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# Are growth and density quantitative indicators of essential fish habitat quality? An application to the common sole *Solea solea* nursery grounds

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## Abstract

Bio-indicators were measured on juvenile fish to assess the quality of eight coastal and estuarine nursery grounds in the Eastern English Channel and in the Bay of Biscay during 3 years. Growth (size and otolith daily increment width), body condition (morphometric index) and abundance of juvenile common soles were analysed together with xenobiotic concentrations (heavy metals and organic contaminants).

Condition indices displayed important variations and did not allow relevant estimation of environmental quality. On the contrary, growth and density indicators showed good steadiness above years but varied among sites. In spite of difficulties of interpreting these indicators on such a meso-scale approach, analyses highlighted the estuaries of Seine and Gironde. In these nursery areas, the levels of contamination were especially high, and the combination of fish growth performances and density was significantly lower than in other sites. The combination of these variables appears to provide reliable indicators of habitat quality and anthropogenic pressure on nursery grounds, especially highlighting contaminated areas. Such indicators may thus contribute to improve assessment of environmental quality of essential fish habitats with the aim of a sustainable management of fisheries resources. A study at a different scale, from this meso-scale nursery approach with more precise analyses, on local habitats, will nevertheless be necessary to optimize the relevance of these indicators for the assessment of essential fish habitat quality.

**Keywords:** quantitative bio-indicators; essential fish habitat quality; flatfish juvenile

## 1. Introduction

As habitat destruction and/or damage to habitat quality can prevent fish stock from recovery (Hall, 1998), one of the main areas of emphasis in current ecological research is the conservation and enhancement of essential fish habitats. Nevertheless, worldwide increasing industrial development and human population growth have led to a rise in environmental damage and human pressure on marine habitats keeps strong. This is especially true in coastal and estuarine marine areas (Costanza et al., 1997) having an important function in fish stock renewal, as nursery grounds for commercially important marine fish species (Miller et al., 1984).

In these coastal habitats, fish can experience numerous stressors including unfavourable natural conditions (fluctuating temperature and salinity, changing substrate in response to river inputs...) in addition to anthropogenic stressors (chemical contamination, digging of channels, clapping of sediments ...). These factors can impose considerable stress to organisms (Wedemeyer et al., 1990). Assessment and monitoring of the quality of nursery areas should aim to prevent alteration and, thus, decline of fish populations (Meng et al., 2000). In this context, the European Union searches for biological indicators to assess environmental quality, and to implement the Habitat Directive (92/43/EC), the Water Framework Directive (2000/60/EC) and the Integrated Coastal Zone Management Program (European Commission, 2000).

On the northern and western coasts of France, and especially in the bay of Biscay and in the Eastern English Channel (Fig.1), inshore shallow waters and estuaries provide nursery areas for a wide variety of fish species and especially for commercially important flatfish (Riou et al., 2001; Le Pape et al., 2003a). The present study about young fish habitat quality focused on the common sole *S. solea*, which is one of the most important commercial species in these areas (Riou et al., 2001; Le Pape et al., 2003ab). Juvenile common sole are concentrated in coastal shallow nursery grounds (Riou et al., 2001; Le Pape et al., 2003a) and are sensitive to environmental changes. Young of the year sole have limited movements between nursery grounds (Koutsikopoulos et al., 1995; Riou et al., 2001), thus bio-indicator responses should properly reflect the quality of the different sampled nursery sites.

The goal of the present study was to find suitable bio-indicators measured on fish to assess the quality of nursery habitat along the coasts of the Eastern English Channel and the bay of Biscay, with a focus on sites heavily impacted by human activities such as the important estuaries of Seine, Loire and Gironde. High quality nursery habitats are those where growth and survival of juvenile fish are enhanced, and where ultimately reproductive potential is optimized (Gibson, 1994). Subsequently, growth, condition, and abundance of juvenile fishes have been used as relevant indicators of nursery habitat quality (Guindon and Miller, 1995; Able et al., 1999; Duffy-Anderson and Able, 1999; Phelan et al., 2000). Growth, body condition and abundances of juvenile fishes were compared to assess the habitat quality of different nursery areas. In the same time, indices of water contamination were used as indicators of human disturbances.

## 2. Materials and Methods

### 2.1 Studied sites and fish sampling

Coastal and estuarine nursery areas of the French coasts, in the bay of Biscay and in the Eastern English Channel (Fig. 1., Table 1) were investigated. The shore of the bay of Biscay is partitioned into open shallow muddy estuarine areas under the direct influence of freshwater inflows: the Vilaine (average river flow:  $91 \text{ m}^3 \cdot \text{s}^{-1}$ ), the Loire (average river flow:  $935 \text{ m}^3 \cdot \text{s}^{-1}$ ) and the Dordogne and Garonne (forming the Gironde estuary) (average river flow:  $780 \text{ m}^3 \cdot \text{s}^{-1}$ ), and semi-enclosed sheltered muddy marsh areas with shellfish-farming, little affected by rivers: the bay of Bourgneuf and the Pertuis Breton and Antioche. In the French part of the Eastern English Channel, sheltered coastal areas identified as nursery grounds are dominated by sandbanks and mudflats in bays and estuaries (Larsonneur et al., 1982). The Seine estuary represents the main freshwater inflow ( $400 \text{ m}^3 \cdot \text{s}^{-1}$ ), and contributes with other smaller rivers (i.e. Somme) to a general decrease in the salinity of coastal waters.

During surveys in September 2000, 2001 and 2002, sole juveniles were collected in 6 nursery sites in the bay of Biscay and in 2 nursery sites in the Eastern English Channel (Fig. 1, Table 1). Autumn was chosen because it corresponds to the end of the growing season of juvenile sole on nursery grounds, which allow better assessments of growth rates at a period when YoY (Young of the Year) fish have not moved since their settlement (Dorel et al., 1991). Moreover, problems of selectivity and size-dependent catchability for trawling, hence bias in estimate of YoY density and size, very important in early summer, are limited at the end of the growing period (Riou et al., 2001). Stratified surveys were carried out with a 3 m beam trawl in depths from 5 to 25 m at each site. This net ( $20 \cdot 10^{-3}$  m stretched mesh in the codend) has been found to introduce no bias in length selectivity during surveys in September (Riou et al., 2001). Depth and sediment were homogeneous for each nursery sector, in each strata (Riou et al., 2001; Le Pape et al, 2003b). An average of 20 hauls were made at each site with duration of 20 min and mean area swept of 4500 to 5000  $\text{m}^2$  per haul. All 0-group and 1-group sole juveniles caught were counted and measured for total and standard length (TL and SL in cm). At each site, a subsample of 30 0-group individuals, was immediately frozen at  $-20^\circ \text{C}$ , for laboratory analyses of recent growth and condition. The identification of 0 and 1-group was performed using length-frequency analysis and confirmed from examination of otoliths.

