

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

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## **Abstract**

Taxonomic Sufficiency (TS), the use of coarser taxonomic resolution in monitoring plans, has been receiving increasing attention in recent years. A dataset of macrobenthic communities from 18 Italian lagoons, representing different typologies, covering a great part of Italian peninsula, from Adriatic to Tyrrhenian Sea, was analysed in order to test how the efficiency of TS varies among benthic assemblages with different taxonomic complexity, and at different scales: local (within each lagoon) and regional (among lagoons). With both univariate and multivariate analyses, most information were retained till family level, at both local and regional scales, despite the different levels of taxonomic heterogeneity. Differences between regional and local scales were observed for higher taxonomic levels and for different type of data transformation. Our results confirm the efficiency of TS till family level, but at the same time support the need of area-specific baseline knowledge prior application of TS in transitional waters.

**Keywords:** Taxonomic Sufficiency, transitional waters, Italian lagoons, benthic community, multi-scale patterns, Mediterranean Sea

## **1. Introduction**

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 impact assessment and monitoring (Olsgard et al., 1998). The basic concept of TS (Ellis, 1985), is that the identification of taxa at taxonomic levels higher than species enables the detection of changes in assemblages exposed to environmental stress without significant loss of information. The use of coarser taxonomic resolution was first suggested by Warwick (1988a, 1988b) who pointed out the theoretical advantages of conducting both multivariate and univariate analyses at various hierarchical levels of taxonomic aggregation in monitoring programmes. Studies supporting the efficiency of TS in marine systems were later performed worldwide in different habitat types, from soft bottom (e.g. Vanderklift et al., 1996, Włodarska-Kowalczyk and Kędra, 2007) to hard bottom (e.g. Mistri and Rossi, 2000, Terlizzi et al., 2002), focusing on strong pollution gradients (Dauvin et al., 2003), and on variation along natural gradients (Terlizzi et al., 2009). In transitional systems, TS has been mainly applied for the description of spatial patterns of macrobenthic community in response to heavy disturbance, such as oil spill (Dauvin et al., 2003), or to compare areas with different levels of anthropogenic disturbance (Mistri and Rossi, 2001; Arvanitidis et al., 2009), but it proved useful also for the discrimination of natural gradients in estuaries (Chainho et al., 2007).

Coastal lagoons represent important and fragile ecosystems in the coastal landscape in many places on the globe. They provide key ecosystem services such as water quality improvement, fisheries resources, habitat and food for migratory and resident animals, protection from storms and touristic

appeal (Mitsch and Gosselink, 2000; Pérez-Ruzafa et al., 2011), but they are also 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; Acquavita et al., 2014; Arienzo et al., 2013; Barhoumi et al., 2014; Pitacco et al., 2018a), fishery, 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 and economic importance of transitional environments, long-term monitoring is fundamental to understand the effect of anthropogenic stress on macrobenthic community and thus to plan efficient management for ecosystem conservation and restoration. Since those projects usually benefit from a limited financial support, TS could be a useful tool for the improvement of 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 multi-scale design researches (Tataranni et al., 2009), but 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).

This paper analysed inventories of macrobenthic communities obtained through research programmes carried out on 18 lagoons, representing all main Adriatic and Tyrrhenian Italian lagoons, with different typologies, from mesohaline to hyperhaline, non-tidal to microtidal lagoons, subjected to different anthropogenic pressures and different levels of protections. The analyses were performed at two different scales: regional (variability among ecosystems) and local (variability within each ecosystem). The aim of the work was to calculate the loss of information in terms of univariate and multivariate analyses with different levels of taxonomic aggregation (from species to phylum), and different data transformations (row data, square root, logarithm, presence/absence). The scope was to understand whether and how the efficiency of Taxonomic Sufficiency in detecting both univariate and multivariate variability of benthic assemblages in transitional waters is influenced by (i) the taxonomic complexity of benthic assemblages, (ii) the scale considered (regional and local).

## **2. Materials and methods**

The analysed dataset was gathered during several sampling campaigns carried out in eighteen Italian lagoons. Samples were collected repeatedly over time, from all main Adriatic and Tyrrhenian Italian lagoons: Baiona, Barbamarco, Caleri, Canarin, Comacchio, Goro, Grado-Marano, Lesina, Marinetta, Palude della Rosa (north easterly part of the Venice lagoon),

Scardovari, and Vallona (Adriatic Sea), Caprolace, Feraxi, Fogliano, Orbetello, Porto Pino, and San Teodoro (Tyrrhenian Sea). This dataset comprise different typology of transitional waters, from large to small, from microtidal to non-tidal, hyperhaline to mesohaline ecosystems (see Table 1). A total of 107 sites, 64 of which were located in Adriatic and 43 in Tyrrhenian Sea (Table 1), have been chosen as representative of the different habitats found within each transitional environment. Samples were sieved on a 0.5mm mesh-size sieve and retained benthic fauna was identified to species level whenever possible.

