The efficiency of Taxonomic Sufficiency for identification of spatial patterns at different scales in transitional waters

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16 Abstract

Taxonomic Sufficiency (TS), the use of coarser taxonomic resolution in monitoring plans, has been 17 receiving increasing attention in last years. A comprehensive dataset of macrobenthos from 18 18 Italian lagoons in a range of different latitude, typology, salinity and surface area, was analysed in 19 order to test the efficiency of TS, in terms of correlation between patterns at level of species and 20 patterns resulting from different levels of taxonomic aggregation. First, TS was applied on a range 21 22 of univariate indices, providing complementary information on macrobenthic community, in order to test the efficiency, in a contest of different taxonomic composition, and different number of 23 24 lower taxa belonging to the same higher taxon in each lagoon. Then, TS was applied on multivariate analyses, in order to test whether the efficiency changes between two different scales: local 25 26 (comparison of sites nested within each lagoon) and regional (comparison among lagoons), and with different data transformation. The patterns resulting from univariate indices and multivariate 27 28 analyses, at both local and regional scales, were retained till family level, despite the different levels of taxonomic composition and different number of lower taxa belonging to the same higher taxon of 29 30 different lagoons. Nevertheless, the correlation values among matrices and the effect of data transformation differed between regional and local scales. Our results support the efficiency of TS 31 32 until family level, but at the same time underline the need of scale- and region-specific baseline 33 knowledge prior application of TS in lagoons.

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35 Keywords: Taxonomic Sufficiency, lagoons, benthic community, multi-scale patterns,

36 Mediterranean Sea

38 **1. Introduction**

39 Taxonomic Sufficiency (TS) has been receiving increasing attention in recent years, in view of avoiding long and difficult precise taxonomic identifications and saving time and costs in marine 40 impact assessment and monitoring (Olsgard et al., 1998). The basic concept of TS (Ellis, 1985) is 41 that the identification of taxa at taxonomic levels higher than species enables the detection of 42 changes in assemblages exposed to environmental stressors without significant loss of information. 43 The use of coarser taxonomic resolution was first suggested by Warwick (1988a, 1988b) who 44 pointed out the theoretical advantages of conducting both multivariate and univariate analyses at 45 46 various hierarchical levels of taxonomic aggregation in monitoring programmes. Furthermore, the identification of organisms at a higher taxonomic level allows the analysis of a larger number of 47 48 replicates, increasing the spatial representativeness of the information. Studies supporting the efficiency of TS in marine systems were later performed worldwide in different habitat types, from 49 50 soft bottom (e.g. Vanderklift et al., 1996, Włodarska-Kowalczuk and Kędra, 2007) to hard bottom (e.g. Mistri and Rossi, 2000, Terlizzi et al., 2002), focusing on strong pollution gradients (Dauvin et 51 52 al., 2003), and on variation along natural gradients (Terlizzi et al., 2009). In coastal transitional ecosystems, TS has been mainly applied for the description of spatial patterns of macrobenthic 53 54 community in response to heavy disturbance, such as oil spill (Dauvin et al., 2003), or to compare regions with different levels of anthropogenic disturbance (Mistri and Rossi, 2001; Arvanitidis et 55 al., 2009), but it proved useful also for the discrimination of natural gradients in estuaries (Chainho 56 et al., 2007). 57

Coastal lagoons represent important and fragile ecosystems in the coastal landscape in many places 58 on the globe. They provide key ecosystem services such as water quality improvement, fisheries 59 resources, habitat and food for migratory and resident animals, protection from storms and touristic 60 61 appeal (Mitsch and Gosselink, 2000; Pérez-Ruzafa et al., 2011). At the same time they are also 62 heavily affected worldwide by human-driven pressures (Newton et al., 2014), such as urban and industrial discharges, agricultural runoff (e.g. Bellucci et al., 2000; Lillebø et al., 2005; Paerl, 2006; 63 Acquavita et al., 2014; Arienzo et al., 2013; Barhoumi et al., 2014; Pitacco et al., 2018a), fishery, 64 65 aquaculture (e.g. Viaroli et al., 2003; Sladonja et al., 2011; Munari and Mistri, 2014), and climate change (e.g. Lloret et al., 2008; Anthony et al., 2009; Pitacco et al., 2018b). Given the ecological 66 67 and economic importance of transitional environments, long-term monitoring is fundamental to understand the effect of anthropogenic stressors on macrobenthic community and thus to plan 68 69 efficient management for ecosystem conservation and restoration. Since those projects usually 70 benefit from a limited financial support, TS could be a useful tool for the improvement of 71 cost/benefits ratio, allowing a more efficient use of available resources (Chapman, 1998).