### 2.2 Data on the chemical contamination of coastal waters

The French national network for the observation of the quality of the marine environment (RNO, French ministry of the Environment, see details and process on <http://www.ifremer.fr/envlit/surveillance/rno.htm>) has been collecting and analysing the whole soft tissue of 2 mollusks: mussels *Mytilus edulis* and oysters *Crassostrea gigas* since 1979, to estimate the levels of chemical concentration in coastal waters (Claisse, 1989). Chemicals measured are heavy metals (Cd, Hg, Pb, Cu, and Zn), pesticides gamma-hexachlorocyclohexane ( $\gamma$ -HCH or lindane) and 4,4 DDT and its metabolites 4,4 DDD and 4,4 DDE (measurements for the three latter are on their sum:  $\Sigma$ DDT), the concentration of a polychlorobiphenyle PCB (CB153) and the concentration of 15<sup>1</sup> polycyclic aromatic hydrocarbons (PAHs).

All the studied nursery sectors correspond to a sampled site investigated by this network. In addition, in the RNO monitoring program, stations are selected to be representative of their surroundings, and point sources of chemical contamination are deliberately avoided (Beliaeff

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<sup>1</sup> Naphtalene, Acenaphtene, Fluorene, Phenantrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Benzo(g,h,i)perylene, Indeno(1,2,3-cd)pyrene.

et al., 1998; Beliaeff et al., 2002). Hence, we considered that each sampled station used in this network is representative of the water contamination of the considered nursery ground. Among the 8 study sites used in the comparisons, 4 (Somme, Seine, Vilaine, Loire) are nursery areas where the mussel *M. Edulis* was collected and 4 (Bourgneuf, Pertuis Breton, Pertuis Antioche, Gironde) are nursery grounds from which the oyster *C. gigas* was taken. The measures between the 2 species are standardized and expressed in mussels species equivalent (O'Connor, 1992).

In the present study, as the RNO program do not yet provide data for the years after 2000 for all chemical compounds, we standardized the chemical data set on the years 1995 to 2000.

The sampling strategy of the RNO program vary between the different chemical compounds and makes it impossible to estimate concentrations of all chemical compounds in a same period or season in the year. In addition, juvenile soles were shown to stay all year long in the nursery areas in the Eastern English Channel (Riou et al., 2001) and at least 6 months in the bay of Biscay (Koutsikopoulos et al., 1989; Dorel et al., 1991). Hence, comparisons of chemical contamination were made using data over the whole year, which means that we did not focus on a particular season of the year.

## **2.3 Biological indicators**

### **2.3.1 Indicators of growth**

#### *Recent growth of 0-group juveniles (RG)*

The recent growth index was determined by measuring the width of the peripheral daily increments of the otoliths (distance between the margin of the otolith back to the 10th ring), to estimate fish growth during the previous 10 days. Sagittal otoliths were used for the microstructure analysis. Daily increment deposition of common sole sagittal otolith was validated on larval stages by Lagardère (1989) and on juvenile stages by Lagardère et al., (unpublished data). The use of the width of otolith daily increments is justified when a linear relationship between the size of the fish and the size of the otolith exists (Campana and Neilson, 1985), which has been verified for 0-group sole (Gilliers et al., 2006). The preparation and the measurements of the otoliths are detailed in Gilliers et al. (2004).

#### *Length of 0-group (L0) and 1-group (L1) juveniles*

Mean total length (cm) was computed at each site for each year, from the catches of 0-group and 1-group sole captured during the surveys.

### **2.3.2 Condition of 0-group juveniles (K0)**

Two condition indices: Fulton's K condition index and R relative condition index, were computed on 0-group juveniles:

$$K = 100 (W.L^{-3}),$$

where W is the body weight (mg) and L is the standard length (mm) which was used to take damages to the caudal fin rays into account.

$$R (mg) = \log W - \log Wc$$

where W(mg) is the observed body weight and Wc (mg) is the computed body weight derived from the logged length-weight relationships of the sample (Blackwell et al., 2000) (i.e. the deviation of predicted weight estimated from the common logged length-weight relationship in terms of fish general condition).

### **2.3.3 Densities of 0-group (D0) and 1-group (D1) juveniles**

For a given nursery ground and a given year, on each stratum, estimated density was multiplied by area size, and the total density was estimated on the weighted sum of these four

strata (Pennington and Grosslein, 1977). Log-transformed densities ( $\text{ind. ha}^{-1}$ ), computed from stratified sampling, of 0- and 1-group individuals have been used as biological indicators.

## **2.4 Data analyses**

### **2.4.1 Development of indicators of the chemical contamination**

Comparisons between sites are based on median values because unlike means, medians are robust with regard to both outliers and concentrations below detection. These medians, based on the whole series of measures from 1995 to 2000, were first computed for each chemical (Cd, Hg, Pb, Cu, Zn,  $\Sigma$ DDT, lindane, PCB, PAH) to obtain a single indicator of pollution by nursery and by contaminant. These medians are considered as indicators of contamination for the study period, as data were not available to provide yearly index of contamination by sector in 2001 and 2002. As spatial variability is very significantly higher than interannual variability, this method appeared reliable to discriminate nursery sectors with regards to differences in contamination (Beliaeff et al., 1998). Claisse and Beliaeff (2000) underlined that statistical results are perturbed by irregular sampling from one year to another and from one chemical compound to another. From a general point of view, few significant increasing or decreasing trends are pointed out in the analyses of the time series. This method appears reliable to provide integrated descriptors based on several years of measurement to compare different coastal sectors .

