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Temporal changes in the spatial coupling between bentho-demersal fishes and their macrobenthic preys in the Seine estuary

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Abstract:

Estuaries are highly dynamic and productive ecosystems that are driven by both physical and biological factors. Trophic interactions are amongst the main biological drivers structuring fish assemblages, yet they are rarely included in estuarine fish habitat models. Using faunal time series (1995-2002) from the Seine estuary, the present study assesses the spatial coupling between a bentho-demersal fish community and their potential macrobenthic preys. The faunal time series were composed of two datasets: a fish dataset in which annual autumn surveys were conducted on the same sampling sites and a benthos dataset for which surveys were conducted at different seasons at varying sampling sites. The two datasets were yearly linked through a neighbour matrix using a three-table approach (i.e. fourth-corner and RLQ analyses). The neighbour matrix was obtained by the intersection of polygons using Voronoï tessellation on each data set every year. Benthic fauna was grouped using various classifications (taxonomic, energetic and functional) to determine the most relevant functional group to assess fish-benthos trophic interactions. Annual variability in the fish-benthos interaction was analysed against environmental factors (i.e. river flow, temperature and salinity) to verify if these factors influenced the spatial fish-benthos coupling. The present study is a contribution to a better definition of fish habitats in the Seine estuary which finds direct application in conservancy and coastal zone management.

Keywords: fish – benthos – communities – estuary – fourth-corner analysis – RLQ analysis – functional habitats

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1. Introduction

Estuaries are highly productive ecosystems that provide a number of ecological services essential to the functioning of the Earth life support and human welfare (Costanza et al., 1997). Among essential ecological functions, they provide reproductive grounds, migratory routes, and nursery habitats for several marine and diadromous species including important commercial species (Beck et al., 2001). Estuaries offer refuges from predators, higher temperatures than in offshore areas, and abundant food (Bergman et al., 1988; Gibson, 1994). Several studies have noticed evidences that habitat conditions (quality and quantity) in coastal nurseries may affect the distribution, growth, condition and survival of the juveniles (van der Veer et al., 2001; Pihl et al., 2005; Gilliers et al., 2006), and thus the recruitment level of fish populations. Coastal-estuarine areas are probably the aquatic ecosystems where natural conditions reach their highest variability and superimpose to anthropogenic pressures, they are under stressful conditions that affect the nursery functions of several marine fish species (Cabral et al., 2007; Le Pape et al., 2007a; Vasconcelos et al., 2007; Courrat et al., 2009). It is thus critical to understand the ecology and the functioning of these coastal areas in order to achieve habitat conservation and sustainable management of fisheries (Rice, 2005).

Habitat models in estuaries have been mostly developed for single fish species and fish assemblages of bentho-demersal species. These models include principally abiotic factors, such as depth, substratum type, salinity, temperature, oxygen, and river flow, as explanatory variables thereby overlooking biotic variables (Rogers, 1992; Eastwood et al., 2003; Le Pape et al., 2003). Species of the epi- and endobenthic community constitute the main food supply for several bentho-demersal fish juveniles. Recent habitat suitability models, developed for flatfish species, have included benthic data in their model, yet they significantly improved the predictive power of their models (Vinagre et al., 2006; Le Pape et al., 2007b; Wouters and Cabral, 2009). However, these studies remain relatively scarce, especially at the fish community level, and they generally describe the benthic compartment using either total abundance or large functional or taxonomic groups. They rarely use information at the species level and have no or few consideration for the spatial organisation of the sampling scheme (Nicolas et al., 2007).

Studies that include a sampling design large enough to have broad environmental variability, the sampling of abiotic factors and potential benthic preys, identified at the species level, definitely require multidisciplinary approaches. These approaches are highly time/cost consuming, for instance the effort involved in the species identification of benthic samples can be extremely high. Therefore, habitat models that include all the aforementioned criteria are rarely observed in the literature. In some cases however, several research or monitoring programs are conducted and historical faunal data with such criteria can be found. The data in such programs often come from several surveys conducted using different sampling designs including different measurements (e.g. fish and benthos) that have been made in different locations. The use of such “combined” surveys inherently requires new methodologies.

The present study is part of an integrated multi-disciplinary project named COLMATAGE (GIP Seine-Aval) bringing together sedimentologists, benthologists and ichtyologists and aiming at understanding the spatial organisation of functional habitats in the Seine estuary – one of the most in Western Europe and the most impacted estuary in France. As part of this project, the historical datasets of each discipline, initially designed for different purposes, have been compiled and made available for joint studies. The faunal (fish and benthos) time series (1995-2002) that could be used indeed covered the estuarine-coastal gradient of the Seine estuary but were not sampled at the exact same locations (see Fig. 4: fish and benthos sampling sites).

An original statistical approach enabling to link two datasets from different spatial sampling schemes have been used (Dray et al., 2002). That method consists in joining two datasets (here fish and benthos datasets) through a spatial neighbourhood matrix using a three-table approach. As a three-table approaches, we used the spatialized version of the RLQ analysis (Dolédec et al., 1996) modified by Dray et al (2002) and the fourth-corner analysis developed by Legendre et al (1997) and recently modified by Dray and Legendre (2008). The RLQ analysis and the fourth-corner method have been recently found complementary in assessing the relationship between fish spatial distributions, fish species traits, and habitat characteristics (Brind'Amour et al., in press). RLQ analysis produces ordinations that can be used to identify the group members of species, species traits, and environmental variables, thereby providing a valuable and complementary tool to the fourth-corner method for the interpretation of the traits-environment relationships. As the spatial-RLQ analysis has never been used in marine environment and the fourth-corner approach has never been spatialized, we adapted the two methods to test the hypothesis of “trophic coupling” between bentho-demersal fish assemblages (mainly juveniles) and their potential macrobenthic preys along a time series in the Seine estuary.

2. Material and methods

2.1 Study area :

2.1.1. The Seine estuary

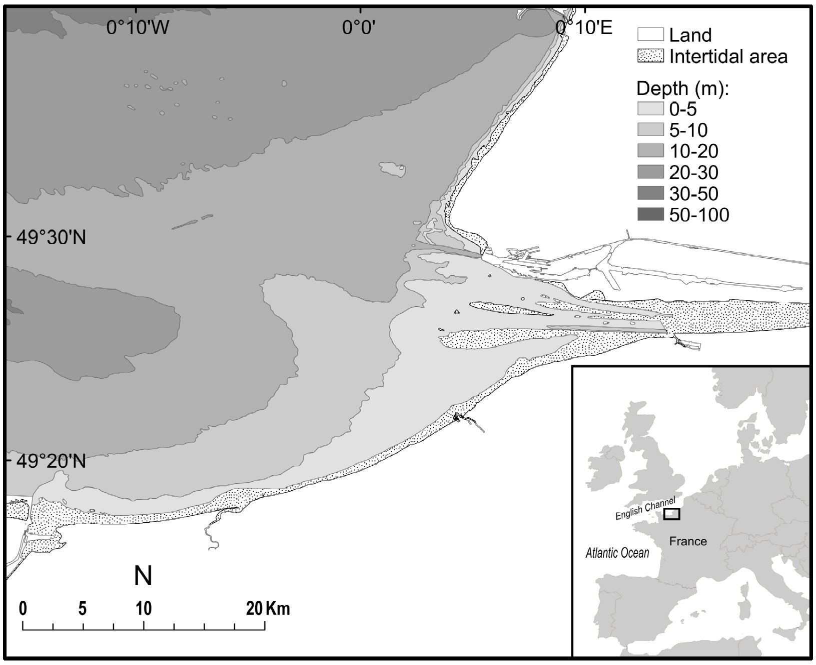


Fig. 1. Map showing the location of the study area with the bathymetry (SHOM 1996)