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

| Lagoon            | Code | Area (km <sup>2</sup> ) | Average depth (m) | Typology   | Salinity        | Number of sites | References   |
|-------------------|------|-------------------------|-------------------|------------|-----------------|-----------------|--|
| Comacchio         | CO   | 100                     | 0.5-1.5           | non-tidal  | euhaline        | 4               | Mistri et al., 2000; Munari et al., 2005                         |
| Goro              | GO   | 26                      | 1.2-1.5           | microtidal | meso/polyhaline | 3               | Corbau et al., 2016; Mistri et al., 2000                         |
| Scardovari        | SC   | 32                      | 1.5-2             | microtidal | meso/polyhaline | 5               | Marchini et al., 2008  |
| Lesina            | LE   | 50                      | 0.8               | non-tidal  | meso/polyhaline | 4               | Marchini et al., 2008; Borja et al., 2011                        |
| Orbetello         | OR   | 27                      | 0.5-1             | non-tidal  | polyhaline      | 11              | Marchini et al., 2008; Munari and Mistri, 2007                   |
| Caprolace         | CA   | 2.26                    | 1.3               | non-tidal  | euhaline        | 5               | Prato et al., 2009   |
| Fogliano          | FO   | 4.08                    | 0.9               | non-tidal  | euhaline        | 4               | Prato et al., 2009   |
| Grado-Marano      | GM   | 160                     | 1.5               | microtidal | poly/euhaline   | 21              | Bettoso et al., 2010; Reizopoulou et al., 2014                   |
| Baiona            | BA   | 10                      | 1                 | microtidal | polyhaline      | 3               | Ponti et al., 2011   |
| Palude della Rosa | PR   | 9                       | 1                 | microtidal | poly/euhaline   | 9               | Ravera et al., 2000; Favero et al., 1996                         |
| Porto Pino        | PP   | 4.4                     | NA                | non tidal  | hyperhaline     | 10              | Rossi and Cannas, 1984   |
| San Teodoro       | ST   | 2.2                     | 0.7               | non-tidal  | polyhaline      | 3               | Munari and Mistri, 2007  |
| Feraxi            | FE   | 0.4                     | NA                | non-tidal  | euhaline        | 10              | Sannio et al., 1997  |
| Canarin           | CN   | 10.0                    | 0.8               | microtidal | meso/polyhaline | 3               | Balasso, 2010; Bianchi and Morri, 1996; Mistri et al., 2018      |
| Barbamarco        | BR   | 8                       | 0.8               | microtidal | meso/polyhaline | 2               | Spillman et al., 2009; Balasso, 2010; Mistri et al., 2018        |
| Vallona           | VA   | 11.5                    | 2                 | microtidal | meso/polyhaline | 2               | Cacciatore et al., 2018; Maggi et al., 2017; Mistri et al., 2018 |
| Marinetta         | MA   | 10                      | 0.8               | microtidal | meso/polyhaline | 4               | Balasso, 2010; Mistri et al., 2018                               |
| Caleri            | CL   | 11.5                    | 2                 | microtidal | meso/polyhaline | 4               | Balasso, 2010; Mistri et al., 2018                               |

Species abundance data were averaged for each site and each sampling occasion. Average density values per m<sup>2</sup> was calculated, and data were aggregated to genus, family, order, class and phylum. Taxonomic classification followed the World Register of Marine Species (WoRMS) (WoRMS Editorial Board, 2018).

For each of the 18 Italian lagoons 'Loss of information  $\alpha$ ' ( $\alpha$ ) was calculated according to Bacci et al. (2009), through the difference  $NT_x - NT_{x+1}$ , when  $NT$  expresses the number of taxa identified at the taxonomic level  $x$ , and  $x+1$  expresses the next higher taxonomic level compared to level  $x$ . ' $\alpha$ ' values give general information about the taxonomic heterogeneity-complexity inside each level considered (higher values correspond to a major 'loss of information' and vice versa). In order to describe the loss of information along the taxonomic structure, the ' $\alpha$ ' value was calculated step by step from the lowest to the highest taxonomic level considered (species-genus, genus-family, family-order, order-class, class-phylum). The percentage was then calculated as follows:  $NT_x - NT_{x+1}/NT_x * 100$ .

For each sample the following indices were calculated: expected taxa richness (ES) calculated with rarefaction method (for number of individuals  $n=10000$ ), Simpson index ( $\lambda$ ), average taxonomic distinctness ( $\Delta^+$ ) calculated on presence/absence data, and variation in taxonomic distinctness ( $\lambda^+$ ). All indices were calculated for matrices at different taxonomic levels, with PRIMER v6 + PERMANOVA software package (Anderson et al., 2008, Clarke and Gorley, 2006). Those indices were chosen because of their lack of dependence from sampling effort (Clarke and Warwick, 2001). For each lagoon the average and standard deviation of each index was calculated, and chi-square test applied to Kruskal- Wallis (KW) ranks (Kruskal and Wallis, 1952) was run to check for significant differences among lagoons. Spearman's rank correlation coefficient ( $r_s$ ) was calculated to test the consistency of each index across different taxonomic levels (from species to phylum). Those calculations were performed using R version 2.4.0 (RDevelopmentCoreTeam, 2008).

For regional scale analyses (differences among lagoons), an abundance matrix with average taxa density for each lagoon was produced for each of the six taxonomic levels (species, genus, family, order, class, and phylum), and for each of four different transformations (none, square-root, logarithm, and presence/absence). The affinities among lagoons were established using Bray-Curtis similarity and a second-stage non-metric Multi-Dimensional Scaling (MDS) ordination was plotted to visualize differences among similarity matrices at different levels of taxonomic aggregation and data transformation. As a measure of similarity between those resemblance matrices, Spearman's rank correlation coefficient ( $r_s$ ) was calculated and tested using RELATE, a non-parametric analogue to the Mantel test, using 9999 permutations. The stress of the two-dimensional plot was calculated using Kruskal's stress Formula 1 (Clarke and Green, 1988). Stress is a measure of the reliability of the representation, a value  $< 0.1$  is considered a good result (Clarke and Warwick,

2001). In order to check the efficiency of TS in discriminating among lagoons, “Hierarchical Clustering Analysis” was performed on each of the six matrices based on average abundances for each lagoon: species, genus, family, order, class, and phylum matrix. Bray–Curtis 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 identified by cluster analysis, permutational multivariate analysis of variance, PERMANOVA (Anderson et al., 2008) was carried out on the six matrices corresponding to the different taxonomical levels. A single factor design (with number of levels corresponding to the number of cluster groups) and the “unrestricted permutation of row data” with 9999 permutations was chosen, as recommended in Anderson et al. (2008). A multivariate analogue of *t*-statistic was used for subsequent pairwise comparisons (Anderson et al., 2008). The same procedure was followed for each type of data transformations.

