Title: Multiscale patterns in the diversity and organization of benthic fauna among French estuaries

Authors:

Hugues Blanchet[1], Benoît Gouillieux[1], Sandrine Alizier[2], Jean-Michel Amouroux[3], Guy Bachelet[1], Anne-Laure Barillé[4], Jean-Claude Dauvin[2,5], Xavier de Montaudouin[1], Valérie Derolez[6], Nicolas Desroy[7], Jacques Grall[8], Antoine Grémare[1], Pascal Hacquebart[9], Jérôme Jourde[8,12], Céline Labrune[3], Nicolas Lavesque[1], Alain Meirland[10], Thiebaut Nebout[7], Frédéric Olivier[14], Corine Pelaprat[11], Thierry Ruellet[2], Pierre-Guy Sauriau[12], Sébastien Thorin[13]

Corresponding author:

Hugues Blanchet ; Université de Bordeaux, CNRS, UMR 5805 EPOC, Station Marine d'Arcachon, 2 rue du professeur Jolyet, 33120 Arcachon, France ; tel : +33(0)5 56 22 39 35 ; fax : +33(0)5 56 84 08 48.

Affiliations:

[1]Université de Bordeaux, CNRS, UMR 5805 EPOC, Station Marine d'Arcachon, 2 rue du professeur Jolyet, 33120 Arcachon, France

[2]Université de Lille1, UMR CNRS LOG 8187, Station Marine de Wimereux, 28 avenue Foch, BP 80, F-62930 Wimereux

[3]UPMC Université Paris 6, CNRS, FRE 3350 LECOB, F-66650 Banyuls-sur-mer.

[4]Bio-Littoral, laboratoire MMS Mer Molécule Santé, université de Nantes, France.

[5]Université de Caen Basse Normandie, Laboratoire Morphodynamique Continentale et Côtière, UMR CNRS 6143 M2C, 2-4 rue des Tilleuls, F-14000 Caen, France

[6]IFREMER Languedoc Roussillon, Pôle "Mer et Lagunes", Bd Jean Monnet BP 171 34203 Sète Cedex

[8]Institut Universitaire Européen de la Mer, UMS 3113, Technopôle Brest-Iroise - rue Dumont d'Urville, F-29280 Plouzané, France

[10]Stareso - Pointe Revellata - BP33, F–20260 Calvi, France

[7]IFREMER LER Finistère-Bretagne Nord, Station de Dinard, 38 Rue du Port Blanc, BP 80108, F-35801 Dinard, France

[9]GEMEL Groupe d'Étude des Milieux Estuariens et Littoraux - équipe Normandie, Station marine - Centre Régional d'Etudes Côtières, 54 rue du Docteur Charcot, F-14530 Luc-sur-Mer, France

[10]GEMEL Groupe d'Étude des Milieux Estuariens et Littoraux - équipe Picardie, Groupe d'Etude des Milieux Estuariens et Littoraux Picardie, 115 quai Jeanne d'Arc, F-80230 Saint-Valéry-Sur-Somme, France

[11]Stareso - Pointe Revellata - BP33, F–20260 Calvi, France

[12]CNRS, Université La Rochelle, UMR LIENSS, UMR 7266, 2, rue Olympe de Gouges, F-17 000 La Rochelle, France
[13]CREOCEAN, Zone TECHNOCEAN, Rue Charles Tellier, F-17000 La Rochelle, France
14MNHN, UMR BOREA Muséum National d'Histoire Naturelle, DMPA, 61 rue Buffon 75005 PARIS, France

Abstract

Based on a parallel sampling conducted during autumn 2008, a comparative study of the intertidal benthic macrofauna among 10 estuarine systems located along the Channel-Atlantic coast of France was performed in order to assess the level of fauna similarity among these sites and to identify possible environmental factors involved in the observed pattern both at large scale (among sites) and smaller scale (benthic assemblages). More precisely this study focused on unraveling the observed pattern of benthic fauna composition and diversity observed at among-sites scale by exploring both biotic and abiotic acting at the among- and within-site scales. Results showed limited level of similarity at the among-site level in terms of benthic fauna composition and diversity. The observed pattern did not fit with existing transitional water classification methods developed in the frame of the WFD. More particularly, the coastal plain estuaries displayed higher among-sites similarity compared to ria systems. These coastal plain estuaries were characterized by higher relative influence of river discharge with lower communication with the ocean and high turbidity. On the other hand, the ria-type systems were more dissimilar and different from the coastal plain estuaries. The level of similarity among estuaries was mainly linked to the relative extent of the "*Scrobicularia plana-Cerastoderma edule*" and "*Tellina tenuis*" or "*Venus*" communities as a possible consequence of salinity regime, suspended matter concentrations and fine particles supply with consequences on the trophic functioning, structure and organization of benthic fauna. Despite biogeographical patterns, the results also suggest that, in the context of the WFD, these estuaries should only be compared on the basis of the most common habitat occurring throughout all estuarine system and suggest that the EUNIS biotope classification might be used for this purpose. In addition, an original inverse relation between γ-diversity and area was put in evidence however its relevance might be questioned.

Keywords: estuaries, benthos, diversity, structuring factors, diversity

Introduction

Whitfield and Elliot (2011) defined estuaries as “semi-enclosed coastal bodies of water which are connected to the sea either permanently or periodically, have a salinity that is different from the adjacent open ocean due to freshwater inputs, and include a characteristic biota”. According to this definition, estuaries should display a characteristic benthic fauna. Benthic organisms are indeed recognized as good indicators of environmental conditions mainly because (1) of their mostly sedentary life as adults, preventing them from escaping changing conditions, and (2) their position at the sediment-water-column interface, allowing them to integrate variations of both sub-systems (Dauvin, 1993). Most estuaries are indeed characterized by a very limited number of benthic species which number decreased as the level of salinity decreases (Remane 1934, Remane and Sclieper 1958). The scheme proposed by Remane (1934), describing the succession of marine, brackish and freshwater species along the salinity gradient in the Baltic sea has been increasingly criticized (; Barnes 1989, Attril and Rundle 2002) and recently reviewed by Whitfield et al. (2012). One of the main objections to this schematic diagram is the existence of truly “brackish species” that were supposed to exclusively dwell within estuaries. Based on works conducted along the full gradient of salinity within estuarine systems (e.g. Attril and Rundle 2002, Rodrigues et al. 2011), there are no evidence of the existence of truly brackish benthic species (Whitfield et al 2012 and references therein). Nevertheless, a pool of typically estuarine species can be recognized. This pool of species would consist in marine euryhaline species that can live in fully marine conditions but which display higher occurrence, abundance and biomass levels in estuarine conditions as the abundance of more stenohaline species decreases with decreasing average level of salinity and increasing level of salinity variations (Attril 2002; Little 2000). Indeed, the other main objection to the Remane scheme is the probably most important consequences of variable salinity conditions compared to its level (Attril 2002). Nevertheless the pattern of increasing abundance and occurrence of typically estuarine species within estuaries compared to fully marine conditions may be explained by the progressive disappearance of more competitive, but more stenohaline, species toward the head of the estuary allowing the increase of populations of typically estuarine, more euryhaline, species as they are released from interspecific competition (Little 2000). As the salinity variations increases toward the head of the estuary, typically estuarine marine species reach their tolerance limit and disappear leading to the generally observed decrease of marine benthic species number from the downstream to the upstream areas. The particularity of this typically estuarine benthic species has lead to define these species as opportunists since they only show high occurrence and abundance levels when other species disappear and they are typically retrieved in area of very low species number. These very features of estuarine benthic fauna have lead to considerable difficulties when applying ecological quality bio-evaluation methodologies based on benthic benthic macrofauna to estuarine systems (Elliot and Quintino 2007, Blanchet et al 2012). The need of appropriate methodologies to evaluate the ecological quality of european estuarine water bodies has been urged since the publication of the European Water Framework Directive

(WFD). One of the main difficulties in estuarine systems is to determine appropriate reference conditions which should correspond to pristine environmental conditions. Several proposals have been made by classifying transitional water bodies into types (e.g. Barbone et al. 2012). For instance Borja et al. (2004) used the WFD-classification to derive theoretical reference conditions for the benthos of each type of water body. More recently, Galvan et al (2010) proposed another classification of transitional water bodies with the same objective: defining reference conditions for each type of estuary. The latter authors however recognized, in accordance with the growing number of studies showing that benthic conditions varied greatly at finer scale within estuarine systems (Bald et al. 2005, de Paz et al. 2008, Rodrigues et al 2011).

Given the characteristics of the typical estuarine benthic fauna and the need for evaluating the ecological quality of estuarine transitional waters through the use of (among other) benthic invertebrates for which appropriate reference conditions has to be derived. Our study focused on comparing the estuarine fauna of ten estuarine systems located along the French Atlantic-Channel coast in order (1) to evaluate the degree of fauna similarity among estuarine systems along the French coasts and to relate observed differences to relevant hydromorphological features at the among-sites scale. The results obtained allowed to evaluate the accuracy of existing typologies developed for the WFD. The second objective was (2) to relate the pattern observed at the among-sites scale to finer (within-site) scale organization of benthic macrofauna and associated environmental factors. This allowed evaluating the possibility of comparing estuarine benthic fauna among sites at a finer biotope-scale (Ducrotoy 2010).

Material and methods

Available data

Hydro-morphological description of estuarine sites

In order to assess resemblance among the ten sites and to relate observed patterns to general hydrological, morphological or sedimentary features of the study sites, several hydro-morphological indices were used. Average river discharges values were retrieved for the October 2007 - October 2008 period from the French water information system database (http://www.hydro.eaufrance.fr/) and from the *Centro de Estudios Hidrográficos* (http://hercules.cedex.es/general/default.htm) or from literature in case of missing data. Estimates of estuarine water volume were obtained from average channel depth estimates based on available depth measures, marine maps or published data (Valencia et al. 2004) and sites areas (Hume et al. 2007). Tidal prism was estimated using the average tidal height (coefficient: 70) at the vicinity of each site using chart datum from the SHOM. Following Hume et al. (2007) and Galvan et al. (2010), ratios between tidal prism and estuarine water volume at high tide (TP:V ratio) and between average river discharge during a 12-H tidal cycle and estuarine water volume at high tide (R12:V ratio) were computed as well the ratio TP:R was computed. Since these values only corresponded to estimates, all values were rounded to nearest 10^5 m^3. Three descriptors of the morphology of the systems were used: EE (TWEI in Galvan et al. (2010)), which is an index reflecting the system elongation; SC (TWCI in Galvan et al. (2010)), an index describing the morphological complexity of the system and CI, which reflects the more or less closed character of the system. Details concerning the computation of these indices can be found in the works of Hume et al. 2007 and Galvan et al. 2010. The main type of sediments occurring in the different estuarine system was calculated as the median value of grain-size measured (in Phi-unit) at each sampled stations (since samples allocation was performed at random or were systematically distributed in each estuary). The variability of sediments type within each site was estimated as the coefficient of variation associated to the mean (in %). The average level of sediments organic content was also computed for each site. Finally, the average river slope was computed as the ratio between the main rivers source elevation (in m) and the length of the river to the mouth of the estuary (in km).

