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Impact of the sampling protocol in assessing ecological trends in an estuarine ecosystem: The empirical example of the Gironde estuary

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Abstract :

In view of the European Union (EU) requirements to diagnose and monitor the ecological status of estuarine ecosystems using fish-based indicators, we assessed the effect of the sampling protocol in meeting these objectives. Using three different sampling surveys, we evaluated the impact of four components, i.e. sampling gear, spatial extent, length of time series and temporal window analysed, on the detection of ecological trends in the Gironde estuary, estimated by different types of fish-based indicator. For each component, temporal trends of several fish-based indicators were analysed and for the final diagnosis these temporal trends were combined. The results showed that sampling gear had significant effects on the assessment of ecological trends, as it was determinant in the functional description of the fish community and also in the final diagnosis obtained. When on the one hand the lower estuary and the estuarine plume and on the other hand the upper-middle estuary, were considered, we were able to visualise the spatialized functional organization of the estuary and to assess its ecological status more precisely. Increased length of marine fish species in the lower estuary and increased delta diversity in the middle estuary suggested that larger individuals of marine species had moved from outside the estuary to the middle estuary. These trends, associated with the spatial change in environmental conditions (salinity and temperature) in the Gironde estuarine ecosystem may be indicative of a functional shift, from diadromous to marine migrant-dominated assemblages in that ecosystem. We hypothesized that the Gironde estuary was actually more suited to providing nursery habitats for the juveniles of marine migrants and marine stragglers than providing migratory corridors and nursery habitats for species seeking low salinities for a period in their life cycle. The present study supported the hypothesis of a marinisation of the Gironde estuary. When the four components of the protocol, i.e. gear, space, and time (length and temporal windows of time series), were compared this study also suggested that fish length and notably the "temporal window" on which the analyses were based were the most "sensitive" components of the sampling protocol when assessing fish ecological trends in estuaries. It clearly underlined the fact that long time series were essential for evaluating changes in the ecological functioning of these estuarine ecosystems.

Keywords : Protocol effects ; Fishing gear ; Ecological trend ; Fish ; Indicators ; Estuarine ecosystem

1. Introduction

Estuaries are naturally dynamic ecosystems characterized by strong variations in the environmental conditions in space and time, which can be observed at various scales (Mc Lusky, 1981). Naturally complex and changing, these ecosystems are very productive but defined by their low specific diversity (Mc Lusky and Elliott, 2004, 2007). They fulfil a variety of essential ecological functions for fish fauna by providing nursery habitats (Able, 2005), spawning grounds, refuges from predators and migratory routes (Elliott and Hemingway, 2002) for many species and notably commercial ones. However, estuarine areas are also subjected to strong anthropogenic pressures (Dauvin et al., 2007; Dauvin and Ruellet, 2009; Elliott and Quintino, 2007), which threaten the integrity of the systems and the fulfilment of the ecological functions they provide. It is thus crucial from an ecological and economic (e.g. human services) point of view to diagnose the status and the evolution of these areas in order to adapt management measurements to help them maintain or recover their ecological functions.

Recent integrated approaches, such as the Water Framework Directive (EU, 2000) and the Marine Strategy Framework Directive (EU, 2005), aim to assess and achieve good environmental status respectively by 2015 and 2020. EU Member States are asked to develop classification tools in order to assess the ecological quality of the different water categories (coastal, transitional and fresh waters). This frequently requires the use of historical and/or geographical data from different sources and thus the combination and standardization of various databases. Indeed, these databases are often composed of several surveys using different gear and sampling strategies. For example, the fish index TFCI developed by the Environment Agency in UK has been developed using long time series of more than 30 years on the Thames river (Steve Coates, com. pers.). The development of the German fish index

FAT-TW was also based on historical data to generate their reference conditions (Jörg Scholle, com. pers.).

Understanding the impact of the sampling protocol when assessing the ecological status of an ecosystem is thus an essential step prior to combining databases and then calculating indicators (Roset et al., 2007).

Numerous estuarine studies have developed multimetric fish-based indices using a spatial approach (Borja et al., 2006; Delpech et al., 2010). These indices have mainly relied on the significant impact of one or several pressure(s) on biological attributes (metric) of the fish assemblage, i.e. pressure-impact models. The relevance of the statistical results from these studies is however highly dependent on the precision and accuracy of the pressure index (Brind'Amour and Lobry, 2009; Courrat et al., 2009), and although these models seem very efficient when comparing multiple ecosystems, they are less so when detecting changes in the same ecosystem. On the other hand, temporal trend analyses have proved relevant for evaluating the ecological state of estuarine-coastal fish communities by assessing indirectly the relationship between metrics and pressures. These analyses are elaborated in a dashboard (Brind'Amour and Lobry, 2009).

Using three different surveys conducted with different sampling gear (the *gear protocol*), covering different spatial extents (the *spatial protocol*) and time periods (the *temporal protocol*), the aim of the present study was to compare ecological trends in the Gironde Estuary as shown by different types of fish-based indicators and to discuss the effects of these components of the sampling protocol on diagnoses of environmental status.

2. Materials and methods

2.1. The Gironde estuary and the study area

The Gironde is a macrotidal estuary of approximately 625 km² at high tide, located in South-West France and opening into the Bay of Biscay (Lat. 45°20′N, Long. 0°45′W, Fig. 1). The salt part of the estuary stretches for 85 km from the coastline to the Bec d'Ambès, where the Dordogne and Garonne rivers join to form the Gironde. It is generally considered that this junction constitutes the upstream salinity limit.

The study area includes the saline part of the estuary and part of the estuarine plume (in front of the estuary mouth; Fig. 1). It is composed mainly of an oligohaline sector (salinity between 0.5 and 5), a mesohaline sector (salinity between 6 and 18) and a polyhaline sector (salinity between 19 and 30) for which the geographic limits vary according to the season (Mauvais and Guillaud, 1994) and the moment of the tide.

2.2. Fish sampling protocols

The datasets used in the present study come from surveys of the Gironde fish communities conducted by Cemagref (French Institute of Agricultural and Environmental Engineering Research) and Ifremer (French Research Institute for Exploitation of the Sea). Three surveys were selected (frame trawl, otter-trawl, and beam-trawl surveys), all with slightly different sampling protocols, i.e. using different gear, or covering different spatial areas or conducted at different time periods (Fig. 1 and Table 1).

The frame trawl sampling survey by Cemagref started in 1979 and is still ongoing. It was first carried out to monitor the fish and shrimp fauna around the Blayais nuclear power plant. Since 1991, the survey has consisted of four transects in the upper and middle estuarine sections of the Gironde. Each transect consists of three sampling sites, one close to each shore and one on the median axis of the estuary. Sampling was carried out near the bottom using a frame trawl with a 2.0 x 1.2 m frame, kept at 0.2 m above the bed by skates. The cod-end is made of 1 mm square mesh. Sampling was carried out monthly, in daytime. Each tow lasted between 5 and 7 minutes and about 144 hauls per year have been recorded. This gear is adapted to sample small species of fish and the younger stages of larger species. All individuals were preserved in 10% formaldehyde, before being identified and counted in the laboratory. They were then weighed by species.

The otter-trawl sampling survey was conducted monthly by Cemagref between 1994 and 2000 over most of the saline part of the estuary to monitor the European sturgeon population (*Acipenser sturio*). Each trawl lasted 30 min on average and 20 benthic trawls were performed per month following a geographical stratification. Samples were collected during the daytime on the flood and ebb tides using a wide-mouth otter bottom trawl (terminal mesh 70 mm, vertical opening 3 m, horizontal opening 13 m). This gear is well adapted to catch large fish, i.e. over 10 cm. The fish were individually identified, counted, measured (total length to the nearest cm) and returned to the water.

