# Feasability study for echo-integration use in the Zeeschelde estuary and comparison with the Garonne river estuary

V. Samedy, Mario Lepage

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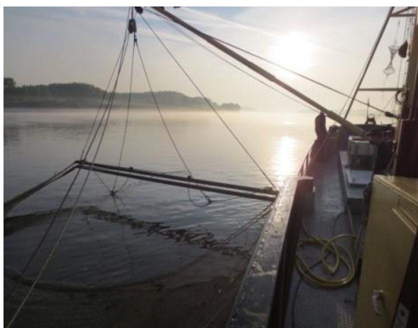
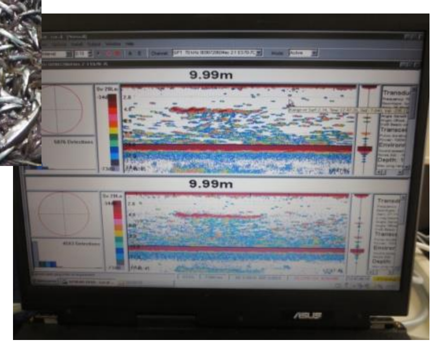
# FEASIBILITY STUDY FOR ECHO-INTEGRATION USE IN THE ZEESCHELDE ESTUARY AND COMPARISON WITH THE GARONNE RIVER ESTUARY






**13 OCTOBER 2014**

**VALÉRIE SAMEDY & MARIO LEPAGE**

irstea

inbo
instituut voor natuur- en bosonderzoek

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# 1. Introduction

At the interface between marine environment and freshwaters, estuarine ecosystems are complex and highly variable natural environments. Because of this, effective assessment of fish densities in these areas imposes a set of challenges. In such a dynamic and huge habitat there is no unique method that can provide a reliable picture of fish populations. A large number of monitoring studies with both active and passive sampling tools showed that the combined use of several different fishing techniques, overlapping in terms of selectivity, providing a relevant picture of the composition and size of fish population.

Today, more and more fishery scientists and stakeholders become involved in ecosystem management and are looking for effective ways to observe and assess environmental damage. Hydroacoustic methods offer high resolution observations through the water column, and provide good values in terms of data richness (Mackinson et al., 2004). This is an alternative or complementary method for studying fish populations in a wide variety of aquatic habitats (Simmonds and MacLennan 2005). Acoustic approach has proven an effective tool providing ecological information on fish distribution, abundance and size-structure which complements more established, but invasive and labour-intensive, methods of biological sampling. This tool is increasingly favoured (Kubečka et al., 2009) over other sampling methods (e.g. scientific and commercial catches data) which are often expensive in terms of time and manpower (Schramm et al., 2002; Simmonds and MacLennan 2005). The use of hydroacoustics to investigate fish populations is compatible with current best practices and successful application in aquatic ecosystem but rarely used in large estuaries.

## 1.1. Presentation

### *1.1.1. Research institutes*

Irstea, the National Research Institute of Science and Technology for Environment and Agriculture, is a public scientific and technical institute in joint supervision with the Ministry of Research and the Ministry of Agriculture. Irstea, the new name for Cemagref, has built a multidisciplinary and systemic approach to three domains – water, environmental technologies and land – which today form the basis of its strength and originality. The Institute has therefore establishing itself as the spearhead of the environment in support of public policies. This led us in 2010 to become the voice of environmental research by founding AllEnvi, the national alliance for environmental research, whose aim is to dedicate time for research in public debate on environmental challenges (www.irstea.fr).

The Research Institute for Nature and Forest (INBO) is the Flemish research and knowledge centre for nature and its sustainable management and use. INBO conducts research and supplies knowledge to all those who prepare or make the policies or are interested in them. As a leading scientific institute, INBO works for the Flemish government primarily, but also supplies information for international reporting and deals with questions from local authorities. In addition, INBO supports organisations for nature management, forestry, agriculture, hunting and fisheries. INBO is a member of national and European research networks. It makes its findings available to the general public (http://www.inbo.be/).

### *1.1.2. Study area*

#### 1.1.2.1. In the Gironde estuary

The Gironde estuary is located in southwest France (Figure 1). Covering an area of over 625 km² at high tide, with a semi-diurnal tidal regime, the Gironde estuary is the largest macrotidal estuary in western Europe (Etcheber et al., 2011).

It is characterized by a high concentration of suspended matter in the turbidity maximum zone (SPM > 50 $g.L^{-1}$) (Allen 1972). Hydrodynamic conditions in the area are extremely variable due to the combined inflows of the sea and tributary rivers, which result in strong temperature and salinity gradients (Selleslagh and Amara 2008). This, combined with a low degree of vertical stratification caused by extensive tidal mixing (Allen 1972) means that the water column is more or less homogenous.

For many years, assessments of fish densities within the Gironde estuary have been based on a number of different fish monitoring techniques, as well as professional and scientific surveys. These assessments were up to now the basis of the monitoring programme for the ecological changes.

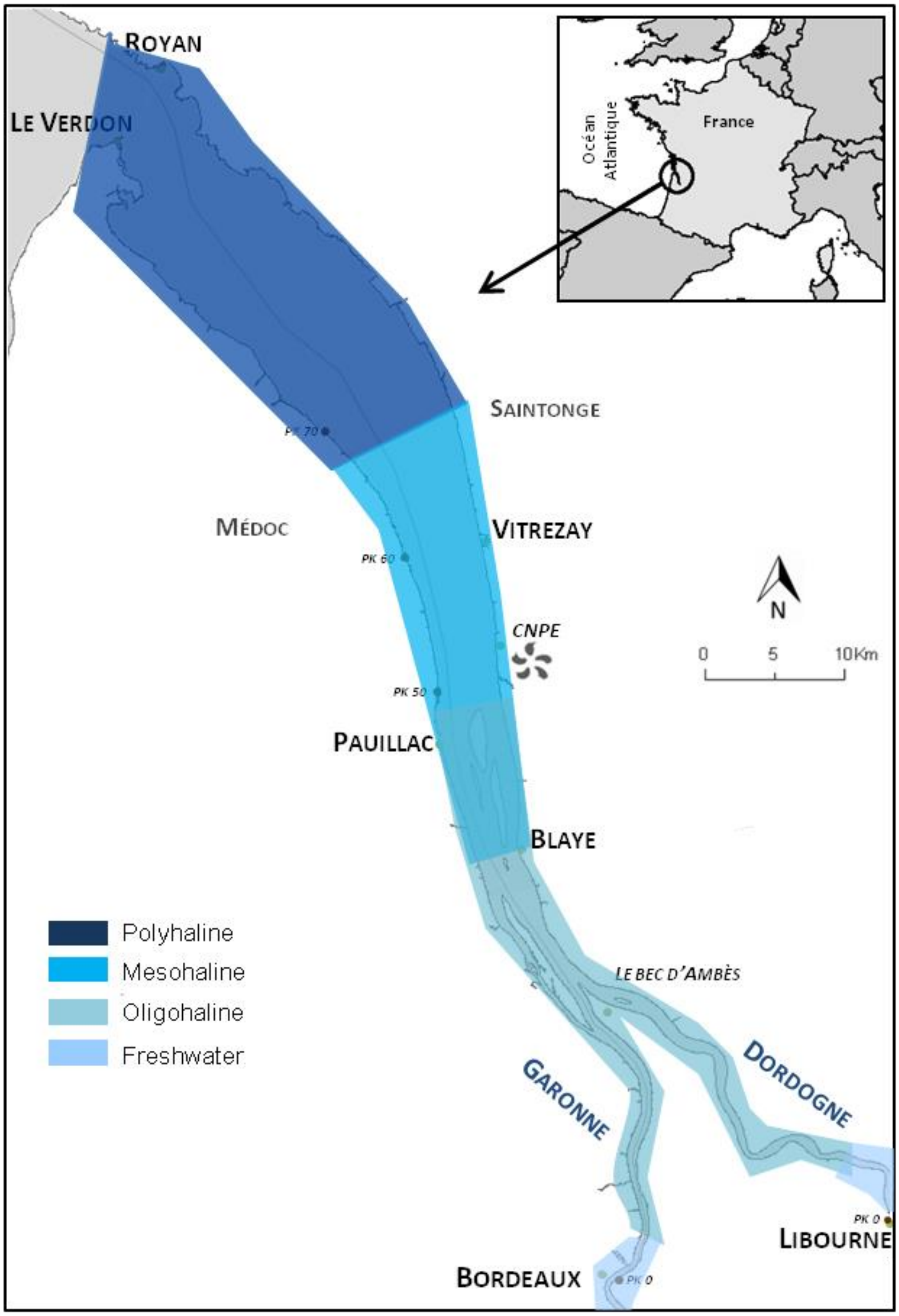


*Figure 1: Map of the Gironde estuary (France).*

#### 1.1.2.2. In the Zeeschelde estuary

The river Schelde is a rain fed lowland river with a length of 355 km from source to mouth. The river is divided in three zones (Figure 2): The Westerschelde in The Netherlands (58 km), followed by the Zeeschelde (105 km) to Gent and the Upper Schelde upstream Gent. The tidal Schelde extends to Gent, where sluices interrupt the tidal wave (Breine 2009).

