ISSN: 2037-7738
GRASPA Working Papers
[online]

Web Working Papers
by
The Italian Group of Environmental Statistics

Gruppo di Ricerca per le Applicazioni della Statistica
ai Problemi Ambientali
www.graspa.org

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GRASPA Working paper n. 49, July 2016

Assessing the role of the spatial scale in the analysis of lagoon biodiversity. A case-study on the macrobenthic fauna of the Po River Delta.

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Abstract

The analysis of benthic assemblages is a valuable tool to describe the ecological status of transitional water ecosystems, but species are extremely sensitive and respond to both microhabitat and seasonal differences. The identification of changes in the composition of the macrobenthic community in specific microhabitats can then be used as an "early warning" for environmental changes which may affect the economic importance of lagoons, through their provision of ecosystem services. From a conservational point of view the appropriate definition of the spatial aggregation level of microhabitats or local communities is of crucial importance. The main objective of this work is to assess the role of the spatial scale in the analysis of lagoon biodiversity. First we analyze the variation in the number of species for alternative aggregations of the monitoring stations in three lagoons of the Po River Delta, then we address biodiversity partitioning by a generalized diversity measure, namely the Tsallis entropy, and for alternative definitions of the local communities. The variation of the corresponding entropy indices is then analyzed by mixed effects models properly accounting for the fixed effects of biotic and abiotic factors and random effects ruled by nested sources of variability corresponding to alternative definitions of local communities. Finally the main results obtained by the proposed statistical protocol are presented, discussed and framed in the Ecological context.

Keywords: Lagoon biodiversity, macrobenthic fauna, Tsallis entropy, biodiversity partitioning, mixed effects models.