The Seine estuary is the largest estuary of the Eastern Channel (Western Europe). It is located on the French coast, covering approximately 150 km^2 at high tide. It is a megatidal estuary with tidal range at the mouth of about 8.5m for spring tides and 4 m for neap tides. It represents the main freshwater inflow in the Eastern Channel, with mean annual river flow > 500 m3.s-1, varying seasonally from a maximum of 2000 m3.s-1 in winter to a minimum of 100 to 200 m3.s-1 in summer (e.g. see Fig.3). The Seine estuary has an extended watershed (79 000 km^2) draining almost exclusively agricultural, industrial and highly urbanized mainly land (40% percent of France industrial and agricultural activity, 50% of national river traffic and 30% of its population). It is subjected to very high anthropogenic pressures of various origin (water contamination, habitat modification). The Seine is one of the most polluted estuaries in Europe (i.e. heavy metals, PCB, PAHs, pesticides and emergent contaminants) (Tronczynski et al., 1999; Chiffoleau, 2001; Miramand et al., 2001), although contaminants levels have dropped substantially (Dauvin et al., 2009). The present day morphology of the estuary is mainly artificial, resulting from man-made modifications since the mid 19th century (dams, navigation channel dredging and harbour constructions). The Seine river has been canalized and dredged up to Rouen (120 km upstream from the mouth) to allow commercial navigation. At the mouth intensive dredging (around 5 millions ton y-1) occur to maintain the central deepened channel. Successive construction of dykes, has reduced the intertidal zone from 130 km2 in the middle of the 19th century, to less than 30 km^2 in 2000 (Rochette et al., 2010). The elongated sandbars typical of tide-dominated estuaries have developed at the mouth of the estuary and superficial sediments have become more and more muddy (Lesourd et al., 2001). The most recent development of infrastructure is the Seine estuary – part of the "Port 2000" project extended the surface of the Le Havre harbour, mainly to permit large container ships access to new extended loading platforms all day. Plans for the North Channel included (1) construction of a 16 m deep, 350 m wide, and 2800 m long channel connected to the Le Havre ports navigational channel and (2) construction of a dam-protected basin (Dauvin et al., 2006).

2.1.2. Sediments types

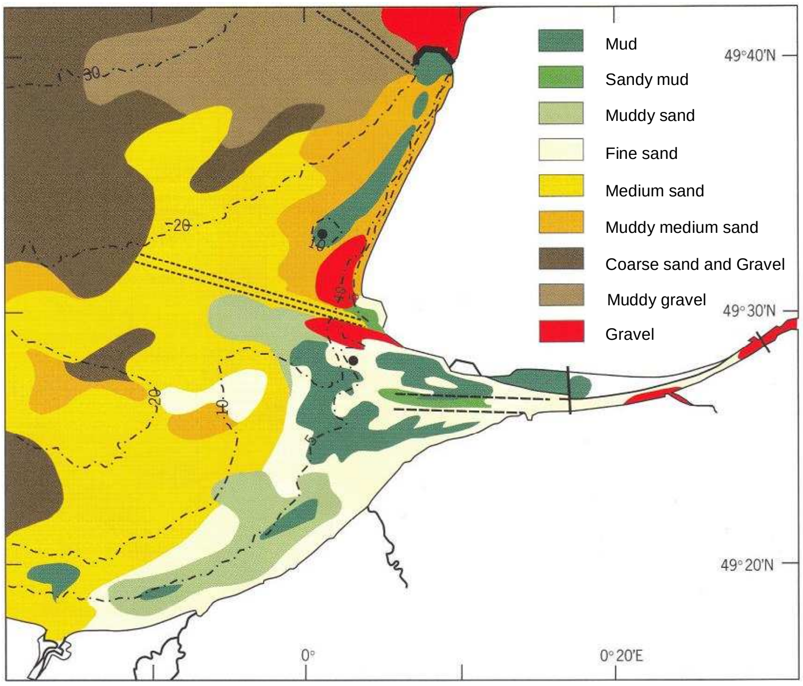


Fig. 2. Map of sediment types in the Seine estuary (Lesueur and Lesourd, 1999)

There is a variety of sediment types in the Seine estuary (Fig. 2), however, it can be divided in two zones: the inner estuary, characterized by fine sand and muddy sediment and the outer estuary characterized by coarser sediment ranging to medium sand to muddy gravel.

2.1.3. River flow

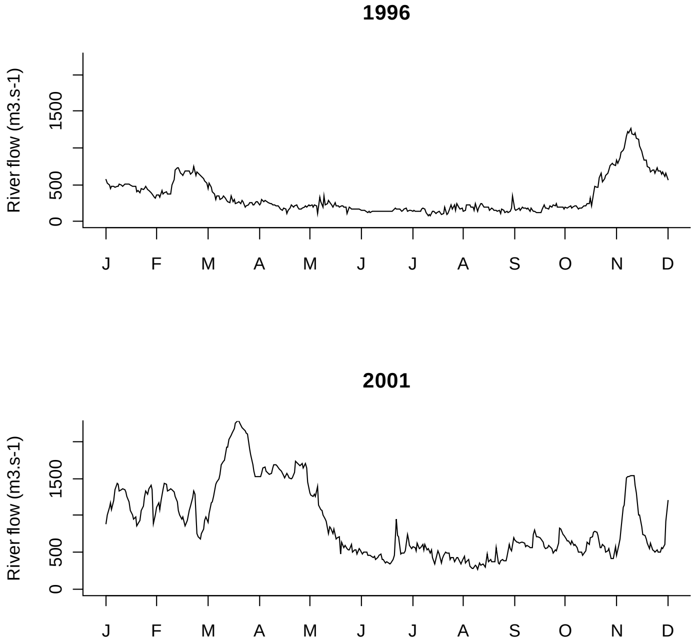


Fig. 3. Annual Seine river flow measured at Poses (160 km upstream from the mouth of the estuary) for 1996 and 2001. These two years were selected as an example for data analysis in the present study – see following section.

The two selected years (1996 and 2001) for data analysis in the present study were very contrasted (compared to normal/average conditions) in term of river flow and thus freshwater input in the Seine estuary system (Fig. 3). 1996 was a drought year with low river flow especially during the winter-spring seasons (less than 500 m3 s-1). In contrast 2001 was a rainy year with high river flow especially at the latter seasons (around 1500 m3 s-1).

2.2. Data description

2.2.1. Fish data

From 1995 to 2002, height series of surveys were carried out in autumn in an area extending in subtidal zones from the coast to a distance of 20 meters depth, between the "Pont de Normandie", Ouistreham and Antifer (Fig. 1). Autumn is the most favourable season to assess juveniles abundance in nurseries areas. Two beamtrawls were used (2 m and 3 m wide) with a mesh size of 20 mm in the codend. The study area was initially divided into 13 strata which were considered homogeneous for bathymetry and environment and between 3 and 6 trawling were carried out in each stratum. At each site all the fish species were identified, and for each species, animals were counted, and weighed. Commercially important fishes were measured (total length in mm). For seven commercial species (*Limanda limanda, Pleuronectes platessa, Solea solea, Trisopterus luscus, Merlangius merlangus, Platichthys flesus, Dicentrarchus labrax*), the age was determined from otoliths or scales (only *Dicentrarchus labrax*). The data set consisted of 414 valid hauls representing between 42 and 54 trawling carried out every year on the study area.

2.2.2. Benthos data

All the available quantitative data on the benthic communities from the Bay of Seine and the Seine estuary are pooled together in a database named MABES (MAcrobenthos of the Bay and Estuary of the Seine). This database includes standardized abundances in number of individuals per m² of macrobenthic species from 1978 to 2006 in its last version and includes the trophic groups of the majority of the species encountered. The MABES database is available from the data administrator of GIP Seine-Aval (nbacq@seine-aval.fr). In this study, a set of benthos data that matched fish data sampling (i.e. years from 1996 to 2002 and the same study area, since no data existed for 1995 in the study area) was extracted, which represented a total of 829 samples.