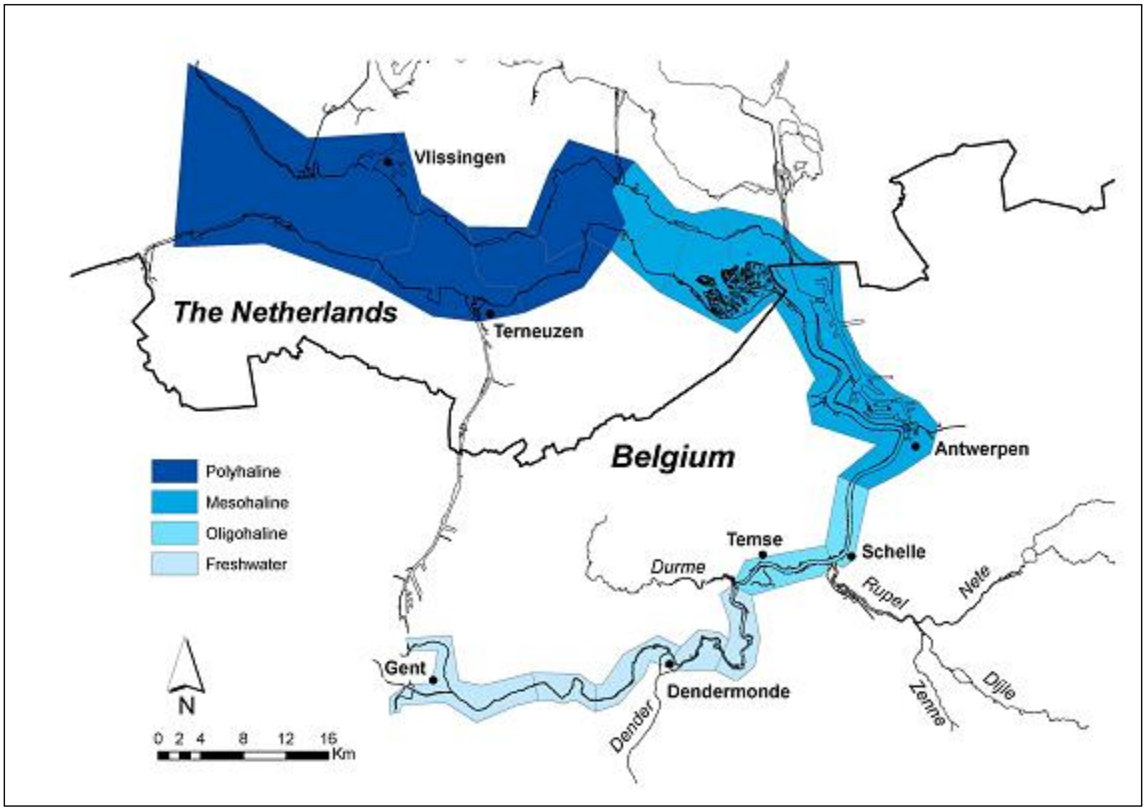


*Figure 2: Map of the Zeeschelde estuary (Belgium).*

Turbidity in the Zeeschelde estuary is high and two maximum turbidity zones might be observed: a first one at the freshwater/seawater interface (mesohaline/oligohaline zone) and a second one originating from tidal asymmetry in the tidal freshwater zone (Herman and Heip 1999; Meire et al., 2005; Van Damme et al., 2005). This turbidity is considered low compared to the Gironde as it reaches a maximum of 300 $mg.l^{-1}$ (Baeyens et al., 1998).

### *1.1.1. Objectives*

Irstea and INBO wish to collaborate in the field of acoustic survey in estuaries. Applying an acoustic approach to this kind of intrinsically changeable environment represents a real challenge. Recently, a research project was carried out to determine the optimum way of applying acoustic sampling in the Gironde estuary (Samedy et al., 2013). The results of this study show that it is possible to use hydroacoustics in estuaries as a complement to traditional methods for monitoring fish populations. The aim of the collaboration is to use the benefits of this experience and to continue efforts for the implementation of an acoustic approach in estuarine environments, especially as internationally-accepted standards need to be created and outlined in order to ensure comparability of results (Godlewska et al., 2011; Guillard et al., 2012) as shown by the European CEN standard guidance (CEN 2009). Acoustic methods can be used to describe the fish biomass and size class distributions at large spatial and temporal scales, without disturbing the environment or the fish fauna (Brandt 1996).

The first main objective was to lead a feasibility research on the use of acoustic surveys to define pelagic fish abundance and to quantify hyperbenthos in the Zeeschelde estuary. To show the potential of this tool in the monitoring of estuarine fish dynamic, acoustic data were compared with fish survey results obtained by anchor net in order to determine whether densities detected acoustically were consistent with the results of fish.

The second objective was to compare the acoustic structure and abundance of the fish population in both the Gironde (France) and Zeeschelde estuaries (Belgium) in order to better understand the ecological functioning of fish population and to move towards the implementation of the standardization of procedures.

This kind of study is necessary in order to take into account the highly variable nature of the ecosystem in question and to improve the knowledge on estuarine systems by using tools that are compatible with current best practices.

## 1.2. Hydroacoustic background

Sonar (originally an acronym for Sound Navigation And Ranging) allows detection of sub-aquatic targets emitting ultrasound and analysing the echoes reflected by the targets. Frequencies typically used in fisheries acoustics vary between 20 and 500 kHz but high frequencies up to 1 MHz and low frequencies down to 1kHz are sometimes used (Simmonds and MacLennan 2005). If the first echo-sounders allowed only to determine the presence or absence of fish, in recent decades, technological developments including miniaturization of electronic components and the increase of the computing power, have facilitated the production of hydroacoustic systems, which can be readily deployed from small vessels on various aquatic ecosystem. The book of Simmonds and MacLennan (2005) provides further details on fisheries acoustics.

Hydroacoustic methods are used to acquire a variety of information in aquatic environments. This tool is increasingly favoured (Kubečka et al., 2009) over other sampling methods (e.g. scientific and commercial data catches) which are often expensive in terms of time and manpower (Schramm et al., 2002; Simmonds and MacLennan 2005). Only few concepts useful to understand hydroacoustics of this study will be discussed and described in this section.

### *1.2.1. Basic principles*

The basic principles behind the use of hydroacoustics for fish population investigations are relatively simple but acoustic systems are the only a way to penetrate the aquatic environment staying at the surface. Echosounders are electronic devices that transmit acoustic pulses through a transducer into water. When a pulse is emitted into the environment, it spreads until it meets a target with a different density from the propagation environment (Martignac et al., 2014). The sound may be directed vertically or horizontally and effectively insonifies a cone of water with each pulse. All types of objects can be detected including fish, suspended matters, wreck ... (Simmonds and MacLennan 2005). The acoustic pulse is reflected from these targets and returns to the transmitter. The acoustic sampling is discrete in time, because of the alternation of the transmission and reception phases (Gerlotto et al., 1999). The shape and intensity of the echo received depends on many parameters, including the frequency of the acoustic signal, the size, the density and the inclination of the insonified target. The detected echoes are displayed on an echogram (Figure 3), on which target echoes are represented by coloured patches whose signal strength is given by a colour scale.

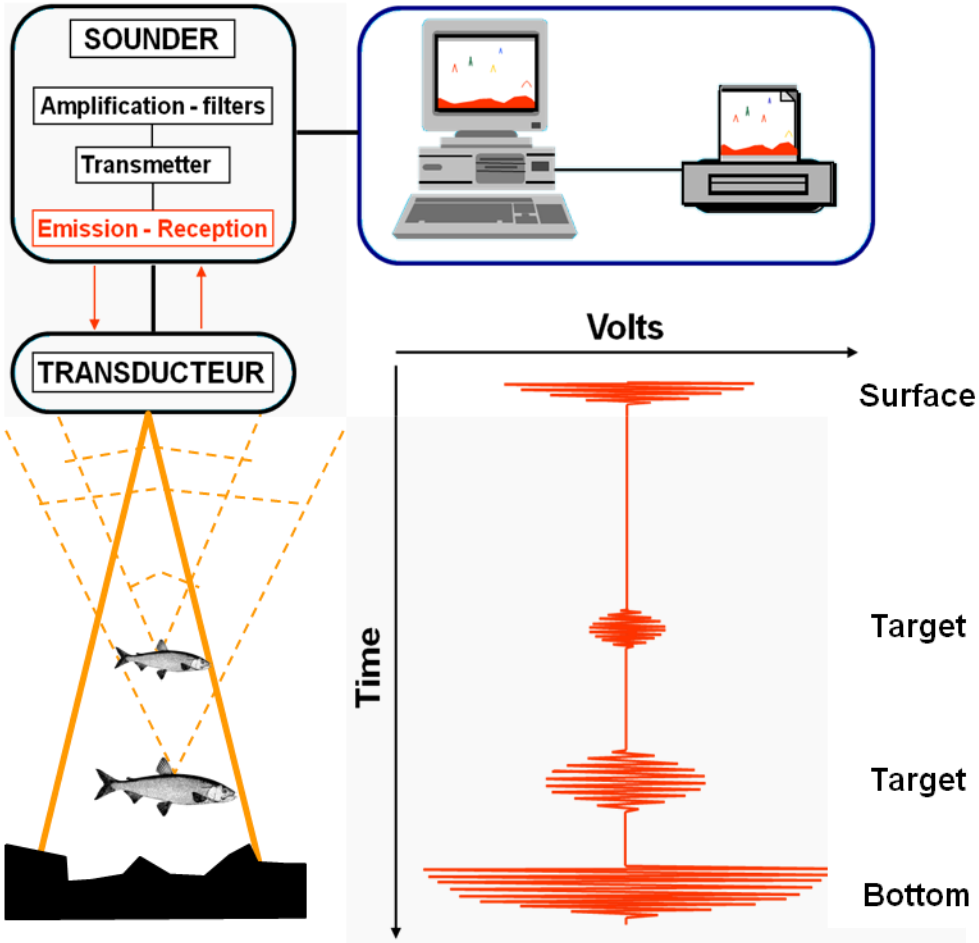


*Figure 3: Set up of echo-sounding*

To reflect acoustic energy of a target, it is essential that one or more elements of its anatomy have acoustic impedance different from the water. In principle, it is possible to calculate the energy returned by an individual fish from acoustic theory and knowledge of the body parts that contribute to the echo. For fish species that possess a swim bladder, 85% from the total sound energy is reflected from this organ and the remaining 15% from other parts of the body (fat, bone and flesh) (Foote 1980; Clay and Horne 1994; Jech et al., 1995). The energy of a detected echo by sonar is thus proportional to the square of the pressure received by the transducer. Foote (1983) confirmed experimentally that the energy received by an echo sounder is proportional to the quantity of insonified fish for frequency ranges and power commonly used in fisheries acoustics.

Transducers are sensitive to echoes from all elements that create noise like turbidity, bubbles in surface, etc. Weak unwanted signals (noise echoes) are very numerous and can seriously bias abundance estimates. Echoes from other targets within the water column can be problematic but they can normally be eliminated during post-survey data processing. The magnitude of the difference between echoes originating from fish and such unwanted echoes is termed 'the signal to noise ratio' (SNR), which can be maximized depending on the setting of the noise threshold (CEN 2009).