The beam-trawl survey is a nursery-dedicated survey carried out by Ifremer in the lower part of the estuary and in the estuarine plume from the end of August to the end of October. Dorel et al. (1991) found that this was a suitable period for sampling the yearly settled flatfish species, thereby providing good estimates for age 0-group fish (i.e. G0). A six-year time series was used in the present study, between 1996 and 2003. The surveys were conducted using a stratified sampling design according to depth and sediment types. Depths ranged from 5 to 35 m. Sampling was carried out using a 2.9 m wide and 0.5 m high beam-trawl with a 20 mm stretched mesh net in the cod-end. A total of 177 hauls were carried out over the six years of sampling. Each haul was made at a speed of 3 kn, lasted 15 min, and covered a mean surface area of 3,790 m^2 (standard deviation = 224.99 m^2). All the individuals that were caught were identified for species, counted and measured (total length to the nearest cm). They were weighed at species level at each haul.

2.3. Protocol comparisons

The effects of different sampling gear on the ecological status assessment were compared using the otter-trawl and frame trawl sampling surveys (Table 1). These two surveys were chosen because they were conducted in the same months of the same years (1994-2000) in the same geographic locations (upper and middle estuary).

To compare the ecological status assessments established in two distinct estuarine areas, the estuarine plume - lower estuary and the upper - middle estuary, we selected the beam-trawl surveys and the frame trawl sampling surveys, respectively. As the two types of sampling gear differed in mesh-size (beam-trawl: 5mm and bottom trawl: 1 mm), and were therefore not exactly the same, we chose to dampen the differences of the mesh selectivity by selecting the same size classes for each species caught (i.e. the juveniles). The two surveys were compared for the same months (August to October) between 1996 and 2003.

The impact of the length of the time-series and the sequence of years used to assess the ecological status of the Gironde was evaluated by comparing different periods in the time-series of the frame trawl sampling survey. Three time periods were considered: 1) the complete time-series from 1979 to 2009, bearing in mind the fact that the protocol evolved slightly during this period (see Girardin and Castelnaud, 2010 for details) although no significant effect was noted in the data and the time-series selected for this study (data not shown); 2) two time-series (1994-2000 and 1996-2003) of almost equivalent length (7 *vs.* 8 years), which included different sequences of years. These two time periods were compared to assess the impact of the temporal window, and 3) the long time-series (30 years) was compared to the two other time-series (7 and 8 years) to verify the impact of length of time-series on the assessment of the ecological status of the estuary.

2.4. Fish-based indicators

Three groups of indicators, corresponding to as many levels of organization (i.e. juveniles, community and functional guild), were calculated and used to assess the ecological status of the Gironde estuary (Table 2). The indicators were chosen based on two criteria: (1) their ecological relevance in assessing the ecological status of estuarine ecosystems (e.g. Breine et al., 2004, 2007; Coates et al., 2007; Courrat et al., 2009; Deegan et al., 1997; Elliott and Dewailly, 1995; Elliott and Quintino, 2007; Harrison and Whitfield, 2004) and (2) the type of data they required (e.g. size or biomass at the individual level).

As the nursery function is one of the most important ecological functions for fish associated with estuaries, we computed the indicators for a fraction of the fish populations, the juveniles (i.e. individuals smaller in size than size at maturity). We propose to focus on two indicators from among the suite of indicators estimated for juveniles (e.g. Le Pape et al., 2003), i.e. density and average length or weight. These indicators were calculated for species that occurred sufficiently frequently i.e. in more than 5% of hauls.

Taxonomic diversity and total density have often been successfully used to assess human impacts in estuarine ecosystems (e.g. Deegan et al., 1997; Delpech et al., 2010). Among the different taxonomic diversity indices developed by Clarke and Warwick (1998), the delta diversity index (Δ) was chosen as it is independent of sampling effort. Total density was also chosen as it appears to be a common surrogate for system productivity and a good metric when assessing the ecological status of an ecosystem (Deegan et al., 1997; Karr, 1981).

Estuaries are associated with a certain number of vital functions for fish such as feeding areas for marine species that regularly enter estuaries in large numbers and particularly as juveniles (marine migrants MM), migratory corridor for diadromous species (CA), permanent habitat for autochthonous species (ER) (Elliott et al., 2007; Franco et al., 2008). These ecological guilds, often selected to assess the ecological status of transitional waters (e.g. Delpech et al., 2010; Harrison and Whitfield, 2004), were selected for the present study (Appendix). We also included the total density of benthic species. This guild was found to be particularly sensitive to siltation and oxygen deficiency and to constitute a good indicator of anthropogenic disturbances for several authors (e.g. Hughes et al., 1998; Kestemont et al., 2000; Oberdorff and Porcher, 1994).

2.5. Time-trend approach

The protocol effect was assessed from trend comparisons of the aforementioned indicators using a time-trend approach adapted from Rochet et al. (2005). In the present study, we conducted three series of analyses, one for each protocol comparison (gear, space and time).The modified time-trend approach is well explained in Brind'Amour and Lobry (2009), and so here we only describe the key features of the methodology. The method can be divided into three steps: the first consists of choosing the relevant indicators which are theoretically or empirically known to respond to human pressures (Table 2), and calculating linear trends for each indicator. Therefore, all the indicators were calculated in each sampling survey, using yearly means to test gear and temporal effects, seasonal means (August to October) to test the spatial effect, and their linear trends were estimated. To take into account the autocorrelation in time series, the modified Mann-Kendall trend test, developed by Hamed and Rao (1998), was used to analyse the indicator trends in the long-term series data (1979-2009) from the frame trawl sampling surveys. For all the trend tests, the significance α threshold considered was 0.05.

The second step is one of the most important in this approach and consists of developing different diagnostic tables that will be further used to interpret the combined temporal trends of the different indicators. However, before building the tables, we needed to evaluate the "reference state" of the studied ecosystem at the beginning of the time series. In the Gironde ecosystem, we knew, from expert knowledge and published information, that the estuary was already impacted by numerous human pressures, e.g. metallic contamination, dredging, fishing (e.g. Durrieu et al., 2005; Féral, 1994; Girardin et al., 2001; Mauvais and Guillaud, 1994). The reference state was therefore assessed as impacted.

Once the reference state is assessed and all the trends have been estimated for all the indicators, the combined trends of the indicators can be interpreted. This is done by pairing indicators at the juvenile and community levels in diagnostic tables. There are as many diagnostic tables as there are possible combinations of indicators, and they are interpreted according to ecological theory and common sense (Table 3). For instance, if the average length of the juveniles within a population decreased, and its abundance also decreased, this means that there were fewer recruits of smaller size. There are several explanations for this: it could reflect increased mortality (fishing or natural mortality or both) concomitant with decreased recruitment; it could also reflect lower growth, which might be the result of environmental changes (food, temperature, competition), or late spawning. The diagnostic tables are filled in this way for the subpopulation (juvenile-based), and community indicators. For diagnosing the state of the functional level, we applied the rule of "one out, all out" as a safety-first principle, i.e. if one indicator trend is decreasing, it means the functional level is degrading. Cells are shaded and qualified as undesirable if it is suspected that one of the potential mechanisms for the trend is related to an anthropogenic pressure, e.g. fishing and pollution (Brind'Amour and Lobry, 2009; Deegan et al., 1997; Delpech et al., 2010; Harrison and Whitfield, 2004). They are hatched if the trend is stationary and thus unsatisfactory with regards to the theoretical trend and left clear if the trend is satisfactory.