For the local scale analysis (differences within lagoons), abundance matrices were produced separately for each lagoon, for each of the same six taxonomic levels, and for each of same four different transformations. The affinities among samples were established using Bray–Curtis similarity and for each data set, a second-stage matrix was created to visualize 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 (as DV), given the low number of sites for each lagoon (2-5). To test the consistency of the effect of different levels of aggregation across the studied lagoons, a third-stage resemblance matrix, was 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 calculation were performed with PRIMER v6 + PERMANOVA software package (Anderson et al., 2008, Clarke and Gorley, 2006).

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

### **3. Results**

Taxonomic heterogeneity, described by total ‘loss of information  $\alpha$ ’, from species to phylum level, was highly variable among lagoons (Fig. 1), showing the highest values (120) at CA and the lowest (16) at VA. Higher values ( $\alpha > 50$ ) were observed at CA, FO, PR, OR, GM, CO and GO. Loss of

information ( $\alpha$ ) descending from species to genus level was observed for every lagoon, with exception of MA, VA, BR and CN, where every genus was represented by only one species. At this level the highest percentage of loss (19%) was observed at CO, and the lowest at SC (2%). Loss of information ( $\alpha$ ) between genus and family levels was observed for every lagoon, with percentages ranging from 5% at FE to 35% at GM, indicating the presence of families with more than one genus. Loss of information ( $\alpha$ ) was observed in every lagoon 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, PR, CA and FE to 19% at CN). In most lagoons the highest percentage of loss was observed between family and order, with the exception of CA, FO and GM, showing the highest loss between genus and family, and of VA, CN, PP, and BA showing the highest loss between order and class levels.

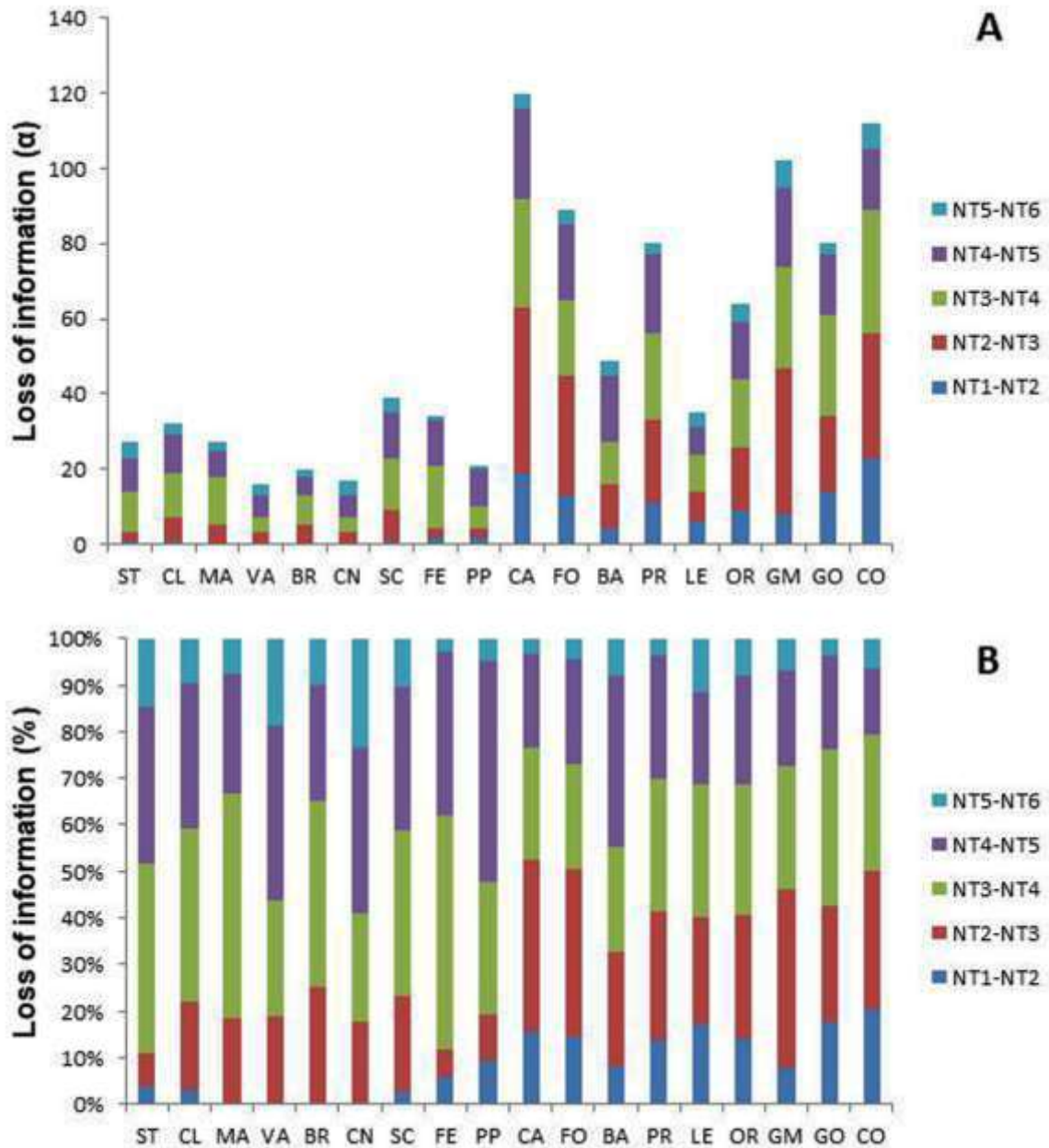


Fig. 1 ‘Loss of information  $\alpha$ ’ from lower to higher taxonomic levels (NT1-NT6) for the eighteen Italian lagoons (see Table 1 for codes). (A)  $\alpha$  values, (B) percentage of  $\alpha$  values.