Most studies have usually compared different levels of TS at a single spatial scale. Recent investigations pointed out the importance of including multi-scale designs. Since processes driving species distributions vary across multiple spatial scales (Underwood & Chapman, 1996; Hewitt et al., 2007) taxonomic surrogates are expected to depend on the same scale-dependent processes (Smale, 2010). To date works investigating the effects of TS on the spatial distribution patterns at multiple scales are still scant (Dethier and Schoch, 2006; Tataranni et al., 2009; Smale, 2010; Mueller et al., 2013).

This paper analysed datasets of macrobenthic communities obtained through research programmes 79 80 carried out on 18 lagoons, representing a random subsampled of all Italian lagoons. Among those lagoons there were all main Adriatic and Tyrrhenian Italian lagoons, with different typologies, from 81 82 mesohaline to hyperhaline, non-tidal to nano- and microtidal lagoons, subjected to different anthropogenic pressures and different levels of protections. The aim of the work was to test the 83 84 efficiency of Taxonomic Sufficiency in detecting different components of univariate and multivariate variability of macrobenthic assemblages in transitional waters. The efficiency of TS 85 86 was estimated in terms of correlation between patterns observed at level of species and patterns resulting from different levels of taxonomic aggregation. With univariate analyses the effect of 87 88 taxonomic aggregation was tested in a contest of different taxonomic composition and number of lower taxa belonging to the same higher taxon in different lagoons. With multivariate analyses 89 (MDS, cluster, PERMANOVA) the effect of taxonomic aggregation was tested in relation with two 90 different spatial scales: regional (spatial variability among lagoons) and local (spatial variability 91 among sites nested within each lagoon). The effect of different data transformations (row data, 92 93 square root, logarithm, presence/absence) was considered, as well.

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2. Materials and methods

96 The analysed dataset was gathered during several sampling campaigns carried out in eighteen 97 Italian lagoons. Samples were collected repeatedly over time, from all main Adriatic and 98 Tyrrhenian Italian lagoons: Baiona, Barbamarco, Caleri, Canarin, Comacchio, Goro, Grado-99 Marano, Lesina, Marinetta, Palude della Rosa (north easterly part of the Venice lagoon), 100 Scardovari, and Vallona (Adriatic Sea), Caprolace, Feraxi, Fogliano, Orbetello, Porto Pino, and San 101 Teodoro (Tyrrhenian Sea). This dataset comprises different typology of transitional waters, from 102 large to small, from microtidal to non-tidal, hyperhaline to mesohaline ecosystems (see Table 1). A total of 107 sites nested within the lagoons, 64 of which were located in Adriatic and 43

104 in Tyrrhenian Sea (

Table 1), have been chosen as representative of the different habitats found within each transitional 105 106 environment. Samples were collected at least in triplicates at each site, sieved on a 0.5 mm meshsize sieve and retained macrobenthic fauna was fixed in 8% formalin. Animals were carefully 107 sorted, and determined in laboratory with the combined use of a stereomicroscope and a 108 microscope, according to basic taxonomic guides (e.g. Fauvel P., 1923; 1927; Ruffo, 1982-1998;) in 109 combination with the most recent taxa-specific relevant publications (e.g. San Martin G., 2003; 110 Viéitez et al., 2004). The identification was performed up to the species level in most cases 111 (exceptions were due to the poor conditions of animals). All specimens were counted and average 112 density values per m^2 was calculated. 113

For the analyses at the regional scale the averages of sampling sites were used as replicates in order to compare the different lagoons, while at local scale samples were used to compare different sites nested within each lagoon. Data were aggregated to genus, family, order, class and phylum. Taxonomic classification followed the World Register of Marine Species (WoRMS) (WoRMS Editorial Board, 2018).

- 119 For each of the 18 Italian lagoons 'Loss of information α ' (α) was calculated according to Bacci et al. (2009), through the difference NTx - NTx+1, when NT expresses the number of taxa identified 120 121 at the taxonomic level x, and x+1 expresses the next higher taxonomic level compared to level x. 122 ' α ' values describes the loss of information on the taxonomic structure of macrobenthic settlement which can be registered at the passage from a lower (x) to a higher (x + 1) taxonomic level (higher 123 values correspond to a major 'loss of information' and vice versa). The 'a' value was calculated 124 step by step from the lowest to the highest taxonomic level considered (species-genus, genus-125 family, family-order, order-class, class-phylum). The percentage was then calculated as follows: 126 (NTx - NTx + 1)/NTx * 100.127
- For each sample the following indices were calculated: expected taxa richness (ES) calculated with 128 rarefaction method (for number of individuals n = 10000), Simpson index (λ), average taxonomic 129 130 distinctness (Δ^+) calculated on presence/absence data, and variation in taxonomic distinctness (λ^+). Those indices were chosen to provide complementary information on macrobenthic community, 131 132 from richness (number of species, ES), to structure (combination of richness and abundances, λ), to taxa identities (taxonomic distance, Δ^+ and λ^+). All those indices are independent from sampling 133 134 effort (Clarke and Warwick, 2001), and were calculated for matrices at different taxonomic levels, with PRIMER v6 + PERMANOVA software package (Anderson et al., 2008, Clarke and Gorley, 135 To test the consistency of univariate indices across different levels of taxonomic 136 2006). aggregation the Spearman's rank correlation coefficient (r_s) was calculated for each index step by 137 138 step from the lowest to the highest taxonomic level (species-genus, genus-family, family-order,

order-class, class-phylum). Those calculations were performed using R version 2.4.0 (R
Development Core Team, 2008).