In the field of fisheries research, acoustic methods convert physical measurements into relevant ecological units describing the fish population (Trenkel et al., 2011). The most commonly used frequencies range from 12 to 430 kHz. Acoustic approaches are generally based on data collected with multiple frequencies as many scientific studies show that the frequency response differs between organisms (Logerwell and Wilson 2004; Korneliussen et al., 2008; Axenrot et al., 2009; Korneliussen et al., 2009). Thus, the multi-frequency approach can be used to identify different species or groups of species (Madureira et al., 1993; Mitson et al., 1996; Horne 2000; Korneliussen and Ona 2002; Korneliussen and Ona 2003; Gauthier and Horne 2004) based on the variability of the acoustic response (Figure 4).

The choice of a frequency for a given study is a compromise between the range of the beam and the energy reflected by the target (Simmonds and MacLennan 2005), and the relevance of a multi-frequency approach depends on ecological questions.

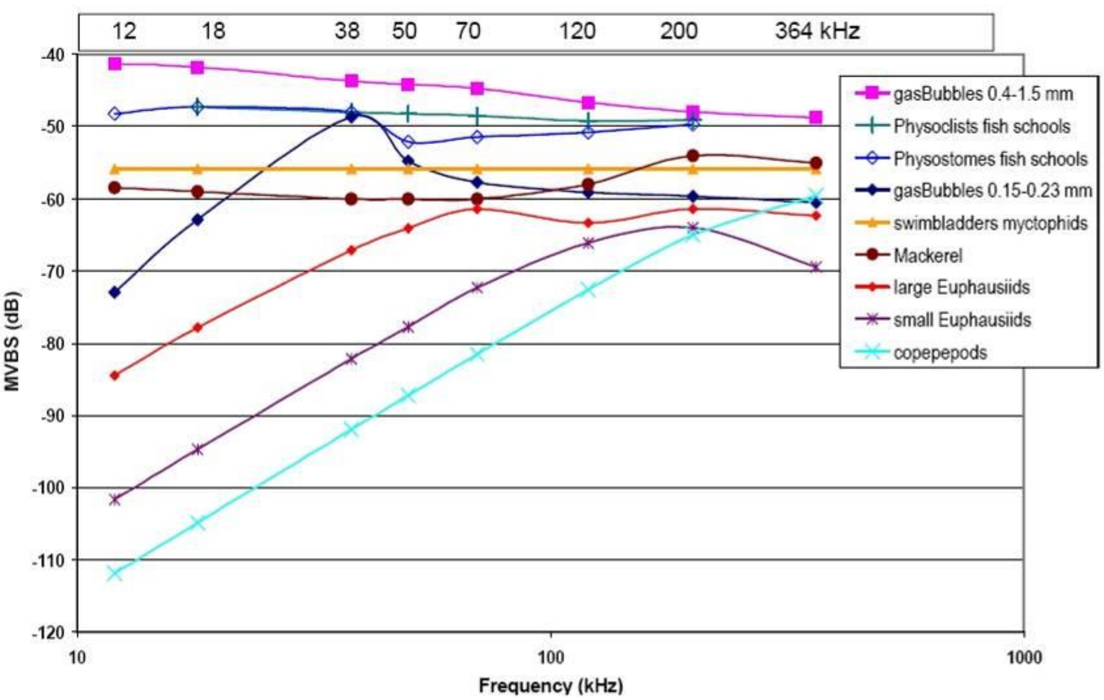


*Figure 4: Examples of the frequency response based on different classes of target (Fernandes et al., 2006).*

### *1.2.2. Acoustic metrics*

#### 1.2.2.1. Target strength

The main property of fisheries acoustic is their capacity of reflection also known as 'Target Strength' (TS). The TS is a logarithmic measure of the proportion of the incident energy that is reflected from the target:

$$TS = 10\log(I_R/I_I)$$ où $I_R$ = incident intensity

$I_I$ = backscattering intensity.

According to Ona (Ona 1999), a relation between target size and echo response exists. However, there are considerable variations between species and even individuals of the same size and species. It depends on the individual behaviour, in particular the orientation of the body relative to the beam. TS is a key factor in the biomass estimates and fortunately it is the easiest factor to measure or estimate (Lévénez et al., 2006). Most existing relationships in the literature were established by linear regression on one or more species of temperate zones (Love 1977; Frouzova et al., 2005). They lead to estimates of TS sometimes very different and so are practically only usable in areas where they were calculated. These relations can, however, be used to estimate a "reasonable" range in which the TS values should lie for the different studied groups of organisms.

#### 1.2.2.2. Echo-integration

Echo-integration, which integrates the return-echo strength (backscattering) from the echosounder's sampled volume, is a very robust method and is a mean to estimate the number of fish in the detection beam (Simmonds and MacLennan 2005). If more than one target is located in the acoustic beam at the same depth, it is usually not possible to resolve them separately. Echo integration assumes that the total acoustic energy scattered by a group of targets is the sum of the energy scattered by each individual target. This assumption holds well in most cases (Foote 1983). The total acoustic energy backscattered by the school or aggregation is integrated together, and this total is divided by the backscattering coefficient of a single animal, giving an estimate of the total number of individuals.

The calculations in the various steps of the echo-integration determine acoustic densities. Two types of backscattering indices can be obtained:

- $s_a$ defined as the *area Scattering coefficient*, expressed in $m^2.ha^{-1}$ of *backscattering cross-section* ($\sigma_{bs}$),
- $S_v$ defined as the *volume backscattering Strength*, expressed in dB (MacLennan et al., 2002).

Acoustic measurements are often quoted in decibel unit. This is done because the numbers involved can be very large or very small, covering many orders of magnitude.

### *1.2.3. Some inherent limitations of hydroacoustics*

The fish fauna is described by its composition, abundance and size structure. However, the major problem with the hydroacoustic is that it is not possible to identify species separately, although many studies worked on this issue (Horne 2000) through the threshold and the multi-frequency. The choice of this threshold is arbitrary and one of the difficulties of the acoustic approach lies in the choice of this threshold, which is a filter (such as the size of the mesh of a net) to retain only that fish compartment.

The recording quality is also dependent on the weather, since the wind friction on the water surface and the movements of the vessel, result in the formation of bubbles near the surface layer that reduces the amplitude of the echoes received by the echo-sounder when the depth of the bubbles layer exceeds that of the transducer (Simmonds and MacLennan 2005).

In acoustics there are two areas where the target cannot be detected: near the surface and near the bottom (Figure 5). Indeed, a blind zone exists near the surface and depends on the position of the transducer and the size of the near field (Demer and Hewitt 1995; Aglen 1996; Simmonds and MacLennan 2005). However, on the bottom, the blind zone begins at the depth from which the echo of a target coincides with that of the background (Guillard 1998). It therefore depends on the morphology of the bottom, the pulse duration and the depth, determining the beam diameter.

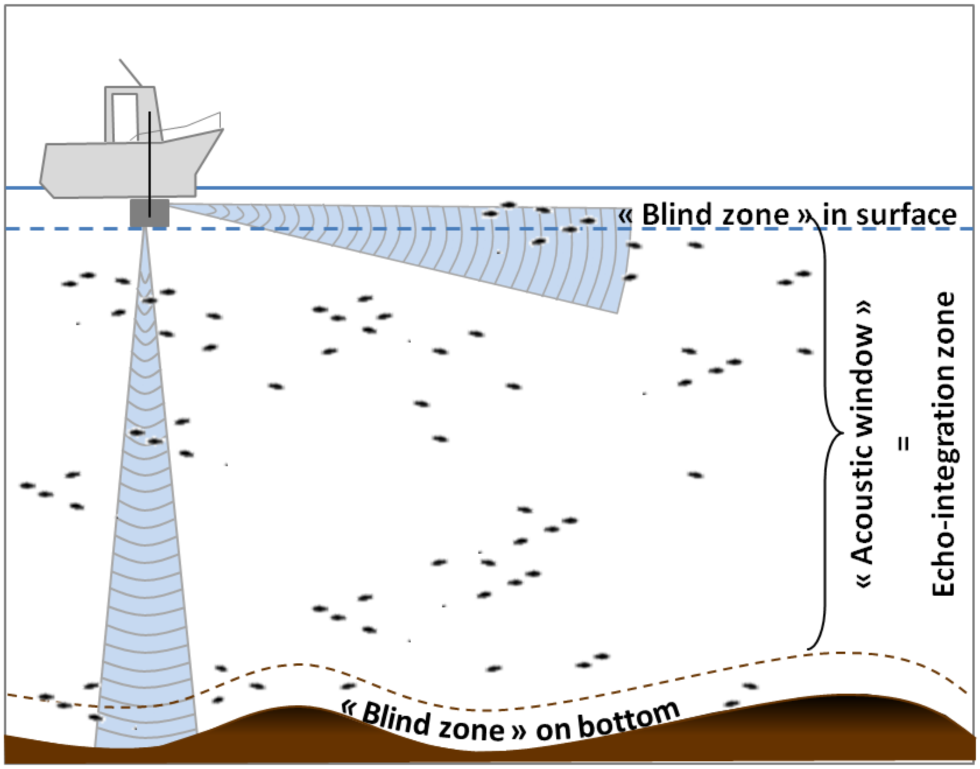


*Figure 5: Sampling window by acoustic approach.*

Although acoustic methods are used for fishery since decades, hydroacoustics still seems innovative in estuarine ecosystems. The implementation of hydroacoustics got there late and this is shown by the "poor literature" on the subject. However, an increasing number of studies (Guillard 1998; Knudsen and Sægrov 2002; Krumme 2004; Boswell et al., 2007) show the potential of hydroacoustics in estuaries despite a number of challenges (Moszynski and Hedgepeth 2000; Guillard et al., 2012).

## 2. Surveys and data acquisition

### 2.1. Surveys

When possible, the use of multiple sampling techniques is preferable to obtain an overview on the fish assemblage structure. Sampling strategies are essential and depend on the scientific objectives, on the study area, on the allotted time and on the spatio-temporal scales of the ecosystem. In this project, we relied on the results of the research project carried out in the Gironde estuary. Through methodological and ecological issues, this work has provided a glimpse into the interests of hydroacoustics in terms of estuarine monitoring but also in terms of analysis of ecological dynamics.