Then, to synthesize the information, we computed indices of overall contamination of coastal waters. Principal Components Analyses (PCA) were undertaken with the contaminants. The 5 heavy metals (Cd, Hg, Pb, Cu, Zn) and the 4 organic chemicals ( $\Sigma$ DDT, lindane, PCB, PAH) were used as variables and the 8 nursery sectors as individuals (rows).

### **2.4.2 Analyses of growth and density indices**

To analyse a standardized dataset, a single value was used per year and per nursery ground for each indicator: the mean value of L0, L1, D0 and D1, computed from the total catches of the individuals, and the mean value of K, R and RG computed from the analysed samples of 0-groups (30 fish). Inter and co-relationships between the 6 bio-indicators and the 8 studied nursery grounds were investigated with Multiple Factor Analysis MFA (Escofier and Pagès, 1994; 1998). The 6 bio-indicators were used as variables (columns), the sites were undertaken as observations (rows); and the year as group of variables (one case of this multi-table is filled with a mean value of 1 indicator for 1 nursery site on 1 year). Instead of using three PCA for each group of variables (one per year), the use of a MFA allows an exploration of the entire data set and highlights relationships between groups of variables.

Overall indices of contamination of coastal waters, obtained separately on PCAs, cf 3.1.), were not taking into account in the MFA analysis but were analysed as supplementary variables.

### 3. Results

#### 3.1 Indicators of overall contamination of coastal waters

Comparisons between annual medians for all the chemical compounds were carried out on the chemical data set using non-parametric Kruskal-Wallis tests to take into account the variability of the sample sizes (due to the irregularity of sampling). Few significant differences between years we found ( $p < 0.05$  in the Loire estuary for Pb and Zn) and no trends were pointed out (Table 1). Hence, to sum up the chemical set including 9 types of contaminants for 8 sites, general indices of water contamination by organic and metal contaminants were computed.

A first PCA (Fig. 2) has showed that, when the 9 contaminants were included all together as variables in a single analysis, the correlations between the organic and the metal compounds were too low to synthesize general indicators of contamination from the principal components, especially because of high discrepancies in spatial trends between organic and metallic contaminants between the different nursery sectors (Fig. 2, Table 2). Nevertheless, it appeared that the 4 organic contaminants were highly correlated with each other, while the 5 heavy metals were highly to moderately correlated to each other (Table 2).

Hence, two separate PCAs were led (Fig. 3, Table 3): one for heavy metals (PCA1) and a second for organic chemicals (PCA2). Two different indicators of contamination were developed: one for heavy metals and the second for organic chemicals. For heavy metals, the first principal component (PC1) explained 67.34% of the total variation. Except for Zn, heavy metals were well and significantly correlated each others (Table 2) and with the first factor of the analysis, (Table 3, Fig. 3). Thus, it was possible to use the 5 variables to synthesize contamination by heavy metals in the 8 nursery areas. Subsequently, coordinates of each site on (PC1) were used as overall index of heavy metals contamination (HM) in the the coastal study sites.

Concerning organics chemicals, PC2 explained 96.62% of the total variation of the PCA (Fig. 3) and the first factor was highly correlated with the 4 contaminants (Table 3, Fig.3). Organic contaminants were strongly and positively correlated each others (Table 2). These results clearly indicated that the organic contamination of water could be synthesized using these 4 variables. Subsequently, we used the coordinates of each site on PC2 as overall index of organic chemicals contamination (OC) in the coastal study sites.

Two sites were pointed out showing high levels, but different typology, of contamination in coastal waters. The Gironde estuary was characterized by high levels of heavy metals whereas the Seine estuary was characterized by high levels of organic chemicals (Fig. 2).

#### 3.2 Multiple Factor Analysis Results

The two first axis of the MFA allowed to explain 54.32 % of the global inertia in the data (respectively 30.18% and 24.14% of the total variation). According to this plan (Fig. 4), there was an important structure in the data with high correlation between variables inside several pools.

The first result of this MFA was that temporal variability appeared to be less important than spatial variability with regards to growth and density variables (Fig. 4). Except for K and R, the position of the variables kept very steady from a year to another on this plan, indicating the good steadiness above years of the structure in variables and the difference between nursery sectors. The first axis of the MFA (F1) appeared to reflect mainly the variation of growth variables, notably the recent growth of 0-group juveniles (RG) (Fig. 4). Length of 1-group (L1) and length of 0-group (L0) also mainly contributed to this axis, except for the year 2000. Densities of 1-group juveniles (D1) mainly accounted for the second axis (F2), whereas densities of 0-group juveniles (D0) contributed moderately to the 2 first two factors.

Hence, F2 could be identified as a density pattern mainly reflecting 1-group juveniles' abundances. The variables of condition (K) and (R) displayed a contribution varying between F1 and F2 among years and did not mainly contribute to one particular axis. Partial and mean observations (as mean value of indicator per site) appeared geographically structured from north to south along F1 that could be identified as a pattern of juvenile soles growth (Fig. 4). This was particularly obvious for the study sites in the bay of Biscay.

Indicators of contamination, illustrative in the analysis, appeared opposed to the variables of growth and densities (Fig. 4). HM was mainly and inversely related to F1 whereas OC, was mainly related to F2 and inversely related to densities.

Two sites were pointed out by the analyses: the bay of Seine and the estuary of Gironde, which are the two most contaminated areas. Common sole juveniles sampled in these two sites seemed to display lower biological performances in term of juvenile sole growth and abundance compared to the other nursery areas (Fig. 4).

### **3.3 Relationships between bio-indicators and environmental indicators**

Biological indicators were moderately related to each other (Table 4). On the contrary, some biological indicators were well related to environmental indicators: RG and L1 were inversely related to HM, while L0 was inversely related to OC. D0 and D1 also displayed moderate negative but significant relationships with this latter indicator of water contamination.