2.3. Data selection and manipulation

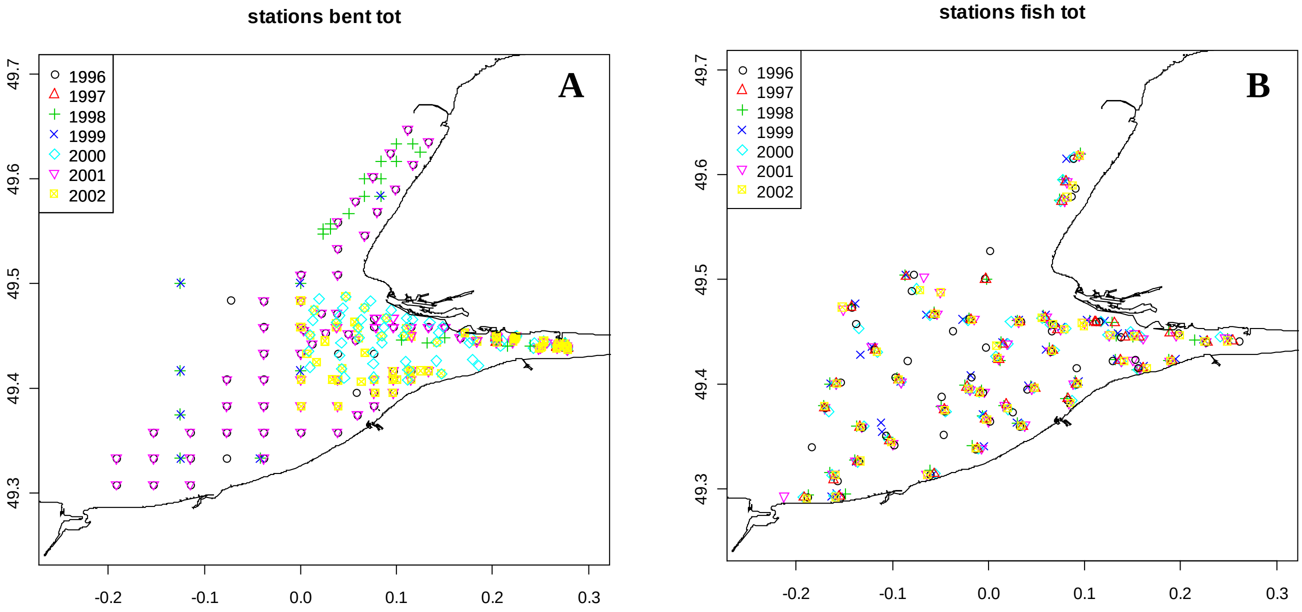


Fig. 4. Location of sampling sites from both fish (A) and benthos (B) datasets in the Seine estuary from 1996 to 2002.

2.3.1. Faunal taxa selection

- <u>fish data set:</u>

Domininant non commercial taxa (*Calionymus lyra*, *Buglossidium luteum*, *Gobiidae*) and Young-Of-the-Year (YOY) from the five commercial species that were aged (*Limanda limanda*, *Pleuronectes platessa*, *Solea solea*, *Trisopterus luscus*, *Merlangius merlangus*) were selected. These height taxa were found on consecutive years on the study area.

- <u>benthos data set:</u>

Taxa that are potentially preys of the selected bentho-demersal fishes (e.g. Darnaude et al., 2001; Amara et al., 2004; Vinagre et al., 2005) with occurrence >= 1% and found on consecutive years were selected.

2.3.2. Fish reference station

Since fish sampling was carried out approximately on the same sites every years, reference sites were calculated. A hierarchical clustering ("McQuitty" method; see: McQuitty, 1966) was applied on Euclidean distances, and groups were defined based on a distance between neighbour points of 2km, which approximately corresponded to the mean trawling distance. Centroïds of such cluster of points were determined, and only centroïds showing at least one haul each consecutive year were selected (Fig. 5). Mean abundances were then calculated for each taxa on each reference site.

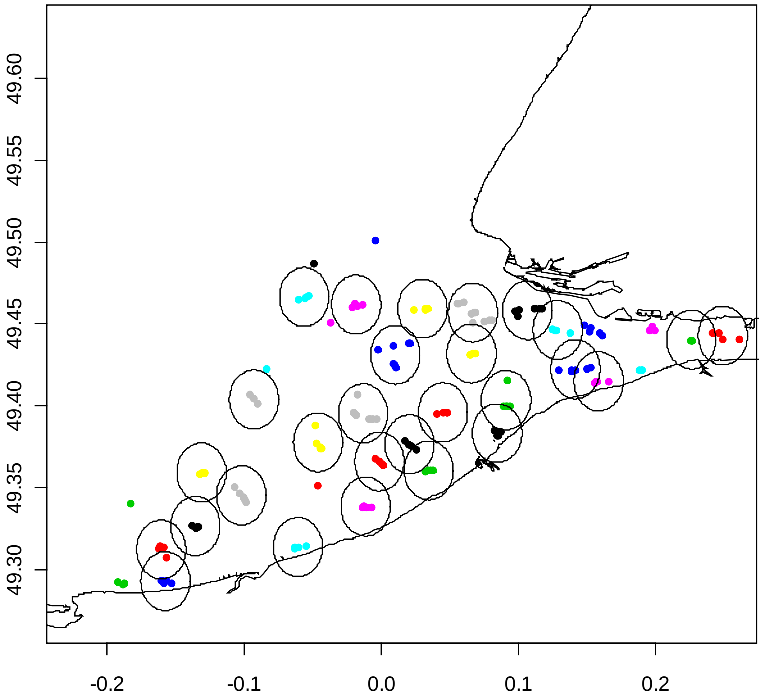


Fig. 5. Spatial grouping of fish sampling sites as reference sites by hierarchical clustering ("McQuitty" method) on Euclidean distances between geo-coordinates. Clusters showing at least one haul each consecutive year are shown as circle drawn from the centroïds, representing 27 reference sites.

2.3.3. Spatio-temporal matching of fish and benthos sampling data

In the benthos time series 1996 – 2002 (no data for 1995), some years (i.e 1997, 1998, 1999) presented very small spatial range of data and were thus removed (see Fig. 4). Only two years (i.e. 1996 and 2001) presented data on the outer estuary. Four years (1996, 2000, 2001, 2002) presented significant spatial sampling in the inner estuary. Thus, the study area could eventually be divided in two distinct zones (inner and outer) to cover a maximum of the spatio-temporal available data. However, for 1996 and 2001, the entire estuary was covered as part of dedicated long term monitoring campaigns (PECTOW) with a regular sampling scheme (exactly same sites sampled each time see Fig. 4). We thus decided to present only these two contrasted years in term of river flow (see Fig. 3) as an example in this presentation.

2.3.4. Benthos seasonal variability

Benthos data were taken from a variety of months from all seasons. On the four years that were first selected, 2000 and 2002 were sampled in Autumn (i.e. same season as for fish data) but 1996 and 2001 were sampled in Winter (February-March). In order to define if such data could be used, the seasonal variability in benthos data in the study area was tested. An appropriate set of data

was selected: data were obtained exactly on the same sites in March, May and September 2002 and located on a wide range of the estuary. First a Kruskal-Wallis test was applied for each taxa to test difference between the three-months, p-value calculation was corrected for the multiple comparisons (Bonferoni), and followed by Conover-Inman post-hoc tests. Different variables were tested (abundance, site specific relative abundance, presence/absence and biomasse calculated with average annual dry weights obtained from specific studies in the Seine estuary). Seasonal differences
in benthos assemblages were also tested using the same space-time method as described in the following section. Results are not shown here, but basically no significant difference was found between winter and autumn seasons in benthos assemblages. Therefore the use of 1996 and 2001 benthos data (sampled in winter) was possible for the spatial coupling analysis with fish data (sampled in autumn). From these different tests, we decided to use calculated biomasses. Biomass is a much better proxy of food availability than abundance since there is a wide range of sizes in benthic organisms. Only the benthos taxa for which seasonal variability could be tested were selected for the final analysis.

2.4. Statistical analyses

2.4.1. Space-time variability for the fish and benthic communities

Analyses of the spatio-temporal variability for the fish and benthic datasets were conducted separately using the same methodology. The spatial variability was assessed by combining a cluster analysis with an approach detecting the species that best characterize the clusters of sites. Whereas the temporal variability was studied by the graphical examination of the species distribution among years and numerically by comparing the different classifications among years.