### 2.1.1. Acoustic equipment

A Simrad EK60 split-beam echosounder controlled by the Simrad ER60 (version 2.2.0) program was used for data acquisition at two frequencies, 70 kHz and 120 kHz. Two circular transducers were used, with beam angles of 7° at -3 dB. An elliptical transducer with a beam angle of 2.5° X 10° was added in horizontal position. All transducers were fixed to the side of the boat, 0.50 m below the surface and vertically or horizontally oriented (Figure 6). The pulse duration was 256 ms, emitting 4 pulses per second, with power set at 100 W.

In multi-frequency configuration, a unique computer synchronizes the ping emissions of the entire system (Figure 6). We used simultaneously the two frequencies of 70 and 120 kHz thanks to special procedures. Observations were carried out by vertical and horizontal echosounders.

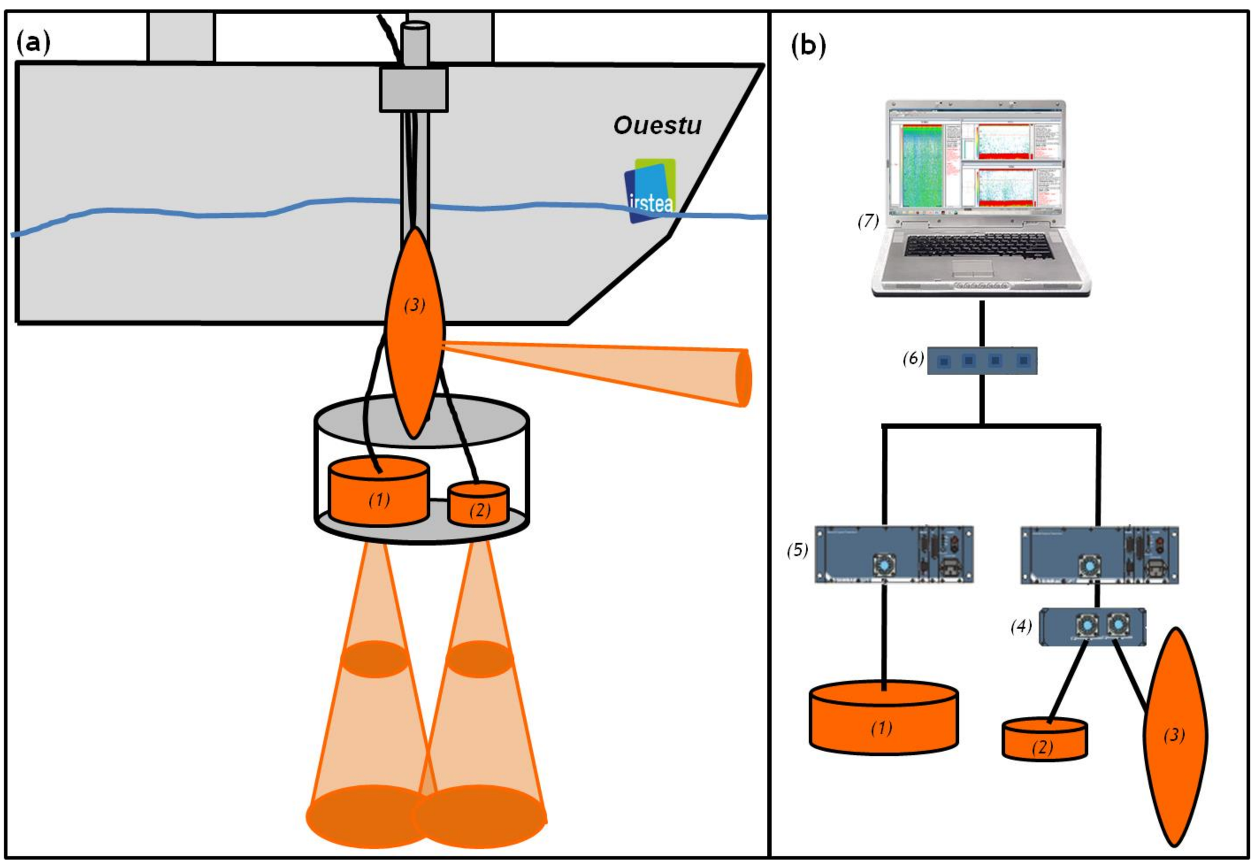


*Figure 6: Schematic installation of echosounders (a) outside and (b) inside.*

Each transducer was calibrated once per year, using standard targets (Foote et al., 1987) and checked for consistency before each survey.

Hydroacoustic surveys typically produce several hundred megabytes of data and so a substantial and robust data archiving system is essential. The safe storage of such data is essential not only in order to allow analyses to be repeated with contemporary software using variations in analytical parameters, but also to be revisited with future developments in new software (Winfield et al., 2011).

### *2.1.2. Sampling design*

The options chosen in the design of a survey could affect both data collection and data analysis. In hydroacoustics, two types of data acquisition are mainly used: fixed stations and mobile sampling. The first method provides values for a specific point, with a limited spatial representativeness of the study area. However, targets can stay longer under the beam, and may allow tracking of individual targets (Simmonds and MacLennan 2005). The second approach is more integrative (Guillard et al., 2004) as the detected volume is larger and can sample different habitats. It therefore provides a more comprehensive view of the environment, distribution and size structure of populations. Acoustic sampling from fixed stations can provide a picture similar to that resulting from a mobile acoustic monitoring (Guillard et al., 2004). However, in estuarine environments, fixed stations are interesting to observe fish individually (Krumme and Hanning 2005; Boswell et al., 2007), minimizing sampling effects on fish behaviour. Although these types of observations are based on a limited representation of the ecosystem compare to a mobile strategy, a stationary sampling design was adopted. Fixed stations provide insight into certain behaviours based on environmental parameters (temperature, depth, flow velocity, direction of current, etc.) and thus to interpret the fish dynamics by freeing some variability of the environment. Moreover, equipment installed at fixed location offers the possibility of continuous observation over a long period of time.

In this study, once the boat is anchored, transducers are set in the water, fixed to a pole on the side of the boat, just in front of the net (Figure 7).

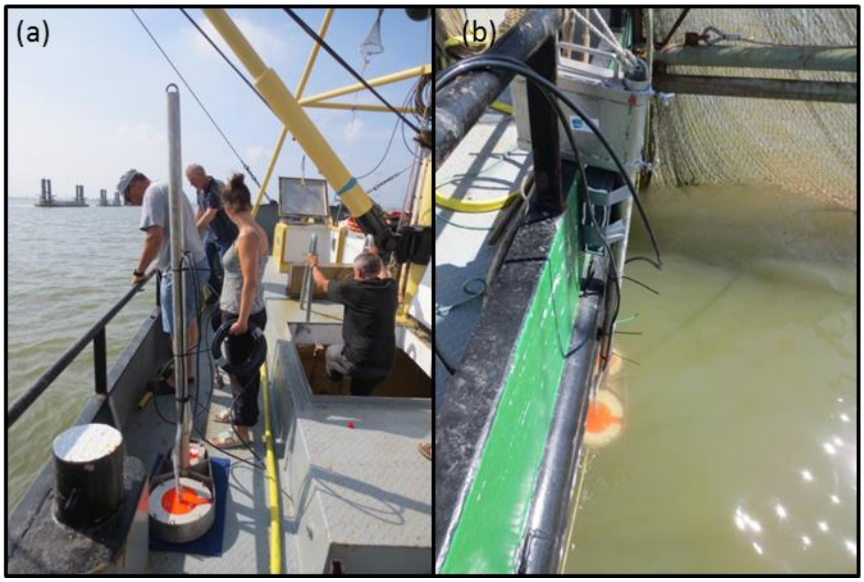


*Figure 7: Transducers installed (a) in a base fixed by a stick and (b) placed on the side of the boat.*

Each survey was carried out under identical conditions, i.e. during the daylight, in fixed location, around high tide (2 hours before and after high tide) in order to obtain the greatest range of depths (Samedy et al., 2013), and data were continuously recorded. During these surveys, acoustic data were collected at different stations along the salinity gradient (Figure 8).

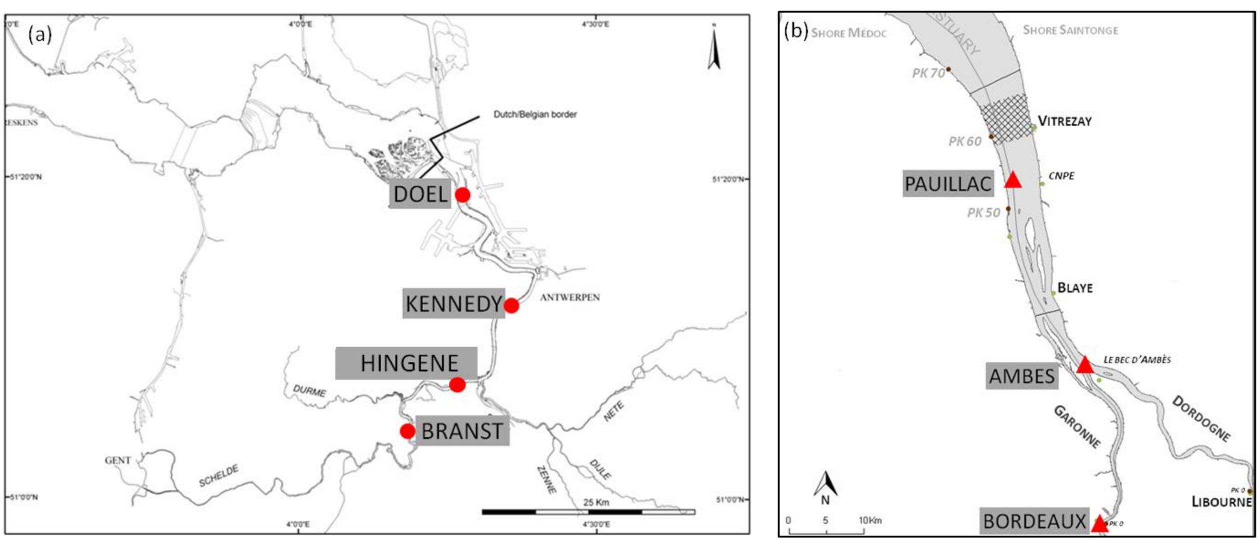


*Figure 8: Localisation of fixed stations (a) in the Zeeschelde estuary and (b) in the Gironde estuary.*

2 series of surveys were conducted:

- in July and September 2013 for the Zeeschelde estuary,
- in August and October 2013 for the Gironde estuary.