Univariate indices varied significantly among the studied lagoons (KW,  $p < 0.05$ ). Species richness weighted to the number of individuals (ES) varied from the highest values of  $31.2 \pm 3.2$  SD in BA to the lowest of  $8.7 \pm 4.8$  SD in OR. Simpson index ( $\lambda$ ) showed the highest dominance at VA ( $0.9 \pm 0$  SD), and the lowest at BA ( $0.18 \pm 0.03$  SD), FE ( $0.19 \pm 0.09$  SD), and GM ( $0.25 \pm 0.17$  SD). Average taxonomic distinctness ( $\Delta^+$ ) showed the lowest value at CL ( $80 \pm 6$  SD) and the highest at BA ( $90 \pm 1$  SD) and PP ( $90 \pm 3$  SD). The highest variation in taxonomic distinctness ( $\lambda^+$ ) was observed at CL ( $503 \pm 196$  SD) and the lowest at FE ( $323 \pm 126$  SD). The consistency of the pattern



of variation across taxonomic levels varied among indices (Fig. 2). Estimated taxa richness (ES) at species level showed very high correlation with ES at genus, family, and order levels ( $r_s > 0.9$ ), and a progressive reduction of similarity with ES at higher taxonomic levels, such as class ( $r_s = 0.79$ ) and phylum ( $r_s = 0.70$ ). Simpson index ( $\lambda$ ) at species level showed very high correlation with  $\lambda$  at genus, family, order and class levels ( $r_s > 0.9$ ), and much weaker correlation with  $\lambda$  at phylum level ( $r_s = 0.55$ ). Conversely the pattern of indices based on taxonomic complexity ( $\Delta^+$  and  $\lambda^+$ ) at species level showed very high correlation only with indices at genus level ( $r_s > 0.9$ ). Average taxonomic distinctness ( $\Delta^+$ ) at species level was highly correlated with  $\Delta^+$  at family, and order levels ( $r_s = 0.85$  and  $r_s = 0.72$  respectively), whereas was weakly correlated with  $\Delta^+$  at class level ( $r_s = 0.23$ ). Variation in taxonomic distinctness ( $\lambda^+$ ) at species level was moderately correlated with  $\lambda^+$  at family, and order levels ( $r_s = 0.59$  and  $r_s = 0.41$  respectively), and was not significantly correlated with  $\lambda^+$  at class levels ( $p > 0.05$ ).

