disturbance gradients should employ

80 several complementary FD indices. As with taxonomic-based indices, trait-based indices also

81 have their pitfalls (e.g. Petchey & Gaston 2006, Verberk et al. 2013). For example, indices that

82 take abundance into account (e.g. Rao's quadratic entropy) measure the amount of trait

83 dissimilarity between two random individuals in a community (Botta-Dukát 2005) and by so doing,

84 might give a differential weight to the traits of the dominant species (Petchey & Gaston 2006). This

85 could provide a distorted picture of functioning in environments where a few species are naturally

86 dominant, as in estuaries. One possible solution when addressing this issue is to measure the

87 amount of trait 'dissimilarity' between 'species' in a community, instead of measuring it between

88 two random 'individuals'. One way of doing so is by measuring the amount of 'functional

89 redundancy' (FRED) among species in a community, i.e. the relationship between species

90 diversity (SD) and FD (*sensu* Rosenfeld 2002, Sasaki et al. 2009). FRED is defined as how much

91 a community is saturated by species with similar trait-categories (Petchey & Gaston 2006) and

92 can range from being non-existent, when all species display different trait-categories (FD = SD),

93 to maximum, when all species share the same trait-categories (i.e. they are functionally identical:

94 FD = 0) (De Bello et al. 2007).

95 As such, FRED has the potential to be used as an indicator of disturbance (Micheli & Halpern

96 2005, Sasaki et al. 2009). The concept behind it goes back to the 'habitat templet theory'

97 (Southwood 1977). FRED is expected to increase (to a certain extent) with increasing disturbance

98 due to the environmental filtering of traits, i.e. rare species with rare trait-categories unsuitable for

99 survival are the first to be filtered out, being substituted by species with less dissimilar trait-

100 categories that can cope with the increase in disturbance. In coastal and marine benthic

101 communities, FRED has been used rather as an indicator of 'ecosystem resilience' (e.g. Van der

102 Linden et al. 2012, Törnroos & Bonsdorff 2012, Darr et al. 2013, Dolbeth et al. 2013, Rodil et al.

103 2013), assuming that FRED acts as a natural 'buffer' against the loss of function in the event that

104 species are lost: the higher FRED is, the greater the probability that at least some of these species

105 will survive changes in the environment and maintain ecosystem functioning (Díaz & Cabido 2001,

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Loreau et al. 2001). These two seemingly contrasting concepts, as an indicator of disturbance or as an indicator of resilience, make FRED difficult to interpret. More so, because FRED can increase or decrease regardless of the number of species in the community (see also Sasaki et al. 2009), and here we argue that this relationship between FRED and species richness (SR) is important when investigating the effects of disturbance on species communities. Based on this relationship, we developed a new index, the SR-FRED index.

The aim of this study was to test the performance of the SR-FRED index in an estuarine environment, alongside nine other indices, seven of which were based on species traits. As descriptors we used subtidal benthic invertebrate communities from the Mondego estuary, Portugal.The lower estuary consists of a north and a south arm, each with different hydromorphological features, causing differences in the hydrodynamics and benthic community composition between both arms (Teixeira et al. 2009, Veríssimo et al. 2013b). The indices were tested according to the hypothesis that the benthic communities in the north arm are more disturbed, mainly due to the stronger hydrodynamic conditions, than the south arm communities. Thus, we expected to find a higher proportion of traits able to cope with that disturbance for the north arm communities, lower values of taxonomic and functional diversity indices, and lower values of the SR-FRED index.

2. Methods

2.1 Study site

The study was conducted in the lower Mondego estuary which is located on the west central Atlantic coast of Portugal (40º08'N, 8º50'E). The downstream part of the estuary consists of two arms with dissimilar hydromorphological features: the north and the south arms (Fig.1).

The north arm has been subjected to several physical interventions over the last few decades, such as river embankment (canalisation) and the construction of the Figueira da Foz harbour, as it is the estuary's main shipping channel. As a result, the north arm is deeper (4-8 m during high tide), handles most of the Mondego river's freshwater discharge, and the combination with fast tidal penetration of seawater results in strong hydrodynamic conditions, i.e. current velocities, turbidity and tidal salinity changes (Teixeira et al. 2008, Veríssimo et al. 2013a).The daily intensity of these conditions is further amplified by seasonal and annual changes in rainfall. The hydrological conditions in the north arm's downstream areas do not change much, i.e. water depth, bottom salinity (30-35) and sediment characteristics (mostly medium-sized sand with low organic matter content (Teixeira et al. 2008). Dredging activities take place only in the most downstream area of the north arm maintain an optimum depth for shipping activities (Ceia et al. 2013).

The morphology of the south arm was less changed, with most of its area (75%) being covered by intertidal mudflats, including seagrass and salt marsh areas. Between the end of the 1980s and 1998, eutrophication was a major threat to the ecological quality in the south arm. During this period, the riverhead connection with the north arm completely silted up, resulting in high water resident time, followed by eutrophication symptoms, which led to several negative impacts on the seagrass and benthic communities in the south arm's upstream stations (Patrício et al. 2009, Dolbeth et al. 2011). In 1998, limited communication between the two arms was re-established which led to a reduction in the water residence time and a general improvement in the ecological quality in the south arm (e.g. Grilo et al. 2011, Dolbeth et al. 2011). In 2006, the riverhead connection was completely restored, resulting in a further reduction of the water residence time (Veríssimo et al. 2013a). Still, most of the river's freshwater discharge flows through the north arm and, as a result, the hydrodynamic conditions are weaker in the south arm.

2.1.1 Disturbance in the benthic communities

154 Previous studies have pointed out that the benthic communities in the north arm are less diverse
155 than those in the south arm (e.g. Teixeira et al. 2008, Teixeira et al. 2009, Veríssimo et al 2013b)
156 and the main causes are the strong hydrodynamic conditions in this arm, making it difficult for
157 species to settle. The communities in the south arm are faced with milder hydro dynamic
158 conditions, and the higher habitat heterogeneity of this arm allows the settlement of different
159 species and higher species diversity compared to the north arm (Teixeira et al. 2008, Teixeira et
160 al. 2009, Veríssimo et al. 2013b). Although eutrophication has not been a major threat since 1998,
161 specific weather events such as floods (Winter 2006), droughts (summer 2005) and occasional
162 engineering works have also impacted the benthic communities of each arm in different ways (e.g.
163 Grilo et al. 2010, Dolbeth et al. 2011, Veríssimo et al. 2013a).