*Table 1: Details of surveys (date, names of stations, tidal coefficient, time of high and low tides in GMT, temperature and salinity) in both estuaries.*

| Date | Station | Tidal coeff | HT/LT (GMT) | Temperature | Salinity |
|---|---|---|---|---|---|
| 15/07/2013 | Doel | 61 | 06:37 / 13:07 | 22 | 12,5 |
| 16/07/2013 | Hingene | 55/53 | 08:32 / 15:04 | 20,7 | 2,5 |
| 17/07/2013 | Branst | 51 | | 21,8 | 0,5 |
| 18/07/2013 | Kennedy | 51/53 | 07:56 / 14:33 | 21,4 | 10 |
| | | | | | |
| 09/09/2013 | Doel | 90 | 04:25 / 11:13 | 20 | 12 |
| 10/09/2013 | Hingene | 83/78 | 06:06 / 12:58 | 19,4 | 2,5 |
| 11/09/2013 | Branst | 72/66 | | 19 | 1 |
| 12/09/2013 | Kennedy | 60/54 | 06:58 / 13:56 | 19 | 10 |

| Date | Station | Tidal coeff | HT/LT (GMT) | Temperature | Salinity |
|---|---|---|---|---|---|
| 12/08/2013 | Bordeaux | 76/72 | 09:28 / 17:08 | 23 | 1 |
| 13/08/2013 | Ambes | 68/63 | 09:29 / 16:46 | 22,8 | 3 |
| 14/08/2013 | Pauillac | 59/54 | 09:39 / 16:34 | 22,4 | 6 |
| | | | | | |
| 09/10/2013 | Bordeaux | 87/81 | 08:48 / 16:37 | 18 | 0,1 |
| 10/10/2013 | Ambes | 75/69 | 08:51 / 16:15 | 18 | 2,5 |
| 11/10/2013 | Pauillac | 62/56 | 09:04 / 16:04 | 17 | 6,5 |

### *2.1.3. Fish surveys*

The use of acoustic tools, combined with traditional fishing equipment, is generally recommended (Kubečka et al., 2009). Many earlier studies involved combining these different methods and comparing the results (Boswell and Wilson 2004; Coll et al., 2007; Emmrich et al., 2012; Guillard et al., 2012). Fishing techniques mainly provide information on the species composition and size spectra of fish (Godø 1998) and have been widely used in fish monitoring due to their simplicity and versatility.

For the Zeeschelde estuary, fish assemblages are surveyed with anchor nets installed on a ship, 'De Harder'; registration number BOU25 property of the company Bout-Van Dijke (Figure 8). Anchor netting is performed in the pelagic zone of the Zeeschelde and provides information on the fish assemblage. The anchor net consists of two 8 meters long steel beams. The lower beam is lowered down to the bottom of the river while the upper beam is maintained at the surface (Figure 9). The net between these two beams covers the total water column on a width of 8 meters. The end of each beam is connected with the anchor that stabilises the boat. The cod end of the net has stretch mesh size of 20 mm. The net is about 70 m long (Breine and Van Thuyne 2013).

Period of fishing is one hour after change of tide until one hour before change of tide. To avoid too much fish-kill, the net remains one hour on one side and two hours on the other side. During fishing, flow velocity is measured to define the volume of water that has been surveyed.

*Figure 9: Boat and fish tools used during surveys in the Zeeschelde estuary.*

Fish are, once on the boat, immediately processed. First, bigger specimens and less commonly caught species are removed and put aside. The rest (i.e. more common species) is subdivided until a manageable representative subsample is obtained. All fish are identified to the species, measured (in mm) and batch weighed (by species). One hundred individuals per species are measured; the rest is only counted and the total mass is recorded (Figure 10). Afterwards all data are digitalised in special datasets.


*Figure 10: Procedure to assess the fishing: (a) sorting of fish and subsampling when necessary, (b) count and weigh and (c) note and record.*

Numbers and biomass are converted to CPUE (catch per unit of effort) and BPUE (Biomass per unit of effort). The unit of effort was set to one hour of fishing. For the analysis, all data were transformed to relative abundance (% of total catch per site, per year and per season).

### 2.2. Acoustic treatment

Acoustic data, recorded in an international format (.raw), requires an important post-processing work before to be analysed (to display the preliminary data, to convert and filter echograms ...). In this study, acoustic data were analysed using Sonar5 Pro software (Balk and Lindem 2006). Only pixels of the echograms above the seabed and below the surface (to remove portions of the echograms affected by air bubbles) were considered. For vertical surveys, this may be simple process of adding up an area estimates or averaging volumetric estimates (Winfield et al., 2011).

The three outputs used in this study are considered as proxies for fish densities ($s_a$ and $S_v$) and fish length (TS) (MacLennan et al., 2002; Simmonds and MacLennan 2005). An instantaneous index of fish biomass ($s_a$ and $S_v$) and the mean target strength were obtained every 30 minutes. The relative distribution of the TS values were divided into three acoustic size classes (small [-60; -55 dB[, medium [-55; -50 dB[ and large [-50; -40 dB[) in order to show the general evolution of targets.

## 3. EVOLUTION OF ACOUSTIC DATA

Several ways of exploring the data exist in hydroacoustics. In this part, we first present the results on the fish biomass detected, then the TS distributions, and finally a comparison between acoustic and fish data. We present results describing the dataset, based on each station according to the salinity gradient. The time is expressed in the GMT +0 (Greenwich Mean Time). Results are mainly based on the descriptive analyses performed with the R statistic program (R Development Core Team 2008) and the Microsoft Excel software. The main difficulty of these results is the lack of continuity in the recorded data introducing complexities in the interpretation.

### 3.1. In the Zeeschelde estuary

#### *3.1.1. Evolution of the acoustic densities ($s_a$ and $S_v$)*

During analyses, a mean value of $s_a$ or $S_v$ was calculated every thirty minutes for the whole water column for each station. Differences in densities were observed between two sampling periods, with the highest values in July, and the lowest in September. In July, the highest values of acoustic densities were observed for Branst station (a) (more than 10 $m^2.ha^{-1}$) and Hingene station (c) (more than 15 $m^2.ha^{-1}$) (Figure 11). Between the two frequencies, the values obtained with the 70 kHz are slightly higher than those obtained with the 120 kHz.

In Doel station (d), the highest acoustic biomass was observed at the slack of the high tide. However, no trend in acoustic densities related to depth was observed for the other stations. To check this hypothesis, the $S_v$ values are used thanks to their independency from the tidal influence as they are expressed per unit of volume (Figure 12).

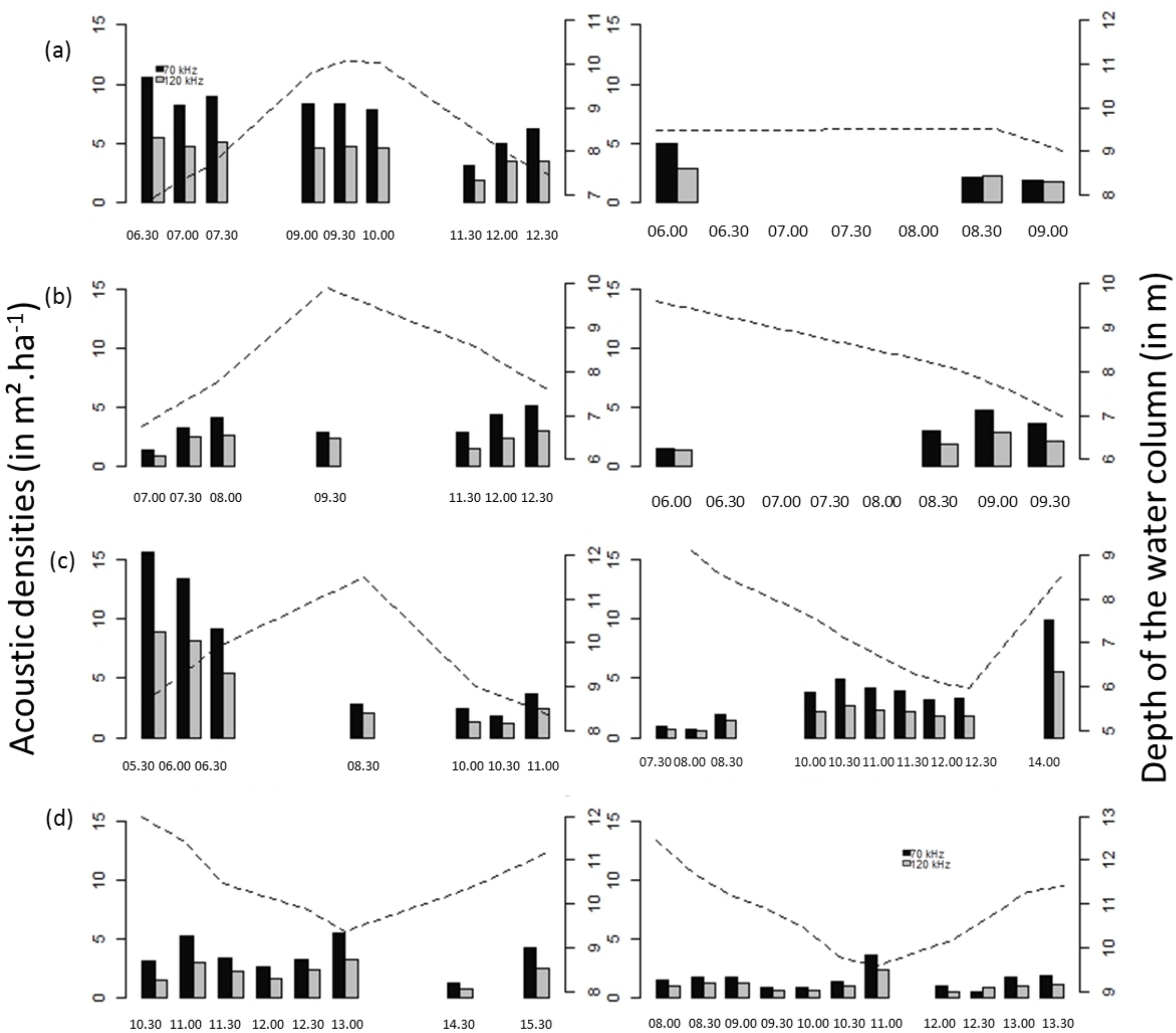


*Figure 11: Evolution of acoustic densities in $s_a$ (m².ha$^{-1}$), at 4 stations (a:Branst, b:Kennedy, c:Hingene and d:Doel) in the Zeeschelde estuary : on the left column in July 2013 and on the right column in September 2013.*

Similar to the $s_a$ values, the highest acoustic densities ($S_v$) are observed for the same stations (Branst and Hingene stations) but seem to be more stable in the two sampling periods (July and September). However, acoustic densities appear to be relatively stable over a tide for the first two stations (Branst and Kennedy stations), with values ranging between -58 dB and -53 dB. At Doel station, the highest value of acoustic densities is observed during the slack of high tide.

