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Multiscale dynamics of zoobenthic communities and relationships with environmental factors in the Lagoon of Venice

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The thesis project was conducted under the supervision of the Ca' Foscari University of Venice (prof.ssa Annamaria Volpi Ghirardini) and the Laboratory of Benthic Ecology of CNR-ISMAR (dott. Davide Tagliapietra).

The aim of the thesis is to outline the spatial and interannual variability of the macrozoobenthic community and the structuring environmental factors in a typical estuarine lagoon. The study site is the Lagoon of Venice.

The activities included a six-month period at the University of Murcia (Spain), under the supervision of prof. Angel Pérez-Ruzafa (Ecology and Management of Coastal Marine Ecosystems Research Group).

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1. INTRODUCTION

1.1 COASTAL TRANSITIONAL ECOSYSTEMS

Estuaries, rias, fjords, coastal lagoons, bahiras, river mouths, tidal creeks, deltas and similar coastal environments are often regarded as a single broad conceptual class (e.g. Guelorget & Perthuisot, 1983; Kjerfve, 1994; McLusky & Elliott, 2007). These water bodies are located within the coastline (e.g. lagoons, fjords) or cross through it protruding into the sea (e.g. deltas). Most of these nearshore, protected environments are related to the main estuarine and lagoonal types. "Brackish", "paralic" and "transitional" are the more inclusive terms used to designate collectively this class of environments. These terms also reveal the environmental models where they originated: "brackish" stresses the importance of freshwater inflow and seawater dilution, "paralic" underlines the proximity of the sea and the role of the marine component, "transitional" points out the presence of gradients and ecotonal traits. Nevertheless every term, generated from different historical perspectives and scientific points of view, excludes some of the above-mentioned environments (Tagliapietra *et al.*, 2009). A diagram showing relationships between the terms is presented in Figure 1.1. The term "Coastal Transitional Ecosystem" (CTE) has been proposed by Tagliapietra *et al.* (2009) with the intention of encompassing the whole class of environments, which in the same paper has been defined in a synthetic form as "coastal water bodies with limited seawater supply".

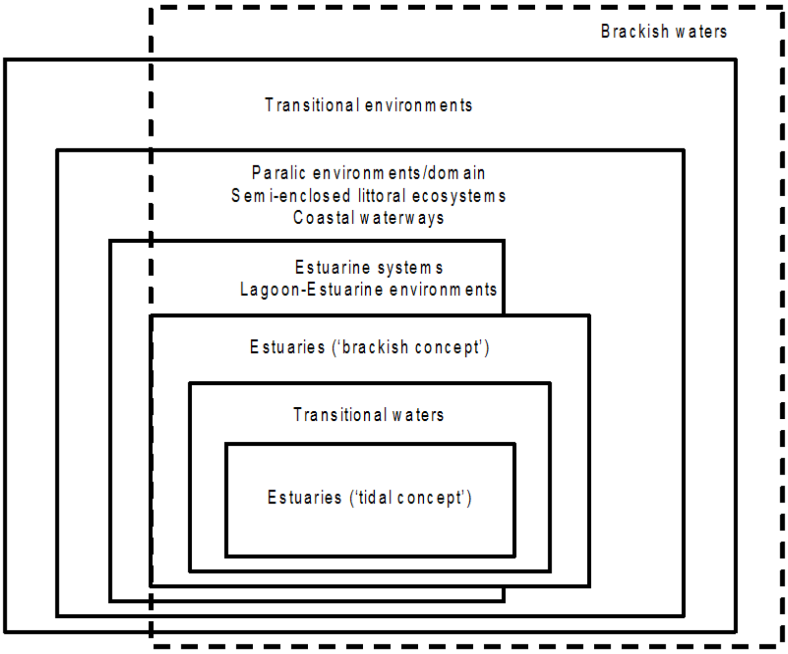


Figure 1.1: Conceptual scheme of the relationships among the terms. The eccentricity of "estuarine system" set results from doubt about its applicability to rocky shores (Tagliapietra *et al.*, 2009).

Estuaries, lagoons and other classes of CTE have many physical and ecological processes in common (Constable & Fairweather, 1999; Ketchum, 1983; McLusky & Elliott, 2004; Thrush & Warwick, 1997).

The main physical factors that contribute to the genesis and characterization of CTE are climate, hydrodynamics and tidal range, coastal typology (Bird, 1994; Pethick, 1984; Tagliapietra & Volpi Ghirardini, 2006), as well as human action. The climate determines the hydrological balance through direct precipitation on the basins and evaporation, controlling the flow of the rivers which in turn cause erosion, sedimentation and the formation of alluvial plains. Climate directly and indirectly affects the saline balance and morphological processes (e.g. Nichols & Boon, 1994). The nature of the coast defines the horizon for the development of a lagoon. The relationships between coastal typology and tidal energy were described by Davies (1964), Hayes (1979), Davis & Hayes (1984). The tidal range determines a series of important features such as sediment dispersal patterns and sediment texture, morphology and residence time (Barnes, 1994b; Brambati, 1988; Kjerfve, 1994; Pethick, 1984). Microtidal low coasts, for example, are apt for coastal lagoon development, as they allow the formation of barrier islands whilst maintaining cyclical water exchange with the sea. The existence of characteristic tidal levels reflects in the vertical and horizontal development of typical landforms and consequently on the vertical and horizontal zonation of communities. In systems subjected to tides, ebbs and floods generate erosional and depositional processes that physically shape the substrate. Typical landforms/habitats, such as channels, subtidal flats, tidal creek and intertidal mudflats and salt marshes are structures generated principally by the tides (Albani *et al.*, 1984). Tides have a direct influence on emersion and submersion times and, consequently, on structure of intertidal biocoenoses. Vertical biological zonation is the result of physical zonation and biological interactions.

CTEs are generated by the merging of sea, land and rivers and mark the passage between marine and non-marine realms. This merging gives rise to new, emergent properties shared by all these environments, including shallowness, shelter, the presence of strong gradients, variability in mesological parameters, prevalent sedimentary bottoms, high spatial heterogeneity in hydrological conditions, high biological production, susceptibility to anoxia and, generally, a significant departure of chemico-physical variables from the normal range of variation measured in the offshore waters or freshwater systems. This reflects on the communities structure and on the presence of a common set of species (Pérez-Ruzafa *et al.*, 2010; Tagliapietra *et al.*, 2009). Levin *et al.* (2001) highlighted their importance as links between land, freshwater and the sea.

In a CTE both landforms (see Harris & Heap, 2003; Pethick, 1984) and biological processes (Sanders 1968) are controlled by chemico-physical processes, which determine large scale patterns such as gradients and patchy structures (Attrill & Rundle, 2002).

Progressive changes in several environmental variables, often mutually dependent or correlated, including salinity, marine water renewal, nutrients, turbidity and sediment structure, generate a composite gradient, which has been referred to as "transitional gradient" (Tagliapietra *et al.*, 2009). The direction of the gradient depends mainly on river or tide energy; therefore, it is generally oriented perpendicularly to the coastline or along the river mouth axis. The shape of the gradient can change in different basins and sub-basins, depending on the relative importance of the environmental variables within the gradients. In very low-energy environments the gradient can be differently oriented, for instance, owing to the presence of wind-driven water circulation. In these situations, gradients with different directions can generate complex fields. The contribution of different variables in distinct systems (e.g. salinity in estuaries, seawater renewal in microtidal lagoons) depends on the main hydrodynamic energy source of the system. In environments with high fluvial energy (Boyd *et al.*, 1992; Dalrymple *et al.*, 1992; Heap *et al.*, 2001) the gradient is structured mainly by the freshwater flows, which dilute the seawater and rearrange the sediments. In this case, salinity can be profitably used as a proxy for the composite gradient. Conversely, in coastal lagoons with weak river input the component that mainly influences the gradient is seawater renewal, which can be considered as a proxy for the whole gradient. Reflecting the ecocline, a coenocline is structured, in which there is a substitution of species along a *continuum* rather than distinct communities (Attrill & Rundle, 2002). In non-tidal systems with reduced or absent freshwater inflow (for example the Mar Menor, Mediterranean coast of Spain) the community doesn't follow a clear gradient, instead it presents a patch distribution which has been related to the nature of the bottom and characteristics of the sediment, wave energy and depth, and which highlights the role of colonization rates and dispersal (Pérez-Ruzafa & Marcos-Diego, 1992).

Environmental structure directly influences benthic community structure and diversity. In natural situations, sites located in different positions along the transitional gradient have community features, such as species number and composition, numeric abundance, biomass, diversity which are inherently dissimilar.

Coastal lagoons are a subset of the CTE. These have been defined on a morphological basis by Kjerfve (1994) as "shallow coastal water bod[ies] separated from the ocean by a barrier, connected at least intermittently to the ocean by one or more restricted inlet, and usually oriented shore-parallel". The term includes "estuarine lagoons" into which rivers flow and "marine lagoons" without a major freshwater input (Barnes, 1980). "Marine lagoons" are often called "coastal lakes", especially when the connection with the sea is reduced or temporarily obliterated. The term "estuarine lagoon" is not univocally defined. Some authors (e.g. Heap *et al.*, 2001) refer to "estuarine lagoons" as "wave-dominated estuaries" and leave the term "(coastal) lagoon" to small, shallow basins that have very low freshwater input (i.e. "marine lagoons"). Kjerfve (1994) suggested a classification of lagoons according to water exchange with the sea: at one extreme there are the "leaky lagoons" characterised by abundant seawater exchange, at the opposite extreme there are "chocked lagoons" with little connection with the sea. Considered separately, lagoons and estuaries show marked differences in physiographical, hydrological and ecological features (Barnes, 1994a, 1994b), nevertheless on sedimentary coasts "marine lagoons" and estuaries are the endpoints of a *continuum*, with "estuarine lagoons" as the midpoint. At the same time, coastal lagoons and estuaries form part of a *continuum* between continental and marine aquatic ecosystems, but in which the former are closer to each other than to continental or marine waters. Main differences among estuaries and coastal lagoons are the fresh water influence and the spatial organization of gradients and environmental variability, with more complex patterns and three-dimensional heterogeneity in lagoons (Pérez-Ruzafa *et al.*, 2010).

1.2 THE BIOINDICATION IN COASTAL TRANSITIONAL ECOSYSTEMS BY MEANS OF MACROZOOBENTHOS COMMUNITY

The Water Framework Directive (WFD; European Community, 2000) establishes a framework for European Community action in the field of water policy. In art. 4 WFD states: “Member States shall protect, enhance and restore all bodies of surface water […] with the aim of achieving good surface water status…”.

Different categories of water bodies are introduced. Transitional Waters are identified as a distinct surface water category, recognizing their unique features. They are defined as "bodies of surface water in the vicinity of river mouths which are partly saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows". This definition stresses on the gradient of salinity as the main feature of the class of environments, and causes coastal lagoons to be assigned to either "transitional waters" (such as the Lagoon of Venice) or "coastal waters" on the basis of freshwater influence (Pérez-Ruzafa *et al.*, 2010; Tagliapietra & Volpi Ghirardini, 2006). The relationship between Transitional Waters as defined by WFD and other CTE categorizations is expressed in Figure 1.1 (Tagliapietra *et al.*, 2009).

For each category, WFD requires at first the Member States to identify and characterize water bodies on the bases of main environmental features. As a second step, water bodies for each water type need to be classified in terms of their "ecological status", which has been defined as "an expression of the quality of the structure and functioning of aquatic ecosystems…" (art. 2). Biological communities have been introduced as quality elements, along with physico-chemical and hydromorphological elements, to evaluate the ecological status. "Type-specific biological reference conditions may be either spatially based or based on modelling, or may be derived using a combination of these methods." In alternative, expert judgment may be also applied (Annex II). High status is achieved when "the values of the biological quality elements [...] reflect those normally associated with that type under undisturbed conditions, and show no, or only very minor, evidence of distortion" (Annex V). WFD does not distinguish between ecological (or biological) integrity, which is associated to "pristine" conditions, and ecological health, which is related more generally to a threshold such as "the preferred state of sites modified by human activity" (Karr, 1996; Karr & Chu, 1999).

Benthic invertebrate fauna is included among biological quality elements.

The benthic community consists in a wide range of organisms from bacteria to plants (phytobenthos) and animals (zoobenthos) and from the different levels of the food web. The definition of macrozoobenthos is at once biological, ecological and dimensional. Benthic animals are generally classified according to the size in different categories. Widely used categories, applied in the present work, include microbenthos < 0.063 mm, meiobenthos, 0.063 mm - 1.0 mm, macrobenthos > 1.0 mm and, occasionally, megabenthos > 10.0 mm. Actually, the boundaries of dimensional classes, and so the very definition of benthos dimensional categories, is closely related to collecting methods. In particular, depending on the studies, the lower boundary of macrobenthos shifts amid 0.5 mm, 0.1 mm and still other values on the basis of the sieving mesh size. Benthic invertebrates can be differentiated, according to the position they occupy on or in the bottom surface, in infauna and epifauna. Seagrass beds and macroalgae host complex communities which are rarely adequately sampled by ordinary methods. Infauna, and generally species with poor mobility, represents the largest component of soft bottom macrozoobenthos samplings.

Macrozoobenthos community of sedimentary coastal transitional ecosystems is predominantly composed (in terms of biomass or abundance) by annelids such as polychaetes and oligochaetes, molluscs such as bivalves and gastropods, crustaceans such as decapods and amphipods, which also are the most studied taxa.

Benthic invertebrates play an important role in transitional ecosystems, by filtering phytoplankton and being predated by bigger organisms such as fish; they link primary production with higher trophic levels, structure and oxygenate the bottom by reworking sediments, play a fundamental role in breaking down organic material before bacterial re-mineralization. At the same time, they are exposed to multiple stressors such as contaminants in the water column and accumulated in the sediment, and low dissolved oxygen levels (hypoxia/anoxia) due to organic matter degradation.

Benthic communities are often used in bioindication either on the basis of the sensitivity of single species (indicator species) or because of some general response at the community level, as they present a number of features which make them appropriate. Benthic organisms are able to integrate environmental signal over a long period of time, because of a limited mobility, a relatively long lifespan, the position in the trophic chain and the location at the sediment-water interface. They can integrate different types of stressors.

Benthic assemblages respond to environmental stress modifying their attributes (e.g. number of species, abundance, biomass, trophic structure) and derived indices (e.g. diversity indices).

The science of bioindication aims to obtain information from modulations of biological attributes induced by environmental stress. Despite a need for clear and unambiguous terminology in ecology, pointed out by various authors (e.g. Dauvin *et al.*, 2007; Tagliapietra *et al.*, 2009), terms and definitions are often used inconsistently in the literature. In order to build a coherent framework for this work, the term "indicator" is defined as “a parameter, or a value derived from parameters, which [...] provides information about [...] the state of a phenomenon..." (OECD, 1993), where parameter is defined as "a property that is measured or observed" (OECD, 1993). Nicolai (1982, mod.) defines the term "indicator" in a similar way as "an aspect of reality that allows to interpret a complex phenomenon". The term "bioindicator", or biological indicator, will be applied to a parameter of a biological system at each level of organization.

The term "index", which has been defined by EPA “a dimensionless numeric combination of scores derived from metrics” (http://www.epa.gov/, 30/11/2010) or “a set of aggregated or weighted parameters or indicators” (OECD, 1993) will be also applied in a wider sense to macrodescriptors and derived indices which historically have been so defined, and which are based on counts (species richness, abundance) or dimensional measures (biomass). For direct measures of number of categories (e.g. species) and importances (e.g. total abundance or biomass) will be used the term "macrodescriptor". The number of indicators and indices based on benthic community is very high. Different classifications have been proposed (e.g. Diaz *et al.*, 2004, Salas *et al.*, 2006), but a consistent system is still missing, also due to the continuous development of new indices independent of distinct disciplines. A far from exhaustive list of "indices" applied on macrozoobenthos of marine and transitional environments is reported in Table 1.1. The overview is circumscribed to univariate indices and macrodescriptors but also distributional methods, such as Abundance/Biomass Comparison curves (ABC curves, Warwick, 1986) or Species/Abundance/Biomass curves (SAB curves, Pearson & Rosemberg, 1978) or multivariate methods (e.g. Principal Response Curves, Pardal *et al.* 2004) has been proposed for evaluation purposes.

Macrodescriptor/Index	Acronym	"Class"	Referencies
Species Richness	S	Macrodescriptors	(various)
Total abundance	A, N		(various)
Total biomass	B		(various)
Margalef index of species richness	R	Diversity indices and related metrics	Margalef (1958)
Shannon-Wiener index	H'		Shannon & Weaver (1949)
Simpson index of dominance	D; D'		Simpson (1949)
Hulbert index (expected number of species)	$E(S_n)$		Hurlbert (1971)
Pielou index of evenness	J		Pielou (1966)
Taxonomic diversity	Δ		Warwick & Clarke (1995)
Taxonomic distinctness	$\Delta^{\bullet}$		Warwick & Clarke (1995)
Amphipod Index of Pollution	-	Biotic indices sensu stricto	Bellan-Santini (1980)
Annelid Index of Pollution	AIP		Bellan (1980)
AZTI Marine Biotic Index	AMBI		Borja *et al.* (2000; 2003; 2004a)
Benthic Index based on Taxonomic Sufficiency	BITS		Mistri e Munari (2008)
Benthic Opportunistic Polychaetes/Amphipods ratio	BOPA		Gomez Gesteira & Dauvin (2000); Dauvin & Ruellet (2007)
Benthic Pollution Index	BPI		Leppäkoski (1975)
Benthic Quality Index	$BQI^{(a)}$		Rosenberg *et al.* (2004)
Benthic Response Index	BRI		Smith *et al.* (2001)
Bentix	BENTIX		Simboura & Zenetos (2002)
Biological Quality Index	$BQI^{(b)}$		Jeffrey *et al.* (1985)
Feeding Structure Index	FSI		Petrov & Shadrina (1996)
Index of r/K strategies	-		De Boer *et al.* (2001)
Indice d'Évaluation de l'Endofaune Côtière	I2EC		Grall & Glémarec (2003)
Indicator Species Index	ISI		Rygg (2002)
Infauna Trophic Index	ITI		Word (1979; 1980); Mearns & Word (1982); Maurer *et al.* (1999)
Macrofauna Monitoring Index	$MMI^{(b)}$		Roberts *et al.* (1998)
Meiobenthic Pollution Index	MPI		Losovskaya (1983)
Mollusc Mortality Index	$MMI^{(a)}$		Petrov (1990)
Nematodes/Copepods Index	-		Raffaelli & Mason (1981)
TWo-stage INdex	TWIN		Marchini & Occhipinti-Ambrogi (2007)

Benthic Condition Index (Benthic Index of Environmental Condition)	BCI	Indices derived from IBI (Karr, 1981) and other multimetrix indices	Engle *et al.* (1994); Engle & Summers (1999); Macauley *et al.* (1999)
Benthic Ecosystem Quality Index	BEQI		Van Hoey *et al.* (2007); Van Damme *et al.* (2007)
Benthic Index of Biotic Integrity	B-IBI		Ranasinghe *et al.* (1994); Weisberg *et al.* (1997); Van Dolah *et al.* (1999)
Benthic Index of Estuarine Condition (Virginia Province Benthic Index)	BIEC		Weisberg *et al.* (1993), Schimmel *et al.* (1994), Strobel *et al.* (1995); Paul *et al.* (2001)
Daphne	Daphne		Forni & Occhipinti-Ambrogi (2007)
Danske Kvalitet Indeks	DKI		Borja *et al.* (2007)
Ecofunctional Quality Index	EQI		Fano *et al.* (2003)
Estuarine QUAlity and condiTION	EQUATION		Ferreira 2000
Fuzzy INdex of Ecosystem integrity	FINE		Mistri *et al.* (2007)
Index of Biotic Integrity	IBI		Nelson (1990)
Infaunal Quality Index	IQI		Prior *et al.* (2004); Borja *et al.* (2007)
Multivariate AMBI	M-AMBI		Borja *et al.* (2004b); Muxika *et al.* (2007)
Norwegian Quality Index; F3	NQI; F3		Rygg (2002; 2006); Borja *et al.* (2007)
Sediment Quality TRIAD	SQ-TRIAD		Long & Chapman (1985); Chapman *et al.* (1987)
Organism-Sediment Index	OSI	Non-taxonomic indices based on Sediment Profile Imaging	Rhoads & Germano (1986)
Benthic Habitat Quality	BHQ		Nilsson & Rosemberg (1997)
Index of Size Distribution	ISD	Non-taxonomic indices based on size	Reizopoulou & Nicolaidou (2007)

Table 1.1: Main univariate macrodescriptors and indices for the macrozoobenthos of transitional and marine environments.

Indices can be divided into two broad categories: taxonomic, for which the taxon identification plays a key role in defining the categories to which the importances are attributed (species, but also wider taxonomical or functional categories), and non-taxonomic, based on other functional or morphological features, such as the size (e.g. ISD, Reizopoulou & Nicolaidou, 2007), or on image-based samples such as Sediment Profile Imaging (e.g. OSI, Rhoads & Germano, 1986).

Taxonomic "indices" proposed for macrozoobenthos community include classical macrodescriptors (number of species, abundance and biomass); diversity indices (e.g. Shannon & Weaver, 1949) and associated metrics; biotic indices (sensu stricto) based on absolute or relative importances of tolerant/sensitive species or ecological strategies (e.g. AMBI, Borja *et al.*, 2000); multimetric indices which integrate different indices based on community (e.g. BQI, Rosemberg *et al.*, 2004), as well as based on other environmental parameters (e.g. indices derived from IBA, Karr, 1981). Moreover, recently introduced indices such as M-AMBI (Borja *et al.*, 2004a, 2004b) use multivariate methods.

About benthic invertebrate fauna, WFD mentions diversity, abundance and presence of disturbance-sensitive taxa (as well as indicative of pollution taxa) (Annex V), not opting for any specific index and so letting the Member States to identify the metrics. This gave a new impulse to the application, development, improvement and revision of “biotic indices” of environmental quality. The process of indices selections and intercalibration for Transitional Waters is still pending for some Member States.

1.**3 CTE AS NATURALLY STRESSED ENVIRONMENTS: THE "ESTUARINE PARADOX"**

Stress has been defined by Selye (1956) as “the state manifested by the specific syndrome which consists of all the non-specifically induced changes within a biological system”. Rapport *et al.* (1985) identify a group of "pathological" signs (syndrome), which they call Ecosystem Distress Syndrome (EDS) that in their opinion is common to all ecosystems affected by anthropogenic stress. This syndrome "is indicated not only by reduced biodiversity and altered primary and secondary productivity but also by increased disease prevalence, reduced efficiency of nutrient cycling, increased dominance of exotic species, and increased dominance by smaller, short-lived opportunistic species" (Rapport *et al.*, 1985). Haskell *et al.* (1992) stated that "healthy" ecosystems should be free from Ecosystem Distress Syndrome (EDS). However, even pristine, or "ecologically integer" CTEs seem to present symptoms of EDS (Wilson, 1994; Elliott & Quintino2007). Stress responses are relatively easy to detect, but it is difficult to attribute them to any cause (Wilson, 1994).

CTEs are characterised by strong heterogeneity, extreme values and ample fluctuations of several environmental variables, such as oxygen, temperature and salinity, often mutually dependent or correlated and structured in gradients by the hydrology of the system. Wilson (1994) noticed that estuarine organisms react in a similar way to pollution and to salinity change, making difficult to separate the responses to anthropogenic stress from natural variation. The emphasis here is put on salinity but reflects the role of the whole estuarine gradient in structuring benthic communities. This is particularly evident when the number of species is considered (biodiversity according to the Rio Convention, 1999). The stress varies along the gradient, so that under “natural conditions” every part of the estuary can host a certain number of species.

Transitional ecosystems can be viewed as naturally stressed environment, particularly if compared to marine conditions (Elliott & McLusky, 2002; McLusky & Elliott, 2007).

Moreover, in the present CTEs, natural and anthropogenic stresses are often associated (e.g. high residence time and low salinity are often associated with high organic content, high nutrient load, and contaminants).

Benthic communities adapted to live in naturally stressed environments have many characteristics similar to assemblages suffering from anthropogenic stress. Organisms are forced to consume energy to face the severity of environmental conditions. Species able to stand the physical selection are favoured by the reduction of biological selection by possible competitors and predators. As a consequence, the saved energy can be devoted to reproduction, increasing the fitness. In the overall balance the disadvantage generated by physiological stress at the individual level is compensated at the population/species level.

Costanza (1992), in an alternative approach to the WFD one, refers to ecosystem "health" in terms of the three constitutive features: vigour, organization and resilience. In fact, in addition to symptoms of EDS, coastal transitional ecosystems present also high vigour in terms of metabolism or productivity; high organization, not in terms of diversity but measured by the number of interactions between system components (e.g. complex food webs, migratory behaviour) and morphological and functional diversity; high resilience considered as the system’s capacity to maintain structure and function in the presence of stress (Rapport *et al.*, 1998).

These features make it difficult to detect anthropogenically-induced stress in transitional ecosystems. The measure of the biological reaction to both natural and anthropogenic stress is not itself a measure of quality. The term "Estuarine Quality Paradox" has been introduced by Dauvin (2007) and developed by Elliott & Quintino (2007) to refer to this concept, which can be extended to the whole CTE category.

The peculiar nature of Transitional Waters made the scientific community re-consider the bioindication tools in use, which are often derived from methodologies developed for the marine environment (Dauvin, 2007). A number of extant or new indices has been proposed for WFD and their application to different geographical locations and ecological conditions has been discussed.

Benthic assemblages respond to environment stress modifying their attributes (e.g. number of species, abundance, biomass, trophic structure) and derived indices (e.g. diversity indices). An attribute is therefore modulated by environmental stress both natural (e.g. residence time or salinity) or anthropogenic (e.g. heavy metals). The debate on the capability of indicators and indices to separate responses to the so-called “natural stress” from anthropogenic stress is still open (e.g. Mistri *et al.*, 2009; Munari & Mistri, 2010).

Elliott & Quintino (2007) suggested two ways to overcome the problem. The first one is to apply an alternate set of methods which needs to integrate traditional structural measures of some ecosystem components with measurements of ecological processes and functional characteristics. This approach was suggested also by Fairweather (1999) who defined “ecoassays” the process-based approach to ecosystem “health”. In fact, in addition to symptoms of EDS, coastal transitional ecosystems present also some signs of high vigour, organization and resilience (Rapport *et al.*, 1998). Processes to be considered were for example nutrient cycle, recolonisation, infestations, mutualisms, competition, bioaccumulation, community metabolism, analysis of trophic guilds.

The second approach requires the quantification of the natural variability and stress and its subtraction from the anthropogenic stress (Elliott & Quintino, 2007). Intrinsic variation in biological attributes (as well as derived quality indices) due to natural stress in fact represents unwanted information that should be subtracted.

Composition, diversity and vigour of benthic communities differ naturally over different scales according to the bioclimatic region, the type of ecosystem and the specific features of the habitat such as salinity, water renewal rate and sediment type. The relationships between community and habitat should be identified at the appropriate scale and investigated parameter "normalized" to this relationship, therefore considering a departure from a model. "Normalization" can be performed using either *continuum* or discrete zonal approaches. Few benthic indices has been already proposed which incorporate in their formulation a correction based on proxy of the transitional gradient, as the salinity (e.g. BCI, Engle *et al.*, 1994; BIEC, Weisberg *et al.*, 1993) or the sediment granulometry (NQI, Rygg, 2002; see Table 1.1). Another approach defers the "normalization" at the end of the process of index calculation, by identifying different limits for quality classes based on different water body types.

1.4 DEPENDENCE OF BENTHIC COMMUNITY ON ENVIRONMENTAL STRUCTURE IN CTE, WITH FOCUS ON ESTUARINE LAGOONS

In CTE chemico-physical processes determines large scale patterns such as gradients and patchy structures (Attrill & Rundle, 2002), which induce similar response in biological systems both spatially and temporally (Legendre & Fortin, 1989; Raffaelli *et al.*, 1993). Spatial heterogeneity of abiotic and biotic variables is therefore functional and not the result of random processes (Legendre, 1993) and can be used to analyse underlying processes (Bolam, 2003; Hall *et al.*, 1993). The identification of environmental gradients and their interactions with biota allow to develop a framework to assess environmental quality. Several conceptual models have been developed describing the relationship between classical macrodescriptors such as species richness, abundance, biomass, species composition (on which are based more complex indices) and the main components of the transitional gradient.

The classical bionomic approach attempted to relate a given biocoenosis to the physical habitat. The scheme for Mediterranean biocoenosis by Pères e Picard (1964) can be applied to the succession of geomorphologic zones in coastal lagoon according to Roy *et al.* (2001). In analogy to the change of the type of sediment, from sand to sandy-silt, silt and finally clay, macrobenthos biocoenosis can be attributed to the Well Calibrated Fine Sands Biocoenosis (SFBC, Biocoenoses de Sables Fins Bien Calibrés) typical of the sandy coast but protruding into the tidal delta; the Superficial Fine Sands Biocoenosis (SFS Biocoenoses de Sables Fins Superficiels) and the Superficial Muddy Sand in Sheltered Area Biocoenosis (SVMC Biocoenoses de Sables Vaseux Superficiels en Mode Calme) in the most dynamic areas of the central basin; different *facies* of the Biocoenosis of Euryhaline and Euritherm Lagoon (LEE, Biocoenose Lagunaire Euryhaline et Eurytherme) on the inner parts and the fluvial delta.

A major attribute of the benthic community which is noticeably modulated by environmental gradients is species richness. The majority of species dwelling in these environments are of marine origin (Barnes, 1989; Cognetti & Maltagliati, 2000). Consequently, moving landward it can be expected that an increasing divergence from marine conditions is tolerated by progressively fewer species (McLusky & Elliott, 2004). From another point of view the environment becomes more stressful for some species and more subsidiary for others. In a river this pattern is mirrored moving downstream towards the fluvial bayhead delta, therefore by species of freshwater origin (Remane, 1934, 1971; Guelorget *et al.*, 1987); the double environmental gradient is thus reflected by a double ecocline (Attrill & Rundle, 2002).

The progressive reduction in the number of species when entering a water body, either from the sea or from the river has been the subject of various conceptualisations, each one emphasising a different aspect of the gradient: salinity (e.g. Remane, 1934; Attrill, 2002), seawater renewal (e.g. D’Ancona *et al.*, 1954; Guelorget & Perthuisot, 1983) or sediment type (e.g. Boesch, 1973; Thrush *et al.*, 2003). Organic enrichment has been recognized as a main factor in structuring communities (Diaz e Rosenberg, 1995; Gray *et al.*, 2002; Pearson & Rosenberg, 1978) and also follows the main gradient. Times of emergence/submergence related to tidal regime are also an important factor in structuring benthic assemblages in this type of environment (Swinbanks & Murray 1981). The degree of connectivity with the sea has strong outcomes on the recruitment of species that require a dispersion phase into the sea (Dye & Barros, 2005; Platell & Potter, 1996). In the inner part of the basins or near the heads of estuaries variability in the physical environment (freshwater discharge, anoxias) can cause periodic mortality of several species, which is followed by recolonisation and restructuring of the communities (Barnes, 1999). A general framework for richness patterns in relation to estuary type was proposed by Roy *et al.* (2001) for Australian estuaries, taking into account tidal exchange, salinity, recruitment and migration.

Conceptualizations are not free of regional influences. British authors pointed out the importance of the saline gradient as if their viewpoint were classical estuaries, whereas French authors supported highlighted the importance of the relation with the sea as if their viewpoint were marine lagoons. Main models regarding salinity, organic enrichment and water renewal (confinement) will be described more in depth in the following paragraphs as they are of particular interest for the case study.

1.4.1 Salinity

A model of benthic invertebrates species richness along a marine-freshwater salinity gradient was proposed by Remane (1934), based on studies performed on the Baltic Sea and associated systems (Figure 1.2, Remane & Schlieper, 1971). He observed an overall trend in the number of species associated with the progressive decrease in salinity. He also distinguished between fresh water, brackish-water and marine species and described qualitatively the change in relative distribution among species belonging to these categories, relating to particular salinity values. The majority of species are of marine origin (see also Barnes, 1989). Consequently, moving landward their proportion decreases. In a river the pattern is mirrored moving downstream by species of freshwater origin. Both the groups of organism reach a species minimum, named "artenminimum", which falls between salinities of 5-8 PSU, suggesting the presence of an ecophysiological barrier caused by salinity (Khlebovich, 1968), later disproved (Deaton & Greenberg, 1986). The Remane model relates brackish-water species to values of salinity below about 18 PSU.

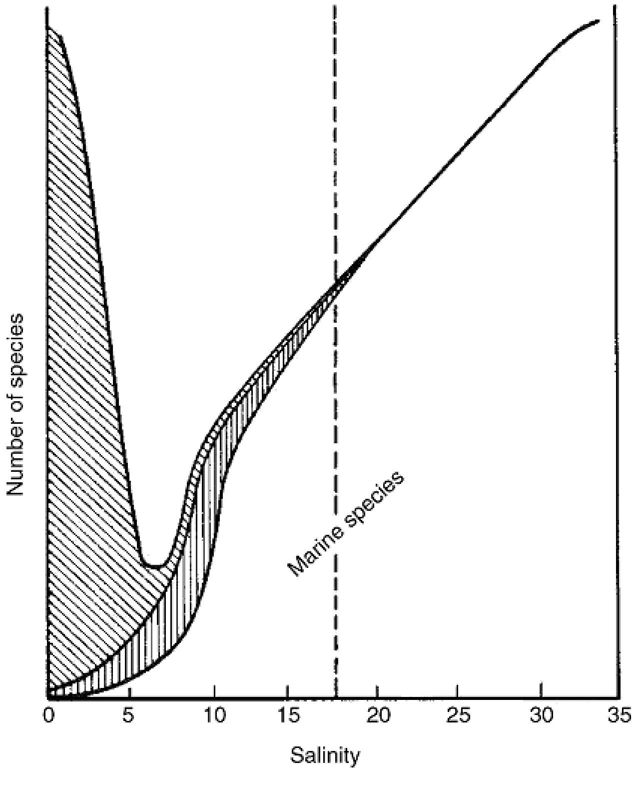


Figure 1.2: Model of species richness along a gradient of salinity (Remane, 1934; 1971). Vertical hashed area: brackish water species; slanted hashed area: freshwater species.

Even though the model has been developed into a particular context, it has been widely applied, basically in the original form, to describe the general structure of estuary communities. The Remane model and subsequent studies on the role of salinity were the starting point for the "Venice system" of 1959 which classifies waters on the basis of salinity (Anonymous, 1959; Segerstråle, 1959).

In fact the model has been developed for the Baltic Sea, which is a brackish-water nanotidal sea, whereas estuaries can be strongly tidal (which is also the base for a well-known definition of the term; see Tagliapietra *et al.*, 2009). Also, where the freshwater inflow is negligible or absent, the reduction in the number of species is only from sea landward. Various authors discussed different aspects of the model, and proposed some modification (e.g. Barnes, 1989; Hedgpeth, 1967; Odum, 1988). Bulger *et al.* (1993) proposed an alternative classification of the salinity gradient in estuaries and presented a zoning scheme based on fish and invertebrate distributions. Wagner (1999) considered the effects of the length of the salinity gradient on diversity. Attrill (2002), in proposing a more quantitative model for alpha diversity (in the sense of the number of species) in estuaries based on salinity, assumed that salinity range should be preferred to salinity absolute value, as variation of salinity (and in general of environmental factors) may be more important in structuring communities than extreme values. He also explicitly used salinity range as a proxy for a set of variable conditions, asserting that "it is not intended that salinity range alone is to be considered causative of any pattern observed".

Salinity varies widely in CTE, from less than 0.5 PSU to more than 140 in β-hypersaline waters or more than 300 in delta-hypersaline ponds sensu Por (1980) (Pérez-Ruzafa *et al.*, 2010). The term "brackish waters" has been used among biologists to describe both waters with a salinity intermediate between salt water and fresh water, and the whole class of CTE, despite the etymology of the word actually limit the applicability at the formers (Tagliapietra *et al.*, 2009). The use of the term stresses the importance of freshwater inflow and seawater dilution in structuring communities. However the Remane model can not be applied where the freshwater inflow is negligible or absent, as for hypersaline marine lagoon.

1.4.2 Organic enrichment

Conceptual models regarding organic enrichment and saprobic processes were first developed for rivers at the beginning of the 20th century (Kolkwitz & Marsson, 1902, 1908, 1909), and then later developed into biotic indices after Pantle & Buck (1955). In marine systems these topics were addressed by Reish (1972), Pérès & Bellan (1972) and Bellan & Bellan-Santini (1972), which proposed the use of marine benthic invertebrates as indicators of organic pollution.

In 1978, Pearson & Rosenberg developed in the Baltic area a conceptual model describing the seriation of benthic invertebrates along a gradient of organic enrichment ("Pearson and Rosenberg model"), refining the work of Leppäkoski (1971, 1975). This conceptual model illustrates the qualitative relationships between magnitude of disturbance and changes in the main macro-descriptors of benthic assemblages, such as the number of Species (S), Abundance (A) and Biomass (B), by means of the SAB curves. The number of species, the abundance and the biomass would vary characteristically according to the organic matter input. A succession of species in benthic assemblages can be identified, both spatially, related to the distance from the impacted site, and temporally, starting after an enrichment episode, such as for instance an eutrophication period (Gray, 1979). The model foresees a seriation of species from "opportunistic species" (Grassle & Grassle, 1974), characterized by the *r* reproductive strategy (Pianka, 1970) that is dominate in organic enriched conditions, toward sensitive species characterized by the *K* reproductive strategy. This change is accompanied by a progressive increase in the number of species. Opportunistic species have short life cycle, small body size, fast growth, often polyvoltine reproduction, the dominant feeding group being detritivores, particularly polychaetes. Pearson & Rosenberg (1978) identified four "zones" corresponding to the changes in fauna and sediment structure (Redox Potential

Discontinuity, RPD) along an organic enrichment gradient. The authors defined five successional states occurring both in space (zones) and/or time (stages), i.e. two endpoints constituted by “Afaunal” and “Normal” (i.e. marine) situations and three intermediate stages, i.e. the “peak of opportunists” with large abundances of few species, the “Ecotone point” with low abundance and high diversity, and the “Transition zone” between them (Figure 1.3).

The Pearson and Rosenberg model relates benthic succession to organic enrichment, giving a strong dependence on the redox conditions of the sediments (Pearson *et al.*, 1983): an excessive organic load exposes the benthos to physiological stress (Diaz & Rosenberg, 1995; Gray *et al.*, 2002). The authors referred to heavy input of organic matter as “pollution".

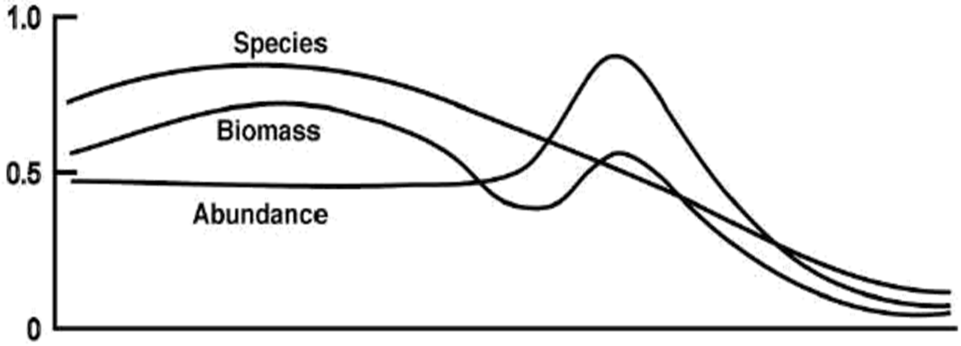


Figure 1.3: Species/Abundance/Biomass (SAB) curves along a gradient of organic enrichment (from left to right) (Pearson & Rosemberg, 1978).

This model is basically descriptive and qualitative (Gray *et al.*, 2002), but recently, some attempts have been made to relate more quantitatively the sedimentary organic matter to the main features of benthic communities (Hyland *et al.*, 2005; Magni *et al.*, 2009).

Expanding upon the Pearson and Rosenberg model, French researchers developed a model for coastal marine environments based on the categorization of benthic invertebrates in five “ecological groups”, according to their relative dominance along a gradient of organic enrichment and oxygen depletion (Glémarec & Hily, 1981; Grall & Glémarec, 1997; Hily, 1984; Hily *et al.*, 1986; Majeed, 1987). The approach thus consisted in the individuation of seven “biotic indices” (BI) defining different stages of community degradation on the basis of the relative dominance of each ecological group (Figure 1.4).

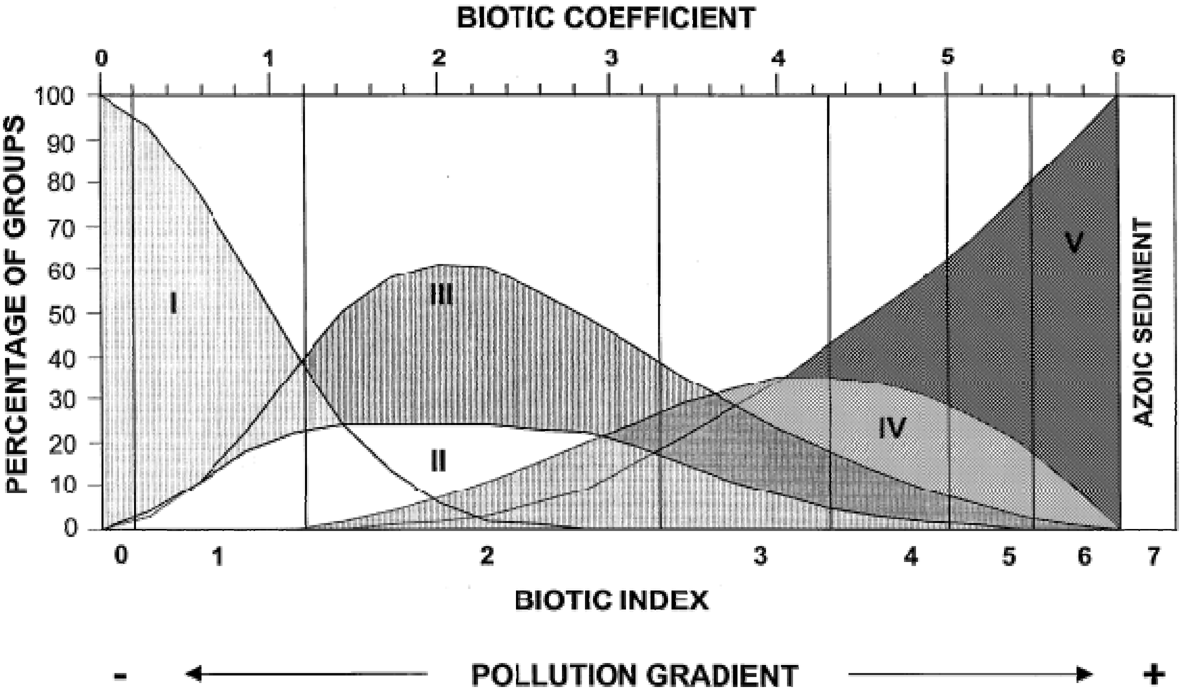


Figure 1.4: Model of ecological groups (I-V) along a gradient of organic enrichment (Glémarec & Hily, 1981; Glémarec & Grall, 2000)

Borja *et al.* (2000, AZTI Marine Biotic Index, AMBI) improved the "biotic indices" method with a formula analogous to Pantle & Buck’s index (1955) which, although does not strictly follow the original distributional model, permits the derivation of a series of continuous values (called “Biotic Coefficient”), by the assignment of a “sensitivity coefficient” to each group (i.e. 0 GI, 1.5 GII, 3 GIII, 4.5 GIV, 6 GV). The “Biotic Coefficient” is then subdivided into 7 classes of quality (called Biotic Index, BI). At this point the application shifted from the organic enrichment to a more generic pollution. Other authors followed this approach (e.g. Simboura & Zenetos, 2002, BENTIX; Grall & Glémarec, 2003, I2EC; Mistri & Munari, 2008, BITS). In addition, several models and indices were based on the faunal successional stages of the Pearson and Rosenberg model (Rhoads & Germano, 1986, OSI; Nilsson & Rosenberg, 1997, 2000, BHQ).

Organic matter accumulates naturally in CTE following hydrodynamics and sedimentary processes and it can be considered a component of the overall transitional gradient. It plays a key role in oxygen availability in this systems. (e.g. Pearson & Rosenberg, 1978; Diaz & Rosenberg, 1995; Gray *et al.*, 2002).

1.4.3 Confinement

The French authors Guélorget and Perthuisot analyzed the biological organization of lagoons at the landscape scale and highlighted the existence of a spatial biological seriation in all lagoons with the substitution of species along an environmental gradient (“Guélorget and Perthuisot model”; Guélorget & Perthuisot, 1983, 1992; Frisoni *et al.*, 1984). The biological seriation are recognizable whatever their state of naturalness, including lagoons with different degrees of anthropogenic impact. The authors stated that their zonal model is a common feature of CTE and also of some very large water basins, such as the Baltic and the Caspian seas, but their scheme is particularly relevant for micro-mesotidal lagoons (see Barnes, 1994b).

The factor that mainly controls the distribution of organisms and the features of living populations was defined as “the time of renewal of the elements of marine origin at any given point” (Perthuisot & Guelorget, 1995) and called “confinement” since it is strictly related with the degree of separation (seclusion) from the sea and the distance from the sea-inlets. They stated that the confinement reduces the availability for the biota of the “life-giving elements” such as mineral salts, trace elements etc. that come from the sea. The authors did not support the rarefaction of these elements with evidence, yet by defining the “confinement” as a “time of renewal”, they drew attention towards those processes that are driven by the hydromorphology of lagoonal systems.

Recognizing the “confinement” as a common, emergent feature of all sedimentary environments “with relation to the sea” they called the whole class of CTE “paralic domain”.

A confinement scale was proposed consisting in six discrete spatial zones. This scale was conceived as valid for Mediterranean lagoons. According to the authors, the succession is recognizable both in hypohaline and hyperhaline environments. The zones were identified mostly using four main groups of indicator species. “Strictly thalassic species”, belonging to the biocoenosis of Sable Fins Bien Calibrés (SFBC; WCFS, Well Calibrated Fine Sands biocoenosis by Pérès & Picard, 1964), are the more “stenohaline” species; “thalassic species” are marine species that colonize lagoonal areas which are in close contact with the sea; “mixed species” are present in both marine and lagoonal environments, and decrease in density as the confinement increases, but have a high or very high biomass inside the lagoon. Finally, “paralic species” or “strictly paralic species” are typical of lagoons, their density generally increasing along the confinement gradient.

As a matter of fact, although giving a wide series of examples and qualitative models (Figure 1.5), Guélorget and Perthuisot did not give a systematic and quantitative description of the distribution of species along the “paralic” seriation. Barnes (1994) criticized the possibility of application of the “confinement” species to estuaries and tidal-flat habitats of the northern macrotidal Europe, characterized by a strong hydrodynamics and water renewal. This author also recognized the non direct dependence of the distribution of “brackish fauna” on salinity. He pointed out that the majority of the species listed by Guélorget and Perthuisot can be considered as euryhaline, or particularly euryhaline, marine species that penetrated also estuaries. However, he stressed the existence of “lagoonal species”, i.e. species that live in nanotidal or microtidal lagoons, but are usually absent from macrotidal estuaries and tidal-flat habitats, that are basically the “strictly paralic species” of Guélorget & Perthuisot (1983).

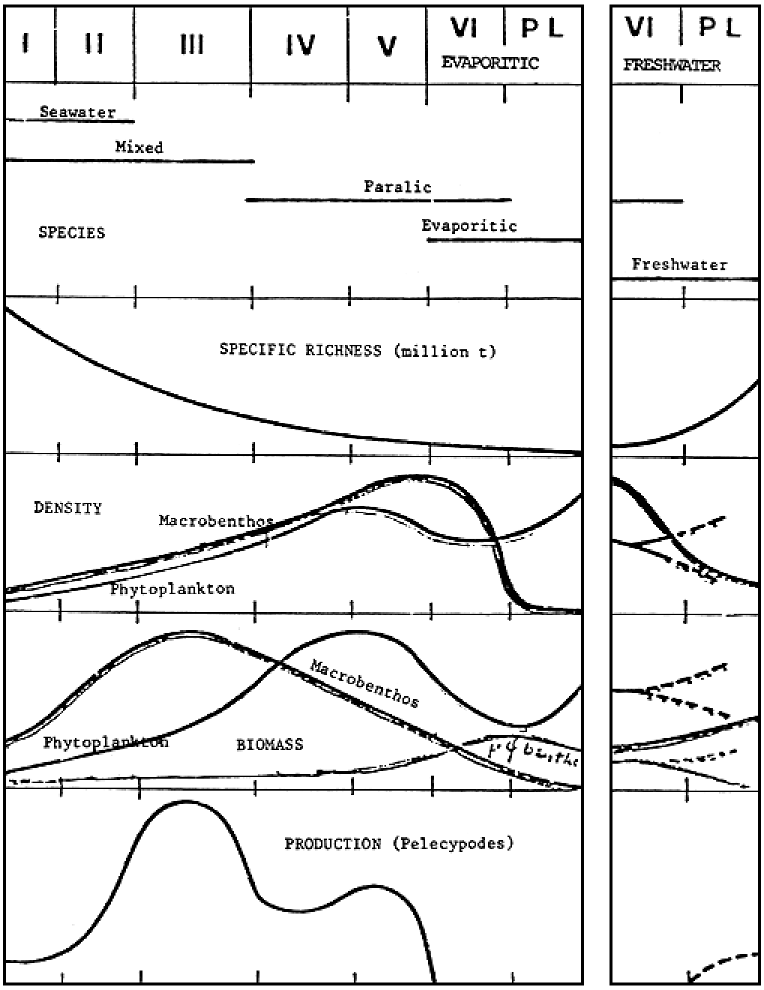


Figure 1.5: Specific richness, density, biomass and production in relation to zonation in paralic systems (Guelorget, 1987)

Guélorget & Perthuisot (1983, 1992) stated that “a confined environment is not synonymous with a reducing environment” since the supply of “vital element”, not saprobity, would be the limiting factor for a community. Nevertheless the model can be related to the Pearson and Rosemberg model (Tagliapietra *et al.*, in press). In fact, they also admitted that “in the paralic domain, confinement often leads to the reducing character of the milieu, notably in the region of the bed” (Guélorget & Perthuisot, 1983, 1989). The authors listed a series of characteristics of confined environments that favour the instauration of a reducing medium such as low hydrodynamics, tendency to oxygen depletion, high biological production and the presence of saprobic microorganisms which contribute to the oxygen consumption and to the production of reduced compounds. They also recognized that reducing environments are more frequent in the innermost parts of the lagoon, the so-called “far paralic” indirectly asserting the presence of a reductive gradient due to organic matter decomposition (i.e. a saprobic gradient) related to the “confinement gradient”. They also recognize that “organic pollution” can induce local variations in the species succession.

Where the freshwater inflow is negligible or absent, the Guelorget and Perthuisot model can be regarded as an operational simplification of a single ecocline lying along a gradient of seawater renewal. In Mediterranean lagoons where the hydroclimate sustains eu-hyperhaline conditions, the decline of species along the sea–land axis is attributed mainly to hydrology and sediment properties, second only to salinity (e.g. Guelorget *et al.* 1987; Reizopoulou & Nicolaidou 2004; Rossi *et al.* 2006).

The Guelorget and Perthuisot model was not followed by indices that quantify the “confinement”, with the exception of a biotic index proposed by Breber *et al.* (2001, 2008) and conceived to assess environmental quality in Mediterranean lagoons.

Since a wide accepted mathematical definition of confinement is still missing, hydrodynamics parameters such as residence time could be used as a proxy.

1.4.4 Biological factors: larval dispersion and colonization

At a very general conceptual level, spatial distribution and structure of species and communities across the system are due to either environmental processes or the dynamics of the species and the communities themselves. Species distribution and community composition are related to spatial contagious processes, such as dispersal, population dynamics, species interactions, intraspecific competition, which determines spatial patterns.

CTE are selecting environments in which the role of chemico-physical factors in controlling biological processes and structures are generally regarded as predominant (Sanders, 1968). Some studies however stress the importance of the biological process as well. Environmental factors can directly induce community structure by species optimum along a gradient of condition and tolerance to extreme values, but can also indirectly act, as for the dispersal of species by tidal currents, despite species could have evolved life-cycle and behaviour to "control" these dynamics.

Despite the concept of confinement as related to the time of renewal of "life-giving elements" has been widely criticized, there is a general agreement about the importance of seawater renewal as a structuring factor for communities, at least for systems with reduced or absent freshwater outflow (Barnes, 1994b). The "confinement" model has since been reinterpreted as related to other factors, such as saprobity (Tagliapietra *et al.*, in press) and colonisation rates and dispersal processes. About the latter hypothesis, Pérez-Ruzafa & Marcos-Diego (1992) linked confinement to the capability of the marine species to colonize the paralic environments, which interact with reproduction and growth rates, as a result of adaptations to chemico-physical conditions, to structure the community at lagoonal scale. The role of dispersion and colonization has been highlighted when considering non-tidal systems with reduced or absent freshwater inflow (as the Mar Menor, Mediterranean coast of Spain; Pérez-Ruzafa & Marcos-Diego, 1992), where communities don't follow a clear gradient, presenting instead a patch distribution.

The degree of connectivity with the sea has strong outcomes on the recruitment of species with a marine dispersion phase, with consequences on the structure of the community (Dye & Barros, 2005; Platell & Potter, 1996).

Interspecific competition between colonizers and strictly paralic species has been proposed as a major factor determining the structure of communities. High immigration rates in the system (for example near the inlets) could compensate for less competitiveness with respect to physically-selected species (see Chapter 1.4.2) (Pérez-Ruzafa & Marcos-Diego, 1992). Species presenting distinct life-cycle (i.e. type of development, direct with respect to pelagic (Mileikovsky,1971), and duration of larval phase) may present different spatial patterns related to dispersal. Also distinct patterns of sessile and vagile fauna could be related to the model (Pérez-Ruzafa & Marcos-Diego, 1992).

1.5 MULTIPLE SCALES IN STRUCTURE AND FUNCTIONING

Ecological phenomena act at different spatial and temporal scales (Levin, 1992). If the concept of scale has previously been widely applied in disciplines such as Landscape Ecology (e.g. Naveh & Liebermann, 1984), the stress on scale in ecological studies has grown since the '80s (Golley, 1989; Schneider, 2001; see for example Gardner *et al.* 2001; Levin 1992; Peterson & Parker, 1998; Wiens, 1989). Scale-dependent spatial patterns and processes have been increasingly analyzed in communities, for example in benthic assemblages (Gimenez & Yannicelli 2000; Thrush *et al.* 1997, 2003; Ysebaert & Herman 2002). These studies have intensified also thanks to the development of new statistical tools (see for a review Perry *et al.*, 2002, more general on spatial patterns, and Bellehumeur & Legendre, 1998).

As an emerging and complex subject, the term "scale" has been used inconsistently across studies and disciplines. Dungan *et al.* (2002) revised its use in ecology, primarily within the spatial context, and identified a number of concepts associated with the term which are not interchangeable, including extent, grain, resolution, lag, support, cartographic ratio. Hierarchical and, more specifically, organization levels (organisms, species, communities, etc.) were also used to express the concept of scale (Allen & Starr; 1982, Schneider, 2001). The authors suggest that the term scale should be avoided to avoid confusion among existing definitions, instead referring to single concepts. Schneider (2001) also reached the same conclusions, recommending that the word be used with an appropriate qualifier. Denny *et al.* (2004), using this approach, introduced in the same paper six different measurable concepts of scale.

Dungan *et al.* (2002) highlighted the different meaning of scale in observation (samplings), analysis and phenomena. Observations and analysis should span the potentially relevant range of space and time scales (Anderson *et al.*, 2005; Perez-Ruzafa *et al.*, 2007) and in turn awareness of scales of variability in ecological phenomena should guide the choice of appropriate scales of observations and analysis (Dungan *et al.*, 2002).

If, generally speaking, the term scale refers to extent and in the present work, scale of variability in ecological phenomena will be operationally defined when necessary. Scale of observations are described by sampling design.

Heterogeneity of ecological patterns and processes can be recognized at multiple scales. Descriptions of observed patterns allow to identify the scales of variation as the first step. The quantification of patterns of variability in space and time can help to understand the underlying ecological processes and their own scales of variation (Levin, 1992; McIntire & Fajardo, 2009; Peres-Neto & Legendre, 2010; Underwood and Chapman, 1996).

Environmental variables as well as species and communities usually show a spatial structure, or "spatial correlation", i.e. a non-random organization across the space (Peres-Neto & Legendre, 2010). Spatial correlation can be either indirectly induced by external forcing ("induced spatial correlation" or "spatial dependence") or due to internal processes ("non-induced spatial correlation", or "spatial autocorrelation"). Spatial patterns in species and community distributions result from a combination of environmental processes and the dynamics of the species, which occur on different scales. Autocorrelation due to contagious processes (dispersal, competition etc.) are expected to occur at smaller scales than induced spatial correlation (Wiens, 1989; Legendre, 1993; Wagner & Fortin, 2005). So, different conceptual models may be applied to observed patterns depending on the scale of observation (McIntire & Fajardo, 2009).

Spatial and temporal variability in chemico-physical conditions follows multiple spatial and temporal scales. Each system may present distinctive scales of variability, and inside a system a same feature or process can present different scales of variability according to the relative location in the system. Biotic communities also present multi-scale variability, primarily related to environmental variation. Different species, and also the same species during different phases of the life cycle, follow different scales. Moreover, the same pattern can be caused by multiple processes which act at different scales. This is of particular interest for bioindication, when the aim is to contrast signals of anthropogenic impacts from natural variability, as natural and man-induced drivers can follow different scales.

The term Coastal Transitional Ecosystem includes a wide spectrum of environments with high diversity in terms of size, morphology, in relationship to the sea, drainage basin, structuring factors and environmental conditions. A CTE has strong environmental variability and internal complexity, so that it is perceived as a "mosaic" of environments rather than a unique environment. This perception is strong at any scale of analysis. In a CTE chemico-physical processes determine large scale patterns such as gradients and patchy structures (Attrill & Rundle, 2002). Communities show high variability both in response to environmental conditions and in their intrinsic dynamics. At smaller scales, with relatively homogenous environmental conditions, biological processes such as reproduction, competition and predator-prey interactions prevail (Bolam, 2003).

According to the role given to main environmental factors in structuring the environment, attention is prevalently directed to a particular scale. Among main models, Guelorget and Perthuissot's confinement model and Remane's salinity model focus on main gradients across the systems. Other authors stress the smaller scale spatial heterogeneity and patchiness, bringing attention to species dispersion and colonization processes as well as competition (Pérez-Ruzafa & Marcos-Diego, 1992). Actually, in different CTEs a particular model can be more appropriate to explain observed scales of variability, and related to distinctive processes.

One possible approach to describe and analyze scale-dependant structures consists in recognizing a hierarchical system of spatial units at a given scale, which at that scale can be considered homogenous in terms of environmental conditions (Farina, 1998; Harris & Heap, 2003; Heap *et al.*, 2001; Naveh & Liebermann, 1984; Zonneveld, 1989). The higher levels should be defined by the stronger environmental factors, starting from the geographical, climatic and tidal factors until the inclusion of communities or *facies* at the lower levels (e.g. Allee *et al.*, 2000; Madden *et al.*, 2002; Madley *et al.*, 2002; Roff & Taylor, 2000; Roff *et al.* 2003). Environmental gradients and related coenoclines may suffer some simplification due to the division into discrete "zones" or "landscape units".

A climatic, bioclimatic or biogeographical classification can guide the definition and placing of transitional ecosystems on a very-wide scale (Tagliapietra & Volpi Ghirardini, 2006). The Geomorphologic Class (Harris *et al.* 2002) indicates the general type of CTE, which can be defined for example on the basis of the relative energy contribution of tides, waves and rivers (Boyd *et al.*, 1992, Dalrymple *et al.*, 1992). Inside each CTE an internal zonation (e.g. Geomorphologic Zones) based mainly on physiographic and hydrographic features can be recognized, indentifying recurrent zones such as the Marine Tidal Delta, the Central Mud Basin and the Fluvial delta (Rochford, 1959, Roy *et al.*, 2001). Alternative approaches including other factors such as salinity have been referred to as Mesological or Hydrogeological Zones (Ferréol *et al.*, 2005; Tagliapietra *et al.*, 2009). Within each Zone, geomorphologic *facies*, or landforms can be located,, such as salt marshes and mudflats. They are related to a model of vertical zonation and constitute the physical substrate for habitat (Heap *et al.*, 2001). Biotic components can be introduced at the last hierarchical levels, in particular those structuring organisms which improve the three-dimensional physical structure of the habitat, such as vegetation (phanerogams or macroalgae) or other bioconstructor (such as polychaetes or shellfish). A relationship between higher levels and the biotic component could follow functional (such as trophic or ecologic) groups rather than species composition or taxonomical structure.

1.6 THE LAGOON OF VENICE

The Lagoon of Venice is located on the North Adriatic coast of Italy, between the mouths of the River Brenta to the South and the River Sile (Piave Vecchia) to the North (Figure 1.6). It originated some 10000 years ago during the Würm post-glacial, coinciding with the rising of the sea level, by alluvial deposits by rivers Adige, Po, Piave and Tagliamento (Gatto, 1980; Gatto & Carbognin, 1981). It is, by extension, one of the most important of the Mediterranean and the most important in Italy. It extends for an overall surface of about 550 km2 and is oriented parallel to the coast, with a maximum length of about 55 km approximately in the direction SW-NE and an orthogonal width of about 15 km. The mean depth of the water column is about 1.2 m, with only the 5% of the lagoon deeper than 5 m (Sarretta, 2008). The surface/volume ratio is considerably high.

The lagoon is a complex system, characterized by a number of gradients and a mosaic of environments and morphologies that are the result of complex environmental and man-induced drivers. Since the days of the Republic of Venice, man has been a major factor in determining the shape and function of the lagoon (Zille, 1955; Ravera, 2000). The lagoonal ecosystem itself is part of a system of three components ("metaecosystem"): the drainage basin, the lagoon and the adjacent coastal waters.

The diversion of main rivers from the lagoon since XIII century is among the most important public works and a major driver of the current state of the system. At present, the drainage basin covers approximately 1850 km2 with 36 freshwater outlets into the Lagoon, either natural or regulated by draining pumps. The average input of freshwater is about 35.5 m^3 s^{-1}, mainly from rivers Silone (23%), Dese (21%), Naviglio Brenta (14%) and Taglio Nuovissimo (13%), with he most important tributaries located in the northern basin (more than 50%) (Zonta *et al.*, 2001; Zuliani *et al.*, 2001). A salinity gradient is produced. For the purposes of WFD, due to the freshwater outflow, the lagoon falls into the category of Transitional Waters for the Mediterranean Ecoregion.

Following Kjerfve (1994), the Lagoon of Venice can be defined as a "restricted" coastal lagoon delimited seaward by a barrier beach which includes the sandbars of Lido and Pellestrina. It is connected to the Adriatic Sea through three inlets (Lido, Malamocco, Chioggia) that allow exchange of water and sediment transported by the tidal cycle. Tidal wave enters each of three inlet and expands into a surface of 418 km2. Four first-rank basins can be identified, from N to S: Treporti, Lido (both pertaining to the Lido inlet), Malamocco and Chioggia (pertaining, respectively, to the Malamocco and Chioggia inlets) (De Bernardi, 1843). Watersheds between basins are not static and consist in belts rather than one-dimensional boundaries. Hydrodynamics between neighbouring basins is reduced but some exchange is allowed (Umgiesser, 1997).

Tides are a main factor in shaping the morphology and structure of habitats and communities. General hydrodynamics in the lagoon is regulated mainly by tidal currents and affects basic parameters such as water exchange, dissolved oxygen, salinity, nutrients and sediment distribution. The amount of seawater that is exchanged during each tidal cycle is about one third of the total volume of the lagoon (Gacic & Solidoro, 2004). North Adriatic tides are the largest in the Mediterranean. The tide is mixed-semidiurnal (Courtier, 1938), with two daily cycles of high and low tides. The range can be defined microtidal, despite the northern basin is actually nanotidal (Sigovini & Tagliapietra, 2009), i.e. < 50 cm following the definition of Tagliapietra & Volpi Ghirardini (2006). Mean tidal range (at the tide gauge of Punta della Salute, 1986-2004) is 61 cm, but it rises to 79 cm during syzygy (Sigovini & Tagliapietra, 2009). Special weather conditions like strong South-East winds and low atmospheric pressure can raise the maximum level, so causing flooding events or "high water" (Canestrelli *et al.*, 2001). The existence of characteristic tidal levels reflects in the vertical and horizontal development of typical lagoonal landforms and, consequently, on the vertical and horizontal zonation of communities.

Typical landforms/habitats, such as channels, subtidal flats, tidal creek and intertidal mudflats and salt marshes are structures generated principally by the tides (Albani *et al.*, 1984). They follow distinctive spatial scales and are unevenly distributed across the lagoon, being part of the whole transitional gradient. Tides have a direct influence on emersion and submersion times and, consequently, on structure of intertidal biocoenoses. Main islands include Venezia, Sant'Erasmo, Vignole (with La Certosa and Sant'Andrea), Chioggia, Murano, Mazzorbo, Burano, Torcello, Santa Cristina. The most recent reclamations on the landward edge include the industrial area of Porto Marghera (since 1913), a former major chemical pole in Italy, and the adjacent areas known as "casse di colmata" (1963-1969), where industrial development was at last stopped. The network of lagoonal channels was modified during the Republic of Venice, but major changes in hydrography occurred in 20th century when the "Canale dei Petroli" was created to serve the industrial port (1968).

The "Conterminazione lagunare" is a border which formally limits geographically the lagoon for regulatory purposes. It was approved in 1784 by the Venetian Senate and it is still in act (with some minor changes made during the years, the last in 1990 with the DMLLPP 9/2/1990). Fish farming water bodies granted to privates ("valli da pesca") are located on the N, NW and SW margins of the lagoon and account for a total of 94.5 km^2. They are included in the "Conterminazione lagunare" but, despite the wide surface and the fact they are (irregularly) connected with the lagoon, very little is known about these systems and their role in the whole lagoon ecosystem. Among main fisheries practiced in the lagoon, a high-impact commercial clam harvesting of *Ruditapes philippinarum* (Adams & Reeve, 1850) (an allochthonous species introduced in 1983), particularly concentrated in the basins of Malamocco and Chioggia, is important in that it causes a direct mechanical (as well as indirect) disturbance on bottom sediments and phanerogams belts. A number of other factors impacted the lagoon ecosystem since the first half of 20th century. They include industrial pollution, mainly related to Porto Marghera (e.g. Guerzoni & Raccanelli, 2003) and secondarily to Murano (glass industries) and other settlements in the lagoon and drainage basin; organic pollution; eutrophication and related anoxia events (especially during the '80s); landform erosion, with deepening and flattening of the lagoon floor, causing a loss in morphological variability and tri-dimensional structure.

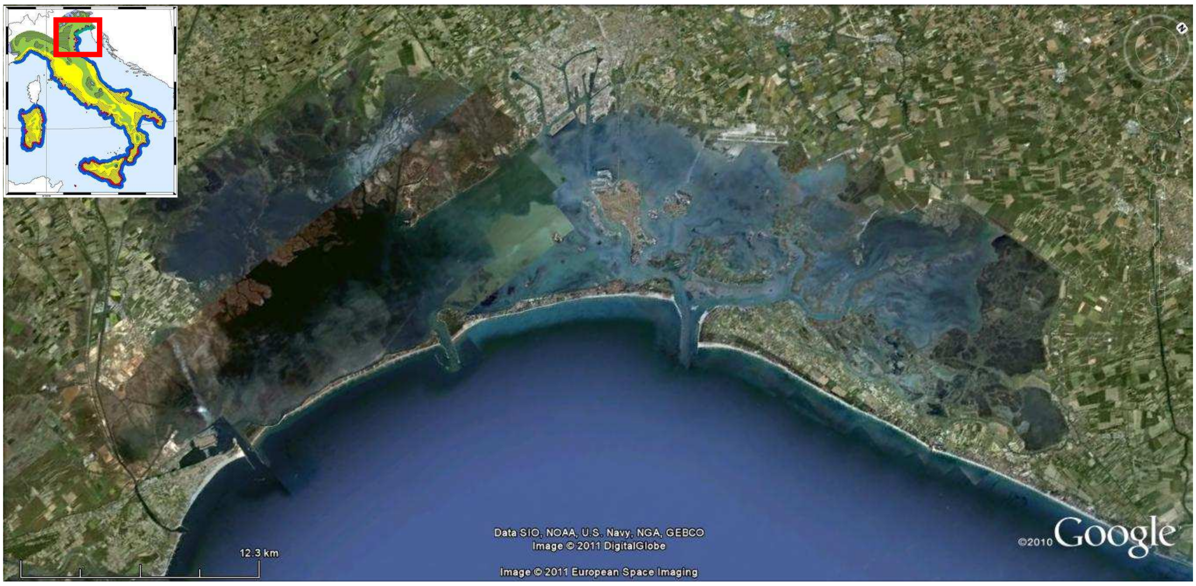


Figure 1.6: The Lagoon of Venice

1.7 BRIEF OVERVIEW OF MACROZOOBENTHOS STUDIES AND MONITORING IN THE LAGOON OF VENICE

The benthic fauna of the Lagoon of Venice has been object of study by naturalists at least since the 18th century. Quantitative studies on the spatial distribution and functionality of the benthic community started in the 20th century. They include the following main steps (Castelli *et al.*, 2003): sampling; sieving and sorting; taxonomic determination; and quantification.

The first quantitative studies were carried out by Vatova since the 1930s on the zoocoenoses of channels and subtidal flats, based on the methods introduced by Petersen. Vatova never published original data. During 1930-1932 he studied the zoobenthos of channels on 142 stations. Data are available as density per each of six zoocoenoses, resulting from aggregation of the actual sampling stations based on expert opinion (Vatova, 1931, 1940). Later in 1944 and 1945 he carried out a study on flats (125 stations), in which zoocoenoses are described quantitatively only for biomass of high rank taxa such as classes and phyla (Vatova, 1949). Thereafter, until the mid-1980s, the only noTable works were carried out by Giordani Soika in 1948 and again in 1968, on 135 and 119 stations, respectively (Giordani Soika & Perin, 1970, 1974). Following important phenomena of macroalgae proliferation and anoxia occurred in the '80s, the Environmental Department of the City of Venice started a series of surveys conducted since 1987 in different areas of the Lagoon. In the same year, the Venice Water Authority (Magistrato alle Acque di Venezia), through its concessionary Consorzio Venezia Nuova, began a series of environmental studies with different temporal and spatial scales and with different aims. In some cases, such studies were carried out at the extent of the whole lagoon, in particular the projects A.3.16/II in 1991 (MAG.ACQUE - CVN - SGS/Ecologia - Biotecnica, 1992), MELa2 in 2002 and 2003 (MAGIS.ACQUE - SELC, 2005) and MELa4 (MAGIS.ACQUE - CORILA, 2009a). The MELa projects consist in a large framework of studies and monitoring conducted since 2000 on various components of the ecosystem. Further studies were carried out generally at a smaller spatial extent by individual researchers or with specific topics, such as fishing and shellfish management (in this case carried out in particular by ICRAM and the Province of Venice).

Morphology investigated, spatial coverage and number of stations vary widely. Most of the studies were carried out on subtidal flats. Intertidal communities were investigated in only three studies, while channels in four studies, including the extensive study by Vatova in 1932-1934.

Those extending all over the lagoon are ten, including the two studies by Vatova, the two by Giordani Soika, five by Venice Water Authority and a study by ICRAM. These all have investigated subtidal bottoms except the first of Vatova. Other studies, while extending all over the lagoon, involved an extremely low number of stations. Several quantitative studies with higher density of sampling stations were performed on a basin or smaller extent (e.g. Maggiore & Keppel, 2007). The density of stations with respect to the extent was found to be highly variable, as well as the type of spatial distribution. A special case is the study A.3.16/II, which was conducted in 18 "areas", each belonging to three transects and consisting of 20 sampling points. Actual density is therefore equal to just 0.06 stations/km^2.

The studies consist in one or more field samplings. The greater the number of stations and the area analyzed, the greater the time required for sampling and laboratory activities. Therefore, despite the purpose of a campaign is to describe an instantaneous condition, in studies covering the whole lagoon, sampling lasted for several weeks to several months. Vatova studies, in particular, as a case limit, have been carried out over several years, and the situation described is somehow "averaged" with respect to seasonal and interannual dynamics. More recently, some studies have tried to highlight the temporal dynamics of benthic communities. In these cases, fieldwork was performed on different time scales at regular intervals such as monthly (Tagliapietra *et al.*, 1998b, 2000a, 2000b) or seasonal (Maggiore & Keppel, 2007). These studies were all performed for a spatial extent inferior to basin. Significant changes during the year at the level of community were highlighted (Maggiore & Keppel, 2007; Tagliapietra *et al.*, 1998a, 2000a, 2000b).

Apart from the spatial (extent, number of stations, *stratum*) and temporal point of view (number and frequency of fieldworks), the studies differ in the techniques used in samplings and subsequent phases, due to the aims, the available resources but also the underlying conceptual approach. In particular, two crucial features when comparing different studies results are total area sampled for station and mesh size.

Total sampled area for stations depends on the area sampled by a single replicate (in turn depending on the size of the instrument) and the number of replicates. The number of species is not a linear function of the sample area, and an adequate minimum area should be sampled. Sampling tools included box corer, grab, corer, Surber and "suction sampler", as well as equipment for commercial fishing. Box corer, grab and corer are particularly suiTable for quantitative studies and allow the sampling of an adequate layer (Castelli *et al.*, 2003). Sample area varied among studies between 400 cm^2 (the studies by Giordani Soika) and 60000 cm^2 (A.3.16/II). Among studies at the lagoonal extent, total sampled area varied between 5.4 m^2 (for the studies of Giordani Soika) and about 50.5 m^2 (1999 study of ICRAM). Both Vatova studies, MELa2 in 2003 and MELa4 in 2007, sampled about 15 m^2; A.3.16/II and MELa2 in 2002 about 40 m^2.

The category of macrozoobenthos is defined operatively according to the sieving mesh size. Different mesh sizes may lead to great changes in density and biomass estimates (Schlacher & Wooldridge, 1996). The studies performed on the Lagoon of Venice used a mesh size of 0.5 mm, 1 mm or 2 mm (the case of A.3.16/II). It is not know for Vatova studies. Other parts of the field and laboratory activities that should be taken into account when comparing studies include specimens preservation (and fixation), taxonomical determination, analytical methods for biomass measurement.

For all these issues, few attempts have been made to compare the structure of benthic communities across a large span of years by implementing different correction techniques (Pranovi *et al.*, 2008; Rismondo & Visintini Romanin, 1997; MAGIS.ACQUE - CORILA, 2008); however more robust results are not quantitative. A full quantitative comparison among different datasets should be limited to studies which share at least total sampled area for station and mesh size.

2. OBJECTIVES

The thesis project has been conducted under the supervision of the Ca 'Foscari University of Venice and the Laboratory of Benthic Ecology of CNR-ISMAR. The aim is to outline the spatial and interannual variability of the macrozoobenthic community and the structuring environmental factors in a typical estuarine lagoon. Understanding these features plays a significant role in quality assessment of transitional ecosystems through benthic indicators and indices. The study site is the Lagoon of Venice, which is the largest coastal lagoon in the Mediterranean Sea and presents a high heterogeneity of environmental conditions.

The main objectives of the thesis are expressed by the following questions:

1. What is the variability over the years of the macrozoobenthic community structure at the lagoon scale?

2. On the bases of an existing hydrogeological zonation in water bodies characterized by homogeneous conditions, what is the spatial and interannual variability of the benthic community?

3. Which is the role of environmental factors in structuring benthic communities?

4. What are the spatial scales of variability of the community, also in relationship to variability scales of environmental factors?

3. MATERIALS AND METHODS

3.1 STUDY AREA

The study area is the Lagoon of Venice (North Adriatic Sea, Italy). The main morphological and environmental characteristics of the area are summarized in Chapter 1.6.

Analysis was limited to the open waters surface, excluding the fish farming water bodies ("valli da pesca") located on the N, NW and SW margin of the lagoon and covering a total of 94.5 km^2. Valle di Ca' Zane (located at the NW of the Lagoon) and Laguna Falconera and Valle Mesola (at the NE of the Lagoon, between the villages of Treporti, Cavallino and Lio Piccolo) were excluded as well.

The tidal wave enters each of the three inlets (Lido, Malamocco and Chioggia) and expands into four first-rank basins, from N to S: Treporti, Lido (both pertaining to the Lido inlet), Malamocco and Chioggia. Basins were variously identified in previous literature on the bases of physiography or hydrodynamical models (Solidoro *et al.*, 2004; Umgiesser, 2000). In the present work the subdivision follows physiographic features (Figure 3.1).

The total surface considered in the present work, which includes intertidal and subtidal planes, covers an area of about 540 km^2. Table 3.1 presents a summary of the total and intertidal surfaces in the Lagoon of Venice, divided by basin. Each basin is approximately a fourth of the Lagoon surface. Total surface of fishing farms is comparable to a single basin surface.

In the present work geographical data are based on the Gauss-Boaga projected coordinates system, fuse E, referred to the datum Roma40. The area is delimited by the following coordinates: 2296105 E to 2328128 E, 5006973 N to 5049175 N. The basic spatial data are based on vector maps provided by the Venice Water Authority. A reference grid for the analysis of raster data has been defined with cell size of 100 × 100 m, which has been considered as the appropriate size to describe local conditions.

	total surface	intertidal surface	
	km2	km2	%
Treporti basin	91.53	10.97	12.0
Lido basin	94.76	3.04	3.2
Malamocco basin	113.69	10.24	9.0
Chioggia basin	108.12	10.76	10.0
Laguna Falconera and Valle Mesola (Treporti basin)	7.56	1.05	13.9
Total open water surface	415.67	36.05	8.7
Fishing farms	94.47	6.77	7.2
Islands	29.96		

Table 3.1: Summary of open water surfaces for the Lagoon of Venice and for each basin (as well as the area pertaining to the Treporti basin which was excluded by analyses), of the area covered by fishing farms and by lagoonal islands. Intertidal surface as a fraction of total surface, and its percentage, is presented.