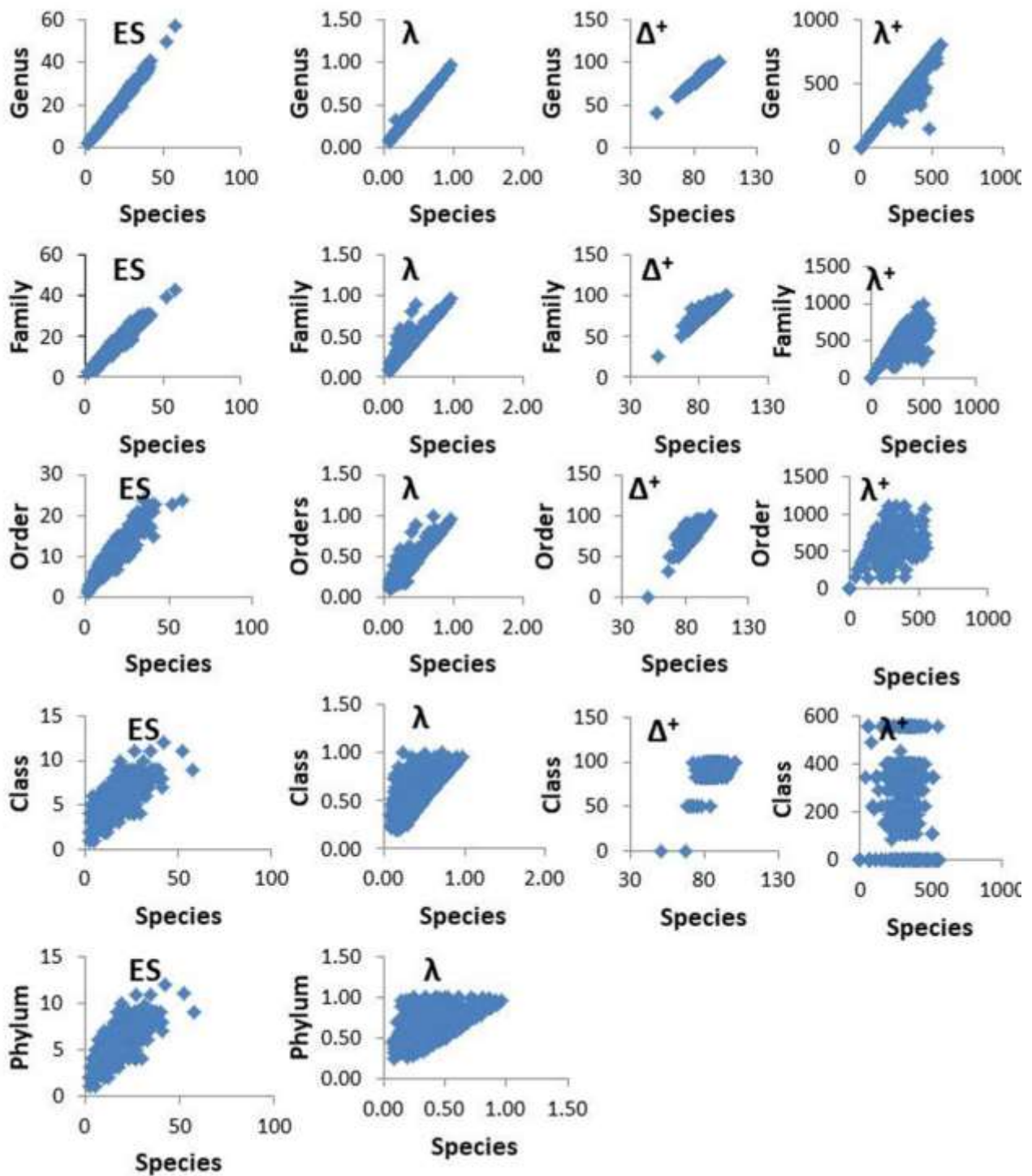


Fig. 2. Correlation between indices calculated at species and higher taxonomic levels (from genus to phylum). ES = Estimated richness for N=10000;  $\lambda$  = Simpson index;  $\Delta^+$  = Taxonomic distinctness;  $\lambda^+$  = Variation of taxonomic distinctness.

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 transformations considered (Fig. 3). Similarity among matrices from species to genus, and from species to family increased with the strength of transformation (with the exception for

presence/absence transformation from species to family level), whereas from species to higher levels (order to phylum), similarity decrease with increasing strength of the transformation. In 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 levels varied from  $r_s = 0.72$  for untransformed data to  $r_s = 0.79$  for logarithmic transformation, 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  $r_s = 0.63$  (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 varied from  $r_s = 0.18$  (presence/absence) to  $r_s = 0.50$  (untransformed), indicating higher loss of information with stronger transformation, in particular between class and phylum levels.

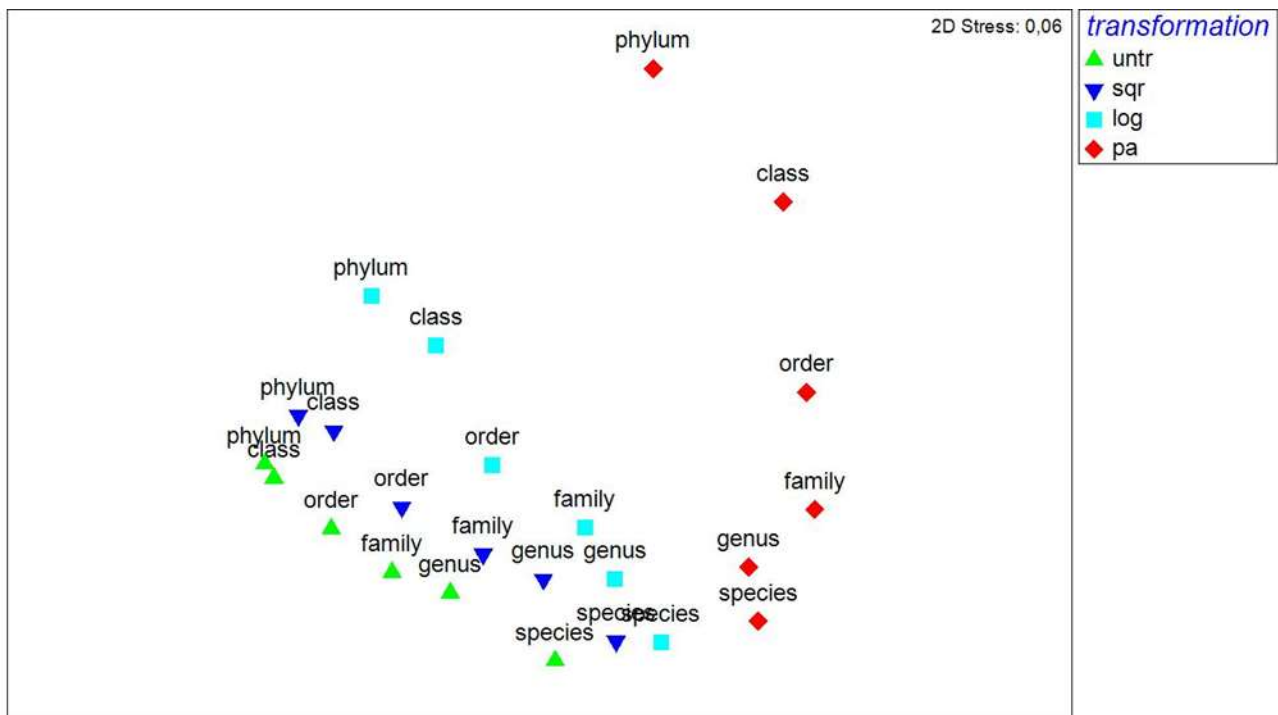


Fig. 3. Second-stage MDS ordination plots of inter-matrix rank correlations based on similarity matrices (with average taxa density for lagoon) at different taxonomic levels and calculated using different data transformation. Untr = untransformed, sqr = square root, log = logarithm, pa = presence/absence.