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165 *2.2 Data collection*

166 *Biological data*

167 We used benthic community data collected from six different subtidal stations in the north and
168 south arms of the Mondego estuary, from 2004 to 2008: three stations in the north arm (10, 11
169 and 12) and three stations in the south arm (4, 6 and 7) (Fig. 1).
170 At each station, three benthic samples (replicates) were taken with a van Veen grab (0.1 m^2) and
171 sieved in situ through a 1 mm mesh bag. Subsequently, the content was preserved in a 4%
172 buffered formalin solution. In the laboratory, the benthic invertebrates were sorted and identified
173 to species level. Biomass was estimated as ash-free dry weight (g AFDW m-2). Mysids and
174 decapods (crabs and shrimps) were removed from the analyses because the sampling method
175 underestimates the size of their populations (Couto et al. 2010, Neto et al. 2010).

176 We only considered spatial differences, since the different level of disturbance between the two
177 arms is for the most part related to the particular hydrodynamic conditions of each arm. Our
178 datasets contained the biological data collected during the spring months (March, April, May) to
179 avoid the months in which extreme climatic events occurred (summer 2005 and winter 2006), and
180 to remove the effects of temporal variations.
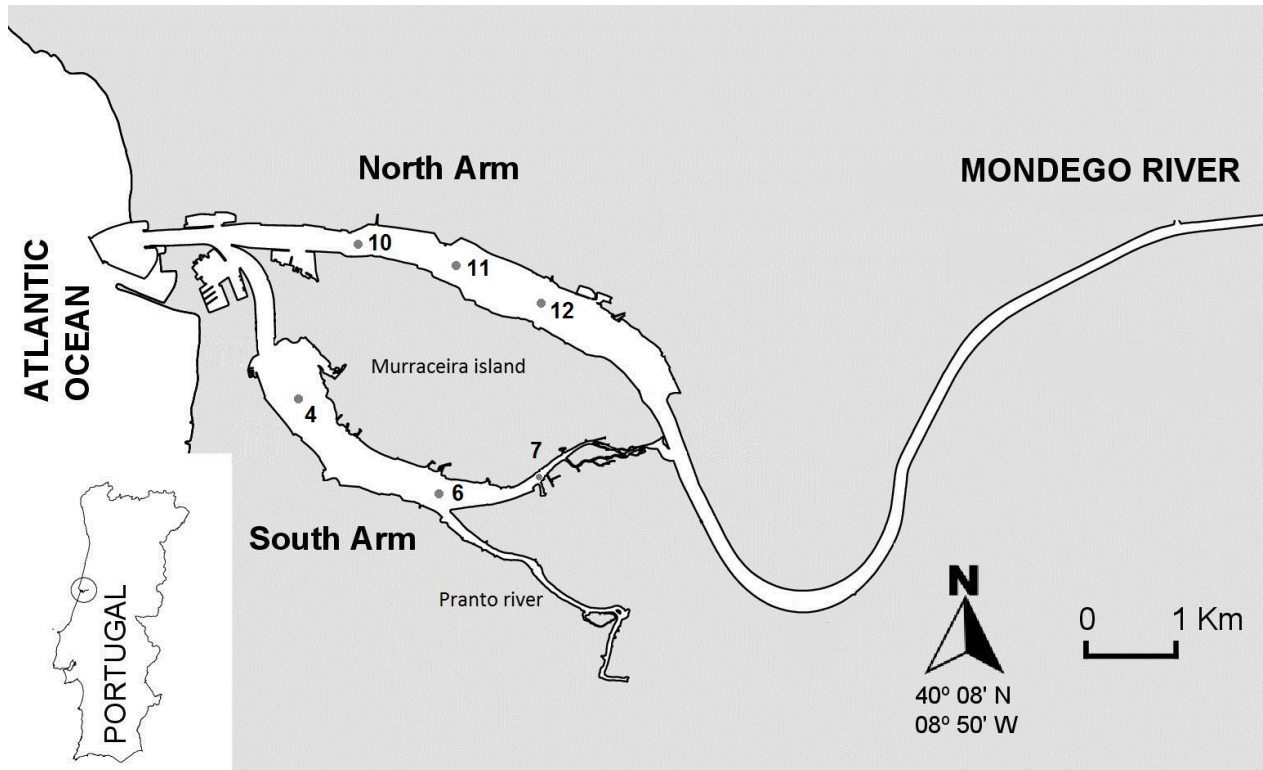
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183   Figure 1. The Mondego estuary and the sampling stations 10, 11, 12 (north arm) and 4, 6, 7 (south arm).

184   *Species traits*

185   We gathered the species traits from a variety of published sources (e.g. species identification
186   guides, scientific papers and established online databases such as MarLIN 2006 and WoRMS
187   Editorial Board 2014). A total of four traits containing 15 trait-categories were chosen for their
188   potential ability to indicate environmental disturbance (Table 1). Each species was assigned to the
189   trait-categories using a 'fuzzy coding' approach (Chevenet et al. 1994). The trait-categories were
190   given an affinity score between '0' and '3', with '0' indicating no affinity of a species to a trait-
191   category, and '3' indicating a high affinity to the trait-category. The fuzzy coding procedure makes
192   it possible to capture variation in the affinity of a given species to the different categories of a given
193   trait, thereby addressing spatial or temporal differences in the traits of a given species (Statzner
194   & Bêche 2010). We compiled these scores into the 'species by traits matrix (47 species and 15
195   trait-categories). To give the same weight to each species and each trait in further analyses, the
196   scores were standardised so that their sum for a given species and a given trait equalled 1 (or
197   100%).

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199   Table 1. Species traits (categories and rationale behind the trait selection).