## **4. Discussion**

### **4.1 Reliability of indicators, differences between populations and geographical gradients**

The interpretation of different indicators is complex and requires cautiousness. In the case of condition indices, as Fulton's K condition index has been criticized for assuming isometric growth and being size-dependent (Suthers, 1998), a second index R, potentially robust and unbiased by size effect, was computed from length-weight residuals. Even if they are correlated, these two morphometric indicators showed relatively different patterns. In addition, they displayed important interannual variations. Variations in condition have been related to the quality and the quantity of food (Lambert and Dutil, 1997) and may vary within weeks. Reflecting feeding conditions on the mid-term and varying upon seasons, morphometric type indices appeared not to be integrative enough to establish a relevant diagnostic of fish condition for the period of the study. In addition, 0-group juvenile soles from Eastern Channel (bay of Seine and Somme but also from other northern sites) displayed systematically higher values than individuals from the bay of Biscay (Gilliers, pers. comm.). Lloret et al. (2002) also demonstrated spatial –effect of latitude- and temporal variability of this type of condition indices measured on demersal fish in the north-western mediterranean. Hence, condition indices did not appear appropriate to develop standardized integrative indicators of habitat quality, in the case of this study at least. These bio-indicators may reflect differences due to stock variability or latitudinal gradients, which may mask the estimate of habitat quality.

On the contrary, spatial comparisons of bio-indicators based on the growth of juvenile soles revealed a spatial heterogeneity. Unlike condition factors, the interpretation of their responses and the diagnostic of quality based on them is facilitated by their good steadiness above years. Juveniles from the Seine estuary and from the 3 southern nurseries presented the lowest growth performances; this was especially obvious in the Gironde estuary and in the Pertuis Antioche. Actually, in such a study, when differences in habitat quality are highlighted, it is necessary to determine the respective influences of variations in natural factors and human-induced changes. In a previous study in the bay of Biscay (Gilliers et al., 2006) concluded that these differences in 0- group soles growth were not probably related to genetic heterogeneity (Exadactylos et al., 2001), to variations in ages (Arbault et al., 1986), nor to temperatures between sites (Le Pape et al., 2003b). Hence, this spatial heterogeneity seems not explained by these natural factors and should be examined with regards to the same north-south differences in chemical contamination. Nevertheless, discriminating natural or anthropogenic factors in the assessment of quality of habitats requires cautiousness and still remains difficult, as these different factors probably act conjointly.

### **4.2 Declining biological performances: influence of anthropogenic disturbances?**

The examination of the results allowed to find a relation between the level of contamination and some indicators of growth and density. When these results were investigated thoroughly, it appeared that these relations came especially from two nursery areas: the Seine and the Gironde, where the coastal water pollution appeared to be related to low biological performances. Though, it is a large estuary characterized by an important water inflows and industrial and harbour activities, we did not find any signs of reduced biological performances in juvenile soles sampled in the nursery areas of the Loire estuary. In addition, the levels of contamination as indicated by the 2 indicators of water contamination did not reached the levels of the Gironde and the Seine estuaries.

These results on Seine and Gironde estuary were not unexpected, as pollutants can impose considerable stress and impairments on the health of fishes. A growing number of studies have attempted to establish linkages between pollutants and fish health. Eastwood and

Couture (2002) have showed that individuals of yellow perch (*Perca flavescens*) from metal-contaminated environment displayed higher metallic concentrations in their tissues but also lower physiological condition and slower growth rates than those from less-contaminated environment. Forrester et al. (2003) revealed possible association between the declining growth rates of a benthic fish (*Gillichthys mirabilis*) and combined concentration of multiple sediment contaminants from different estuaries. In the bay of Biscay, Marchand et al. (2003) found declining genetic variability, probably resulting from chemical stress, of European flounder *Platichthys flesus* populations in contaminated estuaries of Vilaine, Loire, and Gironde compared with a northern estuary assumed to be a reference site in terms of anthropogenic pollution. They also suggested that genotypic and phenotypic responses were specific of the contamination typology (metals or organic chemicals).

A focus on the Seine and the Gironde sectors allowed investigating the consequences of human disturbances on the nursery function. According to RNO data between 1995-2000, these estuarine sites, that provide important nurseries for juvenile flatfishes, appeared heavily impacted with regards to water contamination. The Gironde is characterized by significant levels of trace metals (for example, cadmium concentrations in molluscs are of 27.35 mg.kg<sup>-1</sup> versus 0.729 to 3.07 mg.kg<sup>-1</sup> for all the other sites); whereas the Seine is characterized by significant levels of organic chemicals (PCB concentrations in molluscs are 10 times higher: 321 µg.kg<sup>-1</sup> in Seine than in the other sites 26.20 to 55.65 µg.kg<sup>-1</sup>). Nevertheless, these 2 sites belong to two distinct ecosystems: the Eastern English Channel and the bay of Biscay, and contribute to two distinct stocks of sole. As differences in the biological indicators may be related to different factors from a system to another, it is necessary to consider separately these two highlighted sites to analyze the reasons of their low biological performances.

#### **4.2.1 The Seine estuary**

It seems unlikely that the low biological performances of juveniles caught in the Seine estuary (very low abundances, low growth performances) should result from differences in water temperature or food availability, which are the two main natural factors governing the habitat quality (Gibson, 1994). In the Eastern English Channel, hydrological conditions, such as temperature and salinity, are assumed to be homogeneous along the coastline (Riou et al., 2001). In addition, the seabed environment supports an abundant distribution of benthic fauna (i.e. potential preys; Desroy et al., 2003) and food is constantly available, unlimiting for growth of 0-group soles (Amara et al., 2001). This suggests that lower growth performances and abundance of juvenile soles in the Seine estuary may probably be due to other factors directly related to human activities such as contamination. Our results confirmed those from previous works conducted in the Seine estuary and may help to establish linkages between pollution, biological performances of fishes and habitat quality. A recent work, carried out on the same nursery grounds, has allowed to demonstrate that concentrations of organic contaminants in the liver of the young soles were ten times higher in the bay of Seine (500 ng.g<sup>-1</sup> of 153CB) compared to the other sites (from 135 to 25 ng.g<sup>-1</sup> of 153CB) (Loizeau, unpublished data). Riou et al. (2001) underlined that the bay of Seine, surprisingly displayed low amounts of juvenile sole and plaice despite a potential important carrying capacity as nursery area. Finally, Gilliers (pers.comm.) has showed that indices of condition, growth rates and lipid contents were lower for 0-group juvenile soles from the Seine estuary compared with individuals from other sites in the Eastern Channel (bay of Somme, Canche and Authie estuaries). Hence, we suggest that the Seine estuary provides nursery habitat of lower quality probably due to anthropogenic disturbances and especially pollution impact.