PERMANOVA highlighted significant differences ( $p < 0.05$ ) in macrobenthic assemblages among groups identified by cluster analysis at the species level with all four transformations (none, square root, logarithm and presence-absence), but the number of groups identified by the cluster analysis decreased with increasing strength of the transformation (Table 2, Supplementary material, appendix 5), indicating an increase of similarity among lagoons. For each of the four

transformations, the differences among cluster groups were still significant at higher taxonomic levels, with the only exception of presence/absence data at the level of phylum (Table 2; Supplementary material, appendix 5). Pair-wise comparisons revealed that not all possible pairwise combinations among cluster groups differed significantly, but the number of significant pairwise differences was consistent moving from species to higher taxonomic levels, with the only exception of the level of phylum (Table 2). Cluster plots are available as Supplementary material (Appendix 1-4).

Table 2 Significance of cluster groups (PERMANOVA), and percentage of significant pairwise combinations among those groups.

| Data transformation     | Cluster groups |                                   | Taxonomic resolution |        |        |        |        |        |
|-------------------------|----------------|-----------------------------------|----------------------|--------|--------|--------|--------|--------|
|                         |                |                                   | Species              | Genus  | Family | Order  | Class  | Phylum |
| <b>None</b>             | 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%    |
| <b>Square root</b>      | 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%    |
| <b>Logarithm</b>        | 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%    |
| <b>Presence/absence</b> | 2              | PERMANOVA p-value                 | 0.006                | 0.006  | 0.007  | 0.037  | 0.007  | 0.064  |

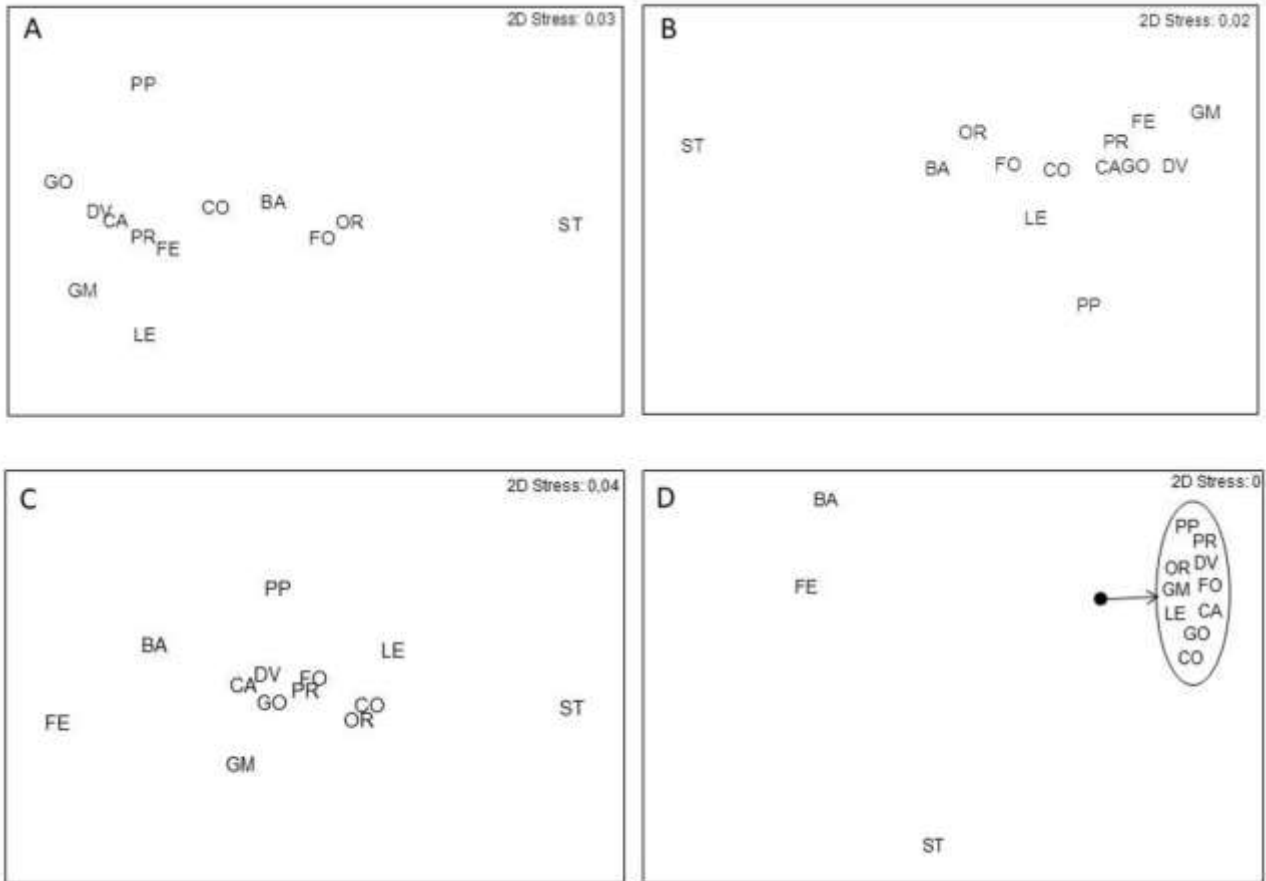


Fig. 4. Ordination MDS plots of ranked intermatrix correlations resulted from the third-stage matrix; matrices involved include information on changes of patterns from the species and higher taxonomic levels, with different data transformation: (A) untransformed data, (B) squared root, (C) logarithm, (D) presence/absence.