Sampling of benthic macrofauna

Intertidal soft-bottom macrofauna was collected during autumn 2008 in ten estuaries. The sampling strategy consisted in sampling stations regulalrly distributed along the downstream-upstream axis of the estuarine systems while restricting to the *ca*. polyhaline and mesohaline areas. The sampling procedure consisted in collecting at least a total area of 0.2 m² using several replicate samples. The sampling effort was higher in the three largest estuaries than in the smaller sites (Table 1). All samples were sieved through a 1-mm mesh. The remaining fraction was preserved in 4% formalin and stained with Rose Bengale. Analysis of fauna was performed in the laboratory where individuals were identified to species level, when possible, and counted. All data collected were organized in a single database, called BET (Benthos in Estuarine Transitional waters). Additional sediment samples were collected in order to characterize the substrate. The sediment samples were sieved through series of meshes of decreasing aperture which allow to determine the sediment grain-size with the proportion of pebbles (particles > 16 mm), gravels (4 to 16 mm),coarse sand (1 to 4 mm), medium sands (0.25 to 1 mm), fine sands (0.063 to 0.125 mm) and mud (particles < 63 µm).

Data analyses

Database management

Prior to the analysis of data, the level of identification of taxa was homogenized throughout the BET database and the small sessile epifauna taxa (e.g. spirorbids and serpulids polychaetes, cirripeds) were removed because it formed only a few taxa collected on some boulders and shells collected in the soft-bottom habitats which were not always taken into account. Abundance data was normalised to a 0.2 m² by pooling or randomly removing replicate samples. Abundance data were first Log_e-tranformed in order to balance the numerical dominance of some particularly abundant taxa such as (eg.) *Hydrobia ulvae* or oligochaetes. Similarity matrix between stations were then computed using the Bray-Curtis similarity coefficient (Clarke et al. 2006).

Comparison of benthic fauna at the among-sites scale and relation to hydromorphological characteristics

Statistically significant difference in benthic fauna among estuaries was tested by way of one-way PERMANOVA performed on the Bray-Curtis similarity matrix using 'sites' as factor (Anderson et al 2008). In case of significant difference, pairwise tests were conducted to assess differences between each pairs of sites. In order to evaluate the degree of fauna ressemblance among sites and to relate the observed pattern to hydromorphological variables, a measure of average fauna similarity among sites was first obtained by computing a matrix of distances among site centroids based on the among-stations Bray-Curtis similarity matrix. The among-sites matrix was obtained using the 'distance among centroids' procedure provided by the PRIMER with PERMANOVA+ package (Anderson et al. 2008). This procedure consisted in calculating a resemblance matrix among site centroids in the space of the Bray-Curtis similarity measure (see Anderson et al. 2008). Ordinations of site centroids were visualized using Principal Coordinates analysis (PCO) and a cluster analysis was performed in order to provide a classification of sites. The obtained classification and ordination was compared to three available typologies issued from (1) the WFD-classification, (2) the fish-based classification of North European estuaries proposed by Nicolas et al. (2010) and (3) the benthos-based typology of transitional water bodies developed by Galvan et al. (2010) for cantabric coast water bodies. Relation between observed pattern of macrofauna and hydromorphological variables was investigated through the BEST procedure (Clarke and Gorley, 2006). This procedure permitted to identify the main hydrological or morphological variables which together displayed the higher level of (rank-) correlation with the distances among centroids matrix. Prior to the BEST analysis, a selection of variables was operated by removing variables showing high level of Spearman rank correlation coefficient.

Identification of benthic assemblages and structuring environmental variables at the within-site scale

Benthic assemblages were determined using hierarchical classification of stations through cluster analysis by group-average method performed on the among-stations Bray-Curtis similarity matrix. The resulting dendrogram was investigated at three levels of increasing similarity: 10%, 20% and 30% similarity levels. The relevance of the station groups obtained was evaluated by the SIMPROF procedure. This procedure performed a series of similarity profile permutation tests at each node of the dendrogram (Clarke and Gorley 2006). At each node of the dendrogram, a test of the null hypothesis that the set of samples to be divided do not differ in multivariate structure is performed. This procedure hence permitted to decide whether further subdivision within a group of stations clustering at e.g. 10% similarity level was relevant at e.g. 20% similarity level.

Since our objective was to evaluate the relative influence of salinity and sediment types in the structuring of macrofauna within each estuary, the further set of analyses was performed separately for each site. Two proxies were used to evaluate the importance of both factors within one site: (1) the relative position of each station along the estuarine gradient of each site was computed as the ratio between the distance from each station to the most downstream station and the distance from the most downstream to the most upstream station following the thalweg and (2) sediments grain-size in Φ units. This relative position of each station along the estuarine axis was expressed as a percentage and was expected to be correlated to the relative level of salinity (and salinity variation) occurring from the lower to the upper reaches of the investigated area. This proxy was preferred to punctual measures of salinity because, in an estuary, a one-time measure of salinity is not relevant to establish the real salinity conditions (average level and variations) occurring at one station in the course of seasons (fluctuations of river discharges), month (spring tide *vs* neap tide) or day (high tide *vs* low tide).

The influence of each of the two variables on the structure of macrofauna was determined by the DISTLM method which consists in partitioning the variation in the data described by the Bray-Curtis similarity matrix using simple or multiple regression models (Anderson et al. 2008). This permitted to evaluate the proportion of variation in among-samples similarity explained by each of the two variables separately and in linear combination. In addition to this procedure, the level of correlation between both variables was measured by Spearman rank correlation coefficient.

Diversity measures

Macrobenthic diversity in the ten estuaries was compared using the three components of diversity, namely α-, β- and γ-diversity. Gamma-diversity is the number at the scale of a large area (e.g. one estuary) whereas α-diversity is the number of species at smaller scale, typically in a collection of samples from one station or one habitat (Gray 2000, Maguran 2004). The γ-diversity at the scale of each site (one of the ten estuaries studied here) was calculated as the total number of taxa recorded in one site (by pooling all stations from a given site). Since this total number of taxa varies as a function of the sampling effort (number of stations), γ-diversity among site was compared using the same number of station (10 stations, corresponding to a sampled area of 2 m^2). The level of γ-diversity obtained was compared to available data from other north European estuarine intertidal areas by retrieving this information from published data obtained on a comparable sampling effort (measured as total sampled area, in m^2). As much as possible, the number of species published was reduced to obtain a similar level of taxonomic level of identification as used in our analysis. For instance, oligochaetes or insects identified to species or family-levels were pooled into one taxon; nematodes, foraminifers, ostracods and small sessile organisms mainly related to the presence of hard substrates (spirorbids, cirripedia) were not considered. The obtained number of taxa and corresponding sampled area were plotted together with the species-accumulation curves obtained for each of the ten sites studied. Observed differences in γ-diversity among studied sites were correlated with hydromorphological variables at the site-scale by way of Spearman rank correlation coefficient. Compared using a similar sampling effort, the total number of taxa in site is dependent on the two components of diversity namely, α-diversity which is the number of taxa in given station, and the variation in the identities of species among stations (β-diversity). In order to measure β-diversity, the classical Whittaker beta diversity index β_w was computed as the ratio between the total number of taxa in a given site (γ-diversity) and the number of taxa in a given station from the same site (α-diversity). This index gave a measure of how much, on average, a whole site was richer than its stations. This index of β-diversity was used in order to give an overview of the general level of β-diversity variations however other complementary methodologies can be used giving more insight on the patterns of β-diversity (Maguran 2004 and recent reviewed by Anderson et al. 2010). Number of taxa per station (0.2 m^2) was used as the measure of α-diversity. Difference in level of α-diversity among sites was assessed by non-parametric Kruskall-Wallis ANOVA and pairwise tests and its pattern within estuaries was described by non-parametric Spearman's rank correlation coefficient with environmental variables (Siegel 1956). Finally, in order to evaluate the contribution of α- and β-diversity on γ-diversity, γ-diversity was plotted against average α-diversity measured in each site. The resulting plot should be more of less linear where site-specific discrepancy from this linear model can be interteppretated as difference in β-diversity when using the multiplicative relation between the different components of diversity ($\gamma=\beta\times\alpha$) (Maguran, 2004). In addition relationship among the three components of diversity and the different measure of α-, β- and γ-diversity used were measured using rank-correlation.

Trophic organization

Species were classified into five trophic groups: subsurface deposit feeders (SSDF) gathered taxa feeding head-down from bulk organic matter within the sediment, interface feeders (IF) gathered species also known as 'surface deposit feeders' that feed from organic matter at the sediment surface and that usually can also shift to suspension feeding, suspension feeders (SF) gathered taxa feeding mainly on suspended organic matter, grazers/herbivores (G) gathered species mainly feeding from microphytobenthos from surface sediments and/or from angiosperms leaves and/or directly from angiosperms or macroalgae, finally carnivores and omnivores (C-O) gathered species which includes fauna as a substantial part of their diet. This classification was established according to literature (Fauchald and Jumars, 1979; Bachelet, 1981; Sauriau *et al*., 1989; Hily and Bouteille, 1999), available informations on WORMS (*www.marinespecies.org)* and/or on unpublished results obtained through stable isotopes data (Dubois et al., submitted; Nzigou et al., in prep.). The study of Tenore et al. (2006) showed that the total number of taxa within different functional groups of macrofauna could be linked to the components (referenced as 'modules') of coastal and estuarine systems. Accordingly, we considered the total number of taxa of the different trophic groups as indicators of each site characteristics. Hence, the average number of taxa from each trophic and each site was compared on the basis of a similar sampled area. This was obtained by computing the species accumulation curves for each trophic group and each site. All species accumulation curves were obtained by randomizing the order of samples (999 permutations) using PRIMER software.

Results

Among-sites comparisons

Benthic macrofauna composition and associated environmental factors

A total of 172 taxa were recorded for the intertidal macrofauna of the ten estuaries studied. Among these taxa, only four taxa were identified in all estuaries namely *Hediste diversicolor*, *Cerastoderma edule*, *Scrobicularia plana* and oligochaetes. Only 15% of the taxa where recorded in at least half the studied sites and more than 50% were recorded in only one site. Among sites, the Belon and Bidassoa estuaries displayed the largest proportion of unique taxa (taxa that were present in only one site) with about two fifth of their total number of taxa as unique. In contrast, the Seine and Loire estuaries displayed the lowest proportion of unique taxa (less than 5%) while the other displayed between one quarter (Trieux estuary) and one tenth (Aiguillon cove, Orne estuary) of their taxa as unique.