When all the tables have been filled, the third step consists of applying a test procedure (e.g. multinomial tests in the present case) to assess whether there was statistical evidence that each component was moving in undesirable directions. From these tests and the functional indicator result, a final diagnosis was made according to the convention that if one level of organization (juvenile, community or functional trend) was found to be undesirable, the whole ecosystem was considered to be deteriorating (i.e. the “one out – all out” rule). All the analyses in the present study were computed using the software R 2.8.1 (R Development Core Team, 2008).

3. Results

3.1. Gear protocol (1994-2000)

The mean relative densities of juveniles were the same for both gear protocols (frame trawl and otter-trawl surveys; i.e. 58%, Fig. 2). Among the more frequently species fished, the frame trawl sampling collected more species (13 species *vs*. 10; see Table 4) and small species (e.g. *Pomatoschistus* spp.). It was also better in sampling benthic fish species (% in densities; see Fig. 2). A total of six species were common to both surveys.

For the frame trawl sampling data, among the thirteen species included in the trend analysis for the juvenile fraction level, three displayed significant trends: a decrease in abundance for *Alosa alosa* (p-value < 0.05) and *Dicentrarchus labrax* (p-value < 0.05), and an increase for *Sprattus sprattus* (p-value < 0.05). *A. alosa* showed the most pronounced trend, from an annual mean of 1.36 fish per 1,000 m^3 in 1994 to 0.24 fish per 1,000 m^3 in 2000. When the multinomial test procedure was applied, the global trend in juvenile fractions was however not significant. Trend-analysis at the community level displayed no significant change for either indicator, the delta diversity index or the total density, whereas indicators at the functional level showed a significant decreasing trend for diadromous fish densities (p-value < 0.05), from 0.07 fish per 1,000 m^3 at the beginning of the time series to 0.04 fish per 1,000 m^3 at the end. According to the rule of "one out – all out", this result suggests a functional degradation of the Gironde estuary using the frame trawl data survey.

Ten species were used in the trend-analysis at the juvenile fraction level for the otter-trawl sampling data. Among them, two populations displayed significant increasing trends in their abundance (*Solea solea*, p-value < 0.05), and their mean length (*Acipenser sturio,* p-value < 0.001). Two decreasing trends were also observed, in the abundance of *Merlangius merlangus* (p-value < 0.01) and in the mean length of *Liza ramada* (p-value < 0.001). However, no significant global trend was obtained after testing with the multinomial test procedure for this group of indicators. No significant change was detected at the community and functional levels. Since the Gironde system was considered impacted at the beginning of the time-series, the global diagnosis suggests that the ecological status of the system is not improving.

Comparison between the two sampling surveys showed different trend results, leading to different diagnoses, i.e. degradation for the frame trawl survey and no improvement for the otter-trawl survey, thereby suggesting an effect of gear selectivity (Table 5). When pulled together, the results from the two surveys emphasized the degradation of the Gironde ecosystem functionality notably for diadromous fish and the juveniles of some fish species.

3.2. Spatial protocol (1996-2003)

Twelve species dominated the fish community sampled in the upper and middle estuary with the frame trawl survey, and most of these were diadromous species (Table 4 and Appendix). Of the ten species dominating the beam trawl survey, seven were marine fish. Only four species (resident and marine fish) were common to both surveys.

Among the twelve species included in the frame trawl survey analyses, only *Syngnathus rostellatus* showed a significant increase in its abundance (p-value < 0.001; Table 4) from 13.24 fish per 1,000 m^3 at the beginning of the time series to 95.54 fish per 1,000 m^3 at the end. Multinomial tests indicated no global significant trend. On the other hand, a significant increase, in the delta diversity index was observed at the community level (Δ: p-value < 0.05). No significant trend was observed with the group of indicators at the functional level.

For the beam-trawl sampling, ten species were included in the calculation of the indicators at the juvenile level. Among them, *Dicologlosa cuneata*, *Mullus surmuletus* and *Pomatoschistus* spp. displayed increasing trends in their mean length (p-value < 0.01, < 0.05 and < 0.05 respectively; Table 4). The multinomial test procedure highlighted a significant improvement for that group of indicators. The community and functional indicators showed no significant trend.

The global diagnosis of both surveys encompassing different geographical areas in the Gironde estuary concluded that there had been no improvement in the system (Table 5). This was however based on significant positive trends at different levels of organisation. The diagnosis of the frame trawl survey, which focused on the upper and middle part of the estuary, displayed an increasing trend for indicators at the community level, while for the beam-trawl survey (located in the lower parts of the estuary) increasing trends were mainly observed for marine species at the juvenile level.

3.3. Temporal protocol

The comparison of three different periods in the time-series analyses using data from the frame trawl sampling survey underlined the impact of the length of the time-series (30 years *vs* 8 years) and the temporal windows (1994-2000 *vs* 1996-2004) used for the analysis. The overall assessment of the ecological status of the Gironde reached similar conclusions, thus different trends were observed according to different effects (length and window, Tables 4 and 5).

From 1994 to 2000, analyses at the juvenile level were conducted on 13 species. Three significant trends were observed: a decrease in abundance for *Alosa alosa* (p-value < 0.05), species displaying the most pronounced trend, and *Dicentrarchus labrax* (p-value < 0.05) and an increase for *Sprattus sprattus* (p-value < 0.05). Linear trend analyses using functional guilds highlighted a significant decrease in diadromous fish densities (p-value < 0.05). At the community level, no significant change was highlighted for the two indicators used in this study. The global diagnosis for the 7-year time series (1994-2000) suggested that the functional status of the Gironde estuary was deteriorating.

From 1996 to 2003, trends at the juvenile level for the 12 species indicated that only the juveniles from the species *Syngnathus rostellatus* displayed a significant increase in abundance (p-value < 0.01, Table 4). No change was observed, however, using the functional indicators, whereas an increase (p-value < 0.05) in taxonomic diversity (Δ) was noticed at the community level. The global diagnosis for the 8-year time series (1996-2003) suggested that the status of the Gironde estuary remained stationary and as the initial state was impacted this implies that the Gironde estuary status was not improving.

3.3.3. From 1979 to 2009

Several trends were observed at the juvenile level using the larger time series, i.e. from 1979 to 2009. Five of the 14 species analysed showed significant decreasing trends in their abundance. This was the case for the two shads (*A. alosa* and *Alosa fallax*), *Anguilla anguilla*, *Osmerus eperlanus*, and *Platichthys flesus* (p-values < 0.05, <0.01, <0.001, <0.001, <0.001 respectively). In this 30-year time series, three species, *O. eperlanus*, *P. flesus* and *A. fallax*, displayed the largest differences in densities between the beginning (12.95 fish per 1,000 m^3, 0.28 fish per 1,000 m^3 and 0.5 fish per 1,000 m^3 respectively) and the end of the series (0, 0.003 and 0.003 fish per 1,000 m^3 respectively). Concurrently, *Gasterosteus aculeatus* juveniles displayed a significant decrease in their mean biomass (p-value < 0.05). On the other hand, two species increased in abundance, *Liza ramada* and *Sprattus sprattus* (p-values < 0.05 and p-values < 0.05) and two others, *Engraulis encrasicolus* and *Pomatoschistus* spp. displayed an increase in abundance (respectively p-values < 0.001 and p-values < 0.05) together with a decrease in mean biomass (p-values < 0.001 and < 0.05).