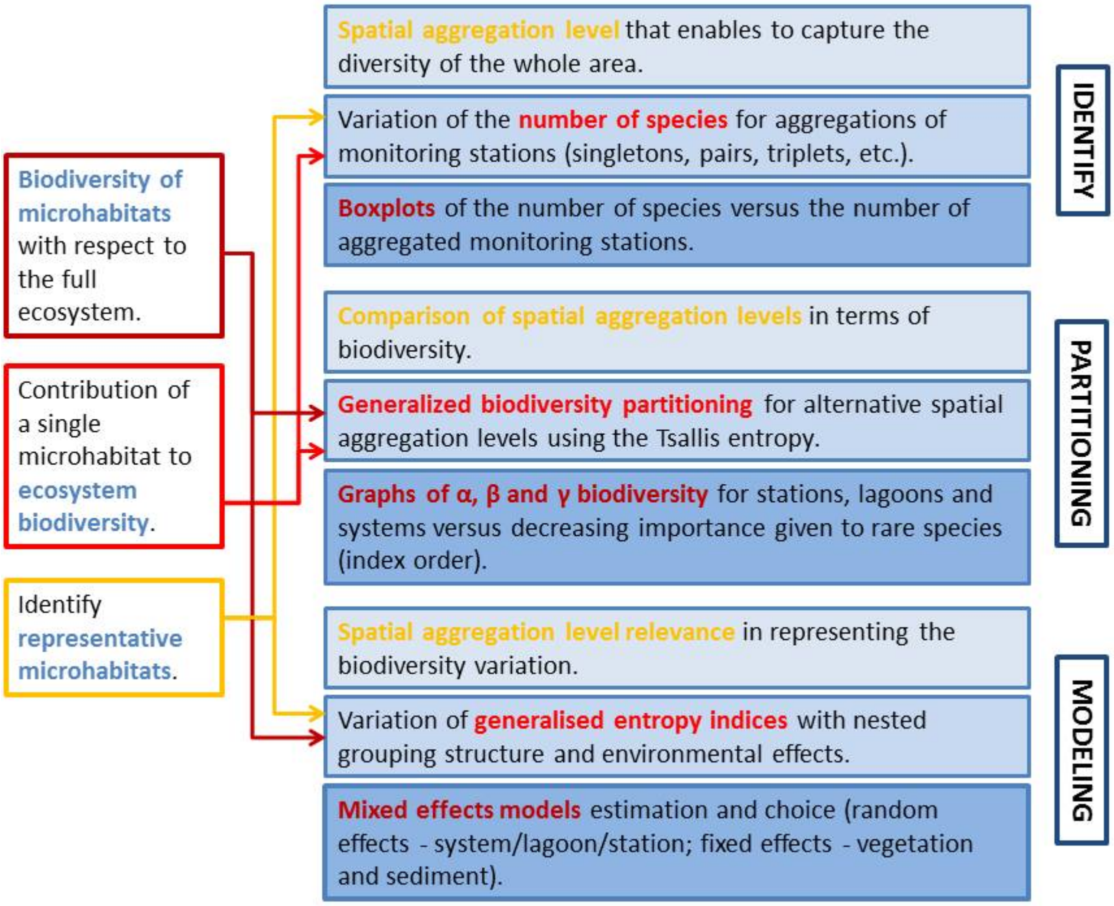
1. Introduction

Transitional waters, such as coastal areas, are highly heterogeneous ecosystems in relation to the high variation of chemical, physical, morphological, hydrodynamic and/or functional factors (Basset et al. 2013). These systems are characterized by high instability (Sousa et al., 2009) which is associated to the instability of their fresh water sources (e.g. floods, droughts of rivers/streams and sediment transport) and to the sea tide. In recent years, phenological shifts have been observed in the vegetation of these ecosystems: in strictly aquatic areas (Viaroli et al., 2008) with the disappearance of rooted macrophytes (e. g. *Zoostera* and *Ruppia*) replaced by phytoplankton and macroalgae ephemeral (e.g. *Ulva*) (Raffaelli et al. 1998), and in limiting areas (shore and shallow waters) with the marked decrease or disappearance of *Phragmites* that were previously abundant in the riparian zones of European coastal areas with relatively low salinity (Van der Putten, 1997; Fogli et al. 2002). These changes are associated to the increase of anthropogenic activities over the coastal areas such as fishing (fish, mussels and clams), sand abstraction, agricultural pollution by nutrients causing eutrophication (Viaroli et al. 2008) etc. Thus, modification of the aquatic vegetation is expected to change the whole community and therefore the ecosystem functionality and provided ecosystem services (Eire and Ferguson, 2002; Smith 2003; Newton et al. 2014). The aforementioned aspects indicate the need to increase our knowledge on transitional areas' functioning in order to improve the development of biodiversity conservation plans. Legislation and actions have been adopted to stop further deterioration and restore these areas to a healthy state. The Water Framework Directive (WFD, 2000/60/EC) requires EU Member States to assess the ecological status of each water body in Europe and to ensure a sustainable management such that good ecological quality of all water bodies will be obtained by 2015. The analysis of benthic assemblages is a valuable tool to describe the ecological status of these transitional ecosystems, since macrobenthic fauna is known to be highly correlated with the sediment, which accumulates the multiple sources of organic enrichment and pollution (Pearson and Rosenberg, 1978). Macrobenthic species are extremely sensitive and respond to both microhabitat and seasonal differences, adding complexity to the variability of these ecosystems (Carvalho et al 2011).

For these reasons, the identification of changes in the composition of the macrobenthic community in specific microhabitats can be used as an "early warning" for environmental changes which may affect the economic importance of lagoons, through their provision of ecosystem services (e.g. nutrient cycling, flood control, shoreline stabilization, water quality improvement, fisheries resources, habitat and food for migratory and resident animals and recreational areas for humans) (Basset et al., 2013). From a conservational point of view several questions arise. What is the importance of the biodiversity of a single microhabitat with respect to the entire ecosystem? Which microhabitats contribute more to the entire ecosystem biodiversity? Is it possible to maintain biodiversity of the entire lagoon preserving only the most diverse microhabitats or should we care more about the conservation of ecosystem peculiarities? Should we consider a lagoon as a combination of microhabitats and can we identify a microhabitat more representative than others? All these questions address the same fundamental issue, i.e. the appropriate definition of the spatial aggregation level of microhabitats or local communities (monitoring station, microhabitat, lagoon, system of lagoon).

The main objective of this work is to assess the role of the spatial scale in the analysis of lagoon biodiversity. We first analyze the variation in the number of species for alternative aggregations of monitoring stations in three lagoons of the Po River Delta, then we address biodiversity partitioning by a generalized diversity measure and for alternative definitions of the local communities. The variation of the corresponding entropy indices is then analyzed by mixed effects models properly accounting for the fixed effects of biotic and abiotic factors and random effects ruled by nested sources of variability corresponding to alternative definitions of local communities.

The work-flow of the paper is displayed in Figure 1. In Section 2 some technical details are given on the sampling procedures, statistical methods and mathematical ideas applied in this work. In Section 3 results are described and finally a discussion is provided in section 4.



Biodiversity of microhabitats with respect to the full ecosystem.

Contribution of a single microhabitat to ecosystem biodiversity.

Identify representative microhabitats.

Spatial aggregation level that enables to capture the diversity of the whole area.

Variation of the number of species for aggregations of monitoring stations (singletons, pairs, triplets, etc.).

Boxplots of the number of species versus the number of aggregated monitoring stations.

Comparison of spatial aggregation levels in terms of biodiversity.

Generalized biodiversity partitioning for alternative spatial aggregation levels using the Tsallis entropy.

Graphs of α, β and γ biodiversity for stations, lagoons and systems versus decreasing importance given to rare species (index order).

Spatial aggregation level relevance in representing the biodiversity variation.

Variation of generalised entropy indices with nested grouping structure and environmental effects.

Mixed effects models estimation and choice (random effects - system/lagoon/station; fixed effects - vegetation and sediment).

Figure 1. Conceptual scheme of the interactions between the ecological processes and the statistical protocol.

2. Materials and methods

2.1 Data Collection

Data on benthic macroinvertebrates were collected in three lagoons (Goro, Fattibello and Comacchio) within the Po River Delta ecosystem (Northern Adriatic Sea, Figure 2). The selected lagoons present from one to three dominant habitat types defined by a factorial classification of sediment granulometry (sand, mud) and vegetation cover/type (without vegetation, submerged macrophytes, emerged macrophytes and macroalgae) as in Basset et al. (2008b). In every lagoon, field sampling campaigns were performed with one to three monitoring stations per habitat type. Three replicates per monitoring station were sampled monthly in the period 1997-2000 with a van Veen grab (area 0.06 m^2). In the laboratory, benthic samples were sorted under a stereomicroscope, identified to the lowest possible taxonomic level and counted. Hereby, we present results including 3 replicates at each of 23 monitoring stations, divided in 10 areas belonging to 3 lagoons (nested grouping structure).