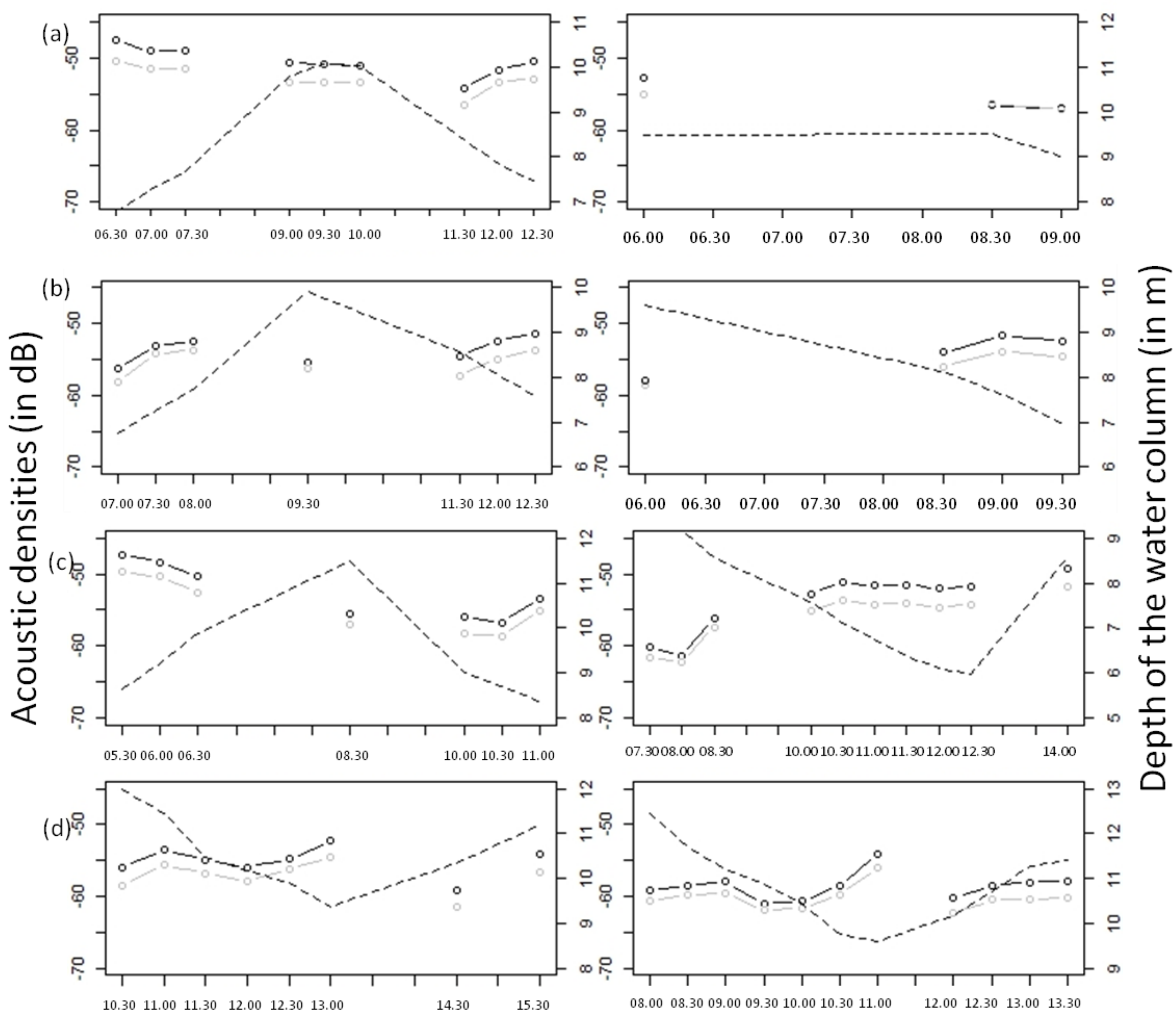


*Figure 12. Evolution of acoustic densities in $S_v$ (dB), at 4 stations (a:Branst, b:Kennedy, c:Hingene and d:Doel) in the Zeeschelde estuary at 70 kHz (in black) and 120 kHz (in grey) in function of the tidal rhythm: on the left column in July 2013 and on the right column in September 2013.*

### *3.1.2. Evolution of the acoustic size classes*

The evolution of mean TS results shows a stability of values along the tidal cycle for both sampling periods and for all stations, with values varying between -60 dB and -50 dB. Both frequencies showed no significant difference and a similar trend.

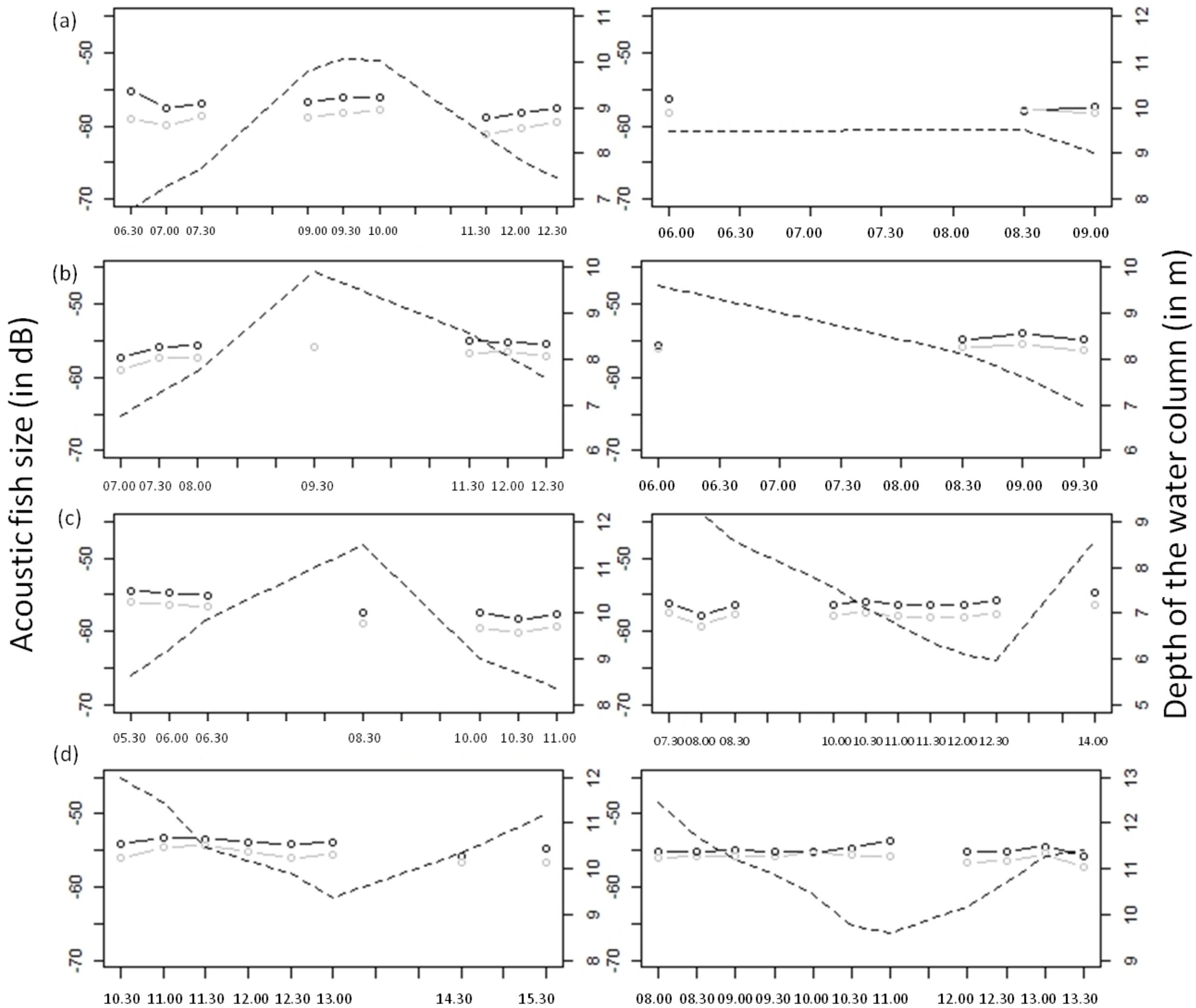


*Figure 13. Evolution of mean TS (in dB), at 4 stations (a:Branst, b:Kennedy, c:Hingene and d:Doel) in the Zeeschelde estuary at 70 kHz (in black) and 120 kHz (in grey) in function of the tidal rhythm: on the left column in July 2013 and on the right column in September 2013.*

However, TS distributions can be tracked and classified in acoustic size classes (by proportion) (Figures 14 and 15). The results for all the stations in the Zeeschelde estuary showed that the number of fish classed as "small" and "medium" represents more than 90% of the total abundance. Larger targets were rarely observed. At 70 kHz, the part of the "medium" targets seems to be slightly higher than the part of the "small" targets, respectively almost 50% and 40% (Figure 14).