For regional scale analyses (differences among lagoons), an abundance matrix with average taxa 141 density for each lagoon was produced for each of the six taxonomic levels (species, genus, family, 142 order, class, and phylum), and for each of four different transformations (none, square-root, 143 logarithm, and presence/absence). The affinities among lagoons were established using Bray-Curtis 144 similarity and a second-stage non-metric Multi-Dimensional Scaling (MDS) ordination was plotted 145 to visualize differences among similarity matrices at different levels of taxonomic aggregation and 146 147 data transformation. As a measure of similarity between those resemblance matrices, Spearman's rank correlation coefficient (rs) was calculated and tested using RELATE, a non-parametric 148 analogue to the Mantel test, using 9999 permutations. The stress of the two-dimensional plot was 149 calculated using Kruskal's stress Formula 1 (Clarke and Green, 1988). Stress is a measure of the 150 151 reliability of the representation, a value < 0.1 is considered a good result (Clarke and Warwick, 2001). In order to check the efficiency of MDS ordination in discriminating among lagoons, 152 153 "Hierarchical Clustering Analysis" was performed on each of the six matrices based on average 154 abundances for each lagoon: species, genus, family, order, class, and phylum matrix. Bray-Curtis 155 similarity was calculated on untransformed data and group average was used as agglomeration method. In order to test the significance of variations in taxa-abundance matrices among the groups 156 identified by cluster analysis, permutational multivariate analysis of variance, PERMANOVA 157 (Anderson et al., 2008) was carried out on the six matrices corresponding to the different 158 taxonomical levels. A single factor design (with number of levels corresponding to the number of 159 cluster groups) and the "unrestricted permutation of row data" with 9999 permutations was chosen, 160 as recommended in Anderson et al. (2008). A multivariate analogue of t-statistic was used for 161 subsequent pairwise comparisons (Anderson et al., 2008). The same procedure was followed for 162 each type of data transformations. 163

For the local scale analysis (differences among sites within lagoons), abundance matrices were 164 produced separately for each lagoon, for each of the same six taxonomic levels, and for each of 165 166 same four different transformations. The affinities among sites within each lagoon were established using Bray-Curtis similarity and for each data set, a second-stage matrix was created to visualize 167 168 differences among different levels of taxonomic aggregation. Lagoons from Po river delta in Veneto region (Barbamarco, Caleri, Canarin, Marinetta, Scardovari and Vallona) were considered together 169 (as DV), given the low number of sites for each lagoon (2-5). To test the consistency of the effect of 170 different levels of aggregation across the studied lagoons, a third-stage resemblance matrix was 171 172 built using second stage matrices constructed for each lagoon. Third-stage resemblance matrix is

defined as a second second-stage resemblance matrix constructed using rank correlations between corresponding elements in the set of second-stage matrices (following Arvanitidis et al., 2009). A third-stage resemblance matrix was built for each data transformation. Spearman's rank correlation coefficient (r_s) was calculated between matrices, and the stress of the two-dimensional plot was calculated using Kruskal's stress Formula 1 (Clarke and Green, 1988). All those calculations were performed with PRIMER v6 + PERMANOVA software package (Anderson et al., 2008, Clarke and Gorley, 2006).

180 For all analyses a p < 0.05 was chosen as significant threshold.

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182 **3. Results**

183 The total 'loss of information α ', from species to phylum level, did not showed a general pattern, but was highly variable among lagoons (Fig. 1), showing the highest value (120) at CA and the 184 185 lowest (16) at VA. The highest percentage of loss (α) from species to genus level was observed at CO (19%), and the lowest at SC (2%). At MA, VA, BR, and CN, loss of information (a) at this 186 187 level was null, because every genus was represented by only one species. Loss of information (α) between genus and family levels was higher than zero in every lagoon, with percentages ranging 188 189 from 5% at FE to 35% at GM, indicating the presence of families with more than one genus. Loss 190 of information (a) was higher than zero also at family-order level (from 19% at CN and BA to 44%) at FE), order-class level (from 13% at CO to 38% at PP), and class-phylum level (from 3% at GO, 191 PR, CA and FE to 19% at CN). In most lagoons the highest percentage of loss was observed 192 between family and order, with the exception of CA, FO and GM, showing the highest loss between 193 genus and family, and of VA, CN, PP, and BA showing the highest loss between order and class 194 levels. 195