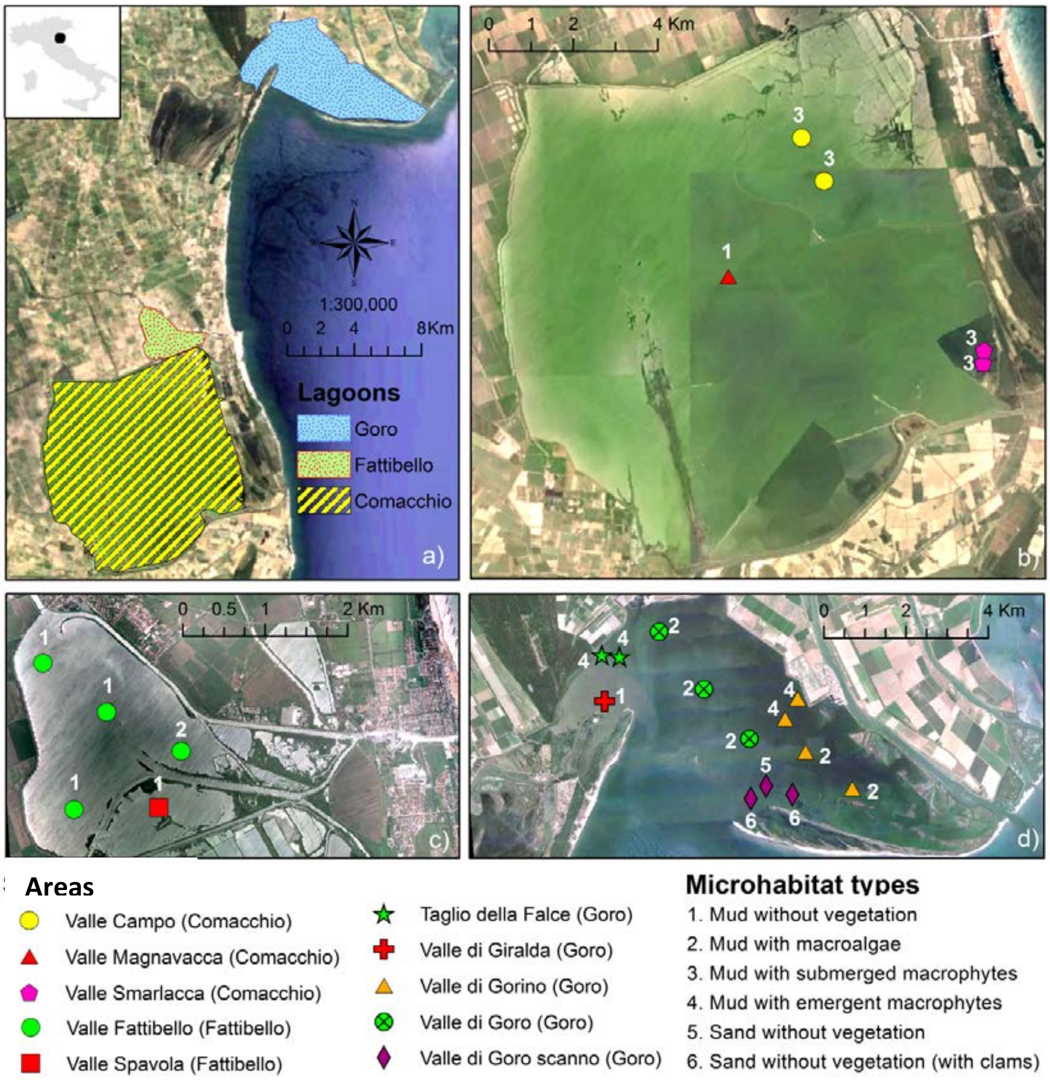


Figure 2 **a)** Location of the three lagoons, monitoring stations and microhabitat types in **b)** Comacchio, **c)** Fattibello and **d)** Goro.

2.2 Statistical Protocol

We address the main ecological questions as summarized in the introduction and sketched in Figure 1 by a specific statistical protocol structured in the three steps corresponding to general and specific objectives as described below.

Identify - A first grasp on the spatial aggregation level that captures the diversity present in the Po River Delta is obtained counting the number of species found considering combinations (singletons, pairs, triplets, etc.) of monitoring stations. Stations are combined in all possible ways, up to the maximum number available in each lagoon and species are counted. Graphical representations of these numbers help to evaluate the "optimal" number of stations to consider at once. Generally speaking, we expect that the number of species tends to stabilize and show smaller variability increasing number of stations considered in the combinations (say singletons, pairs, etc.). Boxplots of the number of species are then plotted versus the number of stations considered in the combinations. We consider optimal the number of aggregated stations above which the central values stabilize and the variability decreases.

Partitioning - We then address the choice of a suitable biodiversity measure and the appropriate definition of the spatial aggregation level or local community (monitoring station, area, lagoon) by biodiversity partitioning into alpha, beta and gamma components. Consider a community where n individuals are sampled. Let $s = 1, \dots, S$ denote the species that compose the community and n_s be the number of sampled individuals of species s, with $\sum_{s=1}^{S} n_s = n$. The probability that an individual belongs to species s is estimated by $p_s = n_s/n$. Given a discrete set of probabilities $p = (p_1, \dots, p_S)$ and any real number q, the Tsallis entropy of order q (Marcon and Hérault, 2015), is defined as

$$H_q(p) = \frac{1}{q-1}\left(1 - \sum_{s=1}^{S} p_s^q\right) \tag{1}$$

The number of species is the Tsallis entropy of order $q = 0$, while Shannon's and Simpson's indices respectively correspond to $q = 1$ and $q = 2$, then the importance given to rare species decreases continuously with q. Corresponding *true diversity* measures $D_q(p)$, or *Hill numbers*, are obtained taking the deformed exponential transformation e_q of the Tsallis entropy:

$$D_q(p) = e_q\left(H_q(p)\right) \tag{2}$$

For $H_q(p) < \frac{1}{q-1}$, the deformed exponential transformation of order q is defined as

$$e_q\left(H_q(p)\right) = \left[1 + (1-q)H_q(p)\right]^{\frac{1}{1-q}} \tag{3}$$

with the standard exponential transformation obtained as a special case when $q = 1$.