Traits	Categories	Rationale behind the trait selection
Feeding strategy	Scavenger Grazer Filter Predator Deposit	Feeding traits determine the species abilities to utilise/tolerate different hydrodynamic conditions, with a switch from predominantly filter-feeders to deposit-feeders indicating a potential reduction in the hydrodynamic conditions (Rosenberg1995, Dolbeth et al. 2009).Grazers are more abundant in areas with high levels of primary producers, usually found in areas with low depth (Dolbeth et al. 2009) and potentially low hydrodynamics. Predators and scavengers will be associated to areas with high availability of prey (Dolbeth et al. 2009), and not specifically with hydrodynamic disturbance.
Living position	Burrow-dweller Tube-dweller Free-living	Tube-dwellers and burrow-dwellers are potentially less vulnerable to strong hydrodynamic disturbance, anoxic conditions and water pollution as opposed to free-living species because they can hide in their fixed tubes or burrows (Reise 2002).
Body size	Very-small (< 1 cm) Small (1-3 cm) Medium (3-10 cm) Large (> 10 cm)	Small-bodied species may characterise environments with high instability, the result of environmental/anthropogenic disturbances imposed on the organisms (Mouillot et al. 2006).
Life span	Short (< 1 year) Medium (1-5 years) Long (> 5 years)	Short-lived species increase in richness and abundance as disturbance increases (Pearson & Rosenberg 1978).

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201   *Data analysis*

202   Two basic matrices were used to perform all analyses and to compute the indices: the 'species-

203   biomass-by-station' matrix and the 'species-by-traits' matrix. We used R statistical software to run

204   the statistical procedures (R Core Team 2013). Data in the 'species-biomass-by-station' matrix

205   was transformed by log (1 + x) in order to reduce the influence of dominant species on the

206   samples.The standard affinity scores for each species in the 'species-by-trait' matrix were

207   multiplied by the species biomass at each station ('species-biomass-by-station' matrix), which

208   resulted in the 'trait-by-station' matrix. Ten indices were tested, including the new SR-FRED index

209   (see Table 2). For the computation of the indices, we used two types of software: R statistical

210   software, (including various packages) and an Excel macro file from Lepš et al. (2006) (available

211   from http://botanika.prf.jcu.cz/suspa/FunctDiv.php) (see Table 2 for details).

212   We tested all indices for significant differences between the stations and the two zones using the

213   Kruskal-Wallis test (Kruskal & Wallis 1952).Two separate Kruskal-Wallis tests were performed;

214   one using the stations as a factor, and the other using the zones as a factor. A p-value ≤ 0.05

215   indicated significant difference. Correlation among the indices was tested with a Pearson

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216 correlation test. We adjusted the reported p-values using the Holm method (Holm 1979)

217 (significantly correlated when p value is ≤ 0.05). Table 3 summarises the expected behaviour of

218 the indices according to the literature, and according to the hypothesis tested in this study.

219 Table 2. List of taxonomic and trait-based indices that were used for this study, with some distinctive

220 features: weighted by abundance, range of values, and the software used to compute these indices.

#	Labels	Index name	References	Weighted by abundance?	Range of values	Software used to compute the indices (reference)
1	SR	Species richness	Gotelli & Colwell 2001	No	$0 - \infty$	R: vegan package (Oksanen et al. 2011)
2	SIMD	Simpson diversity	Simpson 1949	Yes	$0 - 1$	Excel macro (Lepš et al. 2006) http://botanika.prf.jcu.cz/suspa/FunctDiv.php
3	CWM	Community-weighted mean trait values	Garnier et al. 2004	Yes	$0 - 1$	R: Ade4 (Thioulouse et al. 1997)
4	FRIC	Functional richness	Villéger et al. 2008	No	$0 - \infty$	R: FD package (Laliberté & Legendre 2010)
5	FEVE	Functional evenness	Villéger et al. 2008	Yes	$0 - 1$	FD
6	FDIV	Functional divergence	Villéger et al. 2008	Yes	$0 - 1$	FD
7	FDIS	Functional dispersion	Laliberté & Legendre 2010	Yes	$0 - \infty$	FD
8	FRAO	Rao's Quadratic Entropy	Botta-Dukát 2005	Yes	$0 - 1$	Excel macro (Lepš et al. 2006)
9	FRED	Functional redundancy	De Bello et al. 2007, Van der Linden et al. 2012	No	$0 - 1$	-
10	SR-FRED	Species richness-Functional redundancy	Present study	No	$-1 - +1$	-

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1. Species richness (SR) and 2. Simpson diversity index (SIMD)

223 SR measures the number of different species within a community, while SIMD takes into account

224 both the number of species and their abundance. SIMD measures the probability that two

225 randomly selected individuals within a community will belong to the same species, with values

226 constrained between 0 and 1. We expected a decrease in SR and SIMD after disturbance (Gotelli

227 & Colwell 2001).

3. Community-weighted mean trait values (CWM)

229 CWM is the mean trait value in a community weighted by the relative abundance (in our case

230 study, biomass) of the species in a community (e.g. the biomass of filter-feeding species) (Garnier

231 et al. 2004, Ricotta & Moretti 2011).We calculated the CWM values using the 'trait-by-station'

232 matrix. This metric has often been used to define the dominant trait-categories in a community

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233　and is directly related to the mass ratio hypothesis, which considers the traits of the most abundant
234　species to largely determine ecosystem processes (Ricotta & Moretti 2011). CWM can also be a
235　useful indicator of disturbance, because it makes it possible to perceive the shifts in the mean trait
236　values within the community due to environmental selection for certain traits (Vandewalle et al.
237　2010). This index can only be used to analyse single traits separately, making it possible to
238　quantitatively compare trait-categories. A higher proportion of disturbance sensitive traits is
239　expected after disturbance (Statzner & Bêche 2010).

240　*4. Functional richness (FRIC)*

241　FRIC measures the amount of trait space filled by the species in the community (Villéger et al.
242　2008). FRIC is independent from species abundance (Mason et al. 2005), has no upper limit and
243　requires at least three species to be computed (Laliberté & Legendre 2010). FRIC is expected to
244　decrease after disturbance (Mouillot et al. 2013a).