#### **4.2.2 The Gironde estuary**

In term of food limitation, unlike growth variables, the condition parameter K, which has been identified as a good indicator of nutritional condition and lipid reserves (Lambert and Dutil, 1997), did not display differences from Loire to Gironde estuary (Gilliers et al., 2006; this study). This may suggest that nutritional conditions are equal on these nursery areas and confirmed previous results showing that juvenile soles were above the food deprivation threshold in the nursery grounds of the bay of Biscay (Gilliers et al., in press). Hence, despite low negative correlations between parameter of condition and abundances, our results did not provide evidence for density-dependant effects on juvenile sole growth in the southern bay of Biscay at the scale of this study. Nevertheless, as interannual variations of abundances and of condition indices are known to be important, cautiousness is required in the interpretation of such variables.

On the other hand, as described above, the Dordogne-Garonne fluvial system discharges considerable inputs of heavy metals and notably cadmium into the Gironde estuary. The “French mussels watch program” (Claisse, 1989) reported cadmium concentrations in oysters that were about ten-times higher than those found in oysters from French non-industrial areas (Boutier, 1981; Table 1). As a result, the Gironde estuary has been classified as “zone D” by the European Community, prohibiting the sale of mussels and oyster of this estuary. Despite important remediation efforts, heavy metals inputs remain high (e.g. Blanc et al., 1999; cited by Audry et al., 2004). Studies have demonstrated that this contamination is not restricted to the Gironde estuary and spread towards the north, resulting in high levels of metals in the oyster of sites located in the north, as the Pertuis d’Antioche (Boutier et al., 2000). Multiple studies have shown that fish living in metal-polluted environment experienced adverse effects ranging from severe tissue (liver, gill etc...) lesions (Schwaiger et al., 1997) to greater bioenergetics costs (Sherwood et al., 2000) that may affect the multiple levels of the biological organisation such as the whole fish community. Cadmium is one of the more toxic heavy metals (Bryan, 1984) and it has been demonstrated that long-term exposure to this heavy metal reduces the growth and affects the survival of various freshwater and marine fish species (Eaton et al., 1978; Miliou et al., 1998; Hansen et al., 2002). Despite of these numerous studies that allow understanding the role played by pollution on growth and survival of fish, our ability to demonstrate such linkages between growth variables and heavy metals pollution in the bay of Biscay is still limited. At the stage of our investigations, we cannot conclude about the causes of the north-south pattern of growth of common sole juveniles soles, therefore our results could provide a possible explanation for the lower growth performances found in the Gironde estuary.

#### **4.3 The interest of the approach**

Few studies have attempted to use simultaneously a pool of bio-indicators measured on fish both at the individual (growth, size, condition) and at the population (abundances) levels to assess the quality of essential fish habitats on a mesoscale approach. Actually, several authors recommend the use of multiple indicators approach (Suthers et al., 1992; Mc Cormick and Molony, 1993), as no single measure may provide an overall description of habitat quality. Finally, integrating the responses of young fish to a multitude of natural and man-made stressors requires building up a new category of composite indicators. As proposed by Neill et al. (1994), a conceptual framework is necessary to identify the ecophysiological impacts of environmental factors. More than lethal effects, the loss of adaptative capacity may reduce the metabolic scope of fishes and thus the population size in altered habitats.

Finally, if this study demonstrate that growth and density indicators can provide useful tools for the assessment of heavy anthropogenic impact and habitat quality at mesoscale, it also suggests that it would probably be pertinent to work on a more precise scale with the same methods to increase their sensivity and their usefulness, especially to avoid problems related

to geographical gradients and to estimate eventual differences due to anthropogenic perturbations on habitat at a local scale.

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Table 1. Study sites with annual median concentrations (1995-2000) in  $\mu\text{g.g}^{-1}$  of dry weight for metals and  $\text{ng.g}^{-1}$  of dry weight for organic chemicals in bivalves from the RNO program.

			Human activity	Heavy metals					Organic chemicals			
				Cd	Cu	Hg	Pb	Zn	$\Sigma\text{DDT}$	Lindane	PCB	HAP
<b>1</b>	<b>Somme</b>	estuary	moderate	0.73	187.2	0.046	0.30	2989	4.9	3.2	31.8	161.6
<b>2</b>	<b>Seine</b>	large estuary	high	2.40	257.6	0.10	1.04	4013.8	28.4	6	321	385
<b>3</b>	<b>Vilaine</b>	bay	moderate	0.91	211.2	0.06	0.37	3885.7	3.1	3.2	26.4	129.8
<b>4</b>	<b>Loire</b>	large estuary	moderate	2.30	240	0.1	0.68	6191.5	5.35	2.45	55.65	137.2
<b>5</b>	<b>Bourgneuf</b>	bay	moderate	1.14	114.15	0.2	1.05	1563	3.1	3.1	30.7	138.5
<b>6</b>	<b>P.Breton</b>	bay	moderate	2.31	239.2	0.21	1.4	2297	6.4	2.35	26.7	115.9
<b>7</b>	<b>P.Antioche</b>	bay	moderate	3.07	353.15	0.25	1.45	2880.5	5	2.2	26.2	137.2
<b>8</b>	<b>Gironde</b>	large estuary	high	27.35	1275.5	0.24	2.45	5490	6.95	2.7	53	123.25

Table 2 : Pearson's correlation coefficients between the 9 variables as contaminants  $\Sigma$ DDT, lindane, PCB, PAH, Cd, Cu, Hg, Pb, Zn  
 significant correlation for \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

	$\Sigma$ DDT	lindane	PCB	PAH	Cd	Cu	Hg	Pb	Zn
$\Sigma$ DDT	1								
lindane	0.90**	1							
PCB	0.99***	0.93***	1						
PAH	0.97***	0.96***	0.98***	1					
Cd	-0.01	-0.15	-0.05	-0.17	1				
Cu	0	-0.16	-0.04	-0.17	0.99***	1			
Hg	-0.19	-0.44	-0.26	-0.34	0.48	0.48	1		
Pb	0.08	-0.19	0.01	-0.12	0.83**	0.83**	0.85**	1	
Zn	0.16	0.02	0.19	0.07	0.49	0.51	-0.21	0.15	1

Table 3: Correlations between variables and factors (1 to 4) from PCA1 -computed with heavy metals (Cd, Cu, Hg, Pb, Zn) as variables- and from PCA2 - computed with organic chemicals ( $\Sigma$ DDT, lindane, PCB, PAH) as variables-.