Analyses at the functional level underlined the significant decrease in diadromous species densities (p-value < 0.001), and at the same time an increase in marine migrant densities (p-value < 0.01). Diadromous species showed a decline from 0.19 fish per 1,000 m^3 (in 1979) to 0.01 fish per 1,000 m^3 (in 2009), whereas marine migrant densities increased from 0.009 fish per 1,000 m^3 to 0.08 fish per 1,000 m^3 over the same period. A significant increasing trend was also recorded for taxonomic diversity (Δ) analysed at community level (p-value < 0.05). The global diagnosis for the 30-year time series (1979-2009) suggested that the Gironde estuary did deteriorate during the 30-year study period.

Final diagnosis of the ecological status of the Gironde estuary using time series analyses reached similar conclusions, notwithstanding the time periods or the temporal window analysed (Table 5). A general degradation in the estuary was diagnosed for the periods 1979-2009 and 1994-2000 whereas no improvement was diagnosed using the short time series from 1996 to 2003. It is worth noting that at a large time scale (1979-2009), we highlighted a degradation at both juvenile fraction and functional levels, whereas with a shorter scale (1994-2000) the deterioration was only detectable using functional indicators.

4. Discussion

Understanding the effect of the sampling protocol on the assessment of the ecological trends of an ecosystem is an essential step prior to combining and then calculating any indicators to assess the ecological status of that ecosystem. Using three different sampling surveys we evaluated the effect of the sampling gear, the spatial extent, the length of the time series and the temporal window analysed, on the assessment of the ecological fish trends for the Gironde Estuary. Various trends were observed for the four components, leading us to conclude that the ecological status of the Gironde estuary is either deteriorating or at least not improving from its initial impacted state.

4.1. Impact of sampling protocol on the detection of ecological trends

4.1.1. The effect of the sampling gears

The effect of the type of sampling gear on the assessment of ecological trends for fish in the Gironde was tested by comparing data from a dragnet and a bottom trawl. Indicators estimated from the two types of gear displayed similar trends on the juvenile fraction but not on the same species. A difference was also observed at the functional level, with a significant decreasing trend for diadromous fish densities.

It is well known that different types of fishing gear have different capture efficiencies according to various factors such as the dimensions of the gear, tow duration, tow speed, and mesh size (Caverivière, 1993; Godø et al., 1990; Rotherham et al., 2008). Gear efficiency is conditional on the catchability of the fish which in turn is determined mainly by their length and behaviour (Somerton et al., 2007). For instance, large flatfish can easily escape beneath a trawl footrope whereas small individuals may escape through the mesh of the sampling net. In this study, the two gear types differed in almost all the aforementioned factors (tow duration, mesh size, width, etc.) and although the proportion of juveniles was similar in terms of number of specimens, different fish communities and/or fraction of populations were sampled. Thus, the frame trawl captured small benthic species or individuals and diadromous species more efficiently (as seen by the significance of that functional indicator) than the otter-trawl. As the Gironde estuary appears to have the largest migratory diadromous fish assemblage in Europe (Lobry et al., 2003), it seems unequivocal that the ecological status of the Gironde estuary should continue to be monitored using the frame trawl as sampling gear.

4.1.2. Spatial effect

The spatial extent of the sampling area along the estuarine gradient (lower or middle and upper portions) affected the diagnosis of the ecological trends. Differences may be explained by the ecological functioning of estuarine ecosystems. Estuaries can be viewed as spatialized functional ecosystems in which different habitats along the salinity gradient provide a variety of ecological functions for the fish fauna (Bulger et al., 1993; Lobry et al., 2003; Pihl et al., 2002). By considering an external area (lower estuary and estuarine plume) and an internal one (upper-middle estuary) we were able to visualise the spatialized functional organization of the estuary and assess its ecological trends more precisely. Indeed, the fish assemblage in the external area was composed almost exclusively of marine species including marine stragglers (Table 4 and Appendix; see Elliott et al., 2007 for definition). Species from these two ecological guilds (e.g. *Arnoglossus laterna, Buglossidium luteum*) were either absent or appeared in small numbers in the internal zone.

The increases in marine fish species length in the lower estuary and in delta diversity in the middle estuary suggested the colonization of the middle estuary by the larger individuals of marine species coming from the outer estuary into the middle estuary. These trends, associated with the spatial change in environmental conditions (salinity and temperature) in the Gironde estuarine ecosystem (David et al., 2007) may be indicative of a functional shift in that ecosystem. We can then assume that the Gironde estuary is actually more suitable for providing nursery habitats for juveniles of marine migrants and marine stragglers than providing migratory corridors and nursery habitats for species seeking low salinities.

4.1.3. Length of the time series and temporal window analysed

Some indicator trends were significant in the long term series (1979 to 2009) and the short time series. However, they were not consistent between the temporal windows analysed. For instance, the long time series showed great similarities (notably at the juvenile population level) with the 1994 to 2000 period but relatively few similarities with the 1996-2003 period. Nicholson and Jennings (2004) showed that for a number of traditional indicators of fishing impact (e.g. weight, length, and trophic level) the ability to detect meaningful changes was dramatically low for time series of less than 10 years. Were the differences between the two time series of similar lengths due mainly to length of the temporal window or to occasional environmental events? For instance, the increase in *Syngnathus rostellatus* between 1996 and 2003 was partly associated with consecutive droughts in 2002 and 2003 (Girardin et al., unpublished work). It is difficult to answer the question at the moment, but one thing emerges from the differences in the diagnosis of these two time series (1994-2000 and 1996-2003): it clearly underlined that long time series are critical to encompass changes in the ecological functioning of ecosystems and this is clearly not the option taken for surveying the transitional water bodies within the context of the Water Framework Directive in Europe. Most European countries from Atlantic ocean and the Channel will propose to survey at most three years over a period of 6 years. This mean that they will need at least 20 years before having a time series of 10 years long and we demonstrated that 10 years is already limited in order to show a trend. In terms of management a precaution approach should be taken in order to avoid too much deviation from the objective of "good ecological status".

Overall, results from the temporal effect emphasize the problem of the length of the period being analysed and the need to maintain long time series which can be analysed using various methods (linear or non-linear trends, moving windows, spectral analyses, etc.) thereby assessing the natural temporal changes in populations by testing for small-term changes, trends and cycles (Fromentin and Ibanez, 1994; Underwood, 1996). The present study analysed temporal changes using linear trends. Owing to the length of the shorter time series and its related power this is unlikely to be the best way to model temporal relationships. Other approaches, such as monotonous or moving averages (Nicholson and Jennings, 2004; Ridley, 2003; Trenkel and Rochet, 2010) could be used. However, as noted by Rochet et al. (2005), the main objective of the trend analysis method is not to fit the best temporal model but rather to find which temporal trends are probably associated with environmental or anthropogenic changes. Combining trends from these aforementioned alternative approaches would probably help to assess changes and ruptures in the series, which may help detect short-term (e.g. severe anoxia) and long-term events, thereby evaluating the inertia and resilience of fish populations (Underwood, 1996).

4.2. Ecological status of the Gironde estuary

4.2.1. Importance of the initial state and the “leave-one-out” rule in the final diagnosis

The temporal trend analyses used in the present study are based notably on two important assumptions: i) that the initial state of the estuary is known, and ii) if one diagnosis points towards a non-desirable direction then the overall diagnosis is bad (i.e. degrading or not improving, see Brind’Amour and Lobry, 2009 for details). Fortunately, it was easy for the present study to determine the initial state of the estuary at the start of the time series as the state of knowledge of the Gironde estuary is impressive (e.g. Durrieu et al., 2005; Féral, 1994; Girardin et al., 2001; Mauvais and Guillaud, 1994). The Gironde has long-term monitoring programmes for species with high commercial and/or natural heritage values (e.g. sturgeon, Rochard, 1992) which use the estuary for feeding and spawning habitats and migratory routes. This kind of information is however not available for all estuarine ecosystems and this may have an impact on the final diagnosis of the ecosystem status, especially when no trend is significant.