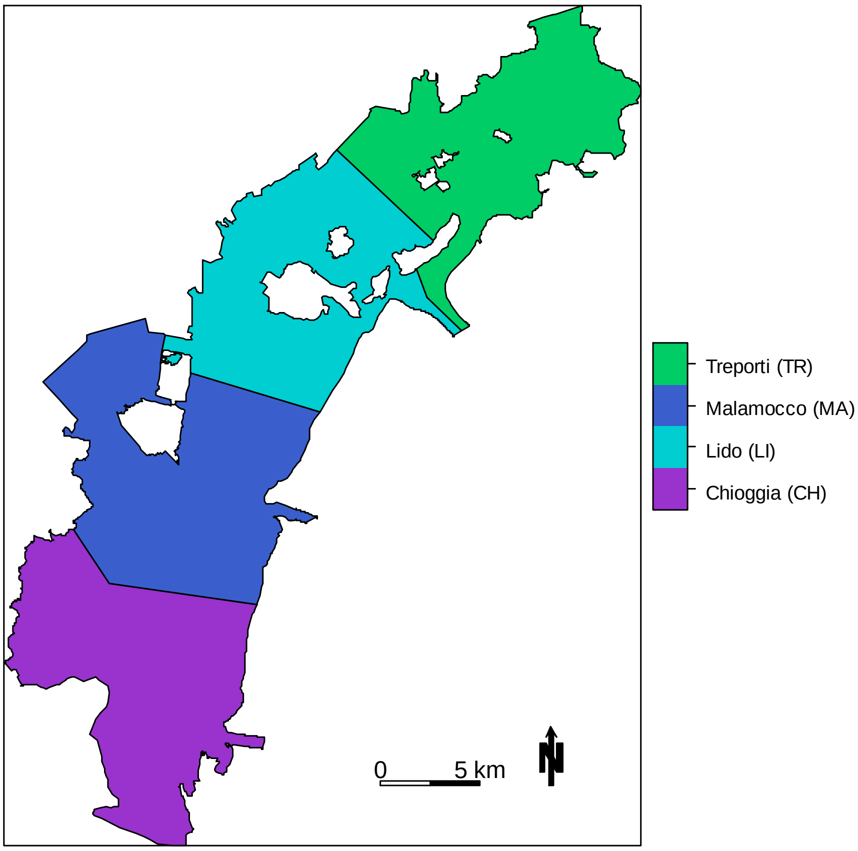


Figure 3.1: First rank basins of the Lagoon of Venice.

3.1.1 Notes on hydrogeological zonation

The open waters surface was classified following a hierarchical system of homogeneous Hydrogeological Zones (HZ) delineated on physiographic and mesological basis (see also Chapter 1.4). This classification is not a product of the present thesis but a result of Research line 3.11 of the 2004-2006 CORILA Research Project (Tagliapietra *et al.*, 2006; Tagliapietra *et al.*, 2009) and of a PhD thesis (Zanon, 2006), both by the unit of Benthic Ecology of CNR-ISMAR led by dott. D. Tagliapietra.

The zones were introduced in order to discretize the surface of the Lagoon into predetermined territorial units, which, at a given "scale", maintain a good homogeneity of environmental factors. The zones are considered as fixed and the succession of patterns was followed during the years and described in spite of the annual variability of environmental conditions. At the same time the suitability of the zonation was "verified" independently on the basis of larger multi-annual environmental and community data sets. The following sections briefly describe the approach and the results of the original works cited above.

The factors considered as the main components of the composite transitional gradient and most relevant ecological drivers for the lagoonal aquatic biota, particularly for benthic invertebrates, are salinity, seawater renewal and sediment characteristics. Water types were obtained through the analysis of the pattern emerging from the combination of the main components of the gradient. The identification of water bodies as discrete patches belonging to the same water type was the result of the transposition of water types into the spatial context. The method consisted in subdividing the

Lagoon into discrete territorial units or “Operational Lagoonal Units” (OLUs), quantifying the basic hydrogeological parameter for every single unit, classifying the units on the basis of the selected hydrogeological attributes.

The analysis was applied only to the shallows, which represent the majority of the lagoon surface, excluding the channels and islands. OLUs were delineated at a suiTable "scale" using natural boundaries (canals, watersheds) and traditional topographic subdivisions. A total of 226 OLUs were obtained. A minimum set of physical variables was selected: salinity, percentage of sand/pelite and water transit time as a proxy for water renewal processes and confinement. Mean salinity was calculated on the basis of the 2001-2003 period, from the data supplied by Consorzio Venezia Nuova on behalf of Magistrato Alle Acque di Venezia; sediment texture was obtained from a CNR-ISMAR data set for the years 2002-2003; transit time was calculated on the basis of a hydrodynamic model (Cucco & Umgiesser, 2006). Transit time was preferred to residence time because of its superior ability in identifying watersheds. Values were standardized before applying multivariate methods. OLUs were, hence, classified into hydrogeological types on the basis of hydrogeological variables through a multivariate approach and mapped. Adjacent OLUs belonging to the same type were regarded as consistent lagoonal “water bodies” or “zones”. In the present work, also not adjacent water bodies characterized by the same water type are considered a single zone.

At a higher level, a first subdivision into two major types was produced, which corresponded spatially to the classic subdivision into Open and Restricted Lagoon. This subdivision can be considered on the basis of its extent as belonging to the "macroscale" landscape level (or "macrochore", 100-500 km^2) (Haase, 1985; Mannsfeld, 1982; Becker, 1992; Schiøtz et al, 2003).

A second-rank subdivision at the "mesoscale" level (or "mesochore", 10-100 km^2) is substantially comparable to the classic hydrogeological zones (Roy *et al.*, 2001) of Wave Dominated Estuaries, i.e. the Marine Tidal Delta, the Central Basin and the Bayhead Estuary. Typical result of such classification is a landward type called the Fringe Zone characterized by a small amount of fresh water inflow and the intermediate Sheltered Lagoon type.

Total surface and surface per basin of macroscale and mesosacale hydrological zones are reported in Table 3.2.

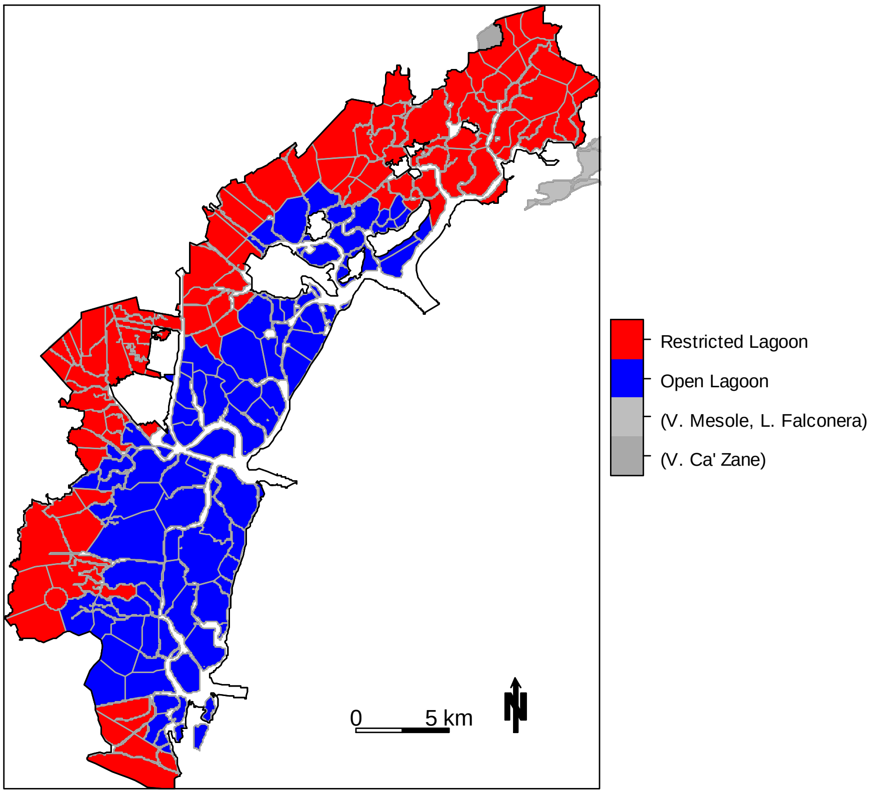


Figure 3.3: First-rank hydrogeological zones (Tagliapietra *et al.*, 2009, mod.).

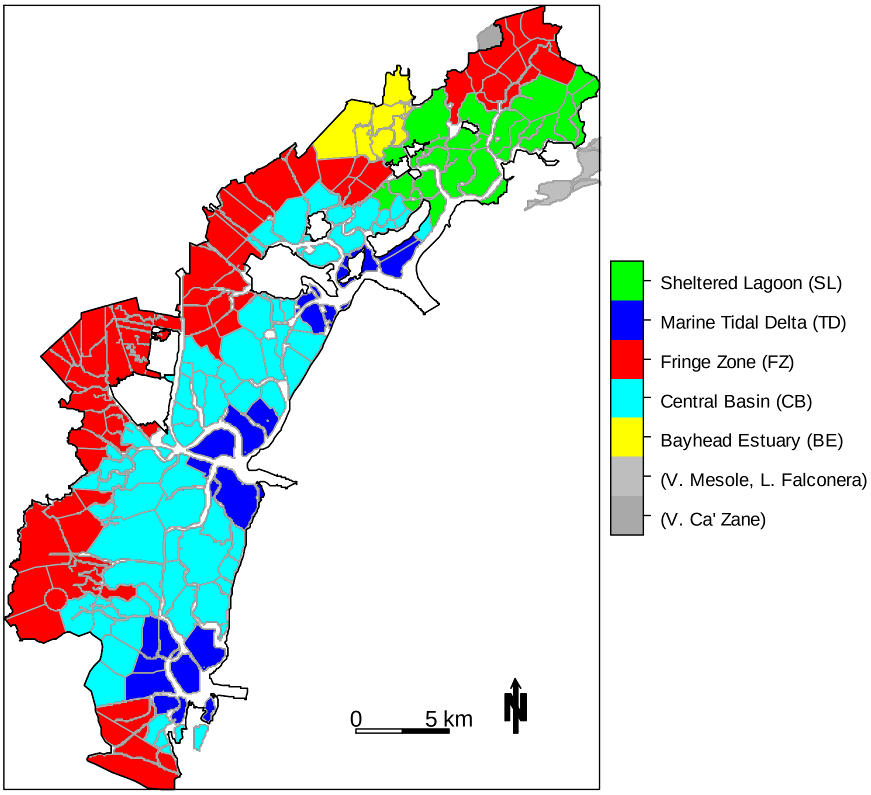
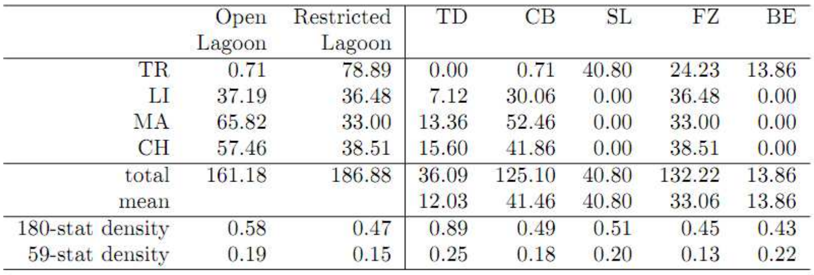


Figure 3.3: Second-rank hydrogeological zones (Tagliapietra *et al.*, 2009, mod.). TD and CB are nested into Open Lagoon and SL, FZ, BE are nested into Restricted Lagoon.



	Open Lagoon	Restricted Lagoon	TD	CB	SL	FZ	BE
TR	0.71	78.89	0.00	0.71	40.80	24.23	13.86
LI	37.19	36.48	7.12	30.06	0.00	36.48	0.00
MA	65.82	33.00	13.36	52.46	0.00	33.00	0.00
CH	57.46	38.51	15.60	41.86	0.00	38.51	0.00
total	161.18	186.88	36.09	125.10	40.80	132.22	13.86
mean			12.03	41.46	40.80	33.06	13.86
180-stat density	0.58	0.47	0.89	0.49	0.51	0.45	0.43
59-stat density	0.19	0.15	0.25	0.18	0.20	0.13	0.22

Table 3.2: Hydrogeological zones: total surface (km^2), surface per basin (km^2) and density of macrobenthos sampling stations (km^{-2}; see Chapter 3.3.2). The Treporti Tidal Delta area is summed with the Lido Tidal Delta area. Sums don't give total open water surface as channels are not considered in zones.

3.1.2 Notes on tidal zonation

Within each zone, geomorphologic *facies* or landforms can be identified, such as mudflats, salt marshes, channels etc. They are related to a model of vertical zonation and constitute the physical substrate for habitat (Heap *et al.*, 2001). As a consequence, a characteristic vertical zonation is displayed by communities as well (Ricketts *et al.*, 1985).

The present work is focused on the subtidal flats or shallows, which represent the majority of the lagoon surface. Channels and islands were excluded from hydrogeological zones on the basis of physiography. In tidal systems, landforms can be divided into *strata* based on characteristic tide levels, which are related to their generating processes (Sigovini & Tagliapietra, 2009). In the lagoon of Venice, tide amplitude is low (Mean Tidal Range at the tide gauge of Punta della Salute, 1986-2004, of 61 cm), but not negligible. The limit between subtidal and intertidal *strata*, which includes primarily salt marshes (above Mean Sea Level) and mudflats (below Mean Sea Level), has been set to the Mean Low Water Spring (MLWS) level (Sigovini & Tagliapietra, 2009). The latter can be defined as the average throughout the year of two successive low waters during those periods of 24 hours when the range of the tide is at its greatest (http://www.pol.ac.uk/ntslf/tgi/definitions.html, 30/11/2010), i.e. during syzygy. The upper limit of the intertidal *strata* has been set to the Mean High Water Spring (MHWS) level, which is the analogous of MLWS for high water. On the other hand, the lower limit of subtidal flats has been defined on physiographic basis and corresponds approximately to the 2 m isobath (although certain deep zones, such as the Fondo dei Sette Morti, reach the 3 m isobath).

MLWS was calculated on the basis of the Harmonic Datum Constant Method (CGS, 1952; Sigovini & Tagliapietra, 2009), which enables an estimate of the number of characteristic tidal levels and ranges for a given location by means of available harmonic constants (extracted from observed tides and routinely applied in astronomical tidal predictions). Tidal ranges are not uniform in the entire Lagoon. Hence the method was applied to the four major harmonic constants (M2, S2, K1, O1) (Ferla, 2006) available for 36 tide gauges of the ISPRA network in the Lagoon of Venice (Sigovini & Tagliapietra, 2009). MLWS were found to vary between -43 and -26 cm. Data were interpolated (IDW, power of 2; see Chapter 3.2.1) and resulting MLWS raster was subtracted from bathymetries. A map of the intertidal *stratum* is presented in Figure 3.4, and a summary of intertidal superficies for each basin is presented in Table 3.1. Although it will not be subsequently mapped, calculations over surfaces will be done taking in account only the subtidal *stratum*.

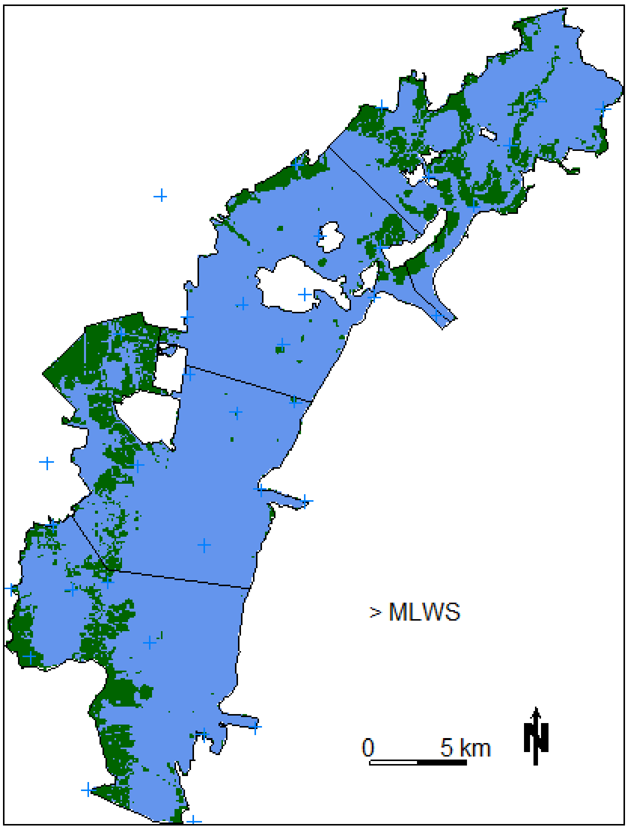


Figure 3.4: Intertidal (green) and subtidal (blue) *strata* (limit at MLWS). ISPRA network of tide gauges is also represented.

3.2 ENVIRONMENTAL DATA SETS

The relationship between the patterns observed in macrozoobenthos community and the environmental factors has been investigated by introducing a set of selected environmental variables. The selection of variables relies on known interactions between biota and environment in transitional ecosystems, on data availability and on exploratory statistical techniques. Available temporal series of hydrological variables was summarized by a central tendency and a dispersion statistic. Substrate variables are based on a single sample per year, i.e. only interannual variability was taken in account. Only spatial variation was considered for intertidal surface and water residence time. The sources of the data are variable. Nevertheless, most of the data sets was produced in the framework of the MELa projects, which consists in studies and monitoring on various components of the ecosystem (included macrozoobenthos), conducted since 2000 on the Lagoon of Venice by the Venice Water Authority (Magistrato alle Acque, MAV) through its concessionary Consorzio Venezia Nuova (CVN).

All the data sets were achieved as raw data. They were checked for errors and anomalies. More details on single variables are given in the following paragraphs.

3.2.1 Sediment

Source of the data

Sediment samples were taken simultaneously to MELa macrozoobenthos field samplings in 2002 and 2003 by CNR-ISMAR operators in a large subset of the samplings stations. The subsequent analyses were undertaken partly by Ca' Foscari University and partly by CNR-ISMAR, resulting in the production of a PhD and an MSc thesis (Frangipane, 2005; Masiol, 2005, respectively). The available data set includes granulometry and organic carbon content. Sediment characterization in terms of granulometry and organic carbon content was afterwards introduced by MAV in the MELa4 project as subsidiary to benthos samplings.

Sampling and laboratory analysis

In concomitance with macrozoobenthos samplings, surface sediment samples were collected at the same stations. During MELa2 2002 fieldwork, 140 out of 180 stations were sampled. The missing stations are mainly located around the island of S. Erasmo, near the inlet of Chioggia, between Venice and Porto Marghera, behind the "casse di colmata" (reclaimed areas) and in front of the fishing farms of the Chioggia basin. In 2003 fieldwork, 52 out of 60 stations were sampled, and two more from the overall MELa network were added (stations 82, 180). During the MELa4 fieldwork, sediment samples were taken in all the 60 stations.

In each station and replicate for macrozoobenthos samplings, a sample of the outermost layer of sediment (0-5 cm) was taken manually with a small polypropylene corer (internal diameter of 3cm) from the undisturbed surface. Each core was directly transferred to sealed PET bags and transferred to a -20°C freezer. The five replicates were then homogenized and sieved at 1 mm mesh size to remove the coarser part, containing fragments of shells.

MELa2 samples were analyzed by Ca' Foscari University. The samples were washed in bi-distilled water to remove the chlorides and in hydrogen peroxide (20 vol. %) to digest the organic matter, then oven-dried at 40°C for 12 h and weighed. The sand fractions were analyzed by dry sieving, and percentages calculated according to weight. Granulometry of the mud component (< 63 µm) was measured with an X-ray sedigraph 5000d Micromeritics. The method relies on settling velocity of particles and mass fraction determination by relative absorption of low-energy X-ray. To avoid particle flocculation, the sample was pre-treated with 6‰ Na-hexametaphosphate solution for 24 hours, and then immersed in ultrasonic bath for 15 minutes (Molinaroli *et al.*, 2009a).

The particle size analysis for MELa4 samples were carried out by SELC (Soc. Coop.) for MAV following the reference standard ISO 13320. All samples were first treated with hydrogen peroxide at 30% diluted in a 1:4 ratio for 48 hours to remove organic matter. Particle sizes were assessed with a laser diffractometer Mastersizer 2000. This method is based on the principle that particles of a given size diffract light through a given angle.

Typically, the three main fractions of sediments are calculated on the basis of the following classes: sand: 2 mm $> \Phi >$ 63 microns; silt: 63 microns $> \Phi >$ 3.9 µm; clay: $\Phi <$ 3.9 µm. Granulometric fractions for three years have been checked visually by means of ternary diagram and box-plots at 59 stations, indicating strong differences between MELa2 and MELa4 data. Many authors showed that laser diffraction, compared with other techniques, underestimates the amount of clay (Blott & Pye, 2006 and references therein). Moreover, no dispersing agent was used before diffractometry in MELa4 analysis, which may have caused aggregates to be interpreted as coarser particles. Because of the biases, these data sets should not be compared directly. However, the analysis might focus more on the interpretation of relative spatial differences as opposed to absolute values.

In the present work, as an attempt to improve the comparability among years, a sill of about 8 µm was chosen between silt and clay, instead of the more common 4 µm, following the estimated transition between cohesive flocks/aggregates and non-cohesive silt particles in coastal lagoon (Molinaroli *et al.*, 2009b). However, with the general trend in the lagoon leaning towards the loss of fine particles, it is not possible to exclude a role of environmental processes in differences between 2002-2003 and 2007.

Total organic carbon (TOC) was measured in the same MELa2 and MELa4 samples which were analyzed for granulometry (140 in 2002, 54 in 2003, 60 in 2007). As for granulometric analysis, different methods for estimation of organic content were applied in the two projects.

The MELa2 samples were analyzed by CNR-ISMAR. The organic carbon content was estimated by measuring the mass loss on ignition (LOI) after 16 hours at 350°C and expressed as percentage on dry weight (Frangipane *et al.*, 2005; Frangipane *et al.*, 2009). Numerous studies found a strong, statistically significant linear correlation between organic carbon as determined by CHN analyser and LOI (Frangipane *et al.*, 2009, and references therein). TOC was then estimated multiplying the LOI by a factor of 0.526 (Frangipane *et al.*, 2009).

For the MELa4 samples, the determination of sediment total organic carbon (TOC) was performed by Thetis (S.p.A.) with a CHN-S elemental analyzer (EPA method 600/R-97/072, 1997, 440.0 rev. 1.4). All organic matter is oxidized to CO_2 by complete combustion of the sample at 1000°C. The amount of CO_2 is proportional to the organic carbon content and is measured by a thermal conductivity detector after chromatographic separation. The samples were previously treated by removing the carbonates by HCl acidification.

As for granulometry, the analyses will focus more on the interpretation of relative spatial differences as opposed to direct comparison of absolute values.

A summary of selected sediment variables is given in Table 3.3. The values for three granulometric classes sum to 100%.

Variables	**Units**	**2002**	**2003**	**2007**
sand (1mm-63um)	% on dry weight	Frangipane, 2005	Frangipane, 2005	MELa4
silt (63-8um)	% on dry weight	Frangipane, 2005	Frangipane, 2005	MELa4
clay <8um	% on dry weight	Frangipane, 2005	Frangipane, 2005	MELa4
TOC	% on dry weight	Frangipane, 2005 (estimated from LOI at 350°C)	Frangipane, 2005 (estimated from LOI at 350°C)	MELa4

Table 3.3: Summary of selected sediment variables

Spatial interpolation and extraction of missing values

Values at sampling stations was interpolated with Inverse Distance Weighted (IDW) algorithm (power of 2), resulting in the production of a raster (100 × 100 m cell size) for each of the four variables. The analyses were performed with R. The estimated value for each cell is the average of the observed values for a maximum of six nearest sampling stations (within a 5 km radius), weighted by inverse square distance. Values were then extracted for macrozoobenthos sampling stations where sediment data were missing

3.2.2 Macrophytes

Main studies regarding macrophytes (both phanerogams and macroalgae) performed in recent years include MELa2 and MELa3 projects carried out by MAV through CVN between 2002 and 2005 (Miotti *et al.*, 2007) and MELa4 in 2007 (MAGIS.ACQUE - CORILA, 2009a). These monitoring were performed independently from macrozoobenthos field samplings and overlap only in part with macrozoobenthos sampling stations. Moreover, MAV carried out a complete mapping of phanerogams in 2002 (Rismondo *et al.*, 2003). Macrophyte distribution and biomasses were also studied at a lagoon scale in 2003 by the Environmental Science Department of Ca' Foscari University (Sfriso & Facca, 2005, 2007). These studies are not consistent in aims and methods, and they were often addressed at either macroalgae or phanerogams. In addition, is difficult to characterize macrozoobenthos samples in terms of coverage on the bases of not simultaneous data sets. Macrozoobenthos stations located on phanerogams prairies were often sampled at nearby bottom without vegetation coverage. For this reason, in the present work the analyses will be limited to records of macroalgae and phanerogams presence collected as ancillary data during macrozoobenthos fieldwork at the same sampling station. Although at times a taxonomic identification was added, this was not the rule. Moreover high turbidity sometimes led to poor description. Original field notes, as well as accessory laboratory notes, were consulted and data for the three years organized consistently. For the majority of stations, data were presented in semi-quantitative classes for each replicate. A procedure was applied to avoid overestimating the minimum presence. Semi-quantitative classes were transformed into a score and the five replicates for each station averaged. If it exceeds a minimum value, the station is considered vegetated. This procedure results in introducing two dichotomous variables describing the presence/absence of macroalgae and phanerogams. Reported phanerogams include *Zostera marina* L., *Nanozostera noltii* (Hornemann) Tomlinson & Posluszny and *Cymodocea nodosa* Ucria (Ascherson). Macroalgae include species belonging to Chlorophyta and Rhodophyta, as well as the Ochrophyta *Vaucheria* sp.

3.2.3 Intertidal surface

The intertidal *stratum* has been defined as the area which elevation is comprised between MLWS and MHWS. Macrozoobenthos samplings were carried out on subtidal bottoms (elevation < MLWS). To explore any interaction between these communities and surrounding intertidal areas, the intertidal surface inside a circular area of 1 km^2, centered at each sampling station, was extracted from the results of the analysis summarized in Chapter 3.1.2. It is assumed that the distribution of intertidal surface across the Lagoon is relatively stable in the considered time span. A single data set was used for all the three years.

3.2.4 Hydrodynamics

Residence time produced by Technital (S.p.A.) (MAG.ACQUE - Technital, 1993) was used as a proxy for water exchange and "confinement". The "eulerian" water residence time is defined as "the time required for each element of the lagoon area to replace most of the mass of a conservative tracer, originally released, with new water" (Cucco *et al.*, 2009).

The same data set was used for all three years. Original data are reported for a regular grid with 511 nodes covering the entire Lagoon. IDW interpolation (power of two) (see Chapter 3.2.1) was performed and value extracted for all macrozoobenthos sampling stations.

3.2.5 Hydrological variables

Source of the data

The source for main hydrological variables is the systematic monitoring of water quality carried out in the framework "MELa" by the Venice Water Authority through its concessionary Consorzio Venezia Nuova (CVN). It is the widest consistent data set available, homogeneously covering the area of the entire lagoon (even if at a low density) and temporally embracing a significant time span of recent years. The overall program (which included MELa1, MELa3 and MELa4) lasted for seven years, from 2000 to 2007, with an interruption in 2006, and consisted in periodical samplings carried out about monthly.

MELa1 (which started in September 2000 and ended in December 2003) and MELa4 (since February 2007) are the specific source for the present data set.

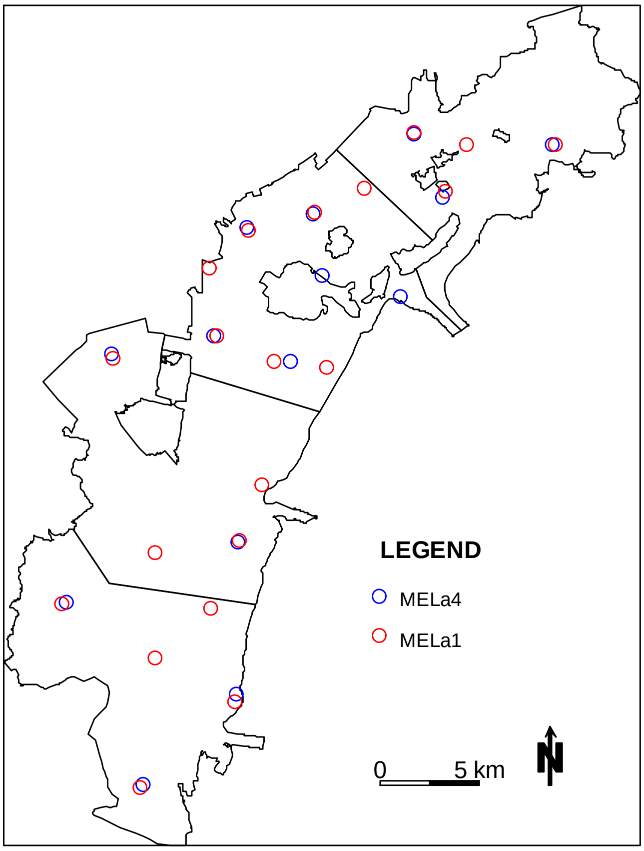


Figure 3.5: MELa water column sampling stations (stations on subtidal flats only displayed for MELa1).

Sampling

MELa1 and MELa4 monitoring were carried out monthly or quasi-monthly, with a sporadic second monthly sampling during MELa4. Sampling stations network homogenously covers the entire surface of the Lagoon. MELa1 monitoring began in September 2000 and ended in December 2003, with a total of 42 sampling events at 30 sampling sites. Among these, 20 sites were located in shallow-water on subtidal flats, 8 in channels and two in the sea just outside Lido and Chioggia inlets. Variables were measured at an intermediate depth between the bottom and the surface (MAGIS.ACQUE, 2004; MAGIS.ACQUE - CORILA, 2009b).

MELa4 "Water quality" monitoring commenced in February 2007, after a year-long interruption in MELa fieldworks, and consisted of one or two samplings per month, with a total of 17 samplings in 2007 alone. Sampling stations were a subset of MELa1 stations: 13 of them were located in subtidal flats, one station in the channel and one in the sea (MAGIS.ACQUE - CORILA, 2009b).

In the present work, the stations located in the sea have been excluded. Of all the 30 MELa1 monitoring sampling stations, only 20 sampling sites located in subtidal flats were retained to assure the comparability with the MELa4 dataset, which presents a minor number of samplings located almost exclusively in subtidal flats, and at the same time to focus on the landform investigated in macrozoobenthos MELa fieldworks. The map of the considered sampling stations is presented in Figure 3.5.

Given that MELa4 water quality samplings began in February 2007, after a year-long interruption of monitoring, only five sampling events (in four months) are available for the purposes of the macrozoobenthos MELa4 fieldwork, which was carried out between May and June. This also affected the 3-year data set, because it was necessary also for the previous fieldworks to choose the samplings on the four months preceding the macrozoobenthos fieldworks. Nevertheless, spring conditions are critical for a number of biological processes.

Chemico-physical and trophic parameters, in number of 24, were monitored through the MELa fieldworks. In addition, eight trace metals were analyzed for only a subset of stations and with less frequency. Variables measured in situ include temperature, salinity, pH, redox potential (Eh), turbidity, dissolved oxygen (DO). Total suspended solids (TSS), total alkalinity, nutrients, organic carbon and metals were measured by laboratory analysis of collected samples. Chlorophyll *a* was measured both in situ and, along with pheophytines, in laboratory. Total alkalinity was measured only during MELa2 fieldworks. Detailed protocols of samplings and analysis are available in MAGIS.ACQUE - Thetis (2008).

In the present work, a subset of the overall set of monitored factors was selected on the basis of their ecological significance for the macrozoobenthos and of the critical evaluation of datasets. Selected variables are presented in Table 3.4 with units of measurement. For a commentary about the choice, see the Results. Nutrients and metals were not taken into consideration.

Variables	Units	2002	2003	2007
Salinity	PSU	X	X	X
Temperature	°C	X	X	X
Dissolved Oxygen (DO) concentration	ppm	X	X	X
Chlorophyll *a*	μg/L	X	X	X
Total Suspended Solids (TSS)	mg/L	X	X	X
Total Alkalinity	μEq/L	X	X	-

Table 3.4: Summary of selected chemico-physical water column variables

Salinity was measured by conductivity and expressed as PSU. DO, as absolute concentration (ppm), was measured with a probe. Total alkalinity is the measure of the ability of a solution to neutralize acids (i.e. of the natural buffer system) and is related to pH (while being more stable), water acidification and shellfish calcification. It was expressed in μEq/L.

In the present work, the main factors responsible for turbidity, i.e. TSS and chlorophyll *a* as a proxy for phytoplankton standing stock, were preferred to the turbidity itself (in situ measurements by turbidimeter) in order to reduce the data set redundancy and focus on the component processes.

Total suspended solids (TSS) was measured as the amount of material filtered at 0.45 µm on a sample of settled water. In the present work, the value of Chlorophyll *a* determined in laboratory using standard analytical procedures (Istituto de Ricerca Sulle Acque (IRSA570.1Q59), 1990) was preferred to in situ fluorimeter measures.

Central tendency and dispersion of monthly series

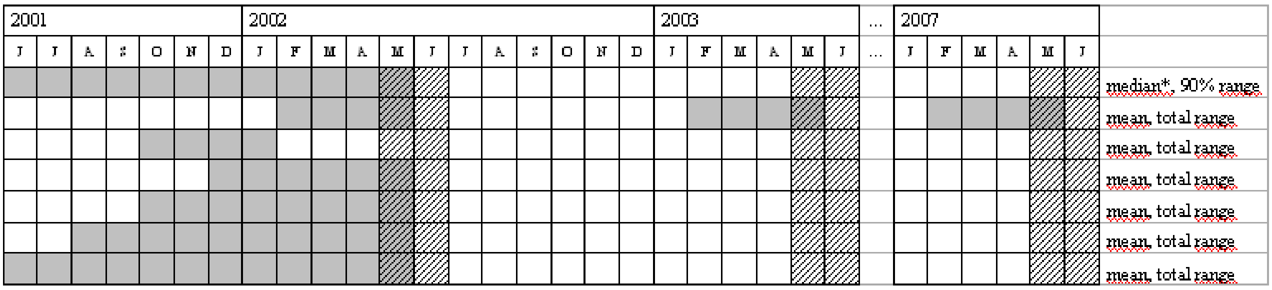
At first, each variable was checked using exploratory techniques. Central tendency and dispersion (i.e. temporal variability) were calculated on monthly time series for each variable at each station and introduced as a new set of variables. Temporal variability of environmental conditions is particularly strong in transitional environment and is recognized as a main factor in structuring community (Attrill, 2002).

Hydrological and generally environmental variables tend to depart from normality in time series (McLeod *et al.*, 1991). With non-normal data, mean and variance may be substituted by more robust, non-parametric statistics, such as median and interpercentile range. Anyway, these are probably inappropriate for very short data sets. Hence, different approaches regarding computed statistics were adopted, depending on the length of the time series (a diagram of time series and calculated statistics is presented in Table 3.5):

1) 180-station 2002 data set: the entire 12-month series was available. Median and 90% interpercentile range were calculated on six variables. However, during exploratory analysis, it was noticed that median might not be an appropriate central tendency statistic for variables which present a strong annual periodicity. Water temperature in particular presents a clear sinusoidal trend as, in shallow water bodies, it presents a high correlation with air temperature. In this case, the mean was selected as central tendency.

2) 59-station 3-year data set: the usable monthly fieldworks for 2002 and 2003 is limited due to the four month period available for 2007. Mean and total range were calculated for February-May 2002, 2003 and 2007. Total alkalinity is not available for 2007 data set and will not be taken into account for the purposes of these analyses.

3) mean and total range were also calculated for the years 2001-2002 on time series of different duration, up to a 12 month maximum: six months; eight months; ten months; 12 months and four months (October 2001-January 2002). The analysis applied to these data sets and the one for February-May 2002 will be described in Chapter 3.6.5.



2001							2002													2003						…	2007						
J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	…	J	F	M	A	M	J	
■	■	■	■	■	■	■	■	■	■	■	▩	▨											▨	▨						▨	▨	median*, 90% range
								■	■	■	▩	▨								■	■	■	▩	▨			■	■	■	▩	▨	mean, total range
				■	■	■	■				▨	▨											▨	▨						▨	▨	mean, total range
						■	■	■	■	■	▩	▨											▨	▨						▨	▨	mean, total range
				■	■	■	■	■	■	■	▩	▨											▨	▨						▨	▨	mean, total range
		■	■	■	■	■	■	■	■	■	▩	▨											▨	▨						▨	▨	mean, total range
■	■	■	■	■	■	■	■	■	■	■	▩	▨											▨	▨						▨	▨	mean, total range

Table 3.5: Diagram of analyzed time series of water column variables. Macrozoobenthos fieldworks are indicated by dashed months. Calculated statistics for all the variables is also shown (*: the mean was calculated in the case of Temperature)

Analysis of collinearity

Multi-collinearity (or collinearity) is the existence of correlation between multiple covariates. In statistical analysis aimed at identifying main driving factors, collinearity could lead to confusing and sometimes misleading results (Zuur *et al.*, 2010) by increasing estimates of parameter variance, lowering statistical significance, producing parameter estimates of the incorrect sign and of implausible magnitude and creating situations in which small changes produce wide shift in parameter estimates (O'Brien, 2007). Environmental variables may present strong collinearity. This is particularly true for tidal-dominated transitional ecosystems with a strong composite gradient. Moreover, average values and variability for the same factor are often highly correlated (MAGIS.ACQUE, 2004). In this case, the same information is introduced into the analysis two times.

Analysis of collinearity was performed on the 12-month 2002 dataset consisting of the average and dispersion of the six selected hydrological variables. Visual inspection was carried out with multiple scatterplots and a correlation-based PCA of standardized variables. Pearson correlation and variance inflation factor (VIF) between all the 12 covariates were checked. VIF quantifies the severity of collinearity as the effects of R^2_i on the variance of the estimated regression coefficient for the i_{th} independent variable. It was calculated by means of the R code provided by Zuur *et al.* (2010). Collinearity is lowered by sequentially dropping the covariates which are highly correlated and present the highest VIF, until a certain threshold for VIF is reached. Some authors propose that the maximum value of VIF be as high as 10 to be taken as evidence of collinearity (see O'Brien, 2007). Following the more stringent approach of Zuur *et al.* (2010), the value of 3 was choosen as the target.

Interpolation and calculation of values for macrozoobenthos sampling stations

The value of hydrological variables for the macrozoobenthos sampling stations was obtained by spatial interpolation (IDW, power of 2; see Chapter 3.2.1), resulting in the production of a raster for each of the selected variables. The estimated value for each cell is the average of the observed values for a maximum of its nearest six sampling stations (within a 10 km radius), weighted by inverse square distance. Because of the limited density of stations, only "wide-scale" spatial variability was retained by the data.

3.3 MACROZOOBENTHOS DATA SETS

3.3.1 Source of the data: the MELa projects

The MELa projects consist in a large framework of studies and monitoring conducted since 2000 by the Venice Water Authority through its dealer Consorzio Venezia Nuova (CVN) on various components of the Venice Lagoon ecosystem.

The macrozoobenthos community was the subject of two main activities: MELa2 project (MAGIS.ACQUE - SELC, 2005), which included extensive and quantitative macrozoobenthos samplings once per year in 2002 and 2003 and MELa4 project (MAGIS.ACQUE - CORILA, 2009a), which included an analogous macrozoobenthos sampling in 2007. MELa2 and MELa4 samplings and laboratory activities followed the same protocol and were both conducted by SELC (Soc. Coop.), hence producing a consistent multi-year series.MELa4 benthos monitoring was supervised (through CORILA) by the Benthic Ecology unit of the CNR-ISMAR, led by dott. D. Tagliapietra, which also collaborated to MELa2. The PhD candidate at that time was part of the Benthic Ecology unit and supported samplings, laboratory activities and data analysis and reporting.

3.3.2 Sampling and laboratory activities

Samplings were performed by SELC operators in late spring within all three studies (7 May - 11 June 2002, 15 - 29 May 2003 and 8 May - 6 June 2007). MELa2 macrozoobenthos samplings were performed in 2002 on 180 sampling stations irregularly but nearly homogeneously spread throughout the Lagoon. The 2003 fieldwork was conducted on a subset of 60 of the total of 180 stations. Almost all of the 60 stations covered in the 2003 fieldwork appear in the MELa4 fieldwork as well. Six station were moved up to 1 km: 55, 59, 108, 120, 122, 139. The station 119 was deleted and a new station (n. 181) located in Val di Brenta, a previously under-represented area. Sampling stations for three years are presented in Figure 3.6. In the present study, part of the analysis was performed on a 3-year data set of 59 sampling stations in common between the three monitoring. The six stations which were moved in 2007 were characterized by the coordinates of the centroids of old and new location.

Five replicates were collected at each station with a 20 × 25cm (500 cm^2) box-corer (penetration depth of 30 cm). The total sampled area per station is 0.25 m^2. Each replicate was subsequently sieved at 1 mm mesh size, and the retained material was placed in an anaesthetic solution, brought to the laboratory at a low temperature and stored in the freezer. In the laboratory organisms were sorted and transferred to a 70% ethanol solution.

For each replicate, animals were identified down to the lowest possible taxonomic rank and counted. Biomass was measured as wet weight (WW), to the nearest 1 mg, and as dry weight (DW), to the nearest 0.01 mg. DW were measured after drying at 60°C for 24 h. No skeletal parts such as shells were removed.

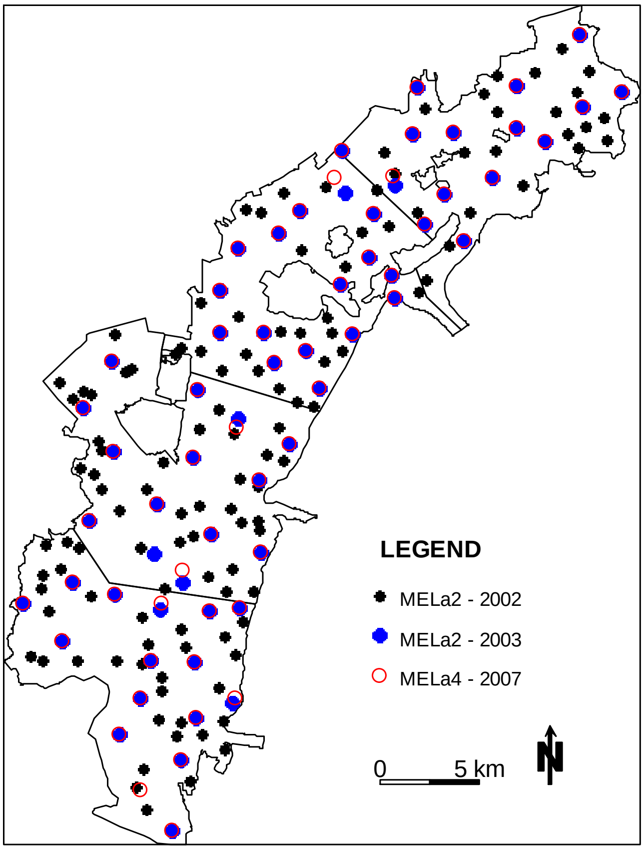


Figure 3.6: Macrozoobenthos sampling stations of the MELa projects.

3.3.3 Taxonomic list

First of all, the raw data for each of three years were organized and checked by identifying and correcting errors and synonyms, following primarily the Checklist of Italian Marine Fauna Volume 1 published by the Italian Society of Marine Biology (SIBM) (Relini, 2009) as well as the online version of Volume 2 (Relini, 2006). Other checklist consulted include the Checklist of the Species of Italian Fauna, online version 2.0 (Minelli *et al.*, 1995), the European Register of Marine Species (ERMS, 2009) and the World Register of Marine Species (WoRMS, 2009). If identified taxa has been subsequently divided or merged, previous synonyms were retained. Some taxa was merged to avoid redundancies, whereas not informative taxa (for example: "Polychaeta indet."), as well as vertebrates, was removed.

A unified and consistent species checklist for the three years was produced, in which different taxonomic ranks up to phylum are also comprised. In the present works, the term "species" will be used in the widest sense of "taxon", in any case saving clarity. An array of biological and ecological traits was associated to the checklist, in particular biomass conversion factors and functional features such as trophic groups.

Species were assigned to trophic groups according to their nutritional needs and their way of feeding. Often a species is assigned to more trophic groups or to different groups by different authors. Moreover some species are classified as "omnivores" or "mixed feeders" which is a somewhat ambiguous definition. In fact, most of the species, in particular in transitional environments, have a variable diet and turn to multiple food sources (Blondel, 2003, Bremner et. al., 2003, Chevenet *et al.* 1994; Statzner *et al.*, 2001; Usseglio-Polatera *et al.*, 2000a, 2000b; Vieira *et al.*, 2006).

3.3.4 Trophic groups

The following trophic groups were considered: carnivores (C), herbivores and micrograzers (H+MG), detritivores and surface deposit-feeders (SDF), subsurface deposit-feeders (SSDF), deposit-feeders with chemoautotrophic symbiotic bacteria (CDF) and suspension- and filter-feeders (SF). The species were assigned to groups according to literature (Bonsdorff & Pearson, 1999; Eaton, 2001; Fauchald & Jumars, 1979; Gaston *et al.*, 1995, Iken *et al.*, 2001; Maurer *et al.*, 1999; and many others). The assignment of a certain species to a certain trophic group was not considered exclusive, but it was divided among multiple trophic groups, with partial assignments summing to one (or 100%). For example, if a species is defined as both C and SDF, the importances of that species were redistributed between the two categories. Species defined as omnivore were allocated to the categories C, H+MG, SDF. Biomasses and abundances for taxonomic categories were redistributed among the trophic groups. Analysis of trophic groups will be in some case performed only on biomass as it is the metabolically active element of organisms.

3.3.5 Operational data sets

Two data sets has been organized: the 180-station 2002 data set and a 59-station 3-year data set, each one composed of two species composition matrices $n \times S$, one with abundances and the other with biomass.

Nine taxa of the MELa4 list, which are organisms with encrusting or colonial forms, were originally quantified by coverage rather than by abundance. An empirical conversion to abundance was then performed, by dividing the coverage by the minimum coverage measured for that taxa.

A high number of species presents calcareous skeletal parts which have very low metabolism, such as the shell of molluscs or the exoskeleton of crustaceans. Biomass was then expressed as "ash-free dry weight" (AFDW) through the application of conversion factors on dry weight (DW). Conversion factors for many species were produced at the laboratories of CNR-ISMAR (Masiero, 2003), while others were taken from literature (in particular Brey, 2001).

Importances are subdivided among *S* identified taxon and *n* sampling station. Identified taxa can be combined to form broader categories, such as higher rank taxon.

3.4 STATISTICAL TOOLS: THE R SOFTWARE ENVIRONMENT

All the analyses (except when otherwise stated) have been performed by R (v. 2.10.1), which is a programming language and software environment for statistical computing and graphics (R Development Core Team, 2010; http://www.r-project.org/). R is available as Free Software under the GNU General Public License. It is a transparent and flexible software and can be extended by means of packages. The packages employed are the following:

Statistical and ecological analyses:
- ade4
- cluster
- clustsig
- nortest
- scatterplot3d
- StatDA
- vegan
- Zelig

GIS, spatial analysis and geostatistics:
- deldir
- gpclib
- gstat
- maptools
- raster
- rgdal
- sp
- spatstat
- spdep

3.5 SPATIAL AND INTERANNUAL PATTERNS OF MACROZOOBENTHOS COMMUNITY AT THE LAGOON SCALE

The structure of the macrozoobenthos community was described at the lagoon scale by univariate macrodescriptors, taxonomic and functional (trophic) composition and multivariate analysis. Spatial and temporal (interannual) patterns were examined on the basis of the following data sets: the 2002 data set, consisting of 180 sampling stations; the 3-year (2002, 2003, 2007) data set, consisting of 59 coincident sampling stations.

3.5.1 Univariate descriptors

For each station, classical univariate macrodescriptors were calculated to represent the significant attributes of the community. These comprise the "SAB" metrics (Species richness, total Abundance, total Biomass) and ecological indices reported in Table 3.6.

These are widely used both as descriptors of the community structure and, in a more stringent sense, as "quality indices". In the present work, they will be used primarily with the purpose of characterizing the structure of the community.

Macrodescriptor	Symbol	Index type	References
Total abundance	A, N		
Total biomass (AFDW)	B		
Species Richness (number of species)	S		
Margalef index	d	Richness	Margalef (1958)
Hulbert index (expected number of species)	$E(S_n)$	Richness, diversity	Hurlbert (1971)
Shannon-Wiener index	H'	Diversity	Shannon & Weaver (1949)
Pielou index	J'	Evenness	Pielou (1966)

Table 3.6: Univariate descriptors and indices considered.

Number of species, total numerical abundances and biomass are the simpler descriptors, as they are the original measured properties. A number of univariate secondary descriptors can be calculated based on them. Distributional methods developed directly from these descriptors in order to understand the community structure include the ABC curves, based on comparison of abundances and biomass (Warwick, 1986), and the SAB curves, based on species richness, abundances and biomass (Pearson & Rosemberg, 1978). Abundances and biomass as AFDW (mg) were expressed as total values for the sample area (0.25 m^2). Species richness includes taxa identified at a higher taxonomic rank than species.

Margalef index was proposed to make richness independent from sample size, by dividing the number of species (minus one) by the logarithm of the total abundance, i.e. $d = (S-1)/\ln N$ (Margalef, 1958). Hurlbert index $E(S_n)$ (Hurlbert, 1971) estimates the number of species expected in a sample of n, say, individuals. Although the index is expressed as estimated richness, it depends on evenness in the same way as diversity indices. However, it is less dependent on the size of the sample compared with classic diversity indices such as Shannon, allowing the comparison of samples of different sizes. It is expressed as:

$$E(S_n) = \sum_{i=1}^{S} \left[1 - \binom{N - N_i}{n} \Big/ \binom{N}{n} \right]$$

where n is the number of individuals to which corresponds the expected number of species S_n. It takes values between 0 and ∞. It has been calculated on abundances, with $n = 50$.

Shannon index H' (or Shannon-Wiener, Shannon & Weaver, 1949), like other diversity indices, takes into account the richness and distribution of importances among the species. The higher the species richness and the more evenly distributed the importances among the species, the higher the value of the index. It can be decomposed into two constituents: richness and evenness (Hurlbert, 1971). It is expressed as:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where $p_i = n_i/N$: relative abundance, N: total abundance, n_i: i-th species abundance. The index takes values between 0 and ∞. High sample size is assumed ($N \rightarrow \infty$). When calculated on abundances, it expresses "uncertainty" in predicting to which species a randomly selected individual belongs.

Evennes measurements quantify the equality of abundances among the species. The evenness index of Pielou J' (Pielou, 1966) was developed in relation to the index of Shannon. It takes values between 0 and 1, the maximum value corresponding to equally-distributed abundances, for which H' = ln(S). Then J' is expressed as H'/ln(S). Shannon and Pielou indexes were calculated both on abundances and biomass (AFDW, in mg).

Pearson correlation was calculated among the macrodescriptors for the two data sets. Abundances, biomass and species richness were summarized through synthetic plots and spatialized through IDW, with power of 2, as reported in Chapter 3.2.1 (calculated on a maximum of six sampling stations within a 3 km radius).

3.5.2 Dominant taxa

Dominant taxa were identified for both the 180-station data set from 2002 and the 59-station data set for each of the three years. Different definitions of "dominant species" exist. In the present work, they are defined as the set of most abundant taxa responsible for (more than) 75% of total abundances for each station. As for abundances, dominant species in terms of biomass were calculated.

3.5.3 Taxonomic and trophic composition

Abundances and biomass were subdivided into a set of supraspecific taxa of different rank which present functional meaning. The following taxa were considered: Polychaeta Sedentaria and Errantia; four orders of Crustacea, i.e. Decapoda, Amphipoda, Isopoda and Tanaidacea; two classes of Mollusca, i.e. Bivalvia and Gastropoda. These groups are the most important in terms of abundances and biomass or number of species. Sedentaria and Errantia are former-taxa not longer accepted, but they are still ecologically meaningful. All other taxa were merged as *Animalia caetera* (AC).

The species were assigned to the following trophic groups according to their nutritional needs and their way of feeding (see Chapter 3.3.4): carnivores (C), herbivores and micrograzer (H+MG), detritivores and surface deposit-feeders (SDF), subsurface deposit-feeders (SSDF), detritivores with chemoautotrophic symbiotic bacteria (CDF) and suspension- and filter-feeders (SF). Next, to represent the trophic structure of the given assemblage, the importances were combined according to the groups. Typically, biomass is used rather than numerical abundances, as it constitutes a metabolically active element involved in the energetic processes. Nevertheless, abundances were calculated as well.

3.5.4 Analysis of hydrogeological zones

The changes in the structure of benthic communities during the three years were referred through a zonal approach to different hydrogeological zones composing the lagoonal ecosystem (Tagliapietra *et al.*, 2009). The hydrogeological types were identified as listed here, following approximately the transitional gradient: the Marine Tidal Deltas (TD), close to the three sea inlets, the Central Basin (CB) and the Sheltered Lagoon (SL), located on the middle ranges of the lagoon, and two landward zones, the Fringe Zone (FZ) and the Bayhead Estuary (BE) (Figure 3.3, Chapter 3.1.2). This approach made it possible to observe year-to-year variations of the characteristics of assemblages in different homogenous areas of the lagoon. The trend of the various areas over the years and a "spatial trend" along the transition gradient were compared.

Mean values of main univariate descriptors for a given year and zone were computed on the 59-station data set and plotted on a chart to follow evolution across time and zones. Abundances and biomass were expressed as densities. Trophic and taxonomic structure as percent composition in terms of biomass and abundances were analyzed.

3.5.5 Analysis of multivariate structure

Next, the structure of the community was analyzed with multivariate methods. Compared to univariate macrodescriptors, models resulting from multivariate analysis show greater sensitivity to small changes in the community structure (Anderson, 2008; Gray *et al.*, 1990; Warwick & Clarke, 1991).

Multivariate data analyses were performed on matrices of importances $N \times S$ obtained from the 180-station 2002 data set and the 59-station 3-year data set, both on abundances and biomass (AFDW), as they carry different signals. Total abundances are count data, which main characteristics are the right-skewed, not normal distribution and the high number of zero-values. Although biomass is expressed in continuous values, its statistical distribution behaves just like the count data.

The analyses include "raw data approach" methods, which are applied directly on the species composition matrices, and "distance approach" methods, through the calculation of a resemblance matrix $N \times N$ between samples. Resemblance measures include the Euclidean distance as well as other distancies or (dis-)similarities, which are more appropriate to species composition data, such as the Bray-Curtis dissimilarity.

Main multivariate statistical methods used are summarized in Table 3.7, with brief notes on their assumptions. Although parametric analyses were occasionally performed as well, non-parametric analysis (no assumptions about data distribution) were generally preferred.

Statistical methods	Assumptions
Parametric:	
General Linear Model (ordinary and multiple linear regression and ANOVA)	Normal distribution of variables, independence of observations, homoscedasticity
Non-parametric:	
PERMANOVA	Exchangeability of observations, homogeneity of dispersion
ANOSIM	Independence of observations, homogeneity of dispersion
Mantel test (second-stage analysis, BIOENV, ANOSIM)	Independence of matrices. See also Chapter 3.6.5.
PCA, RDA	Linear relationship among variables. Euclidean distancies are preserved.
CA, CCA	All data should be non-negative and on the same scale. Unimodal relationship among variables. Chi-squared distancies are preserved.
PCoA, CAP	
nMDS	
Cluster Analysis	

Table 3.7: Summary of applied statistical methods with main assumptions

Transformation of species composition data

Transformations are conventionally applied to count data to reduce asymmetry of distribution and not to underestimate the role of rare species. Between untransformed and presence/absence transformation, there is a range of increasingly severe transformation, including square-root, 4th-root, logarithmic. When data are intended to be positive, as in the case of count data, log-transformation needs to be applied to values higher than 1, and an appropriate constant, usually 1, is summed to the argument. This transformation is not recommended for biomass as its effect is dependent on the scale and unit (e.g. g or mg) (Field *et al.*, 1982, in Thorne *et al.*, 1999; http://ordination.okstate.edu/transfor.htm, 30/11/2010).

Richard *et al.* (1999) suggest a mild transformation or no transformation to discriminate between "polluted" sites, characterized by few tolerant species with very high abundances. On the other hand, they propose strong transformations to enhance the role of rarer species in discriminating between "clean" sites, which present higher richness and evenness. A mid-level transformation is recommended to encompass a range of conditions. These statements can be simply extended to transitional environments covering a whole range of conditions from marine to more selective. On the other hand, when comparing studies performed in different years, it's better to concentrate on the most important species.

The relationship between transformations (natural logarithm, square root, 4th root, presence/absence) of abundances and biomass has been displayed by an nMDS plot (see also in this Chapter "Notes on ordination techniques") on the results of a second-stage analysis (Somerfield & Clarke, 1995) conducted on the 180-station 2002 data set. Second-stage analysis consists in a non-parametric (based on Spearman rank correlation) pairwise Mantel test, which describes the relationship between resemblance matrices, in this case Bray-Curtis dissimilarity matrices. Actually, in this particular case the matrices are not independent, as required, but the test was still recognized suiTable by some authors (e.g. Heino, 2008). Clarke & Warwick (1994, 2001) indicate that the 4th-root and the logarithmic transformation produce similar effects. The nMDS plot of the second-stage analysis (Figure 3.7) shows instead that natural logarithm transforms the biomass similarly to the way a square-root does, whereas on abundances the log-transformed data lie between square-root and 4th-root transformation. The square-root transformation was chosen for both abundances and biomass. 4th-root was judged to overestimate the role of rare species.

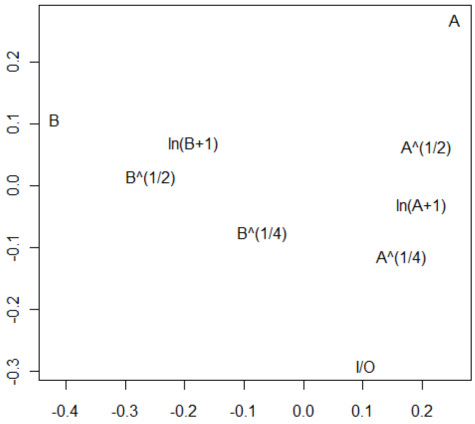


Figure 3.7: nMDS (stress = 0.3) on the results of a second-stage analysis on the 180-station 2002 data set, with different transformation on abundances (A) and biomass (B), up to presence/absence (I/O).

Cluster analysis

The continuous succession of species along the lagoonal gradient (coenocline) was resolved into discrete assemblages. The similarity between stations was explored using hierarchical agglomerative cluster analysis. As measure of resemblance, the Bray-Curtis dissimilarity index was used. As linkage method, "group average" was applied, which evaluates similarities between two groups based on mean similarities between all pairs of objects within the groups. Analysis was performed, as usual, on abundances (transformed by square-root), the results of which are generally inconsistent with those obtained on biomass. Analysis was first of all performed on the 180-station 2002 data set. Subsequently, the 59-station 3-year data set was analyzed. The relationship between different years' assemblages was investigated. Considering each year separately might yield very different results from those obtained by performing a single overall analysis. However, strong patterns can be distinct, and interannual variability directly checked. For the same reasons, result of the 180-station analysis is not directly comparable with the result of the 59-station 3-year analysis. Nevertheless, the role of different spatial resolution in identifying assemblages was discussed.

The resulting dendrograms were cut at given (dis-)similarity levels to obtain homogenous assemblages in a nested system. Reflecting the hierarchical framework, the degree of aggregation in the horizontal spatial distribution is structured at different spatial scales. Voronoi maps for 180 and 59 stations were used to spatialize clusters. In the analysis of the 3-year data, maps were produced for each year. The spatial meaning of the groups was interpreted on the maps on the basis of emerging patterns.

Similarity Profile (SIMPROF) (Clarke *et al.*, 2008) analysis was applied (999 permutations) to evaluate statistical significance ($\alpha = 0.05$) of identified clusters (i.e. assemblages), with the assumption of no *a priori* groups. Non-significant groups at the given (dis-)similarity level were retained aggregate up to the significance level.

Sequential procedure of agglomerative clustering causes distortion in classification at high dissimilarity level. This was explored by a *k*-means cluster analysis on the 180-station data set at the highest hierarchical level (i.e. k = 2) (Legendre & Fortin, 1989). *k*-means is a partitional clustering method which uses an iterative procedure to minimize the within-group variance.

Identified assemblages were characterized in terms of main macrodescriptors (median abundance, median biomass, mean richness), dominant taxa, taxonomic and trophic structure.

Notes on General Linear Model methods and non-parametric alternatives

The general linear model (GLM) is a statistical linear model which includes a number of different models as special cases: ordinary and multiple linear regressions, ANOVA, ANCOVA, MANOVA. Its general structure is $\mathbf{Y} = \mathbf{XB} + \mathbf{U}$ where $\mathbf{Y}$ is a matrix of dependent (response) variables, $\mathbf{X}$ is a matrix of independent (explanatory, or predictor) variables, B is a matrix of parameters to be estimated and $\mathbf{U}$ is a matrix containing errors. $\mathbf{X}$ can be composed by both quantitative variables and qualitative (dummy) variables that identify factors, as for ANOVA. In the GLM framework the coefficient of determination R^2, which corresponds to the variance explained, is extended to ANOVA. Adjusted R^2 (less than or equal to R^2) accounts for the number of explanatory terms in a model.

The general linear model requires normal distribution of variables, homoscedasticity and independence of observations. Techniques that analyze the distribution of the variables and verify for normality include the examination of main statistics (mean and median, skewness, kurtosis), graphical methods (e.g. normal Q-Q plot (Hazen, 1914) and inferential test, such as the chi-square test (Pearson, 1900), the Kolmogorov-Smirnov test and its adaptations (e.g. Lilliefors, 1967), the Shapiro-Wilk test (Shapiro & Wilk, 1965). The tests used to evaluate homoscedasticity include the

Levene's test (Levene, 1960), which assesses the equality of variances among groups. Data transformation could be used to improve normality and to stabilize the variance. Independence of observations is not met in inherently spatially (or temporally) autocorrelated data. However, the problem arises not directly from the correlation per se, but from a biased sampling scheme, in which samples are spatially clustered (Pardo-Iguzquiza & Dowd, 2004). In the present work, we assume that spatial distribution of sampling stations is not clustered.

Environmental and species composition data doesn't usually approach neither normal nor log-normal distribution Reimann & Filzmoser, 2000. If the assumptions are not met even after transformation, robust or non-parametric methods should be considered. In non-parametric statistics independence of observations and homogeneity of dispersion are still assumed. ANOVA (Analysis of Variance) is an extension of the Student's t-test based on the Fisher's F-test, which provides a statistical test of whether or not the means of several groups (levels of a factor) come from the same population, under a null hypothesis H_0 of no differences. The ANOVA design can be quite complex, ranging from one-way to multi-factor design. Multivariate ANOVA (MANOVA) is a generalization of ANOVA to a multivariate data set of dependent variables and, as for ANOVA, requires normality for all the variables making it unsuiTable for species composition data. The main approaches proposed by ecologists as non-parametric alternatives to MANOVA include ANOSIM and PERMANOVA, which both follow a "distances" approach.

ANOSIM (Analysis of Similarities, Clarke, 1993) operates on any (dis-)similarity matrix, comparing the compositional dissimilarities between the groups to those within the groups. As for nMDS, the analysis is performed on the rank order of dissimilarity values. The ANOSIM statistic R is based on the difference of mean ranks between groups and within groups and ranges between -1 and +1, similarly to a correlation coefficient, with value 0 indicating completely random grouping. ANOSIM's R can be directly interpreted as a measure of the size of between-group differences, unlike F statistic which is function of the degree of freedom. The statistical significance of R is assessed by a permutation test. ANOSIM is a form of "Mantel test" (Chapter 3.6.5) and shares all its limitations (Legendre & Legendre, 1998; Legendre *et al.*, 2005).

PERMANOVA (Anderson, 2001; McArdle & Anderson, 2001) tests the response of multivariate dataset to one or more factors in an ANOVA experimental design on the basis of any dissimilarity measure, using permutation methods. Instead of the classical Fisher’s F ratio, a pseudo-F ratio is produced. When PERMANOVA is performed on univariate variable and euclidean distance, the pseudo-F ratio matches the F ratio (Anderson, 2005). p values for statistical significance are obtained using an appropriate permutation procedure choosen on the basis of the design. The method follows closely ANOVA (Underwood, 1997), allowing complex multi-factor mixed models, balanced and unbalanced number of observations per level, nested and crossed design, asymmetric design, analysis of covariance (the effect of a set of covariables is removed before analysis). *A posteriori* pair-wise comparisons among levels of factors can also be performed. PERMDISP is a multivariate non-parametric permutational test of homoscedasticity, developed by Anderson (2006) and associated to PERMANOVA.

Notes on ordination techniques

Ordination methods arrange samples on the basis of their ecological (dis-)similarity and represent them in a Euclidean space with a reduced number of dimensions, such as two-dimensional plots, allowing observation and interpretation of the main patterns. A number of ordination procedures has been developed. They are classified as either constrained or unconstrained, or "direct" and "indirect" ordinations (corresponding to "direct" and "indirect" gradient analysis; Ter Braak, 1995), on the basis of whether or not *a priori* hypotheses about explanatory variables are made. A summary by Anderson (2003) of main unconstrained methods, with their main characteristics, is reproduced in Table 3.8.

Name of method (acronyms, synonyms)	Distance measure preserved	Relationship of ordination axes with original variables	Criterion for drawing ordination axes
Principal Component Analysis (PCA)	Euclidean distance	linear	finds axis that maximizes the total variance (or, equivalently, that minimizes the total residual variation)
Correspondence Analysis (CA, reciprocal averaging, dual scaling)	chi-square distance	unimodal (approximately Gaussian)	finds axis that maximizes dispersion of species scores (which are themselves weighted averages of site scores)
Principal Coordinate Analysis (PCO, PCoA, metric multidimensional scaling, classical scaling, Torgerson scaling)	any chosen distance or dissimilarity measure	unknown; depends on distance measure chosen	Euclidean distances in new full-dimensional space are equal to original distances (or dissimilarities).
Nonmetric Multidimensional Scaling (MDS, NMDS)	any chosen distance or dissimilarity measure	unknown, depends on distance measure chosen	The number of dimensions for the new space is chosen a priori (reduced). Euclidean distances in new space are monotonically related to original distances.

Table 3.8: Methods of unconstrained ordination of a multivariate data set (from Anderson, 2003).

One of the first developed and widely known unconstrained methods is the Principal Component Analysis (PCA). The Euclidean distance among observations is preserved. PCA can be considered non-parametric in that no assumption is made about normality; however, it does assume a linear response of variables (e.g. species) along gradients. If different types of variables compose the data set (as is usually the case for environmental data), they need to be standardized and an analysis needs to be performed on correlation matrix instead of on var/cov matrix. PCA is considered generally unsuited to analyze species composition data (Legendre & Legendre, 1998).

Correspondence Analysis (CA) was developed as an alternative method for dealing with counts data such as species composition matrices. It follows PCA closely, at the same time assuming a chi-square distance and a unimodal response model along gradients, which is more apt to represent community data (Legendre et Legendre, 1998). Anyway, CA efficacy has also been questioned (Faith *et al.*, 1987; Legendre & Gallagher, 2001) and other alternative ordinations proposed. Some authors suggest to use PCA with a proper preliminary transformation of community data, which causes the Euclidean distances to transform into meaningful ecological distances, such as chi-squared or Hellinger distances (Legendre & Gallagher, 2001).

Both PCA and CA are related to single distance metrics. Multidimensional Scaling (MDS) is a method which allows for any dissimilarity index, including ecologically meaningful measures such as the Bray-Curtis dissimilarity index (Bray & Curtis, 1957). The metric MDS, also known as the Principal Coordinate Analysis (PCoA or PCO; Gower, 1966) is a linear method based on the Euclidean distance, even though it can be used with any dissimilarity measures avoiding explicit assumptions regarding the distributions of original variables. If the analysis is directly performed on Euclidean distances, the results are identical to those obtained by the use of PCA.

Non-metric form of MDS (nMDS; Shepard 1962) in particular is considered a robust method and is widely applied. Only the rank of (dis-)similarities among samples is taken into account, and the representation in a dimensionally-reduced space is obtained by an iterative procedure minimizing the stress function, which can be considered as a measure of the "goodness" of the scaling. As it is based on ranks, nMDS is often used in association with other rank-based statistical methods, such as ANOSIM (see in this Chapter "Notes on General Linear Model methods and non-parametric alternatives").

Constrained methods use response (e.g. species composition) variables and explanatory, or predictor (e.g. environmental) variables, both quantitative and qualitative/dummy, in a single, integrated analysis. The analysis relies on *a priori* hypotheses regarding explanatory variables and is addressed to the part of the variance that can be explained by the given constraints. Resemblance matrix is subjected to linear regression on constraining (explanatory) variables, and the fitted values are submitted to the unconstrained ordination. Total variance is partitioned into the variance explained by constrained axes (i.e. by explanatory variables) and the variance explained by unconstrained axes. For any of the previous unconstrained metric methods, a constrained one has been proposed. Redundancy Analysis (RDA) is the constrained version of PCA and Canonical Correspondence Analysis (CCA; ter Braak, 1986) of CA.

Partially constrained methods such as pCCA and pRDA (ter Braak, 1988) can be used to remove the effect of covariables before analysis, in a fashion similar to that of GLM's ANCOVA/MANCOVA. In fact, constrained ordinations such as RDA and CCA are conceptually related to the linear model (Borcard *et al.*, 2004).

The main methods for carrying out constrained ordinations using non-Euclidean dissimilarities include distance-based RDA (dbRDA; Legendre & Anderson, 1999) and Constrained Analysis of Principal Coordinates (CAP; Anderson & Willis, 2003). Both methods perform a PCoA on a given dissimilarity matrix, and the eigenvalues obtained are analyzed with an RDA. CAP is designed to find an axis through the multivariate data cloud which has the strongest relationship with the explanatory variable even in the presence of potentially larger variation due to other measured or unmeasured factors. This differs from RDA and CCA which, instead, attempt to find some combination of environmental variables that best explains patterns in community data (as in BIOENV, Clarke & Ainsworth, 1993; see Chapter 3.6.5).

Multivariate analysis of main spatial and temporal patterns

Ordinations and ANOVA-like methods were applied to the two multivariate data sets. An nMDS ordination was performed to reproduce in two and three dimensions the rank Bray-Curtis dissimilarities between stations. The ordination method was applied to the four square-root transformed community matrices (two data sets and two currencies). Patterns of differences in the location and dispersion were analyzed with the following *a priori* groups:

- year (only the 59-station 3-year data sets);
- basin;
- hydrogeological zones.

Assemblages identified by cluster analysis were also visualized by the means of an nMDS plot.

In order to display indicative relationships with the gradient of species richness, a model of S was automatically fitted as a smooth surface to data clouds (Generalized Additive Model (GAM), a flexible statistical model which is itself a generalization of GLM, is used) and contour plot displayed on the nMDS plot.

Preliminary one-way PERMANOVA (fixed effects model) and ANOSIM (associated to the nMDS) were also performed (999 permutations) on the Bray-Curtis dissimilarities of the square-root transformed community matrices. PERMDISP was applied on the data set to evaluate the dispersion of the variance. The following factors were taken in account:

- year (only the 59-station 3-year data sets): three levels (balanced design).
- basin: four levels (Figure 3.1); the distribution of stations among levels is reported in Table 3.9; the design is not fully balanced.
- hydrogeological zones: five levels (Figure 3.3); the distribution of stations among levels is reported in Table 3.10; the design is not balanced; nevertheless, at least three (six) stations are found (Bayhead Estuary).

	CH	LI	MA	TR	tot
2002	15	16	13	15	59
3-years	48	43	51	38	180

Table 3.9: Number of stations per basin (for the 180-station 2002 data set and the 59-station 3-year data set).

	BE	CB	FZ	SL	TD	tot
2002	3	22	17	8	9	59
3-years	6	61	60	21	32	180

Table 3.10: Number of stations per hydrogeological zone for the 180-station 2002 data set and the 59-station 3-year data set.

One-way PERMANOVA (fixed effects model), ANOSIM and PERMDISP were performed on the untransformed biomass matrices of trophic groups (two data sets) with the same factors year, basin and hydrogeological zones.

One-way ANOVA (fixed effects model) and Levene's test for homogeneity of variance were performed on main univariate macrodescriptors of the community: total abundance, total biomass and richness, with the same factors year, basin and hydrogeological zones. Normality of variables was checked visually (by means of histograms and Q-Q plots) and through the Lilliefors test (which is a modification of the Kolmogorov-Smirnov test) and the Shapiro-Wilk test. Normal distribution was achieved by 4th root transformation of abundance and biomass and square-root transformation of richness (about transformation to improve normality, see Chapter 3.6.1).

CAP ordination was performed on the four square-root transformed community matrices to generate multivariate models of the relationship between benthic assemblages and the qualitative variables (factors) basin, hydrogeological zone and year. The choice of CAP instead of classical RDA is primarily due to flexibility in the use of factors. The analysis was based on Bray-Curtis dissimilarities, with a square-root transformation to avoid negative eigenvalues. A permutational ANOVA-like test was performed to assess the significance of the constraints (i.e. the factors) (Legendre &

Legendre, 1998). Biplot was produced highlighting the groups of station modelled by each CAP and projecting the scores of 5% species with the highest correlation for the plotted axes.

Moreover, a CAP was performed on 2002 abundances using as constraint the results of clusters analysis. The aim is to apply a Procrustean analysis to analyze the relationship between resulting ordination and the analogous ordination constrained on hydrogeological zones. Procrustean analysis rotates and scales linearly (symmetrically) a matrix (or configuration) to the maximum similarity with another matrix (or configuration), minimizing sum of squared differences. It can be applied to compare ordination results. In this case, it enabled us to plot the variation in ordination patterns of the data cloud constrained to the results of clusters analysis (i.e. assemblages) and the hydrogeological zones. The resulting sum of squared differences was considered as a relative measure of match between ordination results. The "significance" of the Procrustes statistic is estimated by a correlation-like statistic derived from the symmetric Procrustes sum of squares.

3.6 RELATIONSHIPS BETWEEN MACROZOOBENTHOS COMMUNITY AND ENVIRONMENTAL FACTORS AT LAGOON SCALE

Spatial and temporal (interannual) patterns of the macrozoobenthos community were related to environmental conditions at the lagoon scale. The presence of spatial structures at different spatial scales was analyzed and linked to environmental conditions.

The following data sets for both macrozoobenthos data and environmental variables were considered: the 2002 data set, consisting of 180 sampling stations; the 3-year (2002, 2003, 2007) data set, consisting of 59 coincident sampling stations.

3.6.1 Normality and data transformation

Classic parametric methods, such as the GLM, assume normal distribution for variables (see Chapter 3.5.5). Environmental data, as a rule, don't show a normal distribution. A number of authors since Ahrens (1953) suggest that geochemical and environmental variables in fact show a lognormal distribution, although some of them point out that neither normal nor lognormal distributions are actually approached (Reimann & Filzmoser, 2000 discuss the problem). Source of non-normality lays both in errors during sampling and analysis and in the very nature of the variables themselves. Spatially dependent data, as for example ecoclines, are not expected to follow normal distribution (Reimann & Filzmoser, 2000) as multiple populations and processes are usually involved.

Normality was checked for each variable of the 180-station 2002 data set and of the 59-station 3-year data set with a set of graphical methods (histogram, Q-Q plot) and inferential tests (Lilliefors normality test, which is a modification of Kolmogorov-Smirnov test, and Shapiro-Wilk normality test). In particular, Kolmogorov-Smirnov test is more robust than Shapiro-Wilk against spatial (as well as temporal) autocorrelation (Durilleul & Legendre, 1992). If the variables were found not to be normally distributed, data transformation were checked to improve normality. Common transformation includes, among others, the inverse, square root, logarithm and power transformation. A same transformation was applied to each single variable in both the data sets. Also if a transformation is found to improve normality, variables could not follow a normal distribution (Reimann & Filzmoser, 2000). Transformed variables were employed only in analysis assuming normal distribution.

3.6.2 Standardization

Since environmental variables are expressed by different units of measure, both transformed and untransformed data sets were standardized by subtracting the mean and dividing by the variance. After standardization, "dummy" variables (presence of macroalgae and phanerogams) were multiplied for $1/\sqrt{2} = 0.7071$, a factor which compensates for their 0/1 coding (M. Greeancre, pers. comm.). Granulometry is expressed through the relative composition data, i.e. a closed system of three variables summing up to 100%. When necessary, one of the three variables (clay) will be eliminated from the following analyses.

3.6.3 Explorative analysis of environmental factors

An explorative analysis was performed on environmental variables data sets for macrozoobenthos sampling stations. Both Pearson and Spearman correlation was calculated among the whole set of variables. CAP analyses based on euclidean distances were applied on (standardized) environmental variables using year, hydrogeological zones and basins as constraining factors, to highlight main interannual and spatial patterns of environmental conditions. The choice of CAP instead of classic RDA is primarily due to flexibility in the use of factors.

3.6.4 Relationship of univariate macrodescriptors of community to environmental data

The three main macrodescriptors, i.e. species richness, total abundance and total biomass (AFDW), were related to environmental variables. At first the Pearson and Spearman correlation were calculated with untransformed environmental variables for both the 2002 and the 3-year data sets.