Cluster analyses were done using Gower's dissimilarity coefficient on the species abundance matrix (Podani and Schmera, 2006). The dendrograms were produced by hierarchical clustering using Ward minimum variance method (Ward, 1963). The number of clusters was identified using the Calinski-Harabasz criterion, a pseudo F (ANOVA) statistic which computes the sum of squared errors (distances) between the kth cluster and the other k - 1 clusters, and compares it to the internal sum of squared errors for the k clusters (i.e. taking their individual squared error terms and summing them (Calinski and Harabasz, 1974; Milligan and Cooper, 1985). Using the results from the classifications, indicator taxa were determined for clusters of sampling sites using the IndVal method (Dufrêne and Legendre, 1997):

$$A_{ij} = \mathrm{Nindividuals}_{ij}/\mathrm{Nindividuals}_{i}$$

$$B_{ij} = \mathrm{Nsites}_{ij}/\mathrm{Nsites}_{j}$$

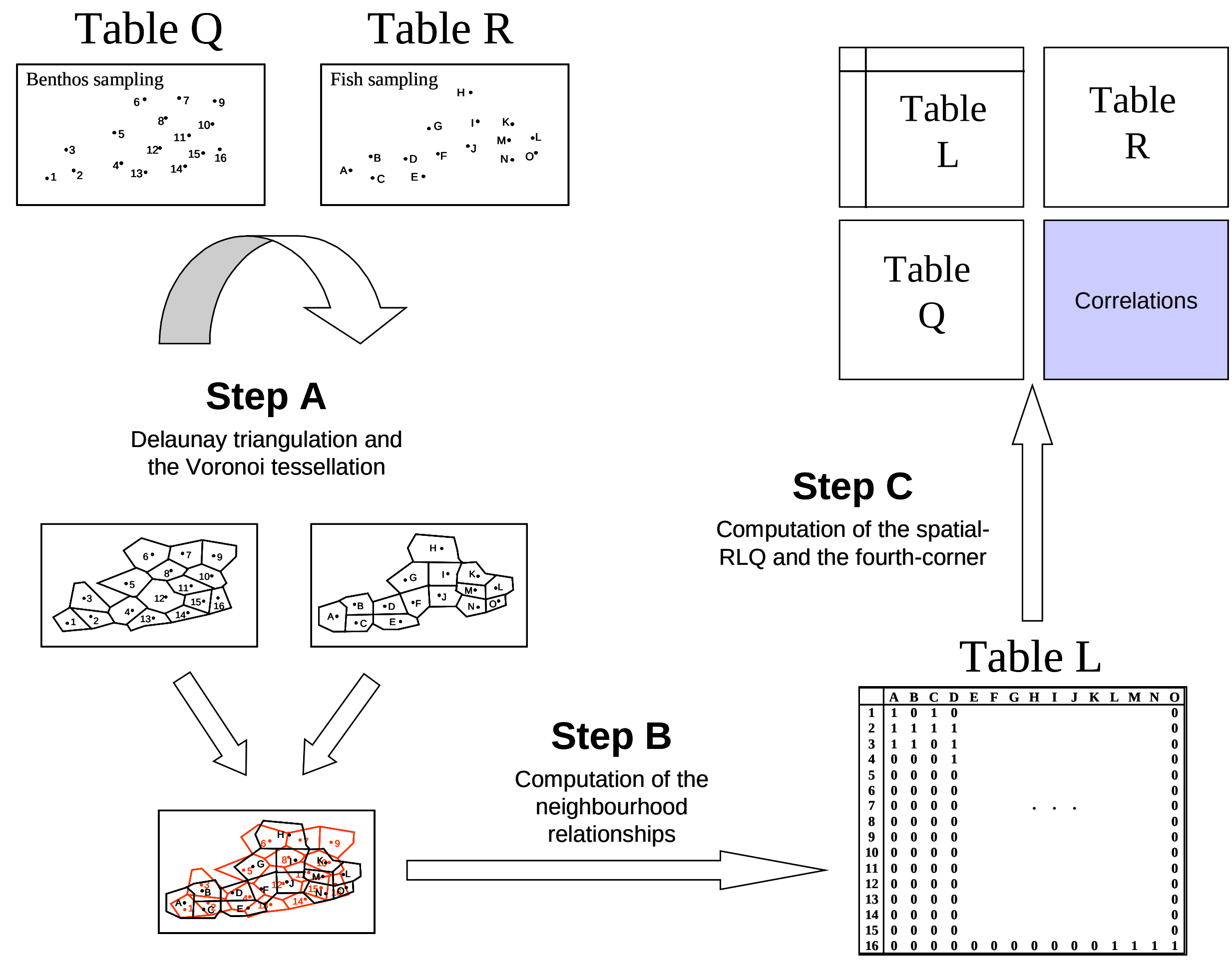
$$\mathrm{IndVal}_{ij} = A_{ij} * B_{ij} * 100$$

where for each taxa *i* in each cluster *j*, we computed IndVal_{ij} which is the product of A_{ij}, the mean abundance of taxa *i* in the sites of cluster *j* compared to all clusters in the study, by B_{ij}, the relative frequency of occurrence of taxa *i* in the cluster *j*. In the formula, A_{ij} was estimated by the quotient of $\mathrm{Nindividuals}_{ij}$, the mean number of individuals of taxa *i* across sites of cluster *j*, and $\mathrm{Nindividuals}_{i}$, the sum of the mean numbers of individuals of taxa *i* over all clusters. As suggested by Dufrêne and Legendre (1997), the mean number of individuals in each site, instead of the sum of the individuals was used, because this removes any effect of the number of sites in the various clusters and the differences in abundance among the sites belonging to a same cluster. B_{ij} was calculated by the quotient between Nsites*ij*, the number of sites in cluster *j* where taxa *i* is present, and Nsites_{j} is the total number of sites in that cluster. The statistical significance of the indicator taxa was evaluated using a randomisation procedure (Monte Carlo randomisation, but see Dufrêne and Legendre, 1997 for details) and only the taxa showing indicator values with an associated alpha of 0.01 were considered as significant. The analyses were conducted using the “duleg” function of the “labdsv” library in the R software (R Development Core Team, 2008).

Comparison of the classifications among years was done in two steps: i) we transformed each year classification (i.e. species*group table) in a dissimilarity matrix using Jaccard’s coefficient (see Legendre and Legendre, 1998), and ii) we computed the correlations between the dissimilarity matrices using Mantel tests (Mantel, 1967). The significance of the Mantel statistic was evaluated by permuting rows and columns of the first dissimilarity matrix. The analyses were conducted using the “mantel” function of the “vegan” library in the R software (R Development Core Team, 2008).

2.4.2 Fish-benthos spatial coupling

The spatial coupling of the fish species and their potential benthic preys was done using the three-table approaches previously described in the introduction (spatial-RLQ and fourth-corner). The use of these approaches in a spatial context required however some adaptations, yet some hypotheses that are described below. A flowchart describes step by step the methodology used in the present study (Fig. 6).



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	1	0	1	0											0
2	1	1	1	1											0
3	1	1	0	1											0
4	0	0	0	1											0
5	0	0	0	0											0
6	0	0	0	0											0
7	0	0	0	0				.	.	.					0
8	0	0	0	0											0
9	0	0	0	0											0
10	0	0	0	0											0
11	0	0	0	0											0
12	0	0	0	0											0
13	0	0	0	0											0
14	0	0	0	0											0
15	0	0	0	0											0
16	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1

Fig. 6. Flowchart describing the different steps for coupling the two datasets: conversion of data points to spatial polygons from Table Q and Table R (Step A); computation of a matrix of the neighbourhood relationships (Table L) between the two polygons sets (Step B); computation of the spatial RLQ and fourth-corner analyses using Tables R, L and Q.

Data conversions. - The spatialized three-table approaches require the computation of a neighbour matrix in which the neighbourhood relationships between the sites of the fishes and the benthos sampling schemes are defined. As datasets in fish and benthic ecology are generally composed of species data collected on different sampling stations (i.e. data points), we first converted the data points to spatial polygons prior to the analyses. That step (step A in Fig. 6 for method and Fig. 7 for 1996 and 2001 fish and benthos datasets) was done using an adapted version of the function *deldir* in the deldir package (http://www.math.unb.ca/~rolf). This function computes the Delaunay triangulation and hence the Voronoï tesselation of a planar point set according to the algorithm of Lee and Schacter (1980). Once the data points were converted in polygons (and afterward in shapefiles), we computed a matrix of the neighbourhood relationships between the two polygons (hereafter named Table **L**: step B in Fig. 6; and see Fig. 8 to visualize intersection between 1996 and 2001 fish and benthos datasets). That matrix is filled considering that **t***ij* = 1 if polygon of the sampling station *i* from the fish dataset intersects with the polygon of the sampling station *j* from the benthos dataset, and **t***ij* = 0 otherwise. We used the function *intersectpolys* available in the spatialrlq.R file (http://pbil.univ-lyon1.fr/members/dray/software.php).