The consistency of the pattern of variation across taxonomic levels varied among univariate indices 196 (Fig. 2). Pairwise comparison between estimated taxa richness (ES) at different taxonomic levels 197 showed very strong correlation up to the order level (species/genus, genus/family, and family/order, 198 $r_s > 0.9$), and a progressive reduction of similarity between ES calculated at higher taxonomic levels 199 200 (order/class and class/phylum, Fig. 2). Pairwise comparison between Simpson index (λ) at different taxonomic levels showed very strong correlation ($r_s > 0.9$) up to class levels (species/genus, 201 genus/family, family/order, and order/class), and much weaker correlation between class and 202 phylum level (Fig. 2). Conversely the pairwise comparison of indices based on taxonomic distance 203 $(\Delta^+ \text{ and } \lambda^+)$ showed very strong correlation only between species and genus level ($r_s > 0.9$). Average 204 taxonomic distinctness (Δ^+) showed strong correlation ($r_s > 0.7$) between genus and family, and 205 between family and order, whereas correlation between Δ^+ at order and class level was weaker (Fig. 206

207 2). Variation in taxonomic distinctness (λ^+) showed weaker but significant correlation between 208 genus and family, and between family and order levels ($r_s < 0.7$), and not significant correlation (p >209 0.05) between order and class levels (Fig. 2).

210 From regional scale analyses (differences among lagoons) the similarity with the original matrix (species-level data) decreased with increasing taxonomic level (from genus to phylum) with all four 211 transformations considered (Fig. 3). Similarity among matrices from species to genus, and from 212 species to family increased with the strength of transformation (with the exception of 213 presence/absence transformation from species to family level), whereas from species to higher 214 215 levels (order to phylum), similarity decreased with increasing strength of the transformation. In 216 particular correlations between ordination plots at species and genus levels ranged from $r_s = 0.81$ for untransformed data to $r_s = 0.93$ for presence/absence transformation; between species and family 217 levels varied from $r_s = 0.72$ for untransformed data to $r_s = 0.79$ for logarithmic transformation, 218 219 indicating a general lower loss of information with stronger transformation. Conversely correlation between similarity matrices at species and order levels varied from $r_s = 0.60$ (presence/absence) to r_s 220 221 = 0.63 (untransformed and logarithm); between species and class aggregation matrices varied from $r_s = 0.44$ (square root) to $r_s = 0.60$ (presence/absence); and between species and phylum aggregation 222 223 varied from $r_s = 0.18$ (presence/absence) to $r_s = 0.50$ (untransformed), indicating higher loss of 224 information with stronger transformation, in particular between class and phylum levels.

PERMANOVA highlighted significant differences (p < 0.05) in macrobenthic assemblages among 225 groups identified by ordination plot and cluster analysis at the species level with all four 226 transformations (none, square root, logarithm and presence-absence), but the number of groups 227 identified by the cluster analysis decreased with increasing strength of the transformation (Table 2), 228 indicating increases of similarity among lagoons. For each of the four transformations, the 229 differences among cluster groups where still significant at higher taxonomic levels, with the only 230 exception of presence/absence data at the level of phylum (Table 2). Pair-wise comparisons 231 revealed that not all possible pairwise combinations among cluster groups differed significantly, but 232 the number of significant pairwise differences was consistent moving from species to higher 233 234 taxonomic levels, with the only exception of the level of phylum (Table 2). Cluster plots are available as Supplementary material (Appendix 1-4). 235

From local scale analyses (differences among sites within each lagoon), the third-stage matrices (Supplementary material, Appendix 5) showed that the pattern of similarity among matrices with different levels of taxonomic aggregation varied among studied lagoon. Those differences, in general, were higher at higher taxonomic levels, and decreased with increasing strength of transformation (with the exception of few lagoons). Considering each lagoon separately, in most