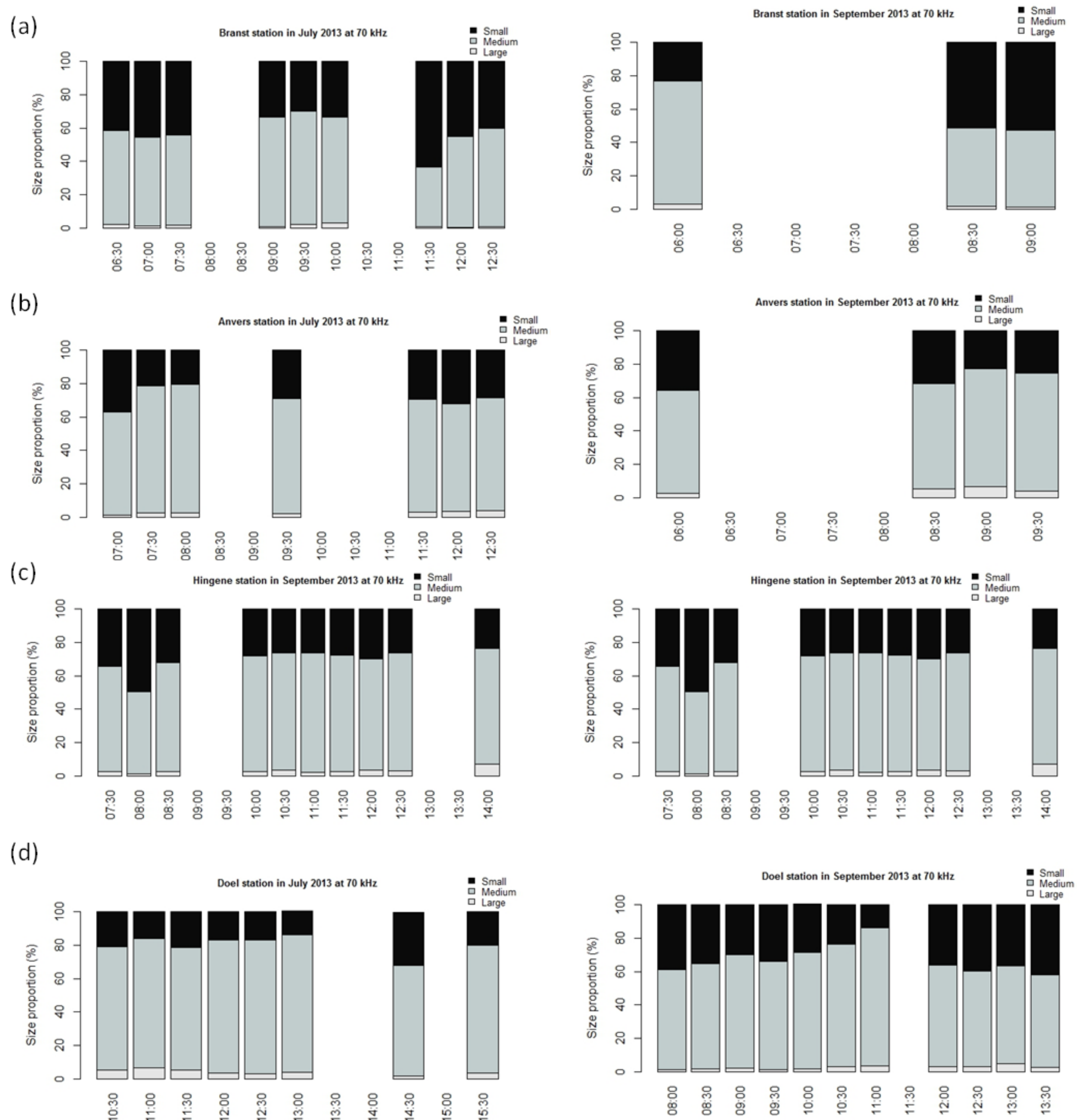


*Figure 14: Evolution of acoustic size class at 70 kHz, at 4 stations (a:Branst, b:Kennedy, c:Hingene and d:Doel) in the Zeeschelde estuary.*

However, this last observation is reversed at 120 kHz, i.e., the proportion of "small" targets is slightly higher than the part of "medium" targets, respectively 60% and 30%, but the trend seems to move towards a more balanced proportion between small and medium targets along the salinity gradient (from Branst to Hingene stations), with finally, more "medium" targets at the last station (Doel station) (Figure 15).

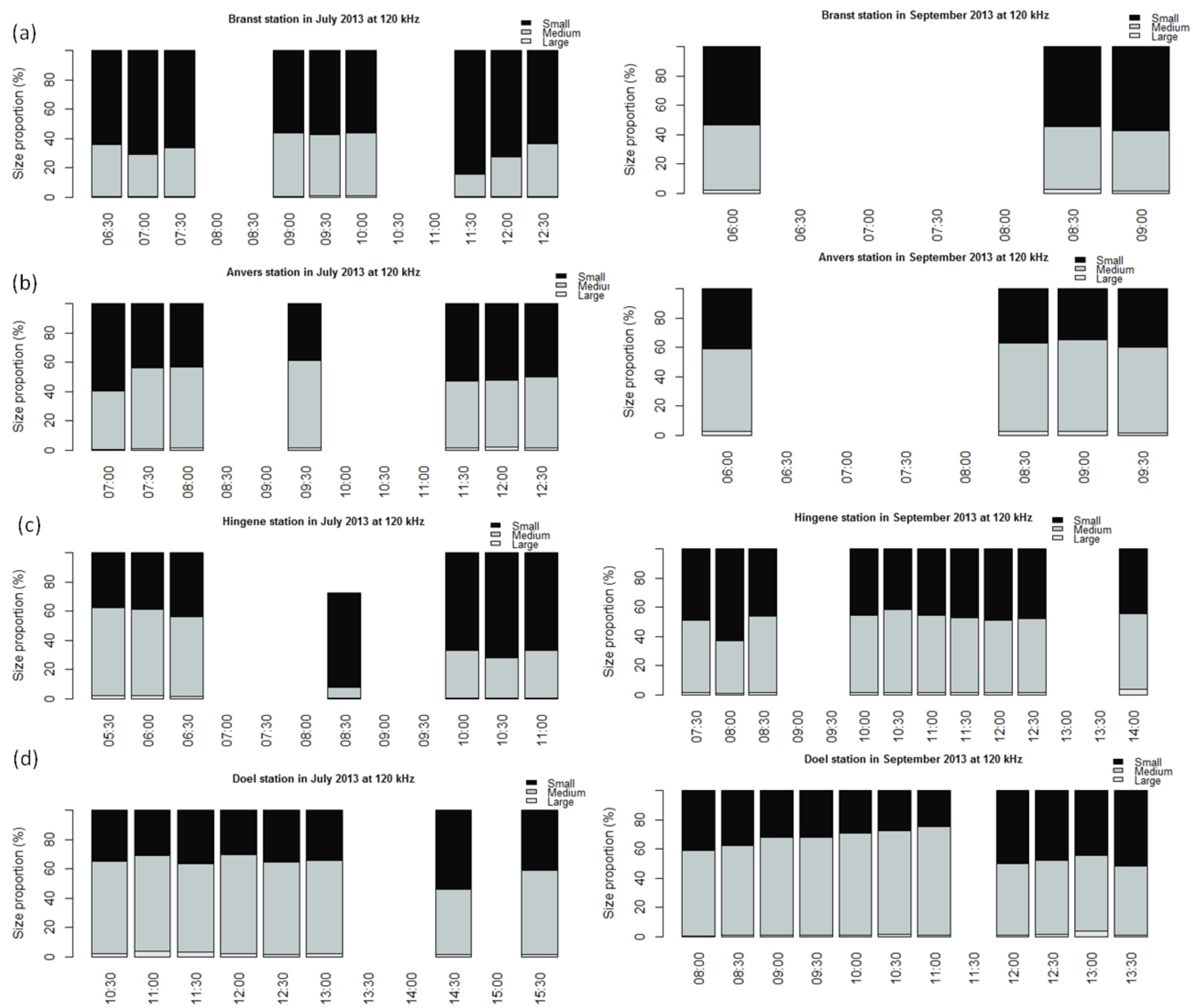


*Figure 15: Evolution of acoustic size class at 120 kHz, at 4 stations (a:Branst, b:Kennedy, c:Hingene and d:Doel) in the Zeeschelde estuary.*

### 3.1.3. Evolution of acoustic densities by stratum

To check the vertical variation of densities in the water column, echograms could be stratified into different horizontal sections. This means that it is possible to refine the assessment by dividing the water column into strata (one value every meter in this study) and this could be done at the timescale previously chosen (every 30 min) for each stratum from the surface to the bottom (Figure 16). This approach allows to locate the highest density of fish in the water column and to identify the relative part of pelagic *vs* benthic species, or to identify specific behaviour due to tide or circadian rhythm.