PERMANOVA test indicated that each of the ten study sites displayed a significantly different benthic fauna (pairwise tests, lowest p-value = 0.038). Ordination of site centroids using PCO coupled to cluster analyses, however put in evidence affinities among the benthic fauna of the Aiguillon-Sèvre niortaise, Gironde, Seine, Loire, Charente , Somme and Orne estuaries and separated the latter sites from the Belon, Bidassoa and Trieux estuaries(Fig. 2). At this distance of 50, the Belon estuary clustered alone whereas the Trieux and Bidassoa clustered together (Fig. 2). At a higher similarity level (i.e. lower distance), the benthic fauna of the Orne and Somme systems were isolated from the main group of sites (Fig. 2). None of the existing classifications tested showed a good agreement with fauna pattern (Fig. 2). The BEST procedure highlighted the relations between the ordination of sites centroids and some of the hydromorphological variables (Table 1). More precisely; the best correlation between environmental and fauna data (Rho = 0.68, p=0.02) was obtained when including River discharge:estuarine volume ratio, Closure Index, Slope and average suspended particulate matter levels (SPM). This result showed that the fauna of these estuarine systems differed according to the combination of the relative importance of freshwater inputs, the relative importance of the connection to sea, the ratio between the main source elevation and the length of the main tributaries and the level of suspended particulate matter. However it should be noticed that, in our dataset, these four variables were correlated with other morphological, hydrological and sedimentary variables. For instance, SPM level was correlated to the absolute value of river discharge, lower intertidal area, lower influence of the tidal prism and finer sediments.

Species diversity

Compared on the basis of ten samples (2 m²), the total number of taxa recorded in each site varied from 58 taxa in the Bidassoa estuary to only 21 in the Loire estuary (Fig. 3). Sites displaying the higher γ-diversity in terms of species density were the Bidassoa, Belon and Trieux with more than 40 taxa whereas lower number of taxa (< 30) were recorded within the Gironde, Loire, Somme and Seine estuaries. The Aiguillon, Charente and Orne displayed intermediate (33 to 40 taxa) levels of γ-diversity (Fig. 3). Correlation between γ-diversity level and environmental variables studied at the site-scale showed that there was significant negative correlations between level o f γ-diversity and both SPM-level (R_S= -0.70, $p<0.05$) and total surface of intertidal area (R_S= -0.86, $p<0.05$).

At the scale of one station, the mean α-diversity was significantly different among sites (K-W test, $p<0.001$). Pairwise tests showed that there was a tendency of decreasing species density from the species-dense stations of the Bidassoa, Trieux and Belon estuaries toward the species-poor Seine, Gironde and Loire estuaries. Other sites displayed intermediate levels of species-density. The level of α-diversity among sites was significantly positively correlated to both relative proportion of intertidal area and ratio between Tidal prism volume and Freshwater discharge volume (R_S > 0.78 and p-values < 0.05). A negative correlation was observed with both SPM-level and total intertidal area (R_S <-0.76 and p-values < 0.05).

In terms of β-diversity, Whittaker's βw values were significantly lower in the bay of Somme compared to the Belon and Orne estuaries. The values of average βw were only positively correlated to the TP:R ratio (R_S= -0.63, $p<0.05$).

Relationship among the three components of diversity at the scale of sites showed that there was a general linear relation between the α-component of diversity and γ-diversity indicating that variations in average α-diversity explained more than 65% of the variations in γdiversity among sites (R^2=0.653) (Fig 4). In addition, discrepancy from the general model indicated higher contribution of (relative) β-diversity to γ-diversity in the Belon, Trieux and Orne estuaries and low β-diversity in the Loire, Somme, Aiguillon-Sèvre niortaise and Bidassoa estuarine systems (Fig 4).

Pattern in trophic organization

Partitioning γ-diversity among trophic groups, there was first a significant linear relationship between total number of species (estimated on 2 m²) and number of taxa for every trophic groups (R > 0.79, all p-values <0.05). Nevertheless, considering the number of interface-feeding and subsurface deposit-feeders taxa, there was a negative relationship with SPM-level (Fig 5). A similar, but probably non-linear tendency was observed for both suspension-feeding and carnivorous/omnivorous specie (Fig. 5). Finally subsurface-deposit feeder diversity was alos negatively correlated to average sediments grain-size (in Φ unit), indicating that the diversity of these organisms was lower in mud than in sandy sediments (R_S=-0.65).

Within-sites patterns

Benthic macrofauna assemblages and associated environmental factors

The observed patterns in benthic assemblages within each estuary have been summarized in Fig 6.

On the basis of fauna similarities among stations, SIMPROF procedure identified 33 homogeneous clusters among which only 22 included more than two stations (Fig 7). At a similarity level of 10%, four main station groups were observed. The largest group (group III) gathered the largest number of stations within each site with the exception of the Belon and the Somme systems. This group of station was mainly characterized by *Hediste diversicolor*, *Nephtys hombergii*, oligochaetes, *Scrobicularia plana*, *Macoma balthica* and *Hydrobia ulvae* (Table 3). Within this group, sediments ranged from pure muds to slightly muddy sands. These stations were either located throughout the Aiguillon, Orne and Somme estuarine systems or occupied most of the Gironde, Loire, Charente and Seine estuaries except the very lower (Gironde) or upper (Loire, Charente and Seine) parts of these system (Fig 6 and 7). Most stations within the Belon estuary gathered into group IV while only two upstream stations gathered in the largest group III (Fig 6 and 7). Station group IV mostly consisted in stations from throughout the Belon estuary where sediments ranged from muddy coarse sediments to sandy muds with less than 40% fine particles, on average (Fig 6 and 7). These stations were mainly characterized by *Nephtys hombergii* and *N. hystricis*, cirratulids, *Owenia fusiformis*, *Spio* spp., oligochaetes and *Tellina tenuis* (Table 3). Within the bay of Somme, half of the stations gathered in group III and the other half in a separate group (group II) (Fig 7). This group gathered stations consisting in clean sands or coarse sediments with very little mud content (< 4%) located in the lower part of the Gironde estuary and upper parts of the Orne and Seine estuaries as well as throughout the bay of Somme. This group was characterized by amphipods of the family Bathyporeiidae and Haustoriidae as together with *Eurydice* spp. (Table 3). Within the Orne estuary, four stations were isolated in group V which was restricted to the lower part of this system on the same kind of clean sand and coarse sediments than in the previous group (group II) (Fig 6 and 7). This group was characterized by the presence of mussel beds (*Mytilus edulis*), *Scolelepis squamata* and *Ophelia rathkei* (Table 3). Station group I only gathered two stations from the Charente and Loire estuaries that were located on muds from the uppermost parts of these sites. In these stations the benthic fauna almost only consisted in *Boccardiella* sp. (Table 3).

At 20% similarity level, additional clusters were identified within group III. These clusters mainly isolated stations within the Bidassoa estuary (group G), the Gironde, Loire, Charente estuaries (group E), the Seine estuary (group F) and the Trieux estuary (group D) while most stations remained within the largest group H. Stations from the Bidassoa (G *vs* H) and Trieux (D *vs* H) estuaries were separated according to both their position within the estuary and different mud content which was lower in the lower part of this system (Table 4, Fig 6 and 7). Within the Gironde, Loire, Charente and Seine estuaries, the separation was correlated to the position of stations within the estuary (E *vs* H, Table 4) as well as difference in mud content in the Seine estuary (F *vs* H, Table 4). Within the Belon estuary, stations from group IV were split into two different groups (I *vs* J) according to slight differences of sediment types (Table 4). Species characterizing each group are indicated in Table 3.

At 30% similarity level, different clusters were identified within group H. However, only stations within the Charente, Loire, Orne and Gironde were separated at this level of similarity. In other sites, all stations remained in the same group. The separation of stations into different groups appeared to be correlated to their position within the Charente and Gironde estuaries (III-H12 *vs* III-H8) or to both mud content and position within the Loire (III-H11 *vs* III-H9) or mostly in relation to mud content within the Orne estuary (H12 *vs* H10 *vs* H9) (Table 4, Fig 6 and 7).

According to DISTLM results, variations in sediments characteristics explained a larger part of the variation in benthic fauna than distance to ocean within the Aiguillon, Orne and Somme estuarine systems (Table 4). It explained a similar part of variation than distance to ocean within the Belon, Trieux and Gironde estuaries however both factor were correlated (negatively) in the latter system (Table 4). Distance to ocean, which represented a proxy of salinity variations, explained a larger part of fauna variations within the Bidassoa, Charente, Loire, Seine estuaries (Table 4). In these systems, this factor explained at least more than 30% of fauna variations while grain-size only explained more than 20% of variations within the Seine, Trieux and Orne estuaries (Table 4). Finally, combination of both factors increased the explained fauna variations of more than 10% within the Gironde, Orne, Seine, Somme and Trieux estuaries (Table 4). As a conclusion, distance to ocean appeared as the only main explanatory variable within the Bidassoa, Charente and Loire estuaries. Variations in sediments appeared as the only main explanatory variable within the Aiguillon and Somme and both factors appeared as additive within the Gironde, Orne, Seine and Trieux estuaries. Within assemblage III, variations in fauna were only explained by station position in the Bidassoa, Charente, Loire and Gironde and mainly explained by this factor, in addition to sediments, within the Orne, Seine and Trieux (Table 4). Within group H, station position in the salinity gradient also appeared as the main explanatory variable in the Gironde and Charente and in addition with sediments within the Loire and Orne estuaries (Table 4).

Within-sites pattern of species diversity

There were significant positive (rank-) correlations between species density (number of species per station) and proximity to ocean within the Bidassoa, Belon, Charente, Seine, Loire, Orne estuaries (Fig. 8). This pattern was also significant (Spearman R= 0.71) within the Gironde estuary when excluding the most downstream stations that corresponded to species-poor exposed mobile sands (Fig. 8). This pattern was significant neither within the Aiguillon –Sèvre niortaise and Somme systems nor within the Trieux estuary (Fig. 8). In addition to this pattern, a lower levels of α-diversity were observed in both the clean sands assemblages (II-C, II-B and V-K) and in the upstream muddy assemblages III-E and I-A compared to assemblages IV-I, IV-J, III-G (K-W and pairwise tests, p-values < 0.05). Assemblage III-H displayed an average level of diversity mainly as a function of its position within each estuary (Fig. 8).

Discussion

Classification of estuarine systems and relation with environmental factors

When considered at the scale of the whole site, each of the estuarine system studied displayed a significantly different benthic fauna. There was however greater similarity of fauna among, on the one hand, estuarine systems characterized by high suspended particulate matter concentrations level (SPM) associated to strong and less variable influence of freshwater discharge and low slope from source to sea. According to Fairbridge classification (1980), these estuaries corresponds to coastal plain estuaries which long tributaries mainly flow through low plains and carry fine sediments forming extensive mudflats (Day et al. 1989, Perillo 1995). In our study these coastal plain estuaries included the Gironde, Charente, Aiguillon-Sèvre niortaise, Loire and Seine estuarine systems. On the other hand, estuarine systems characterized by low SPM, highly variable and generally lower influence of freshwater inputs and high slope, displayed a different benthic fauna. These estuaries can be considered as rias (Fairbridge 1980, McLusky and Elliot 2004) where the main tributary is short and mainly flows through granite substrates (Pyrenees mountains (Bidassoa) or Armorican massif (Belon and Trieux)) (Perillo 1995). Within the coastal plain estuaries, there was however variations according to lower degree of isolation from the sea and lower relative freshwater influence and lower SPM concentrations (Somme) or high slope combined to moderate level of SPM (Orne). In addition, none of the estuarine classifications used here, namely the transitional water bodies classification from the Water Framework Directive (WFD), the classification from Nicolas et al (2010) nor the classification proposed by Galvan et al (2010) were related to observed pattern of benthic fauna among the estuaries studied here. Despite its suitability to reflect the main patterns of benthic fauna among estuary types, the classification of Galvan et al (2010) failed at correctly classifying the estuarine systems studied. The latter study was indeed based on estuaries from the cantabrian coast only. These estuaries, like those of the Basque country, are relatively small estuarine systems with small catchment areas and which sources are located at high altitudes in the nearby cantabric mountains (Valencia et al 2004, Galvan et al 2010 and references therein), as a consequence estuaries of the coastal plain-type were not included in this classification. In accordance with the conclusions of Galvan et al (2010) we propose a modification of its classification system by including slope and SPM level in order to identify coastal plain estuaries.