The other assumption is the leave-one-out decision rule, where one single negative evaluation leads to an overall negative diagnosis. One could argue that this rule is much too severe and that it reduces the probability of diagnosing good ecological status. By selecting only indicators that are relevant to the goal of the study, in this case indicators associated with the ecological functions of the Gironde estuary, we were confident that the trends at each level of organisation were highly informative of the global status of the estuary. The choice of "leave-one-out" was thus done in the context of a cautious approach. Other decision rules, such as scoring the indicators, could have been used and easily implemented in the methodology. In fact, each decision rule has its own limitations and as long as these are known and justified in the context of the study, almost any rules can be used.

4.2.2. Temporal changes of the Gironde estuary

In the present study, significant decreases in diadromous fish densities were observed in the upper and middle parts of the Gironde estuary and significant increases in marine fish (in number of species or in densities) were exhibited in the saline parts of the system. In the middle-upper estuary, these results could be correlated to an increase in salinity (Delpech, Unpublished results). On the other hand, Nicolas et al. (2010) suggested that salinity was the major environmental variable structuring fish assemblages in terms of number of species and density within European estuarine systems. They found higher densities of marine species in the mesohaline area than in the oligohaline and polyhaline areas. They also showed a decrease in catadromous species densities when salinity increased. Together with the spatio-temporal changes of salinity in the upper and middle estuary (Fig. 3), our study strongly supports the hypothesis of the “marinisation” of the Gironde estuary. i.e. an extended intrusion of seawater inside the Gironde estuary due to a decreased river flow (David et al., 2007). This hydroclimatic modification is also observed in other estuaries, notably in the Mondego estuary in Portugal (Martinho et al., 2007). The “marinisation” of an estuary may lead to functional changes in that estuary. River flow in the outer estuary has three main functions that may directly or indirectly affect fish species: diluting and reducing salinity, delivering nutrients and sediment from the watershed, and inducing physical mixing of the bay waters (Pasquaud et al., 2008; Schlacher et al., 2009). In our study the biological evidence supporting “marinisation” are the decrease in anadromous and catadromous densities, the increase in marine migrant species, and the increase in the delta diversity index.

Other studies have recorded significant declines in the abundance of *Alosa alosa* and *A. fallax* in the Gironde-Garonne-Dordogne system (Castelnaud et al., 1999). Apart from the

Gironde estuary, declines have also been observed for many diadromous fish (ICES, 2005) and more specifically for the *Alosa* populations (Aprahamian et al., 2003), *A. anguilla* (Dekker et al., 2003) throughout their geographic range. *Osmerus eperlanus* and *Platichthys flesus* declines have been identified at local scales (Cabral et al., 2001; Pronier and Rochard, 1998). The suggested "marinisation" of the estuary cannot alone explain the decline in the diadromous fish. It could be hypothesized that diadromous fish may be adversely affected by human activities compared with other ecological guilds (McDowall, 1988). The causes of the decline in abundance of these species are evidently various and probably cumulative. There is little firm quantitative evidence for anthropogenic causes in the literature responsible of deteriorations of all the environments, i.e. marine, estuarine and freshwater areas, in which diadromous species spend parts of their lifecycle (Lassalle et al., 2009; Rochard et al., 1990) and much work remains to be done to formally assess the causality between the decline in abundance of diadromous species and specific anthropogenic activities, although dams in the main stem (Gowans et al., 1999; Larinier, 2001), deterioration of preferential habitats (Lepage et al., 2000), water quality (Maes et al., 2007), and unsustainable fisheries (Masters et al., 2006) have been widely cited as the reasons why some of these species have declined. It is also worth mentioning that several diadromous species are now included in the International Union for the Conservation of Nature (IUCN) world red data book and are thus classified as rare or endangered species (IUCN, 2009)

5. Conclusions

This study demonstrates the importance of gear selectivity and the choice of sampling gear in order to most effectively sample a diverse range of fish in estuaries and to make the best assessment of the ecological status of those ecosystems. The analysis of fish-based indicators in an external area (lower estuary and estuarine plume) and an internal one (upper-middle estuary) clearly supported a spatialized functional organization of the estuary so that a more precise diagnosis of its ecological status could be made. The increase in length of marine fish species in the lower estuary and in delta diversity in the middle estuary suggested that larger marine species individuals moved from outside the estuary into the middle estuary. These trends, associated with the spatial change in environmental conditions (salinity and temperature) in the Gironde estuarine ecosystem may be indicative of a functional shift in this ecosystem. We can then hypothesize that the Gironde estuary is actually better suited to providing nursery habitats for juveniles of marine migrants and marine stragglers than providing migratory corridors and nursery habitats for species seeking low salinities. Moreover, all the results of this study support the hypothesis of the marinisation of the Gironde estuary.

When comparing the three components of the protocol, i.e. gear, space, and time, the results suggest that the length and notably the “temporal window” on which the analyses are conducted seemed to be the most “sensitive” component of the sampling protocol and they clearly show that long time series are critical for understanding the ecological functioning of ecosystems. The surveys implemented by European member states for the Water Framework Directive and for the Marine Strategy Directive should assess the length of the time series and the temporal windows needed in order to be able to detect trends and to adopt a precaution approach in order to avoid too much deviation from their goal.

Impacted by numerous human pressures over several decades, the ecological status of the Gironde estuary over the time scale studied seems not to have improved, indeed it has deteriorated. To which extent, we cannot answer but the system was already degraded at the beginning of the time series and the situation is worst at the end of these series. These final conclusions can be drawn because of the existence of a long time-series for the Gironde enabling us to interpret the difference between the indicator trends. It is thus necessary to maintain and/or develop further long time-series to provide a relevant diagnosis of the observed changes in the ecological changes of estuarine ecosystems. Different approaches are giving different results in this study and the importance of the sampling gear was highlighted. While developing fish index this particular effect of sampling gear should be taken into consideration if multiple sampling gear is used. The combination of different sampling gear can enhance the power of trend detection but the analysis should be done gear by gear identifying which metric is best represented by which sampling gear.

The use of historical data needs also an effort of standardization to account for gear-induced biases, prior to calculating indices from such data.

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Appendix.

Species sampled (see the marks) in the three surveys conducted in the Gironde estuary and their guild as detailed in Elliott and Dewailly (1995) and reviewed by Franco et al. (2008). FW: FreshWater species; CA: Catadromous and Anadromous species; MM: Marine Migrants; MA: Marine Adventitious visitors; ER: Estuarine Resident; D: Demersal species; P: Pelagic species; B: Benthic species.