245　*5. Functional evenness (FEVE)*

246　FEVE measures the evenness in the distribution of abundance in the trait space (Villéger et al.
247　2008). FEVE will be maximised by an even distribution of both species and abundances in the
248　trait space. FEVE decreases either when abundance is less evenly distributed among trait-
249　categories or when some parts of the trait space are empty while others are densely populated.
250　FEVE values are constrained between 0 and 1 and need at least three species to be computed.
251　FEVE is expected to decrease after disturbance because species traits will become more
252　unevenly distributed among species (Villéger et al. 2008, Mouillot et al. 2013a).

253　*6. Functional divergence (FDIV)*

254　FDIV measures the degree to which abundance distribution in the trait space maximises the
255　divergence of trait-categories within the community, i.e. FDIV relates to how trait-categories are
256　distributed among individuals (Mason et al. 2005, Villéger et al. 2008). FDIV is low when the most
257　abundant species have trait-categories that are close to the centre of the trait space and high
258　when the most abundant species exhibit extreme trait-categories (Mason et al. 2005). FDIV values
259　are constrained between 0 and 1 and need at least three species to be computed (Villéger et al.
260　2008). FDIV is expected to decrease after disturbance (Mouillot et al. 2013a).

261　*7. Functional dispersion (FDIS)*

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262 FDIS measures the mean distance of individual species to the centre of the trait space occupied
263 by species (Laliberté & Legendre 2010) and accounts for both FRIC and FDIV (Mason et al. 2013).
264 FDIS has no upper limit and requires at least two species to be computed (Laliberté & Legendre
265 2010). FDIS is expected to decrease after disturbance (Mouillot et al. 2013a).

8. Rao's quadratic entropy (FRAO)

267 FRAO is a generalised form of the SIMD index that measures the amount of trait dissimilarity
268 between two random entities (individuals) in the community (Botta-Dukát 2005, Lepš et al. 2006).
269 In fact, if dissimilarity among all species pairs is maximum, then FRAO is identical to SIMD (Botta-
270 Dukát 2005). As a result, the SIMD index represents the maximum potential value that FRAO can
271 reach in a given community where the species completely differ in their trait-categories. FRAO
272 values are constrained between 0 and 1 and need at least two species to be computed (Lepš et
273 al. 2006). FRAO is conceptually similar to FDIS and simulations have shown high positive
274 correlations between the two indices (Laliberté & Legendre 2010). FRAO is expected to decrease
275 after disturbance (Mouillot et al. 2013a).

9. Functional redundancy (FRED)

277 FRED is the relationship between FD and SD (Sasaki et al. 2009) and measures the amount of
278 trait similarity between species in a community. FRED is defined as the extent to which a
279 community is saturated with species with similar traits (Petchey & Gaston 2006). FRED can range
280 from being non-existent, in which case all species have different trait-categories, to maximum, in
281 which case all species display the same trait-categories. FRED can be measured by subtracting
282 SD – FD, i.e. the potential FD minus the observed FD (as in De Bello et al. 2007), or it can be
283 measured by dividing FD/SD (as in Van der Linden et al. 2012). For this study, we calculated
284 FRED as FD/SD, with FD computed as Rao's quadratic entropy (FRAO) and SD computed as
285 Simpson diversity (SIMD). In order to obtain a regularly increasing index, it is necessary to invert
286 the formula into: 1 – (FRAO/SIMD). This way, maximum FRED is indicated by a value of '1' and
287 minimum FRED by a value of '0'. FRED is unaffected by dominant or rare species in the
288 community, since it measures trait similarity between species and not between individuals. In a
289 community of only 1 species, FRED will be 0. Based on its formulation, FRED is expected to
290 increase with disturbance (environmental filtering) until it reaches an asymptote (maximum FRED,
291 occurring when the surviving species share similar traits) (Micheli & Halpern 2005, Sasaki et al.
292 2009). For this reason, FRED is incapable of discriminating among levels of disturbance.

10. Species richness-functional redundancy (SR-FRED)

We hypothesise that in cases of non-disturbance, SR will be high and FRED will be low; when disturbance increases, SR will decrease while FRED will increase until reaching an asymptote. When disturbance increases even further, only SR will decrease, while FRED will remain maximal. Based on this relationship between SR and FRED, a new indicator is being introduced, the SR-FRED index, which measures the relationship between SR and FRED. The formula for SR-FRED is the following: SR-FRED = SR' – (1 – (FRAO/SIMD)), where SR' = SRobs / SRmax is scaled between 0 and 1 by dividing the observed SR value (SRobs) by a value of SR (SRmax) assumed to represent the "reference condition" of no disturbance within the dataset. Following the literature on the assessment of reference conditions for the evaluation of ecological quality (e.g. Andersen et al. 2004, Paganelli et al. 2011), SRmax was computed as the 90th percentile of SR distribution within the dataset; this procedure makes it possible to avoid misrepresentation of index results due to outliers (i.e. very high SR values in a single sample).In case of no disturbance, SR (1) – FRED (0) = 1; in case of medium disturbance SR (.5) – FRED (.5) = 0; in case of maximum disturbance SR (0) – FRED (1) = -1. This index requires at least two species to be computed. Since the observed SR has to be scaled by taking into account a percentile SR value, this index is only suitable for relative comparisons within a dataset.

311 Table 3. Expected (general) behaviour of the index values according to the literature, and their expected
312 outcome according to this study's hypothesis that the north arm communities are more disturbed due to the
313 stronger hydrodynamic conditions, than the south arm communities.