cases the correlation with the original matrix decreased with increasing taxonomic level (from 241 242 genus to phylum) with all four transformations considered (Fig. 4). Correlation between species matrices and matrices at higher taxonomic levels generally decreased with increasing strength of 243 transformation, with two exceptions: ST lagoon, where r_s was more or less constant, and PP lagoon, 244 where the opposite trend was observed. For untransformed data (Fig. 4A) the majority of lagoons 245 showed very strong correlation among matrices (second-stage $r_s \ge 0.9$) until family (GM), order 246 (LE, DV, GO, FO, FE, CA, CO, OR, PR), or even class level (BA). At PP the matrix at species 247 level showed strong correlation (second-stage $r_s \ge 0.8$) with matrices up to order levels. At ST 248 249 lagoon instead, matrices showed an extremely low loss of information untill the class level (secondstage $r_s = 1$). For square root-transformed data (Fig. 4B), all lagoons showed very strong correlation 250 between matrices (second-stage $r_s \ge 0.9$) until family (ST, FE, GM), order (CO, GO, PR, DV, LE, 251 OR, CA, FO) or even class level (BA). At PP matrix at species level showed strong correlation 252 253 (second-stage $r_s \ge 0.8$) with matrices up to order levels. At ST lagoon it showed very strong correlation until class level (second-stage $r_s = 1$), with the exception of correlation between species 254 255 and order (second-stage $r_s = 0.5$). For log-transformed data (Fig. 4C), most lagoons showed very strong correlation among matrices (second-stage $r_s \ge 0.9$) until family level (GM, FO, GO, DV, FE, 256 257 BA) or order level (PR, CA, OR, LE, OR). At PP species matrix showed strong correlation with matrices until order level (second-stage $r_s \ge 0.8$). Species matrices at ST showed very strong 258 correlation (second-stage $r_s = 1$) even up to class level. For presence/absence data (Fig. 4D) lagoons 259 showed very strong correlation between matrices (second-stage $r_s \ge 0.9$) until the family level (FE, 260 GO, GM, LE, OR, CA, PR, FO, CO, and DV). At BA species matrix showed very strong 261 correlation ($r_s \ge 0.9$) with the matrix at genus level, and strong correlation with family matrix ($r_s \ge$ 262 0.7). ST showed a peculiar pattern, with correlation between species and family (second-stage $r_s =$ 263 1) stronger than between species and genus (second-stage $r_s = 0.87$). 264

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266 **4. Discussion**

The present work provides information on the applicability of TS in transitional waters at two 267 268 different scales: regional scale (among lagoons), and local scales (among sites nested within the lagoons). The analysed data set comprises all main Adriatic and Tyrrhenian Italian lagoons. Along 269 Italian coasts there are almost 170 lagoons, but only 30 of them have a surface area bigger than 10 270 km², and most of them (with some exceptions, like Orbetello Lagoon) are located along the Western 271 Adriatic coasts (Mistri et al., 2018). Notwithstanding the recent proliferation of TS studies, present 272 knowledge is still fragmented, and factors influencing the effectiveness of TS still require a great 273 274 deal of clarification, in particular in relation to different spatial and temporal scales (Dethier and

Schoch, 2006; Smale, 2010; Bevilacqua et al., 2018). The use of a wide range of different lagoons, 275 differing for typologies (non-tidal to microtidal), salinity (mesohaline to hyperhaline), and subjected 276 to different anthropogenic pressures and different levels of protections, enabled us to obtain a more 277 general picture of the effectiveness of TS, within and among lagoons, with a supra-local 278 279 perspective. Macrobenthic communities were highly variables among (regional scale) and within the analysed lagoons (local scale), and those differences were observed both with univariate and 280 multivariate analysis. It is known that lagoons exhibit different and peculiar characteristics 281 depending on their geographical, hydrodynamic and ecological features (Tagliapietra et al., 2009). 282 283 Natural gradients of salinity, marine water renewal, nutrients, turbidity and sediment structure, 284 together with the pressures related with human activities, produce changes at levels of macrobenthic 285 community. The present study showed that patterns of variation of macrobenthic community detected with univariate and multivariate analyses were consistent at different taxonomic levels. 286 287 Notwithstanding the differential response of some analysed lagoons to TS, in general, those patterns were consistent until the family level, and the use of higher taxonomic levels lead to more relevant 288 289 changes in observed spatial patterns, consistently with previous investigations (e.g. Mistri and 290 Rossi, 2001; Dethier and Schoch, 2006).

291 Loss of information in terms of taxa richness, described by ' α ' values, varied among the studied 292 lagoons, but did not followed a clear pattern, suggesting a dependence on the specific character of each lagoon, resulting from a combined effect of different factors (e.g. size, salinity, tidal 293 influence). In most lagoons the highest percentage of loss was observed between family and order, 294 or at higher levels, consistently with the suitability of family as a proper taxonomic level for TS, but 295 at CA, FO and GM the highest percentage of loss was observed between genus and family. 296 Notwithstanding the different values of α in different lagoons, strong correlations between levels of 297 taxonomic aggregation until family level, were observed, for both univariate indices and for the 298 299 multivariate space, as already reported from different habitats (Bacci et al., 2009).