However proposing precise thresholds values requires additional comparisons including a larger set of estuaries at the European scale which is beyond the scope of this study, our results suggest that estuaries where SPM concentrations levels are higher than ca. 50 $mg.L^{-1}$ should be considered for inclusion in the ‘coastal plain estuary’ type. Such a threshold-value is not only suggested by our empirical results but this level was also suggested by different authors dealing with limiting factors for water column primary production in coastal areas and estuaries. Theoretically, this level of SPM would indeed correspond to an euphotic depth (Z_{eu}) of less than 2 m (Cloern 1987, Irrigoien and Castel 1997). In shallow estuaries, with a maximum depth of ca. 10 m and assuming that water column is well mixed this would correspond to a maximum Z_m:Z_{eu} ratio of less than 5-6 above which no net phytoplankton production has been observed in estuaries (e.g. Cole and Cloern 1984, Grobelaar 1985, Irrigoien and Castel 1997). Considering its consequence on estuary primary production and, thus, benthic organisms, this rough threshold-value has to be taken into account for an estuarine classification. In addition to its consequence on primary production at the ecosystem-scale, high suspended particulate matter concentrations have a detrimental effect on suspension-feeding organisms especially on bivalves which filtering and respiration apparatus is clogged by too high SPM levels despite the ability of bivalves to cope with increasing SPM level by increasing pseudofaeces production and/or filtration rate, in the long-term the energetic cost and consequences on the scope-for-growth and reproduction (and consequently the occurrence of a species) of these organisms might be too low at this level of SPM concentration (Dame 1996 and references therein). This is suggested by our observation considering the sharp decrease of suspension-feeding species number as a function of increasing SPM-levels. However it is clear that this relation is only based on correlation and on a relatively small number of cases. Moreover confounding factors might occur and complicate this relation such as the effect of salinity on diversity and among-sites differences in the SPM composition (Abril et al 2002) with possible consequence on its nutritional value for organisms (e.g. Bayne and Iglesias 1993, Navarro et al 1998). The influence of high SPM-level and the associated hydromorphologic characteristics has already been evidenced by Warwick et al (1991) through the comparison of the intertidal benthic fauna of six estuaries from southern UK. The latter study evidenced the originality of the benthic fauna of the hypertidal and highly turbid Severn estuary compared to the other five estuaries. In the same way, Ysaebaert et al. (1998) reported few differences in the benthic macrofauna between the Ems-Dollard and Westerschelde estuaries which are both characterized by moderate to high levels of SPM. In addition, Meire et al. (1991) evidenced strong differences of benthic fauna between the Westerschelde and Oosterschelde in relation to low SPM concentrations in the latter ecosystem as a consequence of human-induced modifications of hydrology. High slopes characterized ria-type estuaries such as the Bidassoa, Trieux and Belon estuaries. However, considering a classification methodology, our results suggested that slope should be subordinate to SPM concentration levels. Indeed, the Gironde estuary displayed a high slope (4.5‰) whereas its benthic fauna was typical of the coastal plain estuary type. This observation suggests the preponderant effect of SPM concentrations on benthic fauna.

There were strong differences in the relative influence of river discharge among the estuaries studied here. For instance, the Bidassoa estuary displayed the highest relative river discharge whereas both the Trieux and Belon ranked among the less river-influenced systems. Curiously, the Bidassoa estuary displayed the highest level of number of species compared to all other estuaries studied. This observation is in complete contradiction with our expectation of lower diversity in more brackish estuaries. However, we used yearly-averaged values of river discharge. This estuary is however characterized by the highest yearly variations of river discharge. In addition, this estuary is known to undergo very strong floods suggesting that low salinity conditions may only occur during a very restricted amount of time which is also a characteristic of the other estuaries of the Basque country (Valencia et al. 2004). During our low tide-sampling, water salinity along the channel indeed varied between 33 and 24 in the downstream sector and between 23 and 2 with a median value of 9 in the upstream sector. Hence, the salinity level was not particularly low in this estuary outside of the flood periods. These observations suggest that the temporal pattern of river input should be included in establishing a typology.

Benthic assemblages in estuarine systems

Although a benthos-constrained classification of estuarine systems would be helpful to compare transitional water bodies, for instance, within the frame of the WFD, our results suggest that comparison among estuaries may be conducted at the smaller scale of benthic habitat (*i.e.* assemblages).

Our study indeed showed that all these estuaries shared one common assemblage that was spatially more or less well represented according to sites. This assemblage (assemblage III-H) occurred in all estuarine systems studied here. It displayed a typical set of taxa that have been reported in the literature as characterizing the "*Macoma (balthica)* community" (Petersen 1913, 1918; Thorson 1957) with variations in composition and diversity according to biogeographical patterns and environmental conditions. For instance, a "reduced" *Macoma balthica* community, where *M. balthica* is absent, was observed in the inner part of the Bidassoa estuary. This species indeed reaches its southern limit of distribution south of the Gironde estuary (Bachelet 1980; Hummel et al. 2000) and is therefore absent from the Bidassoa estuary (Garmendia et al. 2003) as well as from the Spanish and Portuguese estuarine systems (Borja et al. 2004). In our study sites, the bivalve *Scrobicularia plana* and *Cerastoderma edule* were the most common bivalve species and occurred in all systems. This was consistent with the proposal of a *Scrobicularia plana – Cerastoderma edule* community by Borja et al. (2004) for the southern part of the North-Western Europe such as the Basque country which biogeographically includes the Bidassoa estuary. This community/assemblage was spatially well represented in all systems except in the less river-influenced system (Belon estuary) where SPM concentration was the lowest. As well, its spatial representation was lower in hypertidal systems where sands were well represented such as in the bay of Somme, Orne and Seine estuaries.

This assemblage displayed different aspects ('*facies*') according to both salinity level and sediment types. More precisely, the most diverse aspect of this assemblage occurred on mud to muddy sands in the lowest part of coastal plain estuaries except when this area consisted in sand substrates such as in the Orne and Seine estuaries. Going upstream, where salinity level decreases (and its variability increases) impoverished aspects of this assemblage occurred on all type of sediments (assemblages III-H 9, III-H 8 or III-H 11). These impoverished '*facies*' are characterized by a reduced occurrence of molluscs. Further upstream, molluscs completely disappeared, as well as the occurrence of polychaetes and the assemblage is characterized by *Corophium volutator* and oligochaetes (assemblage III-E). In two estuarine systems where stations were submitted to human impact, such as in the Loire (dredging in relation to the functioning of the Cordemais powerplant) or in the Charente where these stations were located very close to one of the largest constructed wetland for water treatment in Europe (Moderan et al 2010) the benthic assemblage consisted either almost only in *Boccardiella* sp. or stations were devoid of macrofauna (using a 1-mm mesh sieve). Within ria systems (Belon, Trieux and Bidassoa estuaries), the lower part of the estuary consists in muddy sands or sands where species-rich assemblages occurs. These species-rich assemblages are either characterized by a mixture of a venerid bivalves-rich community ("*Venus* community" (Thorson 1957)) (assemblage II-G) with species from the '*Scrobicularia plana – Cerastoderma edule*' community or by a '*Tellina tenuis*' community (Borja et al. 2004) (assemblages I and J from the Belon estuary) or a mixture between the latter community and the '*S. plana*-*C. edule*' community (assemblages III-D and IV-J from the Trieux estuary). When going upstream, another 'facies' of the '*S. plana – C. edule*' community occurred (assemblage III-H 10). This latter assemblage is also characterized by a reduction of the occurrence of molluscs. The observed pattern of macrofauna are in accordance with previous investigations on the pattern of benthic fauna in the Loire (Marchand 1993), Gironde (Bachelet et al. 1980), Bidassoa (Garmendia et al. 2003), Seine (Ducrotoy and Dauvin 2008) and Somme (Ducrotoy 1987) systems. In addition, there was a very good match with the existing classifications of marine biotopes (Dauvin et al. 2008 and references therein) and more spartcularly with the one proposed in Britain and Ireland by Connor et al. (2004) which has been extended to the European scale and included in the EUNIS classification managed by the European Union Environment Agency (http://eunis.eea.europa.eu/). The occurrence and relatively large extent of the venerid/*Tellina tenuis* assemblage in the lower part of estuaries seems to be a distinguishing feature of ria-like estuaries. Such a pattern is indeed described for rias of the Basque country (Borja et al. 2004, Borja et al. 2006, Junoy and Vieitez 1990) and Galicia (e.g. Ria de Aldan (Lourido et al. 2010)) but were not reported in other coastal plain estuaries such as the Westerschelde (Ysebaert et al. 2003) or the Oosterschelde (Meire et al. 1991). In their comparative study of southern England intertidal estuarine systems, Warwick et al. (1991) reported the presence of *Tellina tenuis* only in the lower part of the Exe estuary. Compared to the other estuaries from this latter study, this estuary is characterized by the shortest river (8.4 km length) combined to the high source elevation (440 m) resulting in the highest slope (5.2‰) among the studied systems. This community might lack or be highly reduced in coastal plain-type estuaries as a consequence of both salinity level and higher SPM concentrations and associated inputs of fine particles in these systems which may represent adverse conditions for these suspension-feeders-rich assemblages and result in sediments consisting in mud. In contrast, considering only the meso-to polyhaline part of estuaries, it appears that coastal plain estuaries with moderate to high SPM-level usually display mostly two main benthic intertidal communities: the '*S. plana/M. balthica*-*C. edule*' community located on most part of the estuary and a mobile sand community characterized by *Bathyporeia* spp. and haustorids amphipods ('*Pontocrates arenarius* –

Eurydice pulchra' community from Borja et al. 2004) restricted to sandy beaches or banks that are exposed to wave action and/or tidal currents. This was observed in the Somme, Gironde, Orne and Seine estuarine systems but not in the Charente nor Aiguillon-Sèvre niortaise systems because the mouths of both systems are sheltered from wave action by islands: Oléron and Ré islands respectively. A similar pattern has been described in the Westerschelde and Ems estuaries (Ysebaert et al. 1998).