#	Labels	Index name	Expected (general) behaviour of the index values after environmental disturbance, according to the references	Expected outcome of the index values, according to this study's hypothesis; that the north arm communities are more disturbed due to stronger hydrodynamic conditions, than the south arm communities	
				north arm	south arm
1	SR	Species richness	Decrease (Gotelli & Colwell 2001)	Lower	Higher
2	SIMD	Simpson diversity	Decrease (Simpson 1946)	Lower	Higher
3	CWM	Community-weighted mean trait values	Higher proportion of trait-categories that are able to cope with the disturbance conditions (see Table 1 for details)	Higher proportion of trait-categories that are able to cope with the disturbance conditions	More even distribution of trait-categories
4	FRIC	Functional richness	Decrease (Mouillot et al. 2013a)	Lower	Higher
5	FEVE	Functional evenness	Decrease (Mouillot et al. 2013a)	Lower	Higher
6	FDIV	Functional divergence	Decrease (Mouillot et al. 2013a)	Lower	Higher
7	FDIS	Functional dispersion	Decrease (Mouillot et al. 2013a)	Lower	Higher
8	FRAO	Rao's quadratic entropy	Decrease (Mouillot et al. 2013a)	Lower	Higher
9	FRED	Functional redundancy	Increase (Micheli & Halpern 2005, Sasaki et al. 2009)	Higher	Lower
10	SR-FRED	Species richness-functional redundancy	Decrease (present study)	Lower	Higher

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314 **3. Results**

315 *3.1 Species biomass distribution*

316 The two arms show some marked differences in the spatial distribution of species mean biomass

317 (Fig. 2). The dominant species in the north arm is the polychaete *Nephtys cirrosa* and the bivalve

318 *Cerastoderma edule* (most dominant at station 10), while the dominant species in the south arm

319 are: *C. edule, Cyathura carinata, Hediste diversicolor* and *Scrobicularia plana.* There are also

320 differences among the stations, for example, *C. carinata* and *H. diversicolor* are more dominant at

321 stations 6 and 7, and *C. edule* and *S. plana* are more dominant at station 4.
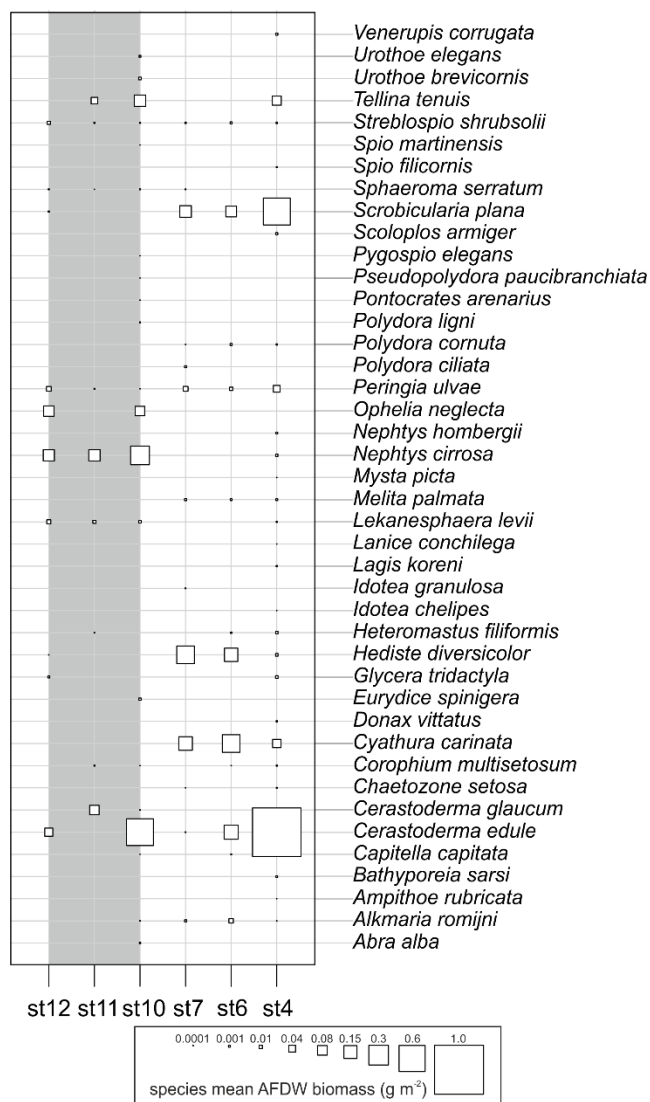
322

323 Figure 2. Spatial distribution of species mean AFDW biomass within the north arm (station 10, 11 and 12)

324 (indicated by the grey background) and the south arm (station 4, 6 and 7) of the Mondego estuary. The size

325 of the squares is proportional to the amount of mean biomass (larger squares equal higher biomass).

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3.3 Performance of the indices