Linear models were produced by regressing the macrodescriptors to the more correlated environmental variables, one at a time, for the 180-station 2002 data set. Multiple linear models were then calculated on all environmental variables, with a stepwise forward selection procedure, which selects a subset of variables by starting with no variables, trying iteratively one by one each variables and adding to the model the ones which minimise the AIC (Akaike Information Criterion; Akaike, 1974). As linear regression requires normality of variables, the models were built over transformed variables.

3.6.5 Relationship of multivariate structure of community to environmental data

Mantel test and BIOENV

The Mantel test (Mantel, 1967; Legendre & Legendre, 1998) compares two triangular resemblance matrices and calculates their Pearson or Spearman correlation. The significance of the statistic is evaluated by permuting rows and columns of the first matrix. It assumes the independence of matrices. This test can be applied to compare a matrix of ecological dissimilarities computed from community data with a matrix of distancies computed from environmental data among the same stations. Mantel correlations are known to be much smaller in absolute value compared to raw data approaches (correlation, regression or ordination) on the same data set (Dutilleul *et al.*, 2000, Legendre, 2000), while the Mantel significance test is known to be less powerful (Legendre *et al.*, 2005).

BIOENV (Clarke & Ainsworth, 1993; Clarke & Warwick, 1994) basically calculates the Mantel test correlation with a stepwise routine. The approach of BIOENV, as for RDA and CCA (see Chapter 3.5.5), consists in finding the combination of environmental variables that best explains patterns in community data. It selects all possible subsets of environmental variables, calculates Euclidean distances and finds the best Spearman correlation with community dissimilarities (usually as Bray-Curtis dissimilarities). BIOENV is related to other rank methods such as nMDS. BIOENV has been applied on the 180-station 2002 data sets in order to (1) identify the combination of environmental variables that best explains patterns in community data at the lagoon scale; (2) analyze how the combination of correlated factors varies by integrating different time spans, i.e. what part of the environmental signal is retained in the community structure with respect to the considered time span.

Matrices of Bray-Curtis dissimilarities calculated on (square-root transformed) abundances and biomass were compared to Euclidean distances calculated on the (standardized) environmental variables data sets. Among environmental variables, water column variables consist of synthetic statistics (average and dispersion) over a given time span of monthly series. As regards 2002, mean and range values for original variables were calculated on different time spans, starting from a four-month period (Feb-May 2002) preceding the macrozoobenthos field samplings, then going back up to a 12-month period (Jun 2001-May 2002), and an additional four-month period (Oct 2001-Jan 2002). BIOENV was also performed on the more robust 12-month 2002 data set based on the median and the 90% interpercentile range. A summary of data sets that were used based on different time span series is presented in Chapter 3.2.5, Table 3.5.

Constrained ordinations

Multivariate relationships between the macrozoobenthos community and the environmental factors at the lagoon scale were also investigated with a number of constrained ordinations: RDA after Hellinger transformation, CCA and CAP (see Chapter 3.5.5). As the response matrices, the species composition matrices in terms of (square-root transformed) biomass and abundances were used. In particular, the analyses performed are presented in Table 3.11, along with their main objectives.

Ordination	Response matrix	Explanatory matrix	Objectives
CCA	2002, 180-stat., A 2002, 180-stat., B	14 variables, 1 year	Analysis of patterns, explained variation by environmental variables, comparison among ordination
RDA (Hellinger transformation)	2002, 180-stat., A 2002, 180-stat., B	14 variables, 1 year	Analysis of patterns, true explained variance by environmental variables, comparison among ordination; variation partitioning
	3-year, 59-stat., A 3-year, 59-stat., B	13 variables, 4 months	Analysis of patterns, true explained variance by environmental variables; variation partitioning
CAP	2002, 180-stat., A 2002, 180-stat., B	14 variables, 1 year	Analysis of patterns, explained variation by environmental variables, comparison among ordination

Table 3.11: Constrained ordination analysis performed on community composition matrices with environmental data as constraining factors, and objectives.

All three ordination methods were applied to the 180-station 2002 data sets (abundances and biomass). A CAP was also applied to the 59-station 3-year data set. Statistical significance of the overall results and of the relative contribution of each variable is evaluated using a Monte Carlo permutation test. However, in case of dummy variables (presence of macroalgae and phanerogams coverage) any statistical test is inappropriate (http://ordination.okstate.edu/envvar.htm, 30/11/2010).

Ordination results obtained through all three methods were compared by means of a Procrustean analysis (Chapter 3.5.5). The resulting sum of squared differences was considered as a relative measure of match among the ordination results. Also, the effects of different transformations applied to community data (logarithm, square-root, 4th-root) on CCA were compared (see also Chapter 3.6.1) and the statistical significance calculated.

Variation partitioning

The variation partitioning method proposed by Borcard *et al.* (1992) enables separating the effects of two (or more) sets of explanatory variables. In the case of two sets, the total amount of variation in the response matrix (e.g. the community matrix) is partitioned into three fractions, two of which are explained by a set of independent variables each, while the third fraction is related simultaneously to both sets. Variation partitioning involves two partial constrained ordinations of the response matrix, each one constrained by a set of explanatory variables while controlling for the effect of the other as covariable. The fractions of the variation explained are obtained by summing canonical eigenvalues for each ordination. This method was proposed to separate "environmental" and "spatial" variations, by introducing "spatial" variables such as the coordinates in the analysis (see Chapter 3.7), but it can also be extended to any other explanatory variable. The method can be extended to several sets, but it is rarely applied to more than three sets (in which case variation is partitioned among seven fractions; see for example Anderson & Gribble, 1998, which take into account time variation as well).

Total variation in ordination methods is expressed by inertia. Depending on the ordination method, it represents different mathematical quantities. In RDA based on covariance matrix, inertia is the true variance, analogous to GLM's R^2. The variance of the community composition matrix is the measure of beta diversity (Legendre *et al.*, 2005). Variation partitioning is based on adjusted R^2. When a single response variable has to be partitioned, partitioning is done by partial regression.

Partition of the variance was performed via RDA on the Hellinger-transformed abundances data for 180-station 2002 data set. Variance was partitioned into three parts: hydrological variables, hydrodynamical variables (i.e. residence time) and sediment/geomorphology variables.

3.7 SPATIAL STRUCTURES AND MULTISCALE ANALYSIS

Spatial structure, or "spatial correlation" (i.e. a non-random organization across the space; Peres-Neto & Legendre, 2010) can be either indirectly induced by external forcing ("induced spatial correlation" or "spatial dependance") or it can be related to internal processes ("non-induced spatial correlation", or "spatial autocorrelation") (see Chapter 1.5). Spatial correlation in itself is the measure of the spatial "scale" of the phenomenon. In case of spatial correlation (i.e. dependence), any ecological phenomenon at a given location may have an influence on contiguous locations (Legendre & Fortin, 1989), and the assumption of independence of observations isn’t met any more. Spatial correlation can invalidate classic statistical tests, such as tests of correlation and regression analyses (e.g. biotic variables on environmental variables) (Legendre, 1993). When variables are spatially structured, a correlation could be observed even if they are uncorrelated, due to the redundancy of information on their relationship when the observations are close in space. In fact, the problem arises not directly from the correlation per se, but from a biased sampling scheme, in which stations are clustered, or their average distance between observations is smaller than the spatial "scale" of the underlying ecological phenomenon (Legendre & Fortin, 1989). Spatial structure of species and communities and its relationships with environmental variables were analyzed. Three approaches were followed to take into account spatial structure at multiple spatial scales: (1) ANOVA-like method using a nested design, (2) MSO for multivariate species composition matrices and semi-variograms for univariate macrodescriptors, (3) classical multivariate analysis (GLM methods, constrained ordination, variation partitioning) in which spatial predictors are introduced among explanatory variables.

PERMANOVA on nested design

A hierarchical design of the sampling stations enables the application of analysis of variance or ANOVA-like methods to estimate the components of variance associated with different spatial "scales" (Bellehumeur & Legendre, 1998; e.g. Pérez-Ruzafa *et al.*, 2007), although a limited number of these scales can be resolved (Gardner, 1997). A nested PERMANOVA analysis (see Chapter 3.5.5) was performed on macrozoobenthos community data with PRIMER6 + PERMANOVA software (Anderson, 2005; Clarke & Gorley, 2006). PERMANOVA was not performed on environmental variables, as the available data sets were considered unsuitable. Mixed models were implemented, in which both fixed and random factors are included (Underwood, 1997). Different scales of spatial variations were introduced in the linear model as successively nested fixed factors. Temporal scale of variation is restricted to the crossed factor year only for the 3-year data set.

Both the 180-station 2002 data set and the 59-station 3-year data set were analyzed. The multivariate matrices and univariate variables were subjected to different experimental designs. The original sampling design of MELa monitoring was not conceived with a nested design. Sampling stations were nearly homogenously distributed in an irregular grid all over the Lagoon. Hence, an "*a posteriori*" nested spatial design was created. The approach relies on a classification multivariate procedure in order to gain objectivity. It is based on *k*-means clustering (see also Chapter 3.5.5) applied on the geographical distance matrix between stations, which was implemented on a hierarchical algorithm. It iteratively searches for the best pattern of a given number (k_1) of subsets (clusters) of stations, maximizing their proximity, then on a given number (k_2) of subsets for each of the k_1 subset, and so on until a chosen level of nested factors (usually the lowest possible) is reached. Geographical proximity within clusters is maximized and proximity to other stations minimized, so they don't manifest any predefined directionality, and are not related to the sea-land direction (i.e. to the transitional gradient).

Different results are possible in *k*-means, so the algorithm chooses the solution which maximizes the balancing (by minimizing the Pielou evenness). However, basins were chosen as the first hierarchical level coincident. The script was implemented in R and is reported in Appendix 1.

The algorithm was applied independently, for each of the four basins, to the 59-station data set (n = 3) and the 180-station data set (n = 4), each recursion with k = 2, resulting in a symmetric, but to some extent unbalanced design, with the number of sampling stations at the lowest nested factor varying between 2 and 10. In Figures 3.8 and 3.9, two hierarchical *a posteriori* designs for macrozoobenthos sampling stations are shown, together with a boxplot of the scale range of each spatially nested factor (expressed as the internal SD). The design proposed for the 59-station data set includes two nested factors at a scale lower than the basin. For the 180-station data set, another nested factor was included. Factors are termed "sectors of rank *n*".

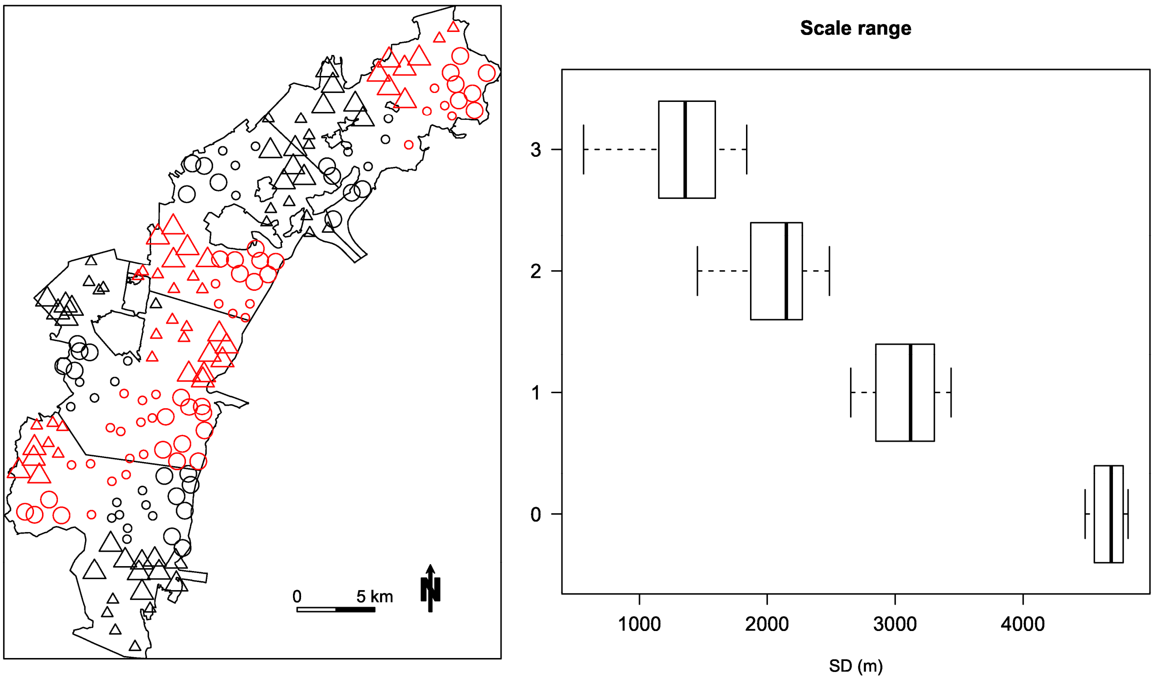


Figure 3.8: Hierarchical *a posteriori* ANOVA design by recursive *k*-means algorithm on the 180-station 2002 data set (on the left). Factors: 4 basins (rank 0); sector rank 1 (nested in basins): colours; sector rank 2 (nested in sector rank 1): shape; sector rank 3 (nested in sector rank 2): shape size. Scale range for each factor (on the right) is expressed as the internal SD (in m).

factors	nested in	model	n. of levels	mean n. of stations	mean SD (km)	mean area (km^2)	mean density (km^{-2})
basin		fixed	4	45	4.75	102	0.44
sector r. 1	basin	random	8	22.5	3.15	51	0.44
sector r. 2	sector r. 1	random	16	10.7	2.15	26	0.41
sector r. 3	sector r. 2	random	32	5.4	1.35	13	0.42

Table 3.12: Factors for the 180-station 2002 data set (mean density of stations: 0.43 km^{-2}). Factors include basin and sectors of rank n.

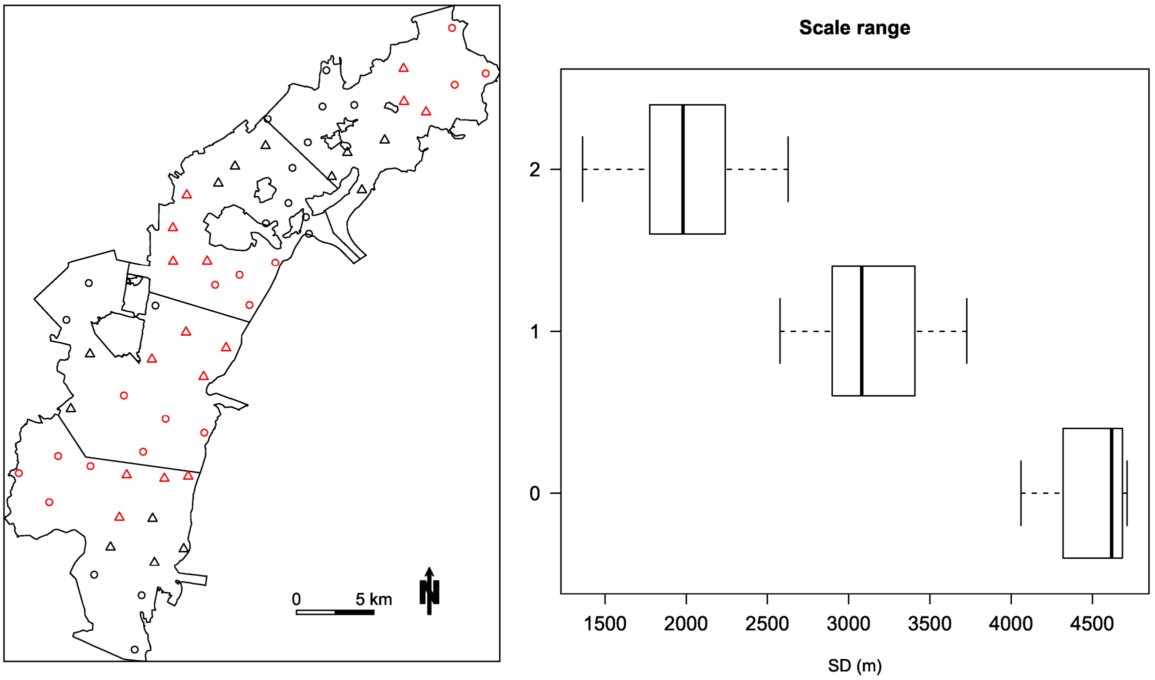


Figure 3.9: Hierarchical *a posteriori* ANOVA design by recursive *k*-means algorithm on the 59-station 3-year data set (on the left). Factors: 4 basins (rank 0); sector rank 1 (nested in basins): colours; sector rank 2 (nested in sector rank 1): shape. Scale range for each factor (on the right) is expressed as the internal SD (in m).

factors	nested in	model	n. of levels	mean n. of stations	mean SD (km)	mean area (km^2)	mean density (km^{-2})
year	(crossed)	random	3				
basin		fixed	4	14.8	4.75	102	0.15
sector r. 1	basin	random	8	7.4	3.15	51	0.15
sector r. 2	sector r. 1	random	16	3.8	2	26	0.15

Table 3.13: Factors for the 59-station 3-year data set (mean density of stations: 0.15 km^{-2}). Factors include basin and sectors of rank n.

The considered linear model for the 180-station 2002 data sets, under the null hypothesis H_0 that each variable tested is homogeneous across all the spatial factors, is:

$$u + Y * [B + Sector1(B) + Sector2(Sector1(B)) + Sector3(Sector2(Sector1(B)))] + e$$

The considered linear model for the 59-station 3-year data sets, under the null hypothesis H_0 that each variable tested is homogeneous across all the spatial and temporal factors, is:

$$u + Y * [B + Sector1(B) + Sector2(Sector1(B))] + e$$

In Tables 3.12 and 3.13, random and fixed factors are presented for each design, along with the number of the factor levels and the spatial scale considered. Basin was considered as fixed factor.

For all the four species composition matrices, analysis was performed on the Bray-Curtis dissimilarity of square-root transformed matrices (9999 permutations) with all the six designs. The two square-root transformed matrices of trophic groups (expressed as biomass) were analyzed on the Bray-Curtis dissimilarities (9999 permutations). The untransformed univariate macrodescriptors total abundance, biomass and richness were analyzed on Euclidean distances (9999 permutations).

Variograms and Multi-Scale Ordination

Omnidirectional empirical semi-variograms (Cressie, 1993) were calculated for the main univariate macrodescriptors (species richness, abundance, biomass). It describes the spatial autocorrelation of the data. A spatial lag of 1 km was selected.

Multi-Scale Ordination (Wagner, 2004) is a variogram-like method which can be applied to community composition matrices. It enables the spatial partitioning of the results of unconstrained or constrained ordination, in particular the inertia. As for variograms, it enables the identification of the spatial autocorrelation of the data. A Mantel test of spatial independence is calculated to evaluate significant autocorrelation (999 permutations). In the case of constrained ordination, such as CCA or RDA, residual inertia and explained plus residual inertia are plotted in the same plot. A confidence interval as a pointwise envelope for the variogram of the total inertia is also plotted on the graph allowing a comparison with the explained plus residual inertia.

MSO was applied to the results of the RDA (after Hellinger transformation) on species composition matrix (180-station 2002 data set, abundances) with one-year (standardized) environmental variables as constraints. In RDA analysis, inertia is the variance, hence the sill is the total variance. A distance class of 2 km has been considered adequate. A Bonferroni-type correction, which divides α by the number of lags ($\alpha = 0.05/13$), was applied to the calculation of the confidence interval. The MSO was performed also on the equivalent PCA after Hellinger transformation to calculate the range of significant autocorrelation of the total variance.

The same analysis was applied to matrices of abundances for main taxonomical groups: Polychaeta Sedentaria and Errantia, Amphipoda, Bivalvia and Gastropoda. Other taxonomical groups were not be analyzed because the number of species was lower than the number of explanatory variables. *Animalia caetera* also was not taken into account. The significant environmental variables for each group, identified by a preliminary RDA analyses (after Hellinger transformation) on taxonomic groups matrices with forward selection, were used as constraining variables. Species composition matrices for taxonomic groups are based on the 2002 180-station data set. Explained variation by each environmental variable was calculated.

Notes on spatial predictors

A number of modelling approaches have been proposed to account for spatial autocorrelation. The presence of spatial structures (or trends) can be assessed by introducing spatial predictors describing the arrangement of sampling stations as a function of geographical coordinates, in order to estimate a model for the observed pattern.

The simplest spatial model is the two-dimensional linear model $Z = a_1 + a_2X + a_3Y$, where (X, Y) are the geographical coordinates, Z the modelled response variable and a_1, a_2, a_3 the parameters to be estimated by regression. The introduction of the geographical coordinates as independent explanatory variables makes it possible (1) to search for a linear trend (space as predictor, or the "spatial legacy" approach), which could be explained by the regression of the model on environmental variables, and possibly (2) to remove it (space as covariable, the "spatial nuisance" approach; Peres-Neto & Legendre, 2010). The same approaches can be extended to a polynomial expression of spatial coordinates to describe more complex spatial trends, by introducing higher-degree polynomial terms as variables (usually no more than 3rd-degree), e.g. X^2, Y^2, XY, X^2Y etc. (Trend Surface Analysis). A stepwise procedure to select significant terms can be associated. Trend analysis focuses on a single scale, and the polynomial predictor presents a number of limitations including the non-independence of spatial variables (Jombart *et al.*, 2009).

A third approach based on geographical variables known as "spatial-filtering methodology" or "eigenfunction spatial analysis" (Griffith & Peres-Neto, 2006) or "spatial eigenvector mapping" (SEVM, Dormann *et al.*, 2007) was recently proposed to account for multiple scales. It includes two main methods which were initially developed independently: Distance-based Eigenvector Maps (DBEM), also known as Principal Coordinates of Neighbour Matrices (PCNM, Borcard & Legendre, 2002; Borcard *et al.*, 2004; Dray *et al.*, 2006) and Topology-based Eigenvector Maps (TBEM), also known as Moran's Eigenvectors Map (MEMs) (Griffith, 2000). They both consist in the introduction of a new set of independent variables accounting for space, which are the eigenvectors resulting from a PCoA (see Chapter 3.5.5) on a centered "connectivity" matrix between stations (only the closer stations are connected). DBEM calculates PCoA on a modified matrix of euclidean distances between stations, which is truncated at a threshold distance *t* that allows for all the stations to be connected; the value 0 is assigned to greater distances and to the diagonal, the value $(1-(\text{dist}/4t)^2)$ is substituted to other distances. The 4*t* stands for an arbitrary value larger than the threshold (Dray *et al.*, 2006). The number of eigenvectors and eigenvalues is equal to the number of stations, but, as the "connectivity" matrix is not euclidean, negative eigenvalues (in a number lower than that of the positive eigenvalues) are produced. Only eigenvectors associated to positive eigenvalues, which represent positive autocorrelation, should be retained. A diagram from Borcard *et al.* (2004, mod.) is reported in Figure 3.10 to describe the procedure. "Space" is then decomposed into different but complementary "scales", in a similar fashion to Fourier analysis. The more regular the sampling transect or grid is, the more regular and symmetrical the spatial patterns of eigenvectors model are. The eigenvectors are usually arranged from "wide-scale" to "small-scale", even though such arrangement is less straightforward in case of irregular grids. To understand the problems related to irregular grids, in Figures 3.11 to 3.12, four arbitrarily selected PCNMs resulting in two DBEMs are presented. These are calculated on a regular grid (Figure 3.11) and on a regularly-spaced grid covering an irregular extent (Figure 3.12). Randomly selected eigenvectors are mapped. Also, an omnidirectional variogram is presented for each PCNM, helping in the interpretation of spatial patterns. The MELa grid adds another degree of complexity, as it is irregularly spaced.

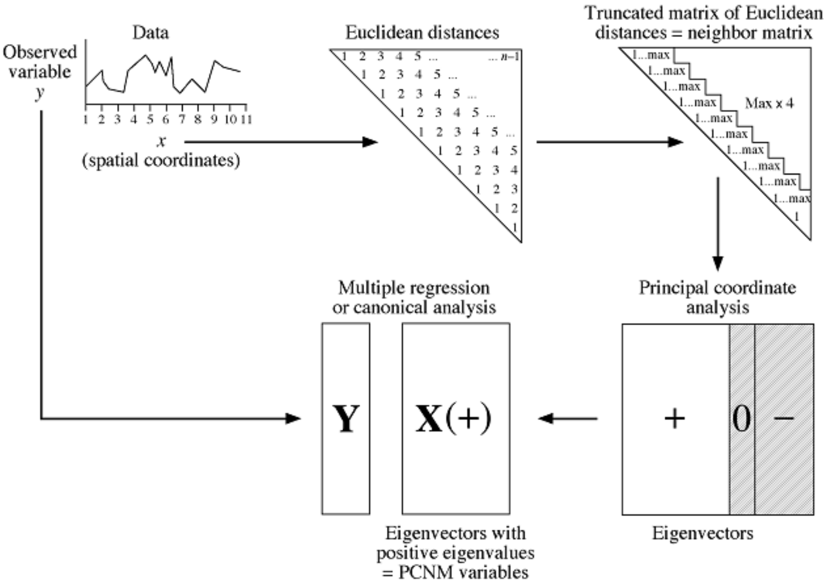


Figure 3.10: Schematic description of DBEM analysis. (Borcard *et al.*, 2004)

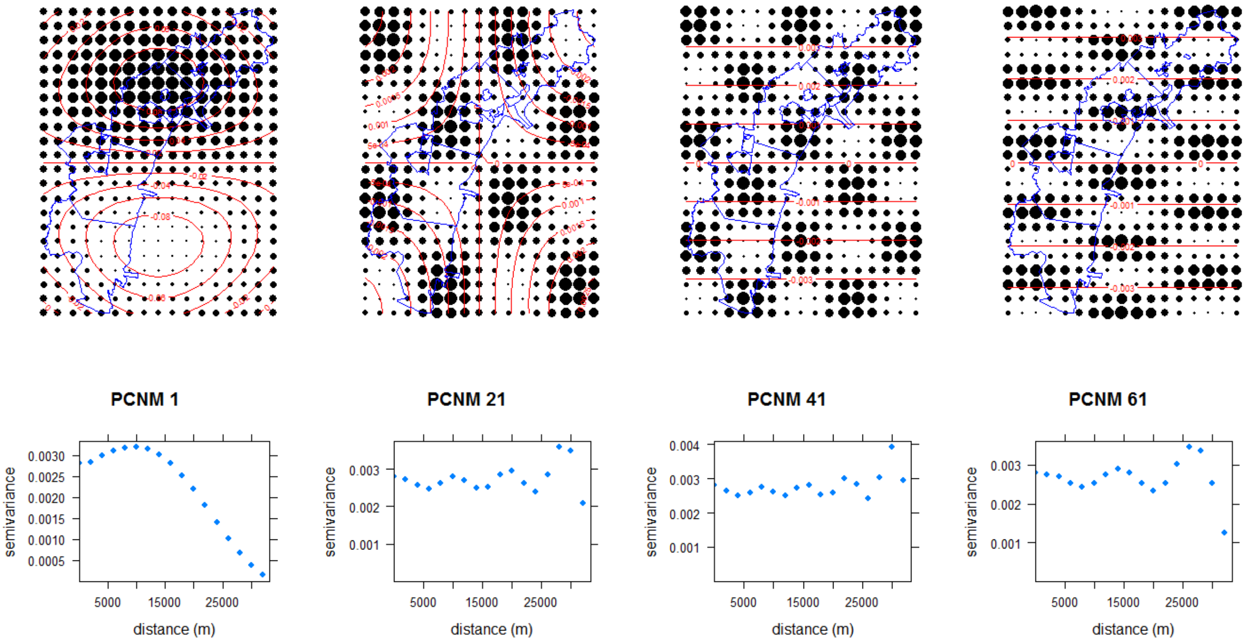


Figure 3.11: Four arbitrarily selected PCNMs (n. 1, 21, 41, 61) of the total of 263 related to positive eigenvalues based on a regular and a rectangular grid with a distance of 2 km between vertices (top) and the respective omnidirectional semi-variograms (2 km lag) displaying variations among pairs of vertices at increasing distances (bottom).

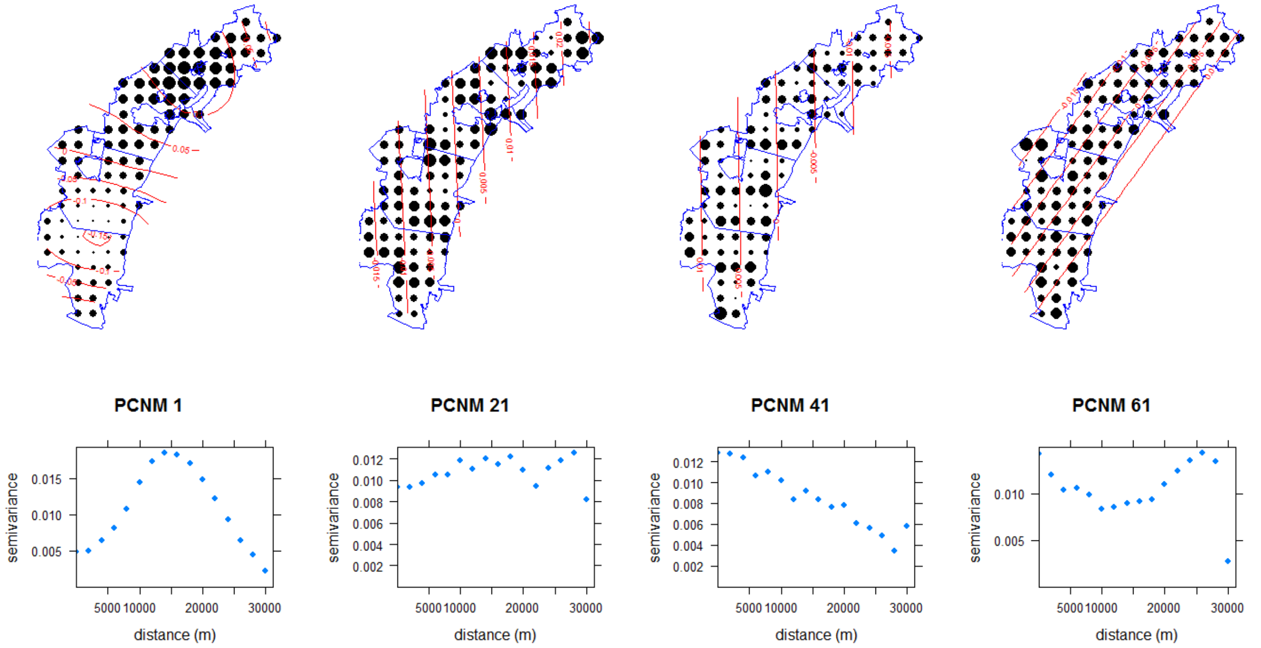


Figure 3.12: Four arbitrarily selected PCNMs (n. 1, 21, 41, 61) of the total of 69 related to positive eigenvalues based on a regular grid covering the Lagoon surface with a distance of 2 km between vertices (top) and the respective omnidirectional semi-variograms (2 km lag) displaying variations among pairs of vertices at increasing distances (bottom).

First of all, a selection of the significant eigenvectors for the model of the given response variable(s) should be applied. This is usually performed by a stepwise forward selection applied to a regression or a constrained ordination. The forward selection method for constrained ordinations is known to overestimate the number of significant eigenvectors (Dray *et al.*, 2006) and other approaches are currently under development (Jombart *et al.*, 2009). The resulting model decomposes the spatial variability of the variable into different significant eigenvectors. The PCoA of a centered matrix produces orthogonal and linearly independent eigenvectors. Therefore, subsets of eigenvectors can be added up to produce sub-models, for example, associated to a given range of spatial scales. The full model, any sub-models or single significant eigenvectors can then be considered as spatial predictors and used as explanatory variables in linear regression, constrained ordinations and variation partitioning (Borcard *et al.*, 2004). As for trend analysis, eigenvectors can be used as explanatory variables in the same way as other environmental predictors, or as covariables to remove the spatial structure from the data and analyze residuals. These two different approaches may produce different results, depending on the collinearity between predictors and space (Borcard & Legendre, 2002).

Multivariate analysis of spatial structure

The presence of spatial structures (or trends) was assessed by introducing spatial predictors describing the spatial arrangement of sampling stations as a function of geographical coordinates, to estimate a model for the observed pattern. This part of the work focuses on the more spatially dense 180-station 2002 data set. The simplest spatial model is a two-dimensional linear model, i.e. a plane which models a linear gradient of a variable in the space. Such model was tested by introducing the geographical coordinates as independent explanatory variables on (1) an MLR on univariate macrodescriptors (A, B, S) and (2) an RDA on Hellinger-transformed (square-root transformed) matrices of abundances.

In the framework of "eigenfunction spatial analysis", PCNMs (Principal Coordinates of Neighbour Matrices, DBEM) were calculated for the coordinates of 180 stations. The matrix of euclidean distances was truncated at t = 2082 m, which allows for all the stations to be connected. The matrix of eigenvectors for positive eigenvalues was introduced as a new spatial predictor.

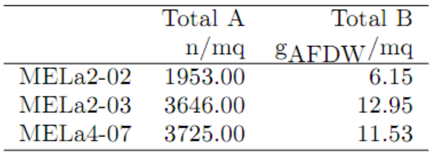
The entire model was tested by introducing all the eigenvectors as independent explanatory variables on (1) an MLR on univariate macrodescriptors (A, B, detrended S) and (2) an RDA on Hellinger-transformed (square-root transformed) matrices of abundances (taking into account the detrending by partialling out the coordinates as covariates) (Borcard *et al.*, 2004). Given that the whole model is significant, stepwise forward selection was applied on the set of PCNMs to select the significant terms. Retained eigenvectors for each of the modelled variables were plotted on a map to explore their spatial pattern. As the PCNMs are orthogonal, four sub-models were introduced as a linear combination of PCNMs chosen on the basis of the spatial pattern ("scale") displayed. Sub-models were plotted on a map and coupled with omnidirectional semi-variograms (with lag of 1 km) to explore the patterns. Spatial autocorrelation is indicated by the increase of semi-variance until the sill is reached. In this context, the range of the variogram indicates the main pattern of variability ("scale") of the sub-model. R^2, adjusted-R^2 and significance of each sub-model were tested. Statistical significance of constrained axes produced by RDA was also tested.

The MLR with environmental variables as explanatory variables (with forward selection) were applied on (1) the linear trend and each sub-model for the univariate descriptors, and on (2) every single axis of the model in its entirety, of the linear trend model and of the four sub-models for the community composition matrix data set (Borcard *et al.*, 2004).

4 RESULTS

4.1 TAXONOMIC LIST

A total of 198452 individuals were sorted and identified during MELa2 and MELa4 studies, 87890 of those in 2002, 54688 in 2003 and 55874 in 2007. Total abundance and biomass (AFDW) were reported to square meter and presented in Table 4.1. From 2002 to 2003 numerical abundances and biomasses show a strong increase, with densities in 2003 and 2007 almost two times 2002 densities.



	Total A n/mq	Total B g_{AFDW}/mq
MELa2-02	1953.00	6.15
MELa2-03	3646.00	12.95
MELa4-07	3725.00	11.53

Table 4.1: Total abundances and biomass (AFDW) densities.

Out of the total number of specimens analyzed during the three studies, only about 49% were identified at the species level, 35% at genus, 12% at family, and the remaining at higher taxonomic levels. The overall list of taxa, which is the basis for the analysis performed in the present work, is presented in Appendix 2. A total of 315 taxa were tabulated, 235 taxa pertaining to MELa2 2002, 194 to MELa2 2003 and 226 to MELa4 2007. Among these species, 12 were defined allochtonous for the Venice Lagoon by Mizzan (1999) and Mizzan *et al.* (2005) and by the Italian Checklist (Relini, 2006; 2009). If a same total sampled area is considered for the three studies, by taking in account only the 60 coincident stations for the three years (15 m^2), the total list includes 298 identified taxa (188 taxa in 2002). The average number of species per station increases from 27.2 in 2002 to 33.2 in 2003 (+ 22%) and to 38.7 in 2007 (+ 43% compared to 2002). Species accumulation curves (SAC) presented in Figure 4.1 show the relationships between species richness and the sampled area. MELa4 2007 in particular has a much higher slope than previous studies. In Figure 4.2 the number of taxa is decomposed into the fractions pertaining exclusively to each year and the fractions which were jointly found during different years. A higher number of species were found during 2007 fieldwork respect to the same area sampled in previous samplings. A total of 130 taxa was jointly found during the three studies. The number of exclusive taxa grows from 2002 (30 on 188 taxa, i.e. 16%; 47 on 235, i.e. 20%) to 2007. In the last year 53 exclusive taxa (23%) were found, with the number of taxa jointly found slightly decreasing from 19 to 9. The overall temporal evolution is characterized by a general increase of species richness and number of species for each station, accompanied by a substitution of species. In Table 4.2 the taxonomic rank of identified taxa is summarized by year of study. The 72% of identified taxa are at the species level.

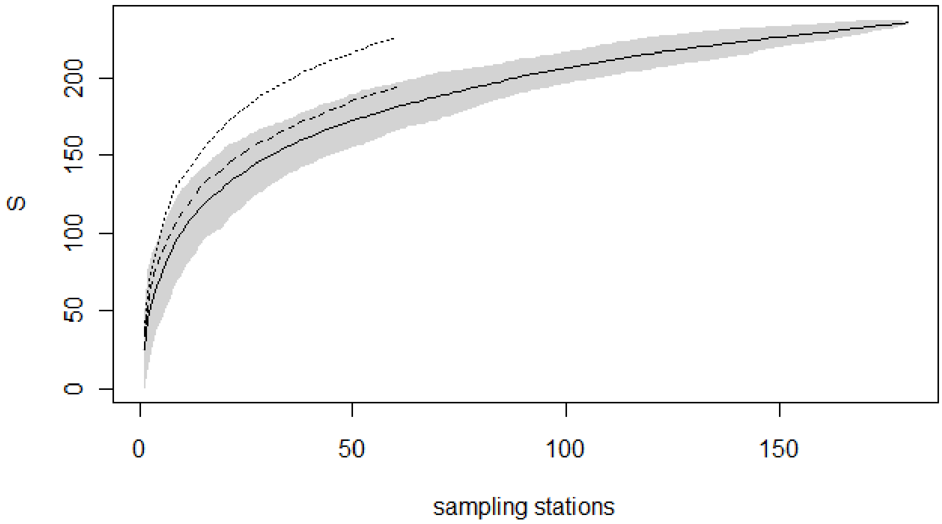


Figure 4.1: Taxa accumulation curves for each year, based on random permutations (solid line: MELa2 2002; dashed line: MELa2 2003; dotted line: MELa4 2007; grey area: MELa2 2002 confidence interval as 2 standard deviation).

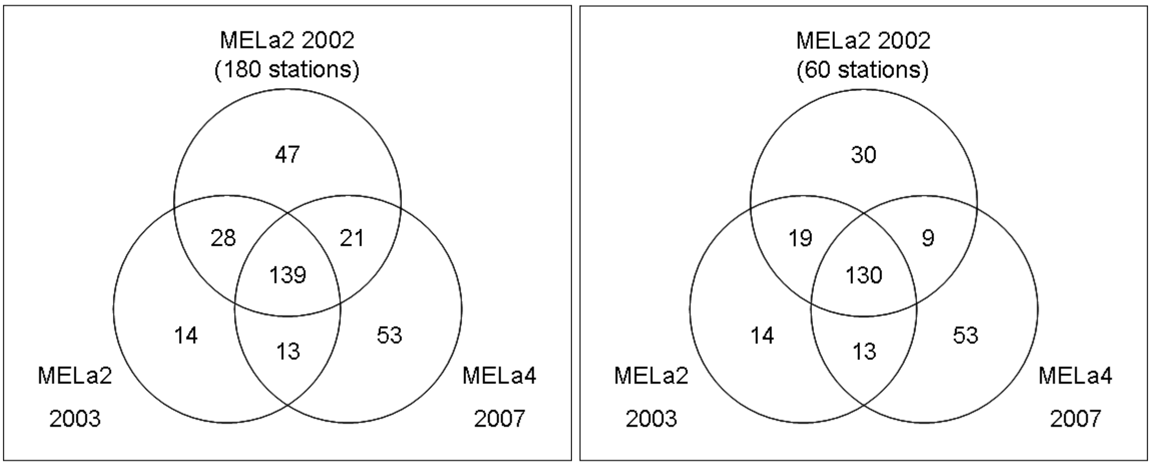


Figure 4.2: Number of taxa for each year and intersections (left: all sampling stations; right: 60 stations).

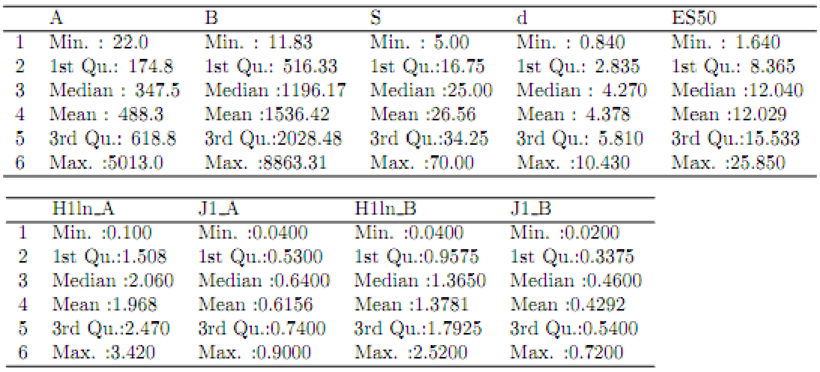
	Total	MELa2-02	MELa2-03	MELa4_07
up to Phylum	5	5	4	3
up to Classis	6	5	5	5
up to Ordo	11	10	8	8
Familia	24	21	20	21
Genus	43	34	26	30
Species	226	160	131	159

Table 4.2: Rank of identified taxa for each year ("up to Ordo" for example indicates all ranks above Familia up to Ordo: Superfamilia, Infraordo and Subordo).

4.2 SPATIAL AND INTERANNUAL PATTERNS OF MACROZOOBENTHIC COMMUNITY AT THE LAGOON SCALE

4.2.1 Univariate descriptors

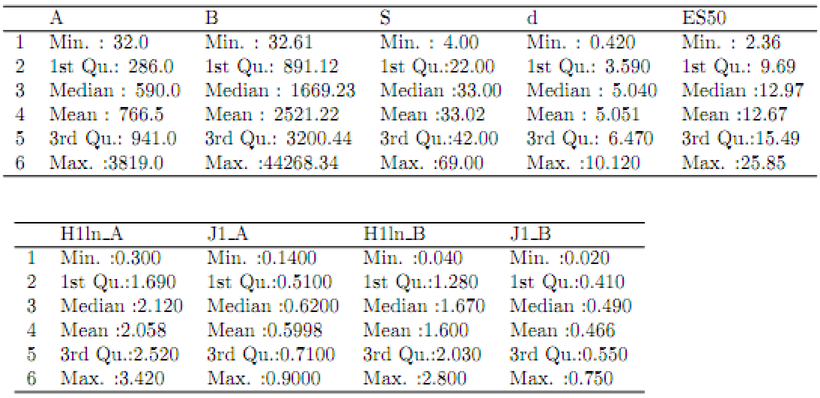
For each station, classical univariate macrodescriptors were calculated for both the 180-station 2002 data set and the 59-station 3-year data set. A summary of the data for the two data sets follows, with main statistics (Tables 4.3 to 4.4). All the macrodescriptors are related to the surface of sampling station (0.25 m^2). Densities for m^2 can be easily obtained for abundances and biomass by taking four times the values.



	A	B	S	d	ES50
1	Min. : 22.0	Min. : 11.83	Min. : 5.00	Min. : 0.840	Min. : 1.640
2	1st Qu.: 174.8	1st Qu.: 516.33	1st Qu.:16.75	1st Qu.: 2.835	1st Qu.: 8.365
3	Median : 347.5	Median :1196.17	Median :25.00	Median : 4.270	Median :12.040
4	Mean : 488.3	Mean :1536.42	Mean :26.56	Mean : 4.378	Mean :12.029
5	3rd Qu.: 618.8	3rd Qu.:2028.48	3rd Qu.:34.25	3rd Qu.: 5.810	3rd Qu.:15.533
6	Max. :5013.0	Max. :8863.31	Max. :70.00	Max. :10.430	Max. :25.850

	H1ln_A	J1_A	H1ln_B	J1_B
1	Min. :0.100	Min. :0.0400	Min. :0.0400	Min. :0.0200
2	1st Qu.:1.508	1st Qu.:0.5300	1st Qu.:0.9575	1st Qu.:0.3375
3	Median :2.060	Median :0.6400	Median :1.3650	Median :0.4600
4	Mean :1.968	Mean :0.6156	Mean :1.3781	Mean :0.4292
5	3rd Qu.:2.470	3rd Qu.:0.7400	3rd Qu.:1.7925	3rd Qu.:0.5400
6	Max. :3.420	Max. :0.9000	Max. :2.5200	Max. :0.7200

Table 4.3: Summary statistics for selected macrodescriptors (2002, 180 sampling stations).



	A	B	S	d	ES50
1	Min. : 32.0	Min. : 32.61	Min. : 4.00	Min. : 0.420	Min. : 2.36
2	1st Qu.: 286.0	1st Qu.: 891.12	1st Qu.:22.00	1st Qu.: 3.590	1st Qu.: 9.69
3	Median : 590.0	Median : 1669.23	Median :33.00	Median : 5.040	Median :12.97
4	Mean : 766.5	Mean : 2521.22	Mean :33.02	Mean : 5.051	Mean :12.67
5	3rd Qu.: 941.0	3rd Qu.: 3200.44	3rd Qu.:42.00	3rd Qu.: 6.470	3rd Qu.:15.49
6	Max. :3819.0	Max. :44268.34	Max. :69.00	Max. :10.120	Max. :25.85

	H1ln_A	J1_A	H1ln_B	J1_B
1	Min. :0.300	Min. :0.1400	Min. :0.040	Min. :0.020
2	1st Qu.:1.690	1st Qu.:0.5100	1st Qu.:1.280	1st Qu.:0.410
3	Median :2.120	Median :0.6200	Median :1.670	Median :0.490
4	Mean :2.058	Mean :0.5998	Mean :1.600	Mean :0.466
5	3rd Qu.:2.520	3rd Qu.:0.7100	3rd Qu.:2.030	3rd Qu.:0.550
6	Max. :3.420	Max. :0.9000	Max. :2.800	Max. :0.750

Table 4.4: Summary statistics for selected macrodescriptors (2002, 2003, 2007, 59 sampling stations).

mean	A	B (mg)	S	H'(A)	J'(A)	H'(B)	J'(B)
2002	452	2001.75	27	2.03	0.63	1.33	0.41
2003	911.5	3213.75	33	1.99	0.58	1.58	0.46
2007	931.25	3245.25	39	2.17	0.60	1.81	0.50

percentage variation	A	B	S	H'(A)	J'(A)	H'(B)	J'(B)
2003 vs 2002	101.6	60.5	22.2	-1.8	-7.5	18.8	13.3
2007 vs 2002	106.0	62.1	40.8	6.8	-4.3	36.0	23.9
2007 vs 2003	2.2	1.0	15.2	8.8	3.5	14.5	9.4

Table 4.5: Mean values for selected macrodescriptors (3-year 59-station data set).

The range of variability of abundances in 2002 has the same order of magnitude of the range in the 3-year data set. Biomass shows a temporal variation across the three years wider than variation among 180 stations in 2002. At first sight, abundances and biomass don't show normality, instead following a highly right-skewed distribution. The distribution of species richness and other indices seems to follow normality. The range of richness in the two data sets is comparable. Azoic conditions were never registered, and a maximum of 70 recorded taxa per station is shared between the two data sets. In Figure 4.3 the species richness calculated for 59-station 3-year data set is presented decomposed into histograms for each year. MELa2 2002 data are right-skewed, with a mode around 10-20 species per station (comparable to the histogram for the whole set of 180 station), whereas the other two years are more normally distributed, with a mode around 30-40 species per station. A general progression over the years toward a higher proportion of richer stations can be recognized. Average station diversity H' and evenness J' are greater for abundances than biomass.

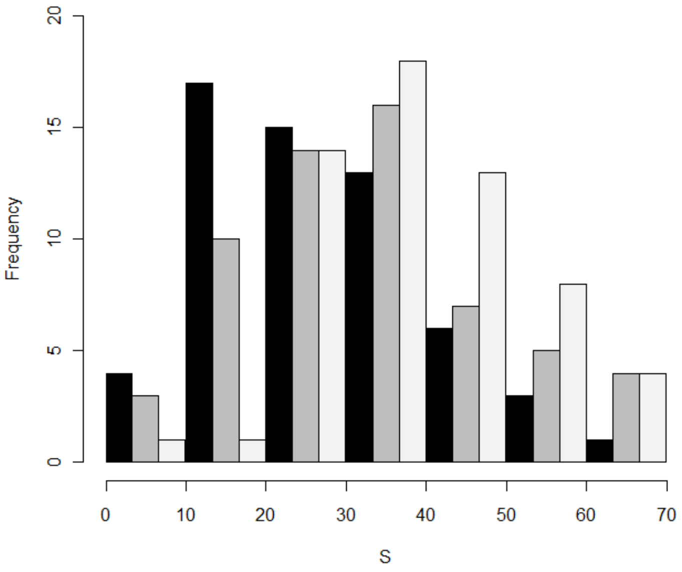


Figure 4.3: Histograms of species richness for the 59-station 3-year data set (black: 2002, dark grey: 2003, light gray: 2007).

Pearson correlation among macrodescriptors was calculated for both the data sets. Only the more rich data set of 2002 is presented (Table 4.6), as the correlation matrices for the two data sets are highly correlated (Mantel tests: $r = 0.93$, $p < 0.001$). Richness (S) and Margalef index (d) are highly correlated ($r = 0.96$), so in the following analyses only the untransformed richness data will be retained. Shannon index (H') and $E(S_{50})$ are also highly correlated between each other ($r = 0.96$). Richness presents also a high direct correlation, around 0.7, with $E(S_{50})$ and H' calculated for both abundances and biomass. Given these high correlations, the majority of subsequent analysis will be restricted on the former only. H' and evenness (Pielou index, J') also show a very high correlation. Diversities on biomass and abundances are to a certain extent correlated ($r = 0.6$), whereas abundances and biomass don't show any strong correlation. In fact, this is probably related to different life-history strategies of species (see Pianka, 1970). An inverse correlation ($r = -0.46$) can be noticed among abundances and abundances evenness, suggesting that high total abundances are often related to uneven distribution among species. This doesn't apply to biomasses, which instead show a correlation with richness ($r = 0.52$).

	A	B	S	d	ES50	H1ln_A	J1_A	H1ln_B	J1_B
A	1.00	0.19	0.10	-0.08	-0.31	-0.29	-0.46	0.02	-0.02
B	0.19	1.00	0.52	0.45	0.28	0.27	0.03	0.23	0.02
S	0.10	0.52	1.00	0.96	0.73	0.72	0.33	0.69	0.38
d	-0.08	0.45	0.96	1.00	0.87	0.83	0.50	0.70	0.39
ES50	-0.31	0.28	0.73	0.87	1.00	0.96	0.79	0.59	0.37
H1ln_A	-0.29	0.27	0.72	0.83	0.96	1.00	0.88	0.60	0.40
J1_A	-0.46	0.03	0.33	0.50	0.79	0.88	1.00	0.36	0.32
H1ln_B	0.02	0.23	0.69	0.70	0.59	0.60	0.36	1.00	0.91
J1_B	-0.02	0.02	0.38	0.39	0.37	0.40	0.32	0.91	1.00

Table 4.6: Pearson correlation matrix between macrodescriptors (180-station 2002 data set).

Temporal variability of abundances, biomass and richness across the three years is presented in Figure 4.4 as synthetic plots for 59 coincident sampling stations, showing the range of values per year. Total abundances and biomass grow in 2003 compared to 2002, and remain high in 2007. The maximum value of biomass (not shown in the plot) was registered in 2003. Figures 4.5 and 4.6 present interpolated maps of spatial patterns of abundances, biomass and richness for 2002 (180 stations). Maps were also produced on the 59 sampling stations for each of the three years, and are reported in Appendix 3. Richness presents a very strong gradient, decreasing from the sea landward and towards the watersheds. Its moderate correlation with total biomass can be also noticed. Abundances seem not to follow any linear gradient, displaying high values both near the inlet and in the inner part of the lagoon.

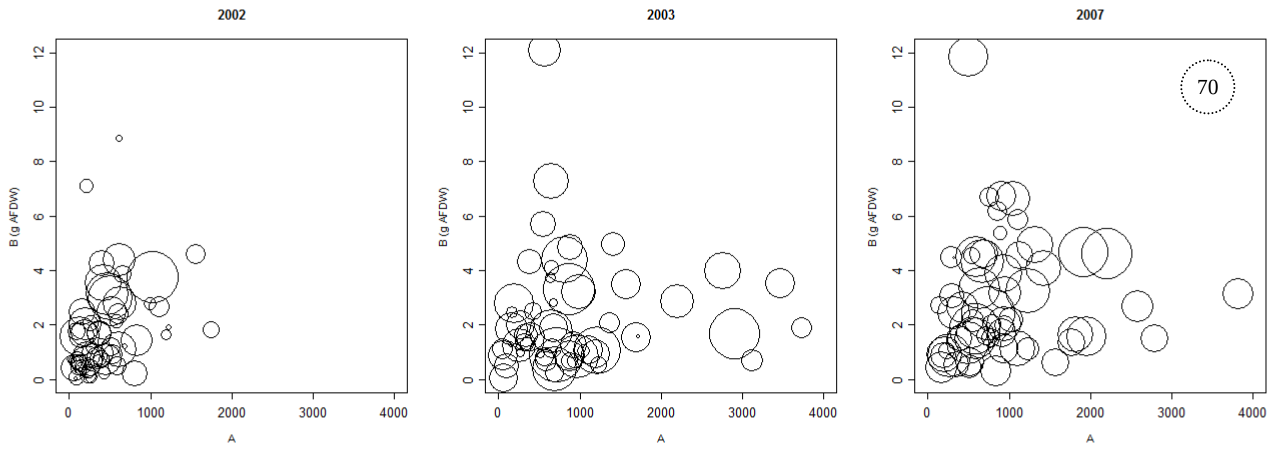


Figure 4.4: Abundances, biomass and richness for each year on 59 sampling stations (for comparison, on the upper right the dimension of the circle indicates 70 species)).

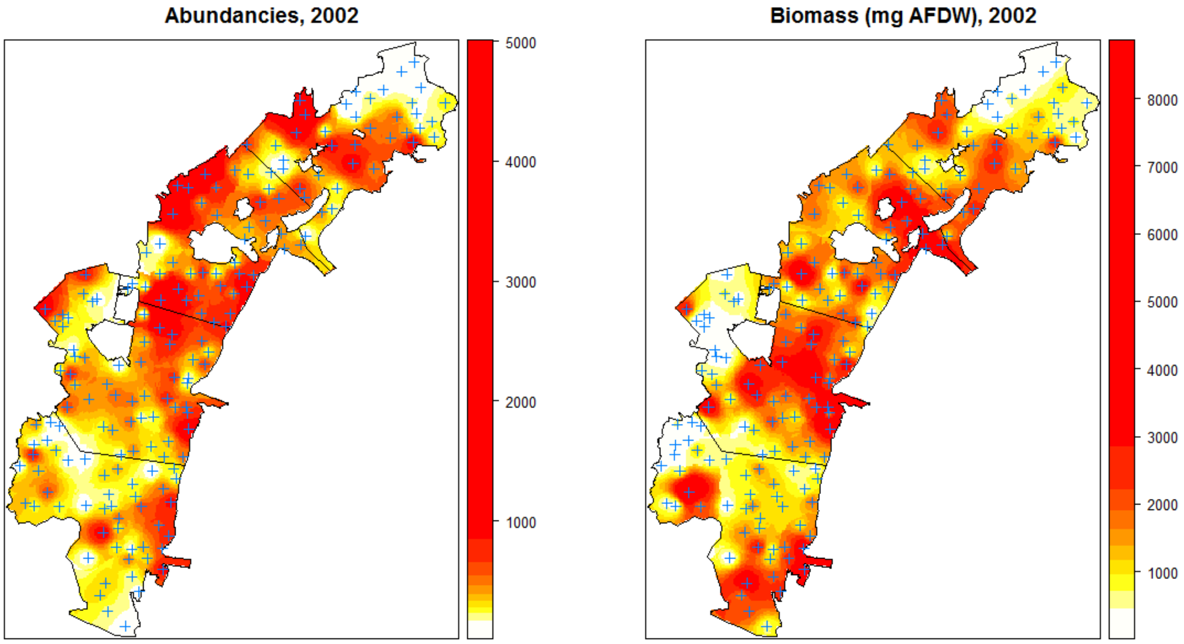


Figure 4.5: Abundances and biomass (180 sampling stations), plotted with 10 quantile classes (IDW interpolation; see Chapter 3.2.1).

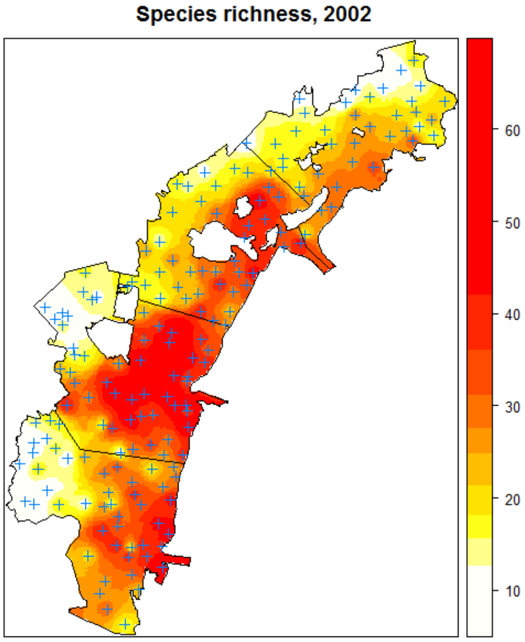


Figure 4.6: Species richness (180 sampling stations), plotted with 10 quantile classes (IDW interpolation; see Chapter 3.2.1).

4.2.2 Dominant taxa

The number of dominant species in terms of biomass is lower than the number of dominant species in terms of abundances (20-30% of the total number of species compared to 30-40%, respectively; Table 4.7). Comparing the 2002 180-station data set with the same year on 59-station data set, with a three times increase in the number of stations, only a 17% increase in the number of most abundant species (i.e. 16 species) was noticed; on the other hand the percentage compared to the total number of taxa remains about the same (0.4). For biomass, 19 other species are added to the set of dominant species. Nearly the 70% of the 3-year most abundant species are dominant both in 2002 and 2007, and the 55% in 2003. About the biomass, these percentages vary between 53% in 2002 to 76% in 2007. The union of the set of abundances-dominant and biomass-dominant taxa led to a set of 123 species for the 180-station 2002 data set and 144 species for the 59-station 3-year data set. Species which are dominant in terms of both biomass and abundances are only 32 (26%) in 2002 and 49 (34%) in the 3-year data set.

currency	year	n stat	n taxa	n dominant taxa	% dominant taxa
A	2002	180	235	95	0.40
	2002	59	187	79	0.42
	2003	59	193	63	0.33
	2007	59	226	80	0.35
	3-years	177	297	115	0.39
B	2002	180	235	60	0.26
	2002	59	187	41	0.22
	2003	59	193	50	0.26
	2007	59	226	59	0.26
	3-years	177	297	78	0.26

Table 4.7: Number of dominant species in terms of biomass and abundances (accounting for (more than) 75% of the total) for all the data sets and years.

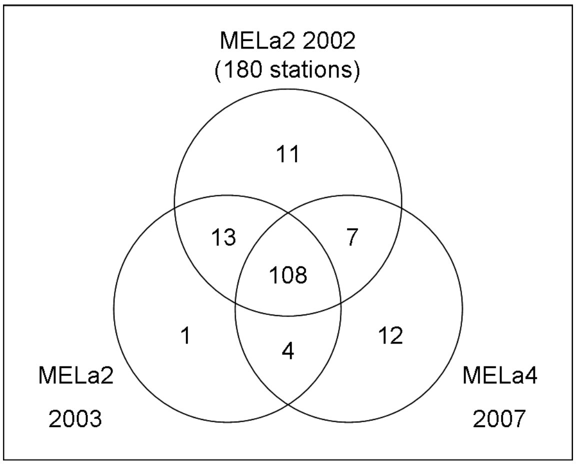


Figure 4.7: Number of dominant taxa (in terms of abundances and biomass) for each year and intersections (total number of stations).

In Figure 4.7 the whole number of dominant taxa is decomposed into the fractions pertaining exclusively to each year and the fractions which were found during different years. A total of 108 taxa was found jointly during the three studies, which is the 69% of the total number of dominant, whereas in the analogous partition for the whole number of species (Figure 4.2), only the 44% was found jointly. About the exclusive taxa, 11 were recorded in 2002, only one in 2003 and 12 in 2007. Despite the dominant taxa shifted from year to year, most of them came from the set of the species which were found in all the studies.

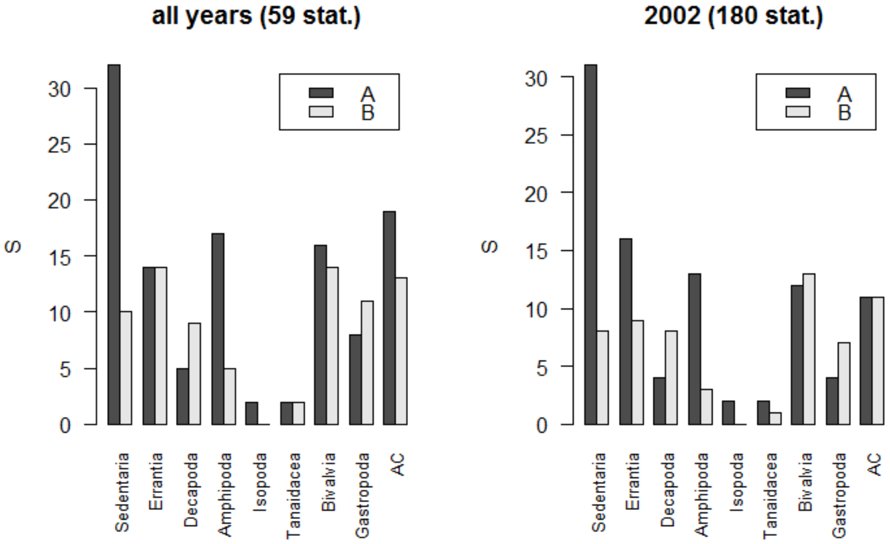


Figure 4.8: Number of dominant taxa in terms of abundances and biomass as distributed among main taxonomic groups for the two data sets.

The number of dominant taxa is presented in Figure 4.8, subdivided in the following high rank taxa: Polychaetes (Sedentaria and Errantia), Amphipoda, Isopoda, Tanaidacea, Decapoda, Bivalvia, Gastropoda and *Animalia caetera* (AC). Some groups have a comparable number of abundances-dominant and biomass-dominant species, such as Errantia (in the 3-year data set) and Bivalvia, whereas other predominate in particular with abundances (Sedentaria and Amphipoda). A list of the dominant species is reported in Appendix 4.

4.2.3 Taxonomic and trophic structure

A better overview of the overall lagoonal community structure is provided by analyzing the division of abundances and biomass into large taxonomic-functional groups and trophic groups. Taxonomic groups are reported in Chapter 4.2.2. Trophic groups which were taken in account are the following: carnivores (C), herbivores and micrograzer (H+MG), detritivores and surface deposit-feeder (SDF), subsurface deposit-feeder (SSDF), deposit-feeder with chemoautotrophic symbiotic bacteria (CDF) and suspension- and filter-feeder (SF). For details about the allocation to different trophic groups, see Chapter 3.3.4. Percentage distribution of abundances and biomass among both taxonomic and trophic groups was plotted for each year (Figure 4.9). Synthetic plots of absolute values are shown in Figure 4.10 and 4.10.

The highest number of individuals in all the three years belongs to Amphipods, followed by sedentary Polychaetes. These two groups are numerically almost the double than other large groups. Biomass is dominated by bivalves in all three years. Other groups which reach the 20% are Decapoda in 2002, Sedentaria in 2003 and Gastropoda in 2007. Errantia also are noteworthy in terms of biomasses. The trophic composition reflects taxonomic composition, with SDF and SF predominant numerically and in terms of biomass (along with SSDF in 2003). Carnivores are not numerous but with high biomass. Species with chemoautotrophic symbiotic bacteria (CDF) have very small population in all the years. Herbivorous and Micro-grazer grow both in abundances and in biomass in 2007, and reflect a growth of the gastropods populations.

The variation in total abundances and biomass along the years was decomposed among groups. In 2003 the biomass of Sedentaria strongly increased, from about 398 mg/m^2 up to about 3423 mg/m^2, although in 2007 it decreases to a level comparable to 2002. Errantia and Bivalvia also increase noticeably. In 2007 Bivalvia still increase up to 4586 mg/m^2, with Errantia returning to previous levels and Gastropoda increasing to become the second group. Changes in abundances are due mainly to Amphipoda (from 750/m^2 in 2002, to 1285/m^2 in 2003, to 1044/m^2 in 2007), Sedentaria (from 522/m^2 in 2002, to 925/m^2 in 2003, to 968/m^2 in 2007) and Tanaidacea (from 138/m^2 in 2002, to 577/m^2 in 2003, to 126/m^2 in 2007). About trophic groups, change in biomass is driven by C, SF and SSDF, whereas in abundances is due to SSDF, SDF, SF, reflecting taxonomic composition.

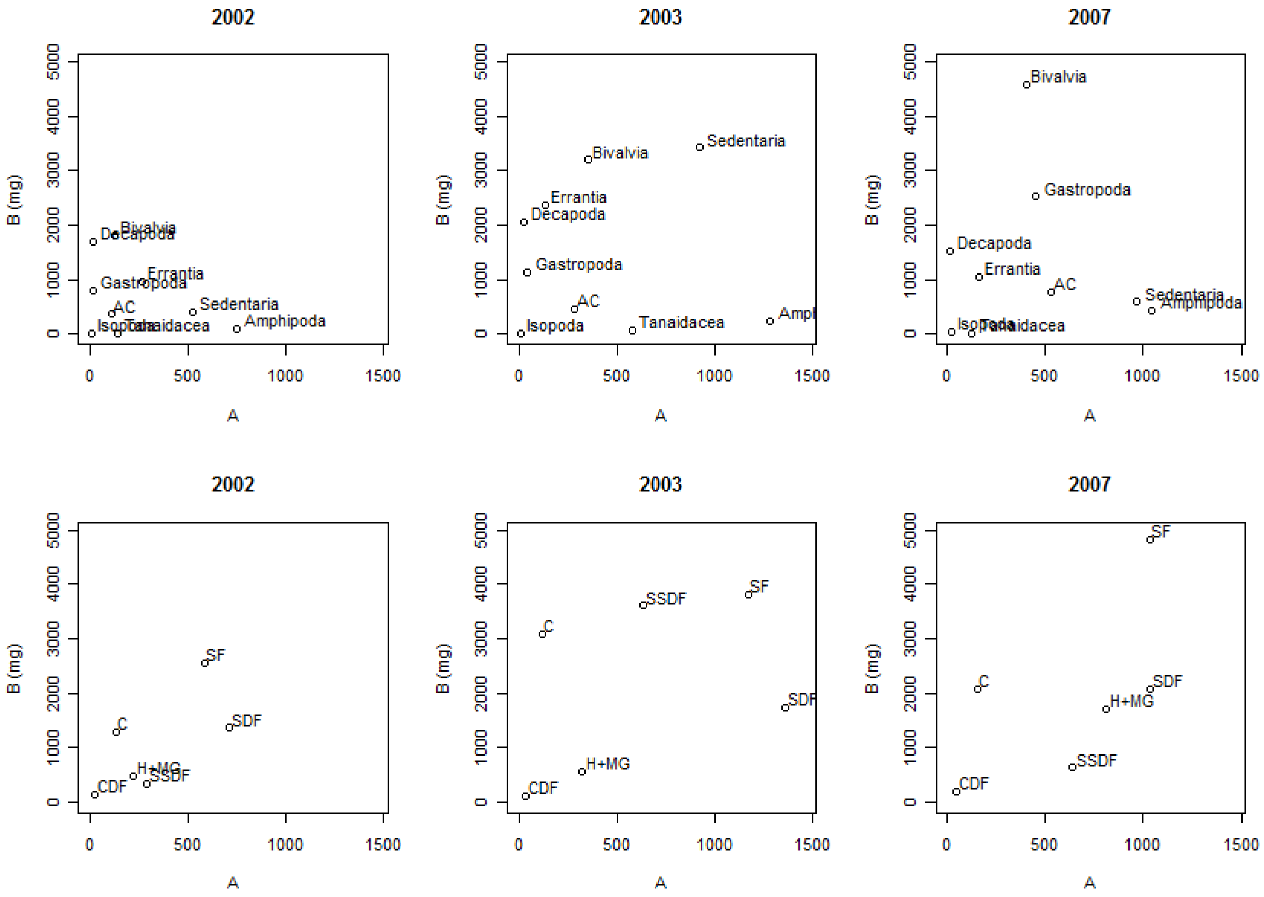


Figure 4.9: Taxonomic and trophic structure of the lagoonal community in terms of abundances and biomass.

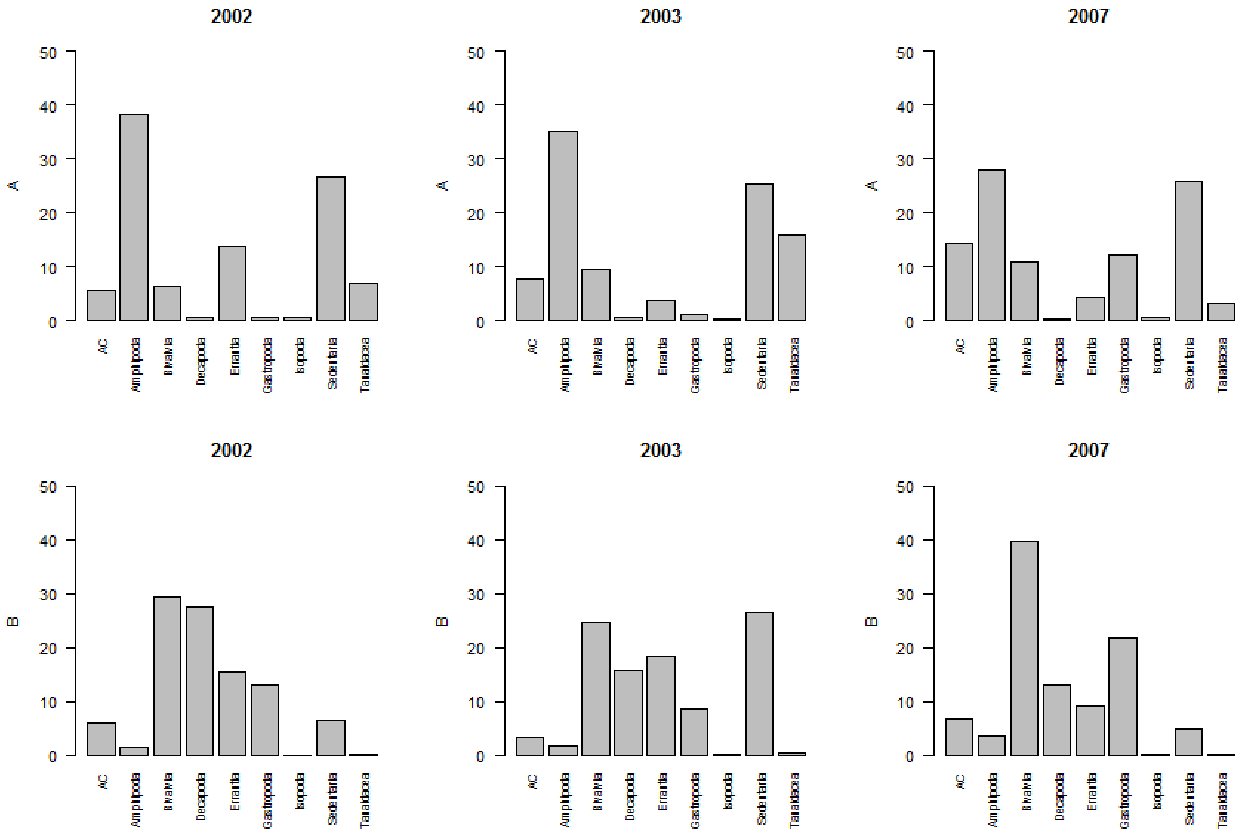


Figure 4.10: Percentage distribution of abundances and biomass between major taxonomic groups.

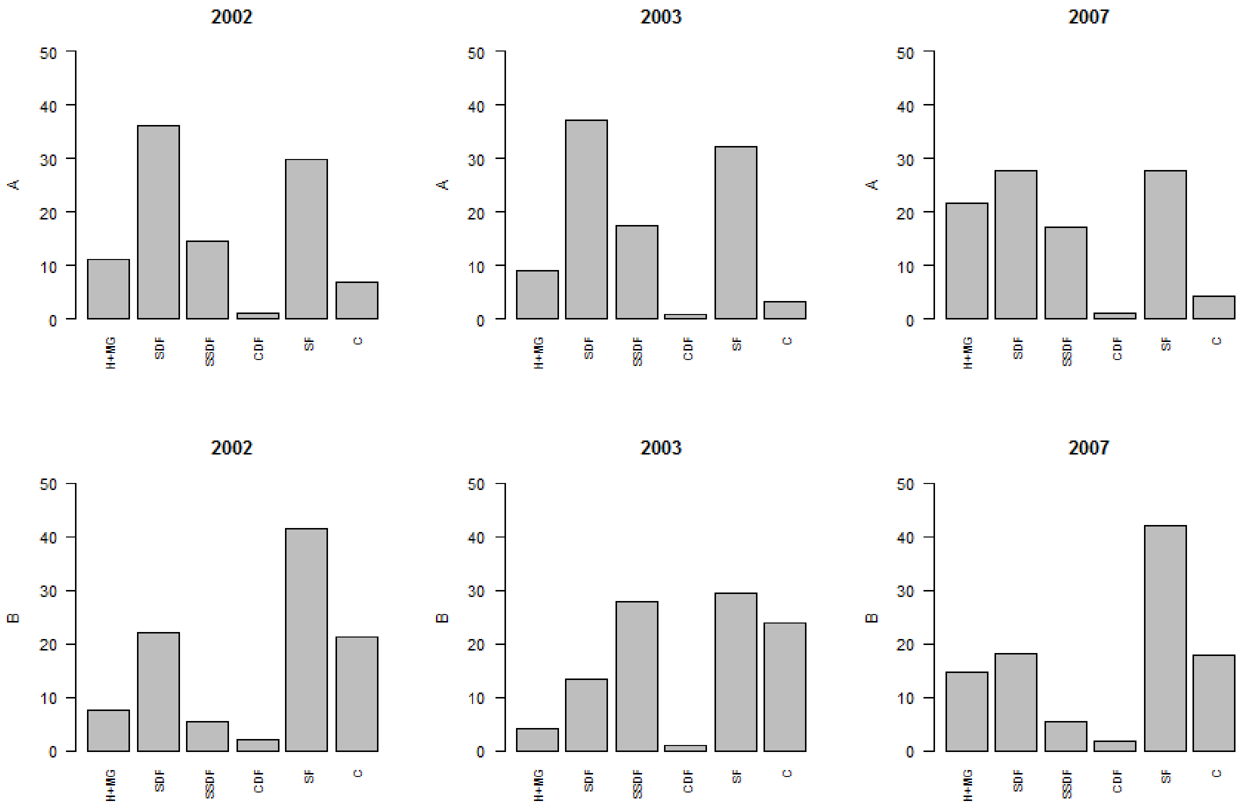


Figure 4.11: Percentage distribution of abundances and biomass between trophic groups.

4.2.4 Multivariate analysis

ANOVA-like analysis, ordination and classification methods were performed on the two multivariate data sets at the lagoon scale to characterize main spatial patterns of community in relation to basins and hydrogeological zones, identify and spatialize main assemblages and evaluate the role of yearly variability.

The results of the one-way PERMANOVA on the community matrices (and the trophic groups biomass matrices), as well as a PERMDISP analysis of homogeneity of variance, are presented in the following Tables (4.8 to 4.9).

Results of analogous univariate parametric analysis on main univariate macrodescriptors (abundances, biomass and richness), i.e. parametric one-way ANOVA on data transformed to normal distribution and Levene's test for homogeneity of variance, are also presented (Tables 4.10 and 4.11).

PERMANOVA results indicate that basins, hydrogeological zones and years present significant differences for all the data sets. Pseudo-F value is a function of the degree of freedom, so it is comparable only for analysis performed among the abundances and biomass composition matrices (which present the same dimension) for each factor. Pseudo-F values (and R^2) for each factor on abundances and biomass are similar in both the data set, but community composition in terms of biomass seems less variable among years. R^2 values allow a comparison among analysis. Around 20% of the variation of community composition matrices is explained by zones in 2002 data set, around 15% in 3-year data set. R^2 for basins and years is lower. R^2 for trophic groups (as biomasses matrix) indicates in general less variability than abundances and biomass, with also a slightly minor significance for the factor year (but this could be related to the low number of categories). PERMDISP analysis indicates significant differences in multivariate dispersion (< 0.05) only for the factor zones (and also on the trophic groups for the factor basins), which may or may not be responsible for PERMANOVA results. This will be subsequently evaluated by analyzing ANOSIM results and nMDS plots. ANOVA performed on univariate macrodescriptors richness, (square-root transformed) total abundances and (square-root transformed) biomass for the 2002 data set indicates statistical significance in respect of factors basin and zones ($p < 0.05$) for each variable. The 3-year data set results show instead that abundances are not significantly different among basin, and the p-value is also high for zones ($p = 0.069$). Heteroscedasticity ($p < 0.05$) by Levene's test was found for richness in 2002 in respect of factor basin; in fact, the number of species ranges between 7 and 70 in the Malamocco basin as a maximum and, as a minimum, between 6 and 33 in the Treporti basin. In the 3-year data set Levene's test was also found statistically significant for abundances and biomass with respect to factor basin and for abundances in respect of factor zones.