Relation with benthic fauna diversity

The level of γ-diversity reported in this study are in the range of values reported on other estuarine systems or habitats in Northern Europe estuaries. The number of taxa scaled to the sampled area showed that the number of species was low compared to, for instance, the intertidal area of a coastal embayment such as the Arcachon bay. In the latter ecosystem, the freshwater influence is very restricted (Plus et al 2009) with, for instance, no clear pattern of species number decrease (pers. obs.). Compared to other estuarine intertidal areas, our estimates of γ-diversity of benthic fauna showed that the γ-diversity of coastal plain estuaries was usually very low with good agreement between our data on the Loire, Gironde, Seine, Somme and Aiguillon and other coastal plain estuaries such as the Westerschelde or the Severn estuaries (Fig 3). On the other hand, the rias displayed higher γ-diversity levels than coastal plain estuaries with similar patterns observed in the habitats of the Ria de Foz (Junoy and Vieitez 1990), estuaries from the Basque country such as Gernika and Plentzia estuaries (Garcia-Arberras and Rallo, 2002) or the Exe estuary in UK (Warwick et al 1991). However, some estuaries did not show the expected pattern, for instance it was not the case for the basque estuary La Arena described by Garcia-Arnerras and Rallo (2002), which displayed one of the lowest levels of γ-diversity. In addition, the Humber estuary as described by Fujii (2007) displayed rather high γ-diversity but is classified as a typical coastal-plain estuary by McLusky and Elliott 2004. Unraveling the underlying environmental factors responsible for these discrepancies would require a more precise study of both the fauna and hydromorphology of all these systems. Finally, the impact of human modification of the hydromorphology as well as pollution would have to be taken into account to explain the full pattern.

Nevertheless at the scale of our study, the observed pattern of γ-diversity was mainly explained by difference in α-diversity among estuaries. The sites which were dominated by species-poor assemblages displayed the lowest γ-diversity. This was exemplified by the Loire estuary which benthic fauna only consisted in different facies of the "*S. plana*-*C. edule*" community associated to high SPM level and strong freshwater influence. Little higher level of γ-diversity was reached in the Somme, Gironde and Seine estuarine systems which displayed only two species-poor assemblages related to the '*S. plana*-*C. edule*' community and the mobile sand assemblage. The Aiguillon-Sèvre niortaise and Charente systems displayed higher level of diversity in association to stronger relative influence of the tidal prism and/or lower influence of river discharge but only displayed assemblages related to the '*S.*

plana-C. edule' community. The Orne estuary reached higher γ-diversity in association to the diversity of benthic assemblages occurring in this system, in accordance to higher level of β-diversity. Finally, the ria systems displayed the higher level of diversity due to the presence and spatial extent of species-rich communities such as the venerid and the "*T. tenuis*" communities in association with the "S. plana-C. edule" community. These communities occurred probably result from the combination of low inputs of fine particles, low SPM concentrations and lower influence of river discharge. As a consequence, a pattern of decreasing γ-diversity with increased total surface of intertidal areas is observed. This pattern is challenging since the relation between number of species and area is one of the fundamental patterns observed in macroecology (Gaston and Blackburn 2000). Moreover, compared to the patterns of fish diversity reported by Nicolas et al. (2010) where it was showed that the number of fish species recorded in estuaries given a comparable sampling effort actually increases with the size of the estuary. Our observations, that should be considered as preliminary since the investigated area is still very limited (for instance, Ysebaert and Herman (2002) reported 106 species in the Westerschelde when including a huge sampling effort (> 30 m²) including both spatial (20 samples × 30 stations) and temporal a (16-year survey) dimensions). Despite a probably insufficient sampling effort, the almost asymptotic shape of the species-accumulation curves however strongly suggest that the recorded number of intertidal macrobenthic species in the Seine, Loire, Gironde, Somme and Aiguillon-Sèvre niortiase is extremely limited and much lower than in other systems. The relevance of this observed pattern might be put in question since it only concerns small macrofauna from soft sediments and do not include subtidal areas nor oligohaline and tidal freshwater areas. Possible explanation might include the historical heavy impact of human activities on estuarine systems or the homogeneity of benthic fauna in the largest intertidal areas which are dominated, in our study, by typical estuarine benthic fauna which very low diversity is one of the main feature in accordance to Elliot and Quintino's 'estuarine paradox' (2007).

Conclusions

This study of ten estuarine systems showed consistent patterns in the organization of benthic macrofauna in permanently open estuaries (Barros et al. 2012). As discussed in the literature, the low level of diversity, the occurring benthic communities and spatial patterns in both assemblage succession and diversity along the estuarine ecotone are classical for these types of estuaries (Attril and Rundle 2002, Elliot and Quintino 2007, Whitfield et al. 2012). In the frame of the WFD our results suggest that estuarine water bodies might be compared providing that the comparison is operated at the level of similar habitats within estuaries. More particularly, our results showed that such a comparison should be based by comparing among biotopes where the "*S. plana*- *C.edule*" occurs. IN this context we suggest that the definition of these comparable biotopes could be based on the existing EUNIS classification. Such an approach implies to define reference conditions at the scale of each biotope at the very least to the level-4 of this classification.

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Table 1: Main hydrological, morphogical and sedimentary characteristics of the ten studied sites. N: number of sampled stations, A: total area (in km²) and intertidal area (in brackets), TH: average tidal height (in m), Rd: average yearly river discharge for the October 2007-October 2008 (when available) ($m^3.s^{-1}$), int: classes of relative intertidal area according to Nicolas *et al.* (2011) (1: 0-10% intertidal; 2: 20-40%; 3: 40-60%; 4: 60-80%; 5: 80-100%), TP:V: ratio between estimated Tidal Prism and estimated estuarine water volume at average high tide, R:V: ratio between the estimated volume of river inputs during a tidal cycle (12H) and estimated estuarine water volume at average high tide, CI : closure index (Hume *et al*., 2007) (low CI values correspond to more closed system while higher CI values correspond to more open systems). SED: Median value of average sediments grain-size (in Φ unit) for intertidal and subtidal area (in brackets), vSED: coefficient of variation of average sediment grain size (in %), slope: average slope of the river-estuary system (in ‰), SPM: level of suspended particulate matter concentrations in water (0: 0-5 $mg.L^{-1}$;1: 5-10 $mg.L^{-1}$;2: 10-50 $mg.L^{-1}$; 3: 50-100 $mg.L^{-1}$;4: 100-500 mg.L-1;5: 500-1000 mg.L-1; 6: > 1000 $mg.L^{-1}$). Sites are AIG: Aiguillon-Sèvre niortaise, BEL: Belon, BID: Bidassoa, CHA: Charente, GIR: Gironde, LOI: Loire, ORN : Orne, SEI : Seine, SOM : Somme, TRI : Trieux.

SITES	N	A	TH	Rd	int	TP:V	R:V	CI	SED	vSED	slope	SPM
AIG	20	56.6 (50.9)	5.7	20.3	5	0.98	0.003	0.1	6	0	0.9	3
BEL	19	2.8 (1.7)	4.5	1.5	3	0.77	0.006	0.04	3.9	20	3.4	1
BID	10	2.8 (2.2)	4	18	4	0.87	0.111	0.01	2.4	40	13	2
CHA	10	25.1 (15.1)	5.7	62.8	3	0.75	0.027	0.05	6	20	0.8	5
GIR	20	530 (53)	5.1	960	1	0.47	0.028	0.06	5.7	20	4.5	6
LOI	20	239 (96)	5.3	939	2	0.63	0.051	0.06	5.3	50	1.4	6
ORN	20	7.2 (4.3)	7	27.5	3	0.81	0.03	0.06	3	50	2.3	4
SEI	20	198 (20)	7.5	435	1	0.63	0.022	0.04	3.1	50	0.6	5
SOM	20	40.5 (36)	9	38	5	0.99	0.005	0.12	3	10	0.3	2
TRI	10	8.4 (6.7)	9.3	8.7	4	0.86	0.005	0.02	3.2	30	3.5	2

Table 2: Spearman rank correlation coefficient (R_s) among variables describing the hydromorphological features of the estuarine systems (Higher values of R_s (> 0.65) are indicated in bold)). TH: average tidal height (m, coeff 70), Rd: average river discharge ($m^3.s^{-1}$), A: area (km^2), int: proportion of intertidal area, TP:V: ratio between tidal prism and estuarine volume at high tide, R:V: ratio between volume of freshwater discharged during one tidal cycle and estuarine volume at high tide, TP:R: ratio between tidal prism and volume of freshwater discharged into the esturine system during one tidal cycle (12H), EE: estuary length, SC: complexity index, CI: closure index, SED: average grain size (in Phi-unit), vSED: variability of sediment grain-size, slope: average slope of the main rivers discharging into the estuary (ratio between river length and source elevation), SPM: suspended particulate matter level.

	TH	Rd	A	int	TP:V	R:V	TP:R	EE	SC	CI	SED	vSED	slope
TH	0												
Rd	-0.24												
A	-0.2	**0.93**											
int	0.17	**-0.76**	**-0.72**										
TP:V	0.32	**-0.85**	**-0.83**	**0.95**									
R:V	-0.5	-0.08	-0.16	0.21	0.1								
TP:R	0.41	-0.43	-0.31	**0.66**	**0.68**	-0.47							
EE	-0.23	0.49	0.39	**-0.68**	**-0.71**	0.35	**-0.95**						
SC	0.26	-0.12	-0.05	0.57	0.52	-0.27	**0.73**	**-0.71**					
CI	0.28	0.02	0.09	0.29	0.32	-0.52	0.63	-0.6	**0.87**				
SED	-0.32	0.51	0.51	-0.25	-0.38	-0.35	0.13	-0.02	0.05	0.28			
vSED	-0.02	0.3	0.07	-0.51	-0.41	0.38	-0.77	**0.73**	**-0.65**	-0.56	-0.35		
slope	-0.5	-0.09	-0.07	0.2	0.03	0.49	-0.4	0.32	-0.28	-0.62	-0.36	0.21	
SPM	-0.13	**0.76**	0.7	**-0.68**	**-0.68**	-0.15	-0.42	0.52	-0.25	0.11	0.62	0.36	-0.3

Table 3: List of the main taxa characterizing each assemblage according to the different levels of the hierarchical classification. The level of occurrence of each taxa within each assemblage (numbered from 1 to 15) is indicated by * (***: taxa occurring in more than two third of stations, **: taxa occurring in more than one third of stations, * taxa occurring in more than one than one fifth of stations, -: taxa occurring in less than one fifth of stations). Taxa identified as contributing together up to 70% to the within-group similarity are indicated in black, taxa which cumulative contribution to group similarity was lower than 70% but higher than 90% are indicated in grey. Taxa contributing together to more than 70% of within group similarity at a similarity level of 10% (groups I through II) are underlined. These taxa were identified through the SIMPER procedure.