PCA factors		1	2	3	4
PCA1	Cd	0.96	0.19	0.21	0.03
	Cu	0.96	0.21	0.18	0.04
	Hg	0.69	-0.67	-0.26	0.09
	Pb	0.95	-0.29	-0.07	-0.14
	Zn	0.40	0.89	-0.32	0.00
PCA2	$\Sigma$ DDT	-0.98	0.18	-0.04	0.05
	lindane	-0.96	-0.26	-0.05	0.01
	PCB	-0.99	0.10	-0.03	-0.07
	PAH	-0.99	-0.02	0.11	0.01

Table 4. Pearson's correlation coefficients between the bio-indicators (mean values per sites for each year) and the indices of overall contamination of coastal waters by organic chemicals and heavy metals.

K: Fulton condition index  $K$  ( $\text{mg}\cdot\text{mm}^{-3}$ ) of 0-group juveniles of *Solea solea*; RG: recent growth index of 0-group juveniles of *Solea solea* ( $\mu\text{m}$ ), L0 and L1 total length (cm), D0 and D1 log-transformed densities ( $\text{ind}\cdot\text{ha}^{-1}$ ) of all the 0-group and 1- group juveniles of *S. solea* caught during the surveys; OC : Organic Contaminants and HM Heavy Metals.

significant correlation for \*  $p < 0.05$ , \*\*  $p < 0.01$

	K	R	RG	L0	L1	D0	D1	OC	HM
K	-	0.48*	-0.028	-0.160	0.202	-0.350	-0.105	0.209	-0.286
R		-	0.06	0.42*	0.27	-0.07	-0.17	-0.17	-0.09
RG			-	0.210	0.281	0.198	-0.009	0.115	-0.670**
L0				-	0.148	0.322	0.493*	-0.734**	-0.179
L1					-	0.240	-0.183	0.301	-0.524**
D0						-	0.316	-0.317	-0.112
D1							-	-0.328	-0.144

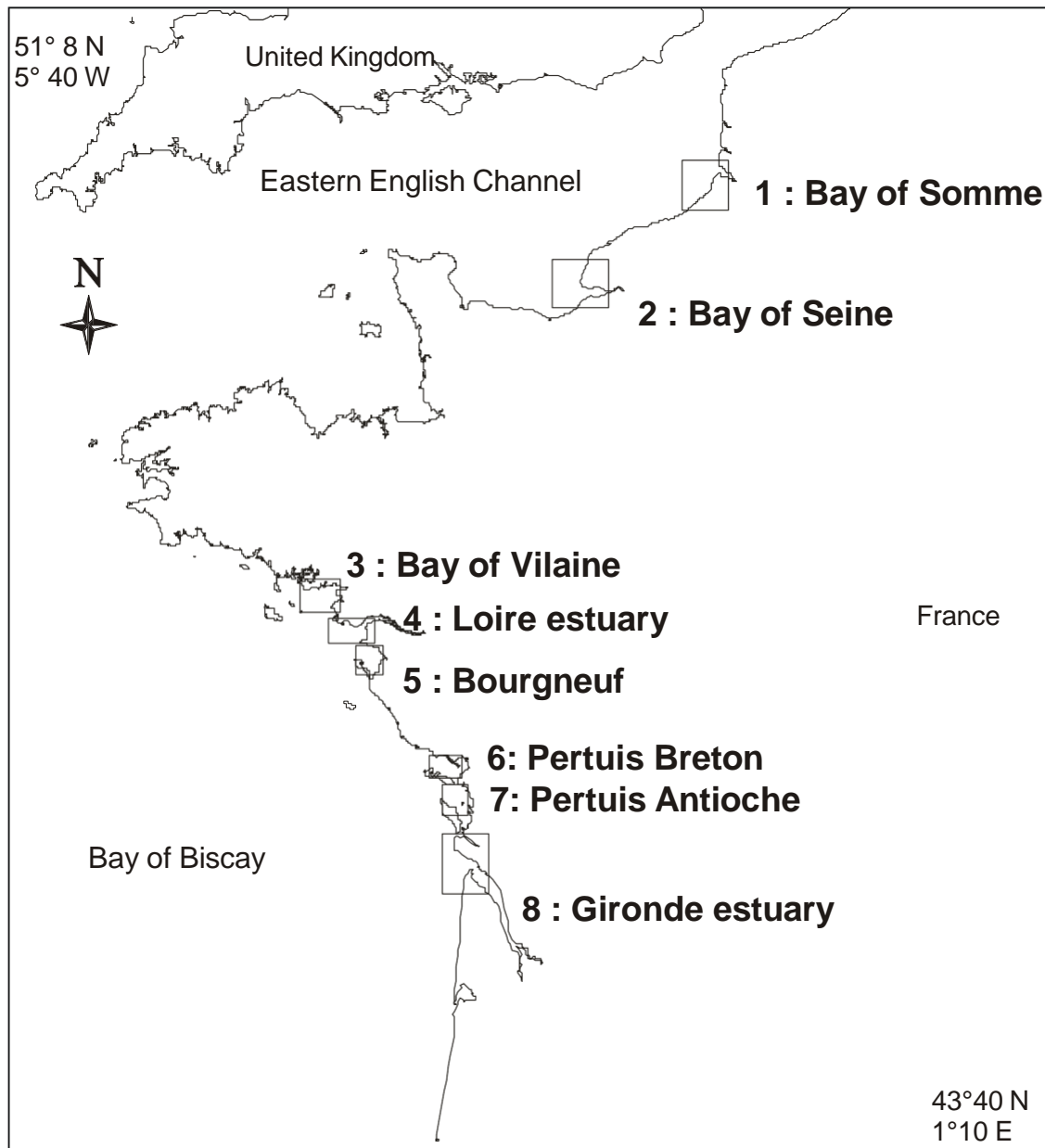


Figure 1. Sampled nursery sites in the Eastern Channel and in the bay of Biscay.