2002 data set (180 stat.)

		A			B			GT(B)		
	Df	pF-value	R2	p(>F)	pF-value	R2	p(>F)	pF-value	R2	p(>F)
basin	3	5.886	0.0912	0.001	4.468	0.0708	0.001	3.130	0.0506	0.001
GZ	4	10.449	0.1928	0.001	10.067	0.1871	0.001	5.059	0.1037	0.001

2002-2003-2007 data set (59 stat.)

		A			B			GT(B)		
	Df	pF-value	R2	p(>F)	pF-value	R2	p(>F)	pF-value	R2	p(>F)
basin	3	4.104	0.0664	0.001	4.120	0.0667	0.001	2.804	0.0464	0.001
GZ	4	6.970	0.1395	0.001	7.563	0.1496	0.001	3.438	0.0740	0.001
years	2	8.327	0.0873	0.001	4.839	0.0527	0.001	2.859	0.0318	0.007

Table 4.8: Results of one-way PERMANOVA (Bray-Curtis dissimilarity, 999 permutations) on species composition data, for abundances, biomass and trophic groups (as biomass). Basin and hydrogeological zone (GZ) were considered as factors for the 180-station 2002 data set (top). Basin, hydrogeological zone (GZ) and year were considered as factors for the 59-station three-year data set (bottom). Number of degree of freedom (Df), pseudoF value, explained variance R^2 and p-value are presented.

2002 data set (180 stat.)

		A		B		GT(B)	
	Df	pF-value	p(>F)	pF-value	p(>F)	pF-value	p(>F)
basin	3	0.950	0.404	2.655	0.054	3.811	0.015
GZ	4	9.625	0.001	17.496	0.001	8.966	0.001

2002-2003-2007 data set (59 stat.)

		A		B		GT(B)	
	Df	pF-value	p(>F)	pF-value	p(>F)	pF-value	p(>F)
basin	3	0.812	0.491	2.061	0.105	1.943	0.113
GZ	4	6.589	0.001	6.157	0.001	3.650	0.01
years	2	2.158	0.137	2.379	0.087	2.210	0.126

Table 4.9: Results of PERMDISP analysis of homogeneity of variance (Bray-Curtis dissimilarity, 999 permutations) on species composition data, for abundances, biomass and trophic groups (as biomass). Basin and hydrogeological zone (GZ) were considered as factors for the 180-station 2002 data set (top). Basin, hydrogeological zone (GZ) and year were considered as factors for the 59-station three-year data set (bottom). Number of degree of freedom (Df), pseudoF value and p-value are presented.

2002 data set (180 stat.)

		A		B		S	
	Df	F-value	p(>F)	F-value	p(>F)	F-value	p(>F)
basin	3	4.748	0.00329	3.409	0.01882	7.409	0.0001058
GZ	4	2.426	0.04977	9.635	4.587e-07	49.949	5.457e-28

2002-2003-2007 data set (59 stat.)

		A		B		S	
	Df	F-value	p(>F)	F-value	p(>F)	F-value	p(>F)
basin	3	0.351	0.7886	4.327	0.005708	7.207	0.0001382
GZ	4	2.220	0.06885	3.711	0.00634	29.424	1.213e-18
year	2	14.044	2.217e-06	6.862	0.001354	10.493	4.981e-05

Table 4.10: One-way ANOVA for main univariate macrodescriptors on 180-station 2002 data set (top) and 59-station 3-year data set (bottom). Basin and hydrogeological zone (GZ) were considered as factors for the 180-station 2002 data set (top). Basin, hydrogeological zone (GZ) and year were considered as factors for the 59-station three-year data set (bottom).

2002 data set (180 stat.)

		A		B		S	
	Df	F-value	p(>F)	F-value	p(>F)	F-value	p(>F)
basin	3	2.593	0.05422	1.100	0.3505	4.510	0.004481
GZ	4	1.918	0.1095	2.412	0.05092	1.649	0.1641

2002-2003-2007 data set (59 stat.)

		A		B		S	
	Df	F-value	p(>F)	F-value	p(>F)	F-value	p(>F)
basin	3	2.843	0.03933	3.074	0.02915	0.715	0.5445
GZ	4	2.955	0.0215	1.610	0.1738	1.041	0.3875
year	2	1.113	0.3309	0.234	0.7913	2.185	0.1156

Table 4.11: Levene's test for homogeneity of variance for main univariate macrodescriptors on 180-station 2002 data set (top) and 59-station 3-year data set (bottom). Basin and hydrogeological zone (GZ) were considered as factors for the 180-station 2002 data set (top). Basin, hydrogeological zone (GZ) and year were considered as factors for the 59-station three-year data set (bottom).

The results of ANOSIM analyses on the community matrices (and the trophic groups matrices in terms of biomass) are presented in Table 4.12. In Figures 4.12 to 4.15 nMDS ordination plots of the community matrices are presented, which are conceptually linked to ANOSIM results. The R statistics can be directly compared among groups, factors and data sets. Results are in general in accordance with those of PERMANOVA analysis. Similarity of community composition (both for abundances and biomass) is higher inside zones, in particular for 2002 data set, but it doesn't reach a value of 0.4. Considering the three years data set, similarities are stronger inside basins than insides zones, suggesting that basins are more stable than zones. Trophic groups have low similarity for every factor, however, as for PERMANOVA results, this could be related to the low number of categories.

2002 data set (180 stat.)

	A		B		GT(B)	
	R	signif	R	signif	R	signif
basin	0.177	0.001	0.107	0.001	0.035	0.002
GZ	0.382	0.001	0.322	0.001	0.041	0.034

2002-2003-2007 data set (59 stat.)

	A		B		GT(B)	
	R	signif	R	signif	R	signif
basin	0.294	0.001	0.299	0.001	0.004	0.409
GZ	0.132	0.001	0.129	0.001	0.044	0.001
years	0.242	0.001	0.131	0.001	0.024	0.01

Table 4.12: ANOSIM R statistic and significance (Bray-Curtis dissimilarity, 999 permutations) on species composition data, for abundances, biomass and trophic groups (as biomass). Basin and hydrogeological zone (GZ) were considered as factors for the 180-station 2002 data set (top). Basin, hydrogeological zone (GZ) and year were considered as factors for the 59-station three-year data set (bottom).

	A		B		GT(B)	
Groups	R	signif	R	signif	R	signif
MA, CH	0.045	1.500	0.015	12.300	0.021	7.000
MA, LI	0.165	0.010	0.078	0.200	0.060	0.700
MA, TR	0.282	0.010	0.149	0.010	0.016	19.500
CH, LI	0.213	0.010	0.144	0.010	0.067	0.200
CH, TR	0.230	0.010	0.126	0.020	-0.019	84.500
LI, TR	0.160	0.010	0.174	0.010	0.067	0.300
FZ, CB	0.447	0.010	0.358	0.010	0.102	0.020
FZ, BE	-0.154	91.100	-0.027	59.000	-0.093	79.500
FZ, SL	0.262	0.010	0.203	0.020	-0.050	82.400
FZ, TD	0.617	0.010	0.579	0.010	0.139	0.300
CB, BE	0.686	0.010	0.681	0.010	-0.210	98.200
CB, SL	0.297	0.010	0.108	2.400	-0.031	69.400
CB, TD	0.091	1.200	0.049	10.400	-0.003	48.700
BE, SL	0.553	0.010	0.718	0.020	-0.037	61.500
BE, TD	0.935	0.010	0.976	0.010	-0.128	86.800
SL, TD	0.599	0.010	0.531	0.010	0.139	0.400

	A		B		GT(B)	
Groups	R	signif	R	signif	R	signif
2002, 2003	0.166	0.010	0.075	0.030	-0.001	45.500
2002, 2007	0.309	0.010	0.172	0.010	0.057	0.090
2003, 2007	0.254	0.010	0.148	0.010	0.019	5.900
MA, CH	0.084	0.100	0.073	0.200	0.011	20.900
MA, LI	0.099	0.090	0.116	0.040	0.029	7.900
MA, TR	0.178	0.010	0.126	0.020	0.045	1.900
CH, LI	0.172	0.010	0.191	0.010	0.074	0.060
CH, TR	0.172	0.010	0.115	0.010	0.009	19.900
LI, TR	0.088	0.020	0.147	0.010	0.087	0.020
FZ, CB	0.319	0.010	0.265	0.010	0.040	14.000
FZ, BE	0.206	1.600	0.337	0.040	-0.156	75.500
FZ, SL	0.141	0.200	0.220	0.010	-0.090	83.800
FZ, TD	0.384	0.010	0.522	0.010	0.040	27.600
CB, BE	0.799	0.010	0.778	0.010	-0.204	87.900
CB, SL	0.175	0.100	0.111	1.600	-0.106	90.000
CB, TD	0.058	10.800	0.064	8.600	-0.114	93.400
BE, SL	0.676	0.010	0.704	0.010	-0.073	61.200
BE, TD	0.913	0.010	0.959	0.010	-0.191	84.500
SL, TD	0.364	0.010	0.343	0.010	0.126	8.800

Table 4.13: ANOSIM pairwise comparison R statistic and significance (Bray-Curtis dissimilarity, 9999 permutations) on species composition data, for abundances, biomass and trophic groups (as biomass). Basin and hydrogeological zone were considered as factors for the 180-station 2002 data set (top). Basin, hydrogeological zone and year were considered as factors for the 59-station three-year data set (bottom).

ANOSIM pairwise comparison was performed for all the factors (Table 4.13). R statistic for zones comparison has the widest range, from value around 0 (or negative) to values near 1. R = 1 indicates that all replicates within sites are more similar to each other than any replicate from different sites. This is approached only when comparing Bayhead Estuary and Tidal Delta with regards to abundances and biomass. Comparison between Fringe Zone and Bayhead Estuary, as well as between Central Basin and Tidal Delta are not significant in all the data sets, Central Basin and Sheltered Lagoon in 2002 biomass data set and in 3-year biomass and abundances data sets, Sheltered Lagoon and Fringe Zone in 3-year biomass data set. All the pairwise tests for Trophic Groups are not significant. In both the data set, contiguous basins are more similar than non-contiguous in terms of both abundances and biomass composition (despite the non-significance of Chioggia-Malamocco comparison), except Lido and Treporti basins with regards to biomass. The similarity between Treporti and Chioggia basins increases in terms of biomass composition. All the basins are highly similar in terms of trophic structure, but almost all the comparisons are not significant. The most dissimilar years are 2002 and 2007. The R statistic on years is doubler when calculated on abundances in terms of biomass, which is a more conservative measure.

nMDS plots, for abundances and biomass and for both the data sets, help to visualize and interpret overall and pairwise ANOSIM results. They have been plotted with different symbols, allowing identifying basins, zones and years. The stress is high for all the nMDS, around 23-25%. If a three-dimensional nMDS is calculated, the stress lower to about 16%. Some author considers unacceptable a level of stress above 20% (Borgatti & al., 1999), whereas others indicate that stress should be lower than 30% (Clarke & Warwick, 1994). Generally, the higher the number of observations, the higher the stress. Two-dimensional nMDS ordinations were displayed despite high stress, but they need to be interpreted carefully. A model of richness (based on GAM; see Chapter 3.5.5) is shown on the ordination plot, but it must be considered only as an aid to the interpretation. Dissimilarities grow as the number of species lowers (as can been observed by the curvature of the species richness isolines). The plot doesn't show clear real solution of continuity between factors. Basins on all the plots are highly superimposed (Figures 4.12 to 4.15). With reference to the 2002 data set, zones appear quite distinct on samples ordination, and follow a sequence from Tidal Delta to the more confined zones (Figure 4.12 and 4.12). Two main successions of zones appear from the sea landward: Tidal Delta to Central Basin to Fringe Zone, and Tidal Delta to Sheltered Lagoon to Fringe Zone. Bayhead Estuary doesn't separate from Fringe Zone. This instead happens in the 3-year data sets (Figure 4.14 and 4.14): Bayhead Estuary partly separates from Fringe Zone whereas other zones appear more superimposed among each other. Temporally, a progressive change of the macrobenthic community is observed, from 2002 to 2007. The two MELa2 studies are more superimposed and a stronger separation between 2002 and 2007 samplings is highlighted (Figure 4.14 and 4.14). A gradual shift can be noticed towards "more marine" assemblages (i.e. more similar to those of the Tidal Delta), with the exception of the Bayhead Estuary samples that continue to maintain their individuality. The shift towards a Marine Tidal Delta community structure was more evident for the Fringe Zone samples, especially for those of the Lido Basin.

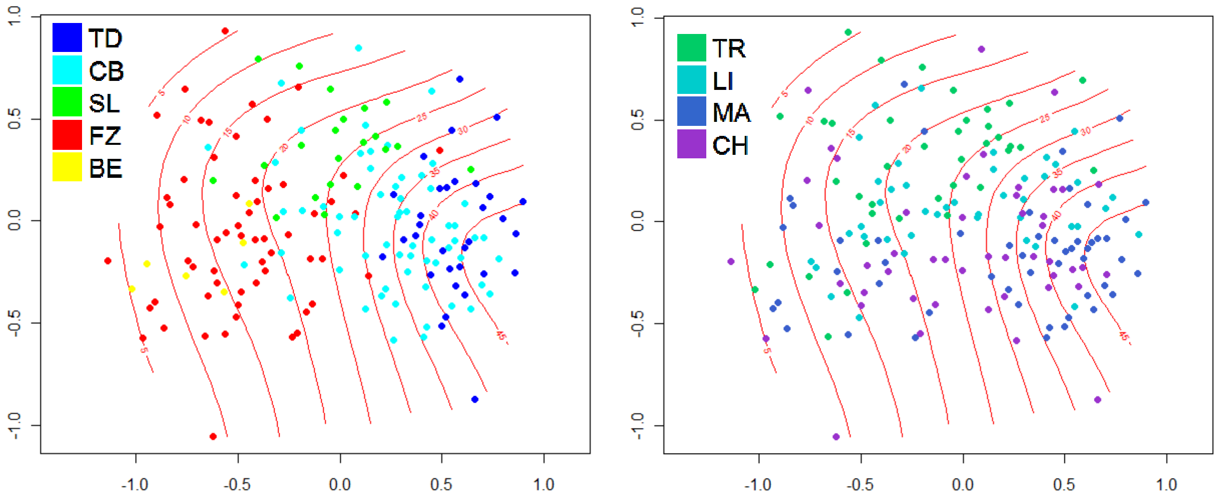


Figure 4.12: nMDS of 180-station 2002 data set, abundances (stress=22.60%); hydrogeological zones (on the left) and basins (on the right) are shown; a model of the richness is also displayed (GCV score=27.75).

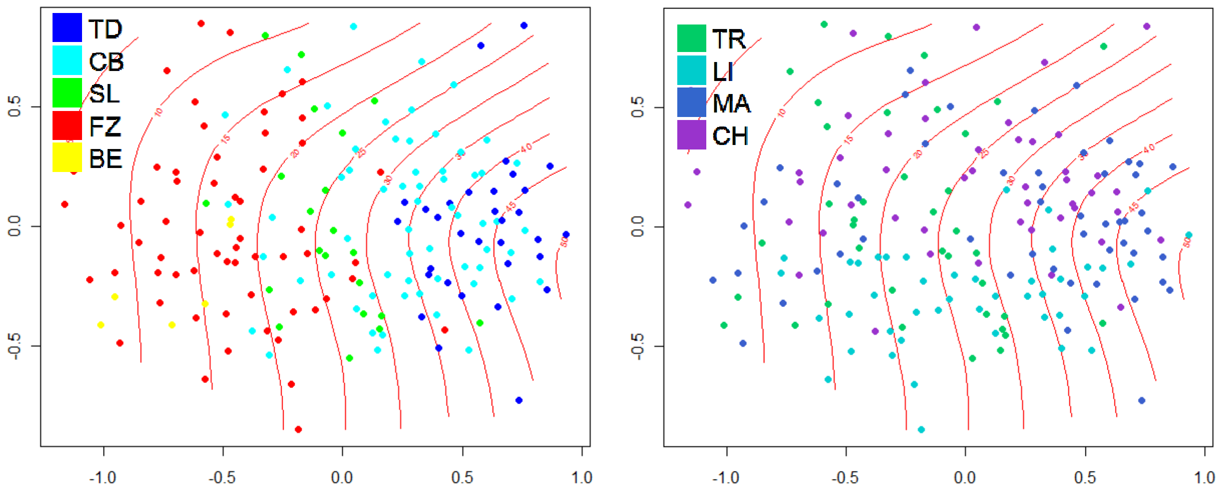


Figure 4.13: nMDS of 180-station 2002 data set, biomass (stress=22.79); hydrogeological zones (on the left) and basins (on the right) are shown; a model of the richness is also displayed (GCV score=25.10).

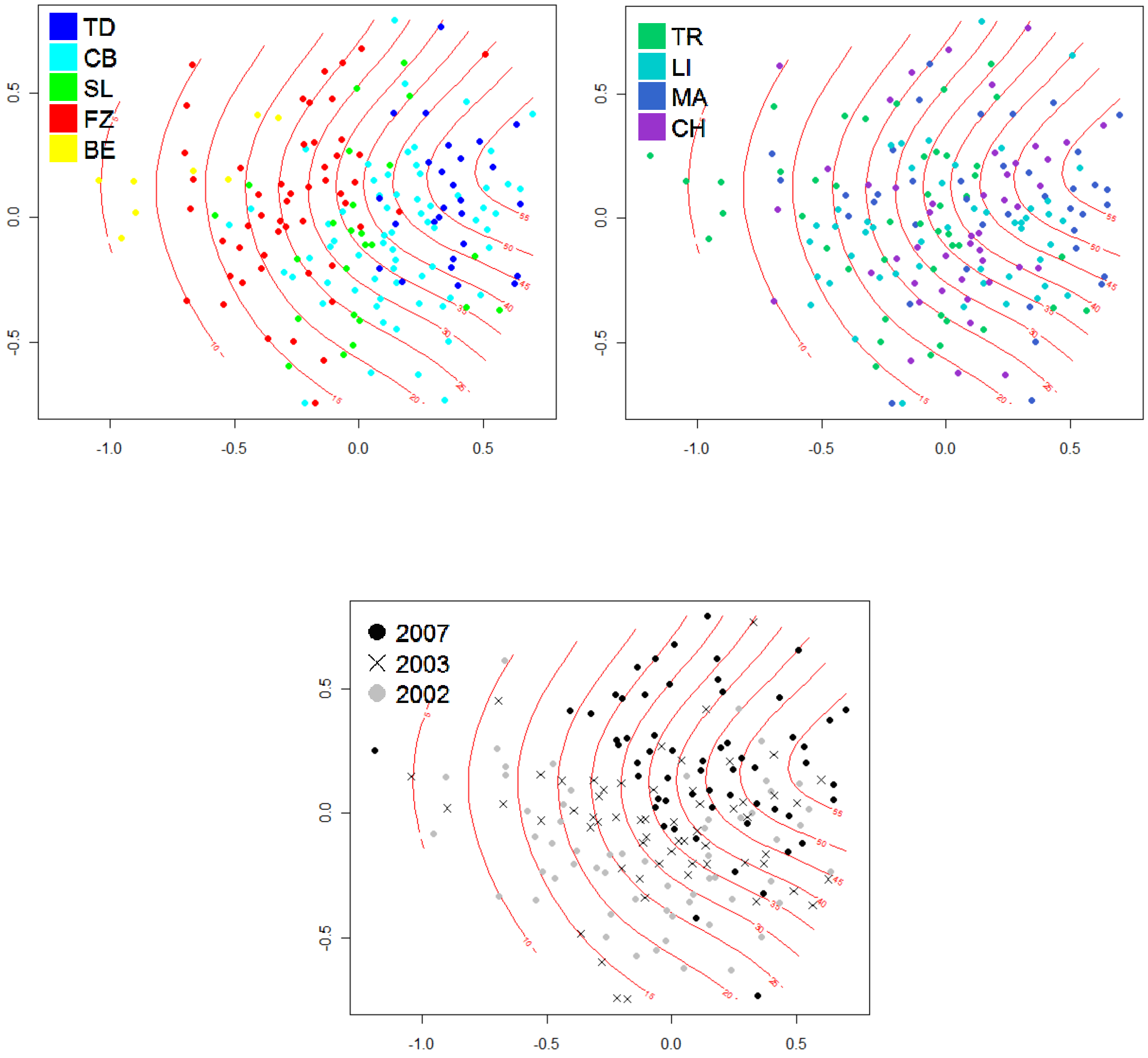


Figure 4.14: nMDS of 59-station 3-year data set, abundances (stress=25.46); hydrogeological zones (on the left), basins (on the right) and years (on the bottom) are shown; a model of the richness is also displayed (GCV score=37.55).

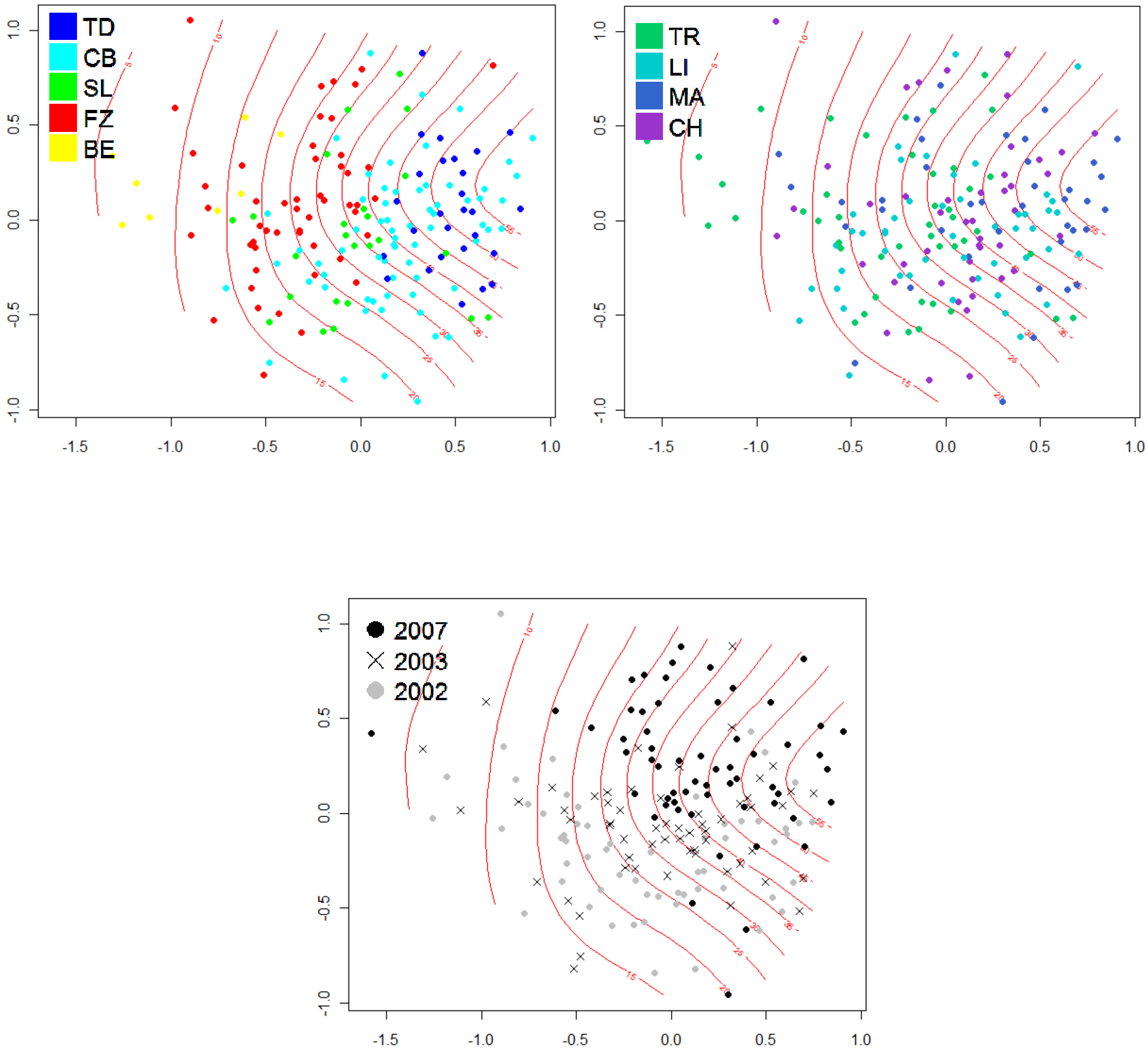


Figure 4.15: nMDS of 59-station 3-year data set, biomass (stress=23.39); hydrogeological zones (on the left), basins (on the right) and years (on the bottom) are shown; a model of the richness is also displayed (GCV score=34.50).

4.2.5 Analyses on hydrogeological zones

Changes in benthic communities structure during the three years were followed in relation to hydrogeological zones: the Tidal Delta (TD), the Central Basin (CB), the Sheltered Lagoon (SL), the Fringe Zone (FZ) and the Bayhead Estuary (BE) (Chapter 3.1.1, Figure 3.3). Mean values of main univariate descriptors for a given year and zone were computed on the 59-station data set and plotted to follow changes (Figures 4.16 to 4.20). Trophic and taxonomic structure as percentage composition in terms of biomass and abundances was analyzed (Figures 4.22 to 4.23). Zones are arranged in the plots along a succession from sea landward (i.e. approximately along the transitional gradient) which was recognized in nMDS and CAP plots (Figures 4.40, 4.40, 4.44 and 4.45 in Chapter 4.2.7) and which also corresponds to a decrease in the number of species.

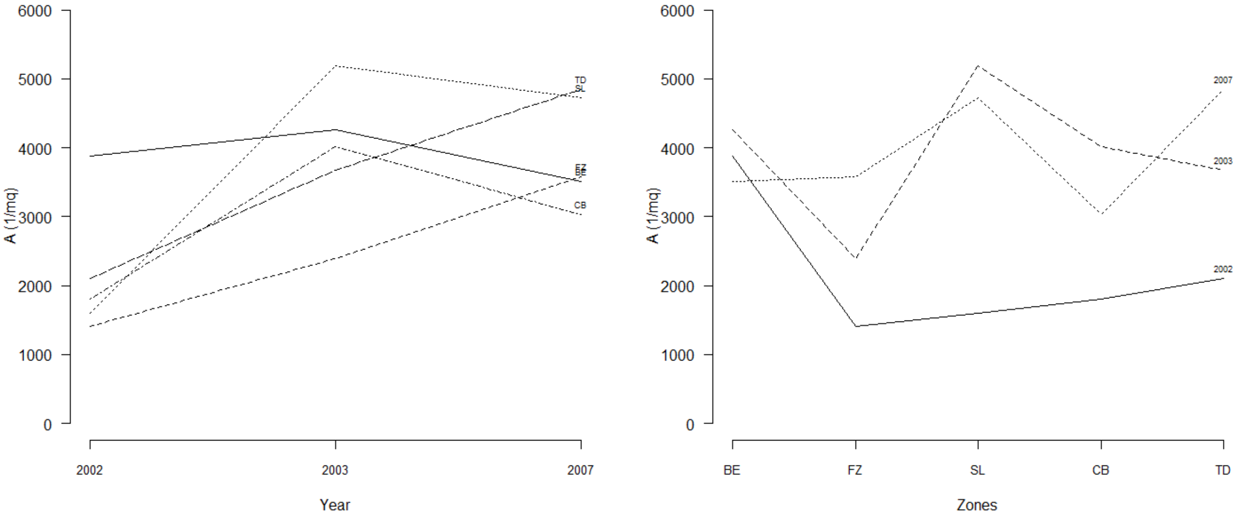


Figure 4.16: Temporal (left) and zonal (right) variation of mean densities based on 59-station data set. The temporal axis on the left plot is not in scale. Zones are arranged in the right plot approximately along the transitional gradient. (TD: Tidal Delta; CB: Central Basin; SL: Sheltered Lagoon; FZ: Fringe Zone; BE: Bayhead Estuary).

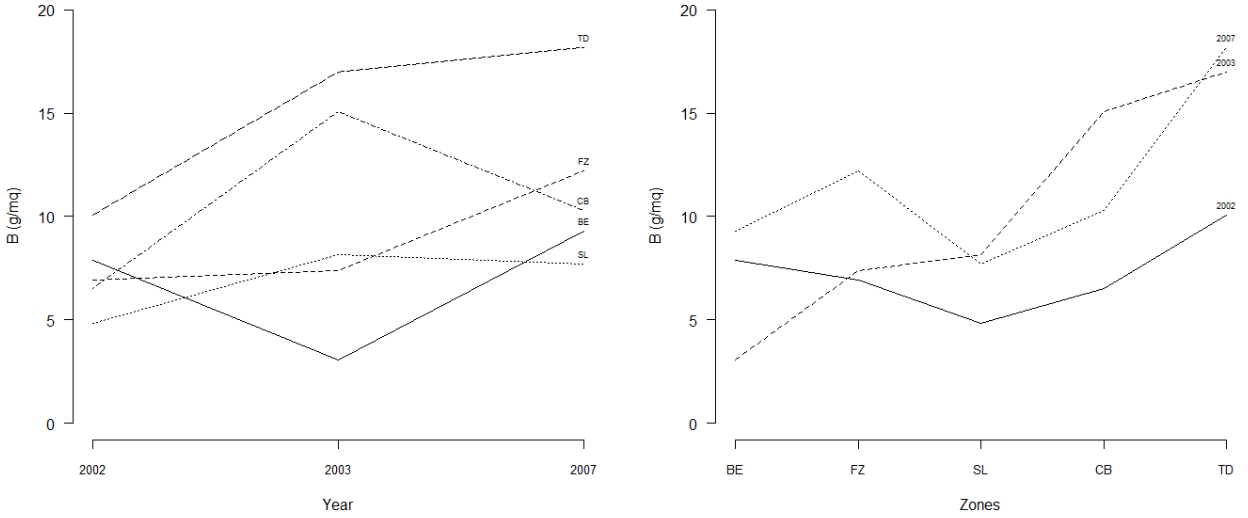


Figure 4.17: Temporal (left) and zonal (right) variation of mean biomass (g/m^2) based on 59-station data set. The temporal axis on the left plot is not in scale. Zones are arranged in the right plot approximately along the transitional gradient. (TD: Tidal Delta; CB: Central Basin; SL: Sheltered Lagoon; FZ: Fringe Zone; BE: Bayhead Estuary).

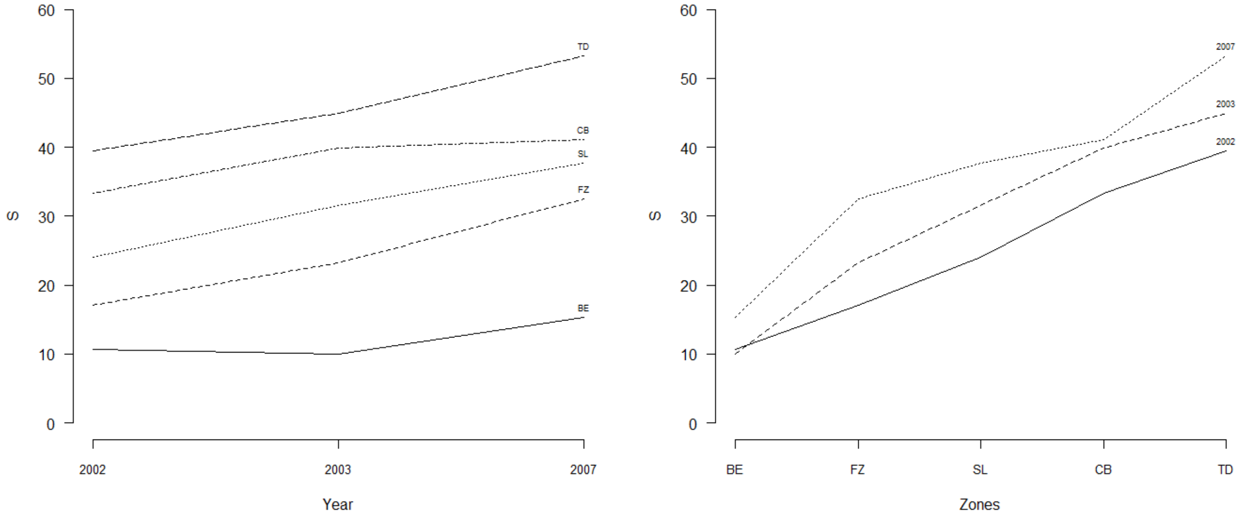


Figure 4.18: Temporal (left) and zonal (right) variation of mean richness S based on 59-station data set. The temporal axis on the left plot is not in scale. Zones are arranged in the right plot approximately along the transitional gradient. (TD: Tidal Delta; CB: Central Basin; SL: Sheltered Lagoon; FZ: Fringe Zone; BE: Bayhead Estuary).

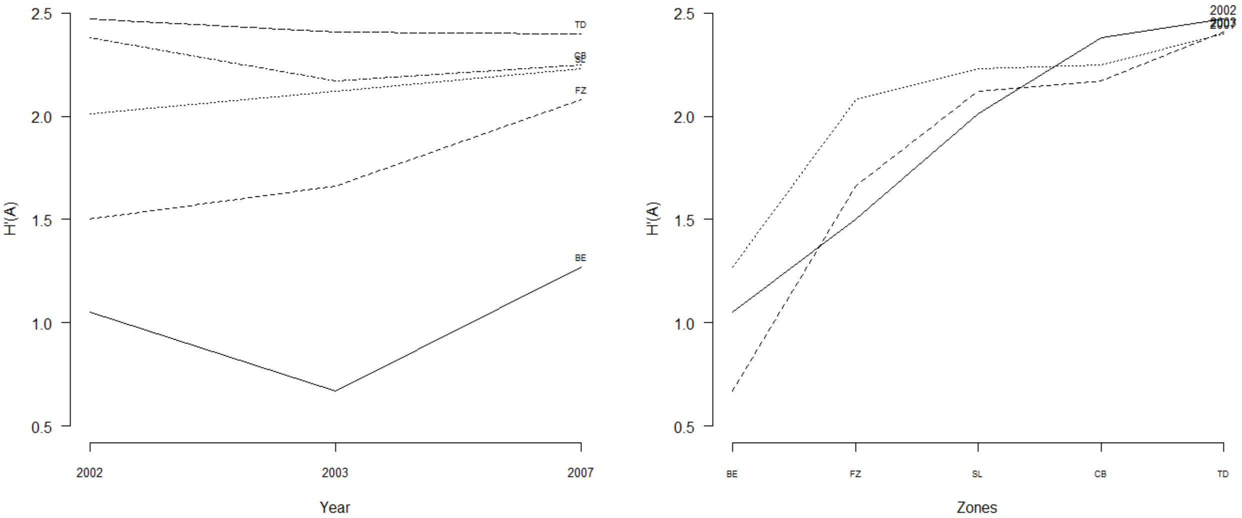


Figure 4.19: Temporal (left) and zonal (right) variation of mean H'(A) based on 59-station data set. The temporal axis on the left plot is not in scale. Zones are arranged in the right plot approximately along the transitional gradient. (TD: Tidal Delta; CB: Central Basin; SL: Sheltered Lagoon; FZ: Fringe Zone; BE: Bayhead Estuary).

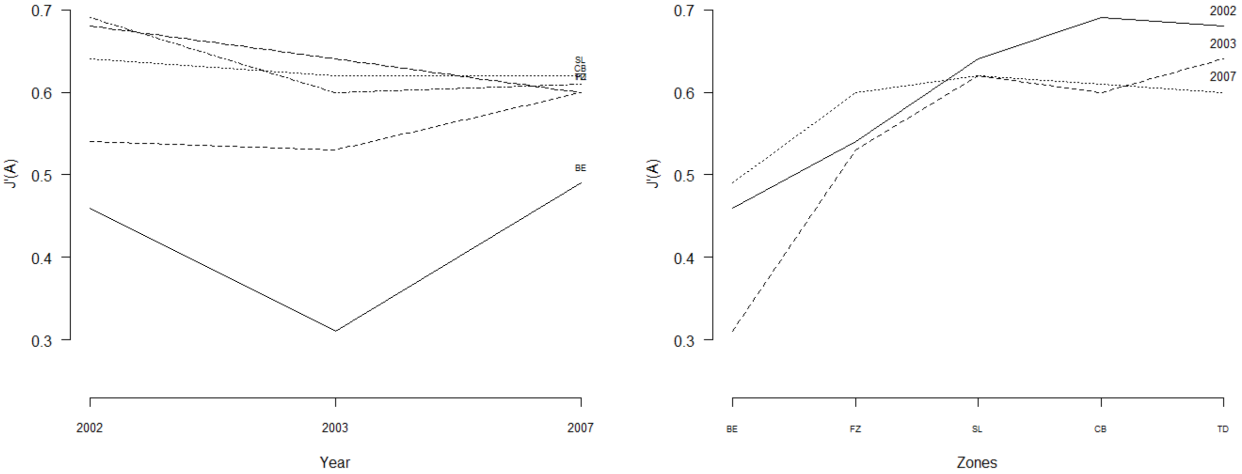


Figure 4.20: Temporal (left) and zonal (right) variation of mean J'(A) based on 59-station data set. The temporal axis on the left plot is not in scale. Zones are arranged in the right plot approximately along the transitional gradient. (TD: Tidal Delta; CB: Central Basin; SL: Sheltered Lagoon; FZ: Fringe Zone; BE: Bayhead Estuary).

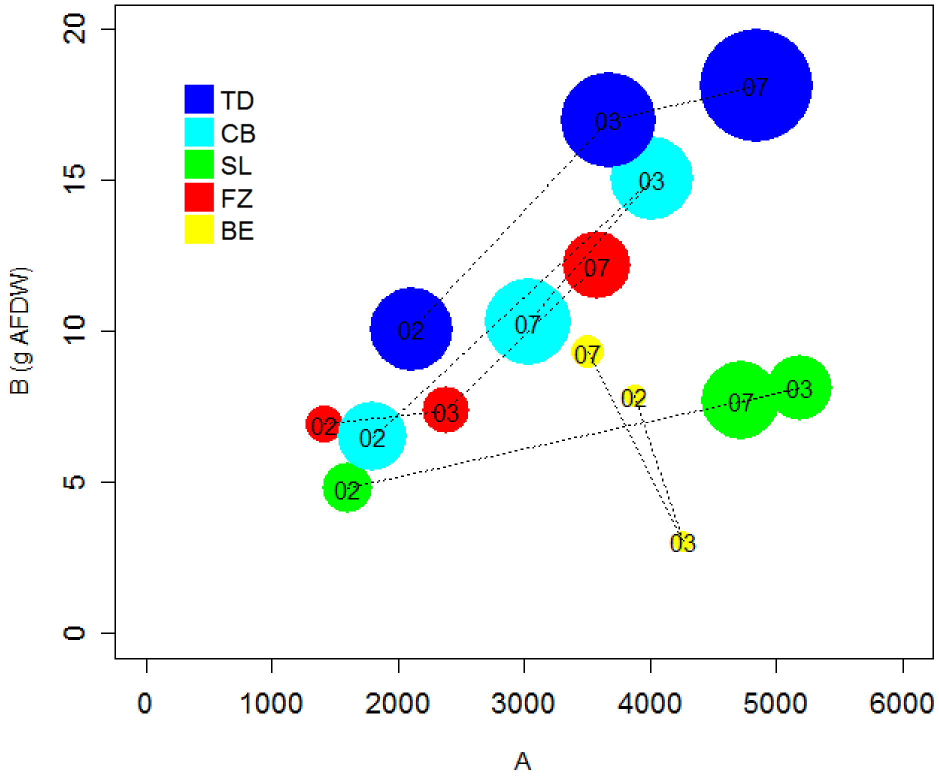


Figure 4.21: Temporal and zonal variation of A, B and S (radius of the circle) based on 59-station data set. (Dotted line: subsequent years for each zone; TD: Tidal Delta; CB: Central Basin; SL: Sheltered Lagoon; FZ: Fringe Zone; BE: Bayhead Estuary).

The average number of species per station for each zone increased over the years, this trend being more pronounced in the Fringe Zone and Bayhead Estuary (Figure 4.18, left). The trend of diversity indices, such as H' (shown for abundances, Figure 4.19, left) or $E(S_{50})$ (not shown), follows generally species richness, except Marine Tidal Delta and Central Basin diversity which remains at about the same levels during the years, probably due to a decrease in evenness (Figure 4.20, left). Evenness on abundances increases in 2007 in the Bayhead Estuary and the Fringe Zone. Meanwhile, H' and J' indices calculated in terms of biomass (not shown) increase for all the zones. There was an increase of the number of individuals from 2002 to 2003 in all zones, especially in the Sheltered Lagoon (Figure 4.16, left). Such an increase was also observed in 2007, but only for the Tidal Delta and the Fringe Zone. The biomass increased considerably from 2002 to 2003 in the Tidal Delta, the Sheltered Lagoon and the Central basin (Figure 4.17, left), whereas in 2007 the increase involved the inner areas such as the Fringe Zone and the Bayhead Estuary. Exceptions to the overall trend were observed in the Central Basin with a reduction of both total abundances and biomass in 2007 compared to 2002-2003, Bayhead Estuary and Sheltered Lagoon, with a reduction of total abundances.

Describing the trends along the succession of zones from land to the sea, the richness increases as expected, followed by diversity indices and evenness (Figure 4.18 to 4.20, right). Following well-known patterns, the higher the richness (i.e. seaward), the lower the variability of diversity indices, which are less sensitive to the increase in rare species. Abundances and biomass show more complex trends. The abundances are typically high in the more confined zones, due to the presence of a large number of individuals of opportunistic species (Figure 4.16, right). In Bayhead Estuary the number of individuals, which remain similar during the three years, is higher (or comparable in 2007) than the Fringe Zone values. The difference between these zones is the strongest in 2002. In this year abundances rise again, monotonically but slowly, up to the Tidal Delta. In 2003 and 2007 abundances increase strongly in the Sheltered Lagoon and then decrease again in the Central Basin. Tidal Delta shows a decrease in respect of the Central Basin in 2002, whereas in 2007 it keeps increasing. Trend in total biomass along the transitional gradient is partly contrasting with the one of abundances (Figure 4.17, right). It shows in the three years a relative maximum in the inner part of the Lagoon (Fringe Zone or, for 2002, Bayhead Estuary), a minimum in the Sheltered Lagoon and an absolute maximum in the Tidal Delta. A bubble plot is reported in Figure 4.21 summarizing abundances, biomass and richness for years and zones.

Figures 4.22 and 4.23 show the percentage contribution of major taxonomic and trophic groups to the abundances and biomass for each year and zone. Numerical abundances are dominated by polychaetes and amphipods. Errantia are typically higher in the inner zones (in particular in 2002) because of the occurrence of opportunistic species, as amphipods for 2003 and 2007. Sedentaria and Tanaidacea percentages increase in the outer zones, particularly in the Sheltered Lagoon and Central Basin. Biomass is generally dominated by bivalves, followed by decapods, with Errantia presenting high percentages particularly in Bayhead Estuary in 2002 and 2003. A dominance of Sedentaria in biomass percentage can be noticed for 2003 in the Central Basin. In 2007 there is a general increase in gastropods abundances and biomass. Temporal changes in dominant taxa are reflected in the trophic composition, which in any case appears more stable. A strong general increase in abundances percentage of herbivores can be noticed for the central and outer zones in 2007. With regards to percentages of biomass, an increase of filter-feeders can be noticed for the Bayhead Estuary in 2007 and sub-surface deposit-feeder for the Central Basin in 2003.

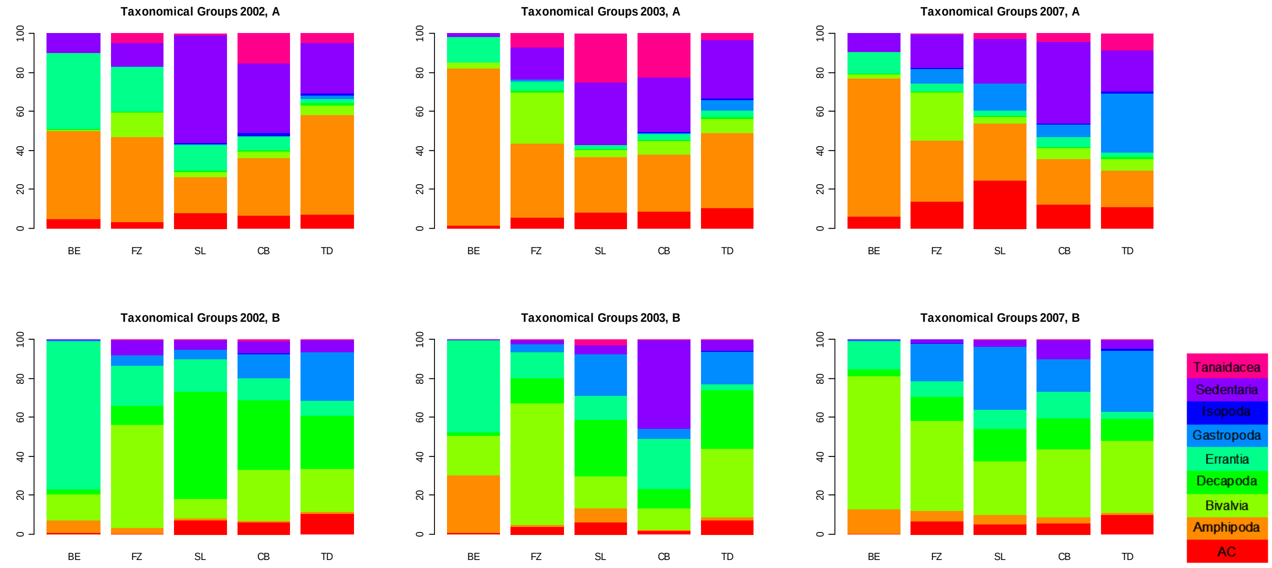


Figure 4.22: Taxonomical structure of community as percentages of abundances (top) and biomass (bottom) in the five hydrogeological zones (arranged approximately along the transitional gradient) for 2002, 2003 and 2007 (from left to right), based on 59-station data set. (TD: Tidal Delta; CB: Central Basin; SL: Sheltered Lagoon; FZ: Fringe Zone; BE: Bayhead Estuary).

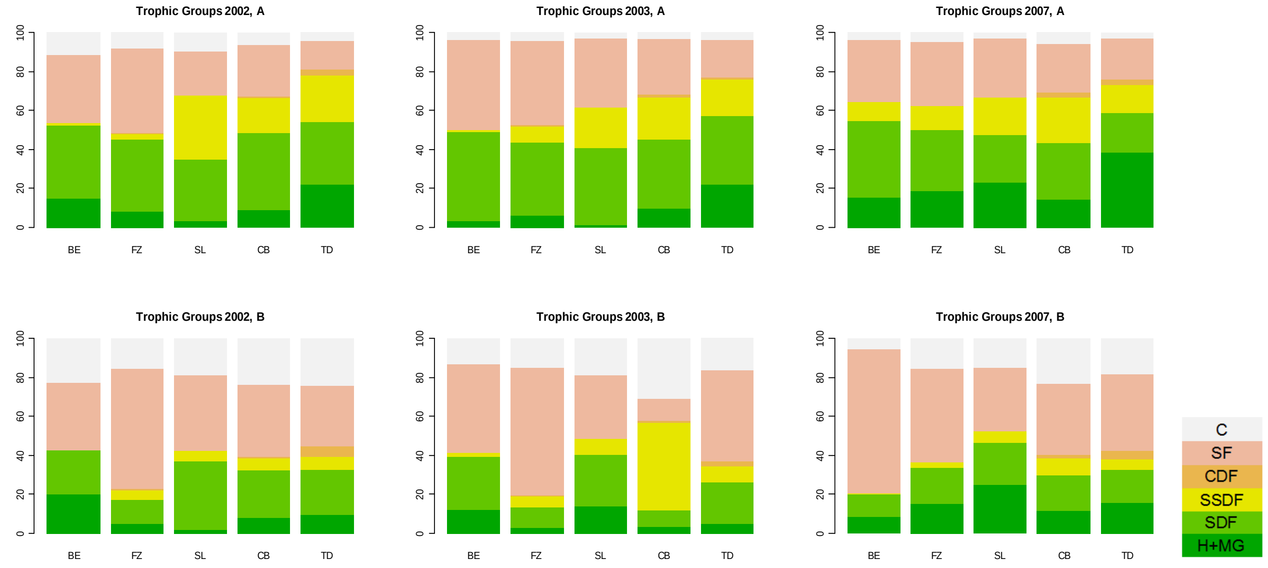


Figure 4.24: Trophic structure of community as percentages of abundances (top) and biomass (bottom) in the five hydrogeological zones (arranged approximately along the transitional gradient) for 2002, 2003 and 2007 (from left to right), based on 59-station data set. Trophic groups are carnivores (C), herbivores and micrograzer (H+MG), detritivores and surface deposit-feeder (SDF), subsurface deposit-feeder (SSDF), detritivores with chemoautotrophic symbiotic bacteria (CDF) and suspension- and filter-feeder (SF). (TD: Tidal Delta; CB: Central Basin; SL: Sheltered Lagoon; FZ: Fringe Zone; BE: Bayhead Estuary).

4.2.6 Cluster analysis

The continuous substitution of species along the lagoonal gradient (coenocline) was resolved into discrete assemblages through hierarchical cluster analysis on abundances. Cluster analysis allowed to identify a number of benthic assemblages and locate them on the Lagoon surface. Dendrograms resulting from analysis on 180-station 2002 data set and 59-station 3-year data set are presented on Figure 4.24 and Figure 4.25 respectively. It was generally verified a low overall similarity between the stations. The total dissimilarity for 180-station 2002 data set is 0.85. The total dissimilarity for 3-year data set is 0.85 as well, however if we apply an cluster analysis to each year individually, a different and higher total dissimilarity is obtained: 0.87 in 2002, 0.88 in 2003 and 0.93 in 2007.

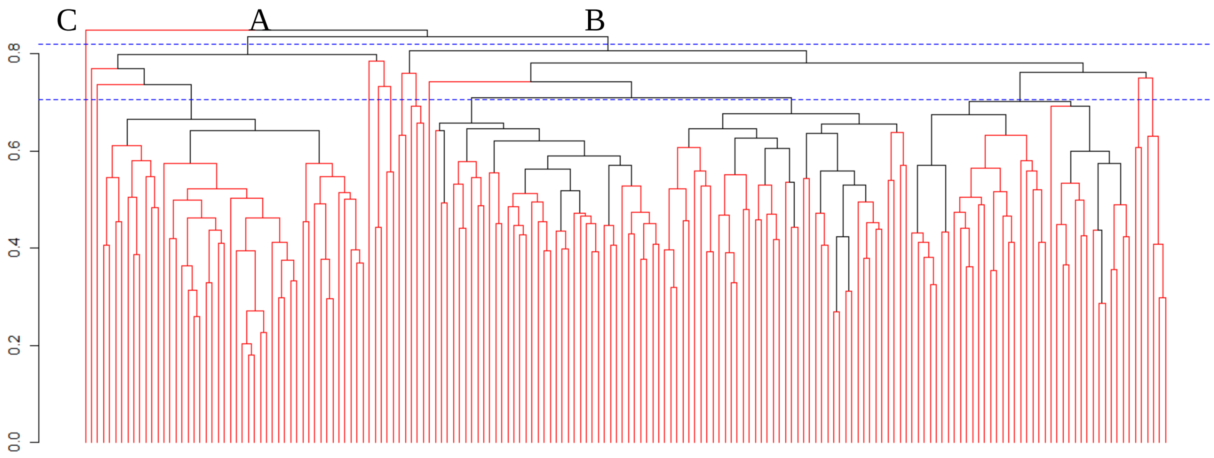


Figure 4.24: Dendrogram of 180-station 2002 data set based on agglomerative cluster (group average) on Bray-Curtis dissimilarity between square-root transformed abundances; red leafs show the level of statistical significance (α = 0.05) based on SIMPROF analysis; blue dotted lines indicate the cutting level.

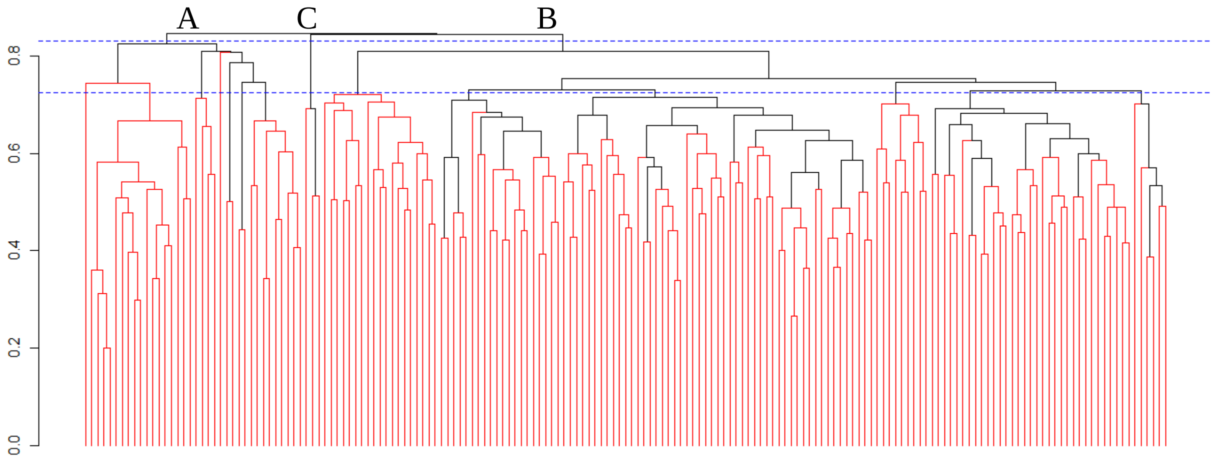


Figure 4.25: Dendrogram of 59-station 3-year data set based on agglomerative cluster (group average) on Bray-Curtis dissimilarity between square-root transformed abundances; red leafs gives the level of statistical significance (α = 0.05) based on SIMPROF analysis; blue dotted lines indicate the cutting level.

Two main levels were choosen to identify homogenous assemblages in a nested system for both the data sets. The two dendrograms were cut approximately at the same dissimilarity levels. When groups were found by SIMPROF analysis to be statistically non-significant at the cutting dissimilarity level, they were retained aggregated up to the significant level.

The dendrogram resulting by the cluster analysis on the 2002 180-station data set was cut at the levels of similarity of 0.82 and 0.71. The highest-dissimilarity cut, at 0.82, groups all the stations into two large clusters (which have been named "A" and "B"), except a single station (number 104) which branches out at highest dissimilarity, forming an independent cluster ("C"). At lower dissimilarity level (0.71), a second cut identifies ten clusters, three of which resulting from the aggregation of not-significant clusters.

The dendrogram resulting by the cluster analysis on the 3-year 59-station data set was cut at the levels of similarity of 0.83 and 0.72. The highest-dissimilarity cut, at 0.83, groups most of the stations into two large clusters (again "A" and "B"), with three others forming an independent cluster (again "C"). In this case one station needs to be merged to another cluster at higher dissimilarity as not-significant. Considered separately, each year may yield very different results from those obtained by performing a single overall analysis. The spatial meaning of the groups was interpreted on the maps on the basis of emerging patterns. Identified clusters at given dissimilarity level were mapped on the Lagoon surface by means of Voronoi map based on sampling stations. Two maps were produced for the cluster analysis on 2002 data set (Figure 4.29 and 4.29), and two per year for the cluster analysis on the 3-year data set, allowing to check for interannual variability among assemblages (Figures 4.31 to 4.33). Results are also visualized on nMDS plots (Figures 4.26 to 4.28).

Assemblages show strong spatial structures related to an overall coenocline. Reflecting the hierarchical framework, the degree of aggregation in the horizontal spatial distribution is structured on different spatial scales. Higher hierarchical levels for both the cluster analyses identify patterns at the Lagoon scale, i.e. at macroscale (about 500 to 100 km^2). Patterns identified when the second hierarchical levels is mapped can be related to mesoscale (about 100 to 10 km^2).

Result of the 2002 180-station analysis is not directly comparable with the result of 3-year 59-station analysis, nevertheless the two data sets were compared with a qualitative approach. Mesoscale clusters were at first arbitrarily named on 2002 data, from A1 to A4 and from B1 to B6. Then, the 3-year clusters were associated on the bases of spatial relationship to 2002 clusters, and named after those (however, with a "*" to stress that correspondences are only indicative). Some of the mesoscale 3-year clusters which are nested in the "A" macroscale cluster bear a resemblance to cluster of the "B" set in 2002 data, in which case a double name is maintained.

The macroscale pattern of assemblages is related to the classical subdivision of the Lagoon into Open and Restricted Lagoon. The agglomerative approach cause high hierarchical levels to depart from the best solution. This was investigated by calculating a *k*-means (k = 2) on the 2002 data set and comparing with the highest levels of classification by the agglomerative method. The two clusters (Figure 4.29), corresponding to "A" and "B", include respectively 63 and 117 stations. *K*-means indicates that 13 stations belonging to cluster "B" in the results of the hierarchical cluster are actually more similar to the stations of cluster "A", and two stations belonging to cluster "A" are actually more similar to the stations of cluster "B". A total of 16 stations on 180 (9 %, which includes the isolated cluster "C") should be reclassified; however this would be to the detriment of the nested structure.

Identified clusters (assemblages) are listed in Table 4.14 with the number of stations and a raw estimation of the area based on Voroni polygons. The number of stations as well as the areas are very variable for both the levels of analysis. To interpret multivariate relationships, two- and three-dimensional nMDS plots based on abundances (Chapter 4.2.4) are presented in Figure 4.26 to 4.27 highlighting classification into clusters.

	stations	area (km2)
A	51	126.13
B	128	274.16
C	1	2.53
A1	1	5.00
A2	1	0.79
A3	44	109.75
A4	5	10.58
B1	5	7.90
B2	1	2.48
B3	38	73.95
B4	41	86.69
B5	37	89.13
B6	6	14.02
C1	1	2.53

				stations			area (km2)
	2002	2003	2007	sum	2002	2003	2007
A	24	11	1	36	179.33	65.80	2.46
B	34	47	57	138	216.98	330.52	391.61
C	1	1	1	3	6.30	6.30	8.54
A3*	13	4	1	18	90.12	19.38	2.46
A4*	2	0	0	2	16.59	0.00	0.00
A5	2	2	0	4	12.67	12.67	0.00
A6	0	1	0	1	0.00	5.22	0.00
A7(B5b*)	6	3	0	9	46.65	20.66	0.00
A8(B6*)	1	1	0	2	13.31	7.86	0.00
B1*	3	3	3	9	16.64	16.64	18.41
B2*	2	1	3	6	16.50	8.48	24.26
B3*	10	9	14	33	60.25	56.84	87.68
B4*	9	28	14	51	55.93	205.50	94.03
B5a*	10	5	5	20	67.66	34.24	37.96
B7	0	1	18	19	0.00	8.82	129.27
C1(A1*)	1	1	1	3	6.30	6.30	8.54

Table 4.14: Number of stations and indicative area (km^2) after Voronoi polygons for each cluster identified by cluster analyses on 2002 180-station abundances (left) and 3-year 59-station abundances (right, divided per year). Two hierarchical levels are showed for both the analyses.

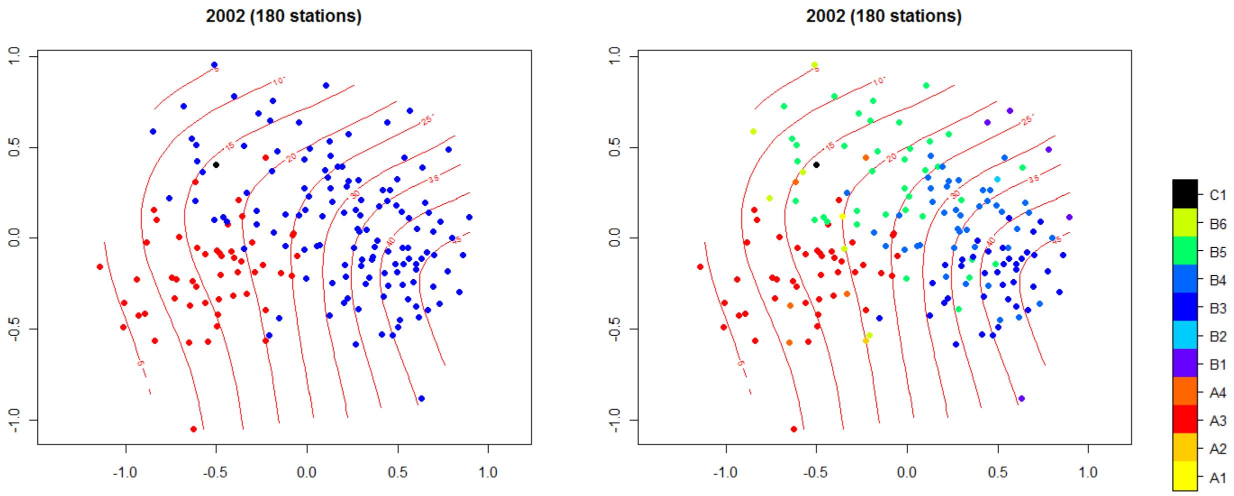


Figure 4.26: 2-d nMDS of 180-station 2002 data set, abundances (stress=22.60%); assemblages resulting from hierarchical cluster analyses on 180-station (abundances). Macroscale (cutting value at dissimilarity = 0.71; on the left) and Mesoscale (cutting value at dissimilarity = 0.82; on the left). A model of the richness is also displayed (GCV score=27.75).

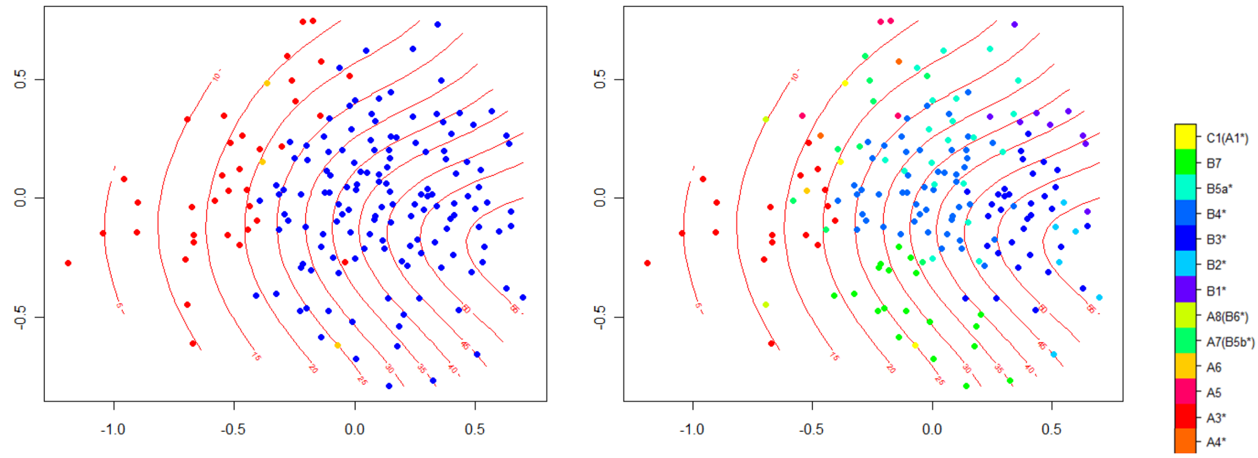


Figure 4.27: 2-d nMDS of 59-station 3-year data set, abundances (stress=25.46%); assemblages resulting from hierarchical cluster analyses on 180-station (abundances). Macroscale (cutting value at dissimilarity = 0.72; on the left) and mesoscale (cutting value at dissimilarity = 0.83; on the left). A model of the richness is also displayed (GCV score=38.57).

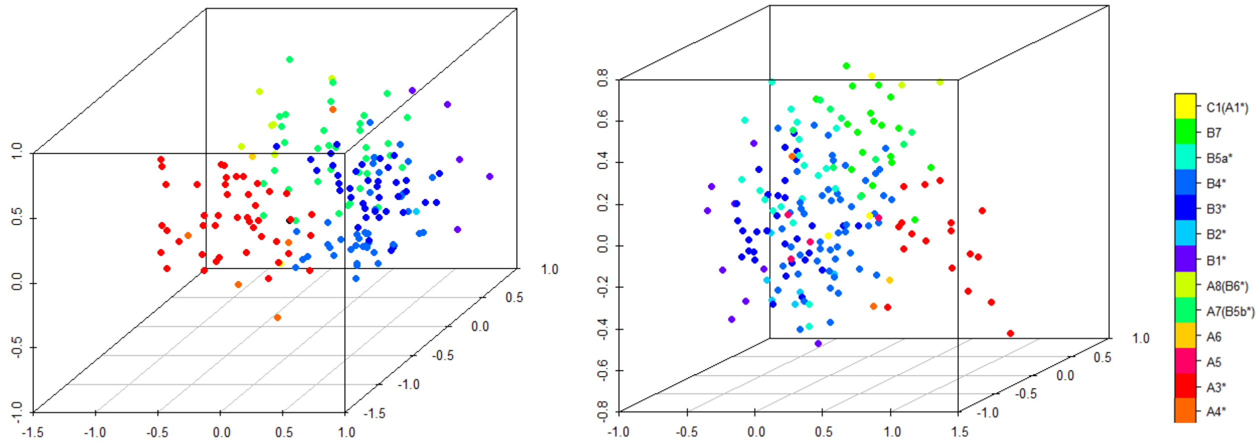


Figure 4.28: left: 3-d nMDS of 2002 data sets, abundances (stress=16.29%); assemblages resulting from hierarchical cluster analyses on 59-station (abundances). Mesoscale (cutting value at dissimilarity = 0.82). Right: 3-d nMDS of 59-station 3-year data set, abundances (stress=19.47%); assemblages resulting from hierarchical cluster analyses on 59-station (abundances). Mesoscale (cutting value at dissimilarity = 0.83).

Comparing the map based on the 180-station data set (Figure 4.30) with that one based on the whole 3-year 59-station data set (Figure 4.31), the patterns displayed are quite similar. Some stations change attribution in the two cluster analyses, nine in number from "B" at macroscale to "A" at mesoscale, and one from "A" to "B". This seems to balance for the reduction in number of stations, unevenly affecting "A" stations, and the general structure is maintained. A noteworthy change can be noticed in the northern part of the Lagoon (inner Palude Maggiore), which is classified by 59-

station cluster analysis as "A", approaching the *k*-means results. In 59-station cluster analysis sampling station 50, located between Venezia and San Giuliano, belongs to a small cluster "C". It branches out at high dissimilarity, and also includes a station in 2003 (again n. 50) and a station in 2007 (n. 11). A strong decrease in the number of stations belonging to assemblages "A" is observed over the years, from 39-41% in 2002 for both the analyses to 19% in 2003 and only 2% in 2007 (one station).

Assemblages identified at the mesoscale level, nested into the previous macroscale clusters, still show a succession of assemblages along a general gradient from the sea landward, despite a major complexity (Figures 4.30 and from 4.31 to 4.33 at the right). A first assemblage, here identified as B3, extends from the inlets into the Lagoon, particularly into the Malamocco basin, following the Canale dei Petroli. Particularly interesting is the assemblage B1 which is characteristically present at one or two stations on the right side of each inlet. B3 and B1 gradually are substituted by B4, particularly in the central and north part of the Lagoon. From B4 towards A assemblages, and in particular main cluster A3, the passage can be direct or indirect through B5 (and secondarily B6). This succession is accompanied by a reduction in the number of species (Figure 4.26). Other identified clusters include B2, localized where more "marine" assemblages (B3) directly encounter confined assemblages (A3), single-station clusters A1 and A2 and finally A4, which, as A1, includes three stations near Porto Marghera.

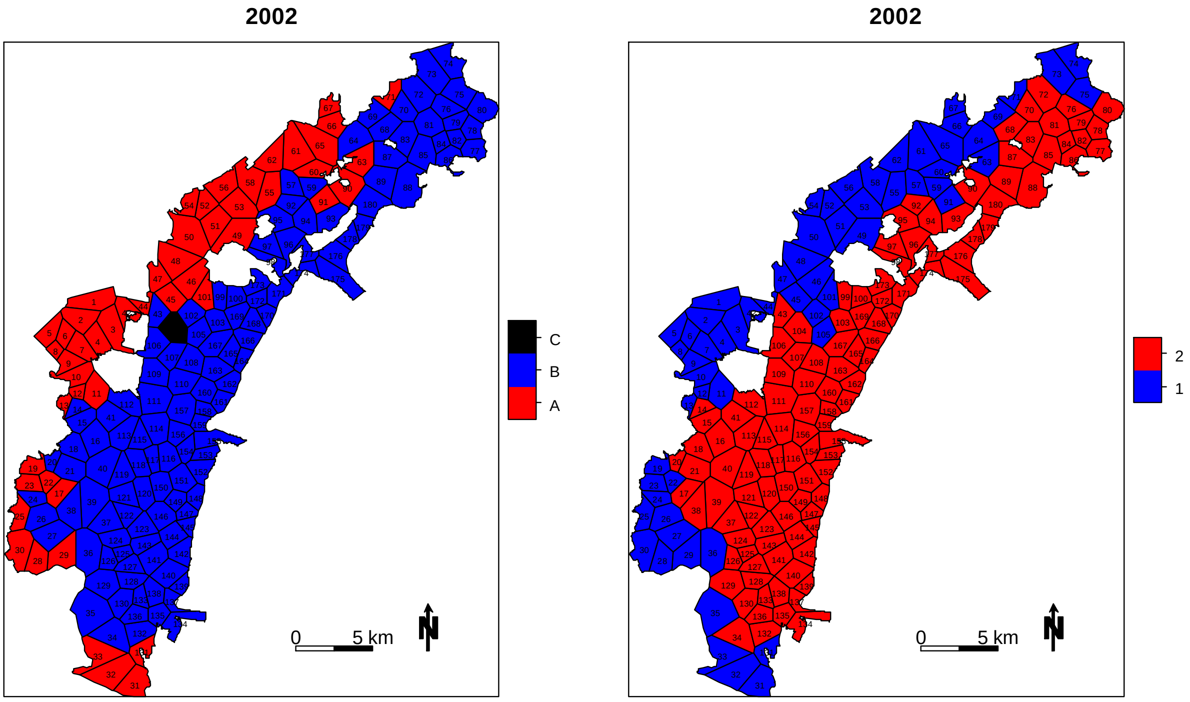


Figure 4.29: Voronoi maps of 2002 assemblages resulting from cluster analyses on 180-station (abundances). Macroscale: hierarchical cluster analyses (cutting value at dissimilarity = 0.83; left) and *k*-means (k=2, right).

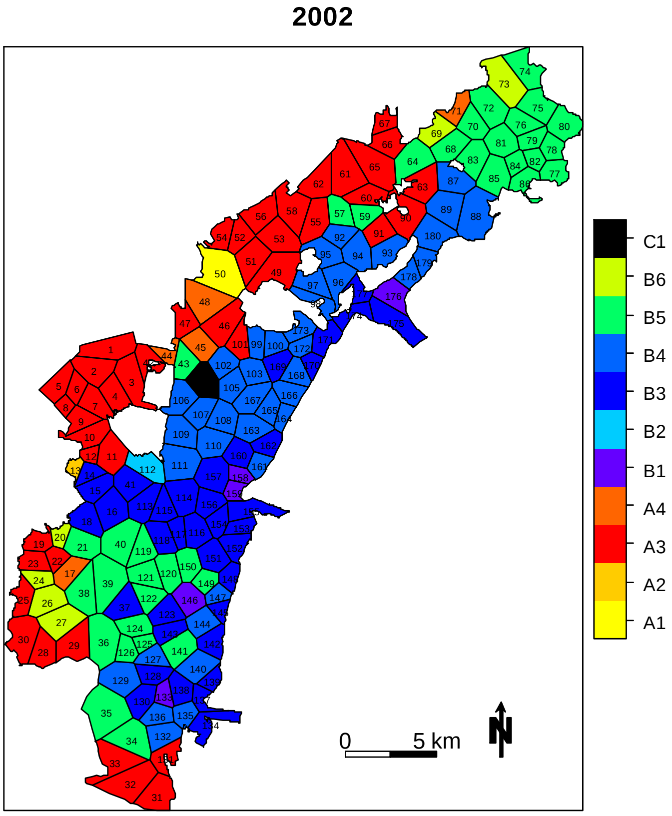


Figure 4.30: Voronoi map of 2002 assemblages resulting from hierarchical cluster analyses on 180-station (abundances). Mesoscale (cutting value at dissimilarity = 0.71).

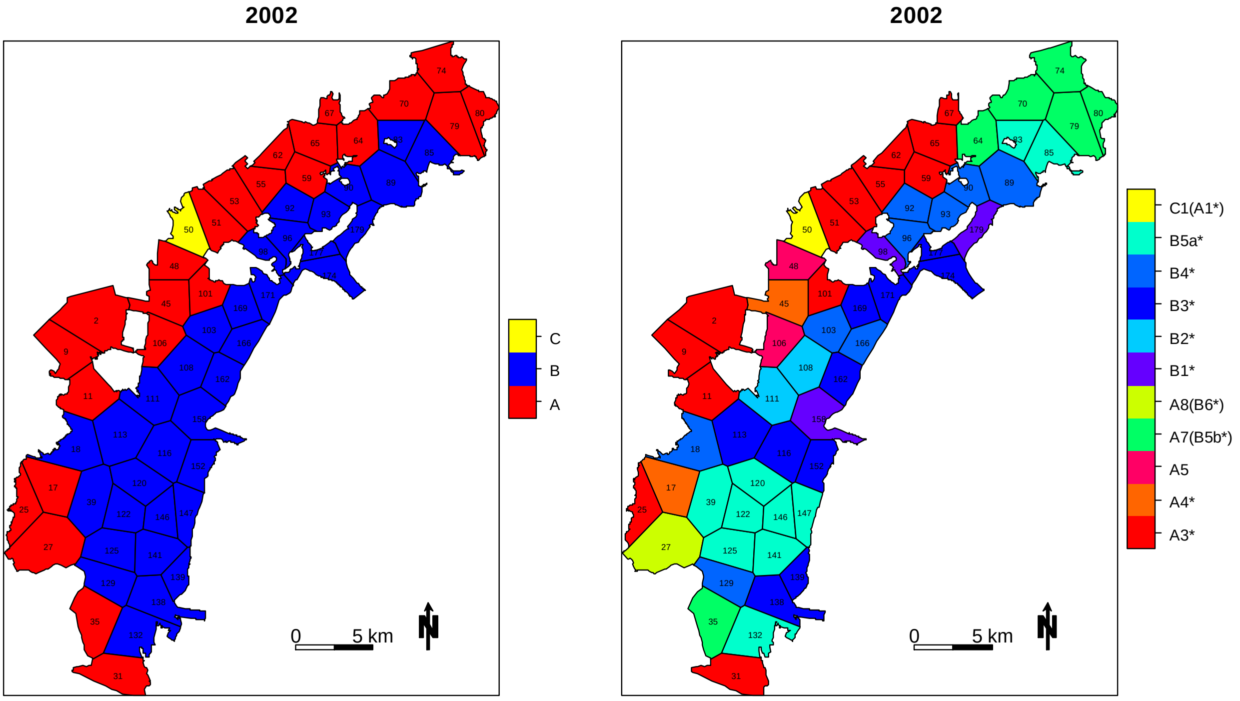


Figure 4.31: Voronoi maps of 2002 assemblages resulting from hierarchical cluster analyses on 3-year 59-station (abundances). Two hierarchical levels are showed: macroscale (cutting value at dissimilarity = 0.83; left) and mesoscale (cutting value at dissimilarity = 0.72, right).

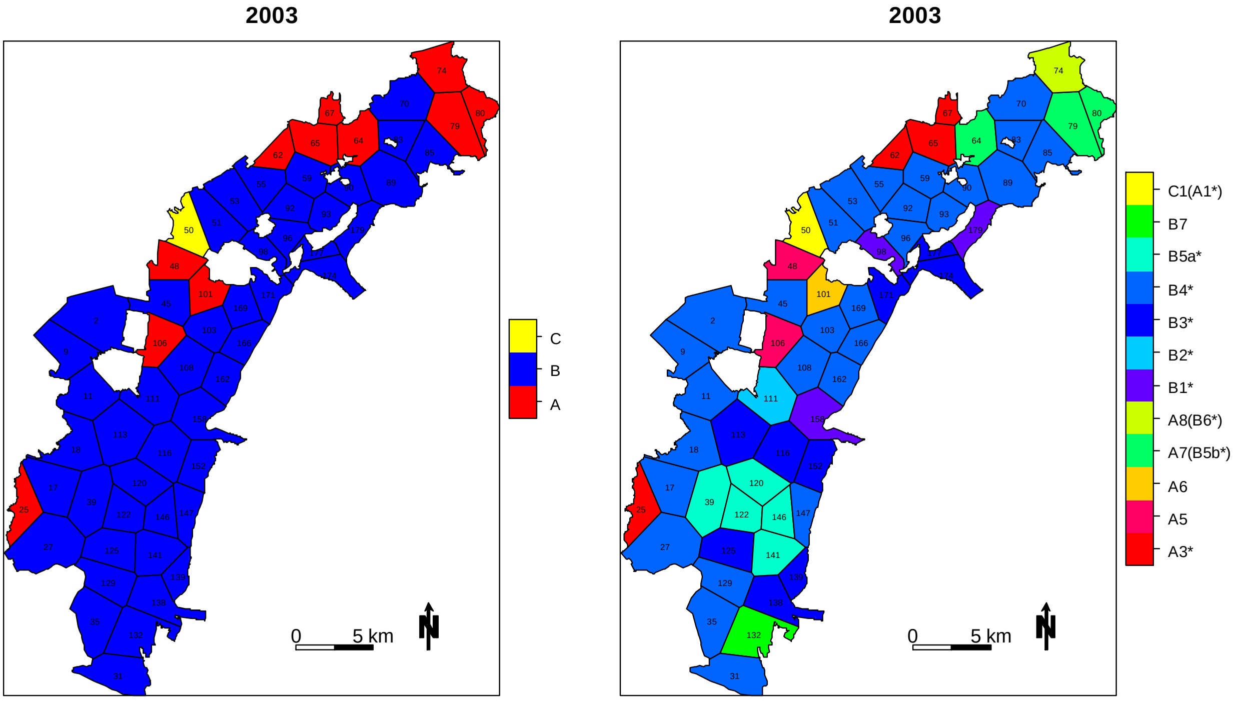


Figure 4.32: Voronoi maps of 2003 assemblages resulting from hierarchical cluster analyses on 3-year 59-station (abundances). Two hierarchical levels are showed: macroscale (cutting value at dissimilarity = 0.83; left) and mesoscale (cutting value at dissimilarity = 0.72, right).