3.3.1 Community-weighted mean trait (CWM)

The CWM values, i.e. the proportion of species biomass in the community with a given trait category, highlighted differences in the trait structure among stations and the two arms (Fig. 3). Most trait-categories showed significant differences between the stations and the two arms, except for predators (not significantly different between the two arms) and very-small sized species (less than 1 cm). In the north arm, most of the community is composed of medium-sized species (3 to 10 cm), with long-life spans (more than 5 years), burrow-dwellers, and scavengers and predators. This tendency maintains practically throughout the three stations of the north arm, with the exception of station 12, which shows a higher percentage of free-living, very small-size species and feeding traits more similar to the ones found in the south arm. Regarding the south arm, there is a higher proportion of small- (1 to 3 cm) and large-sized (more than 10 cm) species, with short (less than 1 year) and medium (1 to 5 years) life-spans, and a higher proportion of tube-dwelling and free-living species. These species are mostly grazers, deposit-feeders and filter-feeders. These tendencies were similar for the three stations, except for station 7, which has the highest proportion of short-lived, large-sized species.
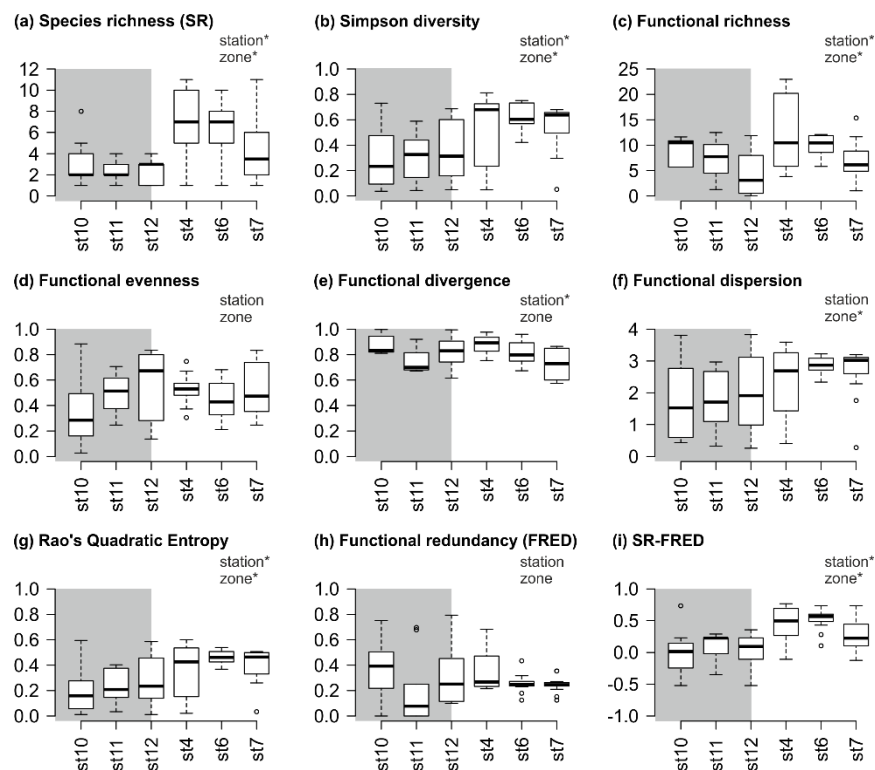
16
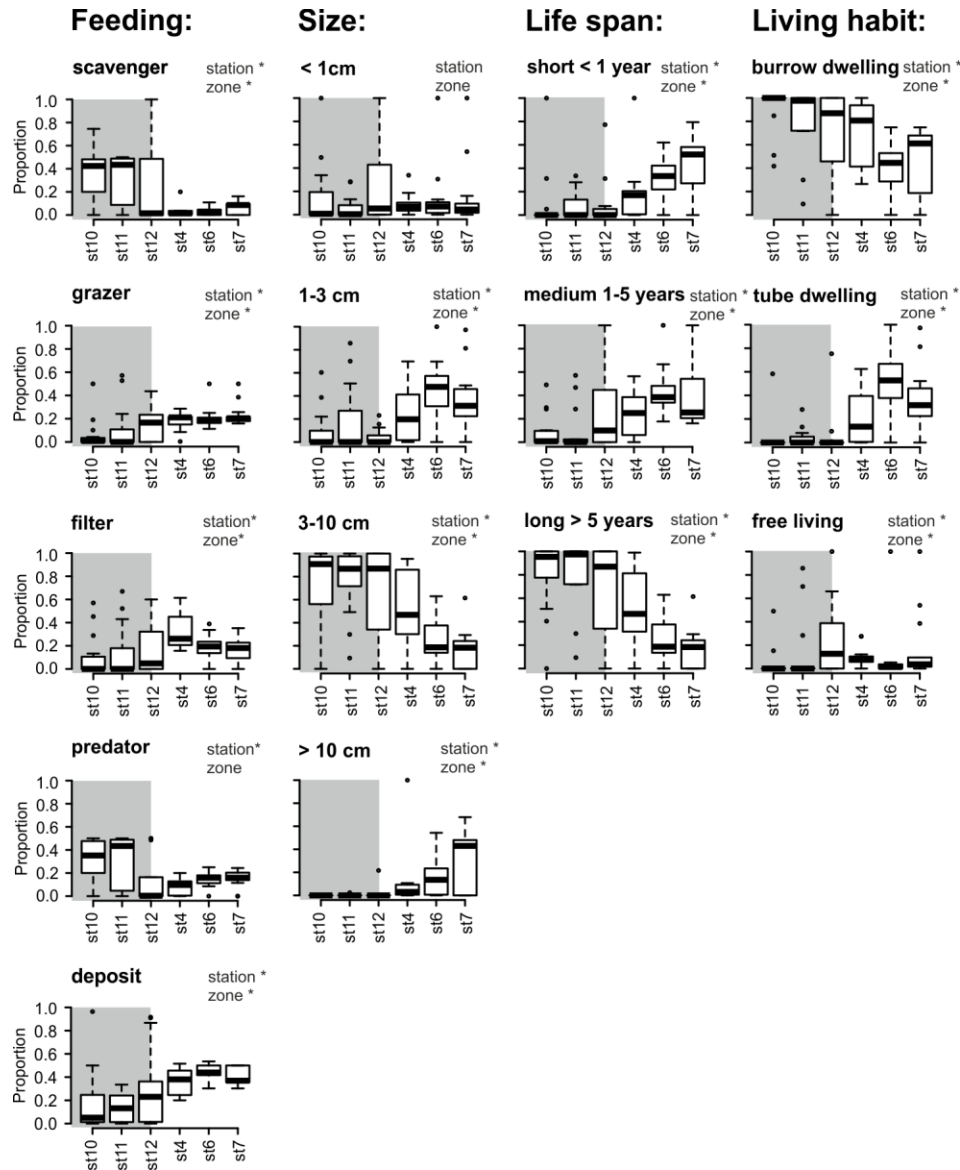
Figure 3. The spatial variability of Community-weighted mean trait values (CWM). The grey background indicates the three stations in the north arm; the white background indicates the three stations in the south arm. * indicates significant differences between the stations and/or the two zones: north and south arm (p-value ≤ 0.05).

3.3.2 Other indices

Fig. 4a-i shows the spatial variability of the index values. Functional dispersion (Fig. 4f) and functional divergence (Fig. 4e) were unable to detect significant differences between stations or zones, respectively. Functional evenness (Fig. 4d) and functional redundancy (Fig. 4h) were unable to detect significant differences between stations and zones; conversely, species richness (Fig. 4a), Simpson diversity (Fig. 4b), functional richness (Fig. 4c), Rao's quadratic entropy (Fig.

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354 4g) and the SR-FRED index (Fig. 4i) made it possible to detect both these differences and all of
355 them provided higher values in the south arm.

356 Most indices, except functional divergence (FDIV) were significantly correlated to two or more
357 other indices (Table 4). For example, species richness (SR) was significantly correlated to
358 Simpson diversity (SIMD), functional richness (FRIC), functional dispersion (FDIS), Rao's
359 quadratic entropy (FRAO) and the SR-FRED index, but this relation was most powerful with FRIC
360 (0.75) and SR-FRED (0.78).The SR-FRED index was also significantly correlated to several
361 indices. The relationship between this index and SR was stronger (0.78) than with FRED (-0.56).
362 There was also a highly significant and positive correlation between FRAO and FDIS (0.96).