Three-table analyses. - The three-table approaches used in the present study required multiple data taking the form of three input tables (R, L and Q) and computed fish-benthos correlations in a fourth matrix (table D). The rows of L correspond to the rows of R and the columns of L correspond to the rows of Q. This section presents the information included in each table used in the spatial-RLQ and fourth-corner analyses.

The first table (**L**: m x k) contains the spatial relationship, i.e. the neighbour matrix previously designed, between the two sample schemes. The second table (**Q**: k x n), includes the abundances of the 8 fish species at the 27 sampling polygons. The abundance data were Hellinger transformed (Legendre and Gallagher, 2001). The third table **R** (m x p) displays information about the 24 benthic species (i.e. potential preys of the fish species) at the 54 sampling polygons. The abundance data were also Hellinger transformed (Legendre and Gallagher, 2001). The last table, the table **D** (n x p) contains the results obtained after conducting the fourth-corner analysis. It is composed of correlations of the 8 fish species (table Q) crossed with the 24 benthic preys (table R). The correlations obtained in individual cells (d*ij*) of table D were tested using 999 permutations, thereby producing p-values. Two permutation models were used (permutation of entire rows and entire columns), and their probabilities combined, to test the null hypothesis (H0) stating that the fish species (table Q) are not related to the benthic preys (table R). The rejection of H0 required the two conditions (or hypotheses), (i) the rejection of the absence of a link between the neighbour matrix and the fish species (i.e. table L and table Q) and (ii) the rejection of the absence of a link between the neighbour matrix and the benthic preys (i.e. table L and table R but see (Dray and Legendre 2008 for more details). Rejection of H0 at significance level $\alpha = 0.05$ required the rejection of the two hypotheses at significance levels $\alpha 1 = \alpha 2 = 0.2236$; in that way, $\alpha = \alpha 1\ \alpha 2 = 0.05$. Only the correlations that remained significant at the 0.05 level after the α adjustment of Holm's procedure for multiple testing (Holm, 1979) and α correction ($\alpha 1\ \alpha 2$) were used for ecological interpretation.

RLQ analyses (Dolédec et al., 1996) were computed using the "rlq" function of the "ade4" package. RLQ performs a double inertia analysis of two arrays (table R and table Q; step C in Fig. 6) with a link expressed by a neighbour matrix (table L). More precisely, it is an extension of the co-inertia analysis that simultaneously finds linear combinations of the variables of table R and linear combinations of the variables of table Q of maximal covariance weighted by the data in table L, which is, in here, a neighbour matrix (Dray et al., 2002). It graphically summarizes and represents the main co-structure in the three tables R, L and Q. The RLQ and fourth-corner analyses were jointly used to identify the fish and the benthic preys that are spatially co-occuring. Graphical representations of the outputs of RLQ analysis (e.g., scores of the fish and benthic preys) were used for interpretation purposes. Absolute contributions of each taxa were calculated and contributions were considered significant when >= overall mean contributions of the taxa to the axis.

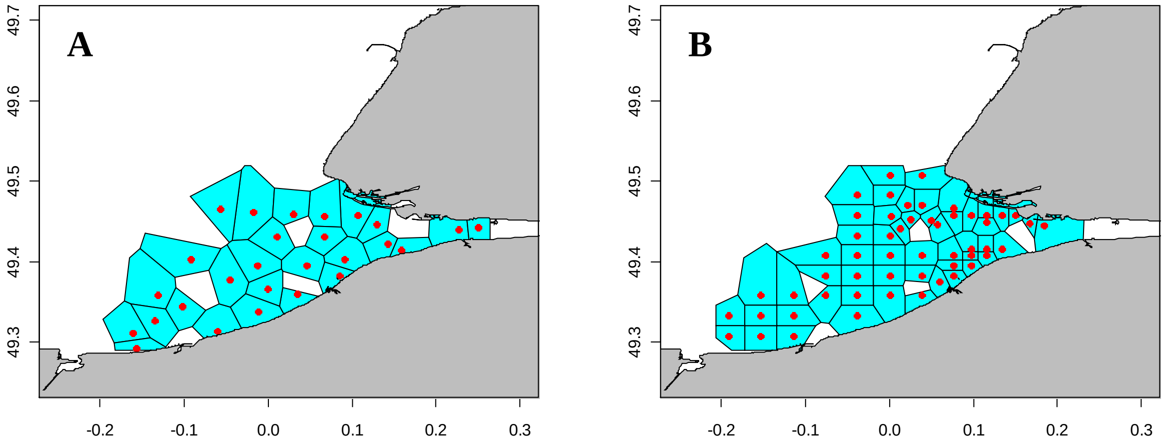


Fig. 7. Polygons calculated around sampling fish (A) and benthos (B) sampling sites in the Seine estuary. Sampling design was the same for the two years 1996 and 2001. Polygons were obtained by Delaunay triangulation and Voronoï tessalation constrained with dummy points on the ridge (5 on X axis and 4 on Y axis).

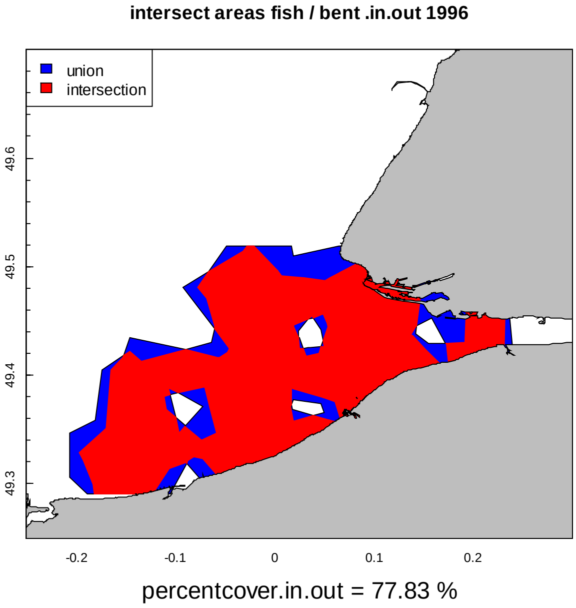


Fig. 8. Overlap of the two sets of polygons (fish and benthos) showing union and intersection. The percent cover of intersection represented 77.83% of the total area covered by the two sets of polygons.

3. Results and Discussion

3.1. Spatio-temporal variability of fish assemblages

Cluster analyses conducted on the fish assemblages classified the sampling sites in two distinct groups: a group defined by the inner estuary and the other by the outer estuary (Fig 9 & 10). Only 5 sites were differently assigned between the two years, showing a progression towards the sea of the inner estuary cluster assemblage in 2001. Outer estuary fish assemblage was mainly characterized by *Callionymus lyra* and *Limanda limanda* for both years and *Buglossidium luteum* only for 1996. Inner estuary fish assemblage, was characterised by *Solea solea* for both years and *Trisopterus luscus* only for 2001. The overall assemblage was significantly similar between the two years (see Fig. 11; Mantel's statistics: 0.373 ; P=0.006).

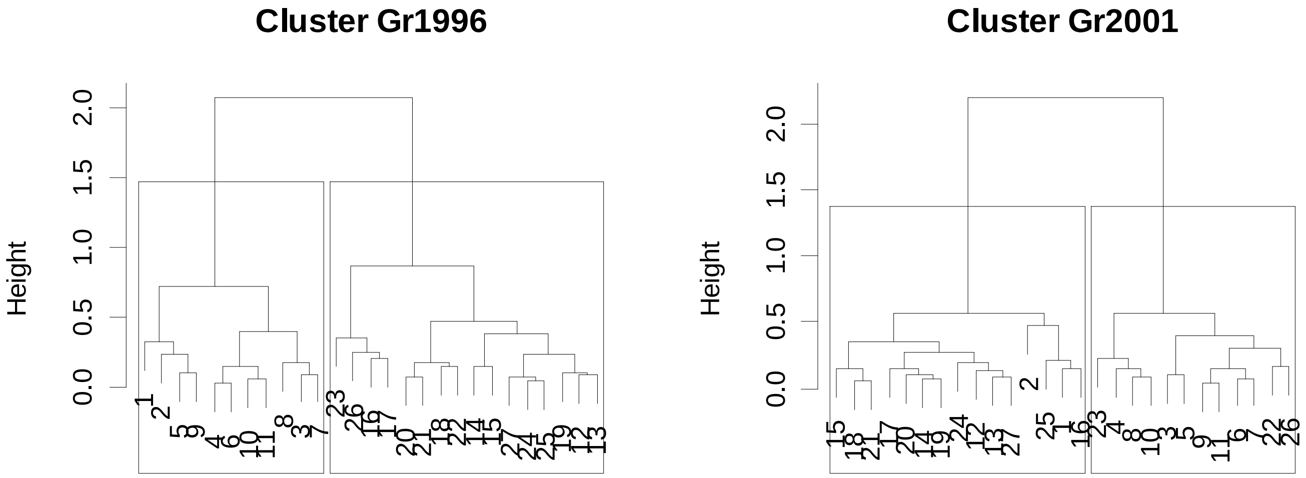


Fig. 9. Hierarchical clustering of the fish sampling sites based on their assemblage composition for 1996 and 2001