	I	II		III									IV		V
	A	B	C	D	E	F	G	H					I	J	K
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Taxa															
Annelida															
Clitellata															
Oligochaeta			-	**	***		**		-	***	-	**	***	***	**
Polychaeta															
Boccardiella spp.	***				-				-	-	-				
Nephtys hombergii			-	***		***	***	-	**	**	-	**	-	***	-
Hediste diversicolor			**	**	**	-	*	***	***	***	**	***		-	
Heteromastus filiformis		*		*		-	***	-	-	**	***	**			
Streblospio shrubsoli				*	**		**	-		***	***	-	-	**	
Cirratulidae				***					-	**	-	-	-	***	
Nephtys hystricis												-	***	**	
Owenia fusiformis													***		
Spio spp.						-				-		-	**	***	
Scolelepis (Scolelepis) squamata			**			-						-			***
Eteone spp.			**	*					**	**	**	-	**		**
Capitella spp.		*	**	*		-	***		-	**		-	-	**	-
Melinna palmata				***						-				-	
Ampharete sp.				**									-		
Glycera convoluta				*			***			-				-	
Pseudopolydora							***			-					

paucibranchiata														
Nephtys cirrosa	**				**	**		-				-	**	
Alkmaria romijni									**					
Pygospio elegans		-	*	-				-	-	-	**			
Phyllodoce spp.			**		-						-	-		-
Pseudopolydora antennata			**		-				-		-			
Paradoneis spp.						***			-					
Phylo foetida						***			-					
Notomastus latericeus			*						-			**		-
Capitomastus minima					-			-	-			-	**	
Arenicola marina					-				-		-		**	
Ophelia rathkei														**
Crustacea														
Amphipoda														
Haustorius arenarius	***	**			-			-						
Bathyporeia sarsi	**	**			-						-			
Bathyporeia pilosa	*	***						-			-			
Bathyporeia elegans		***									-			
Corophium volutator	*	-		***			***	***		-	-			
Corophium acherusicum						***								
Decapoda														
Crangon crangon		-		-	**			-	-		-		-	**
Isopoda														
Cyathura carinata				**			-		***	***	-			
Eurydice spp.	**	**			-					-	-			
Tanaidacea														
Apseudes latreillii												**		
Mollusca														
Bivalvia														
Macoma balthica	*	**		-	***		-	***	-	**	***			
Scrobicularia plana				-		**	***	***	***	***	***		-	
Cerastoderma edule		-	**		***	*		**	-	**	***	-		
Tellina tenuis					-	***					-	***	-	
Loripes lacteus						***						**		

Ruditapes philippinarum				*		-	***			-	-	-	-		
Abra tenuis				*			***			**	-	**			
Parvicardium							***			-					
Mytilus edulis						-			-	-	-	-			***
Lucinella divaricata													**		
Paphia aurea							***								
Thracia spp.				*						-		-	**		
Gastropoda															
Hydrobia ulvae			**			<u>-</u>	<u>**</u>	<u>***</u>		<u>**</u>	<u>**</u>	<u>***</u>			
Nemertina			-		-	-	***		-	-		-	**	-	-

Table 4: Percentage of variation in Bray-Curtis similarity explained by variations in distance to ocean (% downstream), variations in grain-size and combination of both variables (Combined) as estimated by the DISTLM procedure. The level of correlation between both variables is given (corr.). Significant contributions ($p < 0.05$) are indicated by * and in bold. For combinations, bold characters indicate at least 10% increase of explained variation by combining both variables instead of one, when both individual variables explained a significant part of variation.

	% downstream	Grain-size	Combined	correlation
Within sites				
Aiguillon	8% ^{ns}	**15% ***	19% ^{ns}	-0.35
Belon	**15%** *	**10% ***	19% ^{ns}	-0.47
Bidassoa	**56% ***	13% *ns*	56%*	-0.45
Charente	**42% ***	9% *ns*	52%*	-0.08
Gironde	**17% ***	**17% ***	**35%*(+18)**	**-0.64**
Loire	**34% ***	10% ^{ns}	43% ^{ns}	-0.17
Orne	**12% ***	**21% ***	**31%*(+10)**	-0.20
Seine	**41% ***	**23% ***	**56%*(+15)**	-0.37
Somme	10% ^{ns}	**14% ***	25%*	-0.15
Trieux	**30% ***	**25% ***	**48%*(+18)**	-0.36
Within Assemblage III				
Bidassoa (H & G)	**56%***	13%^{ns}	57%*	-0.45
Charente (H & E)	**44%***	< 1%^{ns}	52%*	+0.07
Gironde (H & E)	**23%***	< 1%^{ns}	24%*	-0.47
Loire (H & E)	**37%***	12%^{ns}	49%*	-0.11
Orne (H, F, E)	**20%***	**18%***	**37%*(+17)**	-0.09
Seine (H, F, E)	**46%***	**25%***	**60%*(+14)**	-0.40
Trieux (H & D)	**38%***	**12%***	**54%*(+16)**	-0.41
Within Assemblage H				
Charente (H12 & H8)	**57%***	13%^{ns}	65%*	+0.28
Gironde (H12, H11, H8)	**27%***	< 1%^{ns}	30%*	-0.48
Loire (H11 & H9)	**20%***	**17%***	**37%*(+17)**	-0.09
Orne (H12, H10, H9)	**23%***	**16%***	**39%*(+16)**	+0.09

Figures captions

Fig 1: Location of the ten study sites along the French coast.

Fig 2: (a.) Principal coordinates ordinations of site centroids according to their fauna composition. Contours indicate site centroids gathering together at distances of 50 (full black line) and 45 (grey dotted line) according to cluster analysis (obtained by group average method). Comparisons with existing classification in the scope of the WFD are provided including (b.) fish-based classification developed by Nicolas et al. (2010) (classification mainly related to estuarine-size with estuaries classified from the largest (A) to smallest (G)); (c.) WFD classification of water bodies including T01: polyhaline small estuary with large intertidal area and average turbidity level, T03: small estuary with small intertidal area and low turbidity level, T05: small to medium-size macrotidal estuary with high salinity and average river discharge level, T07: large estuary with mean to high salinity level and high level of river discharge,T08: small estuary with small intertidal area and high to medium turbidity level,T09: small estuary with large intertidal area, low turbidity and high level of salinity, (d.) benthos-based classification of transitional water bodies proposed by Galvan et al. (2010) (ITE: Intertidal Tidal Elongated water body, ITR: Intertidal Tidal Rounded water body).

Fig 3: Species-accumulation curves drawn for each study site (grey lines) showing the number of taxa accumulating over a cumulated sampled area (in m²). These curves are compared to available data of γ-diversity from other intertidal estuarine and coastal areas along the European North Sea – Atlantic coasts (ARC: Arcachon bay (Blanchet et al 2004 and unpublished material); HUM: Humber (Fujii 2007); GER: Gernika, LAR: La Arena, PLE: Plentzia (Garcia-Arberros and Rallo 2002); SCV: Scorff and Blavet estuaries (Le Bris, 1988); OOS: Oosterschelde, WES1: Westerschelde (Meire et al. 1991); AVE: ria de Aveiro (Nunes et al. 2008); EXE: Exe, PLY: Plym, POO: Poole Harbour, SEV: Severn, SHO: Southampton Water, TAM: Tamar (Warwick et al., 1991); WES2: Westerschelde (Ysebaert et al. 1993);WES3: Westerschelde (Ysebaert et al. 2003); TAG1-6: Tagus (Rodrigues et al, 2008).

Fig 4: Relation between estimated γ-diversity (estimated number of taxa over 2 m²) of each trophic group and suspended particulate matter concentration levels (see Tab 1 for the correspondence of SPM-level).

Fig 5: Relation between the α- and γ- components of diversity at the site-scale estimated respectively by the average number of taxa per station (0.2 m²) and estimated total number of taxa on 2 m² (through permutation and species-accumulation). The linear relation between both variables was obtained by linear regression and is indicated together with the R^2 value. Discrepancy between observed level of γ-diversity and α-diversity from the model implies β-diversity effect. Sites-points located under the curve indicate relative lower-than-average level of β-diversity whereas sites-points located above the curve indicate relative higher-than-average level of β-diversity according to a model of multiplicative effect of β-diversity where $\gamma=\beta\times\alpha$.

Fig 6: Schematic representation of the succession of benthic assemblages within the studied estuaries as a function of sediments type on the vertical axis (CS: coarse sediments, S: sands, mS: muddy sands, sM: sandy muds and M: muds (based on the modified Wentworth classification)) and position in the estuarine salinity gradient (horizontal axis).

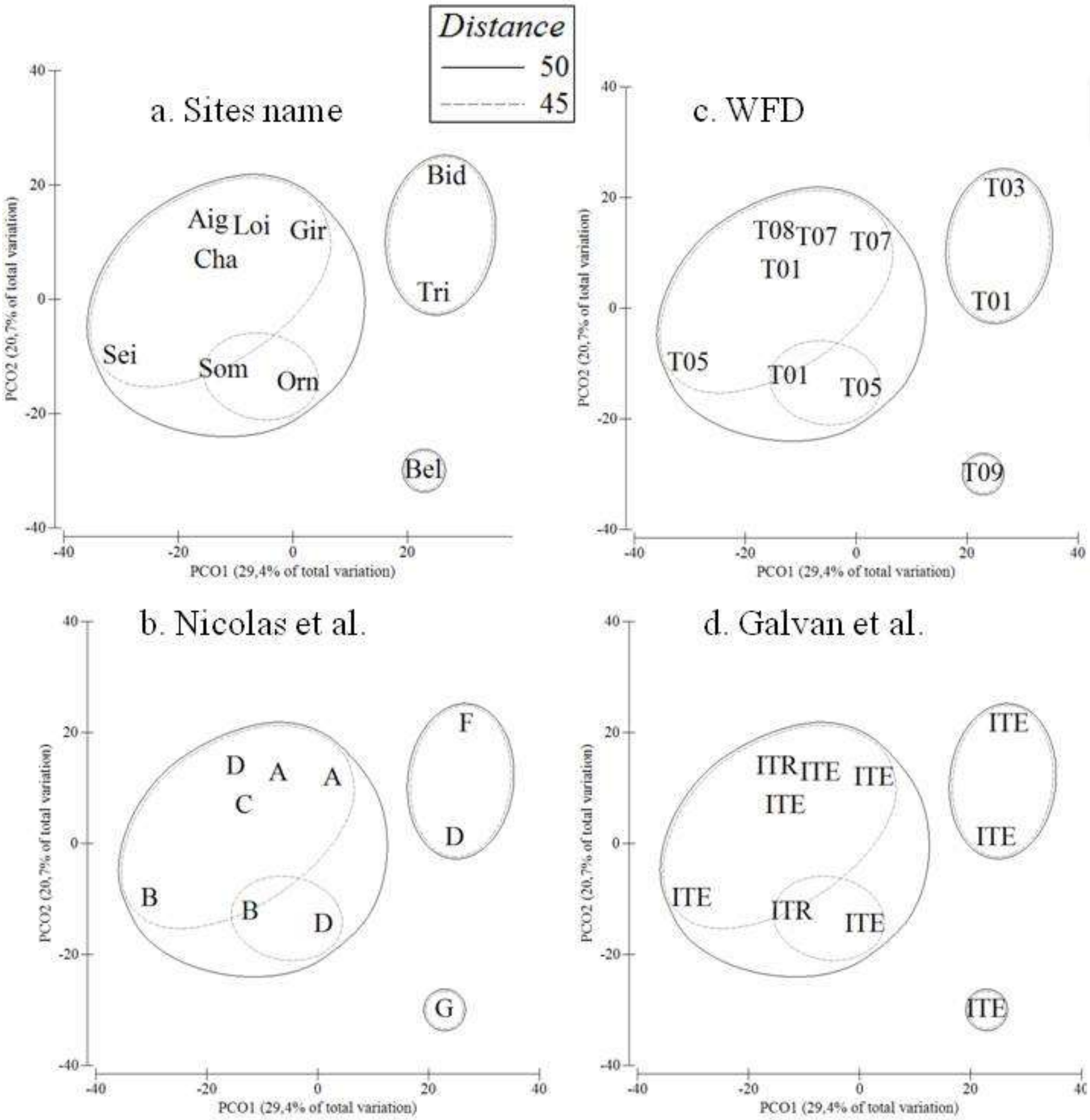
Fig 7: Dendrogram issued from the hierarchical classification of stations from all estuaries. Below the dendrogram, the number of stations belonging to each of the group identified on the basis of the dendrogram structure and SIMPROF procedure is indicated together with (a) sediment type and (b) position (% downstream) within the estuarine gradient. This is indicated for each level of the classification (*i.e.* at 10, 20 and 30 % similarity levels). For clarity, only station groups identified at the 10% level are indicated on the figure.