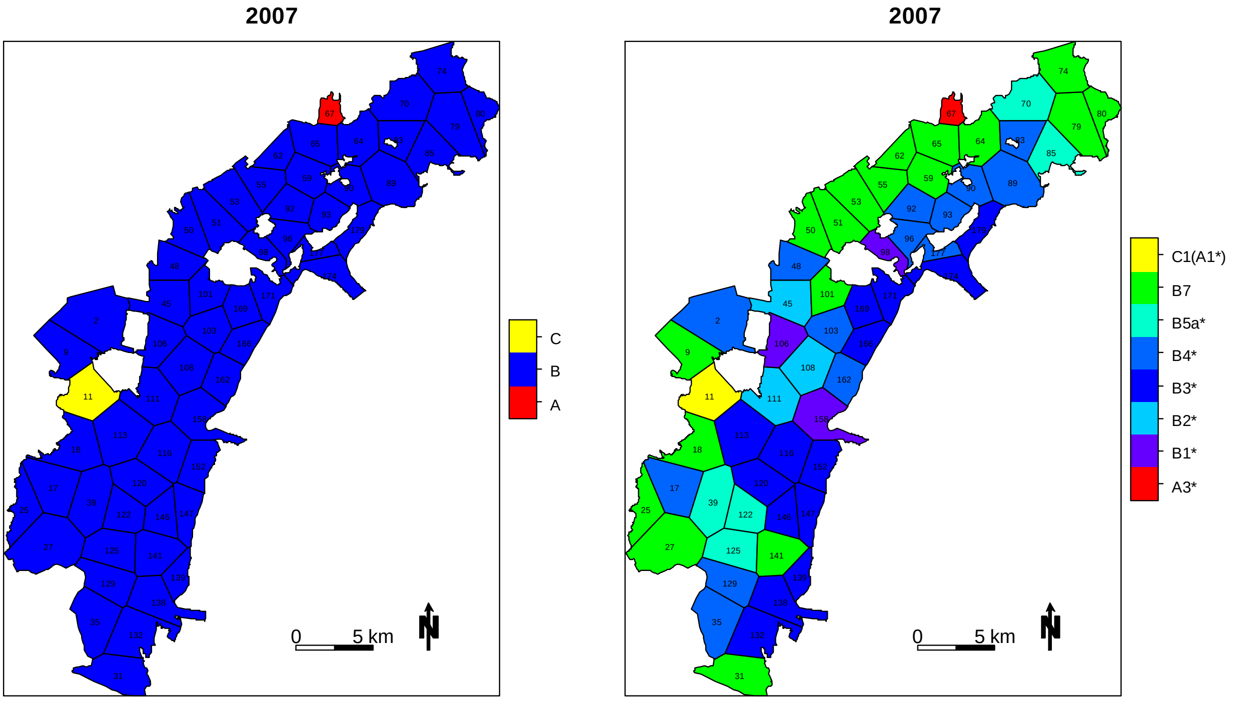


Figure 4.33: Voronoi maps of 2007 assemblages resulting from hierarchical cluster analyses on 3-year 59-station (abundances). Two hierarchical levels are showed: macroscale (cutting value at dissimilarity = 0.83; left) and mesoscale (cutting value at dissimilarity = 0.72, right).

Similarity among stations is to a certain extent preserved on the 59-station results for the same year. In particular, clusters B3* and A3* corresponds to B3 and A3 respectively. B4 divides into B4* and B2*, which also bears resemblances to B2. B5 presents transitional conditions with "A" assemblages, and in fact is divided between A7(B5b*) and B5a* when considering 3-year analysis. B5a* also includes stations from B4. The anomalous isolated cluster in 59-station dendrogram includes station 50 in 2002 and 2003, which corresponds to A1 in 180-station analysis and is named after it as C1(A1*). B6 can be still recognized as the single-station assemblage A8(B6*). A5 is introduced in 59-station analysis including stations proceeding from A4 and B4. A cluster in 59-station analysis can be associated to B1, and named after it (B1*), despite a single station is shared, due to the very strong resemblance in the characteristic spatial pattern, as a transition assemblage between the "marine" assemblages B3/B3* and central basin assemblages. Station 104, which presents an anomalous assemblage (C1), is not part of the 59-station subset.

Strong changes can be noticed over the years following results of the 59-station cluster analysis, as for the macroscale patterns. An assemblage (B7) is observed at a single station in 2003 which doesn't bear resemblances with any 2002 assemblage. It is located in the dendrogram at an intermediate dissimilarity level between "A" assemblages and other "B" assemblages. In 2007, B7 is the predominant cluster in the inner part of the Lagoon, substituting almost completely the "A" assemblages (Figure 4.33). Actually, it indicates that inner Lagoon assemblages variation (i.e. the dissimilarities range) over the years is stronger than outer-inner Lagoon dissimilarity, also due to lower richness, and two groups of assemblages related to Restricted and Open Lagoon could still be recognized. In 2003, stations 48 and 105 maintain their A5 structure, and another new assemblage (A6) appears at station 101, but the Lagoon heterogeneity is reduced by the spread of B4* assemblage, which cover the 47% of the stations. Both A5 and A6 clusters are no more recognizable in 2007, when only eight clusters are identified. "Marine" assemblages (B3* and B1*) are more stable over the years.

Each assemblage for both the cluster analyses (2002 180-station and 3-year 59-station) was characterized in terms of main macrodescriptors (abundances, biomass and richness, as mean and media values per assemblage) and taxonomic and trophic structure. Statistics for main macrodescriptors are summarized by a bubble plot in Figure 4.34 and 4.34. Statistical distribution of richness generally approaches normality, whereas abundances and biomass distribution show skewness, so median values for these two macrodescriptors are also plotted on the graph (which also gives a raw indication of dispersion). When the cluster is composed by a single station, mean and median coincide. Clusters are characterized by a great variability in the number of stations (Table 4.14). The number of stations composing mesoscale clusters in 2002 180-station analysis varies between 1 and 44, whereas 3-year 59-station cluster analysis group up to 28 stations for a single year. Single-station clusters are in total 4 (out of a total of 11 clusters) in 2002 for the 180-station analysis, and, for the three-year analysis, 2 (out of 11) in 2002, 5 (out of 12) in 2003 and 2 (out of 8).

When taking in account cluster analysis on 2002 180-station, assemblages related to Open Lagoon ("B") are in general characterized by high richness (Figure 4.34), except for B6 and, to a lower extent, B5, which in the succession along the coenocline can be both considered as a transition to the "A" assemblages (Figure 4.30). Abundances and biomasses of B6 are both dominated by suspension-feeder Bivalvia (Figure 4.38). B3 and B4 are characterized by high biomass (Decapoda and Mollusca) and abundances (Amphipoda and Sedentaria), and B2 by high biomass only (*Upogebia pusilla* predominates, nevertheless the cluster includes only one station). B1, which bears resemblance in structure with B3 and B4 (Figure 4.36), shows instead lower values for the macrodescriptors, with abundance values quite well-distributed among groups but with a predominance of bivalves (also presenting high biomass percentages) and Sedentaria. A3 presents quite high abundances, which are subdivided among Amphipoda, Sedentaria and Errantia, but relatively low biomass and low richness. A4 and A1 present the highest biomass among "A" assemblages, due to Bivalvia primary contribution and, secondarily, to Errantia in A1 and Sedentaria in A4 (which determines a very high biomass percentage of suspension-feeders), with in any case A4 presenting a strong right-skewed distribution in biomass. A1 is also characterized by the higher registered abundances, due to bivalves and secondarily to amphipods. More anomalous structure can be observed for A2 (abundances dominated by Gastropoda) and C1 (both abundances and biomass dominated by Amphipoda) which are probably characterized by site-specific conditions. Tanaidacea are particularly abundant in B4.

Absolute importances, both abundances and biomass, show a strong variability among years not only considering the whole system but also considering each cluster. Assemblages resulting from cluster analysis on the 3-year data set are described as a whole in Figures 4.35, 4.37 and 4.39. Some raw comparison can still be made with results from 2002 180-station data set analysis. B3* and B4* present similar characteristics (macrodescriptors and taxonomic and trophic structure) to 2002 180-station analogous (B3 and B4), despite an increase in mean importances. B5a* and A7(B5b*) also bear strong resemblance to B5. A strong increase from 2002 analogous was instead registered in B1* for all the descriptors, while stable in terms of trophic and taxonomic structure and number of stations (Table 4.14). Despite the denomination, B2* presents only secondary similarities with B2, which is composed by a single station not included among 59 stations subset. In particular, B2* has stronger mean values for all the macrodescriptors and a more balanced taxonomic and trophic structure, intermediate among B1* and B3* (with a major role of Errantia) (Figure 4.37). Station 50 belong to single-station cluster A1 in cluster analysis on 180 station and to the cluster C1(A1*) in cluster analysis on 59-station. C1(A1*) maintains its characteristics through the years and includes again station 50 in 2003 and station 11 in 2007). A3* maintains its characteristics when comparing with 180-station results as well as over the years. A4* shows instead a strong increase in mean biomass with respect to A4*, but with a similar taxonomic and trophic structure. The assemblage identified as B7*, which at first appears in 2003 and spreads all over the Lagoon in 2007, presents high importances, as well as moderately high richness, that brings it closer to B3* and B4* (Figure 4.35). B7 is characterized by high Bivalvia (and Gastropoda) biomasses and Amphipoda abundances; a high number of individuals belongs to *Animalia caetera*. Trophic structure in particular is more similar to "B" assemblages than "A" assemblages, with importances subdivided among suspension-feeder, surface deposit-feeder and herbivorous. Other clusters exclusive of the 59-station cluster analysis, A5 and A6, presents low importances and richness, similar to A2 and B5 (2002 180-station results), however A5 taxonomic structure, dominated by bivalves, is very similar to B6 (2002 180-station results), and A6 (despite higher abundances of Tanaidacea) to A2, although they have different geographical locations. A8(B6*), which is dominated by bivalves and related to B6 in terms of structure, is characterized by extremely high biomasses (Figure 4.35).

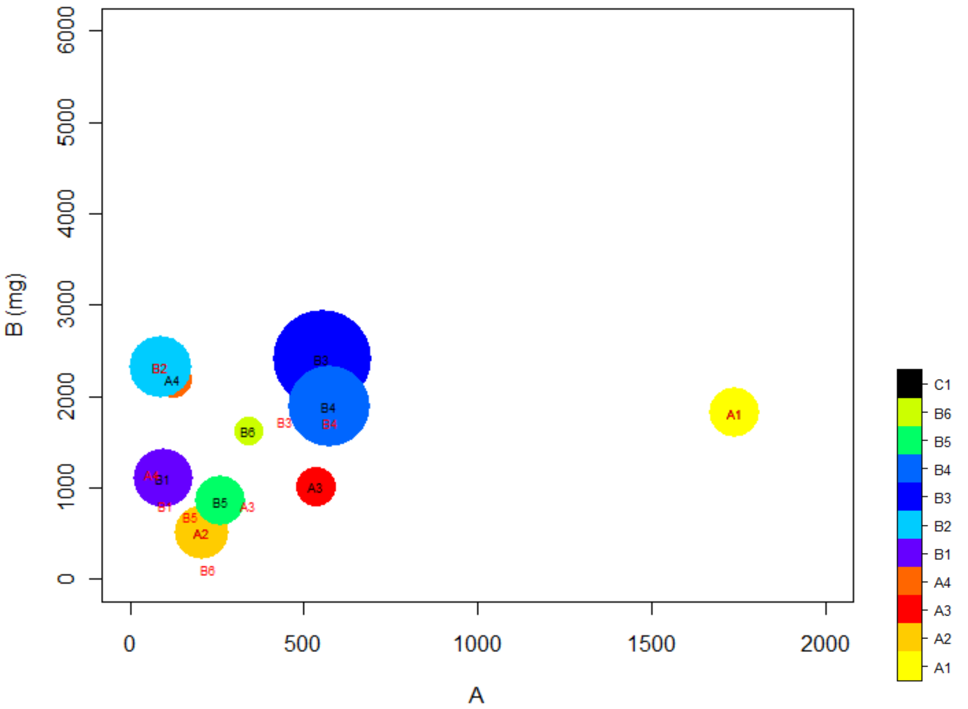


Figure 4.34: Mean abundances, biomass and richness for assemblages identified by cluster analysis on 2002 180-station data set. Species richness is proportional to the radius of circles. Red tags: median value of abundances and biomass; when median coincides with mean values the cluster is composed by a single station. C1 cluster (station 104) not included in the graph (A = 5013 individuals, B = 512.41 mg; S = 15.00).

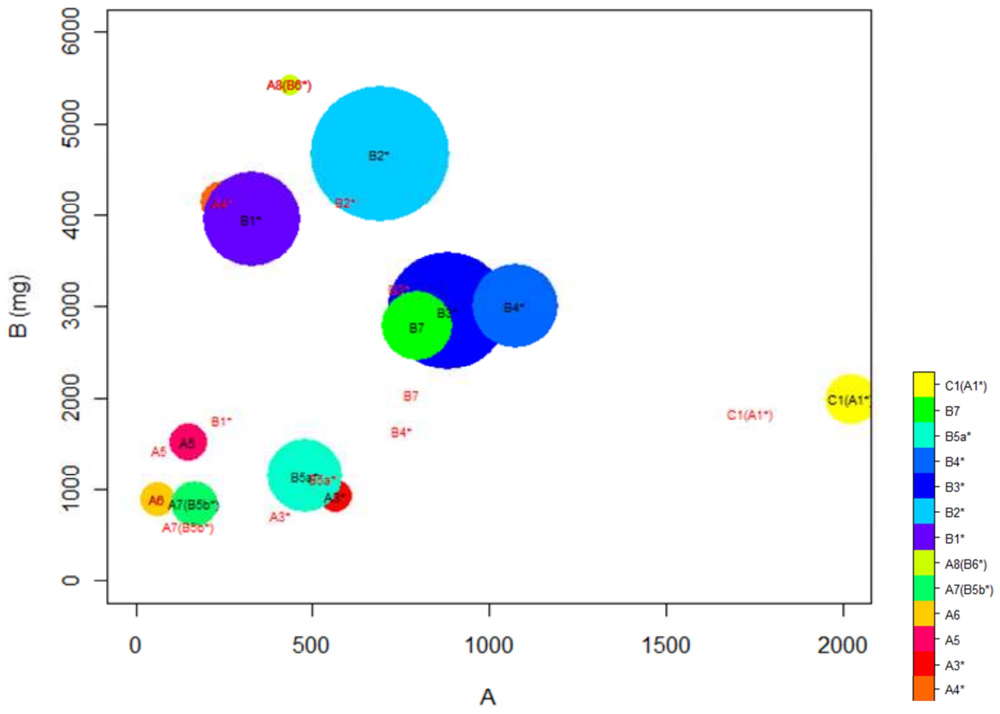


Figure 4.35: Mean abundances, biomass and richness for assemblages identified by cluster analysis on 3-year 59-station data set. Species richness is proportional to the radius of circles. Red tags: median value of abundances and biomass; when median coincides with mean values the cluster is composed by a single station.

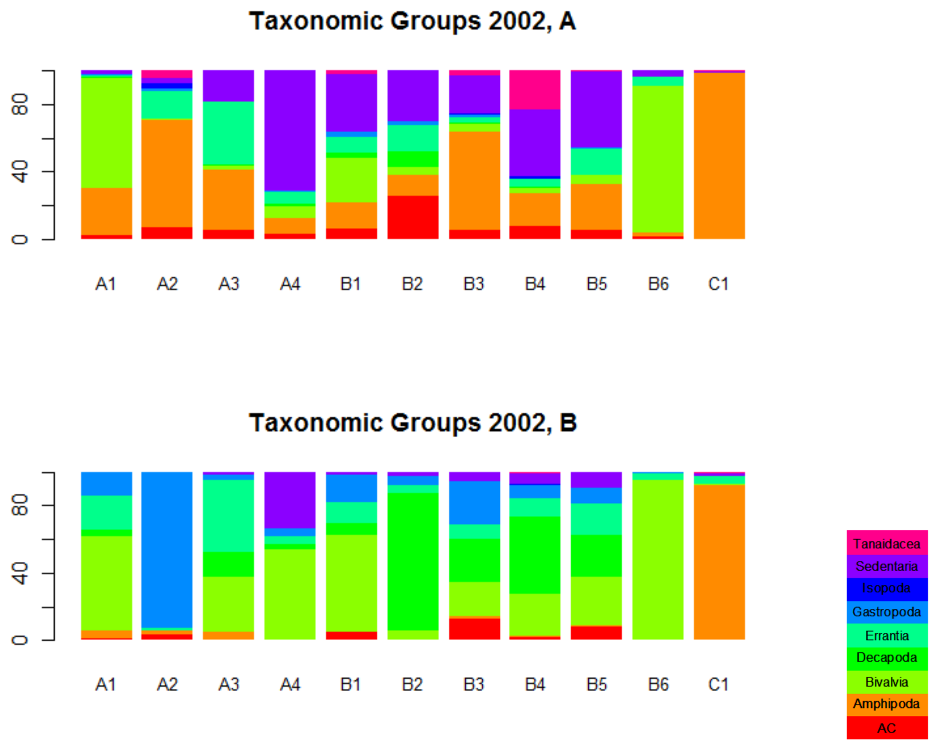


Figure 4.36: Taxonomical structure as percentages of abundances (top) and biomass (bottom) for assemblages identified by cluster analysis on 2002 180-station data set.

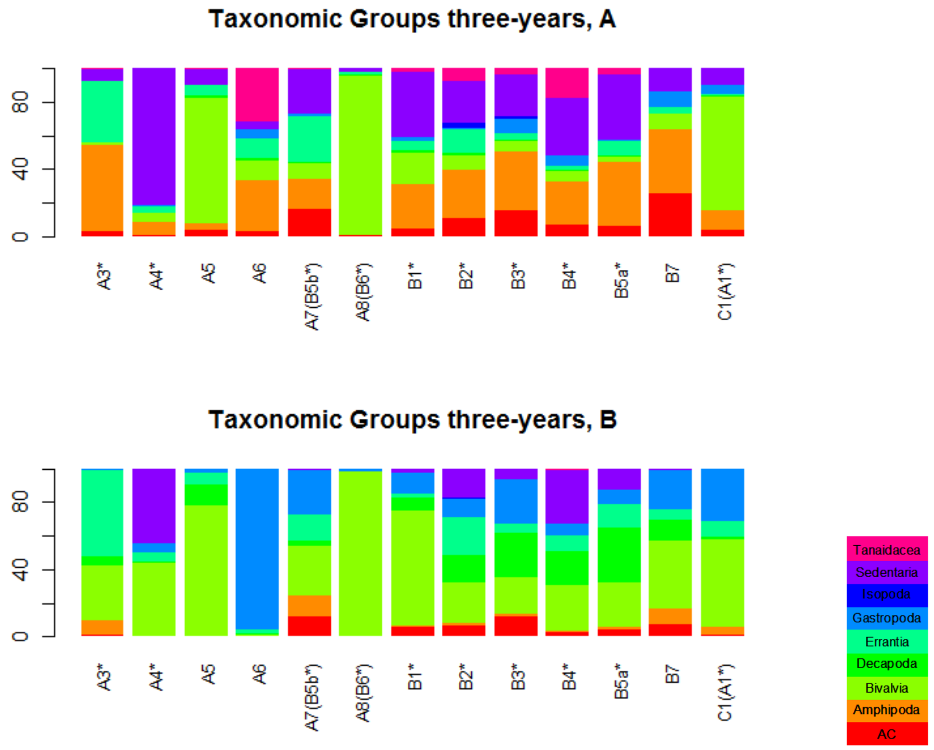


Figure 4.37: Taxonomical structure as percentages of abundances (top) and biomass (bottom) for assemblages identified by cluster analysis on 3-year 59-station data set.

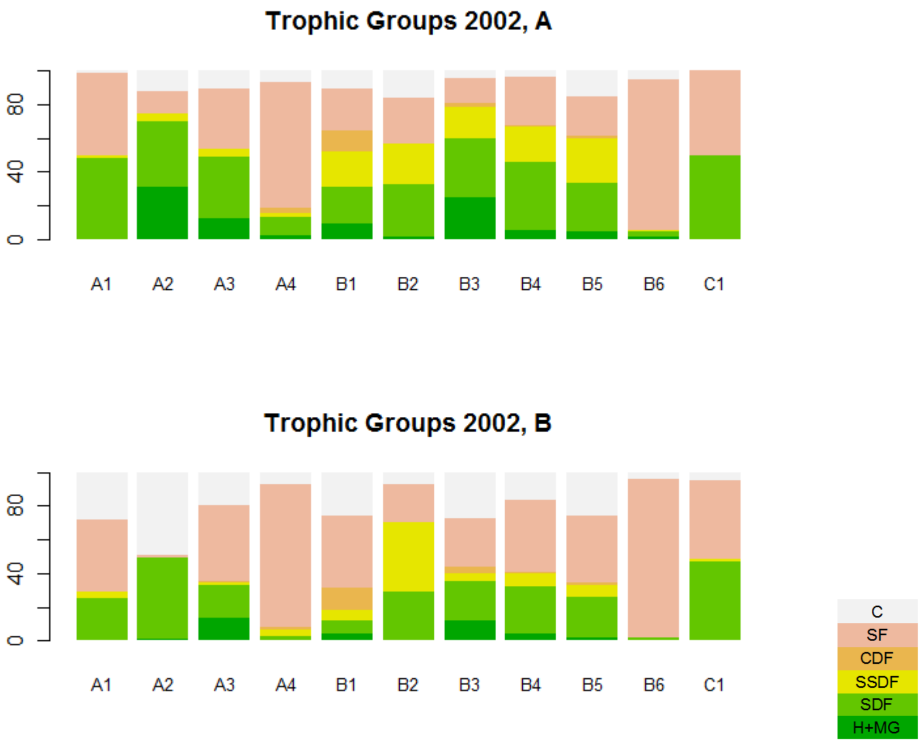


Figure 4.38: Trophic structure as percentages of abundances (top) and biomass (bottom) for assemblages identified by cluster analysis on 2002 180-station data set.

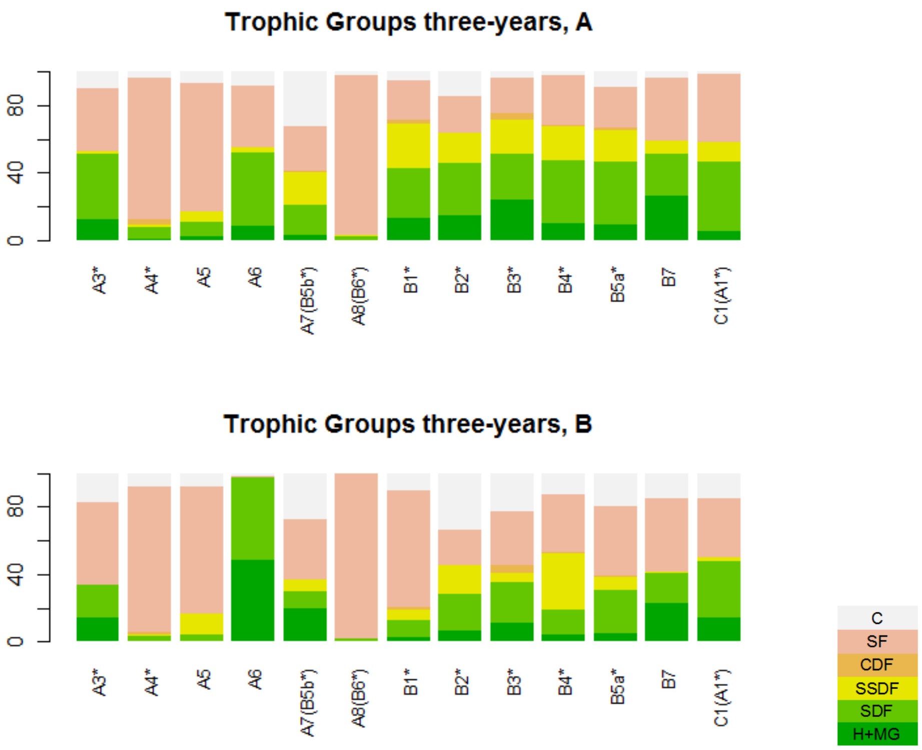


Figure 4.39: Trophic structure as percentages of abundances (top) and biomass (bottom) for assemblages identified by cluster analysis on 3-year 59-station data set.

4.2.7 Ordinations constrained on basins, hydrogeological zones and years

Relationships between benthic assemblages and the basins, zones and years, which was verified as statistically significant by PERMANOVA analysis, were more deeply analyzed with Canonical Analysis of Principal Coordinates (CAP), which find the axes through the multivariate data cloud having the strongest relationship with the given factors. CAP was performed on the abundances and biomass matrices for the 2002 180-station and 3-year 59-station dataset. The resulting plots of the first two axes for each CAP are shown in Figures 4.40 to 4.49. Each plot associates the stations to the level of the constraining factor for that ordination. The complex multivariate configurations of assemblages are hard to summarize in terms of species. In any case, the species that are more correlated with the first two axes were superimposed to the plot as an exploratory tool. Spearman rank correlation was used, and only linear or monotone relationships with the axes were highlighted.

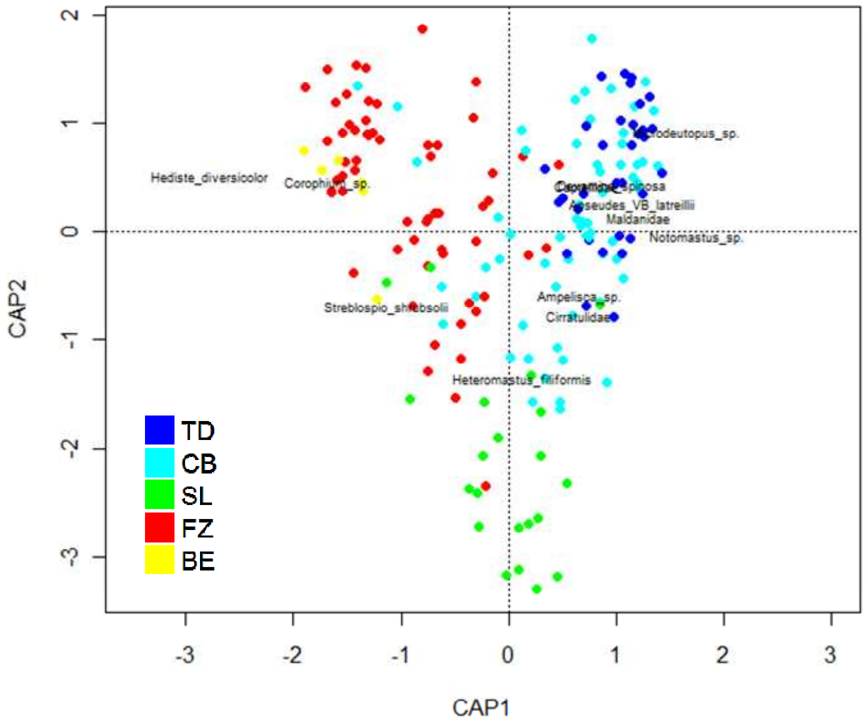


Figure 4.40: CAP ordination of 2002 180-station data set (abundances) ($p < 0.005$). Constraining factor: hydrogeological zones. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 68.57. Constrained inertia: 7.91 (11.5%). Inertia (variation) explained by first two axes: 6.64 (84% of the constrained inertia).

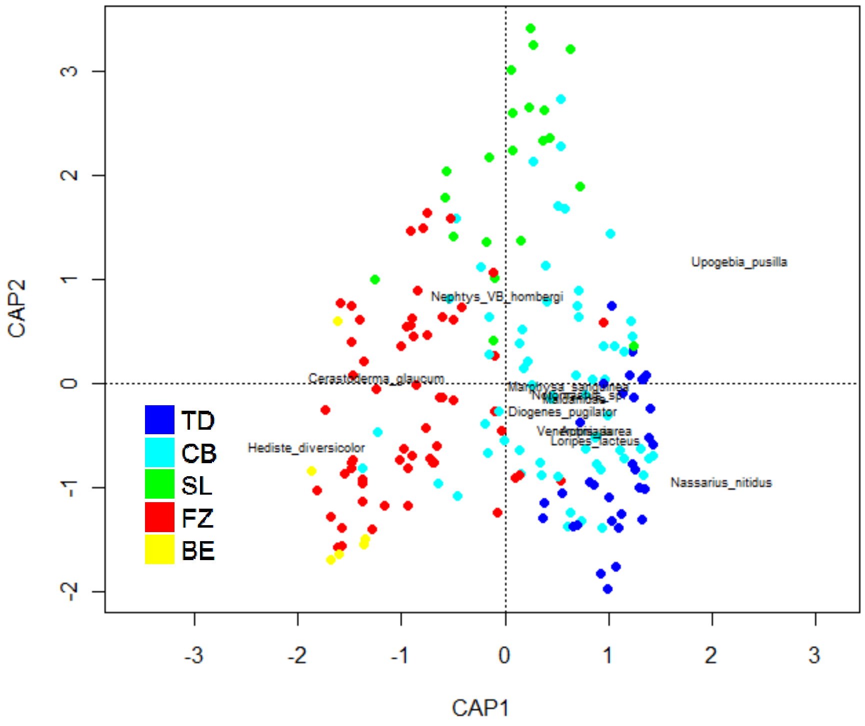


Figure 4.41: CAP ordination of 2002 180-station data set (biomass) ($p < 0.005$). Constraining factor: hydrogeological zones. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 72.77. Constrained inertia: 8.32 (11.4%). Inertia (variation) explained by first two axes: 6.77 (81%% of the constrained inertia).

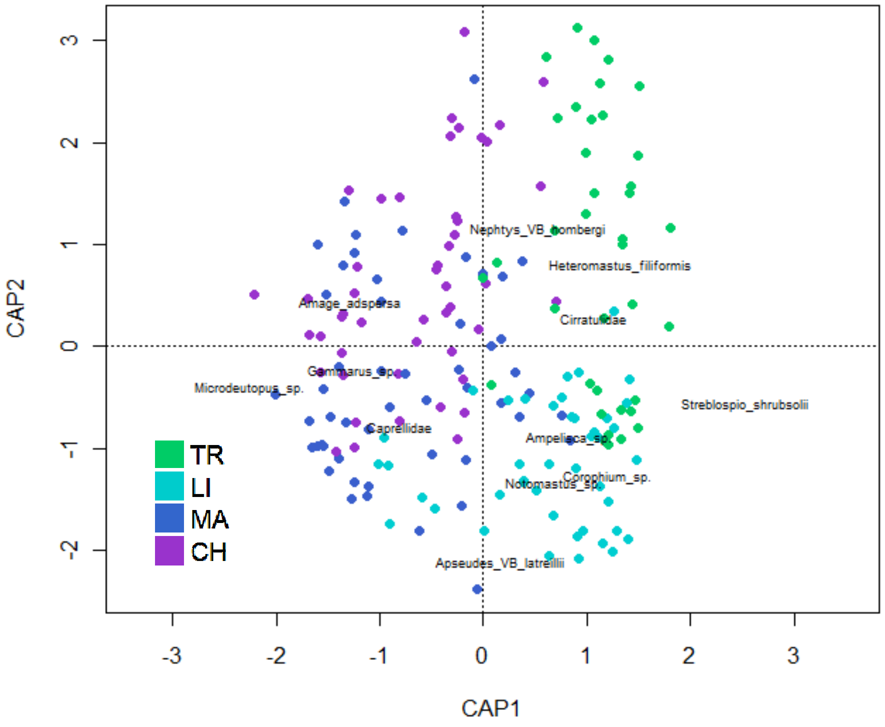


Figure 4.42: CAP ordination of 2002 180-station data set (abundances) ($p < 0.005$). Constraining factor: basins. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 68.57. Constrained inertia: 4.06 (5.9%). Inertia (variation) explained by first two axes: 3.49 (86% of the constrained inertia).

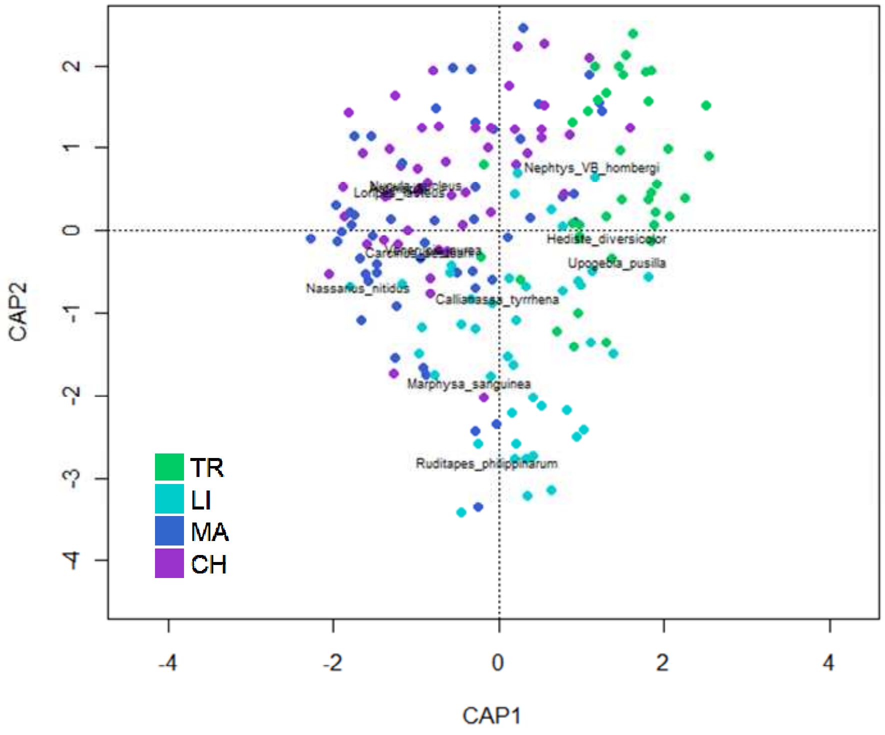


Figure 4.43: CAP ordination of 2002 180-station data set (biomass) ($p < 0.005$). Constraining factor: basins. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 72.77. Constrained inertia: 3.54 (4.9%). Inertia (variation) explained by first two axes: 3.04 (86% of the constrained inertia).

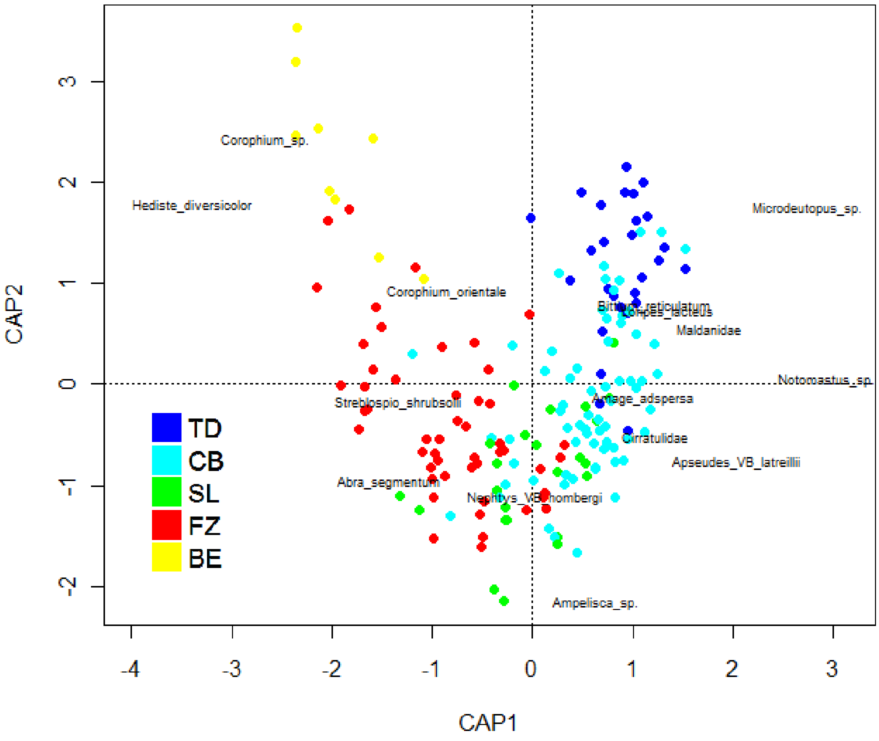


Figure 4.44: CAP ordination of 3-year 59-station data set (abundances) ($p < 0.005$). Constraining factor: hydrogeological zones. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 68.64. Constrained inertia: 5.93 (8.6%). Inertia (variation) explained by first two axes: 4.49 (76% of the constrained inertia).

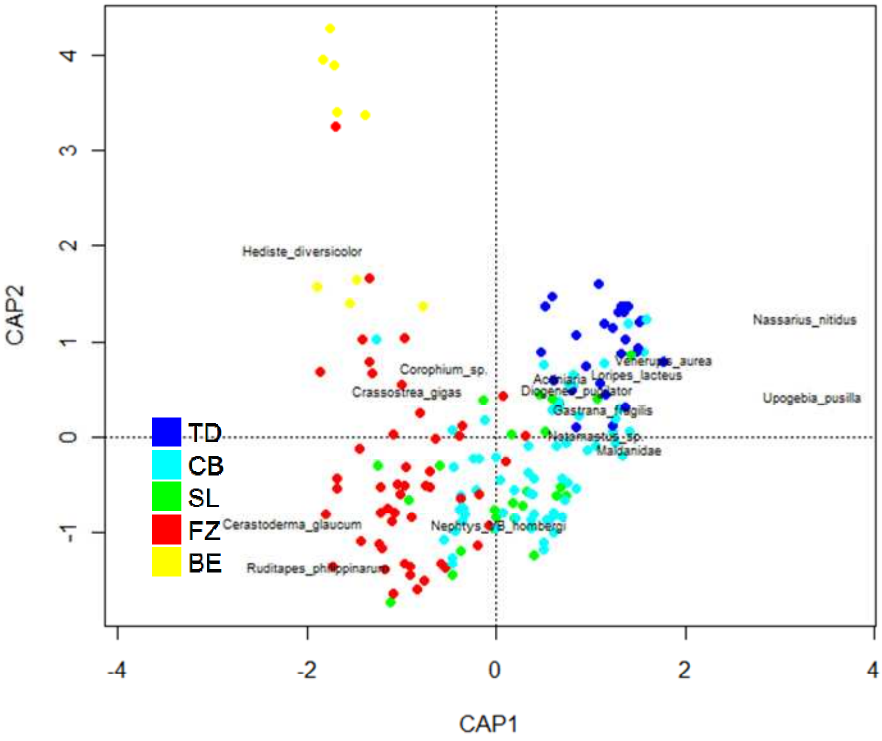


Figure 4.45: CAP ordination of 3-year 59-station data set (biomass) ($p < 0.005$). Constraining factor: hydrogeological zones. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 71.62. Constrained inertia: 6.65 (9.3%). Inertia (variation) explained by first two axes: 5.14 (77% of the constrained inertia).

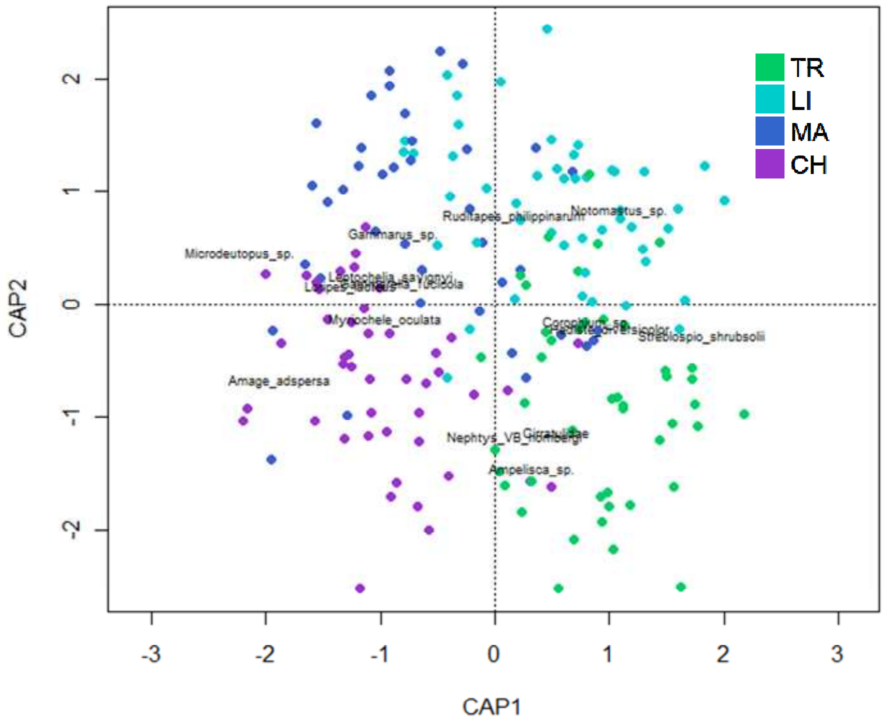


Figure 4.46: CAP ordination of 3-year 59-station data set (abundances) ($p < 0.05$). Constraining factor: basins. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 68.64. Constrained inertia: 3.04 (4.4%). Inertia (variation) explained by first two axes: 2.48 (82% of the constrained inertia).

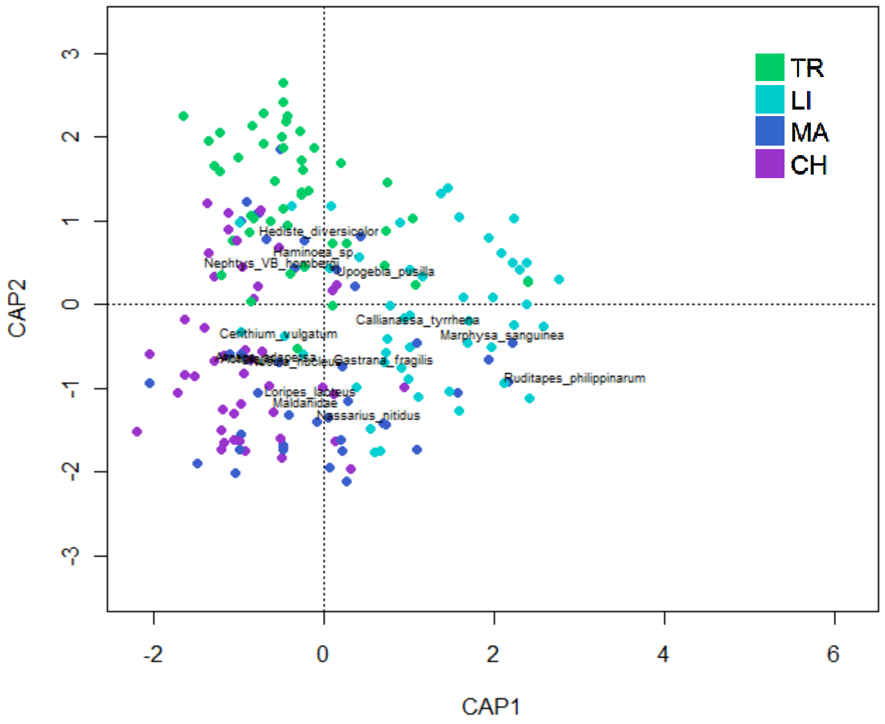


Figure 4.47: CAP ordination of 3-year 59-station data set (biomass) ($p < 0.05$). Constraining factor: basins. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 71.62. Constrained inertia: 3.24 (4.5%). Inertia (variation) explained by first two axes: 2.63 (81% of the constrained inertia).

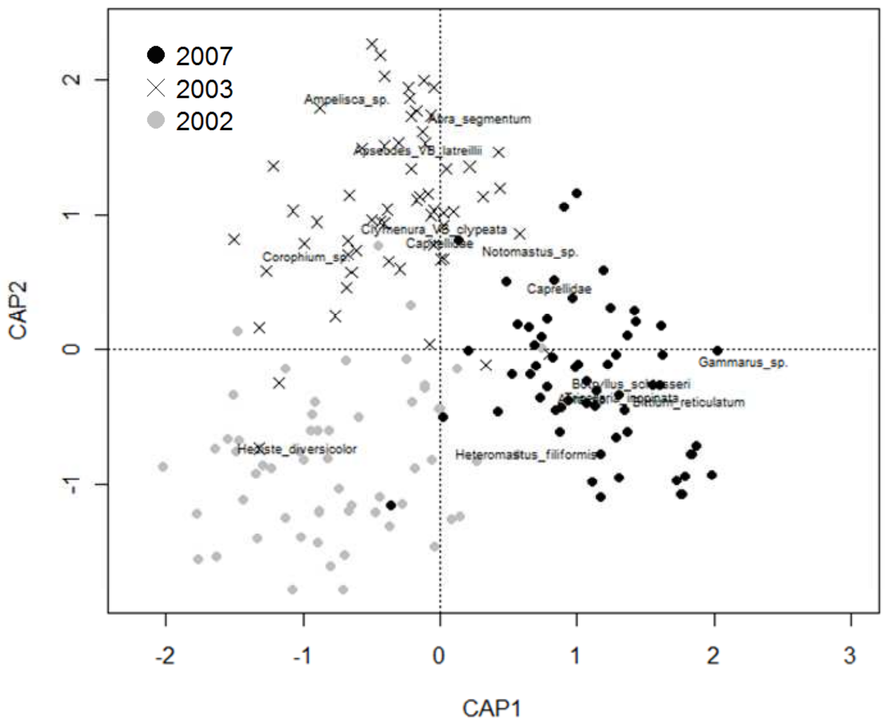


Figure 4.48: CAP ordination of 3-year 59-station data set (abundances) ($p < 0.05$). Constraining factor: years. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 68.64. Constrained inertia: 3.58 (5.2%). Inertia (variation) explained by first two axes: 100%.

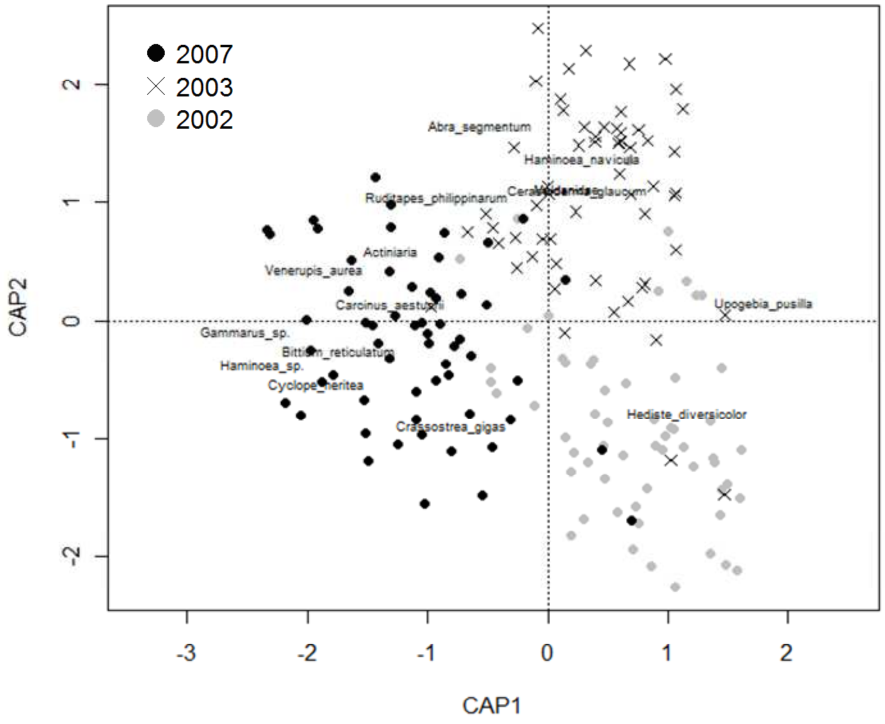


Figure 4.47: CAP ordination 3-year 59-station data set (biomass) ($p < 0.05$). Constraining factor: years. First two axes displayed. 5% of more correlated species are superimposed to the plot. Total inertia: 71.62. Constrained inertia: 2.40 (3.4%). Inertia (variation) explained by first two axes: 100%.

All the CAP analyses were found to be statistically significant ($p < 0.005$). The variation explained by first two axes, displayed in the ordination plots, is always high, from about 80% to 100% (for the three-levels factor year). Explained variation are generally very low. The highest explained variation is obtained when considering hydrogeological zones as factor, but, still, it reaches only the 11.5% for 2002 data set (8.6% for abundances and 9.3% for biomasses with regards to 3-year data set). In this case the coenocline which follows the succession of zones in the sea-land direction, from Tidal Delta to Bayhead Estuary, is displayed on the ordination plots. CAP performed by taking basins as the constraining factor gives an explained variation ranging from 4.4% to 5.9%. The same order of variation magnitude is explained by the factor year: years explain the 5.2% of the variation of the species composition matrix in terms of abundances and the 3.4% of the variation in terms of biomasses.

A CAP was performed on 2002 abundances, with the results of clusters analysis (i.e. assemblages) at mesoscale level as constraining factor, and the results subjected to a Procrustean analysis to visualize the relationship with hydrogeological zones, by plotting the variation in ordination patterns of the data cloud (Figure 4.50). Procrustes sum of squares is 0.32 and correlation-like statistic is 0.82 (1000 permutations, 0.001 significance). The relative position of stations along the first axis of the ordination constrained by zones, which reflects the "transitional gradient", is essentially preserved by the ordination on clusters. Major changes occur along the second axis, and in particular for stations classified as B4, B5 and B6 (and for some of the A3 stations). This can be interpreted as less "power" of zones to explain these assemblages, which are a transition between more "marine" assemblages and "confined" ones.

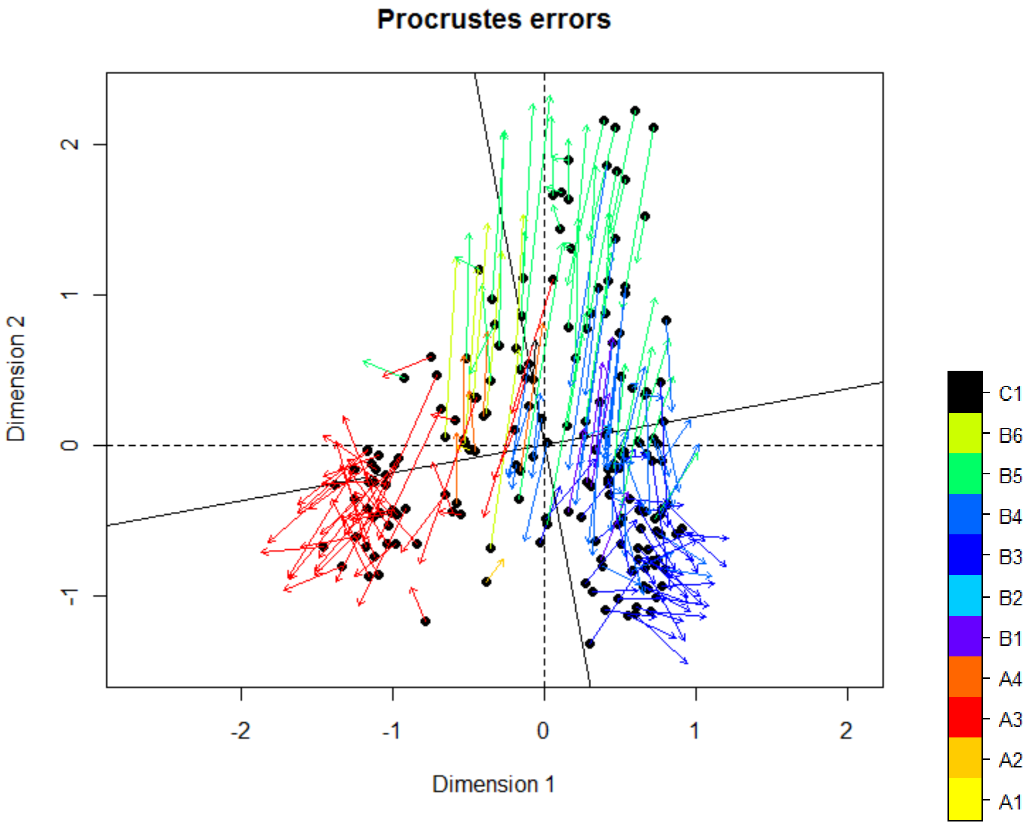


Figure 4.50: Results of procrustean analysis on CAP ordinations (first two axes) constrained by factors hydrogeological zones and mesoscale clusters on 180-station 2002 abundances (sum of squares: 0.32; correlation-like statistic: 0.82, based on 1000 permutations, 0.001 significance). Rotation of axes and configuration for the zones-constrained ordination are plotted. The configuration of station for the cluster-constrained ordination is showed by arrows, and identified by colours.

4.3 RELATIONSHIPS BETWEEN MACROZOOBENTHOS COMMUNITY AND ENVIRONMENTAL FACTORS AT LAGOON SCALE

4.3.1 Environmental variables data sets: exploratory analysis and collinearity

Considered environmental variables include descriptors of the sediment and the water column, presence of macrophytes, surface of intertidal areas and water residence time. Environmental data have been checked with exploratory techniques and spatialized through IDW interpolation. Main characteristics for each data set have been outlined on Chapter 3.2. Interpolated maps of selected environmental variables are presented in Appendix 5.

Sediment

Original granulometric data consist in percentage distribution of sediments among a number of operational particle size classes. Sand, silt and clay percentages were obtained by sum of fractions. Sand is defined (also operationally) by 1 mm boundary. Two classification approaches were explored to identify the more appropriate boundary between clay and silt: classical boundary at 4 μm and at 8 μm. Granulometric classes according to the two approaches were reported on ternary plots (Figure 4.51) and boxplots (Figure 4.52). Whereas 2002 and 2003 MELa2 data sets overlaps, 2007 MELa4 data set doesn't superimpose, in all probability due to different analytical methods. The 8 μm-based classification, which has a sedimentological meaning and improves the superimposition among years, was selected as the boundary between clay and silt. In any case a direct quantitative comparison among 3-year granulometry should be avoided, but relative spatial patterns among each year data set can still be compared. The sand fraction and, therefore, the pelite fraction (silt and clay) as the complement to 100, may be considered more robust for the comparison of granulometry. The increment of sand percentage at the expense of the pelite fraction is noteworthy. Moreover, in 2007 the stations previously rich in sand show an increase in the pelite fraction and vice versa, suggesting a redistribution of finer sediments towards a more homogenous distribution of granulometry across the lagoon. This can be observed in the percentiles represented by the "box" in the boxplot (Figure 4.52): interquartile range of sand in 2007 is 24.5%, considerably smaller than in 2002 (38.4%), and 2003 (46.1%).

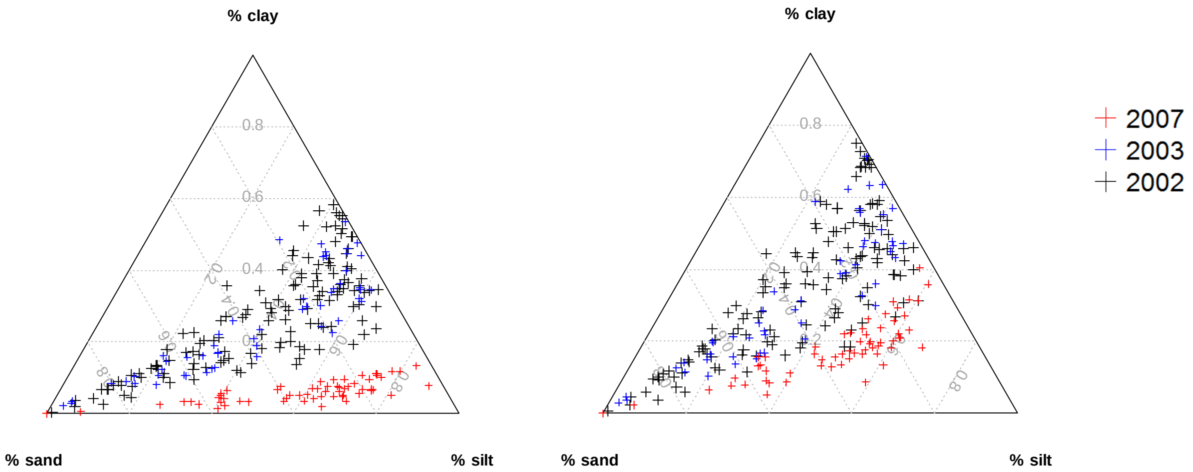


Figure 4.51: Ternary diagrams of granulometric classes sand, silt and clay: silt-clay boundary at 4 μm (left) and 8 μm (right).

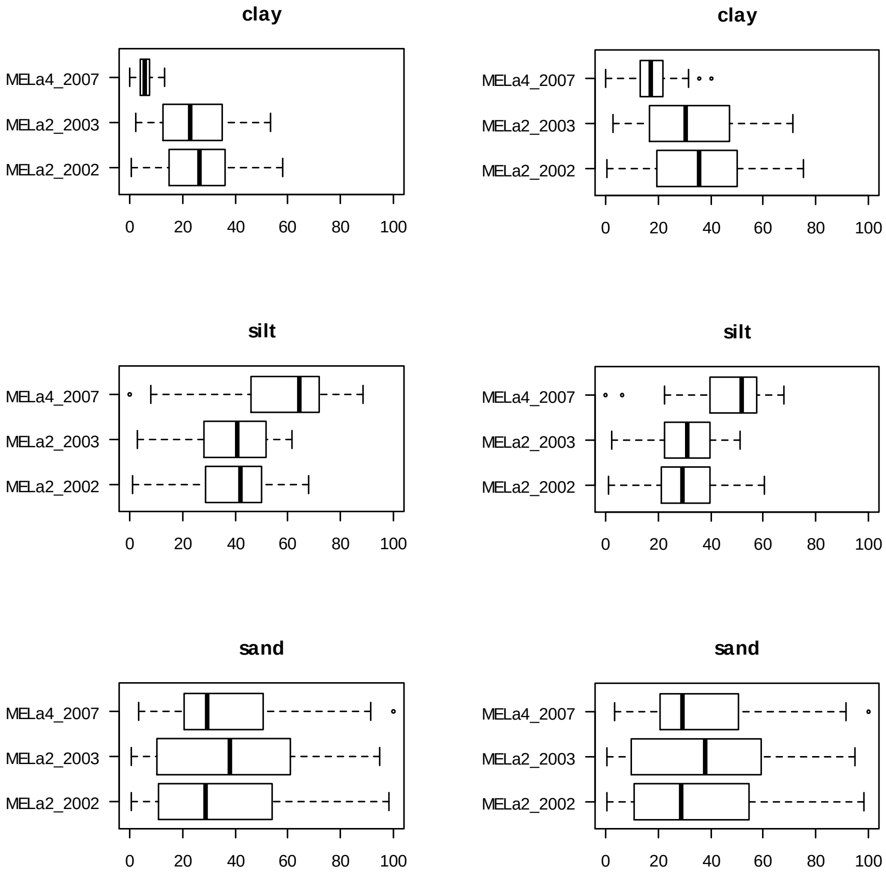


Figure 4.52: Boxplots of granulometric classes sand, silt and clay: silt-clay boundary at 4 µm (left) and 8 µm (right).

As for granulometry, a direct quantitative comparison among 3-year TOC (Figure 4.53) is precluded by the application of different analytical methods. In spatial terms, an explorative analysis reveals an unexpected variation in the distribution on TOC between the years 2002 and 2003, fairly consistent, and 2007, when, in the basins of Lido and Treporti, a depletion along the landward edge can be noticed and a simultaneous increase near the Lido inlet.

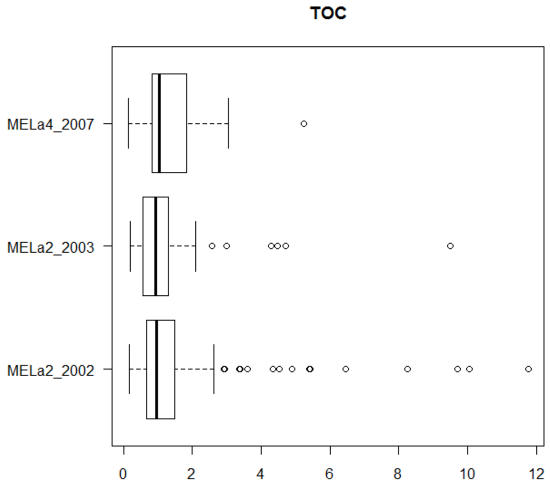


Figure 4.53: Boxplot of TOC on the 59-station 3-year data set

Macrophytes

The presence of macrophyte coverage at the sampling stations in the three years is shown in Figure 4.54. In 2002 48 out of 180 stations, i.e. 26.7%, (16 out of 59 stations, i.e. 27.1%) present a macroalgae coverage, and 42, i.e. 23.3% (12, i.e. 20.3%) a phanerogams coverage; 31 station, i.e. 17.2% (9, i.e. 15.3%) present both macroalgae and phanerogams. The two distribution overlap in particular in Malamocco and Chioggia basin. In 2003 and 2007 the number of stations with macroalgae coverage grow (respectively 23 and 33 stations), with phanerogams remaining approximately stable (respectively 10 and 11 stations). The number of stations with both macroalgae and phanerogams slightly lowers down to 8 in 2003 and 7 in 2007. This is also consistent with the more dynamic distribution patterns of macroalgae respect to phanerogams.

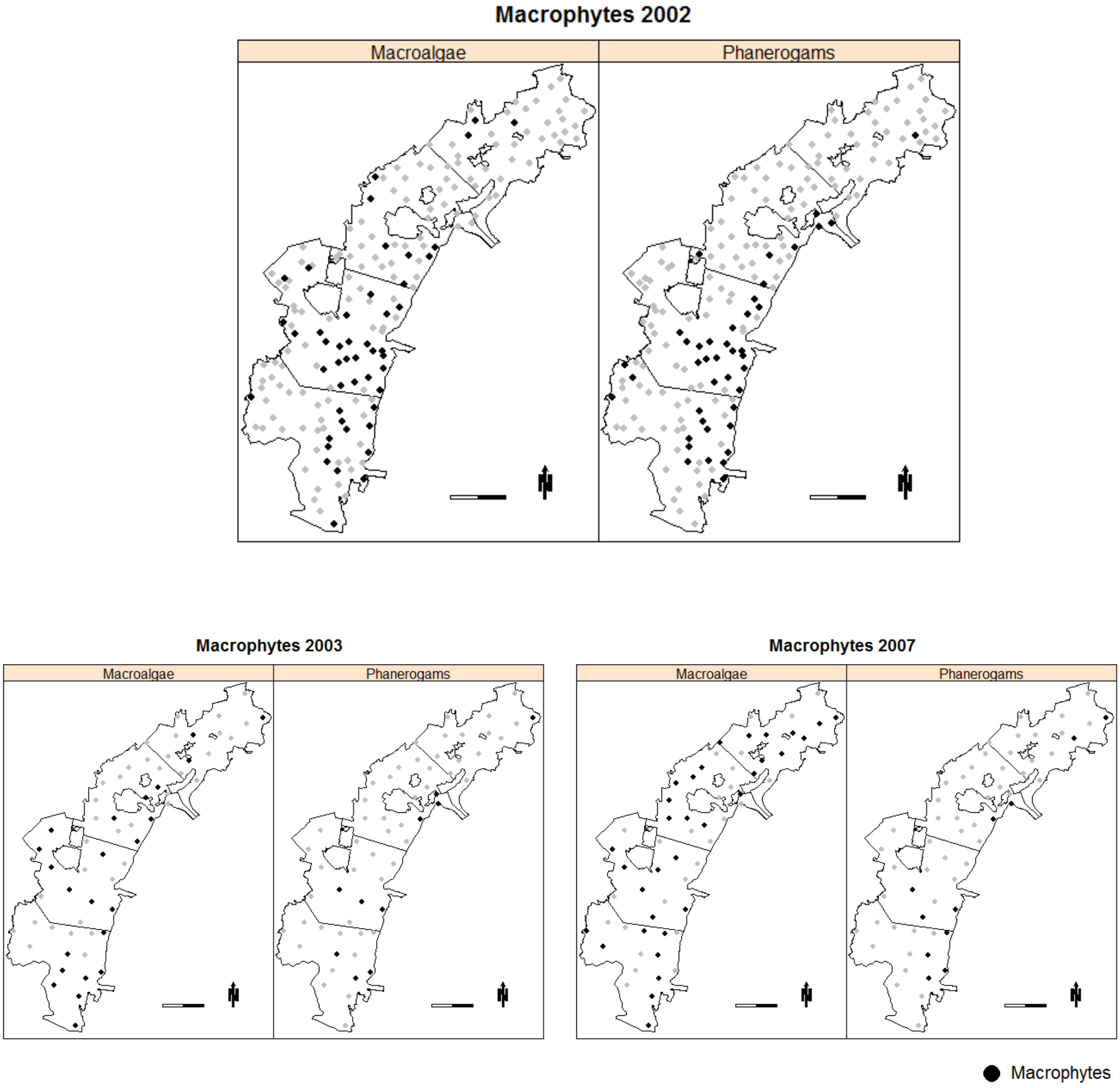


Figure 4.54: Stations with presence of macrophyte coverage.

Intertidal surface and hydrodynamics

The surface of neighbouring intertidal areas (between MLWS and MHWS) (Chapter 3.1.2), as well as water exchange, quantified through the residence time (MAG.ACQUE - Technital, 1993), has been considered constant during the five-year period (2002-2007). Values of these two variables for the 180 stations of MELa2 (i.e. a spatially homogenous sample on the interpolation map) are presented as histograms on Figure 4.55.

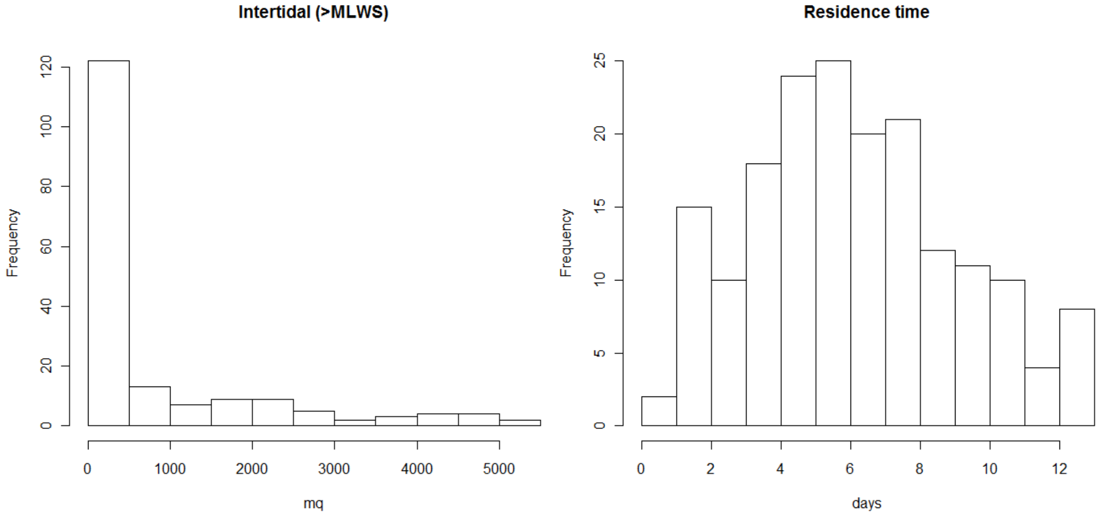


Figure 4.55: Surface of neighbouring intertidal areas (between MLWS and MHWS) (left) and residence time (right).

Hydrological variables

Six hydrological variables was selected and included in the analysis: salinity, temperature, dissolved oxygen (DO) concentration, chlorophyll *a*, total suspended solids (TSS) and, for the 2002 data set only, total alkalinity. Some variables present seasonal trends. Typical monthly trend of temperature is sketched in Figure 4.56. Its strong cyclic trend is the basis for the choice of mean value instead of median to represent the central tendency in 12-month time series. DO concentration also presents a seasonal trend which follows temperature. In the considered years, anoxic events (< 50% DO saturation) were never detected. Other non-cyclic patterns of variability (in absolute values and dispersion) can be recognized in monthly time series of salinity, TSS, chlorophyll *a* and alkalinity.

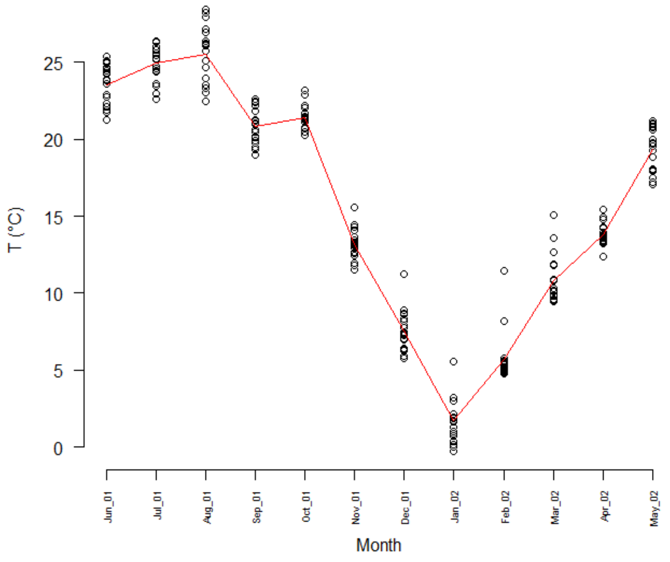


Figure 4.56: Yearly trend of temperature, based on the time series of 2001-2002, at the 20 sampling stations.

Average and dispersion (i.e. temporal variability) of selected hydrological variables have been calculated for the water column sampling stations on monthly time series (related to the 2002, 2003 and 2007 macrozoobenthos samplings) and introduced as new variables. Different monthly time series have been considered (see Chapter 3.2.5), with the introduction of different matrices of environmental variables. Collinearity has been analyzed on the data set composed by averages (median, except for temperature, for which mean value has been used) and dispersion (90% interpercentile range) calculated on the Jun2001-May2002 period for 20 station. A PCA of the 12 new variables is presented in Figure 4.57, and the Pearson correlation coefficient in Table 4.15. These two statistical tools have been used along with the analysis of the variance inflation factor (VIF; Table 4.16) to reduce the number of environmental variables.

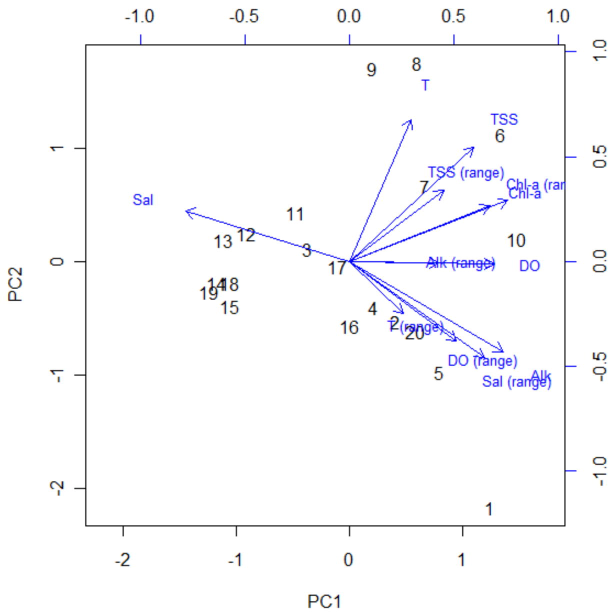


Figure 4.57: PCA of standardized hydrological variables (12-month data sets of 2002): averages and dispersion of temperature, salinity, TSS, DO concentration, chlorophyll *a*, total alkalinity. Proportion of explained variation: 61.3% (I axis: 43.8%; II axis: 17.5%).

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]
[1] mea_T_gC	1.00	-0.17	0.23	0.58	0.37	-0.08	-0.25	0.01	-0.19	0.10	0.62	0.16
[2] med_Sal_PSU	-0.17	1.00	-0.74	-0.37	-0.48	-0.89	-0.09	-0.80	-0.51	-0.19	-0.70	-0.37
[3] med_O2_ppm	0.23	-0.74	1.00	0.49	0.44	0.60	0.07	0.48	0.45	0.28	0.69	0.14
[4] med_TSS_mgL	0.58	-0.37	0.49	1.00	0.65	0.26	0.13	0.07	0.29	0.72	0.63	0.18
[5] med_Chla_ugL	0.37	-0.48	0.44	0.65	1.00	0.45	0.39	0.33	0.28	0.50	0.65	0.41
[6] med_Alc_Tot_uEqL	-0.08	-0.89	0.60	0.26	0.45	1.00	0.35	0.83	0.59	0.21	0.55	0.35
[7] r90_T_gC	-0.25	-0.09	0.07	0.13	0.39	0.35	1.00	0.20	0.25	0.33	-0.10	0.29
[8] r90_Sal_PSU	0.01	-0.80	0.48	0.07	0.33	0.83	0.20	1.00	0.52	0.04	0.53	0.41
[9] r90_O2_ppm	-0.19	-0.51	0.45	0.29	0.28	0.59	0.25	0.52	1.00	0.23	0.30	0.03
[10] r90_TSS_mgL	0.10	-0.19	0.28	0.72	0.50	0.21	0.33	0.04	0.23	1.00	0.38	0.26
[11] r90_Chla_ugL	0.62	-0.70	0.69	0.63	0.65	0.55	-0.10	0.53	0.30	0.38	1.00	0.38
[12] r90_Alc_Tot_uEqL	0.16	-0.37	0.14	0.18	0.41	0.35	0.29	0.41	0.03	0.26	0.38	1.00

Table 4.15: Pearson correlation coefficient of standardized hydrological variables (12-month data sets of 2002): averages and dispersion of temperature, salinity, TSS, DO concentration, chlorophyll *a*, total alkalinity.

A main strong ecocline (the "transitional gradient") was highlighted by PCA, corresponding approximately to the first axis (which alone explains the 44% of the variation). The averages are in some cases highly correlated to temporal variability ("range"), i.e. landward extremes values correspond to high variability. This is consistent to high direct correlation (0.72 for TSS and 0.65 for chlorophyll *a*) or high inverse correlation (-0.80 in the case of salinity, which in fact is the inverse of sea water dilution). Median salinity in particular shows a high correlation with a number of other variables, in particular with median total alkalinity (-0.89), median DO concentration (-0.74) and 90% interpercentile range of chlorophyll *a* (-0.70). DO concentration doesn't show high correlation with chlorophyll *a*. Median salinity was retained despite displaying initially the highest VIF. However, the elimination of other variables, highly correlated with mean salinity, lowered all the VIF down to a value of about 3 (Table 4.16). Order of variability magnitude of median and range among stations was also checked. Range of temperature was not retained in analysis as its absolute variability is low respect to variability of temperature mean. Mean temperature was retained. It presents higher values near the industrial zone of Porto Marghera (Figure 4.58). Mean alkalinity presents the strongest correlation (0.9) with mean salinity, whereas the range of alkalinity is not strongly correlated with any other variable, and was retained as an independent factor (Figure 4.58). Finally, the following seven variables were selected: average values of temperature, salinity, DO concentration, TSS, chlorophyll *a*; and range of DO concentration and total alkalinity.

	I	II
mea_T_gC	9.16	1.96
med_Sal_PSU	14.05	3.15
med_O2_ppm	4.33	2.69
med_TSS_mgL	10.57	2.78
med_Chla_ugL	3.70	2.16
med_Alc_Tot_uEqL	15.14	-
r90_T_gC	4.07	-
r90_Sal_PSU	5.60	-
r90_O2_ppm	2.57	1.88
r90_TSS_mgL	5.32	-
r90_Chla_ugL	13.34	-
r90_Alc_Tot_uEqL	1.90	1.43

Table 4.16: VIF of standardized hydrological variables (12-month data sets of 2002); I: all the variables (averages and dispersion of temperature, salinity, TSS, DO concentration, chlorophyll *a*, total alkalinity); II: retained variables.

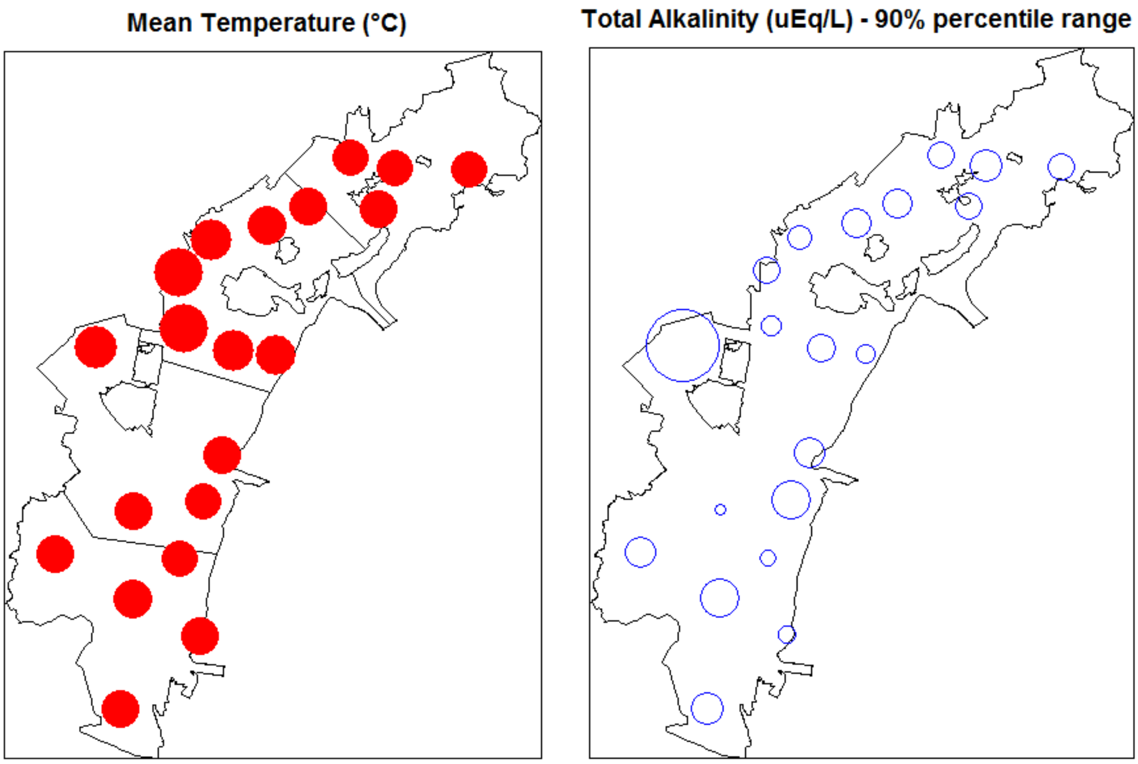


Figure 4.58: Spatial patterns of selected variables calculated on 12-month time series (June 2001 - May 2002): mean temperature (°C) (on the left) and 90% interpercentile range of total alkalinity (on the right).