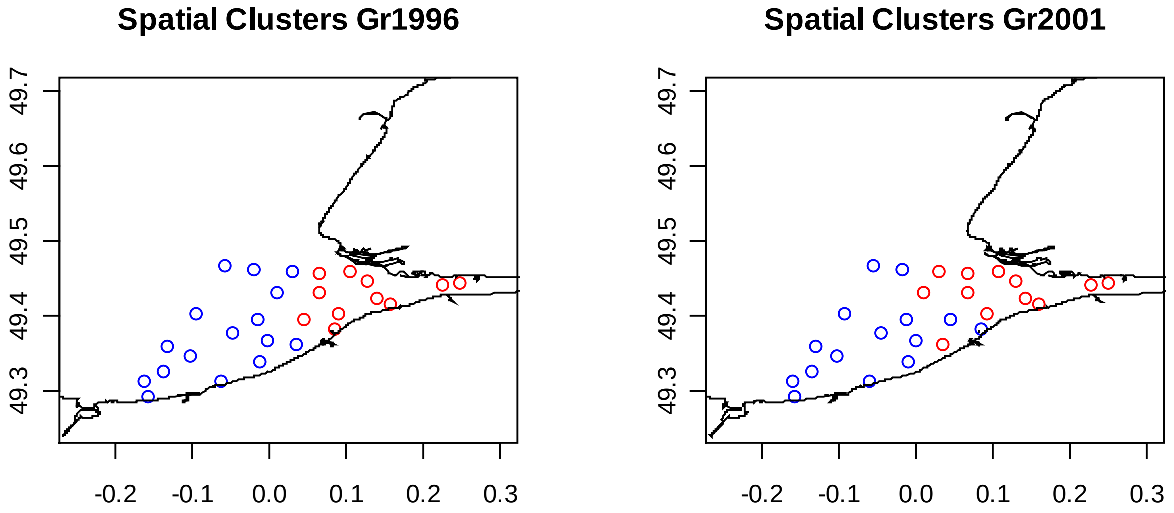


Fig. 10. Spatial representation of fish assemblages clusters for 1996 and 2001

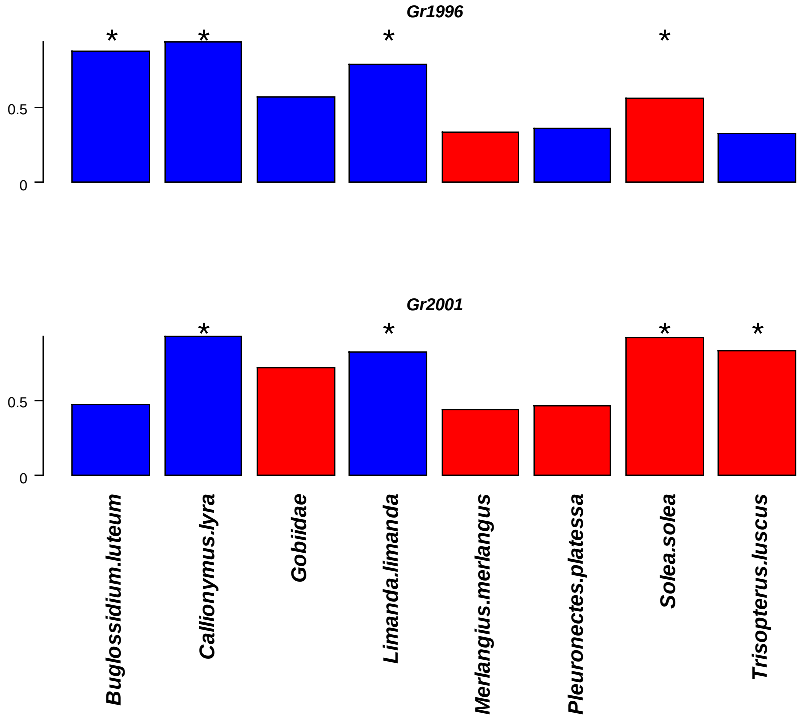


Fig. 11. Indicator taxa for each fish assemblage cluster (same colour as above) for 1996 and 2001. Stars indicate significant indicator taxa (Monte Carlo randomisation test).

3.2. Spatio-temporal variability of macrobenthic communities

Cluster analyses conducted on benthos assemblages classified the sampling sites in two distinct groups: a group defined by the inner estuary but also extending at the southern part of the Bay of Seine and another group for the outer estuary but mostly at depth > 5m (Fig. 12 & 13). Only 8 sites were differently assigned between the two years. Outer estuary benthos assemblage was mainly characterized by *Corbula giba*, *Glycera tridactyla*, *Glycende normanni*, *Leucothoe incisa* and *Phaxas pellucidus* for both years and *Sigalion mathildae* only for 2001. Inner estuary benthos assemblage, was characterised by *Abra alba*, *Macoma balthica*, *Nephtys hombergii* and *Owenia fusiformis* for both years and *Mysella bidendata* only for 1996. The overall assemblage was significantly similar between the two years (see Fig. 14; Mantel's statistics: 0.430 ; P=0.001).

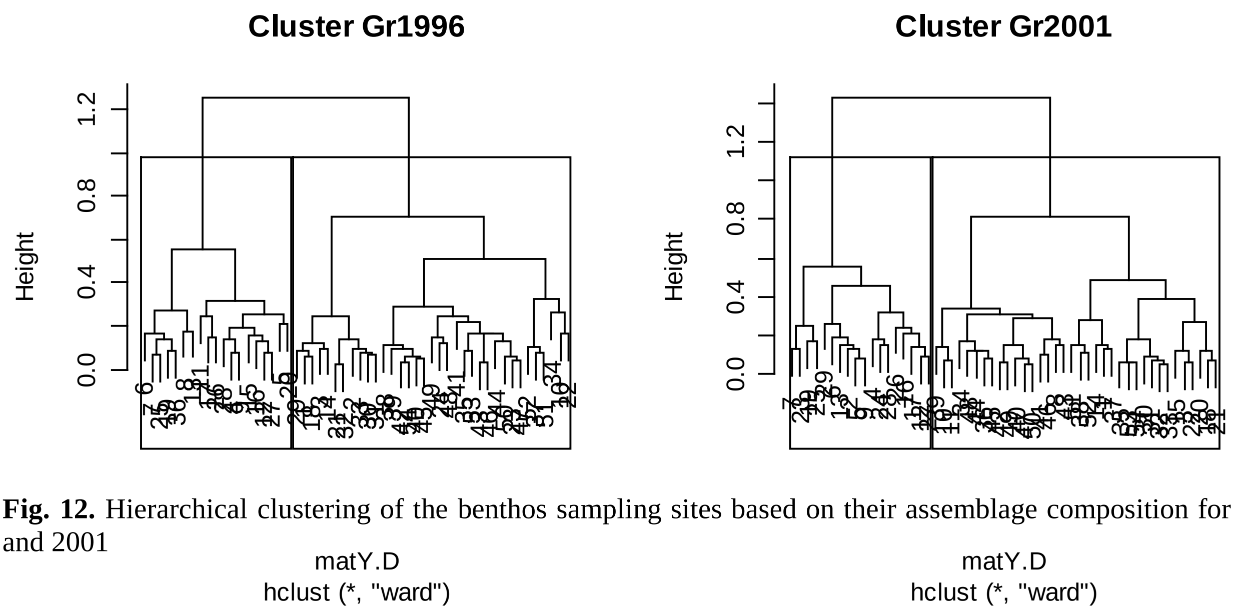


Fig. 12. Hierarchical clustering of the benthos sampling sites based on their assemblage composition for 1996 and 2001