300 Considering univariate indices, estimated taxa richness (ES) and Simpson index (λ) correlated well across the different levels of taxonomic aggregation at least until order level, confirming their 301 suitability in view of analyses based on TS. As it was expected, indices based on taxonomic 302 303 distance lost information more quickly moving to higher taxonomic levels, in particular the 304 variation in taxonomic distinctness (λ^+). Nevertheless, the correlation between species and family levels for both Δ^+ and λ^+ , suggested a reasonably low loss of information at this taxonomic level. 305 306 Their use is still not widespread as classical structural indices, but they could provide a more robust indication in case of biodiversity loss (Clarke and Warwick, 2001). Some attempts to use measures 307 308 of taxonomic distance in transitional waters (Mistri et al., 2001; Salas et al., 2006; Munari et al.,

2009) confirmed that they can add complementary information to more classical measures of 309 310 biodiversity (Munari et al., 2009). Nevertheless, being based on presence/absence data, they could experience reduced discrimination power when changes involve relative abundances of organisms. 311 Therefore, the effectiveness of such measures is still unclear and require further investigations 312 (Bevilacqua et al., 2011), despite Rosser (2017) suggested that better performance in using higher 313 taxa should be expected (i) when the ratio of species to higher taxa is low, (ii) in communities with 314 high evenness and high species turnover, and (iii) when niche conservation within higher taxa is 315 316 preserved.

317 Considering multivariate analyses, our results confirmed that family-level identifications are sufficient to distinguish natural spatial patterns of variability both among sites within lagoons (local 318 319 scale), and among Italian transitional water bodies (regional scale). The loss of information about the general structure of macrobenthic assemblages from species to family taxonomic level is 320 321 limited, irrespective of the transformation used. In fact, correlations between matrices at species and at family levels were very high ($r_s \ge 0.9$) or high (only one case $r_s \ge 0.7$ for presence/absence data at 322 323 BA) across all lagoons (local scale) and data transformations. Correlation was high ($r_s \ge 0.7$) also among species and family matrices with average abundances for each lagoon (regional scale) across 324 325 every data transformation. Our results are consistent with previous investigations proving that 326 analyses based on family abundances were effective in detecting spatial patterns in transitional areas (Mistri and Rossi, 2001; De Biasi et al., 2003; Dethier and Schoch, 2006; Munari et al., 2009; 327 Tataranni et al., 2009). Family level is usually also considered the best compromise between the 328 loss of information and the time gained in sorting and identification at coarser levels than species 329 (Mistri and Rossi, 2001; De Biasi et al., 2003). One of the given explanations for the efficiency of 330 TS in transitional systems is that in brackish environments species are not particularly prone to 331 speciation due to short evolutionary time allowed by the highly fluctuating environment; therefore, 332 sympatric speciation pattern is often reduced or absent, and most families are represented by a 333 single species (Giangrande et al., 2005). Nevertheless, the different values of α (loss of information) 334 observed in the present work suggested different levels of taxonomic heterogeneity, with different 335 336 number of taxa belonging to the same higher taxon, among different lagoons. Those differences did not affect TS efficiency at family level. This result is consistent with investigations in different 337 338 marine ecosystems (Bacci et al., 2009; Bevilacqua et al., 2009), demonstrating that even in presence of high taxonomic heterogeneity, a high number of species, genera and families can make the 339 340 macrobenthic assemblage structure stable and diverse up to family level, compensating the loss of information at the passage to the higher taxonomic level. Warwick (1993), pointed out that families 341 342 generally represent fairly homogeneous groups of organisms sharing basic functional traits.

However, the level of such intra-family functional redundancy could depend on the classification criteria adopted and on taxonomic traditions (Bertrand et al., 2006), which may be not consistent across phyla (Warwick and Somerfield, 2008), and could explain the differential response of different phyla to TS, reported from different habitats (Olsgard and Somerfield, 2000; Bevilacqua et al., 2009). Consequently, transitional waters should be particularly suitable for TS, since they are highly fluctuating environments, with macrobenthic community showing high levels of functional redundancy (e.g. Mistri et al., 2001; Munari et al., 2005; Pitacco et al., 2018b).