Diversity measures are traditionally partitioned into gamma, alpha and beta diversity, respectively ${}^{\gamma}D_q(p)$, ${}^{\alpha}D_q(p)$ and ${}^{\beta}D_q(p)$, with ${}^{\gamma}D_q(p) = {}^{\alpha}D_q(p) \times {}^{\beta}D_q(p)$. Biodiversity partitioning means that the gamma biodiversity of all individuals in a given meta-community may be split into alpha and beta biodiversity that respectively reflect the diversity within and between local communities. The multiplicative partition of biodiversity measures is mathematically equivalent to the additive decomposition of the Tsallis entropy, as stated in Marcon et al. (2014) where the authors define beta entropy as the average generalized Kullback-Leibler divergence between local communities and their average distribution. They also propose estimation bias corrections that can be applied to the Tsallis entropy to obtain, after deformed exponential transformation, easy-to-interpret components of biodiversity. Output interpretation is helped by graphical representation of the diversity components as functions of the order q, together with 95% bootstrap confidence bands (Efron and Tibshirani, 1986). We do expect that if spatial aggregation is uninfluential, then curves corresponding to different spatial aggregation levels tend to overlap.

Modeling - Mixed models, also known as mixed effects models or multilevel models, find application when the data have some sort of grouped and/or hierarchical structure. In the analysis of biodiversity, grouped data are provided by repeated measurements within the same communities, while nested data structures arise due to hierarchically scaled local/spatial communities. If the experimental design implies taking multiple measures per each subject (e.g. monitoring station, habitat, community), then sample data can be considered as made of groups of individual responses. This leads to a violation of the usual independence assumption implied by the linear model, as multiple or repeated measures from the same group cannot be regarded as independent from each other. In mixed models random effects are added to the systematic terms or fixed effects. Adding a random effect for each group of a grouped data structure allows to resolve the non-independence issue and to characterize variations due to group differences by a different random baseline for each group. Fitting mixed effects models implies the selection of relevant fixed and random effects. It is generally based on tools like the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), both made of two terms measuring the fit and the complexity of the model, respectively. The maximized model likelihood is used as measure of fit and can be obtained by two alternative approaches: simple likelihood maximization (ML) and restricted expected likelihood maximization (REML). AIC's and BIC's based on ML are not comparable with those obtained by REML. A complementary approach to model selection is via hypothesis testing, with three options: t-statistics, F-statistics, or likelihood ratio tests. Mixed model selection is generally based on a top-down procedure starting with the so called *beyond optimal model*, where the fixed component contains all explanatory variables and as many interactions as possible. The optimal random structure can be found comparing BIC and AIC values of the REML estimates of the beyond optimal model with alternative nested specifications of the random structure. Then the F-statistic or the t-statistic can be used to find the optimal fixed structure. To compare models with nested fixed effects, but with the same random structure, ML estimation must be used. The final estimates of the selected model are obtained using REML (Zuur et Al., 2009).

Multilevel mixed effects models accounting for fixed and nested sources of variability are used here to analyze the variation of generalized entropy indices corresponding to a set of selected levels of spatial aggregation:

$$h_{rmls} = \beta_1 x_{1,rmls} + \beta_2 x_{2,rmls} + \beta_3 x_{3,rmls} + b_s + b_{ls} + b_{mls} + \varepsilon_{rmls}$$

where h_{rmls} is the value of the generalized entropy index, with $\mathrm{r} = 1, \dots, 12$ for months, $\mathrm{m} = 1, \dots, 21$ for monitoring stations, $\mathrm{l} = 1, \dots, 10$ for areas and $\mathrm{s} = 1,2,3$ for lagoons (systems of areas). The previous model accounts for the fixed effect of the season (factor x_1), of the presence of macroalgae (x_2) and of two sediment types (x_3). Random effects of the lagoon (b_s), of the area (b_{ls}) and of the monitoring station (b_{mls}) reflect the nested grouping structure of the local communities. After model selection and fit, estimates are interpreted and allow finding, among other features, which random effects are more influential in explaining the variability of generalized entropy indices, then returning evidence on the relevance of the spatial aggregation, i.e. the habitat size.