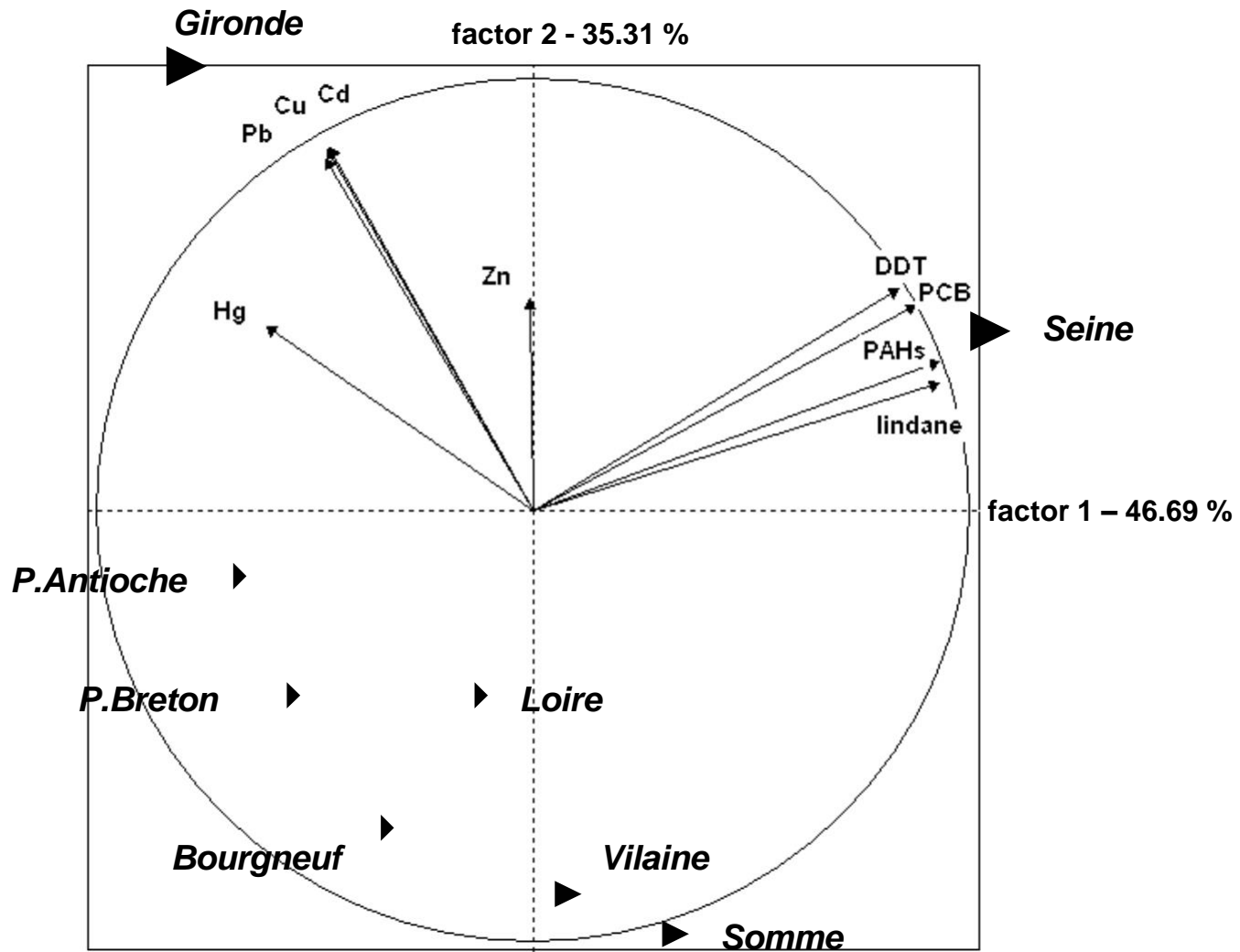


Figure 2. Correlation circle for the ordination of the 9 quantitative variables as heavy metals (Cu, Cd, Zn, Hg, Pb) and organic chemicals (DDT stands for  $\Sigma$ DDT, lindane, PCB, PAH) on the main factorial plan of the Principal Component Analysis.