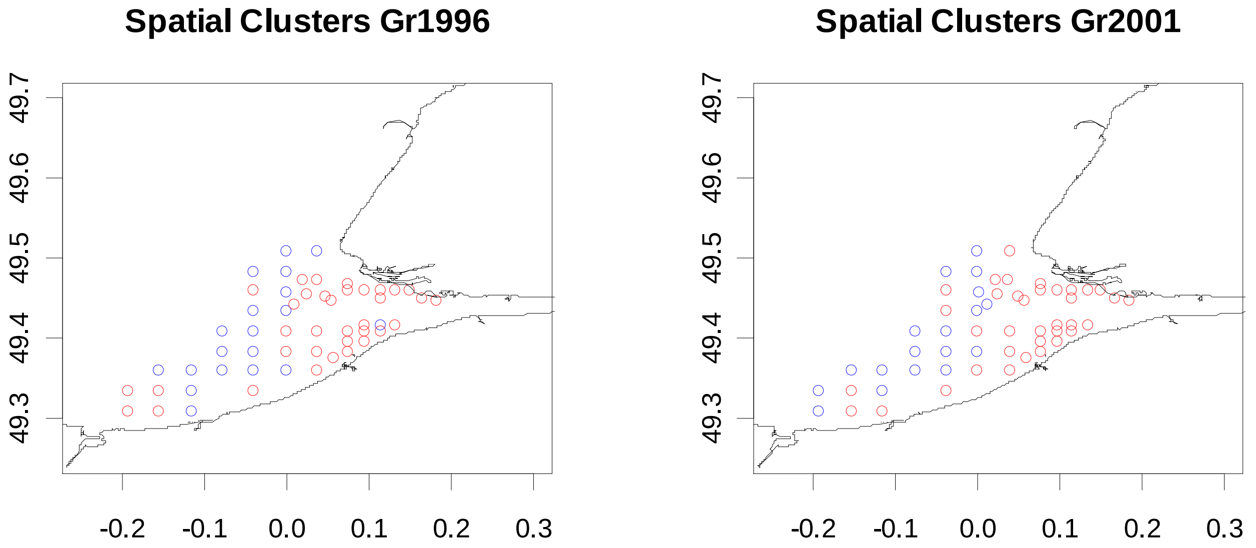


Fig. 13. Spatial representation of benthos assemblages clusters for 1996 and 2001

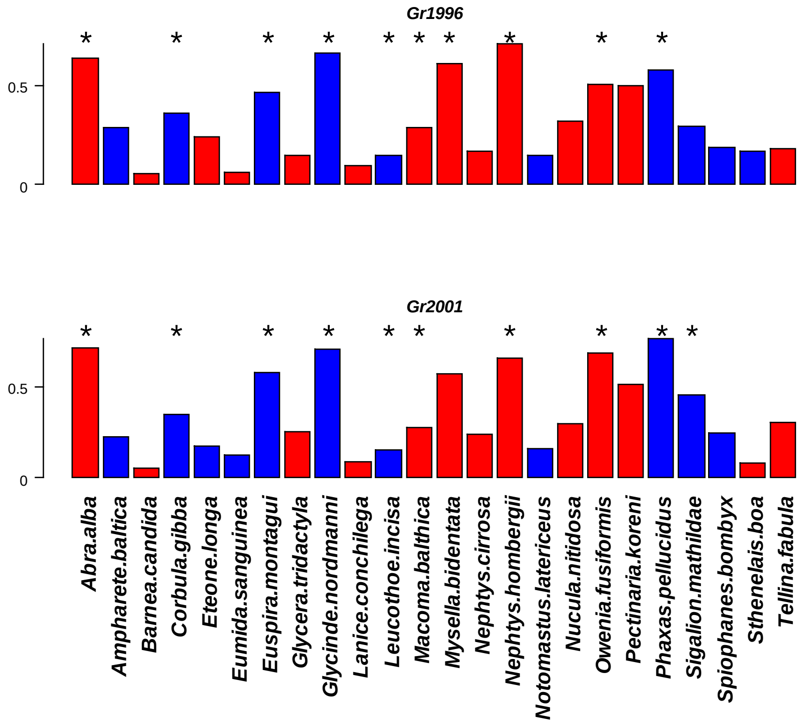


Fig. 14. Indicator taxa for each benthos assemblage cluster (same colour as above) for 1996 and 2001. Stars indicate significant indicator taxa (Monte Carlo randomisation test).

3.3 Spatial coupling between fish and benthos communities

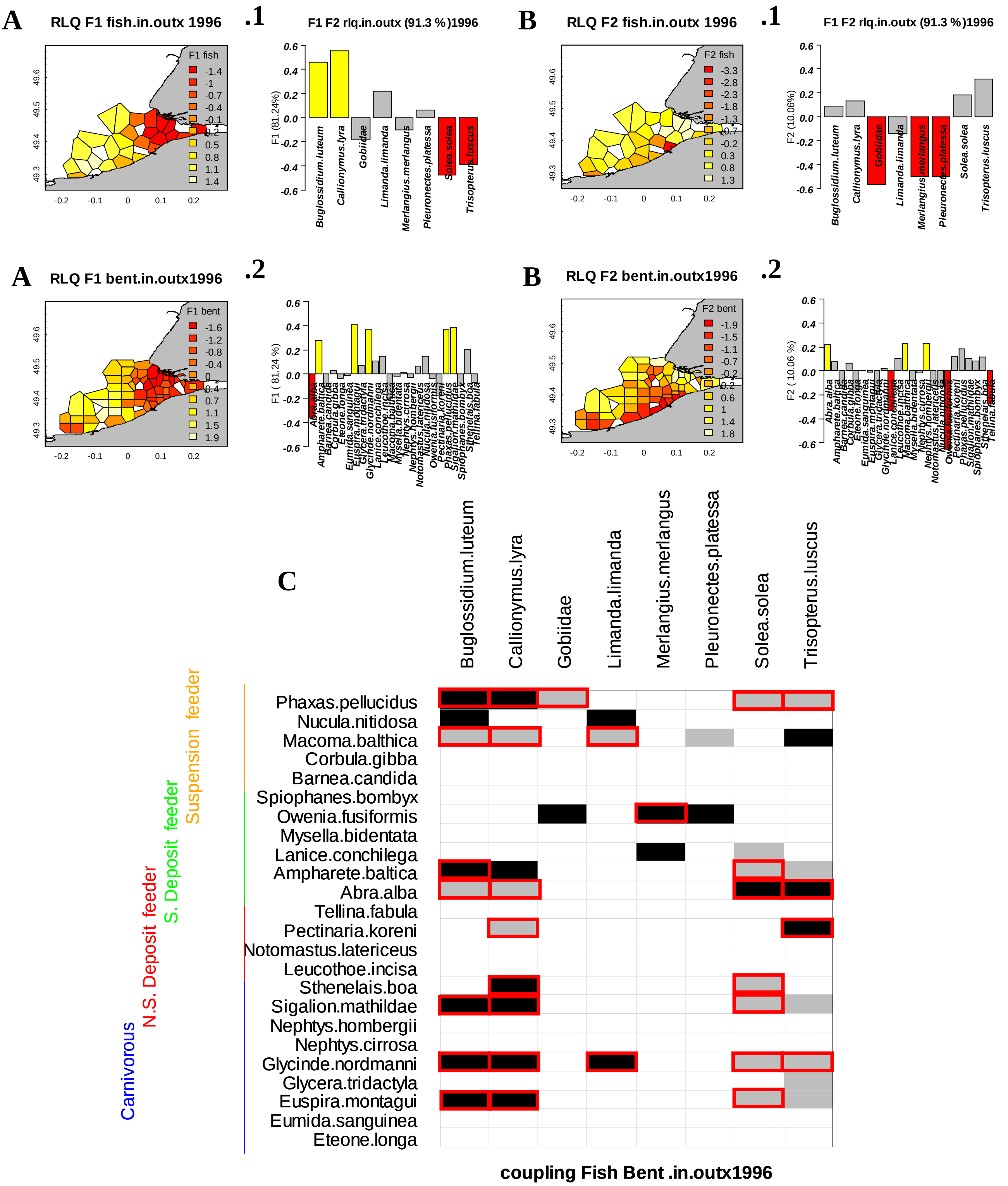
The methodology applied here, using a geometric approach (Delaunay and Voronoï tessellation) on original data points, allowed the spatial coupling between two different datasets (fish and benthos) with a relatively high overlap (see Fig. 8: intersection of around 78% of the total union between the two sets of polygons).