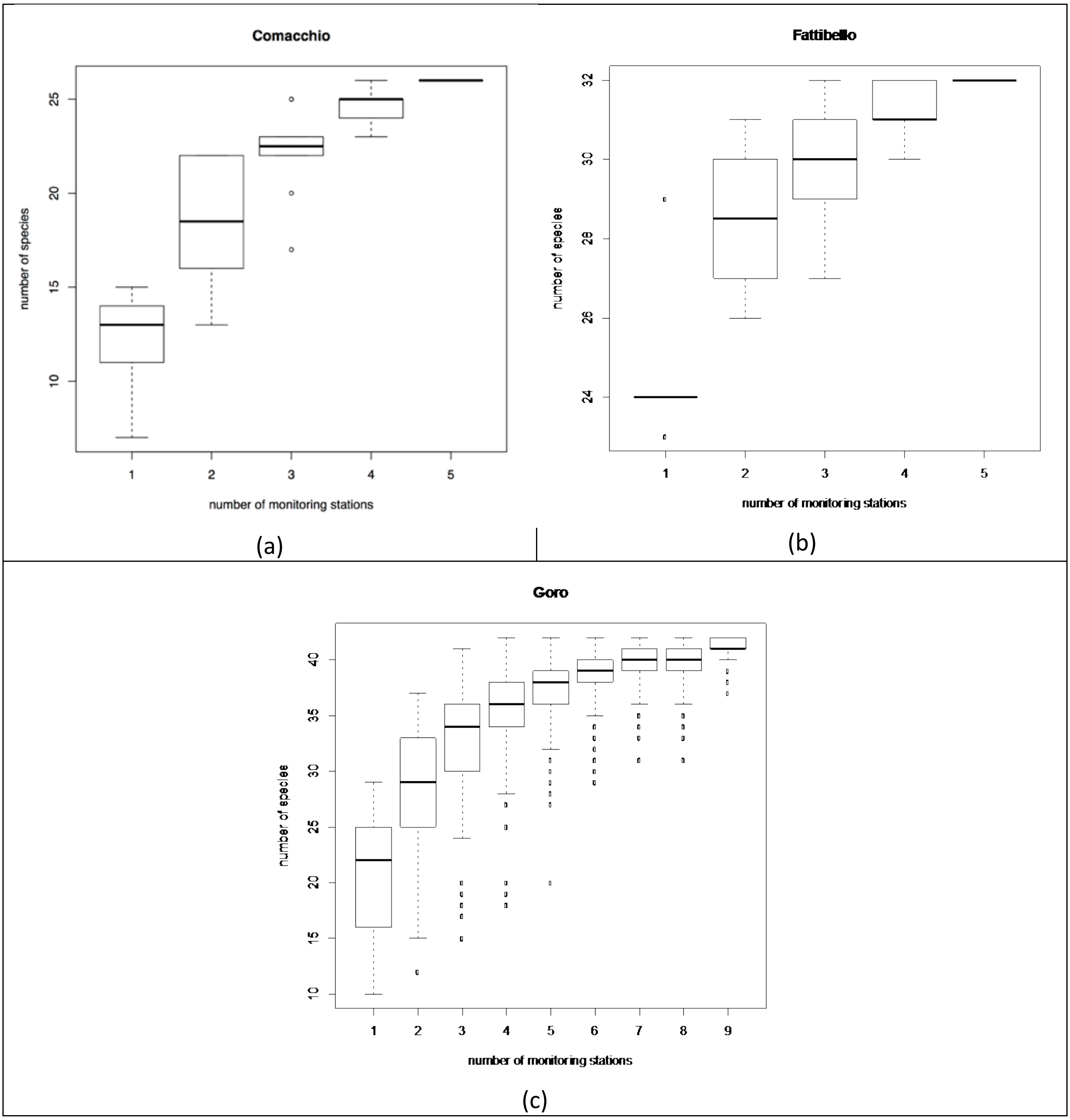
3. Results

A first simple procedure to find out the aggregation level that captures the diversity present in the Po River Delta consists in counting the number of species found for singletons, pairs, triplets, etc. of monitoring stations. We collect all such combinations up to the maximum number of stations that can be aggregated in each lagoon. We expect that increasing the number of aggregated stations most of the combinations return the same number of species. In Figure 3 we report boxplots of the number of species found at each combination of monitoring stations. In the three lagoons it is possible to capture almost all the present species even with a number of stations as small as 4. However those stations should be “suitably” chosen to maximize the unknown species variability. Comacchio and Fattibello lagoons are smaller and have a smaller number of monitoring sites with respect to Goro. Figures 3 (a) and (b) show that the former two systems should be analyzed using all monitoring stations, while in Figure 3 (c) the number of species stops to increase above 6 sites and, on average, it remains stable around 41 species.

In Figure 4 we report the partitioning of the gamma biodiversity of benthic macroinvertebrates into alpha and beta biodiversity for a range of values of the generalized biodiversity index order *q* and 3 alternative definitions of the local communities: monitoring stations, areas and lagoons. 95% bootstrap confidence intervals are also included in the plots.

As expected, gamma biodiversity does not change according to the definition of the local communities and both gamma and alpha biodiversity increase with the importance given to rare species. Alpha biodiversity is obviously larger for larger local communities (lagoons) while the opposite holds for beta biodiversity. Beta biodiversity reaches a minimum for $q \in (0.5, 0.9)$ and increases for extreme values of *q*, i.e. when the importance given to rare species is very high or minimal. This effect is more evident when local communities are small (monitoring stations). An interesting feature of the beta index is that its value decreases with the increasing size of the local community, suggesting that the largest the communities the smallest the differences among them.

We are interested in assessing which spatial scale, i.e. definition of the local communities (lagoons, areas or monitoring stations), seems more relevant in representing the biodiversity variation. Here we measure biodiversity on the log scale analyzing generalized entropies as computed in equation (1). To achieve this goal we estimate mixed effects models with fixed effects defined by vegetation and sediment indicators describing the surrounding environment. Random effects are ruled by the nested effects lagoon/area/monitoring station.