Values of hydrological variables for macrozoobenthos sampling stations were extracted after interpolation (over different time spans). Boxplots for the 59-station 3-year data set (over a four-month time span) are presented in Figure 4.59. High interannual variability subsists for a number of variables. This can be either due to the very short available time span or to real interannual variability of environmental condition. Moreover, during the four-month period, five samplings were carried over in 2007. This in particular should be recognized as the cause of the strong temperature increase for 2007 and the higher spatial and temporal variability (as 90% interpercentile range) of DO percentage. As for sediments, also in this case a direct quantitative comparison among years should not be performed. However the relative spatial distribution among the three years could be still analyzed. Variability of meteorological and flood events among years are in all probability also responsible of the observed patterns.

Salinity presents a negative skewness. The three years are roughly comparable, with the median value slightly higher in the first months of 2002 (30.8 PSU) compared with 2003 (29.6 PSU) and 2007 (29.2 PSU). The mean temperature presents in all the three years a distribution approaching a normal, but the central tendency changes abruptly among the years, in particular in 2007 (probably due to one extra sample in the four-month period). TSS presents a very high variability among stations in 2002 respect to the other two years, and in 2007 it seems to decrease considerably (as well as turbidity, not shown). The DO concentration in 2007 presents a much greater variability among stations, both in mean and range. Chlorophyll *a* present a higher variability and central tendency in 2002 and then both decrease in 2003 and 2007.

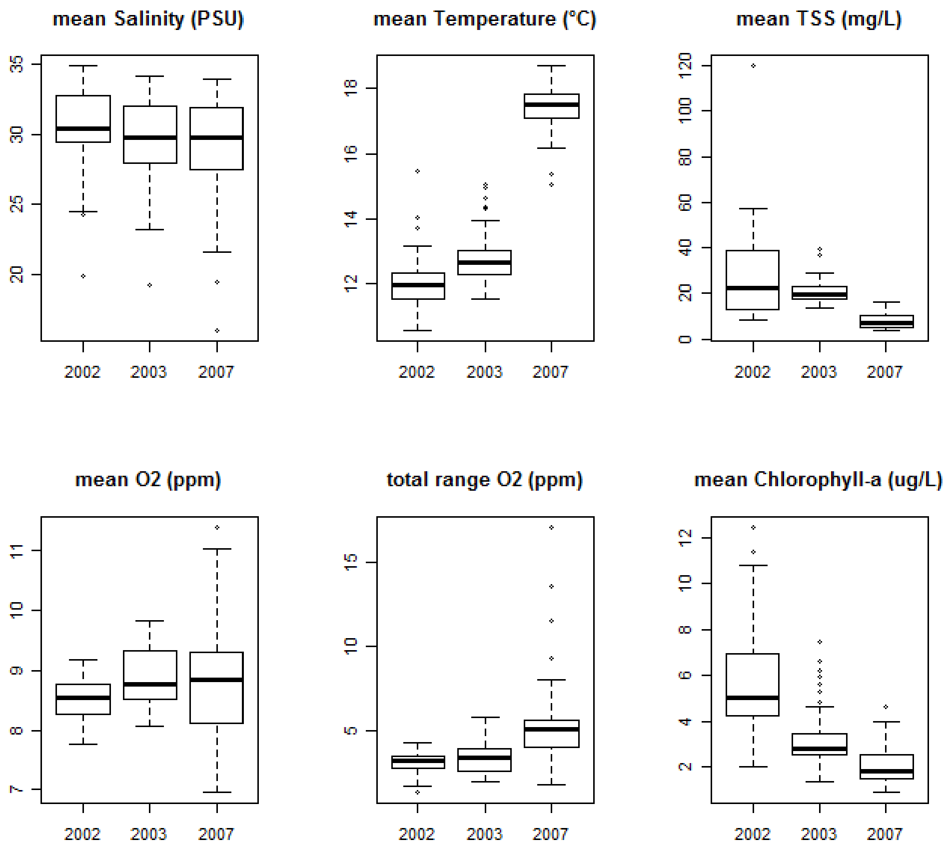


Figure 4.59: Boxplots of selected environmental variables for each year (four-month data set: February-May).

4.3.2 Operational data sets of environmental variables

Finally, 15 environmental variables were selected (Table 4.17) and values extracted after interpolation for all the 180 stations in 2002 and 59 stations for the three years, to be related to two community data sets. 90% interpercentile range of total alkalinity is available only for the 2002 data set. When explicitly mentioned, the clay percentage will be excluded from analysis as linear combination of the other two granulometric classes.

Non-parametric methods were preferred, but part of the analysis was performed with parametric methods requiring normal distribution. Normality was checked on both the 180-station 2002 data set and 59-station 3-year data set with statistical tests (Lilliefors and Shapiro-Wilk tests) and visual methods. Counterparts of data sets were then produced where transformations were applied to improve, if not to achieve, normality. Variables were transformed in the same way in the 180-station 2002 and 59-station 3-year data set. Choosen transformations include power transformation, square- and third-root transformation, and inverse transformation (Table 4.17). Presence of macroalgae and phanerogams is described by dummy variables and was not transformed. Also no appropriate transformation was found for intertidal surface, which has a very high number of zero values and resembles a count variable. Other untransformed variables presented already normality, except for range of total alkalinity, which was not transformed as normality is approached if outliers are not considered.

environmental variable	unit	transformation
Sand (1mm-63um)	% (on dry weight)	$x^{1/2}$
Silt (63-8um)	% (on dry weight)	-
Clay <8um	% (on dry weight)	$x^{1/2}$
TOC	% (on dry weight)	$x^{-1/2}$
Macroalgae	presence/absence	-
Phanerogams	presence/absence	-
Intertidal surface	m^2	-
Residence time	days	-
Average Salinity	PSU	x^3
Average Temperature	°C	-
Average DO concentration	ppm	-
Range of DO concentration	ppm	x^{-1}
Average Chlorophyll *a*	µg/L	$x^{1/3}$
Average TSS	mg/L	$x^{1/3}$
Range of Total Alkalinity	µEq/L	-

Table 4.17: Selected environmental variables, availability for different years and transformation to improve normality (if needed by statistical methods)

CAP analysis was used to highlight main yearly and spatial patterns in environmental variables. A first analysis using year as constraining factor on 59-station 3-year data set evidences the issue regarding comparability between MELa2 and MELa4 data sets (Figure 4.60). The first axis alone account for the 20% of variation explained (the 90% respect to the plotted axes). The second axis, despite still statistically significant, explains only the 2% of the variation. Most of hydrological variables show very high scores for the first axis, in particular mean temperature. However, if temperature variation among years is probably a bias due to samplings considered in different years, mean chlorophyll *a* and mean TSS, which also show very high scores, are probably related to true environmental signals. Also silt and clay are aligned along the first axis, while sand is not (at the same time as silt and clay taken together, i.e. pelite), which suggests to consider sand (as well as pelite) a more robust descriptor of granulometry.

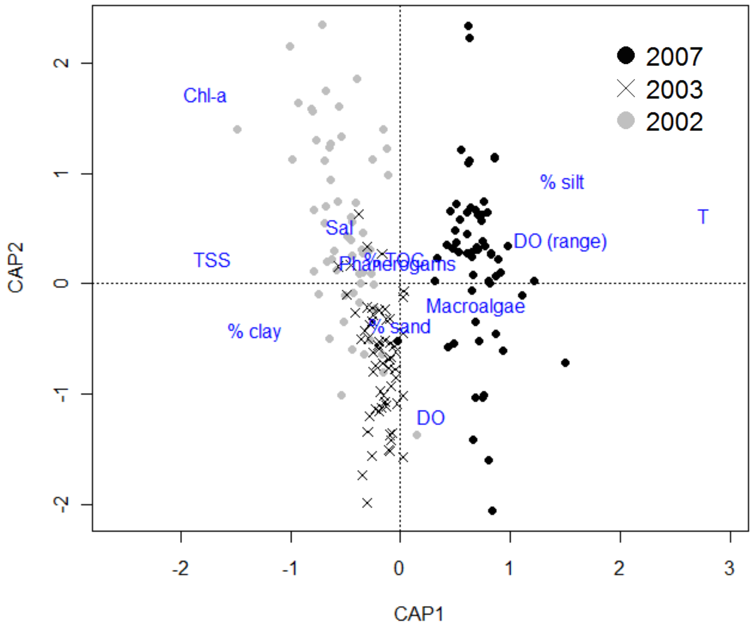


Figure 4.60: CAP of environmental variables on the 59-station 3-year data set, year as constraint factor. First axis: 20.1% of variation explained; Second axis: 2% of variation explained.

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]
[1] pc_sand	1.00	-0.78	-0.89	-0.31	0.23	0.47	-0.21	-0.45	-0.28	-0.31	-0.51	-0.21	0.51	-0.14	-0.41
[2] pc_silt_8um	-0.78	1.00	0.44	0.08	-0.18	-0.40	0.12	0.21	0.01	0.06	0.47	0.22	-0.38	0.06	0.29
[3] pc_clay_8um	-0.89	0.44	1.00	0.29	-0.19	-0.39	0.23	0.47	0.41	0.40	0.42	0.17	-0.49	0.15	0.39
[4] pc_TOC_LOI	-0.31	0.08	0.29	1.00	-0.09	-0.16	0.24	0.47	0.23	0.17	-0.12	-0.17	-0.05	-0.04	-0.11
[5] MA	0.23	-0.18	-0.19	-0.09	1.00	0.59	-0.13	-0.24	-0.06	-0.23	-0.35	-0.25	0.29	-0.11	-0.38
[6] FA	0.47	-0.40	-0.39	-0.16	0.59	1.00	-0.18	-0.37	-0.15	-0.38	-0.42	-0.22	0.43	-0.19	-0.39
[7] iT_mq	-0.21	0.12	0.23	0.24	-0.13	-0.18	1.00	0.20	0.48	0.37	0.34	0.14	-0.51	0.07	0.12
[8] tRes_d	-0.45	0.21	0.47	0.47	-0.24	-0.37	0.20	1.00	0.34	0.48	0.14	-0.10	-0.33	0.43	0.41
[9] r90_Alc_Tot_uEqL	-0.28	0.01	0.41	0.23	-0.06	-0.15	0.48	0.34	1.00	0.51	0.15	-0.09	-0.44	0.34	0.21
[10] med_Chla_ugL	-0.31	0.06	0.40	0.17	-0.23	-0.38	0.37	0.48	0.51	1.00	0.46	0.36	-0.58	0.47	0.68
[11] med_O2_ppm	-0.51	0.47	0.42	-0.12	-0.35	-0.42	0.34	0.14	0.15	0.46	1.00	0.63	-0.85	0.16	0.63
[12] r90_O2_ppm	-0.21	0.22	0.17	-0.17	-0.25	-0.22	0.14	-0.10	-0.09	0.36	0.63	1.00	-0.59	-0.20	0.44
[13] med_Sal_PSU	0.51	-0.38	-0.49	-0.05	0.29	0.43	-0.51	-0.33	-0.44	-0.58	-0.85	-0.59	1.00	-0.18	-0.55
[14] mea_T_gC	-0.14	0.06	0.15	-0.04	-0.11	-0.19	0.07	0.43	0.34	0.47	0.16	-0.20	-0.18	1.00	0.57
[15] med_TSS_mgL	-0.41	0.29	0.39	-0.11	-0.38	-0.39	0.12	0.41	0.21	0.68	0.63	0.44	-0.55	0.57	1.00

Table 4.18: Pearson correlation among the environmental variables (180-station 2002 data set)

Pearson correlations on the 180-station 2002 data set (Table 4.18) indicates, as expected, high inverse correlation among percentages of sand and pelitic fractions silt and clay. Sand correlates with main variables associated to the transitional gradient, such as residence time (-0.45) and salinity (0.51), and also inversely with DO (-0.51) and TSS (-0.41). Inverse patterns can be observed in both or either pelitic fractions, with clay also correlated with chlorophyll *a* and range of alkalinity (both 0.40). TOC is correlated to residence time (0.47) and, only to some extent, to pelite (0.31). The simultaneous presence of macroalgae and phanerogams coverage causes a value of correlation of 0.59 among the two variables. Macroalgae don't show any other remarkable correlation, except for the inverse correlation of -0.38 with TSS and of -0.35with DO. Phanerogams also show the same patterns of correlation (-039 and -0.42, respectively), and moreover are positively correlated to sand (0.47) and salinity (0.43) and negatively to pelitic fractions (both -0.40), residence time (-0.37) and chlorophyll *a* (-0.38). In practice, their spatial distribution is limited to the more "marine" part of the Lagoon. Intertidal surface is inversely correlated to salinity (-0.51), in accordance with the occurrence of intertidal morphologies in the more restricted parts of the Lagoon. In any case, the correlation with residence time, and other variables associated to the transitional gradient, is not so high. Intertidal surface also presents a good correlation with range of alkalinity (0.48). Residence time correlates with similar absolute values to sand (-0.45), clay (0.47), TOC (0.47), chlorophyll *a* (0.48), temperature (0.43) and TSS (0.41). Correlation among hydrological variables was already discussed in Chapter 4.3.1.

About spatial patterns, CAP analysis indicates that 30.6% of the variation of the 12-month 2002 environmental variables data set is "brought" by hydrogeological zones (Figure 4.61). The first axis, in particular, which explains the 22.4%, corresponds to a main gradient of environmental conditions, from the inlet landwards, with the classical changes in salinity, residence time, granulometry, TOC and related variables. The second axis (5.5% of variation explained) separates the more variable internal zones on the bases of high mean (and range of) DO and silt percentage on one side and, on the other, high temperature, residence time, chlorophyll *a*, salinity (and alkalinity), TOC. No clear geographical relationship among stations can be found in the second axis. The same analysis on the 59-station 3-year data set give less clear (variation explained goes down to 19.6%), but consistent results (except for silt percentages, which role is inverted), and the plot will not be presented here.

CAP with basins as constraining factor explains the 25.3% of the variation in environmental variables (180-station 2002 data set). The first axis, which accounts for the 16% of the variation, separates the two northern basin from the two southern. The second axis splits the two northern basins (Treporti and Lido). Malamocco and Chioggia basins are splitted in the third axis. High average (and range of) DO concentration, TSS and silt are related to the north of the Lagoon, whereas high salinity, sand and TOC to the south. TSS, temperature and chlorophyll *a* accounts mainly for the differences among Treporti and Lido. Interestingly, most of these variables (DO, TSS, chlorophyll, silt, temperature) have a very high variation among years, due either to actual variation on bias in average periods. This suggests more stable conditions inside hydrogeological zones rather that inside basins, or, in any case, higher difficulties in characterising basins conditions.

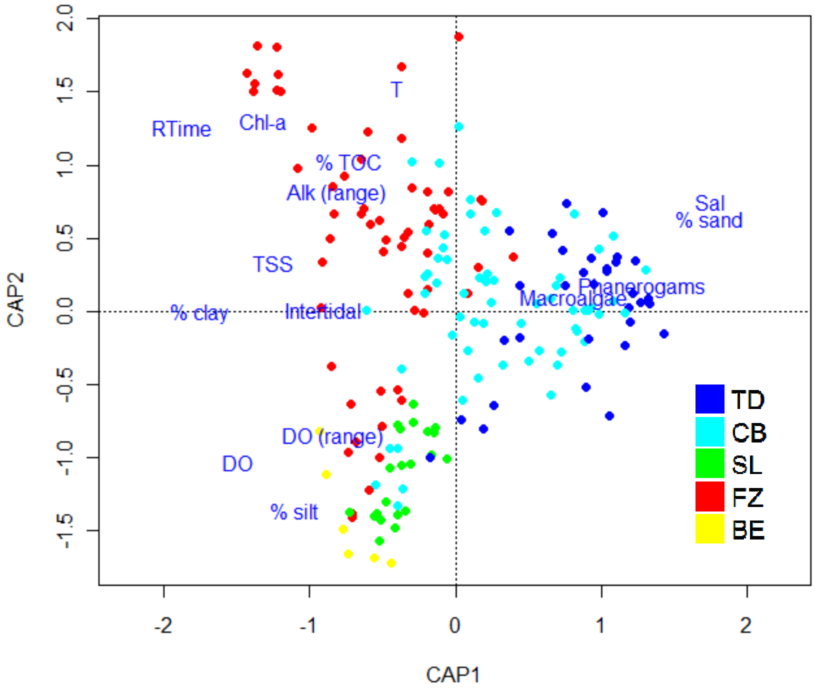


Figure 4.61: CAP of environmental variables on the 180-station 2002 data set: hydrogeological zone as constraint factor (left: I axis: 22.4% of variation explained; II axis: 5.5% of variation explained).

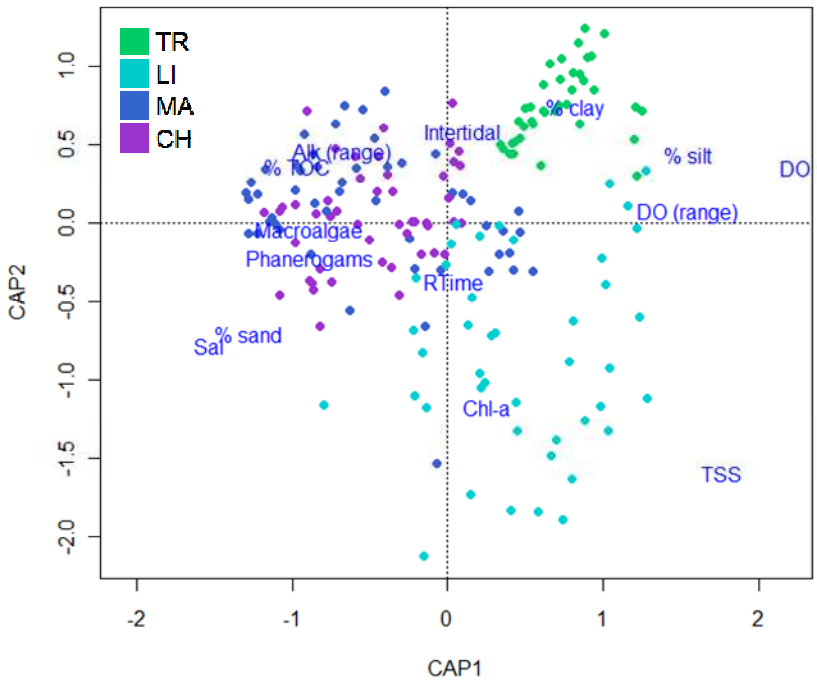


Figure 4.62: CAP of environmental variables on the 180-station 2002 data set: basin as constraint factor (right: I axis: 16.0% of variation explained; II axis: 5.9% of variation explained).

4.3.3 Univariate macrodescriptors of community

Pearson and Spearman correlation were calculated on the 180-station data set among main macrodescriptors (species richness, total abundances and total biomass) and untransformed environmental variables (Table 4.19 and 4.20). The results for the 3-year data set are not presented here as they indicate a same relationship among variables, except that with lower coefficients. With reference to Pearson correlation coefficient (Table 4.19), species richness correlates mainly to residence times (-0.66) and salinity (0.53), then to sediment granulometry (0.49 with sand, -0.51 with clay). Also phanerogams and macroalgae show considerable correlation, r = 0.49 and r = 0.43 respectively. Moreover richness is also inversely correlated to chlorophyll *a* (-0.47), TSS (-0.39) and, unexpectedly, DO (-0.39). Spearman rank correlation with richness is higher than Pearson correlation for all the factors, indicating monotonous but not linear relationships (Table 4.20). For example, correlation with residence time grows up to -0.71 and salinity to 0.60, and also rises the correlation coefficient among richness and TOC (-0.44) and intertidal surface (-0.43). Both abundance and biomass have very low correlation coefficient with considered environmental variables, both Pearson and Spearman, although the later presents higher values with biomass, e.g. residence time (-0.49), TOC (-0.40), sand (0.32).

The number of species at the lagoon scale is mostly affected by the components of the gradient of transition, which also correlate partially and not linearly with biomass.

	pc_sand	pc_silt_8um	pc_clay_8um	pc_TOC_LOI	MA	FA	iT_mq	tRes_d
A	-0.05	0.12	0.00	-0.10	0.12	-0.01	0.07	-0.03
B	0.26	-0.14	-0.26	-0.14	0.12	0.26	-0.08	-0.33
S	0.49	-0.28	-0.51	-0.26	0.43	0.49	-0.31	-0.66

	r90_Alc_Tot_uEqL	med_Chla_ugL	med_O2_ppm	r90_O2_ppm	med_Sal_PSU	mea_T_gC	med_TSS_mgL
A	-0.05	0.07	0.05	0.03	-0.06	0.20	0.14
B	-0.12	-0.12	-0.02	0.03	0.10	0.01	-0.06
S	-0.29	-0.47	-0.39	-0.28	0.53	-0.14	-0.39

Table 4.19: Pearson correlation coefficient among macrodescriptors and environmental variables, 180-station 2002 data set

	pc_sand	pc_silt_8um	pc_clay_8um	pc_TOC_LOI	MA	FA	iT_mq	tRes_d
A	0.03	0.04	-0.04	-0.13	0.20	0.13	-0.05	-0.20
B	0.32	-0.17	-0.30	-0.40	0.19	0.29	-0.14	-0.49
S	0.53	-0.26	-0.53	-0.44	0.42	0.48	-0.43	-0.71

	r90_Alc_Tot_uEqL	med_Chla_ugL	med_O2_ppm	r90_O2_ppm	med_Sal_PSU	mea_T_gC	med_TSS_mgL
A	-0.16	-0.15	-0.05	-0.04	0.13	0.12	-0.00
B	-0.10	-0.16	-0.04	0.03	0.17	0.10	-0.09
S	-0.25	-0.50	-0.42	-0.25	0.60	-0.04	-0.42

Table 4.20: Spearman correlation coefficient among macrodescriptors and environmental variables, 180-station 2002 data set

Salinity and "water renewal" have been widely considered as the main factors in structuring species richness, and generally benthic community, in transitional environments. Each one were considered as the main factor by some authors (see in particular Remane, 1934 for salinity; Guelorget & Perthuissot, 1983 for the confinement theory) but the following debate highlighted their different role according to physiographic/structural and geographic/climatic features of CTE. A linear model was produced on 180-station data set for species richness as function of the more correlated variables, i.e. residence time and salinity. Pearson correlation coefficient between these variables is -0.33 (p-value = $7.22\cdot 10^{-6}$), Spearman correlation is -0.41 (p-value = $1.528\cdot 10^{-8}$), both on 180 sampling stations of 2002. Linear models require normality of variables, therefore transformed salinity (to the third power) was used. Richness was at first regressed to each variable at a time. A model based on residence times only give an $R^2 = 0.43$ (Table 4.21, model 1; Figure 4.63). A model based on (transformed) salinity only gives an $R^2 = 0.37$ (Table 4.21, model 2; Figure 4.63). The linear model considering jointly the two factors improves the (adjusted) coefficient of determination to 0.57 (p-value: $< 2.2\cdot 10^{-16}$; Table 4.21, model 3; Figure 4.64), i.e. at the lagoon scale the 57% of variance in species richness is explained by a linear model of residence time and (transformed) salinity.

A multiple regression analysis among S and (transformed) variables with stepwise forward selection gives an adjusted-R^2 of 0.62 (Table 4.21, model 4). The statistically significant variables (p-value < 0.05) included in the models are residence time, salinity, presence of macroalgae, sand, TSS and TOC.

			independent variable(s)	trasformation	signif.	df	regression equation	(adj-)R2	p
1	y = S	x =	residence time (d)	-	***	178	y = 44.30 - 2.89x	0.43	$< 2 \cdot 10^{-16}$
2	y = S	x =	mean salinity (PSU)	power of 3	***	178	y = -7.57 + 0.0011x	0.37	$< 2 \cdot 10^{-16}$
3	y = S	x1 =	residence time (d)	-	***	177	y = 16.88 - 2.16x1 + 0.00076x2	0.57	$< 2 \cdot 10^{-16}$
		x2 =	mean salinity (PSU)	power of 3	***				
4	y = S	x1 =	residence time (d)		***	172	y = -5.93 - 2.22x1 + 0.00065 x2 +	0.62	$< 2 \cdot 10^{-16}$
		x2 =	mean salinity (PSU)	power of 3	***		6.1x3 + 1.17x4 + 3.02x5 - 5.38 + 1.29		
		x3 =	macroalgae		***				
		x4 =	sand (%)	square-root	**				
		x5 =	mean TSS (mg/L)	3rd-root	*				
		x6 =	TOC (%)	inverse, 3rd-root	*				
		x7 =	mean temperature (C)		.				

Table 4.21: Linear models of species richness as function of (transformed) environmental variables (signif.: ‘***’: < 0.001; ‘**’: 0.001-0.01; ‘*’: 0.01-0.05; ‘.’: 0.05-0.1).

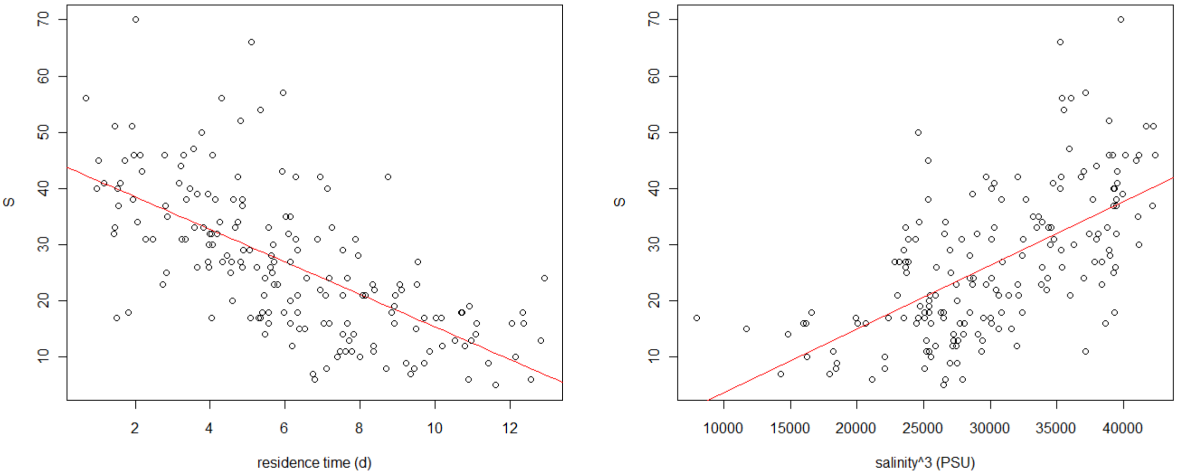


Figure 4.63: Linear model of species richness as function of residence time (left; see Table 4.21, model 1) and (transformed) mean salinity (right; see Table 4.21, model 2).

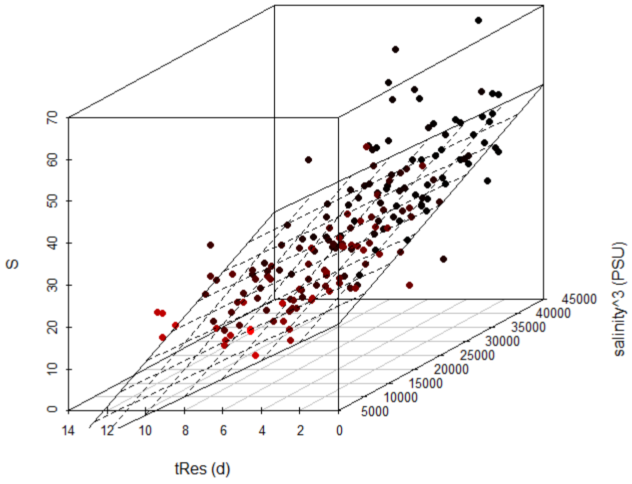


Figure 4.64: Linear model of species richness as function of both residence time and (transformed) mean salinity (see Table 4.21, model 3).

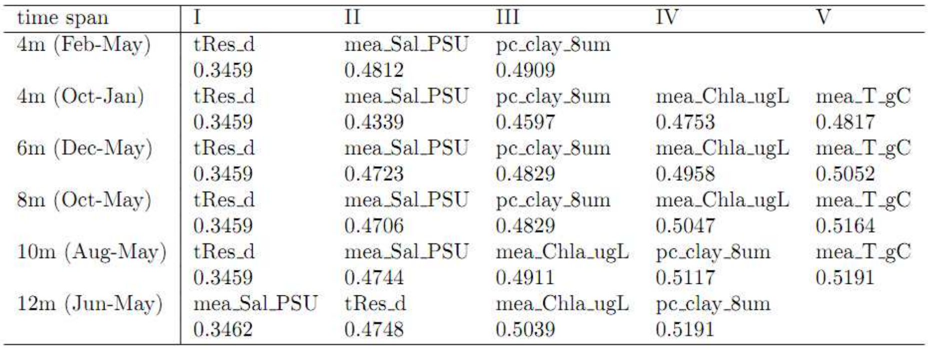
4.3.4 Relationship of multivariate community data to environmental data

BIOENV

BIOENV, which is based on the correlation among resemblance matrices, was applied on 180-station 2002 data sets, comparing the community composition matrices to a number of environmental data sets based on different time spans in order to (1) identify the combination of environmental variables that best explain patterns in community data at the lagoon scale, and (2) analyze how the combination of correlated factors varies by integrating different time spans, i.e. what part of the environmental signal is retained in the community structure compared to the considered time span.

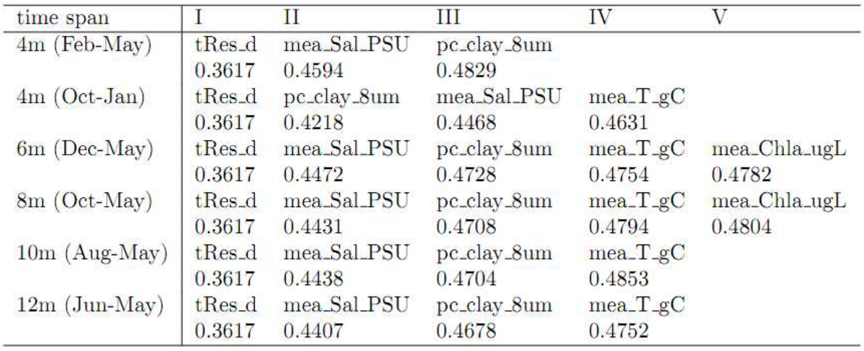
The results of the analysis performed on the matrix of numerical abundances are shown in Table 4.22). Residence time and salinity show higher correlation values for every considered time span. The most important variable is usually residence time (as the hydrodynamical model was based on average conditions, and it was represented by the same values for all the data sets, the correlation value is 0.35 for all the time spans), except when considering 12-month data, in which salinity is at the first place. The 4-month data set preceding the macrozoobenthos fieldwork (from February to May, which is the time span considered when performing analyses on the 3-year data set) shows a correlation of 0.49, adding clay percentage to explanatory variables. Correlation generally increases, although only slightly, considering longer time spans, up to 0.52 for 12-month data sets. Other variables improving correlation with longer time spans are chlorophyll *a* and temperature, the later not found for 12-month data set. Correlation with the data set based on non- contiguous four months, from October to January, is the lowest (0.48).

For the biomass data (Table 4.23), the same overall patterns can be noticed, but the total correlations are in general slightly lower. The higher correlation of 0.49, for 10-month data set, is due to residence time, salinity, clay and temperature. These same variables except temperature are responsible for a correlation of 0.48 for the 4-month data set. BIOENV was also performed on the more robust 2002 12-month data set based on median and 90% interpercentile range (Table 4.24), which give similar results to 12-month data set based on mean and total range, with a total correlation of 0.50 between community composition in terms of abundances and environmental variables, and 0.47 between community composition in terms of biomass and environmental variables.



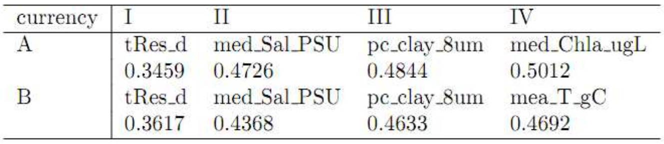
time span	I	II	III	IV	V
4m (Feb-May)	tRes_d 0.3459	mea_Sal_PSU 0.4812	pc_clay_8um 0.4909		
4m (Oct-Jan)	tRes_d 0.3459	mea_Sal_PSU 0.4339	pc_clay_8um 0.4597	mea_Chla_ugL 0.4753	mea_T_gC 0.4817
6m (Dec-May)	tRes_d 0.3459	mea_Sal_PSU 0.4723	pc_clay_8um 0.4829	mea_Chla_ugL 0.4958	mea_T_gC 0.5052
8m (Oct-May)	tRes_d 0.3459	mea_Sal_PSU 0.4706	pc_clay_8um 0.4829	mea_Chla_ugL 0.5047	mea_T_gC 0.5164
10m (Aug-May)	tRes_d 0.3459	mea_Sal_PSU 0.4744	mea_Chla_ugL 0.4911	pc_clay_8um 0.5117	mea_T_gC 0.5191
12m (Jun-May)	mea_Sal_PSU 0.3462	tRes_d 0.4748	mea_Chla_ugL 0.5039	pc_clay_8um 0.5191	

Table 4.22: Mantel correlation calculated by BIOENV. The correlation coefficient is cumulative from I to V, with at each step the contribution of a new variable. The method compared the Bray-Curtis dissimilarities matrix based on community composition (abundances) and Euclidean distancies on (standardized) environmental variables with different time spans based on mean and total range.



time span	I	II	III	IV	V
4m (Feb-May)	tRes_d 0.3617	mea_Sal_PSU 0.4594	pc_clay_8um 0.4829		
4m (Oct-Jan)	tRes_d 0.3617	pc_clay_8um 0.4218	mea_Sal_PSU 0.4468	mea_T_gC 0.4631	
6m (Dec-May)	tRes_d 0.3617	mea_Sal_PSU 0.4472	pc_clay_8um 0.4728	mea_T_gC 0.4754	mea_Chla_ugL 0.4782
8m (Oct-May)	tRes_d 0.3617	mea_Sal_PSU 0.4431	pc_clay_8um 0.4708	mea_T_gC 0.4794	mea_Chla_ugL 0.4804
10m (Aug-May)	tRes_d 0.3617	mea_Sal_PSU 0.4438	pc_clay_8um 0.4704	mea_T_gC 0.4853	
12m (Jun-May)	tRes_d 0.3617	mea_Sal_PSU 0.4407	pc_clay_8um 0.4678	mea_T_gC 0.4752	

Table 4.23: Mantel correlation calculated by BIOENV. The correlation coefficient is cumulative from I to V, with at each step the contribution of a new variable. The method compared the Bray-Curtis dissimilarities matrix based on community composition (biomass) and Euclidean distancies on (standardized) environmental variables with different time spans based on mean and total range.



currency	I	II	III	IV
A	tRes_d 0.3459	med_Sal_PSU 0.4726	pc_clay_8um 0.4844	med_Chla_ugL 0.5012
B	tRes_d 0.3617	med_Sal_PSU 0.4368	pc_clay_8um 0.4633	mea_T_gC 0.4692

Table 4.24: Mantel correlation calculated by BIOENV. The correlation coefficient is cumulative from I to V, with at each step the contribution of a new variable. The method compared the Bray-Curtis dissimilarities matrix based on community composition (abundances and biomass) and Euclidean distancies on (standardized) environmental variables for the 12-month data set based on median and 90% interpercentile range.

Constrained ordinations

Multivariate relationships between macrozoobenthos community (species composition matrices in terms of square-root transformed biomass and abundances) and environmental variables were investigated with constrained ordinations: RDA after Hellinger transformation, CCA and CAP (see Chapter 3.5.5). Percentage of explained variation by environmental variables is reported in Table 4.25 for the analysis performed, even though values from different methods may not be directly comparable. The amount of explained compositional variation is considered by some authors to be underestimated by the eigenvalue-to-total-inertia ratio (Økland, 1999).

dataset	RDA (Hellinger transformation) R^2	RDA (Hellinger transformation) Adj-R^2	CAP	CCA
180-station 2002, abundances	0.31	0.26	0.24	0.21
180-station 2002, biomass	0.29	0.23	0.22	0.17
59-station 3-year, abundances	0.26	0.20	0.19	0.17
59-station 3-year, biomass	0.23	0.17	0.17	0.15

Table 4.25: Fraction of variation explained by environmental variables

Results on 180-station 2002 abundances data are presented in Figures 4.65 and 4.66 with ordination plots of the first two axes (always significant). The first axis of RDA ordination (Figure 4.65) roughly corresponds to the transitional gradient, with classical patterns of environmental variables: high salinity and sand percentage, as well as presence of macrophytes, on one side and, on the other, high values of two groups of variables: a first group including temperature, TSS, median DO, range of DO and silt percentage, and a second one including residence time, chlorophyll *a*, range of alkalinity and intertidal surface. The former group is also mainly responsible for the variation along the second axis in one direction, the other direction explained mainly by TOC and presence of macrophytes. As expected, hydrogeological zones are arranged along the first axis (i.e. the transitional gradient), from assemblages pertaining to Tidal Delta, to Central Basin up to Fringe Zone, all of these equally spread along the second axis. Bayhead Estuary assemblages, which are located at the end of the succession but superimposed to Fringe Zone stations, are in particular characterized by high residence time, chlorophyll *a*, range of alkalinity and intertidal surface, low salinity and sand percentage. The Sheltered Lagoon stations stand out with regard to the second axis, and are characterized by sediments poor in TOC with high silt percentages and no macrophyte coverage, high DO, DO variability and TSS. Total explained inertia is higher for RDA (see Table 4.25), as well as percentage of explained inertia by the first two axis (59% for RDA, 52% for CAP, 46% for CCA). Patterns displayed by CAP (Figure 4.66 left) and CCA (Figure 4.66 right) are qualitatively very similar.

Procrustean analyses among the two first axes of RDA and CAP ordinations in particular superimposes very well, with a sum of squares value of 0.02 and a correlation-like statistic of 0.99 (1000 permutations, significance of 0.001), but also RDA and CCA shows a considerable degree of superimposition, with a sum of squares value of 0.2 and a correlation-like statistic of 0.89 (1000 permutations, significance of 0.001). These values can be compared to the values describing the relationships among ordination results by RDA (after Hellinger transformation) for transformed (log, square-root and 4th-root transformation) and non-transformed community composition matrices. The procrustean sum of squares between non-transformed and square-root transformed is 0.12 and the correlation-like statistic is 0.94 (1000 permutations, significance of 0.001); between square-root and log transformation is 0.02 and 0.99 respectively (1000 permutations, significance of 0.001); between square-root and 4th-root is 0.03 and 0.98 respectively (1000 permutations, significance of 0.001).

Results of a procrustean analysis performed to compare CAP constrained on environmental variables and zones is presented in Figure 4.67, with a sum of squares value of 0.52 and a correlation-like statistic of 0.69 (1000 permutations, 0.001 significance). The succession of stations along the first axis, which corresponds to the transitional gradient, is in general maintained in the two configurations.

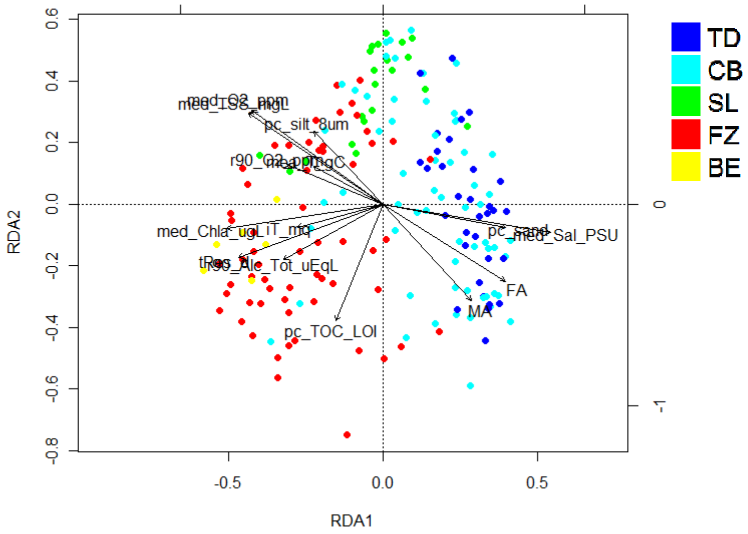


Figure 4.65: RDA ordination of 2002 180-station data set (Hellinger transformed abundances) (p = 0.005). Constraining factor: Environmental variables, 1-year data set. First two axes displayed. Total inertia: 0.64. Constrained inertia: 0.21 (32.5%). Inertia (variance) explained by first axis: 0.14 (42% of the constrained inertia); inertia explained by the second axis: 0.057 (17%). Hydrogeological zones are highlighted by colours.

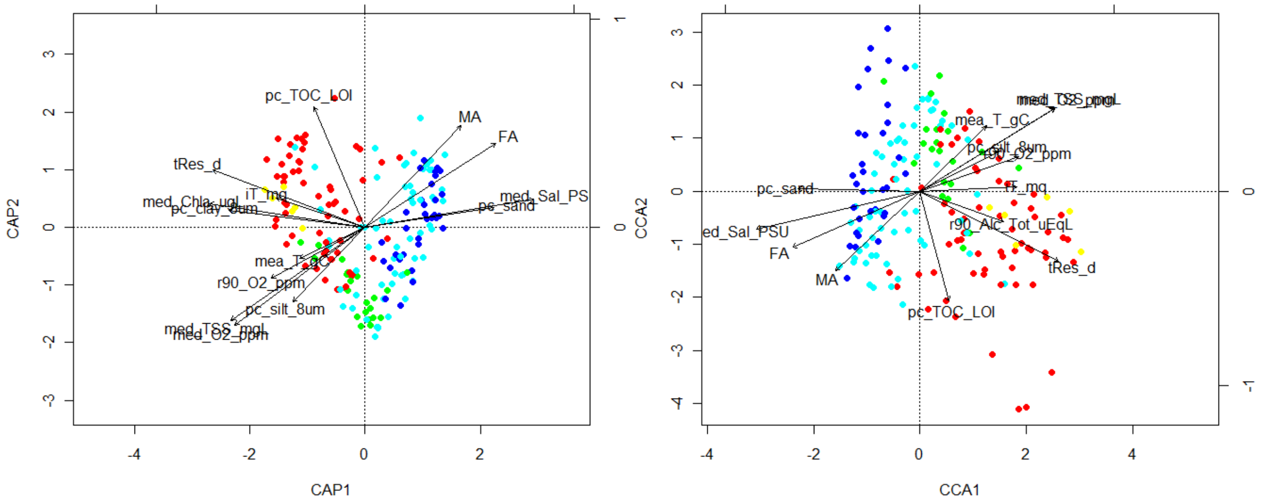


Figure 4.66: left: CAP ordination of 2002 180-station data set (abundances) (p = 0.005). Constraining factor: Environmental variables, 1-year data set. First two axes displayed. Total inertia: 68.57. Constrained inertia: 16.17 (23.6%). Inertia explained by first axis: 6 (37% of the constrained inertia); inertia explained by the second axis: 2.49 (15%).

Right: CCA ordination of 2002 180-station data set (abundances) (p = 0.005). Constraining factor: Environmental variables, 1-year data set. First two axes displayed. Total inertia: 5.84. Constrained inertia: 1.20 (20.6%). Inertia (variance) explained by first axis: 0.37 (31% of the constrained inertia); inertia explained by the second axis: 0.18 (15%). Hydrogeological zones are highlighted by colours.

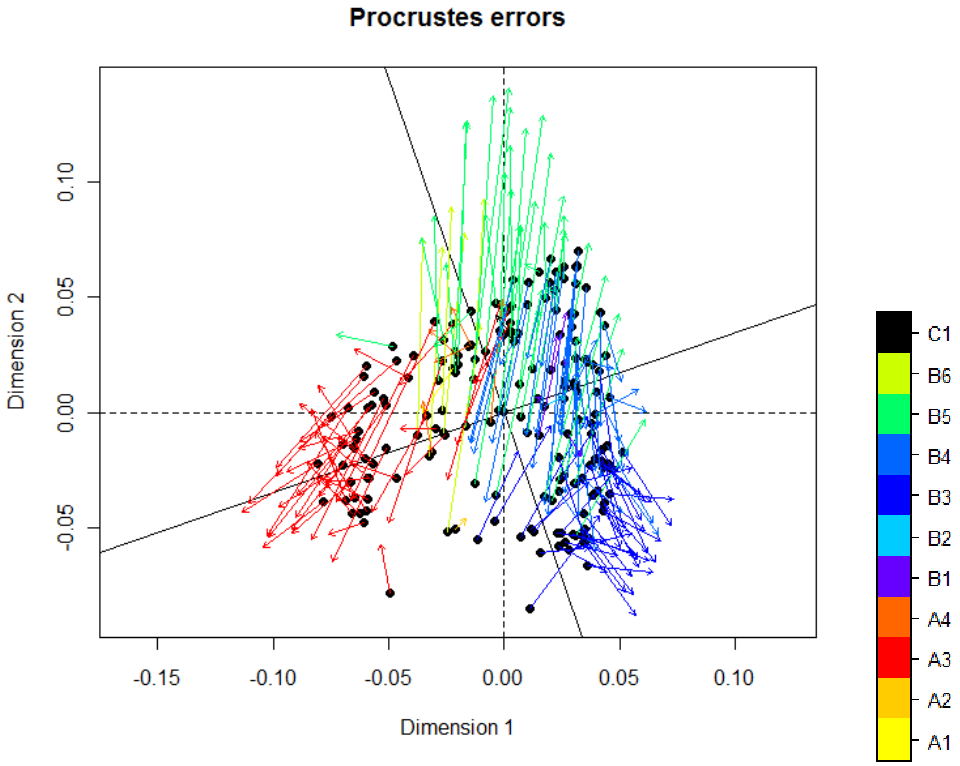


Figure 4.67: Results of procrustean analysis on CAP ordinations (first two axes) constrained by environmental variables and mesoscale clusters on 180-station 2002 abundances (sum of squares: 0.52; correlation-like statistic: 0.69, based on 1000 permutations, 0.001 significance). Rotation of axes and configuration for the zones-constrained ordination are plotted. The configuration of station for the cluster-constrained ordination is showed by arrows, and identified by colours.

With regards to abundances, for ordination methods applied on biomass the variation explained by environmental variables is lower: 28.9% for RDA (after Hellinger transformation), 21.7% for CAP and 17.4% for CCA (Table 4.25). Configurations of the data cloud share partly the same patterns of ordinations based on abundances, in particular with regards to the first axis. About the second axis, RDA and CCA (Figures 4.68 and 4.69, right) doesn't separate the Sheltered Lagoon stations, which remains at the centre of the axes. The second axis in RDA result is characterized by TOC, salinity and macrophytes on one side and, on the other, high temperature, TSS, chlorophyll *a*, DO and range of DO. In CCA results the second axis is less relate to salinity, chlorophyll *a* and range of DO. It recognizes instead range of alkalinity along to TOC and silt percentage as characterizing the second axis on the side opposite to high temperature, DO and TSS, and in particular the Bayhead Estuary stations. CAP analysis instead identifies for the second axis high salinity alongside high TOC on one hand, where are placed most of the Sheltered Lagoon stations, and, on the other, high temperature, TSS, DO, DO range, chlorophyll *a* and intertidal surface (Figure 4.69 left). The relationship among ordinations is again stronger among RDA and CAP (procrustean sum of square of 0.12, correlation-like statistic of 0.94 on 1000 permutations, significance of 0.001), than among CCA and RDA or CAP (both with a procrustean sum of square of 0.39, and a correlation-like statistic of 0.62 for CCA and CAP, and 0.78 for CCA and RDA, both 1000 permutations and significance of 0.001). The procrustean sum of squares between ordinations resulting from abundances- and biomass-based RDA (after Hellinger transformation) is 0.71 (correlation-like statistic of 0.54, 1000 permutations, and significance of 0.001).

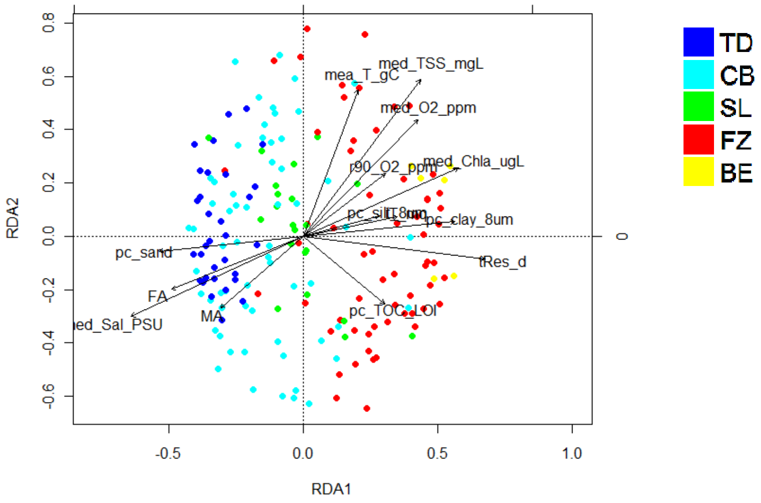


Figure 4.68: RDA ordination of 2002 180-station data set (Hellinger transformed biomasses) (p = 0.005). Constraining factor: Environmental variables, 1-year data set. First two axes displayed. Total inertia: 0.71. Constrained inertia: 0.21 (28.9%). Inertia (variance) explained by first axis: 0.089 (43% of the constrained inertia); inertia explained by the second axis: 0.032 (15%). Hydrogeological zones are highlighted by colours.

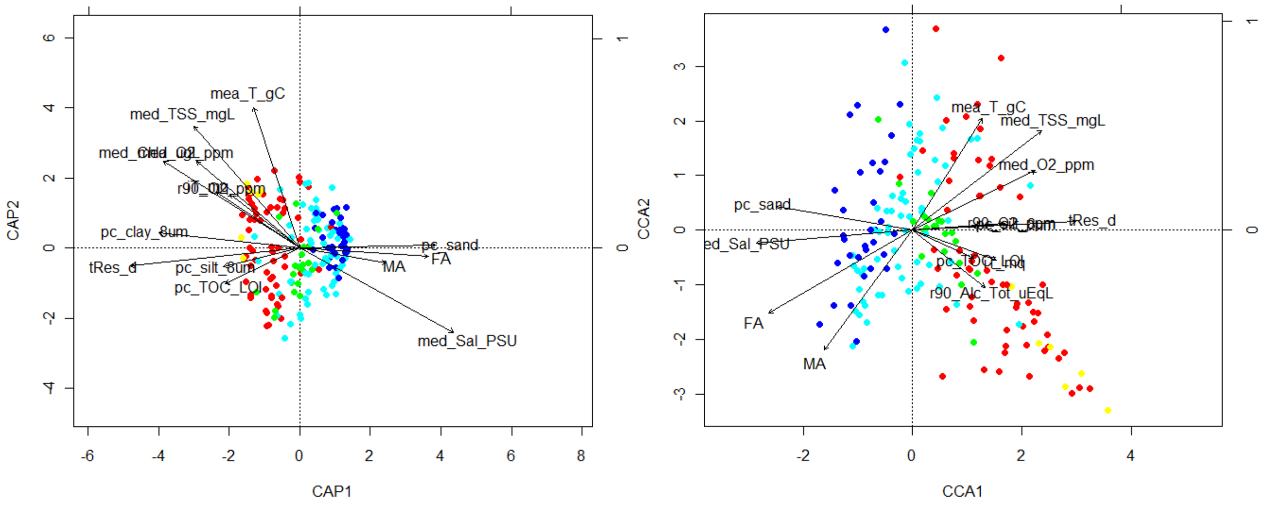


Figure 4.69: left: CAP ordination of 2002 180-station data set (biomasses) (p = 0.005). Constraining factor: Environmental variables, 1-year data set. First two axes displayed. Total inertia: 72.77. Constrained inertia: 15.75 (21.7%). Inertia explained by first axis: 5.86 (37% of the constrained inertia); inertia explained by the second axis: 2.21 (14%).

Right: CCA ordination of 2002 180-station data set (abundances) (p = 0.005). Constraining factor: Environmental variables, 1-year data set. First two axes displayed. Total inertia: 8.88. Constrained inertia: 1.55 (17.4%). Inertia (variance) explained by first axis: 0.44 (28% of the constrained inertia); inertia explained by the second axis: 0.21 (14%). Hydrogeological zones are highlighted by colours.

Variation explained on 59-station data sets is lower than for 180-station data sets, and is reported for all the analyses in Table 4.25. Here only the results of RDA (after Hellinger transformation) on abundances data are presented (with a 25.6% of the variation explained by environmental variables), as the patterns showed by other methods, as well as on biomass data, are in general very similar. The first axis again is characterized by the classical condition following the transitional gradient. The second axis is mostly characterized by high sand percentage, salinity and TSS, low temperature, TOC, residence time, silt percentage, range of DO and absence of macroalgae on one side and vice versa on the other. The patterns displayed by stations belonging to hydrogeological zones (Figure 4.70, left) can be followed over the years (Figure 4.70, right). Zones follow the classical succession from Marine Tidal Delta to Bayhead Estuary mainly along the first axis. The second axis can be mainly explained by interannual variation in environmental variables, with 2007 characterized by high temperature, silt percentage and DO range, low salinity and TSS, presence of macrophytes. Despite part of the variation may be attributableto real interannual variability, a great part is due to sampling and analytical issues (see Chapter 3.2), in particular for temperature, DO and sediment data. The differences among environmental data sets are reflected by zones patterns displayed in ordination plots, with 2007 stations separating from 2002 and 2003 stations. This is particularly evident for Sheltered Lagoon and Fringe Zone.

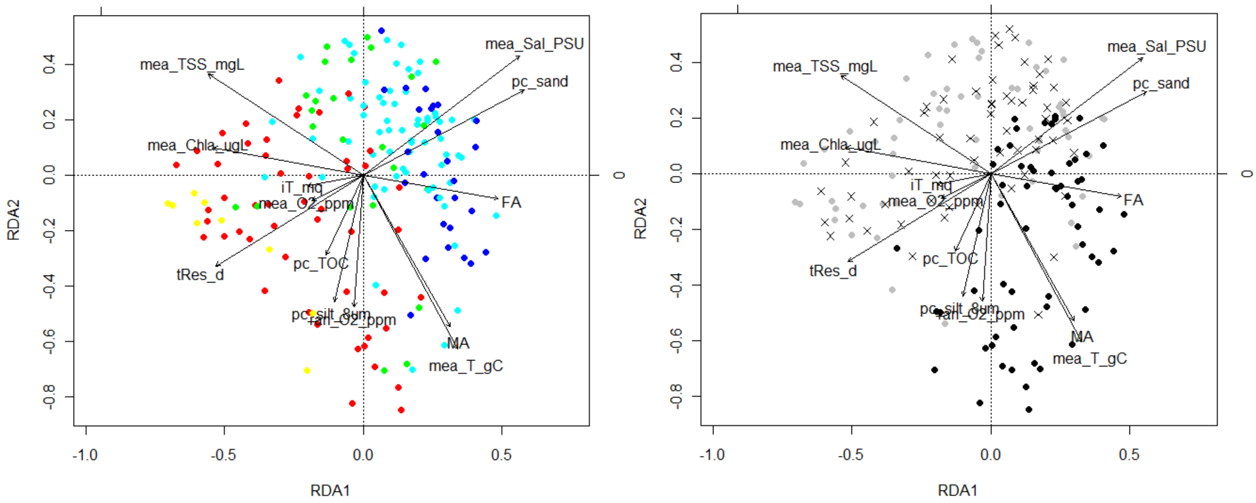


Figure 4.70: RDA ordination of 3-year 59-station data set (Hellinger transformed abundances) (p = 0.005). Constraining factor: Environmental variables, 4-month data set. First two axes displayed. Total inertia: 0.65. Constrained inertia: 0.17 (25.6%). Inertia (variance) explained by first axis: 0.059 (35% of the constrained inertia); inertia explained by the second axis: 0.034 (21%). Left: Hydrogeological zones highlighted by colours; right: years (black dots: 2007; x signs: 2003; grey dots: 2002.).

Variation explained by environmental variables calculated by RDA (adjusted-R^2; see Table 4.25) on abundances and biomass community composition matrices was partitioned among "groups" of variables. Results for abundances and biomass are similar (Figure 4.71). Results will be exposed as percentages of the explained variation (Økland, 1999). Residence time alone explains most of the total constrained variance, about the 30% in both cases. Hydrology alone explains 19% in terms of abundances and 17% in terms of biomass, whereas sediments alone explain 11% in terms of abundances and 13% in terms of biomass. A 15% of the total explained variation for abundances, and 17% for biomass, is explained by all the variables at the same time. In practice, no variation is left which is explained by sediment jointly with either hydrologic variables or hydrodynamics (i.e. residence time). At the same time variation explained jointly by residence time and hydrological variables is 22% of the total constrained variance in terms of abundances, and 17% in terms of biomass. Percentage of constrained variation explained in total by water column (hydrological variables and residence time) is 70% for abundances and 65% for biomasses.

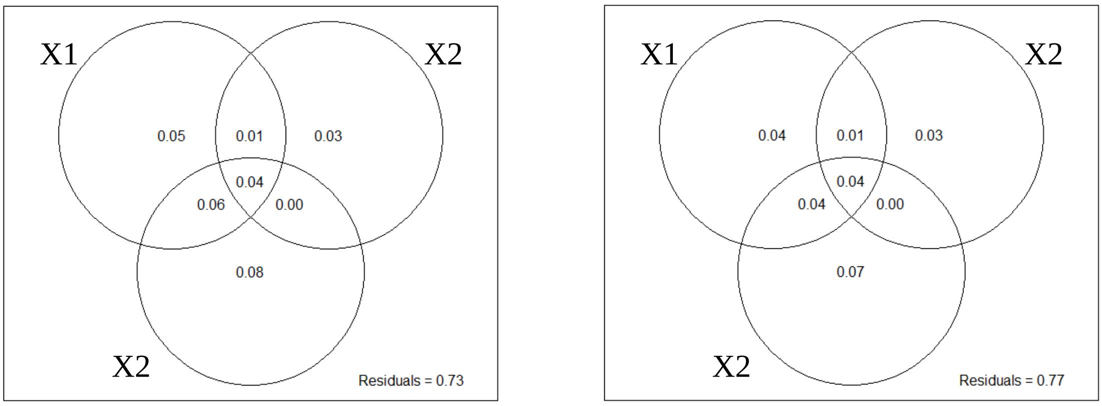


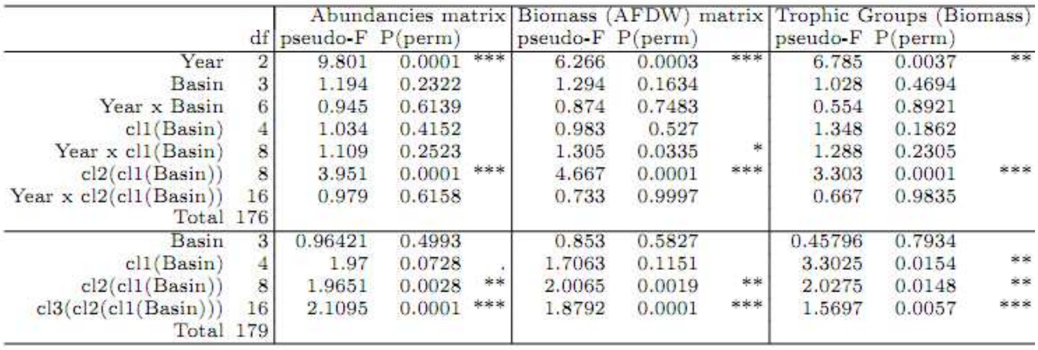
Figure 4.71: RDA-based variance partitioning among categories of environmental variables (180-station 2002 data set) as explanatory variables for community composition data in terms of abundances (left) and biomass (right). X1: residence time; X2: sediment variables: granulometry, TOC, presence of macrophytes, intertidal surface; X3: hydrological variables: salinity, temperature, DO, range of DO, chlorophyll *a*, TSS, range of alkalinity. The calculation applies on adjuste-R^2.

4.4 SPATIAL STRUCTURES AND MULTISCALE ANALYSIS

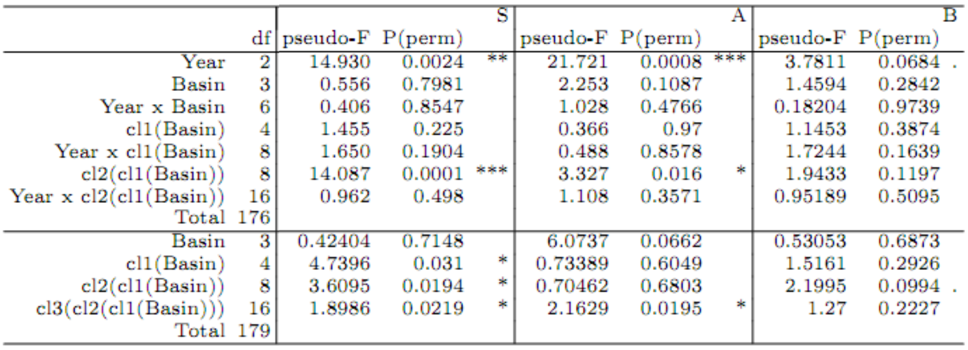
Three approaches were followed to take in account spatial structure at multiple spatial scales: (1) ANOVA-like method using a nested design, (2) MSO for multivariate species composition matrices and semi-variograms for univariate macrodescriptors, (3) classical multivariate analysis (GLM methods, constrained ordination, variation partitioning) in which spatial predictors are introduced among explanatory variables.

4.4.1 PERMANOVA with nested design

The results of the nested PERMANOVA performed on multivariate and univariate community data are shown in Table 4.26. At the top of the table, results refer to the 3-year 59-station data sets. All the investigate data sets show significant variation among years (with p-value for biomass between 0.05 and 0.1). Results of a one-way PERMANOVA design on years (as well as on basin) on community composition matrices (abundances and biomass) and trophic groups matrix (biomass) are presented in Table 4.8. In Table 4.10 are reported the results of a more robust ANOVA test performed directly on univariate macrodescriptors (total abundance and biomass, transformed with 4th-root to follow a normal distribution, and species richness). The very different design causes F-values (as well as p-values) for the factor year to increase respect to one-way model. Spatial hierarchical design implies a different interpretation of the factor basin compared to the one-way analysis. Three nested spatial "scales" (factor basin and two factors of clusters, called "sectors", obtained by an iterative *k*-means method; see Chapter 3.7 and Figure 3.8) were introduced for the 59-station 3-year PERMANOVA design, and four for the 180-station 2002 design (factor basin and three sectors at different rank, due to the largest number of stations; see Figure 3.8). Spatial extent and number of stations for the two designs are reported in Table 3.12 and 3.12.



		Abundancies matrix			Biomass (AFDW) matrix			Trophic Groups (Biomass)		
	df	pseudo-F	P(perm)		pseudo-F	P(perm)		pseudo-F	P(perm)	
Year	2	9.801	0.0001	***	6.266	0.0003	***	6.785	0.0037	**
Basin	3	1.194	0.2322		1.294	0.1634		1.028	0.4694	
Year x Basin	6	0.945	0.6139		0.874	0.7483		0.554	0.8921	
cl1(Basin)	4	1.034	0.4152		0.983	0.527		1.348	0.1862	
Year x cl1(Basin)	8	1.109	0.2523		1.305	0.0335	*	1.288	0.2305	
cl2(cl1(Basin))	8	3.951	0.0001	***	4.667	0.0001	***	3.303	0.0001	***
Year x cl2(cl1(Basin))	16	0.979	0.6158		0.733	0.9997		0.667	0.9835	
Total	176									
Basin	3	0.96421	0.4993		0.853	0.5827		0.45796	0.7934	
cl1(Basin)	4	1.97	0.0728	.	1.7063	0.1151		3.3025	0.0154	**
cl2(cl1(Basin))	8	1.9651	0.0028	**	2.0065	0.0019	**	2.0275	0.0148	**
cl3(cl2(cl1(Basin)))	16	2.1095	0.0001	***	1.8792	0.0001	***	1.5697	0.0057	***
Total	179									



				S			A			B
	df	pseudo-F	P(perm)		pseudo-F	P(perm)		pseudo-F	P(perm)	
Year	2	14.930	0.0024	**	21.721	0.0008	***	3.7811	0.0684	.
Basin	3	0.556	0.7981		2.253	0.1087		1.4594	0.2842	
Year x Basin	6	0.406	0.8547		1.028	0.4766		0.18204	0.9739	
cl1(Basin)	4	1.455	0.225		0.366	0.97		1.1453	0.3874	
Year x cl1(Basin)	8	1.650	0.1904		0.488	0.8578		1.7244	0.1639	
cl2(cl1(Basin))	8	14.087	0.0001	***	3.327	0.016	*	1.9433	0.1197	
Year x cl2(cl1(Basin))	16	0.962	0.498		1.108	0.3571		0.95189	0.5095	
Total	176									
Basin	3	0.42404	0.7148		6.0737	0.0662		0.53053	0.6873	
cl1(Basin)	4	4.7396	0.031	*	0.73389	0.6049		1.5161	0.2926	
cl2(cl1(Basin))	8	3.6095	0.0194	*	0.70462	0.6803		2.1995	0.0994	.
cl3(cl2(cl1(Basin)))	16	1.8986	0.0219	*	2.1629	0.0195	*	1.27	0.2227	
Total	179									

Table 4.26: Results of PERMANOVA nested design on multivariate and univariate community data for the 3-year 59-station data sets (top; crossed design) and the 2002 data sets (bottom).

For the 3-year data sets, spatial variation is statistically significant only for sector of rank 2, i.e. the lowest nested factor, except for total biomass, for which no spatial term is significant (Table 4.26). Total abundances are less significant that other terms (p-value = 0.016). Except a 0.03 value for year × rank 1-sector in matrix of biomasses, no interaction term for the crossed spatial-temporal 59-station design shows statistical significance.

Results for the 180-station analysis are also reported on Table 4.26. Community composition matrices of abundances and biomass show very similar results, with terms decreasing in p-value from the factor "sector of rank 3" (p-value < 0.0001) to the factor "sector of rank 1" (both p-values > 0.05). For trophic groups again rank 3-sector has the highest statistical significance, and rank 2-sector and rank 1-sector have a similar p-value of 0.015, suggesting that trophic groups variability may appear at "wider scale" than assemblages based on species composition. Species richness presents similar levels of significance for all the sectors, with p-values between 0.01 and 0.05. Total abundances present a p-value < 0.05 only for the lowest nested cluster, whereas, with regards to total biomass, higher p-values are yielded, with lowest p-value (0.1) for sector of rank 2.

With regards to the species composition matrices, sector of rank 2 appears for both the 3-year and 2002 data sets as the scale of variation. This is the main scale of heterogeneity for species richness (despite also sectors of other ranks are significant scales for the 3-year data set). Main difference is in total abundances, which may be related to either or both the different spatial density of stations for each sectore and the use of 3-year versus 1-year data. An explorative analysis performed on the subset of 59 stations of 2002 data is comparable to 180-station data set results (i.e. no factor is statistically significant), whereas for trophic groups (biomass) is comparable to 3-year data set results (i.e. only sector of rank 2 is a statistically significant factor).

4.4.2 Multi-Scale Ordination and relationship of main taxonomic groups to environmental variables

Results of RDA (after Hellinger transformation) of community data (180-station 2002 data set, based on abundances), with environmental variables as constraints, were partitioned by distance to distinguish between components of induced spatial correlation and components of spatial autocorrelation (variance explained by single environmental variables and statistical significance are presented in Table 4.27). Empirical variogram of total inertia (i.e. variance) is presented in Figure 4.72. It shows a strong increase with distance until the total variance of 0.65 is reached. The sill is reached at a range of about 7 km (significant autocorrelation found by applying the MSO analysis to a PCA after Hellinger transformation). The "nugget" is 0.4 about the 60% of the total variance. Residual variance is the variogram of the residual inertia related to unconstrained fraction. A significant autocorrelation is found for the first two classes to a distance of about 2 km, indicating that the residuals are spatially correlated. This could be interpreted as the spatial scale of organization of the community, even though it could actually be related to unknown external factors. The sum of variograms of constrained and unconstrained variation exceeds only slightly the envelope for total variance for a couple of distances classes. This suggests considering meaningful the correlation between community and (available) environmental variables independently from scale.

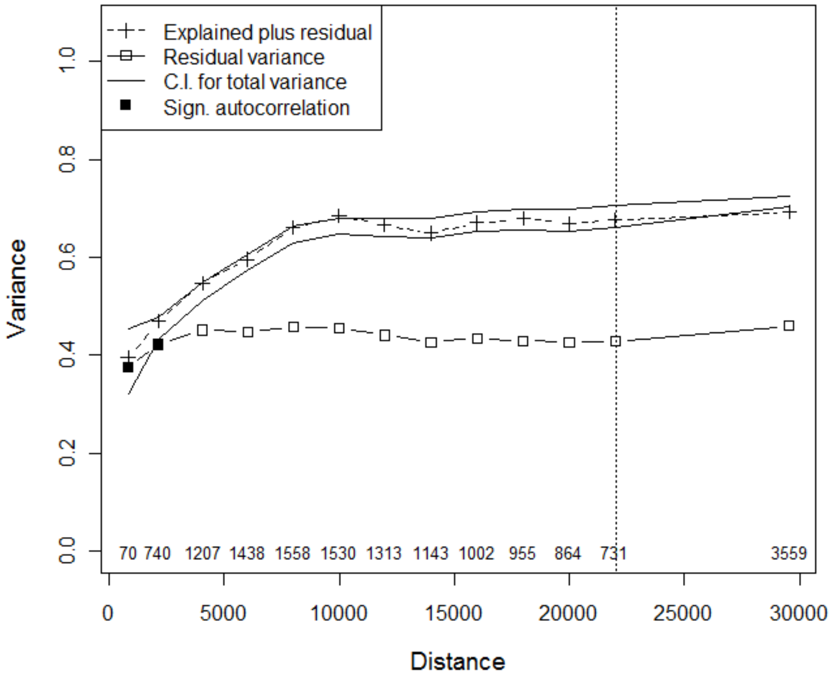


Figure 4.72: Variogram of total inertia (variance): spatial partitioning of RDA results (after Hellinger transformation) on species composition matrix (180-station 2002 data set, abundances) with the environmental variables as constraints. Distance among pairs of stations (number of pairs for each distance class at the top of the x-axis) increases with steps of 2 km. Confidence Interval (CI): point-wise envelope for the variogram of the total inertia (α = 0.05/13, Bonferroni-type correction). Error variance of regression model underestimated by 0.3%.

The same analysis was applied to main taxonomical groups: Polychaeta Sedentaria and Errantia, Amphipoda, Bivalvia and Gastropoda. In Table 4.27 results of the RDA analyses (after Hellinger transformation), which all resulted statistically significant (p = 0.005%), are presented for each group. Total variance of each group assemblages varies among 0.50 and 0.65, with the lowest values related to groups with a lower number of species (Amphipoda and Gastropoda). The percentage of explained variance is in some measure comparable to that one for the whole community (0.31), as it ranges between 0.26 and 0.31, except for Gastropoda which present a 21% of the variance explained by environmental variables. Each group shows statistically significant correlation with a subset of environmental variables. Environmental variables which are significantly correlated to taxonomical groups are reported in Table 4.27. Variation partitioning among substrate (sediment and type of coverage) and hydrological variables can summarize these patterns: if both jointly contribute to a value between 9% and 13% of the total variance for each group, substrate variables by themselves are responsible for the 8% (against 7% due to hydrological variables) in the case of Amphipoda, 5% (against 12%) for Errantia, 4% (against 18%) for Bivalvia and only 2% for both Gasteropoda and Sedentaria (against respectively 8% and 13%).

Variograms of total inertia (i.e. variance) for each of these taxonomical groups are presented in Figure 4.73. All the groups show an increase with distance until the total variance is reached. This happens at about 7 km for Errantia and Sedentaria, and at a slightly lower distance for Amphipoda (about 6 km) and Bivalvia (about 4 km). Variance of Gastropoda increase at a smaller extent and more gradually, with no growth from second to fourth distances classes. Residual variance plots indicate significant autocorrelation for all the groups except for Gastropoda. Sedentaria, Errantia and Bivalvia show spatial autocorrelation for the first two classes up to a distance of about 2 km, whereas Amphipoda show spatial autocorrelation up to about 6 km. In fact, both Sedentaria and Amphipoda show a very low increase in residual variance, which statistically is not evidenced at all for Gastropoda. Significant autocorrelation can be noticed for Errantia and Bivalvia at larger distance classes, between 15 km and 20 km. This is probably due to recurrence of similar condition for each basin, since in fact distance classes wider than 15 km are oriented along the main axis of the lagoon. The sum of variograms of constrained and unconstrained variation is in every case almost entirely contained into the envelope for total variance.

	TOT		Sedentaria		Errantia	
S	235		51		36	
Total variance	0.65		0.65		0.64	
Variance explained	0.20		0.18		0.18	
Multiple R-squared:	0.31		0.28		0.28	
Adjusted R-squared:	0.25		0.25		0.24	
	var	p-value	var	p-value	var	p-value
Sand (1mm-63um)	0.040	$< 2.2 \cdot 10^{-16}$			0.052	$< 2.2 \cdot 10^{-16}$
Silt (63-8um)	0.010	0.0010				
TOC	0.020	$< 2.2 \cdot 10^{-16}$			0.0070	0.013
Macroalgae	0.012	$< 2.2 \cdot 10^{-16}$			0.011	0.0010
Phanerogams	0.012	$< 2.2 \cdot 10^{-16}$	0.039	$< 2.2 \cdot 10^{-16}$		
Intertidal surface	0.0078	0.0010	0.0088	0.0010		
Residence time	0.030	$< 2.2 \cdot 10^{-16}$	0.044	$< 2.2 \cdot 10^{-16}$	0.018	$< 2.2 \cdot 10^{-16}$
Median Salinity	0.0069	0.0050	0.0081	$< 2.2 \cdot 10^{-16}$	0.016	$< 2.2 \cdot 10^{-16}$
Mean Temperature	0.013	$< 2.2 \cdot 10^{-16}$	0.017	$< 2.2 \cdot 10^{-16}$	0.032	$< 2.2 \cdot 10^{-16}$
Median DO concentration	0.016	$< 2.2 \cdot 10^{-16}$	0.034	$< 2.2 \cdot 10^{-16}$		
Range of DO concentration	0.0058	0.027				
Median Chlorophyll-a	0.017	$< 2.2 \cdot 10^{-16}$	0.019	$< 2.2 \cdot 10^{-16}$	0.037	$< 2.2 \cdot 10^{-16}$
Median TSS	0.0065	0.0090	0.0089	$< 2.2 \cdot 10^{-16}$		
Range of Total Alkalinity	0.0052	0.038	0.0064	0.013		

	Amphipoda		Bivalvia		Gasteropoda	
S	21		43		19	
Total variance	0.53		0.65		0.50	
Variance explained	0.16		0.17		0.10	
Multiple R-squared:	0.31		0.26		0.21	
Adjusted R-squared:	0.28		0.23		0.19	
	var	p-value	var	p-value	var	p-value
Sand (1mm-63um)	0.027	$< 2.2 \cdot 10^{-16}$	0.061	$< 2.2 \cdot 10^{-16}$		
Silt (63-8um)	0.013	$< 2.2 \cdot 10^{-16}$				
TOC	0.016	$< 2.2 \cdot 10^{-16}$	0.015	$< 2.2 \cdot 10^{-16}$		
Macroalgae	0.038	$< 2.2 \cdot 10^{-16}$				
Phanerogams	0.017	$< 2.2 \cdot 10^{-16}$	0.019	$< 2.2 \cdot 10^{-16}$	0.050	$< 2.2 \cdot 10^{-16}$
Intertidal surface			0.010	0.0010		
Residence time	0.0082	0.0030	0.022	$< 2.2 \cdot 10^{-16}$	0.028	$< 2.2 \cdot 10^{-16}$
Median Salinity	0.032	$< 2.2 \cdot 10^{-16}$			0.0070	0.026
Mean Temperature	0.013	$< 2.2 \cdot 10^{-16}$	0.013	$< 2.2 \cdot 10^{-16}$	0.017	$< 2.2 \cdot 10^{-16}$
Median DO concentration			0.014	$< 2.2 \cdot 10^{-16}$		
Range of DO concentration			0.017	$< 2.2 \cdot 10^{-16}$		
Median Chlorophyll-a						
Median TSS						
Range of Total Alkalinity						

Table 4.27: Results of RDA analyses (after Hellinger transformation), on the species composition matrices for the whole 2002 180-station data set and for the main taxonomic groups. Variance explained by the whole set of (statistically significant) environmental variables and by each variable with p-values. The number of species for each matrix is also presented.