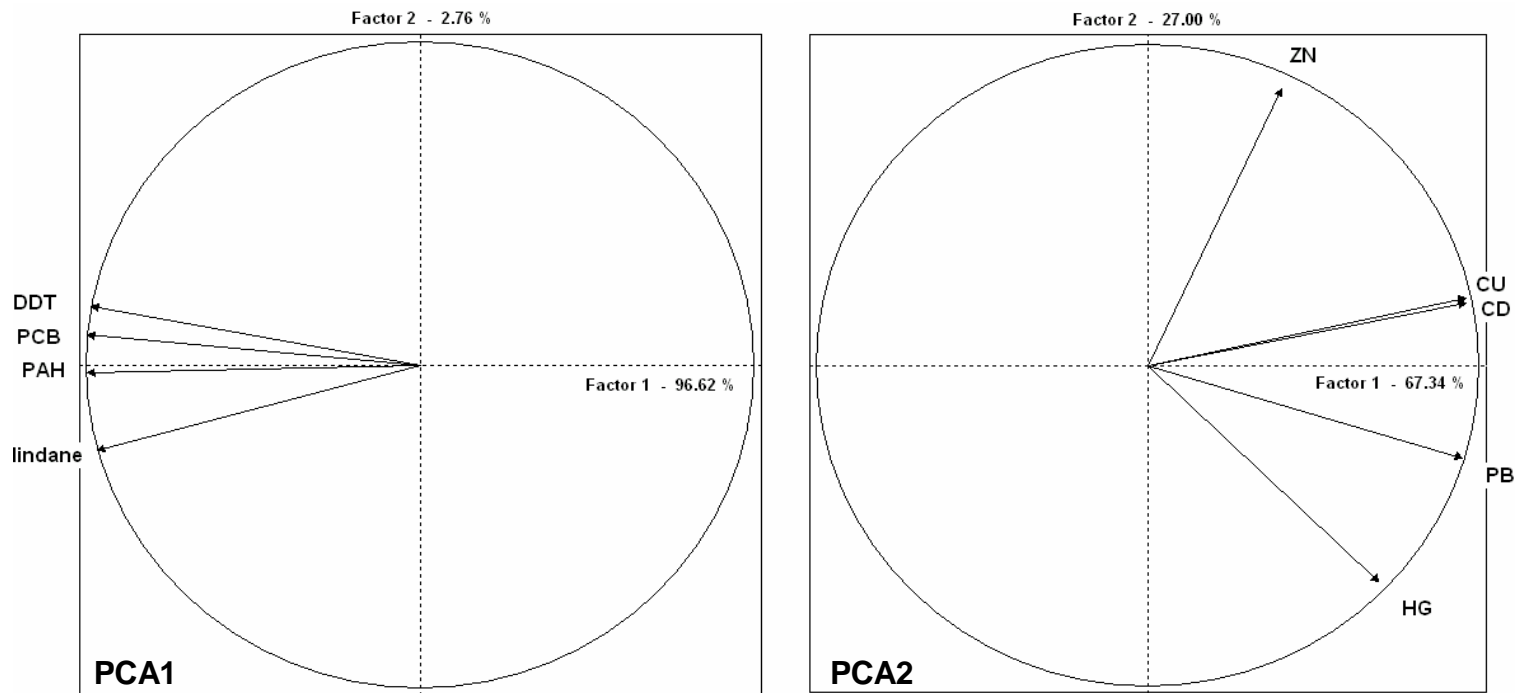


Figure 3. Correlation circles for the ordination of quantitative variables represented by 5 heavy metals: Cu, Cd, Zn, Hg, Pb (PCA1) and by 4 organic chemicals: DDT stands for  $\Sigma$ DDT, lindane, PCB, PAH (PCA2) on the main factorial plan.

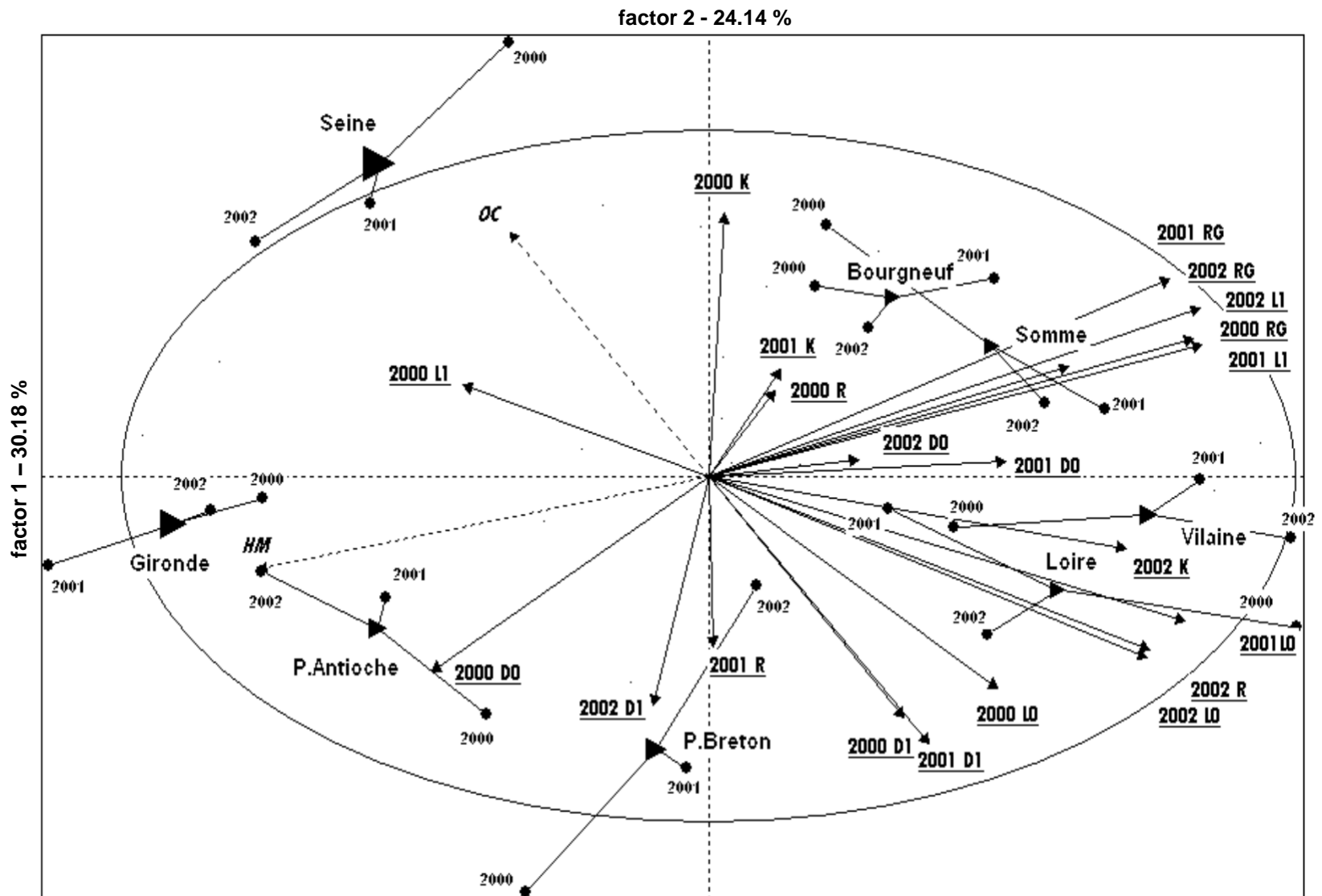


Figure 4. Results of the MFA Analysis on two surimposed figures :

1. Correlation circle for the ordination of quantitative (K: Fulton condition index ( $\text{mg}\cdot\text{mm}^{-3}$ ) of 0-group; R, Residual condition index ( $\text{mg}$ ) of 0-group; RG: recent growth index of 0-group ( $\mu\text{m}$ ); L0 and L1 total length (cm); D0 and D1: log-transformed densities ( $\text{ind}\cdot\text{ha}^{-1}$ ) of 0 and 1- group for the year 2000, 2001 and 2002 on the main factorial plan (F1, F2) of the MFA. Supplementary variables (HM : indicator of heavy metals contamination and OC indicator of organic chemicals contamination in coastal waters) are indicated.

2. Ordination of the 8 sites on the same factorial plan (F1, F2) of the MFA. Each triangle represents the mean observation at each site, with its size proportional to its contribution to the main factorial plan; each point represent the partial observation at each site (2000, 2001, 2002).