The results of the spatial RLQ and the fourth-corner analyses are consistent and very complementary to each other: the spatial RLQ is an ordination method enabling spatial representation of the coupling between the two datasets of interest (fish and benthos) on different axis with the score of each taxa (see Fig. 15 & 16, A & B); the fourth-corner gives relationship between individual taxa from each dataset with an associated statistic and probability (see Fig. 15 & 16 C). Generally the significant relationships found in the fourth-corner results table correspond to the association between fish and benthos taxa that have a significant contribution to the first two axis (see barplots in Fig. 15 & 16, A & B; positive relationship: same colour; negative relationship: different colour). However, since spatial RLQ is only a projection of the total inertia on two axes, more relationships are found with the fourth-corner and it is thus a better synthesis of the spatial relationships between the two data sets.

Results of both RLQ and the fourth-corner analyses demonstrated significant spatial relationship between the overall benthos and fish assemblages for both years. The first axis of the spatial RLQ (Fig. 15 & 16, A) highlighted changing communities along the inner/outer estuary gradient explaining more than 80% of the variability dispatched on the different axes. The second axis of the spatial RLQ (Fig. 15 & 16, B) highlighted an elongated patch located on the southern part of the Bay close to the shore but explained less than 10% of the variability dispatched on the different axes. The fourth-corner analyses highlighted numerous significant relationships between fish and their potential benthic preys distributed widely between the different trophic group of preys (Fig. 15 & 16, C).

The stability in fish and benthos assemblages found between the two years when analysing the spatio-temporal variability of each component individually was observed through the spatial coupling analysis. Numerous potential biological associations through trophic relationships were found in both years. However some other relationships were more variable and demonstrated temporal changes.

In this paper, we test the hypothesis of “trophic coupling” between bentho-demersal fish assemblages (mainly juveniles) and their potential macrobenthic preys along a time series in the Seine estuary. The overall ecological interpretation of the results will discussed in terms of potential trophic relationships and functional habitat for the presentation.



	Buglossidium.luteum	Callionymus.lyra	Gobiidae	Limanda.limanda	Merlangius.merlangus	Pleuronectes.platessa	Solea.solea	Trisopterus.luscus
Phaxas.pellucidus	**■**	**■**	**▨**				**▨**	**▨**
Nucula.nitidosa	■			■				
Macoma.balthica	**▨**	**▨**		**▨**		▨		■
Corbula.gibba								
Barnea.candida								
Spiophanes.bombyx								
Owenia.fusiformis			■		**■**	■		
Mysella.bidentata								
Lanice.conchilega					■		▨	
Ampharete.baltica	**■**	■					**▨**	▨
Abra.alba	**▨**	**▨**					**■**	**■**
Tellina.fabula								
Pectinaria.koreni		**▨**						**■**
Notomastus.latericeus								
Leucothoe.incisa								
Sthenelais.boa		**■**					**▨**	
Sigalion.mathildae	**■**	**■**					**▨**	▨
Nephtys.hombergii								
Nephtys.cirrosa								
Glycinde.nordmanni	**■**	**■**		**■**			**▨**	**▨**
Glycera.tridactyla								▨
Euspira.montagui	**■**	**■**					**▨**	▨
Eumida.sanguinea								
Eteone.longa								

Fig. 15. Spatial RLQ analysis showing spatial scores for Axis 1 (A) and Axis 2 (B) with scores of individual taxa (significant absolute contributions to the axis are coloured in the barplot) of fish (.1) and benthos (.2) 1996 datasets respectively. Fourth-corner results table (C) for 1996, showing significant (positive: black; negative: grey) spatial correlations between fish and benthos taxa (grouped by trophic groups in colour on the left side). Relationships found in both years 1996 and 2001 are highlighted in red.

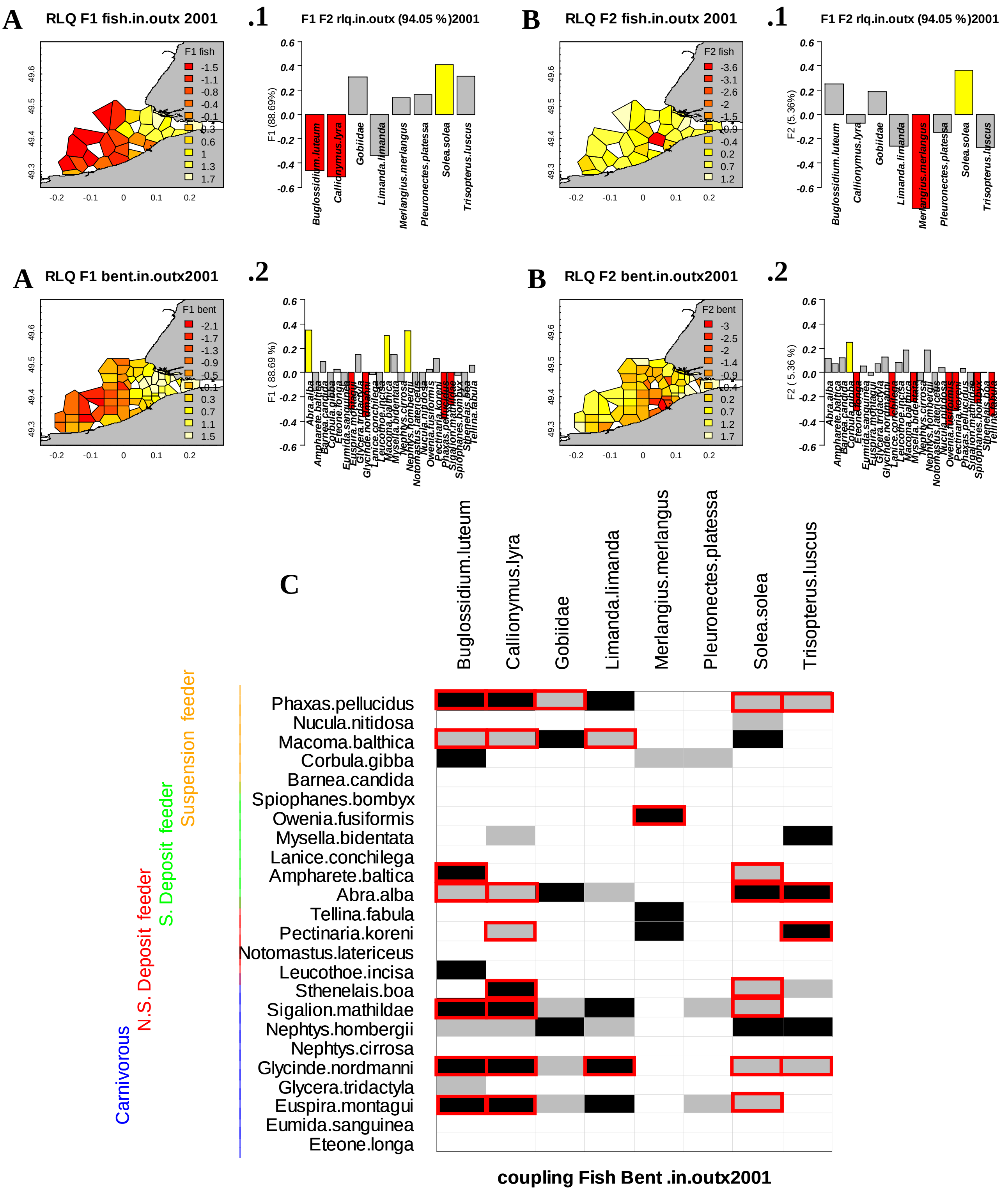


Fig. 16. Spatial RLQ analysis showing spatial scores for Axis 1 (A) and Axis 2 (B) with scores of individual taxa (significant absolute contributions to the axis are coloured in the barplot) of fish (.1) and benthos (.2) 2001 datasets respectively. Fourth-corner results table (C) for 2001, showing significant (positive: black; negative: grey) spatial correlations between fish and benthos taxa (grouped by trophic groups in colour on the left side). Relationships found in both years 1996 and 2001 are highlighted in red.

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