From local scale analyses (differences within each lagoon), the third-stage matrices (Fig. 4) showed that the pattern of similarity among matrices with different levels of taxonomic aggregation varied among studied lagoon. Those differences in general regards high taxonomic levels, and decrease with increasing strength of transformation (with the exception of few lagoons). Considering each lagoon separately, in most cases the similarity with the original matrix decrease with increasing taxonomic level (from genus to phylum) with all four transformation considered (Supplementary material, Appendix 6-9). Similarity between species matrices and matrices at higher taxonomic levels generally decrease with increasing strength of transformation, with two exceptions: ST lagoon, where  $r_s$  was more or less constant, and PP lagoon, where the opposite trend was observed. For untransformed data (Fig. 4A) only ST lagoon differed markedly from the all others (third-stage  $r_s < 0.8$  between ST and all other lagoons except OR). The majority of lagoons showed very high levels of similarity among matrices (second-stage  $r_s \geq 0.9$ ) till family (GM), order (LE, DV, GO, FO, FE, CA, CO, OR, PR), or even class level (BA). At PP the matrix at species level showed

lower similarity (second-stage  $r_s \leq 0.9$ ), with matrices at higher levels, but the similarity between matrices at genus and family/order levels was very high (second-stage  $r_s \geq 0.9$ ). At ST lagoon instead matrices showed an extremely low loss of information till the class level (second-stage  $r_s = 1$ ). For square root-transformed data (Fig. 4B), all lagoons showed high levels of similarity among matrices (second-stage  $r_s \geq 0.9$ ) till family (ST, FE, GM), order (CO, GO, PR, DV, LE, OR, CA, FO) or even class level (BA). At PP matrix at species level showed lower similarity with matrices at higher taxonomic levels (second-stage  $r_s \leq 0.9$ ), but the similarity between genus and family/order was still very high (second-stage  $r_s \geq 0.9$ ). Also in this case only ST lagoon differed markedly from the all others (third-stage  $r_s < 0.7$  in all cases), with higher similarity between species and classes (second-stage  $r_s = 1$ ) than between species and order (second-stage  $r_s = 0.5$ ). For log-transformed data (Fig. 4C), most lagoons showed very high levels of similarity among matrices (second-stage  $r_s \geq 0.9$ ) till the family level (GM, FO, GO, DV, FE, BA) or order level (PR, CA, OR, LE, OR). At PP species matrix showed lower similarity with higher taxonomic levels (second-stage  $r_s \leq 0.9$ ), but the similarity between genus and family/order was high (second-stage  $r_s \geq 0.9$ ). In this case, both ST and FE lagoon differed markedly from all others (third-stage  $r_s < 0.7$  in all cases). Matrices at ST showed very high similarity (second-stage  $r_s = 1$ ) even till class level, whereas at FE similarity among matrices showed a more marked decrease from family to order level compared with other lagoons. For presence/absence data (Fig. 4D) the pattern of similarity among matrices with different taxonomic levels was consistent across most lagoons, with high similarity between matrices (third-stage  $r_s \geq 0.8$ ), with the exception of BA, FE, and ST lagoons (third-stage  $r_s < 0.8$ ). Lagoons showed very high similarity between matrices (third-stage  $r_s \geq 0.9$ ), till the family level (FE, GO, GM, LE, OR, CA, PR, FO, CO, and DV). At BA species matrix showed very high similarity ( $r_s \geq 0.9$ ) only with matrix at genus level, while similarity with family matrix was lower, even if quite high ( $r_s = 0.74$ ). ST showed a peculiar pattern, with similarity between species and family (second-stage  $r_s = 1$ ) higher than between species and genus (second-stage  $r_s = 0.87$ ). At FE similarity between matrices was quite high across all taxonomic levels (second-stage  $r_s \geq 0.7$ ) till the level of phylum, whereas in all other lagoon similarity between species and phylum was always lower (always  $r_s < 0.6$ ).

#### 4. Discussion

The present work provides information on the applicability of TS in transitional waters at two different scales (within and among ecosystems). The analysed data set comprises all main Adriatic and Tyrrhenian Italian lagoons. Along Italian coasts there are almost 170 lagoons, but only 30 of them have a surface area bigger than 10 km<sup>2</sup>, and most of them (with some exceptions, like

Orbetello Lagoon) are located along the Western Adriatic coasts (Mistri et al., 2018). Notwithstanding the recent proliferation of TS studies, present knowledge is still fragmented, and factors influencing the effectiveness of TS still require a great deal of clarification (Dethier and Schoch, 2006). The use of a wide range of different lagoons, differing for typologies (non-tidal to microtidal), salinity (mesohaline to hyperhaline), and subjected to different anthropogenic pressures and different levels of protections, enabled us to obtain a more general picture of the effectiveness of TS, within and among lagoons, with a supra-local 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 multivariate analysis. It is known that lagoons exhibit different and peculiar characteristics depending on their geographical, hydrodynamic and ecological features (Tagliapietra et al., 2009). Natural gradients of salinity, marine water renewal, nutrients, turbidity and sediment structure, together with the pressures related with human activities, produce changes at levels of macrobenthic community. The present study showed that patterns of variation of benthic community detected with univariate and multivariate analyses were consistent at different taxonomic levels. Notwithstanding the differential response of some analysed lagoons to TS, in general, those patterns were consistent till the family level, and the use of higher taxonomic levels lead to more relevant changes in observed spatial patterns, consistently with previous investigations (e.g. Mistri and Rossi, 2001; Dethier and Schoch, 2006).