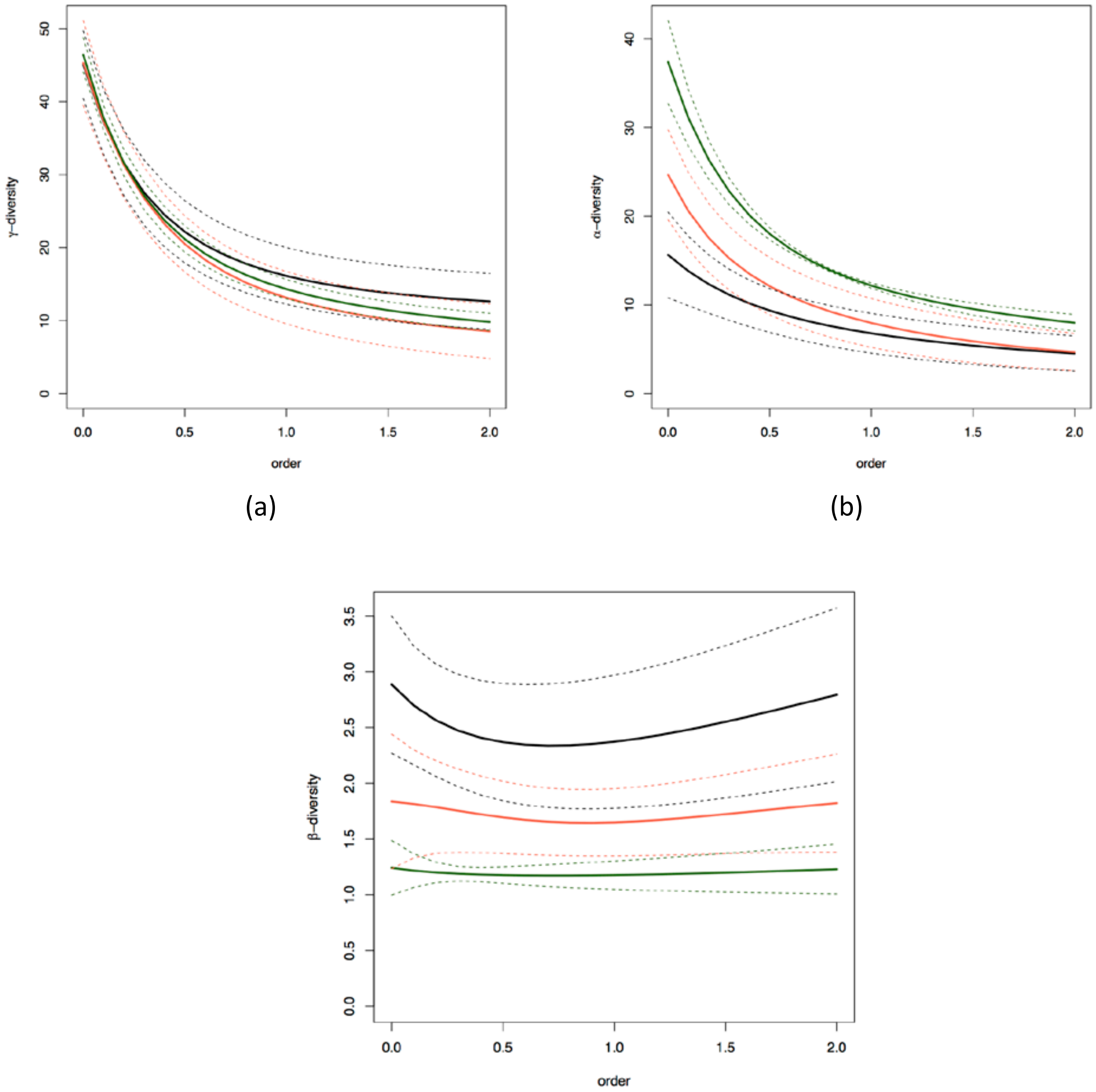


(a)

(b)

(c)

Figure 3. Assessing the number of station required to capture all the species in each lagoon: **(a)** Comacchio, **(b)** Fattibello, **(c)** Goro.



(a) (b)

(c)

Figure 4. Biodiversity indices (solid) and their bootstrap 95% confidence intervals (dashed) for alternative definitions of the local communities (black - monitoring stations, red - areas, green – lagoons): **(a)** gamma diversity, **(b)** average alpha diversity and **(c)** beta diversity.

When the beyond optimal model is estimated with vegetation classified into 4 categories (none, macroalgae, emerged macrophytes, submerged macrophytes), the choice between alternative random structures highlights the relevance of the lagoon and station effects, while the area effect appears to be negligible (with AIC for q = 0, 1, 2, with BIC for q = 0, 1). AIC and BIC comparisons of models with the selected random structure and alternative specifications of the fixed effects leads to ambiguous results for q = 0, 1, 2. The previous considerations (results are not reported here for the sake of space and readability, but are available from the authors upon request) push towards a more aggregate definition of the information on the vegetation coverage, considering the presence/absence of macroalgae.

In Table 1 the “beyond optimal” approach introduced in section 2 for the choice of random effects is reported, with vegetation coded as macroalgae presence/absence. The most relevant feature highlighted is that the intermediate spatial aggregation level (area) is generally not relevant for the description of entropy variability, while the hierarchical combination of lagoon and station effects returns the best value (according to all chosen criteria, except for q = 2 with the BIC).

In Table 2 we report the choice of the fixed effects with the nested random effects lagoon/station chosen above. Again the choice is based on both BIC and AIC criteria. Macroalgae presence together with the type of sediment seem to play a crucial role no matter how we measure biodiversity. Season seems to have a marginal relevance only when rare species are given a relevant role (q=0) and according to only one criterion (AIC).

In Table 3 the estimated fixed effects together with their standard errors and p-values for the chosen models (Table 2) are reported. As both sediment and macroalgae are classified as binary, the model intercept represents the fixed effect of mud and no-macroalgae. The other coefficients are deviations from this intercept value and are significantly different from zero whenever the corresponding p-value is smaller than a fixed error threshold chosen to be 5% (0.05). Hence when q=0 all effects are statistically significant. In presence of a sand sediment and no macroalgae the expected entropy level is obtained adding 6.7984 to the estimated intercept, while if the sediment is sand and macrolagae are present the value 12.6674 (5.864+6.7984) has to be added to the estimated intercept effect. When q=1 and 2 (and less importance is given to rare species) the contribution of the sediment becomes weaker (p-values increase), but it is still important.

Random effects	**q=0**		**q=1**		**q=2**	
	BIC	AIC	BIC	AIC	BIC	AIC
lagoon/area/station	1477.213	1430.775	394.6358	348.1978	-86.4343	-132.8723
lagoon/area	1498.456	1455.590	396.7004	353.8345	-90.1588	-133.0246
lagoon/station	1471.641	1428.775	389.6561	346.7902	-90.8121	-133.6780
area/station	1484.811	1441.945	393.9559	351.0900	-88.2805	-131.1463
lagoon	1494.840	1455.547	400.3243	361.0306	-88.8064	-128.1001
area	1508.481	1469.187	396.0933	356.7996	-91.9428	-131.2365
station	1493.181	1453.887	396.9530	357.6593	-87.0491	-126.3428

Table 1. Choice of the random effects based on both AIC and BIC criteria, for 3 different biodiversity indices: q=0 number of species, q=1 Shannon, q=2 Simpson. In red the chosen combination.