As already found elsewhere (e.g. Olsgard et al., 1998; Anderson et al., 2005; Włodarska-Kowalczuk 350 351 and Kędra, 2007; Bevilacqua et al., 2009), this study showed that the choice of data transformation 352 could influence the results of analyses as well as the level of taxonomic aggregation, or even more. 353 For the analyses at local scale (differences among sites within lagoons), in most lagoons a reduction of similarity between matrices with different taxonomic levels was observed with increasing 354 355 strength of data transformation, and the same was observed also at regional scale (differences among lagoons) for matrices at levels of order, class and phylum. A similar pattern has been 356 357 observed in different habitats (e.g. Chapman, 1998; Clarke and Warwick, 2001; Anderson et al., 2005), suggesting untransformed or mildly transformed data as the best options for TS. Data 358 359 transformation reduces the weight of the most abundant taxa. The structure of macrobenthic 360 communities typical of lagoons is a numeric dominance of few species, so without data transformation this pattern of dominance could be easily transmitted to higher taxonomic levels, 361 since dominant species are usually taxonomically distant (e.g. Marchini et al., 2008). Conversely 362 with a strong transformation such as the conversion to presence/absence data, the dominance effect 363 364 disappeared and taxonomic complexity, considering also rare and less frequent species prevailed. At the same time, analysing species, genus, and family matrices at regional scale (differences among 365 lagoons), the similarity among lagoons increased with increasing strength of transformation, 366 suggesting that in view of TS (with aggregation at family level) data transformation, even strong, 367 should be the best choice. Those differential responses were most probably the results of complex 368 interactions between the effect of taxonomic resolution and data transformation on macrobenthic 369 370 community variability at different scales. Our results are consistent with a previous investigation in 371 another habitat type: Anderson et al. (2005), showed that variability of benthic assemblages associated with kelp holdfast at species level was mainly "compositional" at larger scales, whereas 372 it was driven by changes in relative abundance at medium or smaller scales, particularly by 373 numerically dominant taxa. The relative importance of the two components (composition and 374 abundance) across the different scales, changed at higher taxonomic levels (Anderson et al., 2005). 375 376 Also considering the dataset analysed with the present study, the compositional aspect of the

community had a higher weight on variability at regional scale compared to local scale. Moreover, 377 378 both the scale and the taxonomic level considered had a differential influence on the effect of data transformation. The traditional theory in the field of TS, suggested that in the absence of previous 379 380 knowledge, untransformed data should be analysed, since is often difficult to recognize a priori how environmental variation will affect the structure of assemblages (Bevilacqua et al., 2009). 381 Nevertheless, it was already pointed out that the choice of data transformation should result from 382 biological questions, since it determines the relative contribution of quantitative and qualitative 383 inter-sample differences in the final outcome of multivariate analyses (Tataranni et al., 2009). A 384 385 weak transformation gives a narrow view of the community, deeply influenced by the most 386 abundant taxa, whereas a strong transformation yields a wider view of the community, where all 387 taxa have the same weight (Mistri and Rossi, 2001). Our results, together with other previous investigations (i.e. Anderson et al., 2005) suggested additional care in the choice of data 388 389 transformation, since its effect could vary across different scales, and different taxonomic levels.

Since the most frequent disturbance events (e.g. organic enrichment, eutrophication, chemical 390 391 pollution) are likely related to changes in spatial patterns of assemblages (Warwick and Clarke, 392 1993; Tataranni et al., 2009) TS may be usefully applied to monitoring programs of lagoon systems. 393 TS application would make those programs more cost-effective, since the time necessary to process 394 samples would be markedly reduced. Chapman (1998) estimated that in the same time that it takes to sort a given number of samples to the finest possible resolution, twice or more samples could be 395 sorted to major groups. This improvement of the cost/benefit ratio would be particularly important 396 in highly fluctuating habitat, such as transitional waters, where sampling frequency is particularly 397 398 important. The use of TS in monitoring programs required by national laws, would consequently result in a consistent reduction of cost for Environmental Agencies. 399

400 Our study provides evidences of the suitability of this technique at different scales, and in lagoons with different taxonomic complexity. Nevertheless, a different response to TS, at regional and local 401 402 scales, suggested caution in generalising outcomes, and in the choice of data transformation. Moreover, prior to application of TS, region-specific baseline knowledge of each lagoon system, 403 404 regarding macrobenthic community (at species level), main natural and anthropogenic stressors, and their effect on macrobenthic community, is fundamental. In addition, as suggested by previous 405 investigations (e.g. Dethier and Schoch, 2006, Tataranni et al., 2009; Vieira et al., 2015), analyses at 406 407 finer taxonomic level should be performed periodically and whenever a change in spatial pattern is 408 detected or there are reasons to suspect that a given region is suffering from some new stressor, to which the response of the community is unknown, or when the effects of subtle environmental 409 410 impacts may pass unnoticed with the use of low taxonomic resolution data.