Taxonomic heterogeneity, described by ' $\alpha$ ' values, varied among the studied ecosystems, with clearly higher values at CA, FO, PR, OR, GM, CO and GO, indicating higher loss of information on the taxonomic structure, because of the general presence of more than one taxon within each successive taxonomic level, from genus to phylum. In most lagoons the highest percentage of loss was observed between family and order, or at higher levels, consistently with the suitability of family as a proper taxonomic level for TS, but at CA, FO and GM the highest percentage of loss was observed between genus and family. Notwithstanding the higher taxonomic heterogeneity and the higher loss of information ( $\alpha$  values) in those lagoons, a relevant loss of information till family level, was not observed neither for univariate indices nor for the multivariate space, as already reported from different habitats (Bacci et al., 2009).

Considering univariate indices, estimated taxa richness (ES) and Simpson index ( $\lambda$ ) correlated well across the different levels of taxonomic aggregation at least till order level, confirming their suitability in view of analyses based on TS. As it was expected, indices based on taxonomic complexity lost information more quickly moving to higher taxonomic levels, in particular the

variation in taxonomic distinctness ( $\lambda^+$ ). Nevertheless the correlation between species and family levels for both  $\Delta^+$  and  $\lambda^+$ , suggested a reasonably low loss of information at this taxonomic level. 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 of taxonomic distance in transitional waters (Mistri et al., 2000a; Salas et al., 2006; Munari et al., 2009), confirmed that they can add complementary information to more classical measures of 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. Therefore the effectiveness of such measures is still unclear and require further investigations (Bevilacqua et al., 2011).

Considering multivariate analyses, our results confirmed that family-level identifications are sufficient to distinguish natural spatial patterns of variability both within (local scale) and among Italian transitional water bodies (regional scale). The loss of information about the general structure of benthic assemblages from species to family taxonomic level is limited, irrespective of the transformation used. In fact, correlations between matrices at species and at family levels were very high ( $r_s \geq 0.9$ ) or high (only one case  $r_s \geq 0.7$  for presence/absence data at BA) across all lagoons (local scale) and data transformations. Correlation was high ( $r_s \geq 0.7$ ) also among species and family matrices with average abundances for each lagoon (regional scale) across every data transformation. Our results are consistent with previous investigations proving that 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; Tataranni et al., 2009). Family level is usually also considered the best compromise between the loss of information and the time gained in sorting and identification at coarser levels than species (Mistri and Rossi, 2001; De Biasi et al., 2003). One of the given explanations for the efficiency of TS in transitional systems is that in brackish environments species are not particularly prone to speciation due to short evolutionary time allowed by the highly fluctuating environment, therefore sympatric speciation pattern is often reduced or absent, and most families are represented by a single species (Giangrande et al., 2005). Nevertheless, different levels of taxonomic heterogeneity (measured as  $\alpha$  value), observed in the present work among different lagoons did not affect TS efficiency at family level. This result is consistent with investigations in different 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 benthic 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 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 (Olgard 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. Olgard et al., 1998; Anderson et al., 2005; Włodarska-Kowalczyk and Kędra, 2007; Bevilacqua et al., 2009), this study showed that the choice of data transformation could influence the results of analyses as well as the level of taxonomic aggregation, or even more. For the analyses at local scale (differences within ecosystems), in most lagoons a reduction of similarity between matrices with different taxonomic levels was observed with increasing strength of data transformation, and the same was observed also at regional scale (differences among ecosystems) for matrices at levels of order, class and phylum. Similar pattern has been 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 transformation reduces the weight of the most abundant taxa. The structure of benthic 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, since dominant species are usually taxonomically distant (e.g. Marchini et al., 2008). Conversely with a strong transformation such as the conversion to presence/absence data, the dominance effect disappeared and taxonomic complexity, considering also rare and less frequent species prevailed. Conversely, analysing species, genus, and family matrices at regional scale (differences among ecosystems), the similarity among lagoons increased with increasing strength of transformation, suggesting that in view of TS (with aggregation at family level) data transformation, even strong, should be the best choice. Those differential responses were most probably the results of complex interactions between the effect of taxonomic resolution and data transformation on macrobenthic community variability at different scales. Our results are consistent with a previous investigation in 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 it was driven by changes in relative abundance at medium or smaller scales, particularly by numerically dominant

taxa. The relative importance of the two component (composition and abundance) across the different scales, changed at higher taxonomic levels (Anderson et al., 2005). 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, 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 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). Nevertheless, it was already pointed out that the choice of data transformation should result from biological questions, since it determines the relative contribution of quantitative and qualitative inter-sample differences in the final outcome of multivariate analyses (Tataranni et al., 2009). A weak transformation gives a narrow view of the community, deeply influenced by the most abundant taxa, whereas a strong transformation yields a wider view of the community, where all 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 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 pollution) are likely related to changes in spatial patterns of assemblages (Warwick and Clarke, 1993; Tataranni et al., 2009) TS may be usefully applied to monitoring programs of lagoon systems. TS application would make those programs more cost-effective, since the time necessary to process 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 sorted to major groups. This improvement of the cost/benefit ratio would be particularly important in highly fluctuating habitat, such as transitional waters, where sampling frequency is particularly important. The use of TS in monitoring programs required by national laws, would consequently result in a consistent reduction of cost for Environmental Agencies.

Our study provide 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 scales, suggested caution in generalising outcomes, and in the choice of data transformation. Moreover, prior to application of TS, area-specific baseline knowledge of each lagoon system, regarding benthic community (at species level), main natural and anthropogenic stressors, and their effect on benthic community, is fundamental. In addition, as suggested by previous investigations (e.g. Dethier and Schoch, 2006, Tataranni et al., 2009), analyses at finer taxonomic level should be

performed periodically and whenever a change in spatial pattern is detected or there are reasons to suspect that a given area is suffering from some new stressor, to which the response of the community is unknown.

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