Taxon	Ecological guild	Position in the water column	Frame trawl survey	Otter trawl survey	Beam trawl survey
Abramis brama	FW	D	X	X	
Acipenser baerii	FW	D		X	
Acipenser sturio	CA	D		X	
Alburnus alburnus	FW	P	X		
Alosa alosa	CA	P	X	X	X
Alosa fallax	CA	P	X	X	X
Ameiurus melas	FW	D	X	X	
Anguilla anguilla	CA	D	X	X	X
Argyrosomus regius	MM	D	X	X	X
Arnoglossus laterna	MA	D			X
Aspitrigla cuculus	MA	B			X
Atherina presbyter	MA	P	X		X
Barbus barbus	FW	D		X	
Belone belone	MM	P	X	X	
Buglossidium luteum	MA	B			X
Callionymus lyra	MA	B	X		X
Chelidonichthys lucerna	MA	D		X	X
Chelon labrosus	MM	D		X	
Ciliata mustela	ER	B	X	X	X
Clupea harengus	MM	P	X	X	
Conger conger	MA	D	X	X	X
Ctenolabrus rupestris	MA	D	X		
Crystallogobius sp.	MA	D			X
Cyprinus carpio	FW	D	X		
Dasyatis pastinaca	MA	B		X	X
Dicentrarchus labrax	MM	D	X	X	X
Dicentrarchus punctatus	MM	D	X	X	X
Dicologlossa cuneata	MA	B			X
Diplodus sargus	MA	D		X	
Echiichthys vipera	MA	B			X
Engraulis encrasicolus	MM	P	X	X	X
Eutrigla gurnardus	MM	D			X
Gaidropsarus vulgaris	MA	D			X
Gambusia affinis	FW	P	X		
Gasterosteus aculeatus	ER	D	X		
Gobius niger	MM	B			X
Hippocampus hippocampus	ER	B	X		X
Lampetra fluviatilis	CA	P	X		
Lepomis gibbosus	FW	D	X		
Limanda limanda	MM	B		X	
Liza ramada	CA	D	X	X	X

Lophius piscatorius	MA	B		X	
Merlangius merlangus	MM	D	X	X	X
Merluccius merluccius	MA	D		X	X
Mullus surmuletus	MA	B		X	X
Osmerus eperlanus	CA	P	X	X	X
Pegusa lascaris	MA	B			X
Petromyzon marinus	CA	P	X	X	
Platichthys flesus	CA	B	X	X	X
Pleuronectes platessa	MM	B		X	
Pomatoschistus	ER	B	X		X
Psetta maxima	MM	B	X	X	
Pseudorasbora parva	FW	P	X		
Raja clavata	MA	B		X	X
Raja microocellata	MA	B		X	X
Raja undulata	MA	B		X	X
Rutilus rutilus	FW	D		X	
Salmo salar	CA	P	X	X	
Salmo trutta	CA	P	X	X	
Sardina pilchardus	MM	P		X	X
Scomber scombrus	MA	P		X	X
Scophthalmus rhombus	MM	B		X	X
Solea senegalensis	MM	B	X	X	
Solea solea	MM	B	X	X	X
Sparus aurata	MA	D		X	
Spondyliosoma cantharus	MA	P		X	X
Sprattus sprattus	MM	P	X	X	X
Stizostedion lucioperca	FW	D	X		
Syngnathus acus	ER	D			X
Syngnathus rostellatus	ER	P	X		X
Torpedo marmorata	MA	B		X	X
Trachurus draco	MA	B			X
Trachurus trachurus	MM	B		X	X
Trisopterus luscus	MM	D		X	X
Umbrina canariensis	MA	D		X	X
Umbrina cirrosa	MA	D		X	
Umbrina sp.	MA	D	X		

Tables

Table 1
Characteristics of each of the sampling surveys from which the data analysed in the current study originated.

Characteristics	Frame trawl survey	Otter trawl survey	Beam trawl survey
Objectives	Monitoring of the estuarine fauna near the Blayais nuclear power plant	Monitoring of European sturgeon (*Acipenser sturio*) population	Monitoring of estuarine and coastal flatfish nurseries
Gears	Frame trawl Cod-end 1 mm	Otter trawl Cod-end 70 mm	Beam trawl Cod-end 20 mm
Temporal data series used	1979-2009	1994-2000	1996-2003
Sampling frequencies	≈ Monthly	≈ Monthly	Once per year between the end of August and the end of October
Sampling effort	≈ 144 hauls per year	139-338 hauls per year	About 30 hauls per year
Spatial localisation	Upper and middle estuary	Upper and middle estuary	Lower estuary and estuarine plume
Protocol comparisons			
Gear protocols	X	X	
Spatial protocols	X		X
Time protocols	X [a]		

[a]: The Frame trawl sampling survey was separated in three time series (1979-2009, 1994-2000 and 1996-2003) for the evaluation of the temporal impact of in the assessment of the ecological status of the Gironde estuary.

Table 2. Fish indicators selected for use in the current study.

Level	Indicator	Definition	Expected effect of human pressures
Juvenile fraction	D_{ij}	Density of the juvenile *j* in the population *i*	Decrease
	$\overline{L_{ij}}$ or $\overline{W_{ij}}$	Average length or weight of juveniles *j* of the population *i*	Decrease
Community	Δ	Delta diversity	Decrease
	D_{fish}	Total density	Decrease
Functional guild	D_{CA}	Density of diadromous fish (CA)	Decrease
	D_{ER}	Density of estuarine species (ER)	Decrease or increase
	D_{MM}	Density of marine migrants (MM)	Decrease
	D_{B}	Density of benthic species (B)	Decrease

Table 3

Diagnostic tables for joint interpretation of indicator trends. (a) Juvenile fraction indicators, (b) community indicators. Diagnostic assessment of the combinations when initial state is impacted: cells in grey: undesirable, hatched cells: not satisfactory, unshaded cells: satisfactory. See Table 2 for the definition of the indicator abbreviations. Trends of the indicators are mentioned with the narrows: : increase, ↔ no trend, decrease.

(a)

$\overline{L}_{ij}$ \ $\ln(\overline{D}_{ij})$	↗	↔	↘
↗	GOOD RECRUITMENT FASTER GROWTH More recruits of larger size Early spawning/spatial shift Suitable env. conditions	FASTER GROWTH Early spawning/spatial shift Suitable env. conditions	POOR RECRUITMENT FASTER GROWTH Early spawning/spatial shift Undersized fish mortality *(Un)suitable env. Conditions*
↔	GOOD RECRUITMENT Early spawning/spatial shift Suitable env. conditions		POOR RECRUITMENT Early spawning Increase mortality *Unsuitable env. conditions*
↘	GOOD RECRUITMENT SLOWER GROWTH Late spawning/spatial shift Density dependence *(Un)suitable env. conditions*	SLOWER GROWTH Late spawning/spatial shift *Unsuitable env. conditions*	POOR RECRUITMENT SLOWER GROWTH Mortality of larger individuals Late spawning/spatial shift *Unsuitable env. conditions*

(b)

Δ \ D_{fish}	↗	↔	↘
↗	MORE FISH DIVERSITY OF HABITATS PREY-PREDATOR BALANCE Spatial shift Suitable env. conditions	DIVERSITY OF HABITATS PREY-PREDATOR BALANCE Spatial shift Suitable env. conditions	LESS FISH DIVERSITY OF HABITATS PREY-PREDATOR BALANCE Spatial shift *(Un)suitable env. Conditions*
↔	MORE FISH Spatial shift Suitable env. conditions		LESS FISH Spatial shift Increase mortality *Unsuitable env. conditions*
↘	MORE FISH LOSS OF HABITATS PREY-PREDATOR UNBALANCE Spatial shift Mortality of sensitive species Proliferation of the others *Unsuitable env. conditions*	LOSS OF HABITATS PREY-PREDATOR UNBALANCE Disappearance of sensitive species Spatial shift *Unsuitable env. conditions*	LESS FISH LOSS OF HABITATS PREY-PREDATOR UNBALANCE Disappearance of sensitive species Spatial shift *Unsuitable env. conditions*

Table 4
Summary of the linear trends for all the fish taxa included in the analyses at the juvenile fraction level. The results are presented by sampling survey and time period over which the analyses were conducted. D density, $\overline{L}$ Average length, $\overline{W}$ average weight; <-> no significant trend, ↓ trend significantly decreasing, ↑ significantly increasing; * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$. – : Absence of the species or occurrence frequency < 5%. See Table 2 for the full details for the calculation of the indicators. The last row of the table indicates the total number of juvenile species in each sampling survey.