363

364 Figure 4a-i. Spatial variability of the index values. The grey background indicates the three stations in the
365 north arm; the white background indicates the three stations in the south arm. * indicates significant
366 differences between the stations and/or the two zones: north and south arm (p-value ≤ 0.05).

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Table 4. Pearson correlation values between the indices. Reported p-values were adjusted using the Holm method (significantly correlated when p value is ≤ 0.05*).

	SR	SIMD	FRIC	FEVE	FDIV	FDIS	FRAO	FRED
SIMD	0.65*							
FRIC	0.75*	0.28						
FEVE	-0.07	0.47	-0.07					
FDIV	0.04	-0.23	0.10	-0.12				
FDIS	0.46*	0.93*	0.21	0.51*	-0.19			
FRAO	0.56*	0.92*	0.26	0.39	-0.17	0.96*		
FRED	0.06	-0.23	-0.03	-0.20	0.08	-0.46*	-0.52*	
SRFRED	0.78*	0.70*	0.57*	0.05	-0.02	0.68*	0.80*	-0.56*

4. Discussion

The aim of this study was to test the performance of the SR-FRED index alongside nine other indices, seven of which were based on species traits, in two estuarine arms subjected to different hydrological conditions. We tested the indices against the hypothesis that the benthic communities in the north arm of the Mondego estuary are more disturbed than the south arm conditions due to the stronger hydrodynamic conditions in the north arm. We expected that the indices would indicate this different level of disturbance between the arms. As a result, some indices clearly showed this difference, and others not so well. This section addresses the performance of the indices, having featured the most noteworthy outcomes.

The community-weighted mean trait (CWM)

The CWM revealed patterns in the spatial distribution of the trait-categories associated to each arm with a different disturbance level. Nonetheless, not all trait-categories responded to the disturbance as expected. The trait-categories' long life-span, very small and medium body size, burrow-dwellers and scavengers were most important in the north arm, while the trait-categories short life-span, small and large body size, tube-dwellers, grazers, deposit-feeders and filter-feeders were most important in the south arm.

As expected, deposit-feeders were more important in the south arm, as the milder hydrodynamic conditions allowed their food source (i.e. organic matter) to accumulate. Thus, deposit-feeders potentially indicated a reduction in hydrodynamic conditions, as also discussed by Rosenberg (1995) and Dolbeth et al. (2009). For the same reason, grazers (mostly *Peringia ulvae*) were also more important in the south arm. Grazers feed mostly on benthic algae and epiphytes, which are

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392 potentially more abundant in this part of the estuary due to its lower depth, large intertidal areas,
393 seagrass and salt marsh areas (Baeta et al. 2009).

394 We also expected filter-feeders to be more dominant in the north arm, because strong currents
395 usually provide favourable feeding conditions for bivalves (Rosenberg 1995, Gosling 2004).
396 However, they were more dominant in the south arm; in fact, they were the second-largest feeding
397 group, after deposit-feeders. Several of its dominant species may act both as deposit and filter-
398 feeders (e.g. *Scrobicularia plana,* Baeta et al. 2009), explaining in part the results obtained.
399 Verdelhos et al. (2015) also found this species to be more abundant in the south arm and reasoned
400 that its sediment preference is probably the main reason for this spatial distribution. This burrowing
401 clam has a preference for fine sand or mud, or sand/mud mixtures, such as those found in the
402 south arm (Verdelhos et al. 2015). Another important reason why this species might prefer the
403 south arm is its milder hydrodynamic conditions. Very strong currents may lead to excessive
404 sediment resuspension and water turbidity, which might affect species performance and survival,
405 namely through the clogging up of the feeding structures of these bivalves (Verdelhos et al. 2014).

406 Scavengers and predators were the most dominant trait-categories in the north arm. These traits
407 were mostly expressed by one single dominant species, the polychaete *Nephtys cirrosa*. This
408 species usually prefers more coarse sediments (as those found in the north arm) over more muddy
409 and fine-sand sediments (Clark & Haderlie 1960).

410 The very small-sized (<1cm) and short-lived species (<1 year) were also associated with the north
411 arm, as they may characterise environments with higher instability due to hydrodynamic
412 disturbance. However, the medium-sized (3-10 cm) and long-lived species (>5 years) were the
413 dominant trait-categories in the north arm. Again, these categories were mostly expressed by *N.*
414 *cirrosa*, which contradicts the former assumption. In fact, small-sized (1-3 cm) and short-lived
415 species were highly abundant in the south arm, when we expected the opposite due to the milder
416 hydrodynamic conditions. The species that mostly exhibited these trait-categories were *Cyathura*
417 *carinata* and *Peringia ulvae,* whose preferential habitats occur in the muddy intertidal areas, like
418 those within the south arm, where they can attain high production levels (Dolbeth et al. 2011).
419 Finally, we expected free-living species to be more dominant in the south arm because of the less
420 stressful hydrodynamic conditions. Instead, tube-dwellers and burrow-dwellers were the dominant
421 groups. Here too, the dominance of certain species clearly affected these results, and the
422 relationship between these traits and hydrodynamic disturbance was not totally clear. In this
423 estuarine system, deposit-feeders and grazers seem to be the best indicators of hydrodynamic
424 disturbance, whereas size and in particular life-span are not.