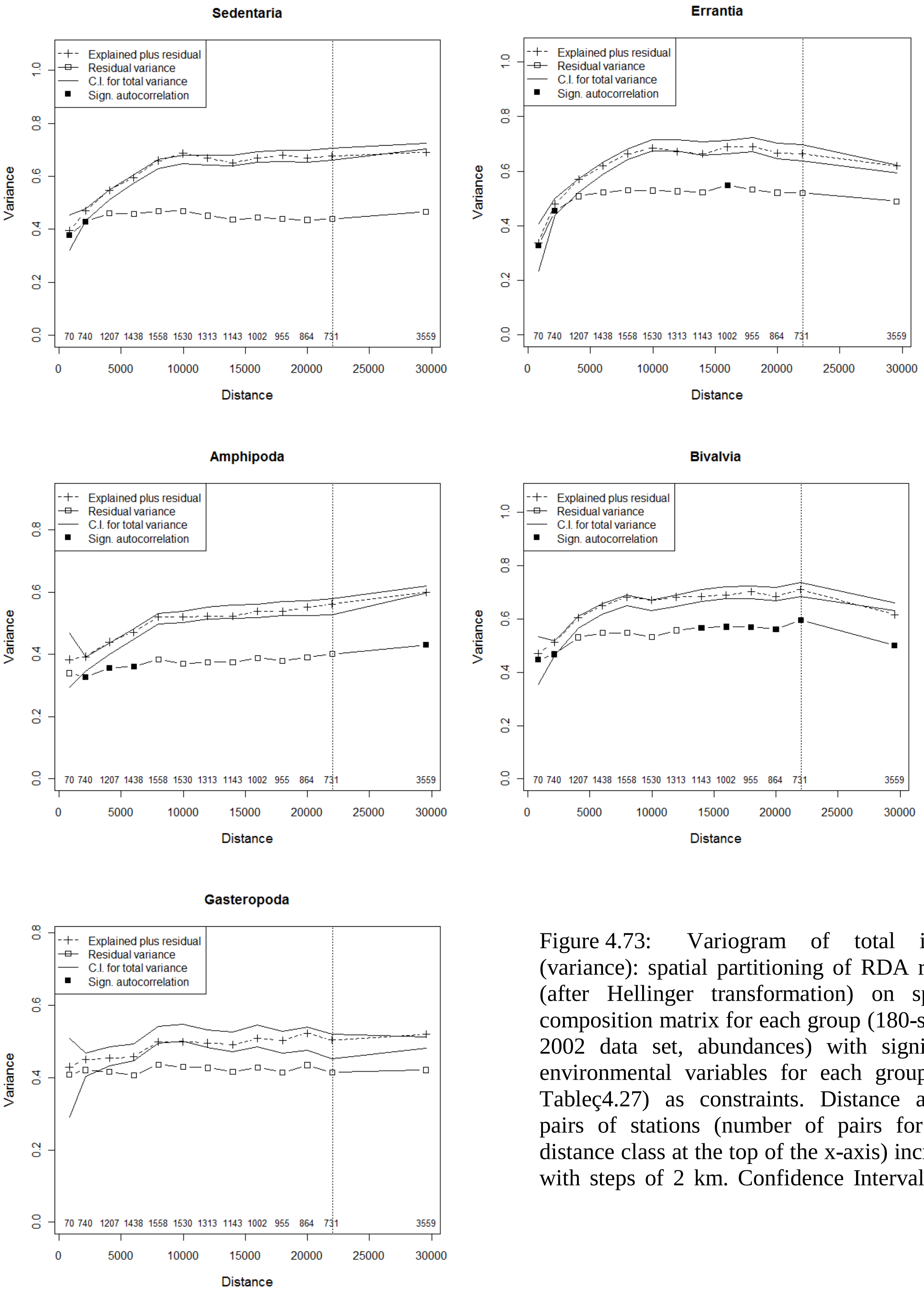


Figure 4.73: Variogram of total inertia (variance): spatial partitioning of RDA results (after Hellinger transformation) on species composition matrix for each group (180-station 2002 data set, abundances) with significant environmental variables for each group (see Tableç4.27) as constraints. Distance among pairs of stations (number of pairs for each distance class at the top of the x-axis) increases with steps of 2 km. Confidence Interval (CI):

4.4.3 Spatial predictors: linear and PCNM models

The presence of spatial structures (or trends) can be assessed by introducing spatial predictors describing the spatial arrangement of sampling stations as function of geographical coordinates, to estimate a model for the observed pattern.

A linear model was tested by introducing the geographical coordinates as independent explanatory variables for three main univariate macrodescriptors: total abundances, biomass, species richness. Only for species richness a linear model of the coordinates was found to be statistically significant (adjusted-R^2: 0.19, p-value: $2.84 \cdot 10^{-9}$). The direction of the plane corresponds to the sea-landward gradient. Partition of the variance was applied with environmental variables and coordinates (i.e. the linear trend) as explanatory variables, to evaluate their relationship. The variance due to environmental variables is partitioned into a strictly linear component (R^2: 0.19) and a non linear component for the remaining 0.44, i.e. a third of the variation of richness across the Lagoon due to environmental variables is spatially linear. No part of the variation related to linear model remains unexplained by environmental variables. This does not prevent that other non-linear spatial structures may be recognized, caused or not by known environmental factors. In the framework of "eigenfunction spatial analysis", PCNMs (Principal Coordinates of Neighbour Matrices, DBEM) were calculated and introduced as spatial predictors to decompose spatial structures at multiple scales. A matrix of euclidean distancies was calculated on the coordinates of the 180 stations of 2002 data set and then truncated at a threshold distance of 2082 m, which is the minimum distance allowing for all the station to be connected. PCoA ordination applied on this matrix produced 179 eigenvectors, with 115 associated to positive eigenvalues and retained in analyses. "Space" is decomposed into different complementary "scales". As the sampling stations are not located into a regular grid, PCNMs doesn't display regular sine-shaped patterns. Nevertheless, it is still possible to recognize "scales of increasing fineness" (Borcard *et al.*, 2004) from the first PCNM onward (the first three PCNMs are reported as example in Figure 4.74). Contour plots are superimposed on maps to help the interpretation of patterns. The set of 115 PCNMs was introduced as explanatory variable to analyze patterns in univariate macrodescriptors (total abundances, biomass, species richness) and community composition matrix (based on squared-root transformed abundances).

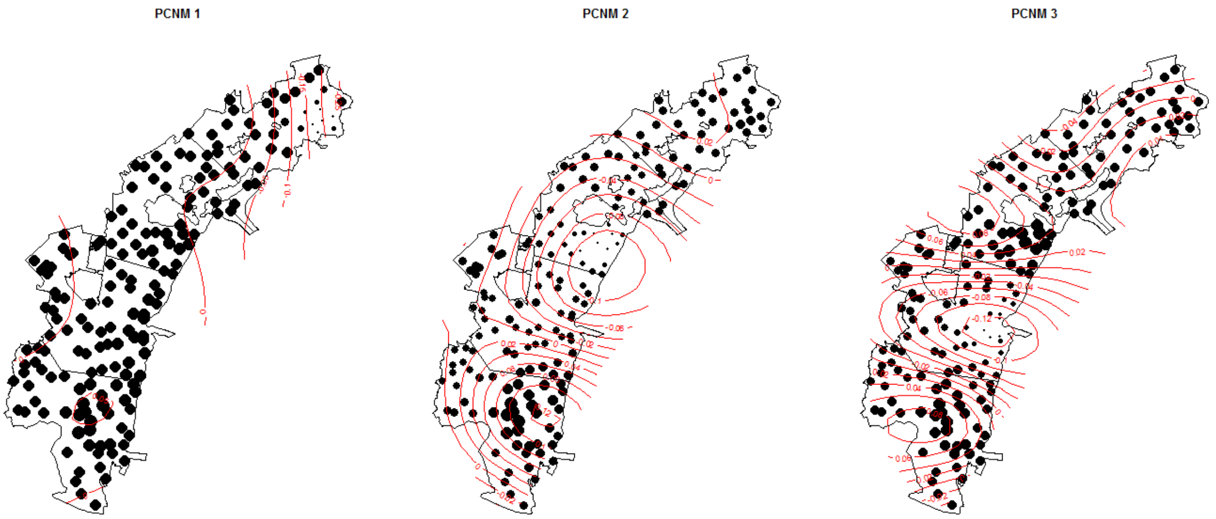


Figure 4.74: First three of 115 PCNMs associated to positive eigenvalues, calculated on 180 stations of 2002 data set. A contour plot is displayed to help the interpretation of patterns.

The resulting adjusted-R^2 of multivariate linear regressions with stepwise forward selection, applied on each of the univariate macrodescriptors with PCNMs as explanatory variables, are reported in Table 4.29. The linear trend identified for richness was removed by considering coordinates as covariable in MLR. Only statistically significant (p-value < 0.05) PCNMs were retained in the model (causing a slight decrease in the R^2 values for the whole models). A list of all significant PCNMs is presented in Table 4.28. A total of 20 PCNMs were retained by the model of total abundances, 15 for biomass and 29 for richness. Resulting models for the three macrodescriptors, as linear combination of PCNMs, are presented near to actual values in Figures 4.79 to 4.81. The PCNM model is unable to correctly represent extremely high importances, which are underestimated.

Plots are coupled with empirical omnidirectional variograms for each of the variables, which contribute to the interpretation of the main pattern of variability of the sub-model (Figure 4.82). Cyclic patterns, as indicated by the ranges (from 10 to 20 km), are related to the basins and watersheds. The clearer pattern is observed in the species richness variogram, which presents a range of about 8 km, that can be also recognized for biomass. Abundances variogram is more confused, but it seems to increase until a range of about 15 km. This range can be considered as the limit of omnidirectionality in variogram, as wider lags can be calculated only along the main axis of the lagoon. The nugget of species richness is lower than the one of biomass, indicating lower variability at the minimum lag, i.e. 1 km, whereas an aggregated distribution of biomasses is suggested.

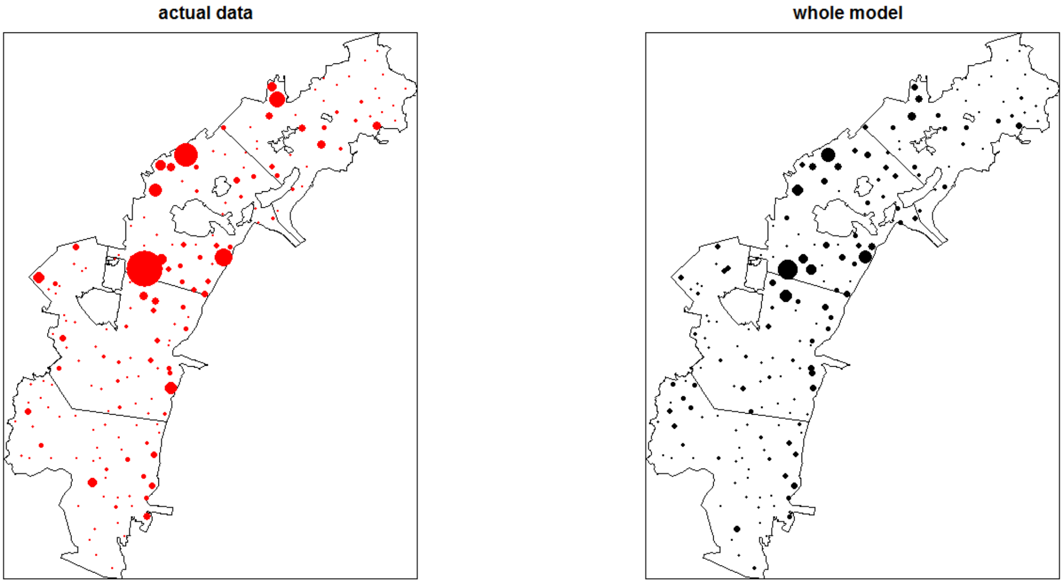


Figure 4.75: Plots of abundances based on actual data (left) and the whole model of significant PCNM (right).

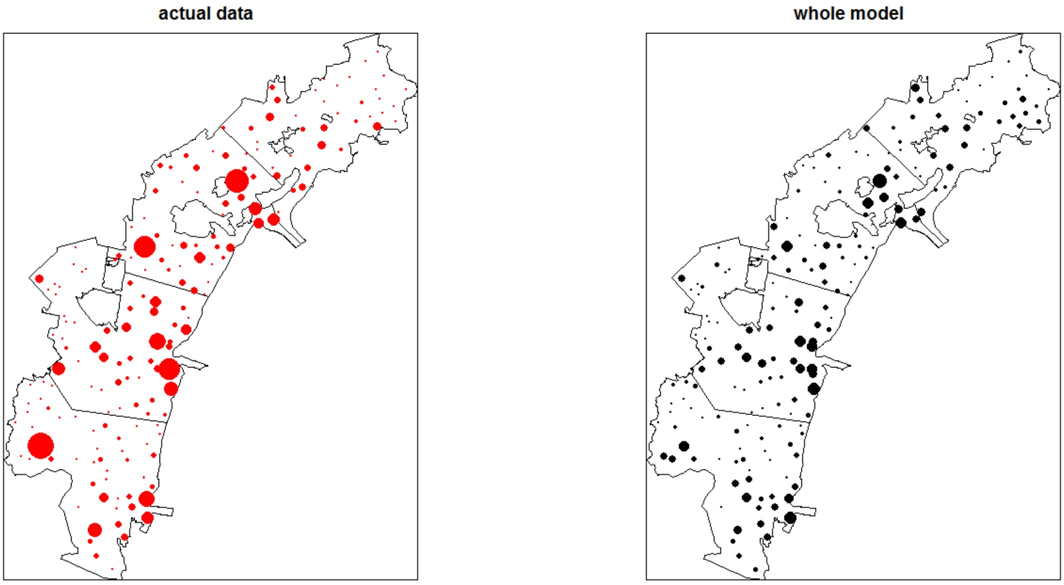


Figure 4.76: Plots of biomass based on actual data (left) and the whole model of significant PCNM (right).

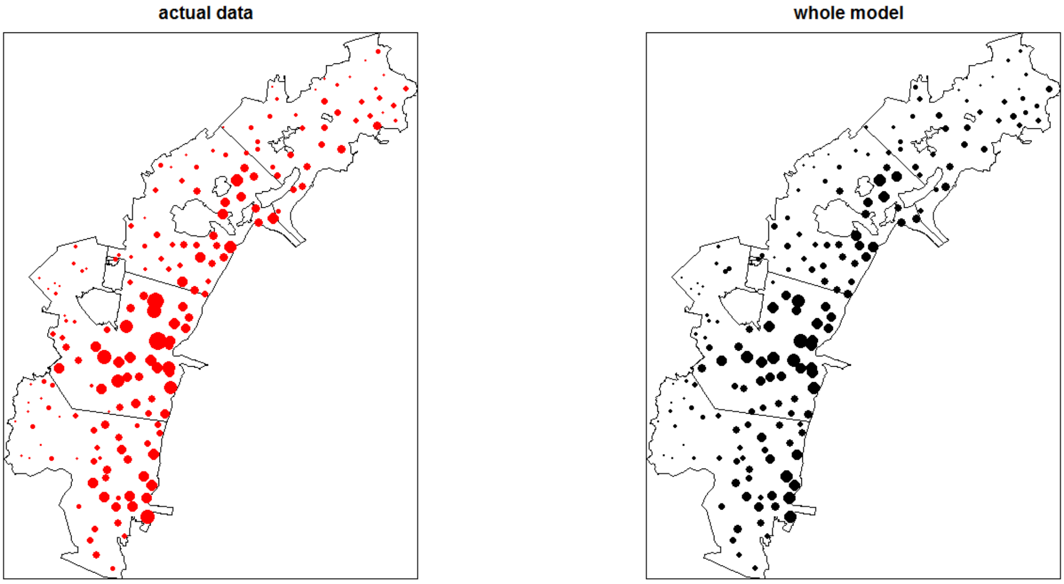


Figure 4.77: Plots of species richness based on actual data (left) and the whole model of significant PCNM (right).

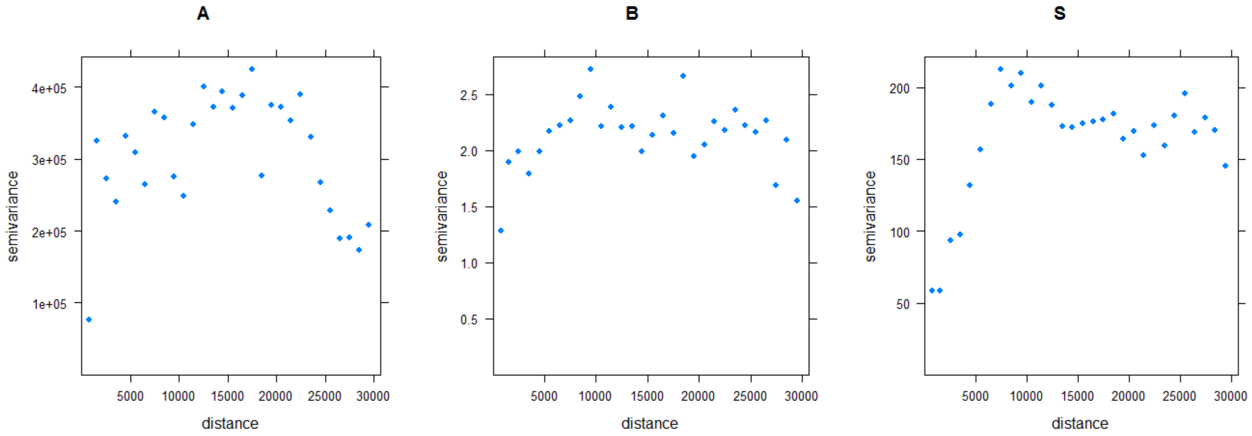


Figure 4.78: Empirical omnidirectional variograms for univariate macrodescriptors abundances (A), biomass (B) and species richness (S), helping the interpretation of main pattern of variability.

The same approach was applied to multivariate community composition data (abundances) for the 2002 data set. A partial RDA analysis (after Hellinger transformation) with forward selection of variables was performed, with PCNMs as explanatory variables and coordinates as covariables. A statistically significant linear trend was previously identified (R^2: 0.12; see Table 4.30), by considering coordinates as explanatory variables. A total of 34 statistically significant PCNMs were considered in the whole model (Table 4.28), explaining the 36% of the total variance (Table 4.30).

As PCNMs are orthogonal, sub-models can be introduced which are a linear combination of given PCNMs, allowing to discretize the *continuum* of "scales". Four sub-models were introduced based on arbitrary subdivision of PCNMs in four subgroups following an order of represented scale, from the first PCNMs which generally corresponds to "wider scale" patterns down to the lowests, related to "small scale" variability (see Table 4.28).

Due to "irregular" structure displayed by PCNMs, eigenvectors for the first two sub-models were selected visually on the bases of the displayed patterns, with patterns wider than basins forming the first sub-model and patterns describing basins configurations forming the second sub-model. Another outcome of the grid irregularity is the coexistence of different "scales" into a same sub-model. Sub-models for each of the univariate macrodescriptors are presented in Figures 4.79 to 4.81. They are coupled with empirical omnidirectional variograms for each of the sub-model. Variograms showing cyclical patterns are related to the cyclic structure of PCNM, and only the first range should be used to describe the scale. Flat variograms indicates the absence of autocorrelation, or that lags are too large compared to the existing autocorrelation (with irregularity of sampling grid also reducing the interpretability of small lags). Finally, decreasing variograms indicate anisotropy in the pattern of variability over the extent and the coexistence of different scales, possibly coupled with larger lags compared to the existing autocorrelation. Moreover, variograms are omnidirectional but the shape of the lagoon causes directionality in lags wider than 15 km. In this approach, variograms should be used to help in interpreting the patterns more than in strictly identify a single range (i.e. scale) (Denny *et al.*, 2004).

The model of species richness is the fittest, allowing to explain the 73% of the variance of detrended data (based on significant PCNMs; 67% in terms of adjusted-R^2), with 50% and 46% of the variance explained by abundances and biomass respectively (Table 4.29). Sub-model 2 is not significant for species richness. Species richness pattern (after detrending) is explained mainly by sub-models 1 and 3. Abundances is explained mainly by sub-models 1, 4, 3 and biomass by sub-models 4 and 1.

	A	B	S	community composition matrix	
PCNM 1			X	X	sub-model 1
PCNM 4		X	X	X	
PCNM 7		X		X	
PCNM 10				X	
PCNM 11		X	X	X	
PCNM 12		X		X	
PCNM 13	X			X	
PCNM 14			X	X	
PCNM 17		X	X	X	
PCNM 18			X	X	
PCNM 2	X		X	X	sub-model 2
PCNM 3		X	X	X	
PCNM 5		X	X	X	
PCNM 6				X	
PCNM 8	X			X	
PCNM 9			X	X	
PCNM 15	X		X		
PCNM 19				X	
PCNM 20	X		X	X	sub-model 3
PCNM 21			X	X	
PCNM 22			X	X	
PCNM 23				X	
PCNM 24			X	X	
PCNM 25	X		X	X	
PCNM 26		X	X		
PCNM 27				X	
PCNM 28			X	X	
PCNM 29			X	X	
PCNM 30			X	X	
PCNM 31	X			X	
PCNM 37	X			X	
PCNM 39	X				
PCNM 40				X	sub-model 4
PCNM 44	X				
PCNM 47		X			
PCNM 48	X		X		
PCNM 51				X	
PCNM 56		X			
PCNM 58	X				
PCNM 59			X	X	
PCNM 60			X	X	
PCNM 65			X		
PCNM 68		X	X		
PCNM 70			X		
PCNM 74		X			
PCNM 75		X			
PCNM 77	X				
PCNM 79	X				
PCNM 80	X				
PCNM 81	X				
PCNM 90			X		
PCNM 91	X				
PCNM 92	X	X	X		
PCNM 95	X		X		
PCNM 101	X				
PCNM 104		X			
PCNM 107				X	

Table 4.28: Statistically significant PCNMs ($p < 0.05$) for models of univariate macrodescriptors (total abundances, biomass, species richness) and community composition matrix (based on abundances) of the 180-station 2002 data set.

	A			B			S		
	R^2	adj-R^2	p-value	R^2	adj-R^2	p-value	R^2	adj-R^2	p-value
Linear trend							0.20	0.19	<2.84e-09
Whole model after forward selection	0.68	0.56	***	0.61	0.51	***	0.81	0.73	***
Whole model with significant PCNMs	0.50	0.44	***	0.46	0.41	***	0.73	0.67	***
sub-model 1	0.033	0.022	.	0.14	0.11	***	0.21	0.18	***
sub-model 2	0.071	0.06	**	0.05	0.039	*	0.19	0.16	
sub-model 3	0.17	0.15	***	0.081	0.076	***	0.20	0.16	***
sub-model 4	0.23	0.18	***	0.19	0.16	***	0.13	0.083	**

Table 4.29: Linear trend and PCNM model of abundances, biomass and species richness: R^2 and p-value for whole models after forward selection, whole models with significant PCNMs ($p < 0.05$) and sub-models (signif.: '***': < 0.001; '**': 0.001-0.01; '*': 0.01-0.05; '.': 0.05-0.1).

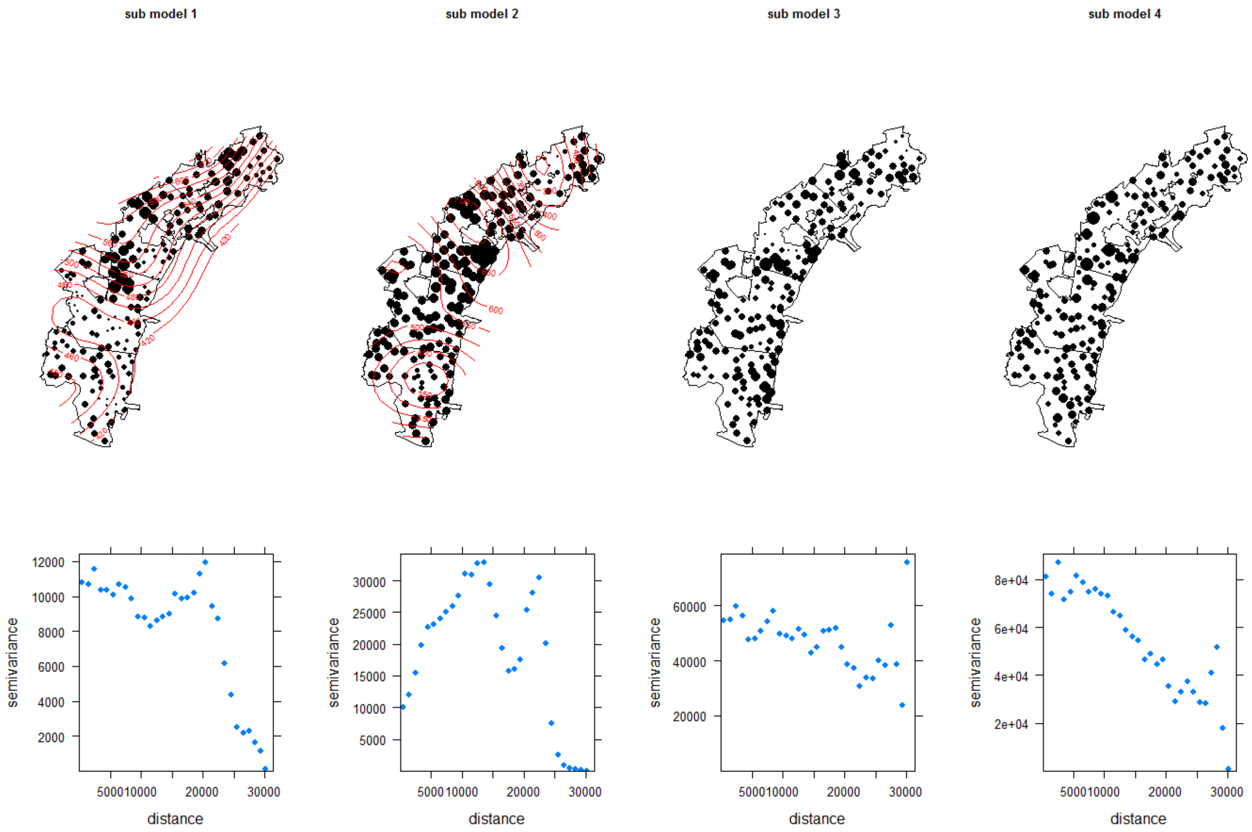


Figure 4.79: Sub-models on abundances. The size of bubbles is proportional to the values. For wider-scale sub-models, a contour plot is displayed to help the interpretation of patterns. An empirical omnidirectional semi-variogram (lag: 1 km) is showed for each of the sub-model.

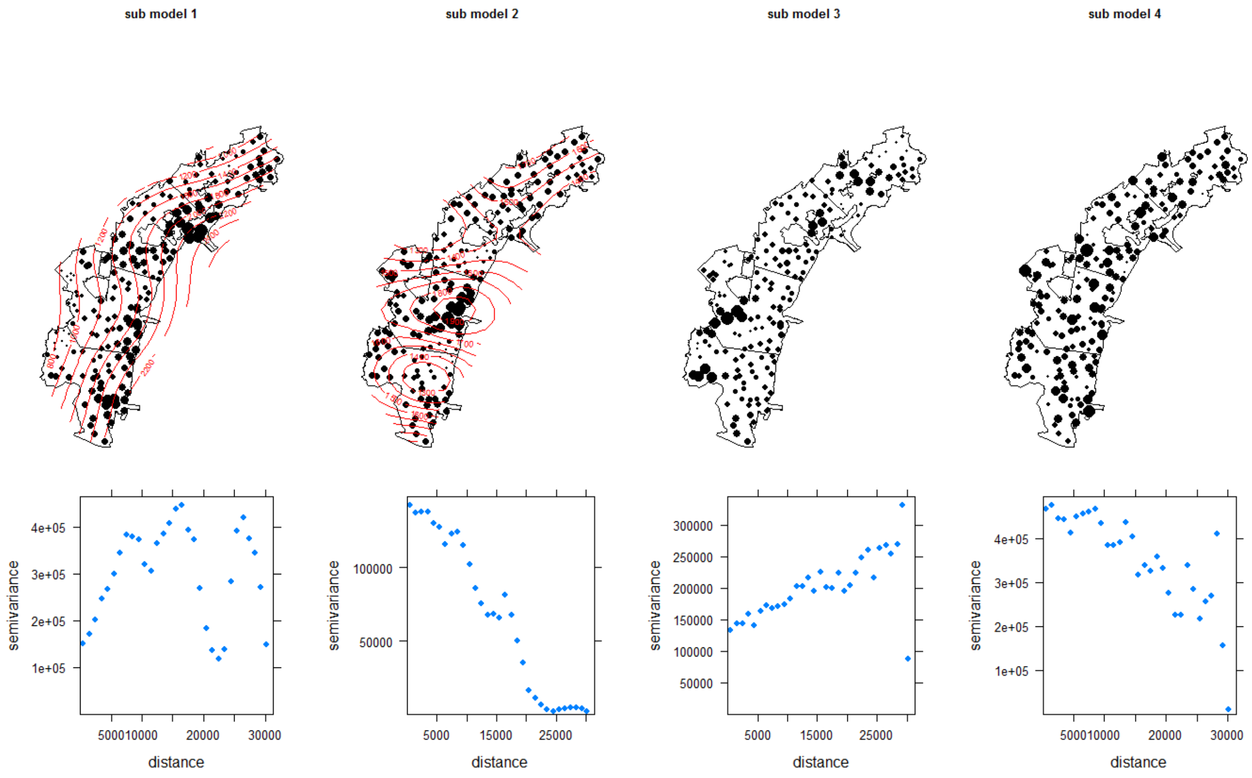


Figure 4.80: Sub-models on biomasses. The size of bubbles is proportional to the values. For wider-scale sub-models, a contour plot is displayed to help the interpretation of patterns. An empirical omnidirectional semi-variogram (lag: 1 km) is showed for each of the sub-model.

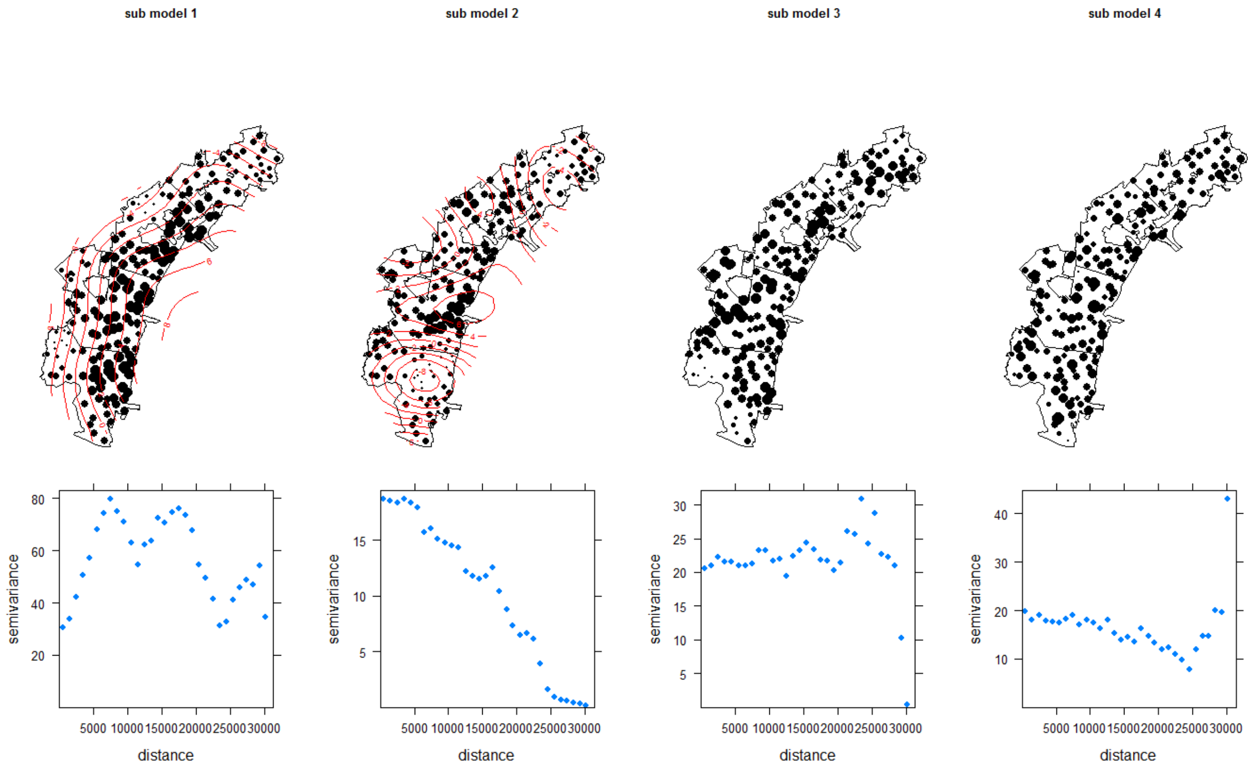


Figure 4.81: Sub-models on species richness. The size of bubbles is proportional to the values. For wider-scale sub-models, a contour plot is displayed to help the interpretation of patterns. An empirical omnidirectional semi-variogram (lag: 1 km) is showed for each of the sub-model.

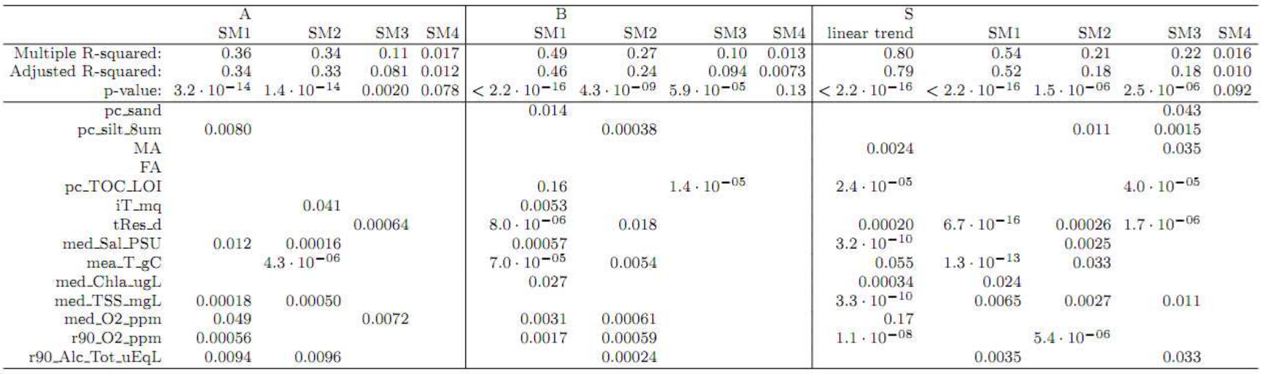
	variance	R^2	adj-R^2	p-value
Total	0.64			
Linear trend	0.08	0.12	0.11	0.05
Whole model with significant PCNMs	0.23	0.36		0.05
sub-model 1	0.088	0.14		0.05
sub-model 2	0.045	0.071		0.05
sub-model 3	0.078	0.12		0.05
sub-model 4	0.026	0.04		0.05

Table 4.30: Linear trend and PCNM model of community composition matrix (abundances): R^2 and p-value for the whole model, after forward selection, with significant PCNMs ($p < 0.05$) and sub-models.

Table 4.30 presents the results of the variation partition among the four sub-models for the species composition matrix. A significant linear trend was found and controlled for by using coordinates as covariables. It explains the 12% of the community matrix variance. A same amount is explained by sub-models 1 and 3.

Each RDA (after Hellinger transformation) applied on the coordinates, on the whole detrended model and on the four sub-models identified significant constrained axes (Table 4.31). The two only axes resulting from the RDA on coordinates are both significant. The whole detrended model presents three significant axes, as well as the sub-models 1 and 2. The sub-model 3 present two significant axes and the sub-model 4 a single one.

The regression to the environmental variables (with forward selection) of each sub-model for the univariate descriptors and each axis of sub-models for the community composition matrix data set, allowed identifying significant explanatory variables for the decomposed patterns. Results are presented in Table 4.31 for macrodescriptors and Table 4.32 for community composition matrix.



	A				B				S				
	SM1	SM2	SM3	SM4	SM1	SM2	SM3	SM4	linear trend	SM1	SM2	SM3	SM4
Multiple R-squared:	0.36	0.34	0.11	0.017	0.49	0.27	0.10	0.013	0.80	0.54	0.21	0.22	0.016
Adjusted R-squared:	0.34	0.33	0.081	0.012	0.46	0.24	0.094	0.0073	0.79	0.52	0.18	0.18	0.010
p-value:	$3.2 \cdot 10^{-14}$	$1.4 \cdot 10^{-14}$	0.0020	0.078	$< 2.2 \cdot 10^{-16}$	$4.3 \cdot 10^{-09}$	$5.9 \cdot 10^{-05}$	0.13	$< 2.2 \cdot 10^{-16}$	$< 2.2 \cdot 10^{-16}$	$1.5 \cdot 10^{-06}$	$2.5 \cdot 10^{-06}$	0.092
pc_sand					0.014							0.043	
pc_silt_8um	0.0080					0.00038					0.011	0.0015	
MA									0.0024			0.035	
FA													
pc_TOC_LOI					0.16		$1.4 \cdot 10^{-05}$		$2.4 \cdot 10^{-05}$			$4.0 \cdot 10^{-05}$	
iT_mq		0.041			0.0053								
tRes_d			0.00064		$8.0 \cdot 10^{-06}$	0.018			0.00020	$6.7 \cdot 10^{-16}$	0.00026	$1.7 \cdot 10^{-06}$	
med_Sal_PSU	0.012	0.00016			0.00057				$3.2 \cdot 10^{-10}$		0.0025		
mea_T_gC		$4.3 \cdot 10^{-06}$			$7.0 \cdot 10^{-05}$	0.0054			0.055	$1.3 \cdot 10^{-13}$	0.033		
med_Chla_ugL					0.027				0.00034	0.024			
med_TSS_mgL	0.00018	0.00050							$3.3 \cdot 10^{-10}$	0.0065	0.0027	0.011	
med_O2_ppm	0.049		0.0072		0.0031	0.00061			0.17				
r90_O2_ppm	0.00056				0.0017	0.00059			$1.1 \cdot 10^{-08}$		$5.4 \cdot 10^{-06}$		
r90_Alc_Tot_uEqL	0.0094	0.0096				0.00024				0.0035		0.033	

Table 4.31: MRA of the linear trend model and the sub-models of abundances, biomass and species richness with environmental variables as explanatory variables: R^2 and p-value for overall regression model and significance of environmental variables as explanatory variables of the regression are presented.

	linear trend		Whole PCNM model			PCNM sub-model 1		
total var	0.64		0.64			0.64		
var constrained	0.08		0.23			0.09		
R2	0.12		0.36			0.14		
	axis 1	axis 2	axis 1	axis 2	axis 3	axis 1	axis 2	axis 3
Var	0.051	0.028	0.057	0.033	0.023	0.032	0.019	0.010
% of explained var of the model	0.65	0.35	0.25	0.14	0.10	0.36	0.21	0.11
cum % of explained var of the model	5.20	1	0.25	0.39	0.49	0.36	0.57	0.68
Multiple R-squared env:	0.73	0.67	0.5747	0.4461	0.5469	0.6082	0.455	0.3725
Adjusted R-squared env:	0.72	0.65	0.5441	0.4269	0.523	0.5826	0.4295	0.3545
p-valuE:	¡ 2.2E-16 ***	¡ 2.2E-16 ***	¡ 2.2E-16	¡ 2.2E-16	¡ 2.2E-16	¡ 2.2E-16	¡ 2.2E-16	3.61E-16
FA	0.11	0.040 *			7.89E-08 ***	0.15		1.09E-10 ***
iT_mq	.			0.00037 ***			0.00046 ***	
MA		0.00046 ***	0.071 .	0.00061 ***	8.41E-06 ***	0.04168 *	0.080 .	
mea_T_gC		0.0080 **	0.0064 **	1.80E-11 ***	0.00030 ***	6.91E-05 ***	8.86E-10 ***	2.35E-07 ***
med_Chla_ugL	3.4E-06 ***	4.27E-06 ***	0.00027 ***		0.042 *	2.17E-06 ***		
med_O2_ppm		6.34E-05 ***	0.065 .			0.024 *		
med_Sal_PSU	1.1E-15 ***				1.04E-05 ***		0.019 *	0.011 *
med_TSS_mgL		6.41E-08 ***	6.17E-05 ***			2.48E-05 ***		
pc_sand			0.0096 **		0.00090 ***	0.0011 **		
pc_silt_8um		0.005002 **	0.062 .		0.0020 **	0.047 *	0.0081 **	
pc_TOC_LOI		4.51E-07 ***	5.05E-05 ***		0.0032 **	9.93E-06 ***		0.0059 **
r90_Alc_Tot_uEqL								0.0071 **
r90_O2_ppm		0.0046 **	3.38E-09 ***	0.00014 ***		4.23E-08 ***	0.00026 ***	
tRes_d	8.4E-11 ***	0.017 *	1.17E-07 ***	6.80E-15 ***		4.03E-11 ***	1.82E-10 ***	

	PCNM sub-model 2			PCNM sub-model 3		PCNM sub-model 4
total var	0.64			0.64		0.64
var constrained	0.05			0.08		0.03
R2	0.07			0.12		0.04
	axis 1	axis 2	axis 3	axis 1	axis 2	axis 1
Var	0.014	0.010	0.009	0.024	0.014	0.011
% of explained var of the model	0.32	0.22	0.20	0.31	0.18	0.43
cum % of explained var of the model	0.32	0.54	0.74	0.31	0.49	0.43
Multiple R-squared env:	0.6157	0.5251	0.4712	0.5579	0.3843	0.338
Adjusted R-squared env:	0.593	0.5029	0.4366	0.5345	0.3555	0.3111
p-valuE:	¡ 2.2E-16	¡ 2.2E-16	¡ 2.2E-16	¡ 2.2E-16	6.56E-15	5.99E-13
FA	0.080 .	2.00E-06 ***	0.0050 **	0.032 *		
iT_mq			0.027 *		0.00012 ***	
MA	0.0082 **	0.00011 ***	0.079 .	0.0060 **		0.043 *
mea_T_gC	2.70E-08 ***		1.00E-06 ***	0.0041 **	8.39E-08 ***	
med_Chla_ugL	2.67E-11 ***		0.012 *	3.46E-06 ***	0.056 .	
med_O2_ppm	0.020 *					
med_Sal_PSU		2.94E-09 ***	0.017 *		0.041 *	
med_TSS_mgL	1.44E-05 ***		0.0090 **	0.00013 ***		0.014 *
pc_sand	0.0017 **					0.0011 **
pc_silt_8um		0.026 *			0.064 .	0.012 *
pc_TOC_LOI	5.56E-05 ***	0.00017 ***	0.042 *	2.85E-05 ***		
r90_Alc_Tot_uEqL		0.090 .				0.00022 ***
r90_O2_ppm	0.00042 ***		9.94E-07 ***	1.64E-07 ***	0.023 *	2.51E-11 ***
tRes_d	1.95E-09 ***	3.92E-10 ***	0.0039 **	8.27E-11 ***	4.17E-10 ***	

Table 4.32: MRA of the significant axes of the RDA with coordinates (linear trend) and PCNMs whole model and sub-models with environmental variables as explanatory variables. Only significant axes accounting for a noTable amount of variance were considered. R^2 and p-value for overall regression model and significance of environmental variables as explanatory variables of the regression are presented.

From sub-model 1 to 4 the variance explained by environmental variables decreases. As previously noticed, the main part of the model of abundances lies in sub-models 3 and 4 (Table 4.29). Sub-model 4 is not explained significantly by environmental variables, whereas sub-model 3 is explained significantly in particular by residence time and DO, but these explain only about the 10% of the sub-model (Table 4.31). The first sub-model of biomass (which mainly explains the whole model, being $R^2 = 0.46$) is explained by residence time, mean temperature, salinity, intertidal surface, DO average and range, sand percentage, and chlorophyll *a*; hence, as it can be observed in Figure 4.80, it represents the transitional gradient. Species richness linear trend is also explained by transitional gradient variables, such as salinity, TSS, range of DO, chlorophyll *a*, residence time, mean temperature and macroalgae. PCNM sub-model 1 also reflects both the part not along the main trend and the non-linear part of the transitional gradient; it is explained (about the 50%) by residence time, main temperature, TSS, chlorophyll *a* and range of alkalinity. Sub-model 2, which seems again related to the gradient (e.g. it describes the non linear part of the salinity) and is explained (about 20%) by range of DO, residence time, salinity, TSS and mean temperature, is actually not significant (see Table 4.29). Sub-model 3, which is significant (Table 4.29) explain another 20%. As an example, a model of species richness has been created which sums the linear trend model and significant PCNM sub-models 1 and 3, explaining 53% of the total variance, and mostly explained by environmental variables. It is presented in Figure 4.82, together with the residuals from the original data that still maintains a spatial structure.

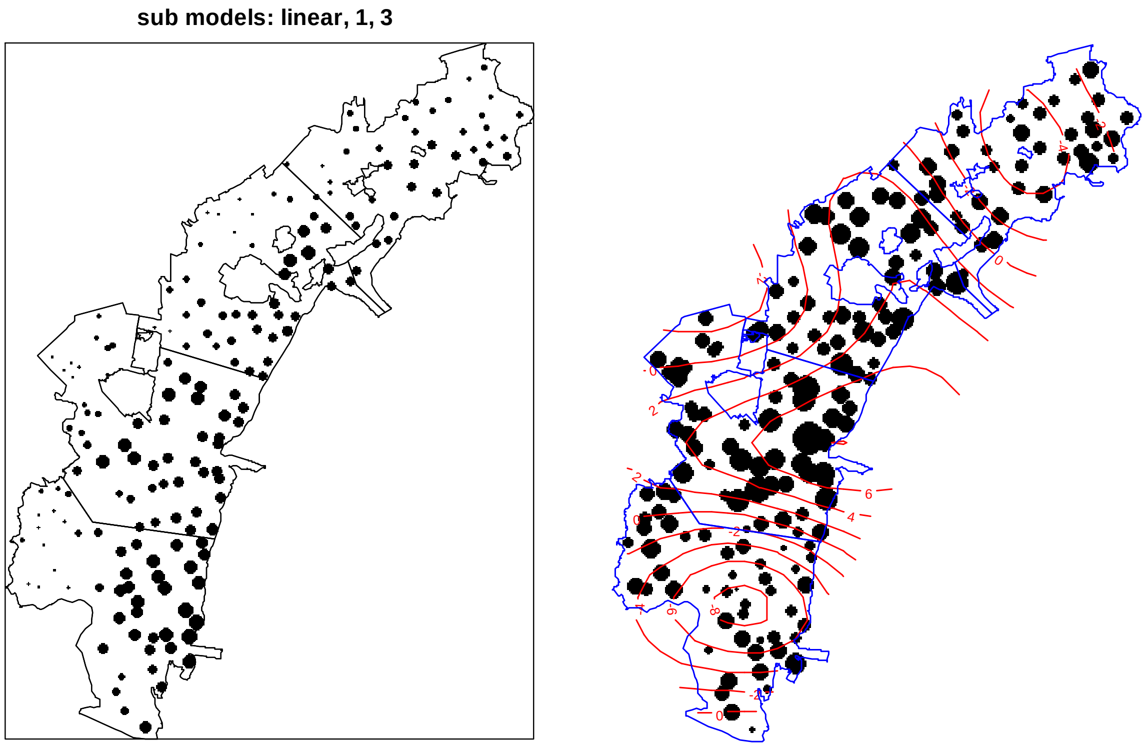

sub models: linear, 1, 3

Figure 4.82: Left: Model of species richness composed by linear trend model and significant sub-models 1 and 3 (see Figure 4.81, Tables 4.29 and 4.31). The size of bubbles is proportional to the values.

Right: residuals of the model (original value - model) with contour plot to help the interpretation of patterns.

The linear trend (R^2 = 0.12) for the multivariate structure of community (on abundances) is explained by almost the totality of the environmental variables (Table 4.32), indicating once again the role of transitional gradient. Each axis of the whole models of the detrended data has a high percentage (about the 50%) of variance explained by environmental variables. The first and the third axes again are related to the variables of the transitional gradient, whereas the second axis is explained by intertidal surface, macroalgae, mean temperature and range of DO (together with residence time). Sub-models 1 and 3, which account for the main part of the whole model, fails to identify spatial patters particularly related to single variables.

5 DISCUSSION AND CONCLUSIONS

The present work analyzes the macrozoobenthos community of the Lagoon of Venice. This coastal transitional ecosystem, one of the most important of the Mediterranean, can be considered an estuarine lagoon, located at a midpoint of a *continuum* with marine lagoons and estuaries as the endpoints, but highly modified by man over the centuries (Chapter 1.1). It is a complex system characterized by abundant seawater exchange due to sensible tides and large inlets and presenting a great variety of patterns and processes at multiple spatial and temporal scales (Chapter 1.6). These kinds of environments are characterized by strong gradients of both abiotic and biotic components (McLusky, 1993; Tagliapietra *et al.*, 2009).

Spatial and temporal variability of the communities and the structuring environmental factors were analyzed. Understanding the relationships between communities and the environment plays a central role in quality assessment through indices and indicators (Chapter 1.2). This is particularly true for coastal transitional ecosystems as they are naturally stressed environments with confounding effects in the identification of anthropogenic stresses (the "Estuarine Paradox"; Chapter 1.3).

The specific objective is the analysis of the benthic macroinvertebrates communities (here defined operationally by mesh size of 1 mm; see Chapters 1.2 and 3.3.2) of the subtidal flats, which represents the greater part of the lagoon surface (Chapter 3.1). Intertidal areas, as well as other landforms, are characterized by distinctive ecological patterns and processes, and they are not considered in the present work. All the analyses and results were performed on data sets referring to the 2002-2007 period.

Main results are presented in response of the following scientific questions, which were initially stated in the Objectives.

5.1 What is the variability over the years of the macrozoobenthic community structure at the whole lagoon scale?

An overall list of 315 taxa was produced for the three years. To describe the community structure, univariate macrodescriptors and multivariate data sets were analyzed. For each station, considered univariate macrodescriptors include species richness, total abundance, total biomass and main ecological indices.

As expected, both richness and diversity indices, such as the Margalef index, Shannon index and $E(S_{50})$, present a strong correlation ($r \geq 0.7$) with the number of species (Chapter 4.2.1). Diversity indices integrate the number of categories, i.e. species, and the distribution of objects among them, expressed as evenness. Positive correlation among richness and diversity is due to both the major importance given to richness in the indices and the positive correlation among richness and evenness, which is a pattern recurrently found in community ecology, suggesting that species richness is related to variation in relative abundances (Stirling & Wilsey, 2001). Major attention was then given to species richness, along with abundance and biomass.

These findings are in line with classical ecology. Diversity usually varies with the harshness of the environment. Low richness and high dominance (low evenness) indicate the most stressful or most recently stressed areas, conversely, areas with the highest richness and evenness represent most stable environments (Engen, 1979). Disturbances (such as floods, hypoxias, etc.) and pollution are classically found to reduce the diversity of communities. These simple relationships are due to the fact that few species are able to tolerate the conditions of a harsh or disturbed environment. However, at the same time, species that are tolerant of harsh environmental conditions have less competition for resources by other species and their numbers will increase, thereby increasing the dominance (or decreasing the evenness) of a few species. Relationship between richness and dominance is reflected on the functionality and some key processes such as trophic relationships (Dangles & Malmqvist, 2004).

A gradient from high richness/high evenness to low richness/low evenness (high dominance) was evidenced, indicating a range of "degree of stress" in the lagoon. The number of species decreases considerably from the inlets landward, a pattern more or less explicitly recorded in the Lagoon of Venice in previous studies (Maggiore & Keppel, 2007; Rismondo & Visintini Romanin, 1997). A similar pattern richness/evenness was also pointed to a smaller scale in the Venice Lagoon, in the presence of a quite strong estuarine gradient (Tagliapietra *et al.*, 1998a, 2000a).

The range of richness among the stations is comparable for all the three years, with a maximum of about 70 species per station. The reduction in species richness from sea landward can be considered the main sign of the coenocline related to the transitional gradient, i.e. the progressive change of the community structure, related to a succession of species (and assemblages). This is recognized as a distinctive feature for a wide range of CTEs, including Mediterranean lagoons (e.g. Guelorget *et al.* 1987; Reizopoulou & Nicolaidou 2004; Rossi *et al.* 2006), and which deserves an explanation.

These patterns can be related to the salinity gradient (Remane, 1934), stronger in estuaries than in lagoons, sediment type (e.g. Boesch, 1973; Thrush *et al.*, 2003) and seawater renewal (Gamito, 1997, 2006). On the other hand, a gradient of saprobity generated by the interplay of organic matter accumulation and seawater renewal was recently advocated (Tagliapietra *et al.*, submitted). This latter consideration derives by a clear parallelism between the biological succession presented in the Guélorget and Perthuisot’s (1983) and the Pearson and Rosenberg’s (1978) models. The structure described by Guélorget and Perthuisot (1983) was actually evidenced by a number of researches in lagoons worldwide (see for example Evagelopoulos *et al.*, 2007; Munari & Mistri, 2008; Palacin *et al.*, 1991; Victor & Victor, 1997; Reizopoulou, 2004). The model has found, so far, some difficulties to be quantified, being the confinement defined in terms of mean time that non specified “vital” elements of marine origin take to reach any given point of the basin (Perthuisot & Guelorget, 1995), although some effort was made in this direction (Frénod & Goubert, 2007).

Most species are of marine origin (Barnes, 1989; Cognetti & Maltagliati, 2000) and, consequently, it can be expected that an increasing divergence from marine conditions is tolerated by progressively fewer species (McLusky & Elliott, 2004). However, in non-tidal systems with reduced or absent freshwater inflow such as some marine lagoon is possible that the community doesn't follow a strong and clear gradient (see for example the Mar Menor, Spain, Pérez-Ruzafa & Marcos-Diego, 1992, or the Varano Lagoon, Italy, Specchiulli *et al.*, 2010).

The overall temporal evolution is characterized by a significant variability (Chapter 4.2.4) and a general increase of species richness, accompanied by a substitution of species (Chapter 4.2.6) over the three years. Only the 44% of the 315 taxa is shared among the three MELa studies, which possibly suggests the presence of colonization processes by marine species, mainly in the Open Lagoon, by means of migration or, most likely, transport of planktonic stages from the sea (Pérez-Ruzafa & Marcos, 1992), due to the high seawater renewal rates. Another possibility, not yet fully investigated, is that the deeper lagoon channels, characterized by water masses larger and more oxygenated than the shallow mudflats, act as *refugia* for invertebrate populations, so the re-colonization could take place through a lagoonal rather than a marine pool of organisms.

Most of the dominant taxa (69%), despite shifting from year to year, come from the set of the species jointly found in all the three studies (Chapter 4.2.2), indicating that the taxa structuring the communities (at least in terms of dominance) are mostly established in the lagoon. About the 60% (191 out of 315) of the taxa were not recorded in previous main extensive and quantitative studies in the Venice Lagoon (Vatova study in 1930-1932, Giordani Soika studies in 1948 and 1968, A.3.16/II in 1991), but it should be noted that these studies are characterized by different methods and effort (Chapter 1.7) and that in any case most of the species were already recorded by other ecological or more detailed zoological studies (Coen, 1937; Fauvel, 1938; Giordani Soika, 1948; 1950 among others).

A significant variability (Chapter 4.2.4) and a general increase of numerical abundance and biomass were also registered, with main differences between 2002 and 2003 (Chapter 4.2.1). Biomass shows a mild direct correlation with species richness (r = 0.52), as expected from known relationships (e.g. Guélorget, 1987; Pearson & Rosemberg, 1978). Both high diversity and high biomass are generally assumed a sign of thriving communities and stable environments. Under the classical diversity-stability hypothesis, biodiversity enhances stability and biomass of the ecosystem (Elton, 1958 MacArthur, 1955; Odum, 1953). An increase in the biomass diversity was found to causes an increase in the whole systemic stability in aquatic ecosystems (Aoki & Mizushia, 2001). Nevertheless this rule is not always applicable mainly because instability of diversity-stability relationships (McNaughton, 1977). Manipulative experiment on marine benthos (Bolam *et al.*, 2002) suggested complex relationships between diversity/abundance/biomass and ecosystem functions, supporting the null-hypothesis of weak or no relationships between ecosystem function, diversity and biomass and linking processes more on functional groups than species richness. High biomass can be related to the predominance of *K*-selected species, characterized by larger body size and lower abundances. High values of total abundance indicate instead the predominance of *r*-strategists. These are opportunists characterized by small body size, shorter life span and rapid development (Heip, 1974; Pianka, 1970). Nevertheless, it should be stressed that there is a *continuum* between *K*- and *r*-strategies driven by the habitat typology, as well as between opportunistic and non-opportunistic species (Cognetti, 1978), and that features of both life-styles can be combined in the fauna of lagoons (Barnes, 1980).

According to classical theories (Pearson & Rosenberg, 1978), the increase in *r*-strategists reflects an increase in environmental stress. The occurrence or *r*-strategists can be explained by their ability of quick reproduction/invasion and exploitation of empty niches. They are capable to grow rapidly up to large numbers in case of low competition and predation due to selective condition or abrupt changes, such as anoxia events, but also undergo catastrophic mortalities, hence forming assemblages which are subjected to rapid and frequent changes. Despite at some extent high abundance are spatially complementary to high total biomass, they show more complex patterns, with no clear gradient and high variability between years. This can also be related to analytical issues. Samplings were performed once per year during spring, and describe a synoptic but instantaneous situation. Patterns displayed are instead caused by processes which occur at a range of temporal scales, from days to months, responding to different dynamics and previous state. In particular, *r*-strategists populations follow more fast and sudden colonization dynamics, that can or cannot be recurrent, than processes involving *K*-strategists, which compose more stable assemblages. Hence the measure of biomass integrates a wider temporal signal than abundance. High temporal variability in univariate descriptors and multivariate structure was already verified for the Venice Lagoon at the seasonal and monthly scale (Maggiore & Keppel, 2007; Tagliapietra *et al.*, 1998a, 2000a). Processes acting at different time scales tend to superimpose and increase complexity of spatial patterns.

According to Mageau and Costanza (1999) the health of an ecosystem should be assessed taking into account the resilience, the organization and the vigour of a system through a comprehensive, multiscale, hierarchical and dynamic evaluation. This is a quite hard task since the lagoon is a complex and variable ecosystem for which set of criteria and benchmarks implies an awareness of a high inherent uncertainty (Kay & Regier, 2000). Nevertheless, while an increase in abundance is primarily dependent on *r*-strategist species, an increase of biomass is generally due to *K*-strategist (or, at least to species having a more "K behavior"). We may consider the increase in species richness as an increase in organization of the system and the increase in biomass as an increase in vigour. This can be either a sign of "improved" conditions of the lagoon or a shift of the system towards more "marine" conditions (such as, for example enhanced seawater renewal, reduced continental loads of freshwater and sediments). In the absence of reliable references about a shift in physical condition of the system, then, very carefully, we could recognize, in the benthic community, some improvement between 2002 and 2007.

Mistri (2002) suggests that the perception of persistence, or variability, of communities changes, when focusing to different "levels of numerical resolution", i.e. to the presence/absence of taxa or their relative or absolute importances, but this reasoning is applicable also to high-rank taxa. The community structure was analyzed by means of high-rank taxonomic and trophic composition (Chapter 4.2.3). Despite absolute values change, Sedentaria and Amphipoda characterize in all the years the abundance, with Tanaidacea achieving a major role only in 2003. Biomass is mainly related to Bivalvia (and secondarily to Decapoda) in all the years, with other important groups (Sedentaria, Errantia, Gastropoda) varying over the years. The taxonomical groups maintain a functional role in the community. The first two years are characterized by a higher number of organic-tolerant species, whereas 2007 presents more sensitive ones. The overall trophic structure is dominated by filter-feeders and deposit-feeders in terms of abundance and biomass. Their relative role changes considerably over the 3-year period. In 2002, biomasses were dominated mainly by filter-feeders and secondarily by surface deposit-feeders, which also dominated in terms of abundance, and carnivorous. In 2003 all the groups underwent an increase in both or either abundance and biomass, but with relative importance of filter-feeders in terms of biomass decreasing compared to sub-surface deposit-feeders. In 2007 the community is again dominated by filter-feeders, with sub-surface deposit-feeders decreasing considerable in absolute terms. Stabilizing properties of filter-feeders were evidenced by Ott and Fedra (1977), converting water column biomass into macrofauna biomass with a lower respiration:biomass ratio.

A significant variability among years was also recognized in multivariate community composition (Chapter 4.2.7). The 2007 samples are quite different from those of 2002 and 2003, which largely overlaps, as expected due to different lag. Pairwise comparison indicates that similarity is higher between subsequent samplings, suggesting a continuous and unidirectional shift in communities composition over the years.

5.2 On the bases of the hydrogeological zonation, what is the spatial and interannual variability of the benthic community?

A zonal approach was adopted, by which changes in benthic communities structure are referred to hydrological zones with relatively homogeneous environmental conditions which characterize the lagoon ecosystems (Rochford, 1951; Roy *et al.*, 2001). An existent hydrogeological zonation, which identifies five zones, was introduced into analyses (Zanon, 2006; Tagliapietra *et al.*, 2009; Chapter 3.1.1). Some author suggests to consider the basins as functional units of a coastal lagoon (Marani *et al.*, 2004; Tagliapietra & Volpi Ghirardini, 2006), even though the degree of connectivity between them should be taken in account (Tagliapietra & Volpi Ghirardini, 2006). Basins are characterized by different hydrodynamics and inflow, but maintain roughly the same succession of zones. The zones are the Marine Tidal Deltas (TD), close to the three sea inlets, the Central Basin (CB) and the Sheltered Lagoon (SL), located in the middle section of the lagoon and two landward zones, the Fringe Zone (FZ) and the Bayhead Estuary (BE). Zones follow a succession along the sea-landward direction, with main sequence being TD - CB - FZ. In the Treporti basin the sequence include the distinctive zone SL and, landward, the BE. This succession follow the species richness gradient (Chapter 4.2.4), and was used to simplify the ecocline in descriptive analyses (Chapter 4.2.5).

Variability of the main univariate macrodescriptors over the years previously described at the lagoon scale was decomposed among the zones. The species richness curves show the typical monotonous trend in all the years, with a more pronounced increase in 2003 and 2007 in the inner zones. The communities of Restricted Lagoon are typically characterized by low richness and numerically dominated by tolerant species, such as opportunistic polychaetes and amphipods. Between 2002 and 2003 abundance and biomass increased in the seaward zones, but especially in the Sheltered Lagoon. In 2007 an increase in abundance occurred in the Tidal Delta and the Fringe Zone. With regards to biomass, the Tidal Delta was stabilized, while for the inner zones (Fringe Zone and Bayhead Estuary) the increase in biomass continued in 2007. For 2007 it is registered an increase of diversity all over the lagoon, except in the Central Basin where a sensible reduction of both abundances and biomass was recorded (Chapter 4.2.5). An increase in abundances accompanied by a decrease of biomass may be therefore indication of more stressed situations. This situation occurred in 2002 at the Sheltered Lagoon stations and in 2003 at the Sheltered Lagoon and Bayhead Estuary stations.

The factor "zone" was verified, by CAP analysis, to explain only a value about the 12% of the variation of community composition matrix. Basin also is a significant factor but explains a still lower percentage of variability (about the 5%). Variability among years too is lower than among zones.

Spatial structure of community in terms of species compositions follows a main axis of variation which is less or more parallel to the decrease in species richness. This gradient is enhanced by the role of the number of categories (i.e. species) in multivariate analyses. Stations with few species have lower similarity among themselves and also with respect to stations with higher richness. This phenomenon is due to both the correlation between richness and evenness above reported but also to the small number of categories (species). It can be observed for example in nMDS plots when a model of the number of species is superimposed, and a characteristic convolute configuration around richer stations is observed (Chapter 4.2.4).

To describe operationally the spatial variation in community composition, the coenocline was resolved into discrete assemblages, identified by means of a cluster analysis on abundance (Chapter 4.2.6), with no assumption about the inner relationships among species. The most widely known definitions of biological community stress in a different way on interactions among components, with at one extreme the "biocoenosis" approach by Möbius (1877), ("a collection of species, and a massing of individuals, which find here everything necessary for their growth and continuance.[…] Any change in any of the relative factors of a biocoenosis produces changes in other factors”), and on the other the use of term in a more descriptive way, defining the community “A group of population of plants and animals in a given place” (Krebs, 1985). The idea of biocoenosis characterized by strong and characteristic relationships between components was rejected by many authors for macrobenthos communities in transitional ecosystems, which instead stress on a continuous substitution of species along the lagoonal gradient, a concept that was applied to the Lagoon of Venice at first by Giordani Soika (1951). The presence of a biological seriation with the substitution of species along an environmental gradient was described by Pérès and Picard (1968) in terms of natural communities and by early authors (Grassle & Grassle, 1974; Gray, 1979; Leppäkoski, 1975; Pearson, 1975; Pearson & Rosenberg, 1978; Reish, 1971; Rosenberg, 1976) in terms of species seriation after anthropogenic impacts. Guélorget & Perthuisot (1983) explicitly described a seriation in lagoons and proposed a confinement scale for Mediterranean lagoons consisting in six discrete zones. However, as reported above, they did not give a systematic and quantitative description of the distribution of species along the “paralic” seriation, with only a few taxa listed.

Assemblages were considered invariant over the years, and changes in spatial patterns and covered surface were assessed. Agglomerative cluster analysis allowed identifying two nested levels which are related to spatial patterns at different scales (Chapter 4.2.6). At the highest level, stations are grouped together into two main clusters which describe inner and outer lagoon assemblages. This partition of the lagoon into two belts normal to the transitional gradient changed over the years towards the predominance of seaward assemblages. In assemblages of the confined lagoon, Errantia and Amphipoda dominate in terms of abundances whereas Errantia and Bivalvia in terms of biomass. Sedentaria overcome Errantia in terms of both abundances and biomass in the open lagoon assemblages. Differences in trophic structure are slight, with 50% of the biomass in the open lagoon assemblage composed of filter-feeders, which reduces to about 40% with a corresponding increase in deposit-feeders in confined area assemblages.

A more complex mosaic of taxonomic and trophic structures appears when considering clusters at a lower statistically significant level. Assemblages still show a succession along a general gradient from the sea landward, despite a major complexity. At this level it is possible also to appreciate differences among basins. A very interesting pattern brought to light consists in the distinctive assemblage of the right side of the main inlet channels (also identified for the area between Venice and Murano), which was generally stable over the three years. Despite this patterns is not explained, it can be hypothesized an indirect dependence on hydrodynamic factors (probably in relation to Coriolis Effect), for example the processes of sediment transport. Assemblages configuration in multivariate coenospace was compared with the hydrogeological zones.

In spite of the great complexity of their spatial patterns, assemblages resulting from cluster analysis follow an overall coenocline. The succession along the transitional gradient is essentially preserved, but zones have more "power" to explain outer and inner assemblages than the intermediate assemblages (Chapter 4.3.4). Inner (close to the landshore) and outer (close to the sea-inlets) assemblages can be considered as “poles of attraction”, the one related to the more stable marine condition, which is also a source of species in terms of colonization processes, the other to related to the selective inner conditions tolerated by a reduced number of species. The two extremes are therefore mainly characterized by biological control on the sea side, and environmental control on the mainland side. Between the two extremes, the succession of species and assemblages can follow different paths, which are possibly related to both environmental and biological factors and vary over the years.

A clear temporal succession from 2002 to 2007 towards more "marine" assemblages is evidenced, both by zonal analysis and cluster analysis. Near the inlets assemblages are more stable over the years, whereas central and inner areas for every basin present a clear temporal succession towards higher richness and more "marine" assemblages. The shift is evident for the Fringe Zone samples too, especially for those of the Lido basin. In 2007 only the more estuarine area of the Lagoon (the Dese estuary) still maintains its characteristics. This process was already recognized by the study of Giordani Soika & Perin (1974) for the 1948-1968 changes in the area where, during the late '60, the Canale dei Petroli was excavated, and where still now there is the greater influence of marine conditions on the communities.

Richness and diversity indices are a measure of the variation in species composition on an individual site (or sampling stations), the so-called alpha diversity. The variation in species composition among sites within a given area at a given spatial scale was termed "beta diversity" by Whittaker (1960, 1972; see also Anderson *et al.*, 2006). A number of measures of beta diversity have been proposed (Anderson *et al.*, 2006; Whittaker, 1960; 1972), including total variance of the species composition matrix (Legendre *et al.*, 2005). Mean dissimilarities was suggested as a measure of beta diversity by Whittaker (1972). Mean Euclidean distance was related to total variance by (Legendre & Anderson, 1999), but any ecologically meaningful dissimilarity matrix is itself a measure of beta diversity (Legendre *et al.*, 2005). So the mean dissimilarity value characterizing assemblages is a measure of beta diversity. Assemblages of the inner lagoon in 2007 are characterized by a lower mean dissimilarity, i.e. beta diversity, than assemblages of the inner lagoon in the previous years.

5.3 Which is the role of environmental factors in structuring benthic communities?

A set of environmental variables was identified to explain the relationships between community and environment. These include sediment data collected simultaneously with benthos samplings, hydrological variables measured monthly at independent sites, water exchange described by residence time and nearby intertidal surface. The procedure of selection includes the evaluation of the suitability of the original data sets and an analysis of collinearity among variables (Chapter 4.3.1). The set of selected variables is known not to be exhaustive as regards to important environmental driving factors. Actually, benthic organisms are expected to respond to a more complex set of environmental factors related to water column, sediment, interstitial water, and interface layer (e.g. Maurer *et al.*, 1985; Whiteman *et al.*, 1996). Part of the information about the system is not available, or not suitable. Eh and pH values, for example, while measured during MELa water column surveys, were excluded from the analyses due to strong spatial and temporal variability and lower instrument reliability. Some unfavourable aspects of the available data sets that will be discussed point by point.

Environmental variables, in general, have proved to be highly correlated among each other, in particular following a main gradient of conditions from sea landward, both for water column and sediment variables, confirming the conceptual use of the term "transitional gradient”. Along this gradient, salinity and sand percentage decrease, whereas residence time, chlorophyll *a*, alkalinity, pelite and TOC percentages, and intertidal area increase. DO average and variability also increase in the inner part of the lagoon (Chapter 4.3.1). Environmental conditions are less clearly defined and less stable among basins than among zones. The northern basins (Treporti and Lido) are characterized by high DO concentration, DO variability, TSS and silt percentage, whereas Malamocco and Chioggia basins by high salinity, sand and TOC percentages. Assessment of interannual environmental variability presents some issues. A number of variables may not be directly comparable between years due either to the application of different methods among MELa2 and MELa4, or short averaged temporal data sets in the case of hydrological variables (Chapter 3.2). The analyses were therefore centered more on the interpretation of patterns of spatial variability as opposed to changes in absolute values over the years.

All the selected environmental variables have proved to be statistically significant in describing the community structure (Chapter 4.3.4). When comparing the rank similarities among stations, a subset of variables (in particular residence time, salinity, clay percentage and chlorophyll *a*) is able to explain about the 50% of the structure of the community. This approach (which includes BIOENV, nMDS, ANOSIM) assumes only a monotonous relationship between variables. Similarities between stations are maintained in time compared to assemblage changes.

About the same percentage and variables are maintained irrespectively of the period considered to integrate the hydrological data, from 4 to 12 months, indicating that stations maintain during the year their similarities due to the same responsible variables (Chapter 4.3.4).

Constrained ordinations were also introduced into the analyses to relate multivariate community structure to environmental variables (Chapter 4.3.4). Widely applied ordination methods assume a given model of relationship between species, such as the unimodal relationship (CCA). Actually, the relationship between species is not known *a priori*. RDA after Hellinger transformation was verified to produce very similar results to CAP, which doesn't rely on any assumption (Chapter 4.3.4). This indicates that the "Hellinger model" of relationships among species is valid, as long as it represents the most of the variability. RDA was selected as it calculates real matrix variance and can be employed in variation partitioning and other techniques. However, the effectiveness of the Hellinger transformation method is still debaTable (Minchin & Rennie, 2010).

Only about 20-30% of the variance of the community matrices is explained by constrained ordinations (Chapter 4.3.4). However, the amount of explained variation is considered by some authors to be underestimated by the eigenvalue-to-total-inertia ratio (Økland, 1999). This means that in variation partitioning the fraction of explained variance should be evaluated in relative terms among explaining factors and not with absolute values.

In every ordination, the main axis represents the coenocline explained by main transitional gradient variables, and is characterized by very high percentages of explained variation. Abundances matrices are "explained" more than biomass matrices. In particular, water column factors, which are more variable, are less capable to describe biomass matrices.

Univariate descriptors were correlated to environmental variables, and regression model were proposed (Chapter 4.3.3).

Species richness is mainly correlated to residence times and salinity, and secondarily to granulometry, presence of macrophytes, chlorophyll *a* and TSS. When correlation is expressed by Spearman's rank coefficient, it increases for all the factors and in particular for TOC and intertidal surface, suggesting that these variables could be related to non-linear monotonous relationships. A linear model was produced only for species richness. Residence time and salinity alone account for a value of $R^2 = 0.57$, which can be improved just to 0.62 using seven variables. Other significant variables include macroalgae, sand percentage, TSS and TOC.

Low values of correlation, slightly increasing when rank correlation is considered, were found for abundances and biomass. These basic macrodescriptors show more complex patterns than species richness, as highlighted by the analysis of SAB curves produced for the the zones succession (Chapter 4.2.5) and in agreement to literature (e.g. Pearson & Rosemberg, 1978).

The results are consistent with both the salinity (Remane, 1934) and the confinement conceptual models (Guélorget & Perthuissot, 1983), at least at the scale which highlight the whole transitional gradient. Both the models predict a monotonous decrease in species richness moving landward (Chapter 1.4). The minimum values of salinity (11.5 PSU) were recorded at the station of the Dese Estuary, which presented a mean annual value of 27.8 PSU. The analyzed data sets don't allow to observe the freshwater communities and to obtain any information about the position of the species minimum ("*Artenminimum*") along the salinity gradient, which was estimated by Remane (1934) to fall between salinities of 5-8 PSU (“*horohalinicum*” of Kinne, 1971; but was later subject to debate e.g. Deaton & Greenberg 1986).

Previously to analyses, salinity was transformed to the third power to achieve normality. High correlation and explorative calculation with untransformed data suggest that in fact salinity and richness are related not linearly but by a third-degree term. Attrill (2002) argued that variation of salinity (and in general of environmental factors) may be more important in structuring communities than extreme values, but the two variables in the present study result highly correlated. Salinity (i.e. the opposite of seawater dilution) constitutes a main gradient across the lagoon. It is a conservative parameter and a tracer for water masses of different origin, retaining signals of different water column variables (Chapter 4.3.1).

Considering the primary role played by hydrology in structuring the environmental gradient, clear relationships between biological variables and the hydrodynamics of the system was expected. Residence time was considered as a measure of water renewal along the transitional gradient and is here considered as a proxy for the "confinement". In Mediterranean lagoons where the hydroclimate sustains eu-hyperhaline conditions, the decline of species along the sea-land axis is attributed mainly to hydrology and sediment properties and only secondarily to salinity (e.g. Guélorget *et al.* 1987; Reizopoulou & Nicolaidou 2004; Rossi *et al.* 2006). In the Lagoon of Venice salinity variation is not negligible, but it is inherently related to water renewal and their gradients largely overlap. However the orthogonal component between them in the multiple models suggests that both salinity and water renewal play a major role in structuring the community.

Residence time generally presents the highest correlation and regression coefficient (along with salinity and granulometry) to univariate and multivariate descriptors of community structure. Percentage of constrained variation of the community matrix explained by hydrological variables and residence time is 70% (on abundance matrix; Chapter 4.3.4), almost half of which attributableto residence time alone.

The scheme for Mediterranean biocoenosis proposed by Pères and Picard (1964) couples biocoenosis to bottom sediment texture, and can be applied to the succession of geomorphologic zones in coastal lagoon (Roy *et al.*, 2001). Spatial distribution of clay, silt and sand partly follows the transitional gradient, with pelite percentages increasing from sea landward, accompanied by an increase in the TOC fraction (Chapter 4.3.1). Organic enrichment is a major causal factor of stress in communities, and associated with tolerant *K*-strategist species (Hyland *et al.*, 2005; Magni *et al.*, 2009).

Species richness is directly correlated to sand and inversely with clay and TOC (as well as with neighbouring intertidal surface). Also, biomass shows negative rank correlation with TOC (Chapter 4.3.3). Measured sediment variables seem to have a minor role than water column variables in structuring communities, in spite of measurements made simultaneously with macrozoobenthos samplings. Their role accounts for a total of 26% in terms of abundance and 30% in terms of biomass of the explained variance (Chapter 4.3.4).

The higher correlation with biomass is probably due to the role of *K*-selected species, which compose more stable assemblages, hence maintaining a more strong relationship with sediment variables, which are, in turn, more stable if compared to water column variables. Clay is among main variables explaining rank similarity of communities in BIOENV analysis. A convergence with classical models of relationships between community and sedimentary environments characterized by high organic matter and fine sediment content, could be envisaged, and in particular the Pearson & Rosemberg model. However, the complexity of patterns at the lagoon scale should be further investigated.

Chlorophyll *a* and TSS plays a major role as explanatory variable for multivariate and univariate data as well. Chlorophyll *a* can be considered as a proxy for phytoplankton standing stock and possibly integrates the signals of nutrients, DO, temperature and residence time. Chlorophyll *a* and TSS are main factors responsible for turbidity and was preferred to turbidity to reduce the redundancy of the data set and focusing on the constituent processes, even though it was verified that they are actually poorly correlated (Chapter 3.2.5). Turbidity is enhanced by sediment resuspension and, indirectly, by eutrophication through plankton stimulation. High turbidity reduces the amount of light available for photosynthesis, affecting generally primary production (Monbet, 1992; Cloern, 1987). Sediment resuspension is related to meteorological events and runoff, sediment dredging, clam harvesting using illegal gears (in particular to exploit the Manila clam *Ruditapes philippinarum*, introduced in the 1980s), waves induced by motorboats and other irregular or occasional disturbance of bottom sediments. TSS can smother and clog benthic organisms (particularly filter feeders) and habitats when it settles. Moreover, suspended sediment particles link the bottom, the water column and the food chain, and control the transport, reactivity and availability of contaminant and other substances (Turner & Millward, 2002).