	Gear protocol (1994-2000)		Spatial protocol (1996-2003)		Temporal protocol (1979-2009)
Taxa	Otter trawl survey	Frame trawl survey	Frame trawl survey	Beam trawl survey	Frame trawl survey
Acipenser sturio	$\overline{L}$ ↑ ***	-	-	-	<->
Alosa alosa	<->	D ↓ *	<->	-	D ↓ *
Alosa fallax	<->	<->	<->	-	D ↓ ***
Anguilla anguilla	-	<->	<->	-	D ↓ **
Argyrosomus regius	<->	-	-		<->
Dicentrarchus labrax	<->	D ↓ *	<->	-	<->
Dicologlossa cuneata	-	<->	<->	$\overline{L}$ ↑ *	-
Engraulis encrasicolus	-	<->	<->	<->	D ↑ ***, $\overline{W}$ ↓ ***
Gasterosteus aculeatus	-	-	-	-	$\overline{W}$ ↓ *
Liza ramada	$\overline{L}$ ↓ ***	<->	<->	-	D ↑ *
Merlangius merlangus	D ↓ *	-	-	<->	-
Mullus surmuletus	-	-	-	$\overline{L}$ ↑ *	-
Osmerus eperlanus	-	<->	<->	-	D ↓ ***
Platichthys flesus	<->	<->	-		D ↓ ***
Pomatoschistus spp.	-	<->	<->	$\overline{L}$ ↑ *	D ↑ *, $\overline{W}$ ↓ *
Solea solea	D ↑ *	<->	<->	<->	<->
Sprattus sprattus	-	D ↑ *	<->	-	D ↑ *
Syngnathus rostellatus	-	<->	D ↑ **	<->	<->
Trachurus trachurus	-	-	-	-	-
Trisopterus luscus	<->	-	-	<->	-
Total number of species	10	13	12	10	14

Table 5

Results of the combined trend diagnostics for the different data series studied in the Gironde estuary (initial state being impacted). ↔: no significant trend, ↑: significant increase, ↓: significant decrease.

Sampling survey	Time period	Localisation	Trends in juvenile fractions	Trends in community	Trends in functional guilds	Overall diagnostic	Protocol comparison[1]
Otter-trawl survey	1994-2000	Upper and middle estuary	↔	↔	↔	Not improving	Gear
Frame trawl survey	1994-2000	Upper and middle estuary	↔	↔	↓	Degradation	Gear, Time
Frame trawl survey	1996-2003	Upper and middle estuary	↔	↑	↔	Not improving	Spatial, Time
Beam trawl survey	1996-2003	Lower estuary, estuarine plume	↑	↔	↔	Not improving	Spatial
Frame trawl survey	1979-2009	Upper and middle estuary	↓	↑	↓	Degradation	Time

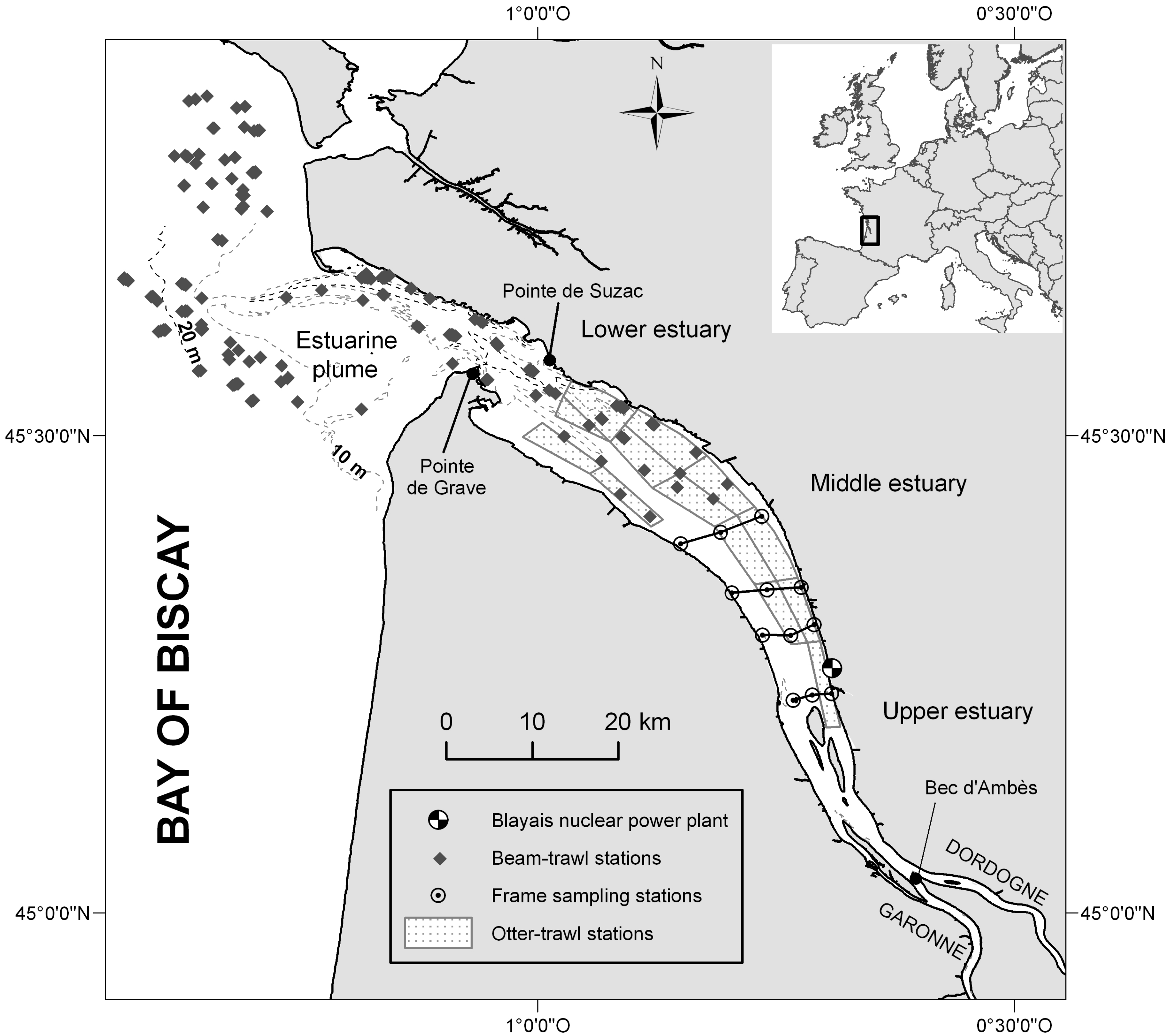
[a] The protocol for which each survey was used for the comparison. Some surveys were used in two protocol comparisons. This was notably the case for the Frame survey for which we used different time periods to compare the different components of the protocol (gear, spatial and time).