Fixed effects	**q=0**		**q=1**		**q=2**	
	BIC	AIC	BIC	AIC	BIC	AIC
Season*Macroalgae+Sediment[1]	1486.813	1443.543	366.0261	322.7565	-132.0346	-175.3042
Season* Macroalgae	1498.510	1458.846	366.5817	326.9179	-131.5525	-171.2163
Season+ Macroalgae +Sediment	1478.230	1445.778	351.1506	318.6984	-147.5589	-180.0111
Season+ Macroalgae	1489.923	1461.076	351.7047	322.8583	-147.0784	-175.9248
Macroa+Sediment	1473.405	1451.770	339.4166	317.7817	-159.9924	-181.6272
Season+Sediment	1492.917	1464.071	358.8131	329.9666	-138.9411	-167.7875
Season	1490.706	1465.466	353.7882	328.5476	-144.1383	-169.3789
Macroalgae	1485.118	1467.089	339.9750	321.9460	-159.5090	-177.5381
Sediment	1488.114	1470.085	347.0858	329.0568	-151.3701	-169.3991
Intercept	1485.908	1471.484	342.0621	327.6389	-156.5669	-170.9901

Table 2. Choice of the fixed effects given the random effects lagoon/station. The choice is based on both BIC and AIC criteria, for 3 different biodiversity indices: q=0 number of species, q=1 Shannon, q=2 Simpson. In red the chosen combination.

q=0	**Value**	**Std.Error**	**p-value**
No Macroalgae and mud	8.1057	3.1652	0.0110
Macroalgae	5.8640	1.0337	0.0000
Sand	6.7984	1.3401	0.0001
q=1			
No Macroalgae and mud	1.2586	0.2023	0.0000
Macroalgae	0.4825	0.1147	0.0005
Sand	0.3934	0.1488	0.0165
q=2			
No Macroalgae and mud	0.5800	0.0578	0.0000
Macroalgae	0.1762	0.0393	0.0003
Sand	0.1364	0.0510	0.0153

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[1] The * notation denotes interaction between two variables.

Table 3. Estimated fixed effects and their standard errors for the chosen model according to BIC and AIC criteria, for three levels of the generalized entropy measure. P-values refer to coefficient significance.

Information on the relative relevance of the spatial scale of the local communities on the expected generalized entropy is obtained by the estimates of the random effects standard deviations. In Figure 5 the percentage of total standard deviation explained by each effect are depicted. The barplots show that the lagoon level is always the most relevant for these data, no matter the importance given to rare species. The role of monitoring stations becomes more important for larger values of *q*, i.e. when rare species are less and less considered.

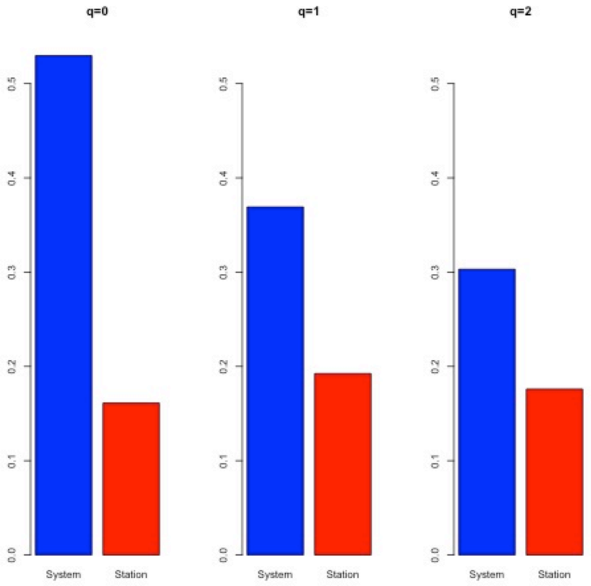

Figure 5. Percentage of total standard deviation explained by the nested random effects (lagoon – blue, monitoring station – red) in the chosen models, for 3 levels of the order *q* of the generalized entropy measure

4. Discussion

The main findings obtained following the Identify step of the proposed protocol highlight that even with a number of stations as small as 4 it is possible to capture all the species present in each of the three lagoons; however those stations should be “suitably” chosen to maximize the unknown species variability. Cautiously Comacchio and Fattibello should be analyzed using all 5 available monitoring stations, while 6 out of 13 stations are already enough to capture all species in the Goro lagoon.

The Partitioning step shows that diversity at the metacommunity level (gamma diversity) doesn't change when the spatial aggregation level (i.e. the definition of the local communities) changes. As expected gamma biodiversity increases with the importance given to rare species. Mean species diversity at the habitat level, (diversity at the local community level or alpha diversity) depends on the definition of the local communities, i.e. on the spatial aggregation level. It is larger at the lagoon level and significantly smaller when evaluated at the area level. Again, as expected, alpha biodiversity increases with the importance given to rare species. Differentiation among microhabitats (beta diversity) shows an opposite behavior with respect to alpha diversity. Coherently beta diversity slightly decreases when the level of spatial aggregation, i.e. the size of the local communities, increases (in fact it is smaller at the lagoon level), suggesting that the largest the communities the smallest the differences among them. Beta biodiversity increases when the importance given to rare species is extreme, i.e. very high or minimal, this effect being more evident when local communities are small (monitoring stations). Naturally, beta diversity is the measure that is mostly influenced by changes in the spatial definition of microhabitats as it measures the differentiation among them. Notice that by widening the spatial scale, beta diversity does not increases, confirming the relevance of small-scale habitat structures highlighted in Hewitt et al. (2005) for coastal marine environments. The considerable functional specificity of the macrobenthic community with respect to food items presence can be seen as the main driver to small-scale analyses at microhabitat level (Barnes and Hendy, 2015).