Mean water temperature presents the highest values near the industrial areas of Porto Marghera and Fusina, where a main thermal power plant is located, due to the input of cooling waters. In the rest of the lagoon, the range of variability follows the transitional gradient. The importance of temperature increases when time span is longer than four months, in particular for biomass (Chapter 4.3.4), and this is probably symptomatic of the role of seasonal changes in population dynamics.

Macrophytes distribution contributes to increase the richness and explain the community structure, even though secondarily to chemico-physical variables of the transitional gradient (Chapter 4.3.4). These results are based just on qualitative data sets (presence/absence data) and higher values of correlation are expected if a more detailed and complete information about phanerogams and macroalgae coverage is achieved. Macroalgae and phanerogams belong to the biotic component of the system but can be considered as habitats for the benthos community, at a lower spatial scale and hierarchical level than landforms. In turn, they are conditioned by environmental conditions and anthropogenic disturbance. The importance of vegetation in lagoons with its rolt in constructing a tridimensional environments suiTable to host a thriving benthic community is acknowledged, as well as its function in stabilizing sediments, increasing transparency and oxygenation (Gamito, 2006; Nicolaidou, 2007; Ponti et al, 2007; Pranovi *et al.* 2000; Sfriso *et al.* 2001; Tagliapietra *et al.*, 1998a).

Organisms react to the environmental conditions according to species and life stage. The variables at which different taxonomical groups are correlated vary: Sedentaria are correlated with residence time, phanerogams and water column variables. Errantia are correlated to sand percentage (or the complementary pelitic fraction), residence time and, among hydrological variables, salinity, chlorophyll *a* and temperature. Amphipoda show significant correlation to sediment variables and macroalgae, as well as to salinity and temperature. Bivalvia are correlated to residence time, sediment and water column variables, but not to chlorophyll *a* or TSS, as could be expected. Finally Gastropoda are correlated to phanerogams, residence time and temperature (Chapter 4.4.2).

Variability which is not explained by our set of environmental variables could be related to one or more of the following causes: unknown environmental processes, including patterns of variability at scales not represented by data and not considered environmental factors; biological interactions, including larval dispersion and colonization as well as intraspecific and interspecific relationships such as predation and competition; stochastic processes, not spatially structured, which are expected to prevail in inner zones subjected to recurrent but not regular harsh events (Barnes, 1980); anthropogenic stress and disturbance; and, finally, the system memory of previous states and events. The importance of these factors in explaining the community structure depends on the temporal and spatial scale of observation.

5.4 What are the spatial scales of variability of the community, also in relationship to variability scales of environmental factors?

The perception of variability of communities changes in relationship with different spatial and temporal scales (Frost *et al.*, 1995). The concept of scales cannot be univocally defined (Chapter 1.5). Scales of phenomena which can be analyzed are related to scales of observations and analysis (Dungan *et al.*, 2002).

Scales of variability were approached in two ways. The first approach consists in defining the scale of observation and then describing the patterns that can be observed at the chosen scale. The perception of scale depends on the observations (Dutilleul, 1993). Sampling and analysis scales (Dungan *et al.*, 2002) are in this approach defined by the grain size (the size of the elementary sampling unit), the extent and the density of stations.

From this perspective, a hierarchy of scales was developed on the basis of hydrological zones (Chapter 3.1.1), considering sampling station area (0.25 m^2) as grain size:

1) Whole Lagoon. The study area can be considered on the basis of its extent as belonging to the macroscale landscape level (100-500 km^2). The extent of the open water surface is about 408 km^2 (which reduces to 371 km^2 if the intertidal area is not considered). Mean density of stations is 0.44 km^{-2} for the 180-station data set and 0.14 km^{-2} for the 59-station data set.

2) The two first-order hydrogeological zones, corresponding to Open and Restricted Lagoon, again at the macroscale level. Their mean extent is 174 km^2.

3) The five second-order hydrogeological zones, at the mesoscale level (10-100 km^2).

Hydrogeological zones approximately follow one another along the transitional gradient. Total extent, mean extent per basin and density of stations for first-order and second-order zones are reported in Table 3.2. Station density is roughly maintained across the scales.

Patterns in community structure are distinct across the three hierarchical levels. However, the intermediate scale is less information that other levels and then main attention was given to the lagoon scale (see in particular Chapter 5.1) and the mesoscale zones (Chapter 5.2). Patterns of variability of univariate macrodescriptors and taxonomic and trophic composition, and their variation over the years, are described in Chapters 4.2.1 to 4.2.3.

At the lagoon scale main patterns of variation were identified between years in terms of species richness and importances. Down-scaling the whole lagoon trends at the zones scale allows the interpretation of patterns and processes structuring the benthic community. Variation of biological macrodescriptors, as well as species composition matrices and trophic groups, were found to be significant at the level of hydrological zones.

Cluster analysis and identification of assemblages at different hierarchical level evidence the strong spatial dependence of communities. Clusters were represented by means of a tessellation of the lagoon surface with Voronoi polygons, i.e. a portion of the lagoon which includes all the points closer to a given station than any other station. The same assemblage patterns are generally maintained when analyzing 59-station or 180-station data set for 2002, hence changing the density of observations.

Variability at a lower spatial scale is highlighted when stations have higher density. First-level clusters for 2002 (Figure 4.29) reflect clearly the Open and Restrict Lagoon zones (Figure 3.3), in the following years, the Restricted Lagoon assemblages regress, and the two clusters assume very unbalanced extent.

However, looking at the second-level clusters, it is possible to have a better insight of assemblage behaviour in space and time (Figure 4.30). Second-level clusters are composed by a very different number of stations, from single station clusters to a maximum of 15 contiguous polygons, and clusters have very different extent. Spatially isolated clusters could be related to local patterns of unique environmental conditions or anthropogenic disturbance.

Patterns presents spatial anisotropy: similarity between stations varies along the transitional gradient (e.g. Figure 4.12) not only due to species composition, but also to number of species and evenness (i.e. the two components of diversity), causing stronger dissimilarities in stations poor in species and with a very high abundance of few opportunistic species.

Another hierarchical system of zones, in this case termed "sectors", was introduced, this time not on the bases of *a priori* knowledge of the system (on which rely the hydrogeological zones), to be subjected to a PERMANOVA analysis with nested design (Chapter 4.4.1). In this case, spatial scales are represented by nested clusters of stations (sectors of rank *n*) characterized by similar density and extent, identified by a recursive approach, for each basin, only on the basis of their closeness (see Appendix 1). Extents and densities for the two data sets are reported in Tables 3.11 and 3.12.

When the 59-station data set is considered (i.e. station density of 0.15 km^{-2}), the main significant spatial scale of variability is the sectors of rank 2, with an extent of about 26 km^2. The matrices of species composition based on abundances and biomass show statistically significant heterogeneity at that scale, as well as the matrix of trophic groups, number of species and total abundance. With a higher density of stations (0.43 km^{-2}) and one more nested factor, sector of rank 2 generally maintains the statistical significance (except for abundance), but sector of rank 3, about 13 km^2 in extent, presents this time the highest significance for species richness, species composition and trophic groups matrices. Heterogeneity is also found significant in sector of rank 1 (52 km^2) for richness and trophic groups.

The same-rank sectors have no relationship with the transitional gradient (two sectors at the same rank could be located parallel or normally to the gradient, see Figures 3.8 and 3.9), and PERMANOVA results are independent of geographical location.

Both methods are based on *a priori* choice of a discrete number of relevant scales and a simplified design, which could obscure complex patterns and the interaction of factors at scales not considered (Denny *et al.*, 2004). The second approach consists in not considering *a priori* scales (and evaluating their significance for the variables) and instead "resolving" the continuous spatial heterogeneity of the data into main scale(s) of variation by the direct observation of patterns. A different operative definition of scale was applied to this approach, which is the geostatistical concept of variation range (Chapter 3.7). It should be stressed that also in this case the scale of observation determines the identification of scales of phenomena; therefore station density and grain should be taken into account when interpreting the results. However, there could be more interest in describing the whole spectrum of spatial scales rather than identifying a single scale (Denny *et al.*, 2004).

This approach involves the use of variograms, MSO and PCNM models.

MSO is a variogram-like method, which was applied to partition the variance of the community composition matrix along increasing spatial lags. The scale of analysis changes with respect to the scale of observations, as spatial lags of 2 km are introduced, the stations being irregularly spaced. The significant range of variability for the community is about 7 km (with density of stations of 0.43 km^{-2}). The two approaches used are not directly comparable, however this value is roughly comparable to the scale of mesoscale zones (measured according to their extent per basin) and to an intermediate level between rank 1 and rank 2 sectors in PERMANOVA design. This emerges as the main spatial scale of communities variability in terms of species composition, mostly driven by environmental factors. An extent of about 40 km^2 can be estimated, which roughly corresponds to subtidal flats as delimited by natural boundaries such as channels and watersheds (called “paludi”, recognized also by traditional topographic subdivisions, see Tagliapietra *et al.*, 2000).

The results of PERMANOVA suggest that trophic groups follow scales of variation characterized by wider extent, hence encompassing different assemblages.

When the community is decomposed into taxocoenosis, different ranges of variability are found. Errantia and Sedentaria show a comparable range (about 7 km) and Bivalvia and Amphipoda a slightly reduced range (6 km and 4 km, respectively). Both Errantia and Sedentaria are characterised by high abundance and biomass, so probably they are responsible for the overall variability pattern displayed by the whole community. Only Gastropoda show a noTable departure, with 2 km of range of variability, whereas intermediate range of Amphipoda could be related to the two main functional groups they belong to, that is free living grazers and shredders, associated to macrophytes, and tubicolous deposit-feeders mostly associated to sediment. This suggests that a classification of species by means of a mixed system between taxonomical and functional groups could improve in understanding the ecological meaning of distribution patterns.

Empirical variograms were directly calculated for main univariate macrodescriptors (Chapter 3.6.5). Species richness presents a range of variability of about 8 km, which can be also recognized for biomass (although showing a more complex pattern). Both are comparable to that one for community variance. The pattern of total abundance is more complex, as probably the variogram fails in depicting lower ranges of variability. This small-scale variability is highlighted by PCNM sub-models 3 and 4 (Chapter 4.4.3), which are described by flat and decreasing variograms that indicate lags larger than existing autocorrelation and anisotropy in the pattern of variability. First-rank PCNM sub-model of biomass and species richness is comparable to the overall empirical variogram, whereas other main sub-models don’t show any single range of variation, being related to non homogeneous patterns at the lagoon extent. Both MSO and variograms are formally omnidirectional, but, actually, for ranges greater than about 15 km they follow the main axis of the lagoon, perpendicular to the transitional gradient and the watersheds, showing periodical behaviour due to repeated structure of basins.

Complex and multiple scales of environmental heterogeneity, both temporal and spatial, are present in coastal transitional ecosystems. Depending on the variable, these scales differ, on the basis of involved processes. Spatial and temporal variability could be correlated, but factors related to the same spatial scale may act in different time scales, and vice versa.

Spatial and temporal scale of variability of hydrological factors respond mainly to climate, interannual fluctuations and meteorological events, exchanges with the sea, inflow from drainage basins, mixing processes, and chemical and biochemical processes. For example, seasonal and interannual variations in salinity depend on air temperature (through evaporation) and rainfall in the drainage basin. Samplings may not detect variability related to short and intensive events, such as meteorological events and floods. Temporal variability is also related to tidal cycles, which can determine a vertical stratification in the inner part of the lagoon, near to fresh water inflow, and depending on water mixing.

Water temperature in transitional ecosystems, and especially microtidal and nanotidal lagoons, is mainly subjected to diurnal and seasonal fluctuations, following air temperature, due to the low bathymetry and limited water exchange (Dejak *et al.*, 1992; Dupra *et al.*, 2001). A number of hydrological variables, such as DO and variables related to metabolic processes of organic matter (including Eh and pH) are known to vary mainly at smaller spatial scale. Their spatial variability is related to the consumption of oxygen by saprobic processes, so a general variation along the water renewal gradients is coupled to smaller scale processes, particularly in the inner part. Their vary according to multiple temporal scales, including diurnal, due to photosynthesis process, as well as related to tidal cycles due to water renewal, and at wider scale on the basis of temperature fluctuations and phytoplankton blooms.

Sediment characteristics vary across spatial scales, but they are more temporally stable.

Whereas the scale of observation of sediment variables is the same of communities, hydrological measurements were carried out on a smaller number of stations.

All the analyzed variables present a significant variability at "wide" spatial scale, if not the most important. Beside the main axis of variability that follows the transitional gradient direction, a secondary south-north gradient can be noticed. This secondary axis divides the lagoon into two semi-lagoons, the first in the south characterized by an extensive renewal of sea water and the second one, in the north, characterized by more confined and estuarine conditions. Benthic community also presents variability according to these directions.

MSO analysis indicates that the correlation between community and environmental variables are maintained across the scales (Chapter 4.4.2). It is instead possible to recognize spatial patterns at different scale in the community structure and to hypothesize the existence of scale-dependent relationships between community and environment. As indicated by PCNM analysis, different variables explain the community structure across different scales, depending on the descriptor. Variables linked to the transitional gradient, which act at a wide scale, are always the most important factors, in particular for richness, biomass and community structure, dominating over any other small scale relationships (Chapter 4.4.3). Total abundance is mainly related to small scale variability. It is more difficult to describe the relationships at the "local" scale, because even the temporal dynamics of populations have shorter time scale; in particular, abundances are more variable than biomass. This small scale includes sediment and water column variables but also microtopography and vegetal coverage. Small scale variability of communities is retained as residuals of the analyses performed on wide scale environmental variability.

The fraction of variability which are not related to environmental variables still have a spatial structure (Chapters 4.4.2, 4.4.3), which could be attributed to other factors as well as community processes. The unexplained fraction of the PCNM spatial patterns (Chapter 4.4.3) is mainly at small scale and could be related to residual autocorrelation. A significant autocorrelation in the community is found by MSO to a distance of about 2 km, indicating that the residuals are in fact spatially correlated. This could be interpreted as the scale of autocorrelation of the community, i.e. when biological interactions and colonization processes prevails in structuring the community, even though it could be related to unknown factors at that scale (Chapter 4.4.2). The value varies between taxonomic groups, with Gasteropoda not showing any residual autocorrelation. Sedentaria, Errantia and Bivalvia show spatial autocorrelation to a distance of about 2 km, whereas Amphipoda up to about 6 km, suggesting the some other wide scale process is not explained by environmental variables data set (Chapter 4.4.2).

5.5 Final considerations and perspectives

The present work confirmed the major role of environmental factors in structuring communities and the importance of scale in identifying and interpreting these relationships. Scale is a complex concept and it doesn't exist a unique way to address it.

The benthic communities follow a main gradient from more "marine" and stable conditions to more confined and selective environments, tolerated by a decreasing number of more opportunistic species. The system can be considered as naturally stressed.

Communities are related to both water column and substrate factors, which are connected into a complex system. The community structure appears mainly related to hydrodynamical and hydrological variables at wide scale. These results should be carefully interpreted, as scale resolution cannot actually be higher than the measurements scale. Relationships to sediment variables, which at the main scale seems to have a secondary role, could be mediated by water column processes and/or act primarily at different scales. Not explained variation can be related to unknown environmental factors and biological interactions, as well as to anthropogenic factors.

The structure of community can be represented by different univariate and multivariate descriptors, which are characterized by different scales of spatial and temporal variability, and are associated to different ecological processes. Biological assemblages respond to environmental conditions modifying their attributes (e.g. species richness, abundance, and biomass). A number of widely applied benthic indices of quality are based on species richness or diversity, whereas other on relative or absolute importances (mainly abundances) of indicator groups. As an example, the AMBI index (Borja *et al.*, 2000) and the BENTIX index (Simboura & Zenetos, 2002) are based on relative abundances of some groups of indicator species. Both these indices were originally developed for the coastal environment and are based on the Pearson and Rosemberg model. Therefore, such indices respond to abundances variability, which follows small scale spatial and temporal dynamics, changing in intensity along the transitional gradient. Biomass should be a more stable measure capable to integrate the environmental conditions in a longer period. When community is resolved into main taxonomical-functional groups, ecological processes which structure communities could be highlighted. In this sense, the molluscan taxocoenosis, which is composed by a high number of species characterized by long lifespan and high biomass is a good candidate group for bioindication.

The science of bioindication aims to obtain information from modulations of biological attributes induced by environmental stress. Analogously with communications, biological attributes are the carriers whose modulations can be read as information signals, in this case information on environmental stress. The "Estuarine Quality Paradox" can be addressed in terms of signal and interference (Tagliapietra & Sigovini, 2008).

Biological responses to both natural and anthropogenic stresses are often correlated or coherent with each other and modulate in a similar way the carrier; the superposition of the two kinds of signals generates composite information. Both the natural signal and anthropogenic signal could be classified as a disturbance for the other. In bioindication, the desired signals are the responses to anthropogenic change; therefore the biological response to natural variation can be regarded as “unwanted information” and treated as an “interference", which is additional "unwanted information" that reduces the intelligibility of the wanted signal. Differently from noise, interference has some temporal or spatial structure similar to that of the signal.

The ratio between natural stress and anthropogenic stress differs among habitats; the strongest the “natural stress” the higher the need to filter out the composite signal. In confined, organically enriched, oligo- and hyperhaline habitats the signal-to-interference ratio is low and often the wanted signal (due to anthropogenic stress) is not sufficient to overcome interference (due to natural stress).

However, in transitional environments biological responses to natural stress follow spatial or temporal information patterns of natural dynamics, which represent a key for their identification.

The interference should be filtered out from the composite signal. A "filter" is a function or procedure which removes the interference from the wanted signal. For example, Moran Eigenvector Maps (Getis & Griffith, 2002) are equivalents of PCNM which were developed independently and initially used as spatial filtering functions in a statistical context, to remove spatial autocorrelation from residuals of a model, allowing for classical statistical tests.

Filter procedures for each carrier/attribute (e.g. number of species, diversity indices, other indices) imply the detection of the "normal" pattern of variation of the responses to natural stress at the appropriate scale and its subtraction from the overall signal, therefore considering a departure from a model. This approach could be applied to bioindication to filter environmental signal and try to isolate other factors which modulate the parameter's response. An example of filtering procedure is reported in Figure 4.82, Chapter 4.4.3, where a model of species richness mainly explained by environmental variables is subtracted from the original data. The residuals reveal spatial patterns hidden by natural structures, such as the decrease in richness along the transitional gradient, which could be interpreted, and their sources investigated also in terms of anthropogenic stressors.

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LIST OF ABBREVIATIONS

A:	Total Abundance (also N)
AC:	*Animalia Caetera* (see Chapter 3.5.3)
AFDW:	Ash Free Dry Weight (see Chapter 3.3.5)
Alk:	Total Alkalinity (see Chapter 3.2.5)
ANOSIM:	Analysis of Similarities (ANOVA-like Mantel test method: see Chapter 3.2.5)
ANOVA:	Analysis of Variance (see Chapter 3.2.5)
B:	Total Biomass (see also AFDW)
BE:	Bayhead Estuary (hydrogeological zone; see Chapter 3.1.1)
BIOENV:	BIO-ENV analysis (Mantel test method: see Chapter 3.2.5)
C:	Carnivores (trophic group; see Chapter 3.3.4)
CA:	Correspondence Analysis (ordination method; see Chapter 3.2.5)
CAP:	Constrained Analysis of Principal coordinate (ordination method; see Chapter 3.2.5)
CB:	Central Basin (hydrogeological zone; see Chapter 3.1.1)
CCA:	Canonical Correspondence Analysis (ordination method; see Chapter 3.2.5)
CDF:	Deposit-Feeders with Chemoautotrophic symbiotic bacteria (trophic group; see Chapter 3.3.4)
CH:	Chioggia basin (see Chapter 3.1)
Chl-a:	Chlorophyll *a* (see Chapter 3.2.5)
CNR:	Consiglio Nazionale delle Ricerche (National Research Council)
CVN:	Consorzio Venezia Nuova (concessionary of Water Authority of Venice)
d:	Margalef index (see Chapter 3.5.1)
DBEM:	Distance-Based Eigenvector Maps (spatial predictors; see Chapter 3.7)
dbRDA:	distance-based RDA (ordination method; see Chapter 3.5.3)
DO:	Dissolved Oxygen concentration (see Chapter 3.2.5)
$E(S_n)$, $E(S_{50})$:	Hulbert index (expected number of species; see Chapter 3.5.1)
FZ:	Fringe Zone (hydrogeological zone; see Chapter 3.1.1)
GAM:	Generalized Additive Model (statistical model; see Chapter 3.2.5)
GLM:	General Linear Model (statistical model; see Chapter 3.2.5)
H':	Shannon-Wiener index (see Chapter 3.5.1)
H+MG:	Herbivores and MicroGrazers (trophic group; see Chapter 3.3.4)
IDW:	Inverse Distance Weighting (interpolation method; see Chapter 3.2.1)
ISMAR:	Istituto di Scienze Marine (Institute of Marine Sciences; CNR)
J':	Pielou index (evenness; see Chapter 3.5.1)
LI:	Lido basin (see Chapter 3.1)
LOI:	Loss On Ignition (TOC analysis method; see Chapter 3.2.1)
MA:	Malamocco basin (see Chapter 3.1)
MANOVA:	Multivariate Analysis of Variance (see Chapter 3.2.5)
MAV:	Magistrato alle Acque di Venezia (Water Authority of Venice)
MELa:	Monitoraggi Ecosistema Lagunare (Lagoonal Ecosystem Monitorings; MAV-CVN)

MLR:	Multiple Linear Regression (statistical model; see Chapter 3.2.5)
MLWS:	Mean Low Water Spring, lower limit of intertidal (see Chapter 3.1.2)
MSO:	Multi-Scale Ordination (variogram-like multivariate method; see Chapter 3.7)
N:	Total Abundance (also A)
nMDS:	non-metric MultiDimensional Scaling (ordination method; see Chapter 3.2.5)
PCA:	Principal Components Analysis (ordination method; see Chapter 3.2.5)
pCCA:	partial CCA (ordination method; see Chapter 3.2.5)
PCNM:	Principal Coordinates of Neighbour Matrices (spatial predictors; see Chapter 3.7)
PCoA:	Principal Coordinate Analysis (ordination method; see Chapter 3.2.5)
PERMANOVA:	Permutational Multivariate Analysis of Variance (see Chapter 3.2.5)
PERMDISP:	Permutational test of multivariate Dispersion (non-parametric statistical test; see Chapter 3.2.5)
pRDA:	partial RDA (ordination method; see Chapter 3.2.5)
RDA:	Redundancy Analysis (ordination method; see Chapter 3.2.5)
S:	Species richness (number of species; see Chapter 3.5.1)
Sal:	Salinity (see Chapter 3.2.5)
SDF:	detritivores and Surface Deposit-Feeders (trophic group; see Chapter 3.3.4)
SF:	Suspension- and filter-Feeders (trophic group; see Chapter 3.3.4)
Simprof:	Similarity Profile analysis (non-parametric statistical test; see Chapter 3.2.5)
SL:	Sheltered Lagoon (hydrogeological zone; see Chapter 3.1.1)
SSDF:	SubSurface Deposit-Feeders (trophic group; see Chapter 3.3.4)
T:	Temperature (see Chapter 3.2.5)
TBEM:	Topology-Based Eigenvector Maps (spatial predictors; see Chapter 3.7)
TD:	Marine Tidal Delta (hydrogeological zone; see Chapter 3.1.1)
TOC:	Total Organic Carbon (see Chapter 3.2.1)
TR:	Treporti basin (see Chapter 3.1)
TSS:	Total Suspended Solids (see Chapter 3.2.5)

APPENDICES

1 R Script: Recursive *k*-means algorithm *(see Chapter 3.7)*

2 List of taxa *(see Chapter 4.1)*

3 Interpolated maps of A, B and S for 2002, 2003, 2007 (59-stations data sets) *(see Chapter 4.2.1)*

4 Dominant taxa *(see Chapter 4.2.2)*

5 Interpolated maps of selected environmental variables for 2002 *(see Chapter 4.3.1)*

```
#======================================================================================================
#  bkm
#  NESTED AND PARTIALLY BALANCED k-means
#  v.1.2 (22/06/2010)
#======================================================================================================

bkm <- function(x, k = 1, p = 999, IDstaz = 1, prev = 0, map = NULL){
        require(vegan, quietly = T)
        require(sp, quietly = T)
        ev <- function(y){diversity(t(y))/log(specnumber(t(y)))}
        normvec <- function(X){sqrt(X%*%X)}
        xy <- x
        if(IDstaz>0) xy <- xy[, -IDstaz]
        if(prev>0){
                prev.num <- as.data.frame(data.matrix(as.numeric(x[, prev])))
                if((IDstaz==0)|(IDstaz>prev)) xy <- xy[, -prev]
                if((IDstaz!=0)&(IDstaz<prev)) xy <- xy[, -(prev-1)]
                }
        Jmax <- 0
        Jlist <- NULL
        name <- deparse(substitute(x))

#       ------------------------------------------------------------------------------------------------
        for(j in 1:p){
                if(prev==0) staz <- data.frame(xy, cl_0 = rep(1, dim(xy)[1]))
                if(prev>0) staz <- data.frame(xy, cl_0 = prev.num)
                sint <- NULL
                if(prev>0){
                        size_0 <- as.vector(table(as.factor(x[, prev])))
                        cenx0 <- NULL
                        ceny0 <- NULL
                        SS0 <- NULL
                        for(c0 in 1:length(size_0)){
                                cenx0 <- append(cenx0, sum(xy[prev.num==c0, 1])/size_0[c0])
                                ceny0 <- append(ceny0, sum(xy[prev.num==c0, 2])/size_0[c0])
                                SS0 <- append(SS0, sum(apply(as.matrix(cbind(xy[prev.num==c0,
                                        1]-cenx0[c0], xy[prev.num==c0, 2]-ceny0[c0])), 1, normvec)^2))
                                }

                        sint <- data.frame(rep(name, max(prev.num)), rep(0, max(prev.num)),
                                rep(dim(xy)[1], max(prev.num)), rep(max(prev.num), max(prev.num)),
                                size_0, cenx0, ceny0, SS0, round(sqrt(SS0/size_0), 0))
                        names(sint) <- 1:9
                        }
#               ----------------------------------------------------------------------------------------
                for(i in 1:length(k)){
                        sol <- NULL
                        code <- rep(name, dim(xy)[1])
                        for(w in 1:i) code <- paste(code, staz[, dim(xy)[2]+w], sep = "-")
                        key <- data.matrix(data.frame(code, stringsAsFactors = TRUE))
#                       --------------------------------------------------------------------------------
                        for(l in 1:max(key)){
                                ris <- kmeans(xy[key==l, ], k[i])    # algorithm: "Hartigan-Wong"
                                sol <- append(sol, ris$cluster)
                                sint.tmp <- data.frame(rep(name, k[i]), rep(i, k[i]),
                                                rep(sum(ris$size), k[i]), rep(k[i], k[i]), ris$size,
                                                ris$centers, ris$withinss,
                                                round(sqrt(ris$withinss/ris$size), 0))
                                names(sint.tmp) <- 1:9
                                sint <- rbind(sint, sint.tmp)
                                }
#                       --------------------------------------------------------------------------------
                        sol <- data.frame(cl = sol)
                        cl <- data.frame(sol[match(row.names(staz), row.names(sol)), ])
                        names(cl) <- paste("cl", as.character(i), sep = "_")
                        staz <- data.frame(staz, cl)
                        }
#               ----------------------------------------------------------------------------------------
                code.car <- rep(name, dim(xy)[1])
                code.car <- paste(code.car, staz[, dim(xy)[2]+1], sep = "-")
                for(w in 1:length(k))code.car <- paste(code.car, staz[, dim(xy)[2]+1+w], sep = "-")

                lev <- table(as.factor(code.car))
                J <- ev(lev)
                Jlist <- append(Jlist, J)

                if(J>Jmax){
                        Jmax <- J
```

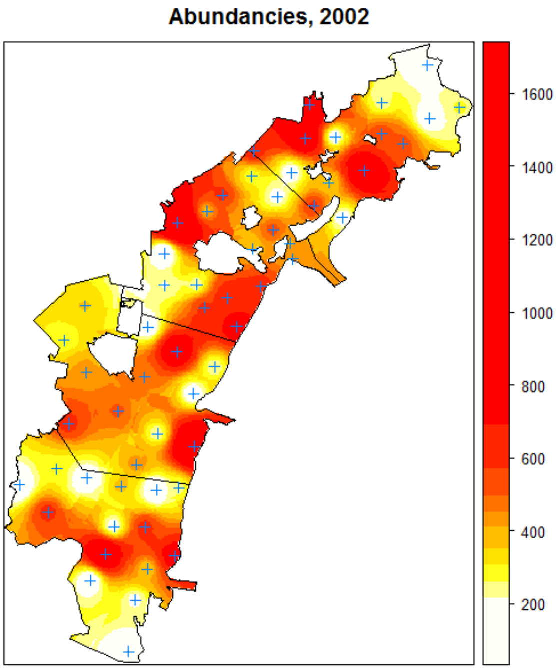
```
                        staz.fin <- staz
                        code.fin <- code.car
                        sint.fin <- sint
                        }
                }
#       -----------------------------------------------------------------------------------------------

        code.fin <- data.frame("code" = code.fin, stringsAsFactors = TRUE)
        code.num <- as.data.frame(data.matrix(code.fin))
        names(code.num) <- "c-num"
        staz.fin <- cbind(staz.fin, code.fin, code.num)
        if(sum(prev)>0){names(staz.fin)[3] <- "cl_0"}
        sint.names <- c("object", "ger_level", "n_stat", "k", "size", "X_centr", "Y_centr", "SS",
                        "SD")
        names(sint.fin) <- sint.names
        pdf(file = "c:/R_data/plot.pdf", width = 21, height = 7)
        par(mfrow = c(1, 3))
                hist(Jlist)
                if(length(k)==1) plot(xy, col = staz.fin[, 4])
                if(length(k)==2) plot(xy, col = staz.fin[, 4], pch = staz.fin[, 5])
                if(length(k)>2) plot(xy, col = staz.fin[, 4], pch = staz.fin[, 5],
                        cex = staz.fin[, 6])
                if( class(map)=="SpatialPolygonsDataFrame") plot(map, add = T)
                points(sint.fin[which(sint.fin[2]==1), 6:7], col = 6, pch = 10, cex = 4)
                title(main = "Map of cluster")
                boxplot(sint.fin$SD~as.factor(sint.fin$ger_level), horizontal = T)
                title(main = "Scale range")
        dev.off()

        if(sum(IDstaz)>0){
                staz.fin <- cbind(x[, IDstaz], staz.fin)
                names(staz.fin)[1] <- "ID"
                }
        tot <- list(staz.fin, sint.fin, Jmax)
        return(tot)
        }
```

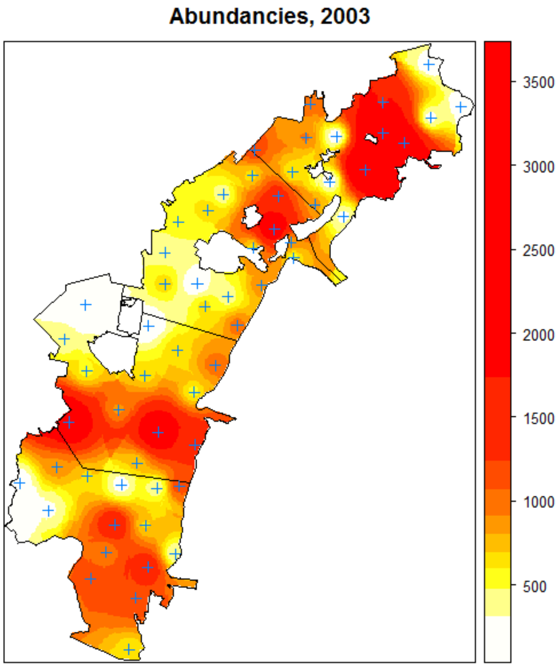
Phylum	Classis	Ordo	Familia	Species	Authority	02	03	07
Annelida	Oligochaeta			**Oligochaeta**		X	X	X
	Polychaeta	Capitellida	Capitellidae	**Capitellidae**		X	X	X
				Capitella capitata	(Fabricius, 1780)	X	X	X
				Capitomastus minimus	(Langerhans, 1880)	X	X	X
				Heteromastus filiformis	(Claparde, 1864)	X	X	X
				Mediomastus fragilis	Rasmussen, 1973	X		X
				Notomastus sp.		X	X	X
			Maldanidae	**Maldanidae**		X	X	X
				Clymenura clypeata	(Saint-Joseph, 1894)	X	X	X
				Euclymene sp.			X	
				Euclymene lumbricoides	(Quatrefages, 1865)			X
				Euclymene oerstedi	(Claparde, 1863)	X	X	
				Petaloproctus terricolus	Quatrefages, 1865	X		
		Cirratulida	Cirratulidae	**Cirratulidae**		X	X	X
				Cirriformia tentaculata	(Montagu, 1808)	X	X	X
		Cossurida	Cossuridae	*Cossura soyeri*	Laubier, 1962	X	X	X
		Eunicida	Dorvilleidae	*Dorvillea rudolphii*	(Delle Chiaje, 1828)	X	X	X
			Eunicidae	**Eunicidae**		X	X	X
				Eunice sp.		X		X
				Eunice pennata	(O. F. Mller, 1776)	X		
				Eunice vittata	(Delle Chiaje, 1828)	X	X	X
				Lysibranchia paucibranchiata	Cantone, 1983	X	X	X
				Marphysa bellii	(Audouin & Milne-Edwards, 1833)		X	
				Marphysa sanguinea	(Montagu, 1815)	X	X	X
				Nematonereis unicornis	(Grube, 1840)	X		X
				Palola siciliensis	(Grube, 1840)			X
			Lumbrineridae	*Lumbricalus adriatica*	(Fauvel, 1940)			X
				Lumbrineris sp.		X	X	X
				Lumbrineris gracilis	(Ehlers, 1868)	X	X	X
				Lumbrineris latreilli	Audouin & Milne-Edwards, 1834	X	X	X
			Oenonidae	*Arabella sp.*		X		
			Onuphidae	*Onuphis eremita*	Audouin & Milne-Edwards, 1833	X	X	X
		Flabelligerida	Flabelligeridae	*Pherusa monilifera*	(Delle Chiaje, 1841)	X	X	X
		Magelonida	Magelonidae	*Magelona papillicornis*	F. Mller, 1858			X
		Opheliida	Opheliidae	*Armandia cirrhosa*	Filippi, 1861			X
		Orbiniida	Orbiniidae	**Orbiniidae**		X		X
			Paraonidae	**Paraonidae**		X	X	X
				Cirrophorus furcatus	(Hartman, 1957)	X		
		Oweniida	Oweniidae	*Myriochele oculata*	Zachs, 1923	X	X	X
				Owenia fusiformis	Delle Chiaje, 1841	X	X	X
		Phyllodocida	Aphroditidae	**Aphroditidae**		X	X	X
			Glyceridae	*Glycera sp.*		X	X	X
				Glycera alba	(O. F. Mller, 1776)	X		
				Glycera cfr. fallax	Quatrefages, 1850	X		
				Glycera tridactyla	Schmarda, 1861	X	X	X
				Glycera unicornis	Savigny, 1818		X	
			Hesionidae	**Hesionidae**		X	X	X
			Nephtyidae	*Micronephtys sphaerocirrata*	(Wesenberg-Lund, 1949)	X	X	X
				Nephtys hombergi	Savigny, 1818	X	X	X
			Nereididae	**Nereididae**		X	X	X
				Ceratonereis costae	(Grube, 1840)	X		X
				Hediste diversicolor	(O. F. Mller, 1776)	X	X	X
				Neanthes caudata	(Delle Chiaje, 1828)	X	X	X
				Neanthes irrorata	(Malmgren, 1868)	X		
				Neanthes succinea	(Frey & Leuchart, 1847)	X	X	X
				Perinereis cultrifera	(Grube, 1840)	X	X	X
				Platynereis dumerilii	(Audouin & Milne-Edwards, 1833)	X	X	X
			Phyllodocidae	**Phyllodocidae**		X	X	X
				Eteone picta	Quatrefages, 1865	X	X	X
				Phyllodoce sp.				X
				Phyllodoce mucosa	rsted, 1843	X	X	
				Phyllodoce schmardaei	Day, 1963			X
			Sigalionidae	*Sthenelais boa*	(Johnston, 1833)			X
			Syllidae	**Syllidae**		X	X	X
				Eusyllis sp.		X		
				Syllis sp.		X		
		Sabellida	Sabellidae	**Sabellidae**		X	X	X
				Desdemona ornata	Banse, 1957	X	X	
				Megalomma lanigera	(Grube, 1846)	X	X	X
				Sabella pavonina	Savigny, 1820			X
			Serpulidae	**Serpulidae**		X	X	X
				Ficopomatus enigmaticus	(Fauvel, 1923)			X
				Hydroides dianthus	(Verrill, 1873)	X	X	X
				Hydroides pseudouncinatus	Zibrowius, 1968		X	X
				Pomatoceros triqueter	(Linnaeus, 1767)	X	X	
				Serpula vermicularis	Linnaeus, 1767			X
				Vermiliopsis sp.		X	X	X
			Spirorbidae	**Spirorbidae**		X	X	X
		Spionida	Spionidae	**Spionidae**		X	X	X
				Malacoceros sp.		X	X	
				Malacoceros fuliginosus	(Claparde, 1868)	X	X	
				Microspio mecznikovianus	(Claparde, 1868)	X		
				Polydora sp.		X	X	X
				Polydora ciliata	(Johnston, 1838)	X		
				Prionospio sp.		X	X	
				Prionospio caspersi	Laubier, 1962	X	X	
				Prionospio cirrifera	Wiren, 1883	X	X	
				Pseudopolydora antennata	(Claparde, 1870)	X		
				Scolelepis sp.		X		
				Scolelepis cantabra	(Rjoia, 1918)	X		
				Spio sp.		X	X	
				Spio decoratus	Bobretzky, 1870	X	X	
				Spio filicornis	(O. F. Mller, 1766)	X	X	
				Spiophanes bombyx	(Claparde, 1870)	X	X	
				Streblospio shrubsolii	(Buchanan, 1890)	X	X	X
		Sternaspida	Sternaspidae	*Sternaspis scutata*	(Ranzani, 1817)	X	X	X
		Terebellida	Ampharetidae	**Ampharetidae**		X	X	
				Amage adspersa	(Grube, 1863)	X	X	X
				Ampharete acutifrons	(Grube, 1860)	X		
			Pectinariidae	**Pectinariidae**			X	
				Pectinaria koreni	(Malmgren, 1866)	X	X	X
			Sabellariidae	*Sabellaria sp.*		X	X	X
			Terebellidae	**Terebellidae**		X	X	X
				Lanice conchylega	(Pallas, 1766)	X	X	X
Arthropoda	Insecta	Diptera	Chironomidae	*Chironomus salinarius*	Kieffer, 1915		X	X
	Malacostraca	Amphipoda		**Gammarida**		X	X	X
			Ampeliscidae	*Ampelisca sp.*		X	X	X
			Amphilochidae	*Amphilochus neapolitanus*	Della Valle, 1893	X	X	X
			Ampithoidae	*Ampithoe sp.*		X	X	X
			Aoridae	*Microdeutopus sp.*		X	X	X
			Caprellidae	**Caprellidae**		X	X	X
				Caprella penantis	Leach, 1814			X
			Corophiidae	*Corophium sp.*		X	X	X
				Corophium acutum	Chevreux, 1908			X
				Corophium orientale	Schellenberg, 1928			X
			Dexaminidae	*Dexamine spinosa*	(Montagu, 1813)	X	X	X
			Gammaridae	*Gammarus sp.*		X	X	X
			Iphimediidae	*Iphimedia sp.*		X	X	X
			Ischyroceridae	*Jassa sp.*		X		
			Leucothoidae	*Leucothoe venetiarum*	Giordani Soika, 1950	X	X	X
			Liljeborgiidae	*Idunella sp.*		X		
			Lysianassidae	*Lepidepecreum longicorne*	(Bate & Westwood, 1861)		X	
			Melitidae	*Elasmopus sp.*		X		X

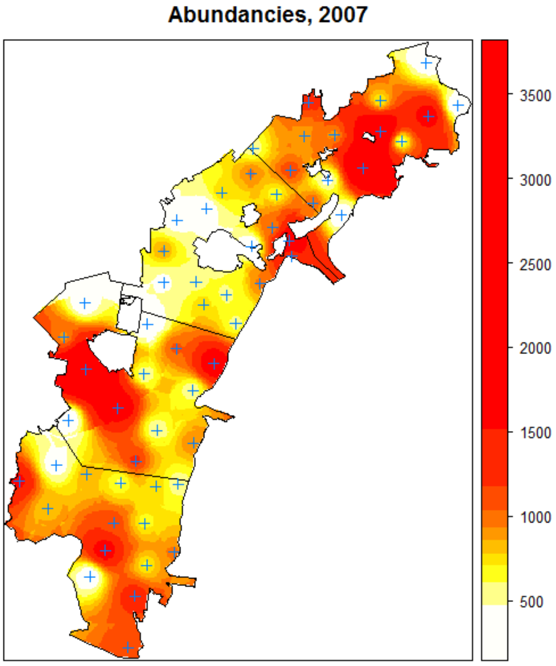
Phylum	**Classis**	**Ordo**	**Familia**	**Species**	**Authority**	**02**	**03**	**07**
				Gammarella fucicola	A. Costa, 1853	X	X	X
				Melita sp.		X	X	X
				Melita palmata	(Montagu, 1804)			X
			Oedicerotidae	*Monoculodes sp.*			X	X
				Perioculodes sp.			X	X
				Perioculodes aequimanus	(Kossman, 1880)	X	X	
				Perioculodes longimanus	(Bate & Westwood, 1868)	X		
				Synchelidium haplocheles	(Grube, 1864)	X		
			Phoxocephalidae	*Metaphoxus simplex*	(Bate, 1857)	X	X	X
			Stenothoidae	*Stenothoe sp.*		X	X	X
		Decapoda		**Natantia**		X	X	X
				Paguroidea		X		
			Alpheidae	*Athanas nitescens*	(Leach, 1814)	X		X
			Callianassidae	*Callianassa tyrrhena*	(Petagna, 1792)	X	X	X
			Crangonidae	*Crangon crangon*	(Linnaeus, 1758)	X	X	X
			Diogenidae	*Diogenes pugilator*	(Roux, 1829)	X	X	X
			Hippolytidae	*Hippolyte sp.*				X
				Hippolyte longirostris	(Czerniavsky, 1868)	X		
			Palaemonidae	*Palaemon sp.*		X		
				Palaemon adspersus	Rathke, 1837	X	X	X
			Panopeidae	*Dyspanopeus sayi*	(S. I. Smith, 1869)	X	X	X
				Rhithropanopeus harrisii	(Gould, 1841)	X	X	X
			Pasiphaeidae	**Pasiphaeidae**		X		
			Portunidae	*Carcinus aestuarii*	Nardo, 1847	X	X	X
				Liocarcinus depurator	(Linnaeus, 1758)			X
				Liocarcinus maculatus	(Risso, 1827)	X		
				Liocarcinus navigator	(Herbst, 1794)	X		
			Processidae	*Processa sp.*		X	X	X
				Processa edulis	(Risso, 1816)			X
			Upogebiidae	*Upogebia deltaura*	(Leach, 1815)			X
				Upogebia pusilla	(Petagna, 1792)	X	X	X
			Varunidae	*Brachynotus sexdentatus*	(Risso, 1827)	X	X	
		Cumacea		**Cumacea**		X	X	X
			Bodotriidae	*Iphinoe serrata*	Norman, 1867	X	X	X
			Nannastacidae	*Cumella limicola*	Sars, 1879			X
		Isopoda		**Flabellifera**		X	X	
				Valvifera			X	X
			Anthuridae	*Cyathura carinata*	(Kryer, 1847)	X	X	X
			Bopyridae	**Bopyridae**				X
				Bopyrus squillarum	Latreille, 1802		X	
				Ione thoracica	(Montagu, 1808)	X		
			Idoteidae	*Idotea sp.*		X		X
				Idotea baltica	(Pallas, 1772)	X		
				Idotea chelipes	(Pallas, 1766)	X		
				Idotea linearis	Linnaeus, 1767	X		
				Synisoma sp.		X	X	
			Janiridae	*Ianiropsis breviremis*	(Sars, 1883)	X		
				Jaera sp.				X
			Sphaeromatidae	*Cymodoce truncata*	Leach, 1814	X		
				Paracerceis sculpta	(Holmes, 1904)			X
				Sphaeroma hookeri	Leach, 1814			X
				Sphaeroma serratum	Fabricius, 1787		X	
		Leptostraca	Nebaliidae	*Nebalia bipes*	(O. Fabricius, 1780)	X		X
		Mysidacea	Mysidae	**Mysidae**		X	X	X
				Diamysis bahirensis	(G. O. Sars, 1877)			X
				Mesopodopsis slabberi	(van Beneden, 1861)	X	X	
				Mysidopsis angusta	G. O. Sars, 1864	X	X	
		Tanaidacea	Apseudidae	*Apseudes latreillii*	(Milne-Edwards, 1828)	X	X	X
			Leptocheliidae	*Leptochelia savignyi*	(Kroyer, 1842)	X	X	X
	Maxillopoda			**Copepoda**		X		X
		Thoracica	Balanidae	*Balanus improvisus*	Darwin, 1854	X	X	
	Ostracoda			**Ostracoda**		X	X	
	Pycnogonida	Pantopoda		**Pantopoda**		X		X
Brachiopoda				**Brachiopoda**		X		
Bryozoa	Gymnolaemata	Cheilostomatida	Bugulidae	*Bugula neritina*	(Linnaeus, 1758)			X
			Candidae	*Tricellaria inopinata*	d'Hondt & Occhipinti Ambrogi, 1985	X	X	X
			Tendridae	*Tendra zostericola*	Nordmann, 1839			X
Chordata	Ascidiacea			**Ascidiacea**		X	X	X
		Enterogona	Ascidiidae	*Ascidia mentula*	O. F. Müller, 1776			X
				Ascidiella aspersa	(O. F. Müller, 1776)			X
				Phallusia mammillata	(Cuvier, 1815)	X	X	
			Cionidae	*Ciona intestinalis*	(Linnaeus, 1767)			X
		Plerogona	Molgulidae	*Molgula sp.*			X	X
			Styelidae	*Botrylloides leachi*	(Savigny, 1816)			X
				Botryllus schlosseri	(Pallas, 1776)	X	X	X
				Styela plicata	(Lesueur, 1823)			X
Cnidaria	Hexacorallia	Actiniaria		**Actiniaria**		X	X	X
	Hydroidomedusa	Conica	Kirchenpaueriidae	*Ventromma halecioides*	(Alder, 1859)			X
Echinodermata	Holothuroidea	Apodida	Synaptidae	*Labidoplax thomsoni*	(Hrapath, 1865)	X	X	X
		Dendrochirotida	Cucumariidae	*Trachythyone sp.*		X	X	X
				Trachythyone elongata	(Düben Koren, 1844)	X		X
	Stelleroidea	Ophiurida		**Ophiurida**		X	X	X
			Amphiuridae	*Acrocnida brachiata*	(Montagu, 1804)			X
				Amphipholis squamata	(Delle Chiaje, 1828)	X	X	X
				Amphiura chiajei	Forbes, 1843	X	X	X
				Amphiura filiformis	(O. F. Müller, 1776)			X
			Ophiothricidae	*Ophiothrix fragilis*	(Abildgaard, 1789)	X		X
			Ophiuridae	*Ophiura albida*	Forbes, 1839			X
		Valvatida	Asterinidae	*Asterina gibbosa*	(Pennant, 1777)	X	X	X
Hemichordata	Enteropneusta	Enteropneusta		**Enteropneusta**		X		
Mollusca	Bivalvia	Arcoida	Arcidae	*Barbatia barbata*	(Linnaeus, 1758)	X		
				Scapharca demiri	Piani, 1981			X
				Scapharca inaequivalvis	(Bruguière, 1789)	X		
		Myoida	Corbulidae	*Corbula gibba*	(Olivi, 1792)	X	X	X
				Lentidium mediterraneum	(O. G. Costa, 1839)			X
			Gastrochaenidae	*Gastrochaena dubia*	(Pennant, 1777)		X	X
			Hiatellidae	*Hiatella arctica*	(Linnaeus, 1767)		X	X
			Pholadidae	*Pholas dactylus*	Linnaeus, 1758	X		
		Mytiloida	Mytilidae	**Mytilidae**		X	X	X
				Gregariella petagnae	(Scacchi, 1832)	X		
				Modiolarca subpicta	(Cantraine, 1835)		X	X
				Modiolus adriaticus	(Lamarck, 1819)		X	
				Modiolus barbatus	(Linnaeus, 1758)	X		X
				Musculista senhousia	(Benson in Cantor, 1842)	X	X	X
				Mytilaster lineatus	(Gmelin, 1791)		X	
				Mytilaster minimus	(Poli, 1795)	X		
				Mytilus galloprovincialis	Lamarck, 1819	X	X	X
		Nuculoida	Nuculidae	*Nucula nucleus*	(Linnaeus, 1758)	X	X	X
		Ostreoida	Ostreidae	*Crassostrea gigas*	(Thunberg, 1793)	X		X
				Ostrea edulis	Linnaeus, 1758	X	X	X
		Pholadomyoida	Thraciidae	*Thracia papyracea*	(Poli, 1791)	X	X	X
		Pterioida	Anomiidae	*Anomia ephippium*	Linnaeus, 1758		X	
			Pectinidae	*Chlamys flexuosa*	(Poli, 1795)	X		
				Chlamys glabra	(Linnaeus, 1758)		X	X
				Chlamys varia	(Linnaeus, 1758)	X		X
		Veneroida	Cardiidae	**Cardiidae**				X
				Acanthocardia sp.				X
				Acanthocardia tuberculata	(Linnaeus, 1758)			X
				Cerastoderma glaucum	(Poiret, 1789)	X	X	X
				Parvicardium exiguum	(Gmelin, 1791)	X	X	X
				Plagiocardium papillosum	(Poli, 1795)	X		
			Donacidae	*Donax semistriatus*	Poli, 1795			X
			Leptonidae	*Hemilepton nitidum*	(Turton, 1822)	X	X	X

Phylum	Classis	Ordo	Familia	Species	Authority	02	03	07
			Lucinidae	*Anodontia fragilis*	(Philippi, 1836)			X
				Ctena decussata	(O. G. Costa, 1829)			X
				Loripes lacteus	(Linnaeus, 1758)	X	X	X
				Lucinella divaricata	(Linnaeus, 1758)	X	X	X
			Mactridae	*Mactra stultorum*	(Linnaeus, 1758)			X
				Spisula subtruncata	(Da Costa, 1778)		X	X
			Montacutidae	*Montacuta ferruginosa*	(Montagu, 1808)	X	X	X
				Kurtiella bidentata	(Montagu, 1803)	X		
			Petricolidae	*Mysia undata*	(Pennant, 1777)		X	
			Pharidae	*Pharus legumen*	(Linnaeus, 1758)			X
				Phaxas adriaticus	(Coen, 1933)		X	
			Scrobiculariidae	*Scrobicularia plana*	(Da Costa, 1778)	X		X
			Semelidae	*Abra sp.*		X	X	X
				Abra alba	(W. Wood, 1802)	X	X	
				Abra prismatica	(Montagu, 1808)	X	X	X
				Abra segmentum	(Rcluz, 1843)	X	X	X
			Solecurtidae	*Azorinus chamasolen*	(Da Costa, 1778)			X
			Solenidae	*Solen marginatus*	Pulteney, 1799	X	X	X
			Tellinidae	*Gastrana fragilis*	(Linnaeus, 1758)	X	X	X
				Tellina sp.		X	X	X
				Tellina balaustina	Linnaeus, 1758		X	
				Tellina distorta	Poli, 1791	X	X	X
				Tellina fabula	Gmelin, 1791	X	X	X
				Tellina nitida	Poli, 1791	X	X	
				Tellina pulchella	Lamarck, 1818	X		X
				Tellina tenuis	Da Costa, 1778	X	X	X
			Veneridae	*Chamelea gallina*	(Linnaeus, 1758)	X	X	X
				Dosinia lupinus	(Linnaeus, 1758)	X	X	X
				Pitar rudis	(Poli, 1759)	X	X	X
				Ruditapes decussatus	(Linnaeus, 1758)	X	X	X
				Ruditapes philippinarum	(Adams & Reeve, 1850)	X	X	X
				Venerupis aurea	(Gmelin, 1791)	X	X	X
	Gastropoda	Cephalaspidea	Haminoeidae	*Haminoea sp.*				X
				Haminoea navicula	(Da Costa, 1778)	X	X	X
		Neogastropoda	Muricidae	*Hadriania oretea*	(De Gregorio, 1885)	X		
				Hexaplex trunculus	(Linnaeus, 1758)	X	X	X
			Nassariidae	*Cyclope neritea*	(Linnaeus, 1758)	X	X	X
				Nassarius mutabilis	(Linnaeus, 1758)		X	
				Nassarius nitidus	(Jeffreys, 1867)	X	X	X
				Nassarius pygmaeus	(Lamarck, 1822)	X		
		Neotaenioglossa	Calyptraeidae	*Calyptraea chinensis*	(Linnaeus, 1758)	X	X	X
			Cerithiidae	*Bittium reticulatum*	(Da Costa, 1778)	X	X	X
				Cerithium alucaster	(Brocchi, 1814)	X	X	
				Cerithium vulgatum	Bruguire, 1792	X	X	X
			Hydrobiidae	*Hydrobia acuta*	(Draparnaud, 1805)	X		X
			Naticidae	*Polinices guillemini*	(Payraudeau, 1826)			X
			Rissoidae	*Rissoa sp.*		X		
			Skeneopsidae	*Skeneopsis pellucida*	(Monterosato, 1874)	X		
		Nudibranchia		**Nudibranchia**		X	X	X
		Vetigastropoda	Trochidae	*Calliostoma virescens*	Coen, 1933	X	X	
				Calliostoma zizyphinum	(Linnaeus, 1758)			X
				Gibbula sp.		X		X
				Gibbula adriatica	(Philippi, 1844)	X	X	X
				Gibbula albida	(Gmelin, 1791)	X	X	X
				Gibbula divaricata	(Linnaeus, 1758)			X
				Gibbula magus	(Linnaeus, 1758)			X
				Gibbula varia	(Linnaeus, 1758)			X
			Turbinidae	*Tricolia pullus*	(Linnaeus, 1758)	X	X	X
	Polyplacophora			**Neoloricata**		X	X	X
			Acanthochitonidae	*Acanthochitona fascicularis*	(Linnaeus, 1767)	X		
			Ischnochitonidae	*Lepidochitona cinerea*	(Linnaeus, 1767)	X		
	Scaphopoda			**Scaphopoda**			X	X
Nematoda				**Nematoda**		X	X	X
Nemertea				**Nemertea**		X	X	X
Phoronida			Phoronidae	*Phoronis muelleri*	Selys-Longchamps, 1907	X	X	X
Platyhelminthes				**Platyhelminthes**		X	X	
Porifera				**Porifera**		X	X	X
	Calcarea	Leucosolenida	Sycettidae	*Sycon raphanus*	Schmidt, 1862		X	X
Sipunculida	Sipunculidea	Sipunculiformes	Sipunculidae	*Sipunculus nudus*	Linnaeus, 1766	X		X

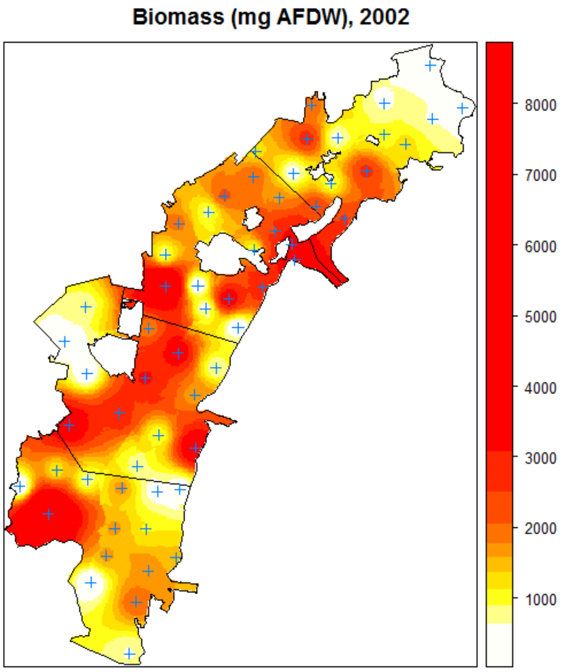
A3 Interpolated maps of A, B and S for 2002, 2003, 2007 (59-stations data sets)

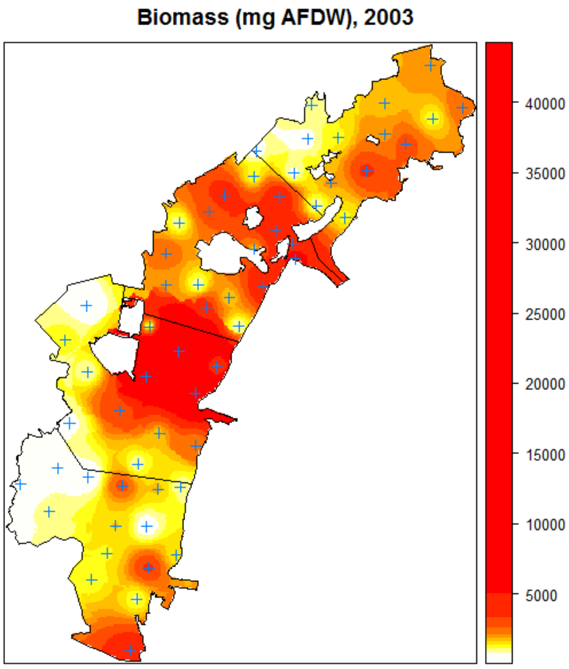


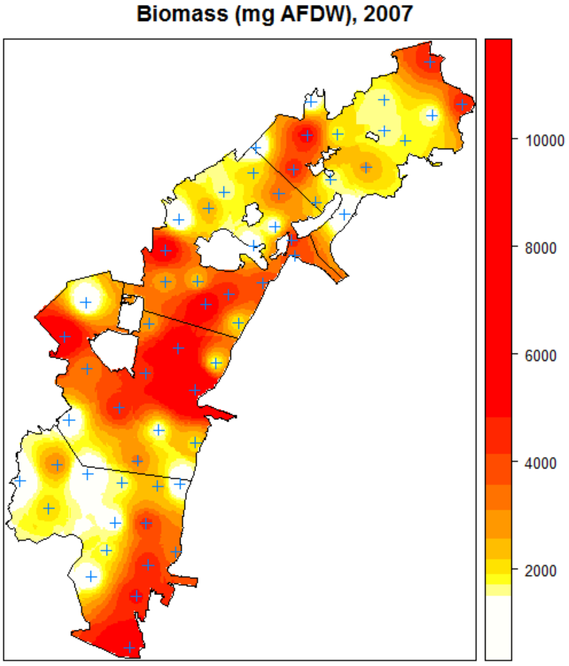




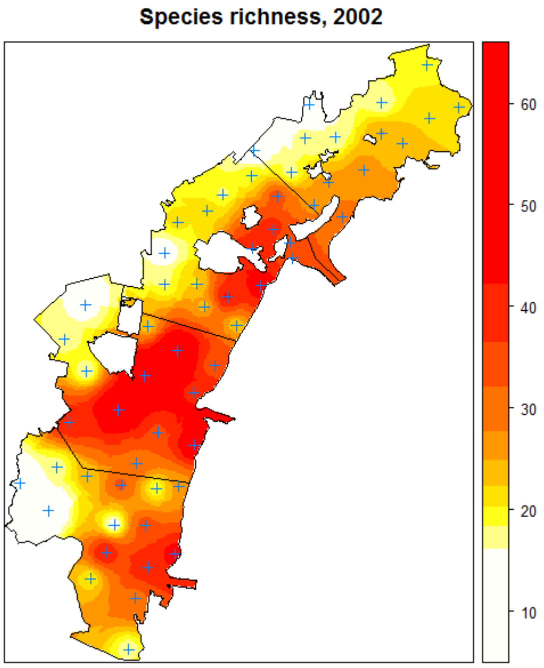
A3 Interpolated maps of A, B and S for 2002, 2003, 2007 (59-stations data sets)

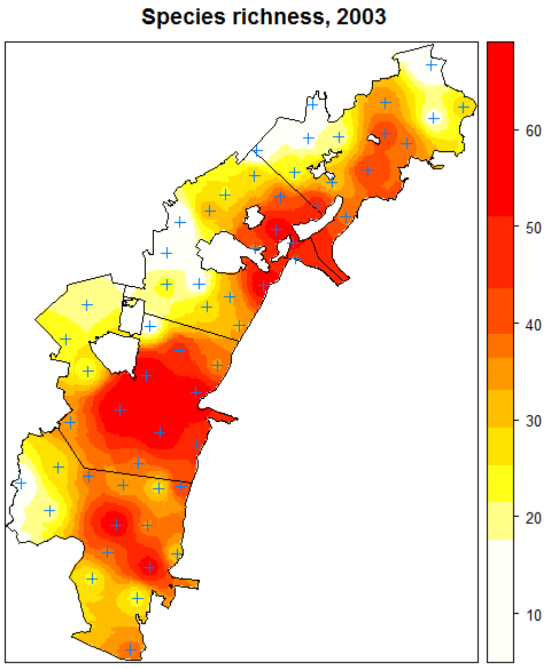

Biomass (mg AFDW), 2002

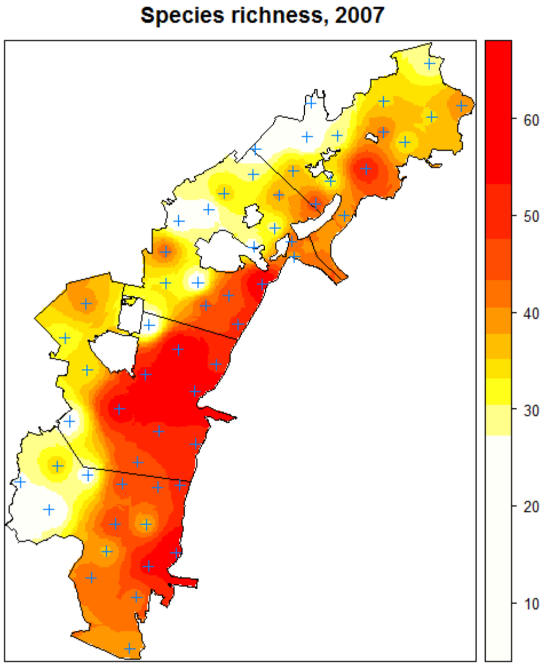

Biomass (mg AFDW), 2003


Biomass (mg AFDW), 2007

A3 Interpolated maps of A, B and S for 2002, 2003, 2007 (59-stations data sets)


Species richness, 2002

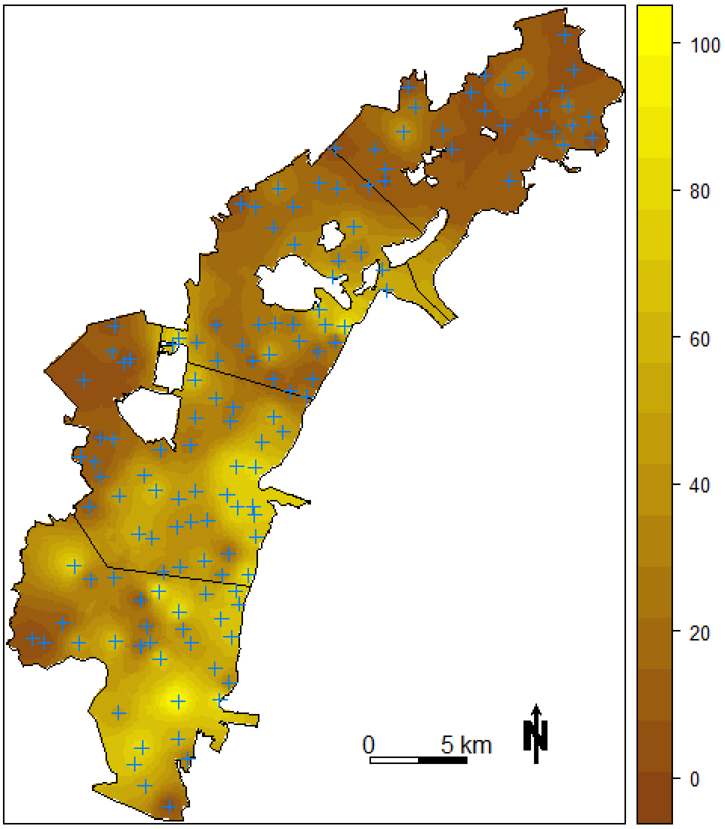

Species richness, 2003


Species richness, 2007

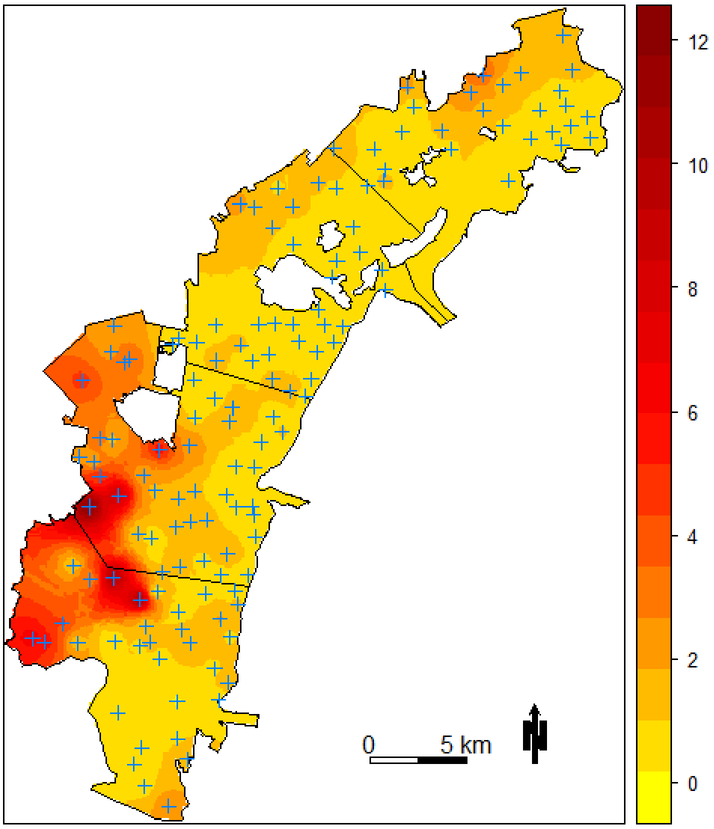
A4 Dominant taxa (> 75% Total Abundances and Biomass per station)

	A	B
Abra prismatica	X	
Abra segmentum	X	X
Abra sp.	X	
Actiniaria	X	X
Amage adspersa	X	X
Ampelisca sp.	X	X
Amphipholis squamata	X	X
Amphiura chiajei	X	
Apseudes latreillii	X	X
Ascidiacea	X	X
Asterina gibbosa		X
Azorinus chamasolen		X
Bittium reticulatum	X	X
Botrylloides leachi	X	
Botryllus schlosseri	X	
Bugula neritina	X	
Callianassa tyrrhena	X	X
Capitella capitata	X	
Capitellidae	X	
Capitomastus minimus	X	
Caprella penantis	X	
Caprellidae	X	
Carcinus aestuarii	X	X
Cerastoderma glaucum	X	X
Cerithium alucaster		X
Cerithium vulgatum		X
Chamelea gallina	X	X
Chironomus salinarius	X	
Chlamys flexuosa		X
Chlamys glabra		X
Ciona intestinalis	X	X
Cirratulidae	X	X
Cirriformia tentaculata	X	
Cirrophorus furcatus	X	
Clymenura clypeata	X	X
Corophium orientale	X	X
Corophium sp.	X	X
Crassostrea gigas		X
Cumacea	X	
Cyathura carinata	X	
Cyclope neritea	X	X
Desdemona ornata	X	
Dexamine spinosa	X	X
Diogenes pugilator	X	X
Dorvillea rudolphii	X	
Dosinia lupinus	X	
Dyspanopeus sayi	X	X
Elasmopus sp.	X	
Enteropneusta		X
Eteone picta		X
Euclymene lumbricoides	X	X
Eunice pennata		X
Eunice vittata	X	X
Eunicidae	X	X
Gammarella fucicola	X	
Gammarida	X	
Gammarus sp.	X	X
Gastrana fragilis	X	X
Gibbula adriatica		X
Gibbula albida	X	X
Gibbula sp.	X	X
Glycera cfr. fallax	X	
Glycera tridactyla	X	X
Glycera unicornis		X
Haminoea navicula	X	X
Haminoea sp.	X	X
Hediste diversicolor	X	X
Heteromastus filiformis	X	
Hexaplex trunculus		X
Hydroides dianthus	X	X
Hydroides pseudouncinatus	X	
Ione thoracica	X	
Iphinoe serrata	X	
Labidoplax thomsoni		X
Lanice conchylega		X
Leptochelia savignyi	X	X
Leucothoe venetiarum	X	
Liocarcinus navigator		X
Loripes lacteus	X	X
Lucinella divaricata	X	
Lumbricalus adriatica		X
Lumbrineris latreilli	X	X
Mactra stultorum		X
Malacoceros fuliginosus	X	
Malacoceros sp.	X	
Maldanidae	X	X
Marphysa sanguinea	X	X
Megalomma lanigera	X	X
Melita palmata	X	
Melita sp.	X	
Mesopodopsis slabberi	X	
Metaphoxus simplex	X	
Microdeutopus sp.	X	
Microspio mecznikovianus	X	
Myriochele oculata	X	
Mysidae	X	
Mysidopsis angusta	X	
Mytilus galloprovincialis		X
Nassarius nitidus	X	X
Neanthes caudata	X	X
Neanthes succinea		X
Nematoda	X	
Neoloricata		X
Nephtys hombergi	X	X
Nereididae	X	
Notomastus sp.	X	X
Nucula nucleus	X	X
Oligochaeta	X	
Onuphis eremita	X	
Ophiothrix fragilis		X
Orbiniidae	X	
Owenia fusiformis	X	
Palaemon adspersus		X
Paraonidae	X	
Perinereis cultrifera	X	X
Perioculodes longimanus	X	
Perioculodes sp.	X	
Petaloproctus terricolus		X
Phallusia mammillata		X
Pholas dactylus		X
Phoronis muelleri	X	
Phyllodocidae	X	
Platyhelminthes		X
Platynereis dumerilii	X	X
Polydora sp.	X	
Porifera	X	
Prionospio cirrifera	X	
Prionospio sp.	X	
Processa sp.		X
Rhithropanopeus harrisii		X
Ruditapes decussatus		X
Ruditapes philippinarum	X	X
Sabellaria sp.	X	
Sabellidae	X	
Sipunculus nudus		X
Solen marginatus	X	X
Spio decoratus	X	
Spio filicornis	X	
Spio sp.	X	
Spionidae	X	
Spirorbidae	X	
Stenothoe sp.	X	
Sternaspis scutata		X
Streblospio shrubsolii	X	
Syllidae	X	
Tellina distorta	X	
Tellina fabula	X	
Tellina tenuis	X	
Terebellidae	X	
Trachythyone elongata		X
Trachythyone sp.		X
Tricellaria inopinata	X	X
Tricolia pullus	X	
Upogebia deltaura		X
Upogebia pusilla	X	X
Venerupis aurea	X	X

A5 Interpolated maps of selected environmental variables for 2002

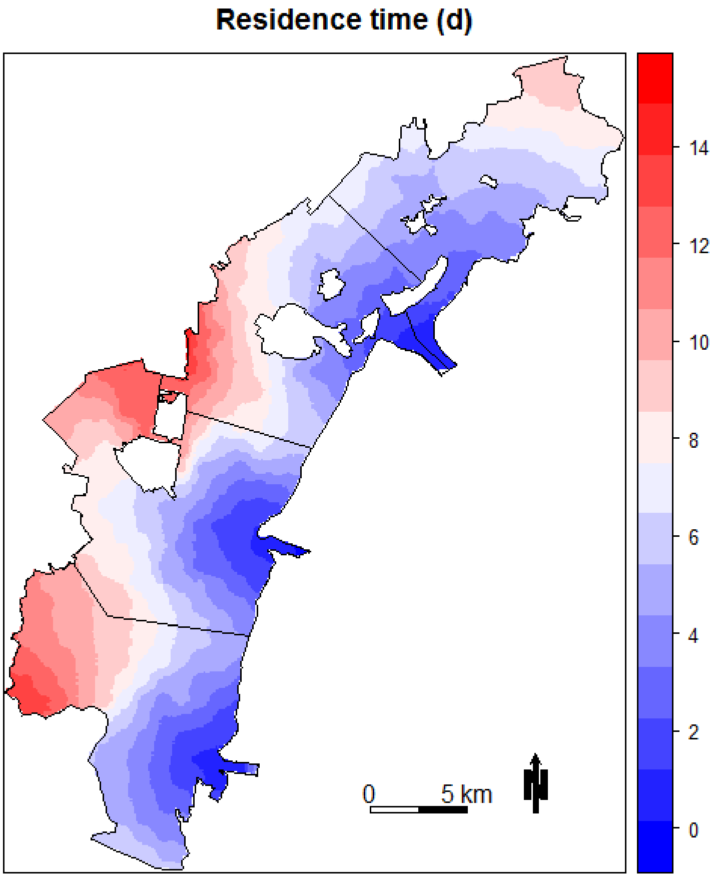


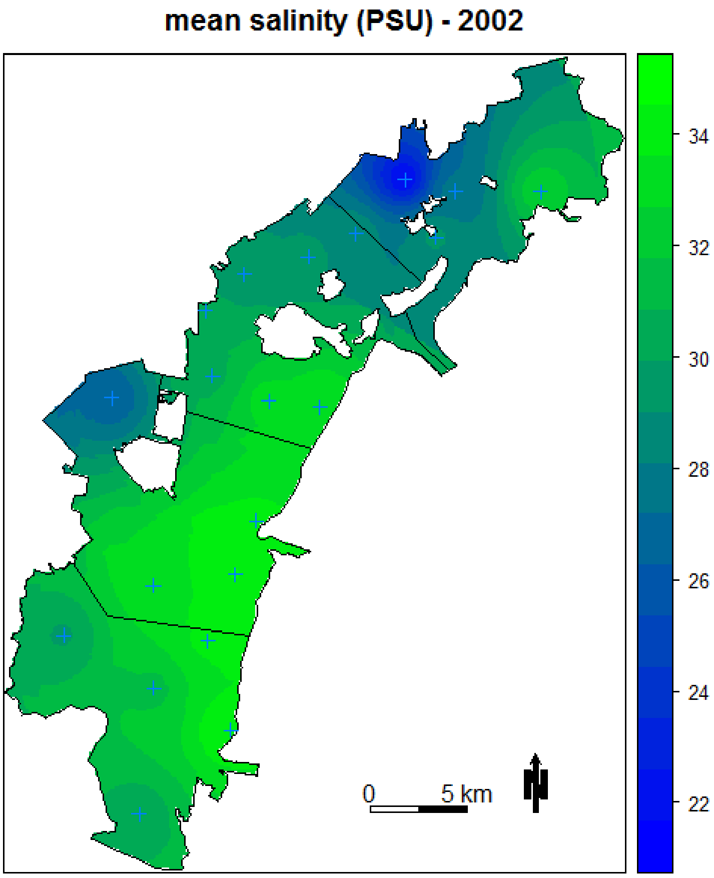
% sand



% TOC

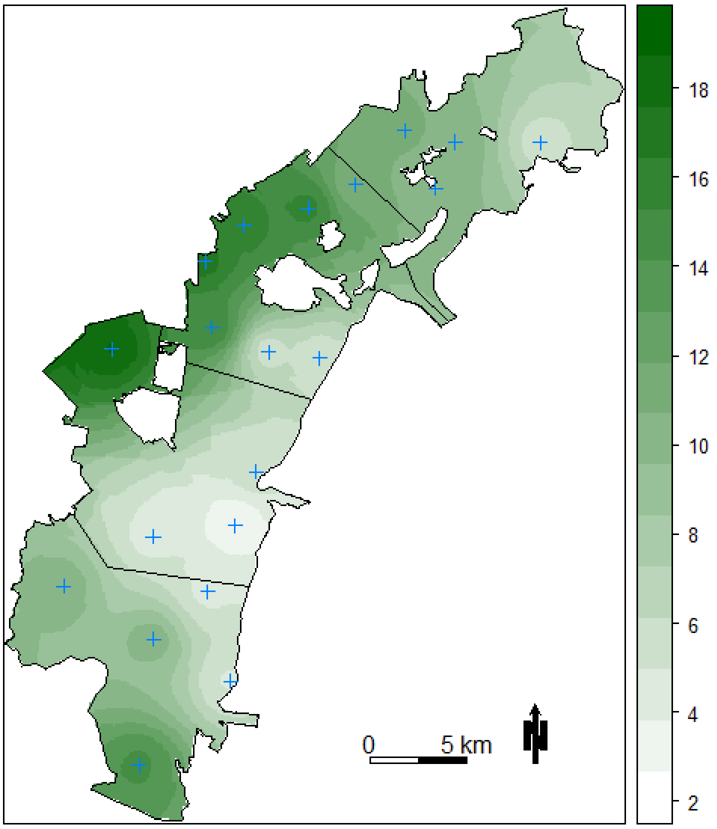
A5 Interpolated maps of selected environmental variables for 2002





A5 Interpolated maps of selected environmental variables for 2002

mean Chlorophyll a (ug/L) - 2002



mean TSS (mg/L) - 2002