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| Lagoon | Code | Coordinates | Area (km ²) | Average depth (m) | Typology | Salinity | Sites | References | |
|-------------------------------|------|------------------|----------------------------|----------------------|------------|---------------------|-------------------------------|-------------------------|--|
| Venice (Palude della Rosa) | PR | 45°31'N; 12°30'E | 5.5 | 0.3-0.8 | microtidal | poly/ euhaline | 9 | Sabil et al., 1994 | |
| Grado-Marano | GM | 45°42'N; 13°20'E | 160 | 1.5 | microtidal | meso/poly/ euhaline | 21 | 1Bettoso et al., 2010 | |
| Caleri | CL | 45.09°N; 12.31°E | 11.5 | 2 | microtidal | meso/ polyhaline | 4 | Mistri et al., 2018 | |
| Marinetta | MA | 45°04'N; 12P22'E | 10 | 0.8 | microtidal | meso/ polyhaline | 4 | Mistri et al., 2018 | |
| Vallona | VA | 45°02'N; 12°23'E | 11.5 | 2 | microtidal | meso/ polyhaline | 2 | Mistri et al., 2018 | |
| Barbamarco | BR | 45°00'N; 12°46'E | 8 | 0.8 | microtidal | meso/ polyhaline | 2 | Spillman et al., 2009 | |
| Canarin | CN | 44°92'N; 12°49'E | 10.0 | 0.8 | microtidal | meso/ polyhaline | 3 | Bianchi and Morri, 1996 | |
| Scardovari | SC | 44°86'N; 12°42'E | 32 | 1.5-2 | microtidal | meso/ polyhaline | 5 | Marchini et al., 2008 | |
| Goro | GO | 44°82'N; 12°31'E | 26 | 1.2-1.5 | microtidal | meso/ polyhaline | 3 | Corbau et al., 2016 | |
| Comacchio | СО | 44°61'N; 12°17'E | 100 | 0.5-1.5 | non-tidal | euhaline | 4 | Munari et al., 2005 | |
| Baiona | BA | 44°50'N; 12°25'E | 10 | 1 | microtidal | polyhaline | 3 | Ponti et al., 2011 | |
| Lesina | LE | 41°53'N; 15°26'E | 50 | 0.8 | non-tidal | meso/ polyhaline | 4 | Marchini et al., 2008 | |
| Orbetello | OR | 42°26'N; 11°12'E | 27 | 0.5-1 | non-tidal | polyhaline | 11 | Lardicci et al., 1997 | |
| Caprolace | CA | 41°20'N; 12°58'E | 2.26 | 1.3 | non-tidal | euhaline 5 | | Prato et al., 2009 | |
| Fogliano | FO | 41°24'N; 12°54'E | 4.08 | 0.9 | non-tidal | euhaline | 4 | Prato et al., 2009 | |
| San Teodoro | ST | 40°48'N; 09°40'E | 2.2 | 0.7 | non-tidal | polyhaline | naline 3 Munari and Mistri, 2 | | |
| Feraxi | FE | 39°20'N; 09°35'E | 0.4 | NA | non-tidal | euhaline | 10 | Sannio et al., 1997 | |
| Porto Pino | PP | 38°58'N; 08°36'E | 4.4 | NA | non tidal | hyperhaline | 10 | Rossi and Cannas, 1984 | |

Table 1 Characteristics of sampled lagoons and number of sampled sites.

- 614 Table 2 Significance of cluster groups (PERMANOVA), and percentage of significant pairwise
- 615 combinations among those groups. Percentages are not shown for presence/absence data because of
- 616 low number of comparisons.

| Data transformation | Cluster | | Taxonomic resolution | | | | | |
|---------------------|---------|---|----------------------|--------|--------|--------|--------|--------|
| Dum transformation | groups | | Species | Genus | Family | Order | Class | Phylum |
| None | 6 | PERMANOVA p-value | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| | | significant pairwise combinations | 21% | 21% | 21% | 21% | 21% | 14% |
| Square root | 3 | PERMANOVA p-value | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |
| | | significant pairwise combinations | 67% | 67% | 67% | 67% | 67% | 67% |
| Logarithm 3 | | PERMANOVA p-value | 0.0001 | 0.0002 | 0.0004 | 0.0005 | 0.0004 | 0.0191 |
| | | significant pairwise combinations | 67% | 67% | 67% | 67% | 67% | 33% |
| Presence/absence | 2 | PERMANOVA p-value | 0.006 | 0.006 | 0.007 | 0.037 | 0.007 | 0.064 |

618 Figure Legend

- 619 Fig. 1 'Loss of information α ' from lower to higher taxonomic levels (NT1-NT6) for the eighteen
- 620 Italian lagoons (see

- 621 Table 1 for codes).
- Fig. 2. Correlation between indices calculated at consecutive taxonomic levels (from species to phylum). ES = Estimated richness for N=10000; λ = Simpson index; Δ^+ = Taxonomic distinctness; λ^+ = Variation of taxonomic distinctness.
- Fig. 3. Second-stage inter-matrix rank correlations (r_s) based on similarity matrices based on average taxa density for lagoon, at different taxonomic levels and calculated using different data transformation. Untr = untransformed, sqr = square root, log = logarithm, pa = presence/absence.
- Fig. 4. Second-stage inter-matrix rank correlations (r_s) based on similarity matrices based on
- average taxa density for site nested within lagoon, at different taxonomic levels and calculated
- using different data transformation: (A) untransformed data, (B) squared root, (C) logarithm, (D)
 presence/absence.