The Modeling step of the proposed protocol allows to highlight several relevant features related to the influence of the spatial scale of microhabitats on the definition of the alpha diversity on the log scale (entropy). The hierarchical combination of lagoon and station effects is generally a relevant source of variability of the entropy measure, while the intermediate spatial aggregation level (area) has a poor performance in terms of model fit. This highlights the possible role of the two habitat sizes (lagoon and station). The presence of macroalgae together with the type of sediment seem to play a crucial role no matter how we measure biodiversity. When q=1 and 2 (and less importance is given to rare species) the contribution of the sediment becomes less important, while the season seems to have a marginal relevance only when rare species are given a relevant role (q=0). These results confirm the main role of the vegetal community structure in influencing the macrozoobenthic component and determining differentiation in the community. It is well known that the composition of macrobenthic communities can vary on small spatial scales in transition systems (Ysebaert and Herman, 2002). This can be due to abiotic factors such as salinity, grain size of the substrate, the hydrology (Mannino and Mountain 1997; Barbone and Basset 2010), and biotic such as trophic relationships and the availability of food sources (Carvalho et Al. 2011). Among the biotic factors, the vegetal community seems to deeply influence the structuring of the macrobenthic community. In particular the presence of seagrass bed (Blanchet et Al. 2004), macroalgae bed (Carvalho et Al. 2011), emergent macrophytes (Yuhas et Al. 2005) have the most relevant impact. The results of the estimate of mixed effects models also show that the geographical location doesn't contribute to this feature: analyzing geographically distant lagoons all characterized by the same seagrass bed structure leads to similar results most likely due to a strong homogeneity in the macrozoobenthic component. Macrobenthic communities accordingly respond to such mosaic-type lagoon environments structuring metacommunities with patches specific to available microhabitats. The degree of diversification reflects this structure, highlighting that all types of microhabitats play the same role in contributing to the diversification of the community as a whole (Davis et Al., 2014). We can definitely say that the use of mixed models facilitates understanding the influence of the spatial scale of the local communities on biodiversity. Results emphasize that also in the Po River Delta, as in other transitional water ecosystems (Basset et Al. 2008a), biodiversity conservation cannot be managed locally but requires a large scale process of governance to be effective. This need to preserve higher levels of biological organization has long been recognized and is reflected in the emergence of a recent IUCN Red List of Ecosystems (Keith et Al., 2013). In view of the conservation of transition systems, there is then the need for careful management of all types of microhabitat given their high value of specificity in term of both species and processes.

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Appendix

Species List	
Abra segmentum	Lentidium mediterraneum
Actiniari spp	Littorina littorea
Alitta succinea	Lucifer typus
Ampelisca sp	Microdeutopus gryllotalpa
Anadara inaequivalvis	Monocorophium insidiosum
Arcuatula senhousia	Mytilaster minimus
Ascidiacei spp	Mytilus galloprovincialis
Balanidae spp	Nassarius reticulatus
Bittium reticulatum	Nephtys hombergii
Brachynotus sexdentatus	Oligochaeta spp
Capitella capitata	Ostrea edulis
Carcinus aestuarii	Palaemon elegans
Cerastoderma glaucum	Phyllodoce lineata
Chironomus salinarius	Polydora ciliata
Crassostrea sp	Prionospio multibranchiata
Cyclope neritea	Ruditapes decussatus
Ensis siliqua	Ruditapes philippinarum
Ficopomatus enigmaticus	Sphaeroma serratum
Gammarus aequicauda	Spio decoratus
Haminoea hydatis	Streblospio shrubsolii
Heteromastus filiformis	Tellina sp
Hydroides dianthus	Turbellaria
Idotea balthica	Ventrosia ventrosa
Lekanesphaera hookeri	

Main findings	
IDENTIFY	By appropriately selecting a number of stations as small as 4 it is **possible** to capture all the species present each lagoon.
	Cautiously, Comacchio and Fattibello should be analyzed using all 5 stations, while 6 out of 13 stations are **enough** for the Goro lagoon.
PARTITIONING	**Diversity at the metacommunity level** (gamma diversity) doesn't change when the definition of local communities (spatial aggregation level) changes. Gamma biodiversity increases with the importance given to rare species.
	Mean species diversity at the habitat level (alpha diversity) depends on the definition of the local community (its spatial aggregation level). It is larger at the lagoon level and significantly smaller when evaluated at the area level. Alpha biodiversity increases with the importance given to rare species.
	Differentiation among microhabitats (beta diversity) decreases with the level of spatial aggregation, i.e. with the increasing size of the local communities, suggesting that the largest the communities the smallest the differences among them. It increases when the importance given to rare species is very high or minimal, this effect being more evident when local communities are small (monitoring stations).
MODELING	The hierarchical combination of lagoon and station effects returns the best value in terms of model fit, while the intermediate spatial aggregation level (area) is not relevant for the variability of the entropy measures. This highlights the possible role of **two habitat sizes** (lagoon and station).
	The presence of **macroalgae** together with the type of **sediment** seems to play a crucial role. When q=1 and 2 (and less importance is given to rare species) the contribution of the sediment becomes less important. The **season** seems to have a marginal relevance only when rare species are given a relevant role (q=0).
	The **lagoon** spatial aggregation level is the most relevant for assessing biodiversity in this system, no matter the importance given to rare species. The heterogeneity of **monitoring stations** becomes more evident when rare species are less and less considered.