Fig 8: Relations between species density (S: number of species per station) and position of station within the estuarine gradient (proximity to ocean (%)). R_S is the Spearman rank-correlation coefficient between number of species and relative proximity to downstream boundary of the estuarine system (%)).The level of statistical signification of R_s is given (^{ns}: non significant ($p>0.05$), *: significant ($p<0.05$)).

Fig1


North Sea
Bay of Somme
Seine estuary
English Channel
Orne estuary
Trieux estuary
Belon estuary
Loire estuary
Aiguillon-Sèvre niortaise estuary
Charente estuary
Bay of Biscay
Gironde estuary
Bidassoa estuary

Fig 2



Distance
50
45
a. Sites name
b. Nicolas et al.
c. WFD
d. Galvan et al.
Bid
Tri
Aig Loi Gir
Cha
Sei
Som
Orn
Bel
T08 T07 T07
T01
T03
T01
T05
T01
T05
T09
D A A
C
F
D
B
B
D
G
ITR ITE ITE
ITE
ITE
ITE
ITE
ITR
ITE
ITE
PCO1 (29,4% of total variation)
PCO2 (20,7% of total variation)

Fig 3

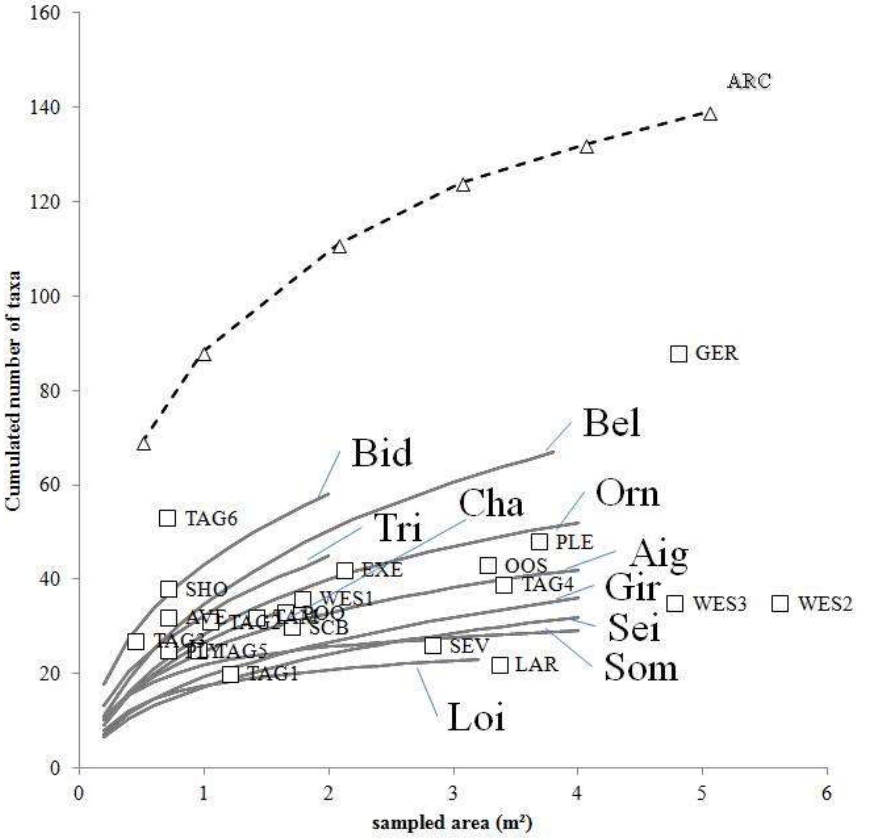

Cumulated number of taxa
sampled area (m²)
ARC
GER
Bel
Bid
Orn
Cha
Tri
Aig
Gir
Sei
Som
Loi
TAG6
PLE
EXE
OOS
TAG4
SHO
WES1
WES3
WES2
SCB
SEV
LAR
TAG5
TAG1

Fig 4

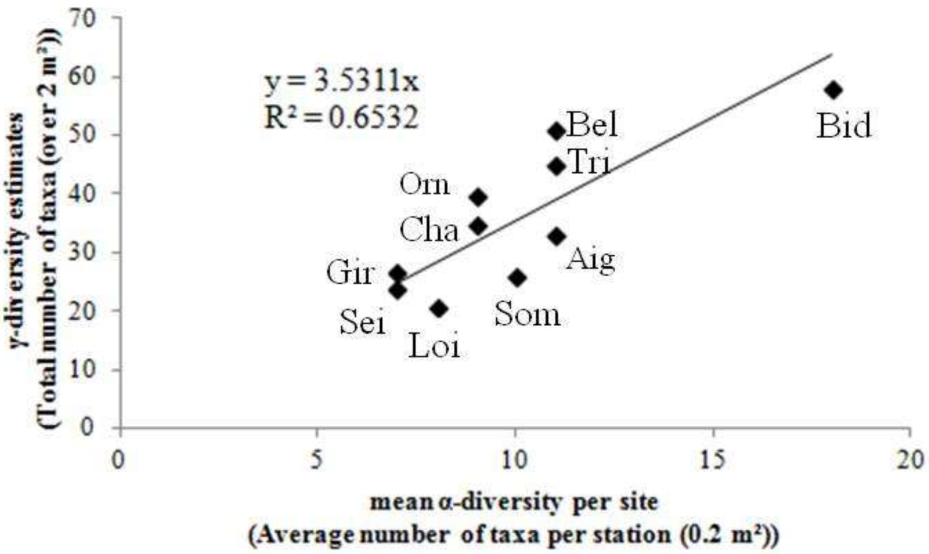

y = 3.5311x
R² = 0.6532
γ-diversity estimates
(Total number of taxa (over 2 m²))
mean α-diversity per site
(Average number of taxa per station (0.2 m²))
Bel
Tri
Bid
Orn
Cha
Aig
Gir
Sei
Loi
Som

Fig 5

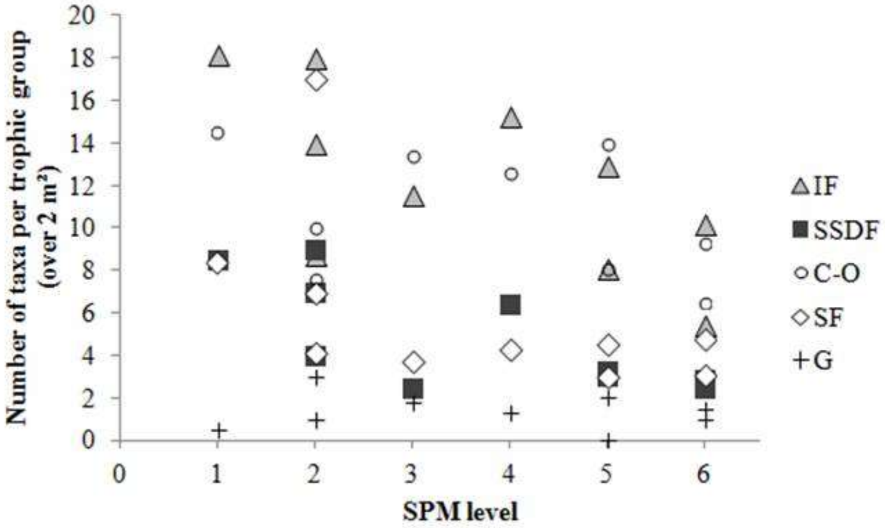

Number of taxa per trophic group (over 2 m²)
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
SPM level
IF
SSDF
C-O
SF
G