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Functional evenness (FEVE) and functional divergence (FDIV)

It was expected that FEVE and FDIV would decline in the presence of disturbance (Mouillot et al. 2013a), and yet we found no significant differences between the two arms. FEVE and FDIV values were unexpectedly high in the north arm, suggesting that the traits were regularly distributed in the community (especially obvious at stations 11 and 12) and that the most abundant species had dissimilar combinations of traits in comparison to the rare species. In the case of FEVE, its high values could have been related to the low amount of SR. Podani et al. (2013) found FEVE to be negatively correlated with SR, and reasoned that fewer species are more likely to produce an even distribution of traits than many species, due to decreasing functional redundancy (FRED) (see also Mouillot et al. 2013a). This study seems to support their reasoning; SR and FRED values were low, which was especially obvious at station 11. In the case of FDIV, its high values might have also been related to the low amount of SR in combination with the high abundance of a few dominant species. The chance that the most abundant species have dissimilar combinations of traits in comparison to the few rare species will be high. Another reason for the high values of both these indices can be related to their mathematical algorithm. For the computation of these indices (and for functional richness–FRIC), at least three species are required. However, some of the communities, especially the ones from the north arm, often have fewer than three species, and in these cases, FRIC, FEVE and FDIV values could not be computed. As in this study, several other studies also found FEVE to perform poorly. Mouchet et al. (2010) demonstrated that the power of FEVE to detect assembly patterns occurring in (theoretical) species communities was poor with SR values lower than 40, and very poor when SR was 10. Mason et al. (2013) also found low variation in FEVE, and mentioned the possibility that FEVE is simply not associated with changes in assembly processes. In the case of FDIV, Mouchet et al. (2010) observed medium to high power among all SR values: (FDIV was most powerful when SR was higher than 30).Contrary results were obtained in the studies by Mason et al. (2013) and Pavoine & Bonsall (2010), who found low power using FDIV. Taking the above into consideration, our main message is that caution is required when using these indices in environments where SR is naturally low, such as in estuarine environments.

Taxonomic versus trait-based indices

Functional-richness (FRIC) and Rao's quadratic entropy (FRAO), the functional counterparts of species-richness (SR) and the Simpson-diversity index (SIMD), were able to indicate the different level of disturbance between the two arms. As expected, all these indices showed higher values

457 in the south arm than in the north arm, and spatial patterns were significantly similar. The only
458 exceptions were the slightly different FRIC values in comparison to the SR values in the north
459 arm. This indicated that FRIC can increase or decrease regardless of SR; FRIC will show a higher
460 rate of increase or decrease when rare species with rare traits are added to or lost from the
461 community (Mouillot et al. 2013a). Most other studies also found a strong positive relation between
462 these two indices with different types of communities (Villéger et al. 2008, Schleuter et al. 2010,
463 Mason et al. 2013, Podani et al. 2013). The same accounts for FRAO and SIMD, which showed
464 similar patterns (Lepš et al. 2006, Vandewalle et al. 2010). FRAO was also significantly correlated
465 with functional-dispersion (FDIS), which was expected, because both these indices have a similar
466 mathematical background (Laliberté & Legendre 2010, Mason et al. 2013). Overall, the trait-based
467 indices FRIC and FRAO performed similarly to their taxonomic-based counterparts SR and SIMD,
468 which indicated that with the loss or addition of a species, unique traits were being lost or added
469 to the community. Again, in communities with few and dominant species, such as those occurring
470 in estuarine environments, abundance-weighted diversity indices like SIMD, FRAO and FDIS
471 should be interpreted with caution. These indices measure the amount of (trait) dissimilarity
472 between two random individuals in a community. As a result, the chance of these individuals
473 belonging to a particular dominant species is very high, resulting in low (trait) dissimilarity (low FD
474 and SD). By so doing, these indices fail to take into account rare species and their traits, which
475 could lead to an underestimation of FD and SD. Rare species often have distinct combinations of
476 traits, thus increasing the FD of communities (Mouillot et al. 2013b).

477 According to Southwood's 'habitat templet concept' (1977), rare species with rare combinations of
478 traits are the first to be filtered out when disturbance increases, with the remaining species usually
479 being the ones with traits that can cope with this increased disturbance. Therefore, the extinction
480 of rare species and their traits might provide an advance warning to increasing disturbance
481 (Mouillot et al. 2013a). Considering this, indices that indicate the extinction of these rare species
482 and their traits might be especially useful to investigate the effects of disturbance, especially in
483 environments where a few dominant species naturally occur.

484 *Functional redundancy (FRED and SR-FRED)*

485 FRED measures the amount of trait-dissimilarity among 'species' and not among 'individuals' the
486 way that FRAO and FDIS do. As a result, FRED is not weighted by abundance, i.e. all species are
487 equally important. When rare species with rare combinations disappear because of increasing
488 disturbance, FRED will increase, because the remaining species share traits that are more similar.
489 Thus, FRED might be a potential 'early warning' indicator for increasing disturbance. Nonetheless,

FRED should be used with caution as a disturbance indicator. In highly disturbed environments where very few species can survive (low SD), the FD of the community might rapidly approach the value of SD, thus resulting in low FRED. For this reason, FRED was not significantly different between the two arms, in contrast to expectations (higher FRED was expected for the north arm). This result was especially due to station 11, featuring low FD in combination with low SD. Moreover, FRED can increase or decrease regardless of the amount of SR in the community; for this reason, we developed the new SR-FRED index which takes into account the nonlinear relationship between FRED and SR.SR is a critical variable for the interpretation of FRED (Petchey & Gaston 2002, Sasaki et al. 2009)

The SR-FRED index succeeded in indicating potentially higher levels of disturbance in the north arm, as opposed to the south arm. We argue that this new index provided the clearest picture of the potential changes in the benthic functioning regarding the different level of hydrodynamic disturbance. The strength of this index is that it is based on both SD and FD, it is not affected by dominant species and it is able to give an indication of the level of disturbance. However, one important limitation of this index is that it uses reference values to normalise SR into the range 0-1. Consequently, results strongly rely on the size and quality of the available dataset. To test the full potential of this index, it has to be tested in other environments subjected to different types and levels of disturbance conditions.

Concluding remarks

Some indices responded clearly to the different level of hydrological disturbance in this estuarine ecosystem and others not so well. We argue that the community-weighted mean trait (CWM) in combination with the new SR-FRED index provided the best overall picture of how the benthic communities might have been affected by a different level of disturbance. The CWM index is useful for revealing patterns in the spatial distribution of the trait-categories, while the SR-FRED index makes it possible to combine and synthesise the taxonomic and functional structure of the communities. This study also showed that some indices should be used with caution when dealing with communities with few and dominant species, which often occurs in estuarine ecosystems.

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