Figure captions

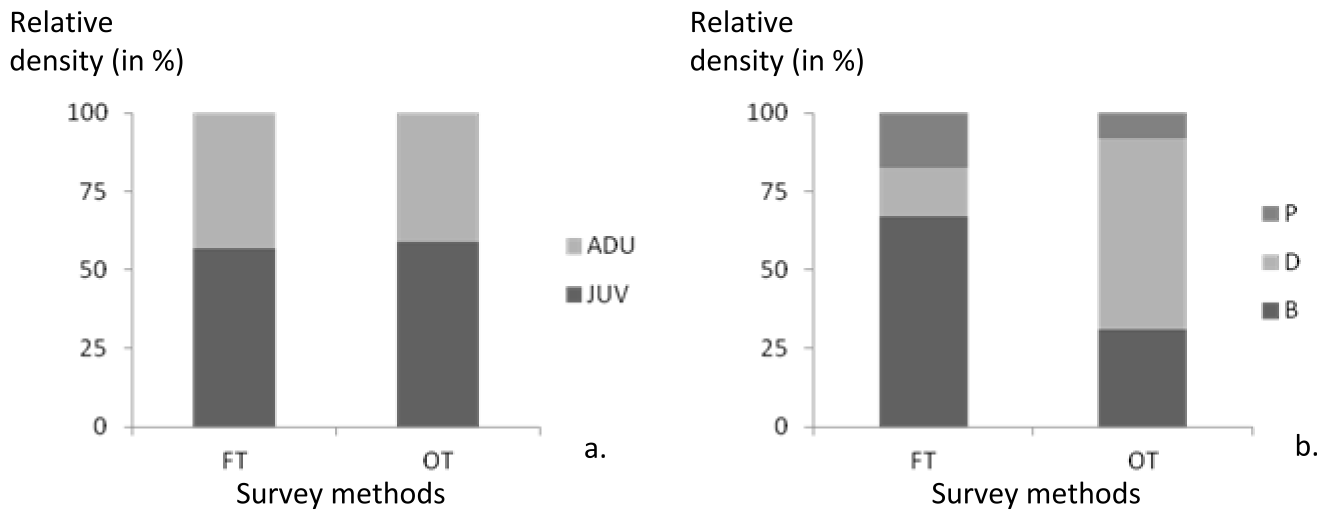
Fig. 1. Location of sampling stations considered in the three sampling surveys in the Gironde estuary.

Fig 2. Relative densities - **a.** of adults (ADU) and juveniles (JUV) - **b.** of the benthic (B), demersal (D) and pelagic (P) species, sampled by (FT) the frame trawl survey and (OT) the otter trawl survey between 1994 and 2000.

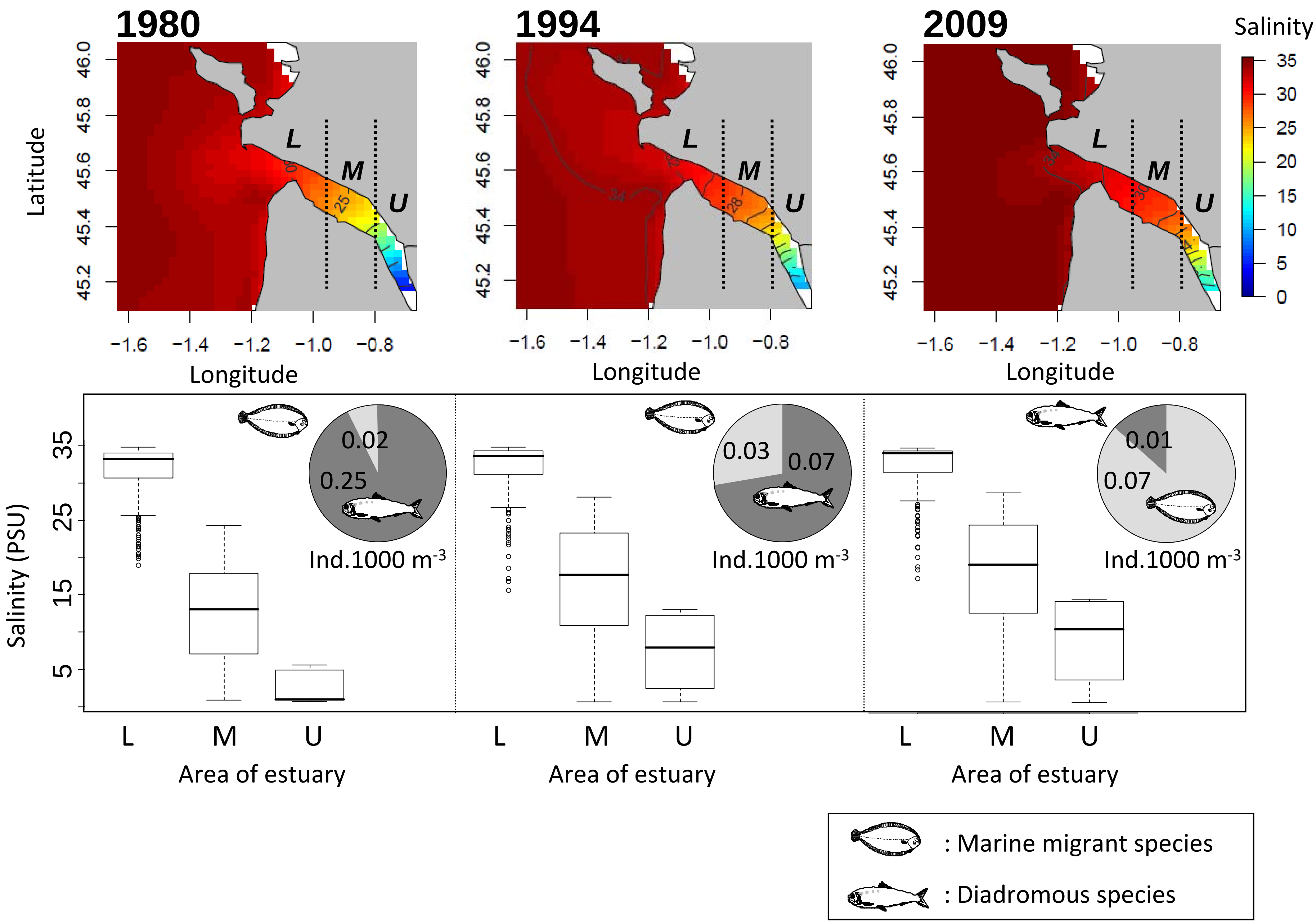
Fig 3. Spatial and temporal variability of the salinity and relative density of diadromous and marine migrant species observed between 1980 and 2009 in the Gironde estuarine ecosystem. The upper panels display the temporal and geographic evolution of the salinity as we move from the lower (L) to the middle (M) and upper estuary (U). Lower panels indicate the mean salinity values estimated in the three portions of the estuary for the three years shown in the upper panels. Shaded circles in the lower panels illustrate the functional shift of the fish densities going from a diadromous-dominated system in 1980 to a marine migrant-dominated system in 2009.



1°0'0"O
0°30'0"O
N
Pointe de Suzac
Lower estuary
Estuarine plume
20 m
10 m
Pointe de Grave
Middle estuary
Upper estuary
45°30'0"N
45°0'0"N
BAY OF BISCAY
0
10
20 km
Bec d'Ambès
DORDOGNE
GARONNE
Blayais nuclear power plant
Beam-trawl stations
Frame sampling stations
Otter-trawl stations



Relative density (in %)
100
75
50
25
0
ADU
JUV
FT
OT
Survey methods
a.
Relative density (in %)
100
75
50
25
0
P
D
B
FT
OT
Survey methods
b.



1980
1994
2009
Salinity
Latitude
Longitude
L
M
U
Salinity (PSU)
Area of estuary
0.02
0.25
0.03
0.07
0.01
0.07
Ind.1000 m-3
: Marine migrant species
: Diadromous species