Fig 6

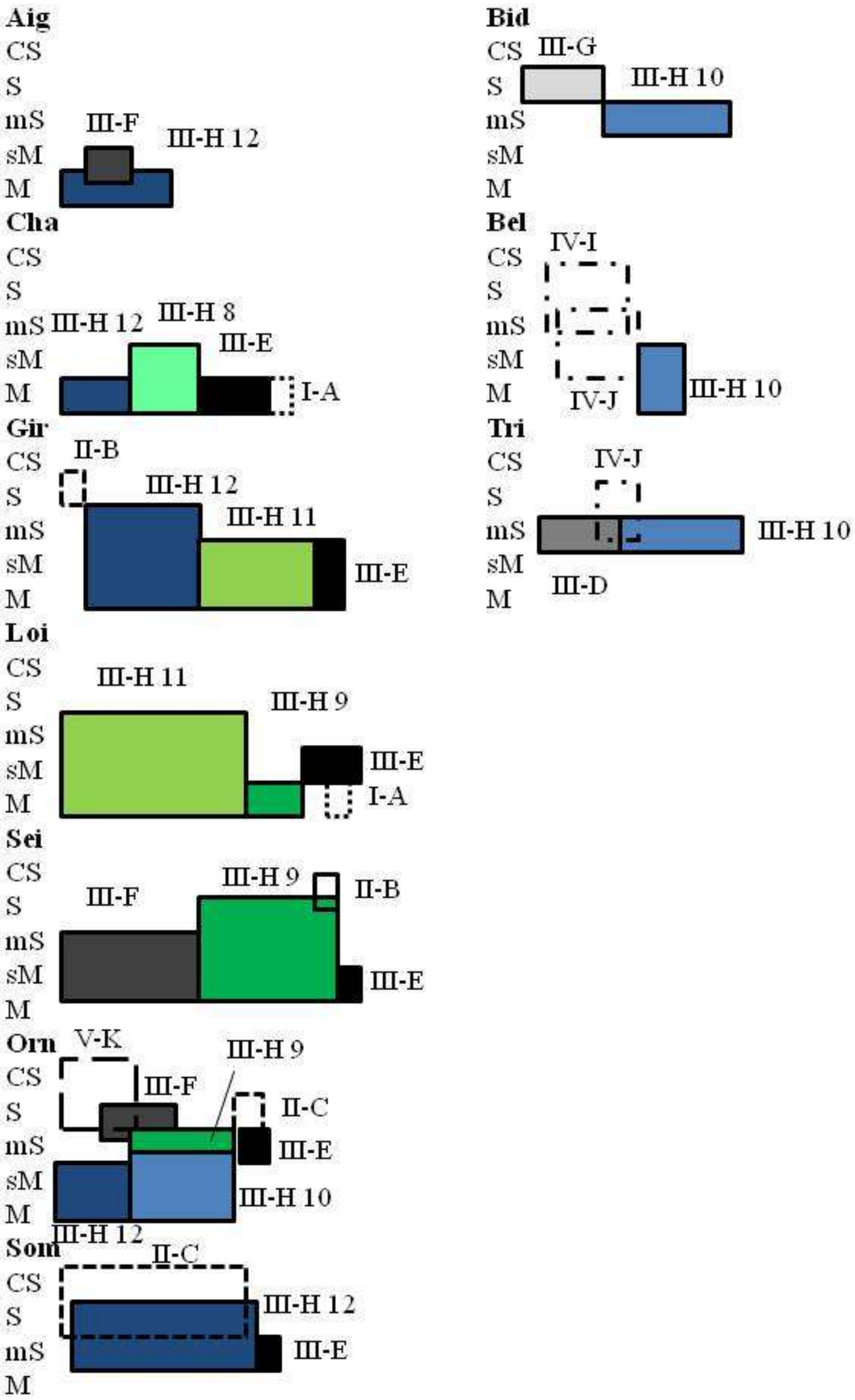
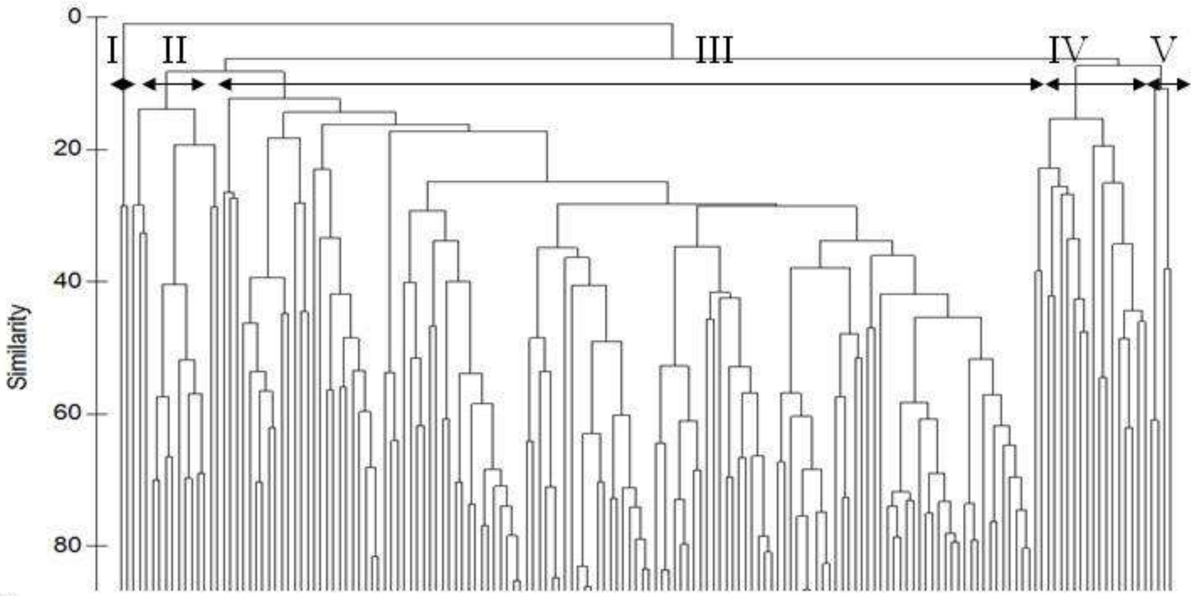

Aig
CS
S
mS
sM
M
III-F
III-H 12
Cha
CS
S
mS
sM
M
III-H 12
III-H 8
III-E
I-A
Gir
CS
S
mS
sM
M
II-B
III-H 12
III-H 11
III-E
Loi
CS
S
mS
sM
M
III-H 11
III-H 9
III-E
I-A
Sei
CS
S
mS
sM
M
III-F
III-H 9
II-B
III-E
Orn
CS
S
mS
sM
M
V-K
III-F
III-H 9
II-C
III-E
III-H 10
III-H 12
Som
CS
S
mS
M
II-C
III-H 12
III-E
Bid
CS
S
mS
sM
M
III-G
III-H 10
Bel
CS
S
mS
sM
M
IV-I
IV-J
III-H 10
Tri
CS
S
mS
sM
M
IV-J
III-H 10
III-D

Fig 7



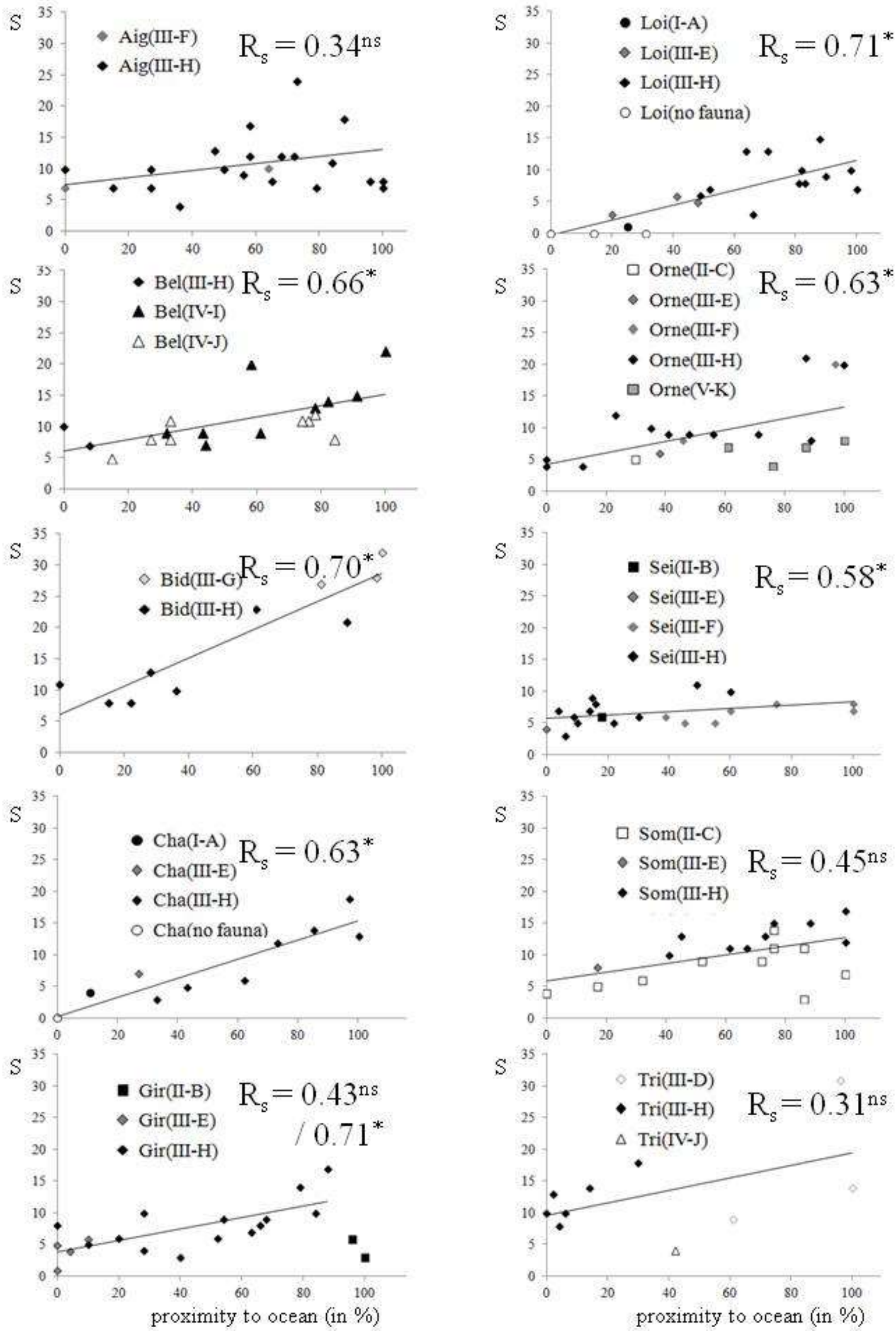
(a)

10%	I		II			III																								IV						V	
20%	A		B	C		D	E			F			G	H																I			J			K	
30%	1		2	3		4	5			6			7	8		9				10			11			12				13			14			15	
sed.	M	sM	S	S	CS	mS	M	sM	mS	M	mS	S	S	M	sM	M	sM	mS	S	sM	mS	S	M	sM	mS	M	sM	mS	S	mS	S	CS	mS	S	CS	S	CS
Aig										2																17	1										
Bel																				1	1									3	4	2	5	2	1		
Bid													3								6	1															
Cha	1						1							2	1											4											
Gir			2				1	3							1								3	6		2	1	1									
Loi		1						3								2							1	5	4												
Orn				1					1			2						1	1	1	3	1					2	3								2	2
Sei			1					1			1	6					2	6	3																		
Som				9	1				1																			3	6								
Tri						3															6													1			

(b)

10%	I		II			III																								IV						V	
20%	A		B	C		D	E			F			G	H																I			J			K	
30%	1		2	3		4	5			6			7	8		9				10			11			12				13			14			15	
sed.	M	sM	S	S	CS	mS	M	sM	mS	M	mS	S	S	M	sM	M	sM	mS	S	sM	mS	S	M	sM	mS	M	sM	mS	S	mS	S	CS	mS	S	CS	S	CS
Aig										32																60	73										
Bel																				0	8									45	78	70	36	80	78		
Bid													93								34	61															
Cha	11						27							48	43											89											
Gir			98				10	1							20								19	53		67	88	68									
Loi		25						36								58							100	78	80												
Orn				30					38			72						48	23	0	6	41					62	76								69	94
Sei			18					0			45	72					20	26	13																		
Som				57	86				17																			41	78								
Tri						86															9													42			

Fig 8


S
Aig(III-F)
Aig(III-H)
$R_s = 0.34^{ns}$
Loi(I-A)
Loi(III-E)
Loi(III-H)
Loi(no fauna)
$R_s = 0.71^{*}$
Bel(III-H)
Bel(IV-I)
Bel(IV-J)
$R_s = 0.66^{*}$
Orne(II-C)
Orne(III-E)
Orne(III-F)
Orne(III-H)
Orne(V-K)
$R_s = 0.63^{*}$
Bid(III-G)
Bid(III-H)
$R_s = 0.70^{*}$
Sei(II-B)
Sei(III-E)
Sei(III-F)
Sei(III-H)
$R_s = 0.58^{*}$
Cha(I-A)
Cha(III-E)
Cha(III-H)
Cha(no fauna)
$R_s = 0.63^{*}$
Som(II-C)
Som(III-E)
Som(III-H)
$R_s = 0.45^{ns}$
Gir(II-B)
Gir(III-E)
Gir(III-H)
$R_s = 0.43^{ns}$ / 0.71^{*}
Tri(III-D)
Tri(III-H)
Tri(IV-J)
$R_s = 0.31^{ns}$
proximity to ocean (in %)
proximity to ocean (in %)