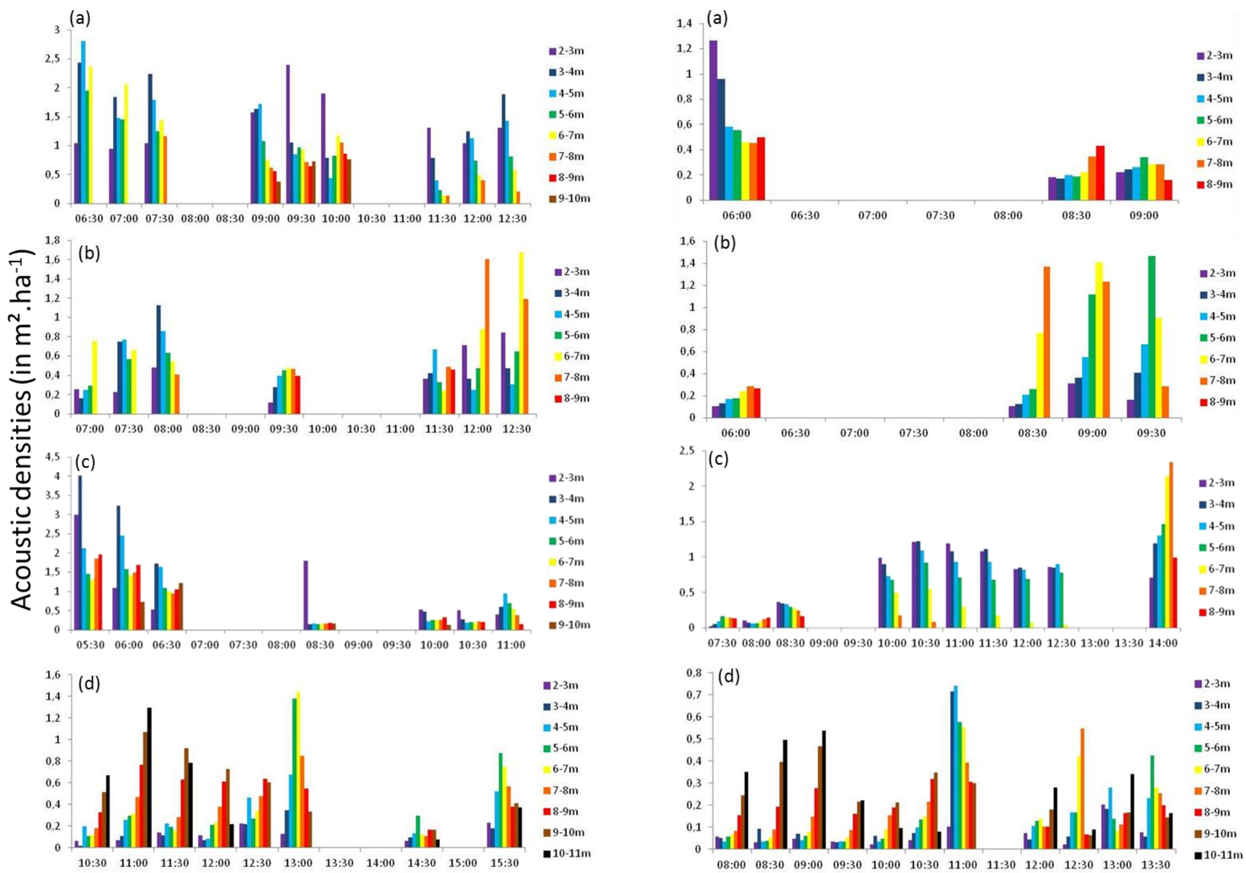


*Figure 16: Evolution of acoustic densities in $s_a$ ($m^2.ha^{-1}$) by strata (each meter) at 70 kHz, at 4 stations (a: Branst, b: Hingene, c: Kennedy and d: Doel) in the Zeeschelde estuary: on the left column in July 2013 and on the right column in September 2013.*

In figure 16, one can note that the highest values of $s_a$ occur in July compared to September. The acoustic densities are nearly twice as much in July except for Hingene station where the values are in a similar range. No clear trend can be identified from the stratified $s_a$ analysis. Nevertheless, it appears clear that the highest densities found at Doel (Fig. 16d) were in the deepest strata (7-11 m) while for the other stations, Branst, Hingene and Kennedy, it changed between July and September and even during the sampling window of the day. In July densities appeared to be greater in the subsurface layers (main $s_a$ peaks between 1.8 and 2.8 $m^2.ha^{-1}$ from layer 2-5m depth) at Branst station (a) and in September the stratum 2-3m reached a $s_a$ value of 1.25 $m^2.ha^{-1}$. All other values are more or less equally distributed and do not exceed 0.6 $m^2.ha^{-1}$. There seems to be a little higher density in the surface layers (2-4 m) in the early morning but later on, the densities are homogenous and low. At Hingene station (b) the deeper layers show the highest densities but again, in the early morning the highest density is located in the upper half of the water column. In September at Kennedy station (c), there is a large proportion of the fish densities in the upper layer (2-5 m) but that was not observed in the July survey.

### 3.1.4. Comparison with the fishing data

Acoustic methods have proven to be effective tools providing information on fish distribution, abundance and size-structure at the population scale but without taxonomic distinction. In order to get information on the species composition, acoustic sampling generally needs to be conducted in parallel with traditional fish sampling methods. Although hydroacoustics differ significantly in terms of equipment, costs, and manpower requirements, the concomitant use of acoustic and other direct sampling methods has been extensively tested in a wide variety of ecosystems (Hansson and Rudstam 1995; Emmrich et al., 2012; Guillard et al., 2012). However, the main limit to acoustic tools is their difficulty in identifying species, especially if they are not schooling.

While the use of fishing gear integrates targets from a small area (considered as spot samples), hydroacoustics rapidly integrates abundance over a larger area, using continuously recorded data.

Before incorporating these technological advances into standard procedure, the useful methods should be field-tested to demonstrate accuracy and precision. Combining information from different sources is the greatest challenge for research (Godø 1998). One form of accuracy is exhibited by comparing the evolution of acoustic data with data from another assessment tools. Data were analysed to determine whether densities detected acoustically were consistent with the results of fishing. This allows to define if different monitoring tools are complementary or provide the same information.

During the surveys in the Zeeschelde estuary, the period of fishing was established according to tides: flow period and ebb period. The estimation of fish showed that the biomass in July is lower than the biomass in September (Figure 17). A significant difference was observed between flow and ebb tides in July for Kennedy and Doel stations) and in September for Hingene and Kennedy stations.

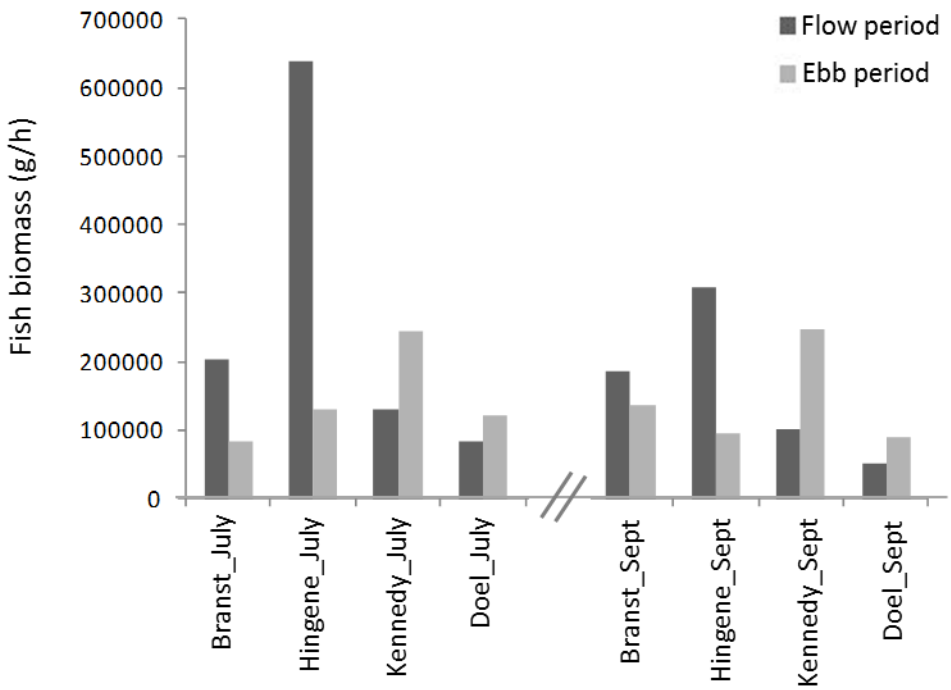


*Figure 17: Evolution of fish biomass at different stations in the Zeeschelde estuary estimated from anchor netting.*

Fish densities (anchor net g/h and acoustic data $s_a$) varied from one station to another. The largest acoustic fish densities were recorded in July whereas the largest fish biomass was recorded in September. However, overall variations seemed to follow the same pattern for both sampling periods along the salinity gradient (Figure 18).

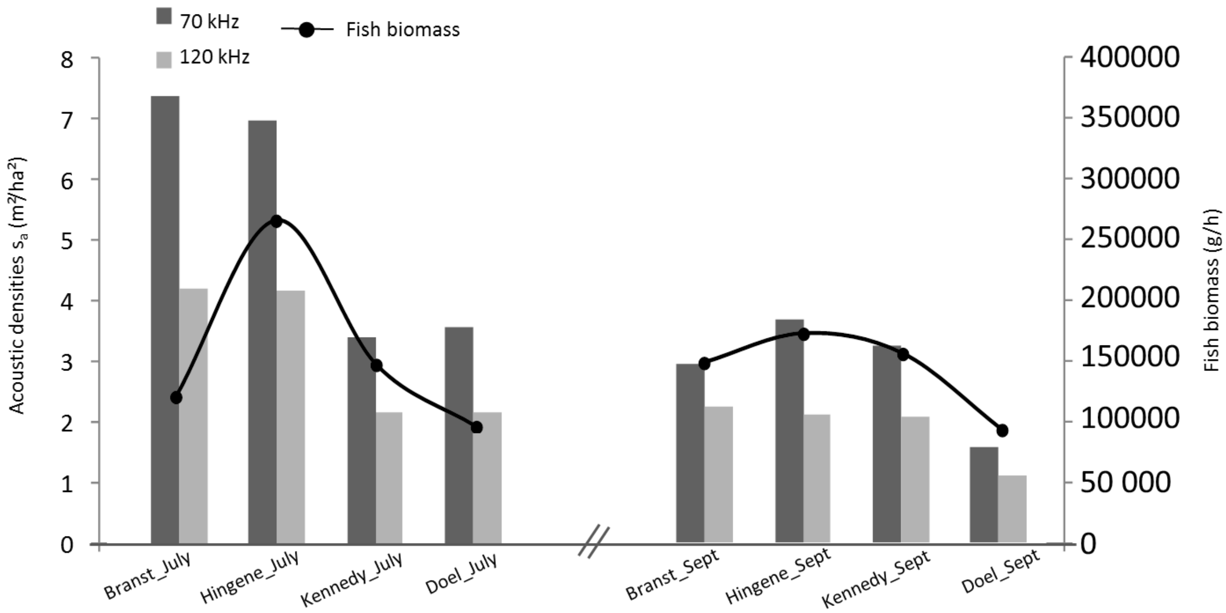


*Figure 18: Variations of densities estimated by acoustic and anchor net surveys in the Zeeschelde estuary.*

A non-significant correlation (Figure 19) between fish biomass and acoustic densities was observed at both frequencies (Spearman rank correlation: $rho_{70kHz}$ = -0.548, p>0.05; $rho_{120kHz}$ = -0.548, p>0.05) throughout both study periods.

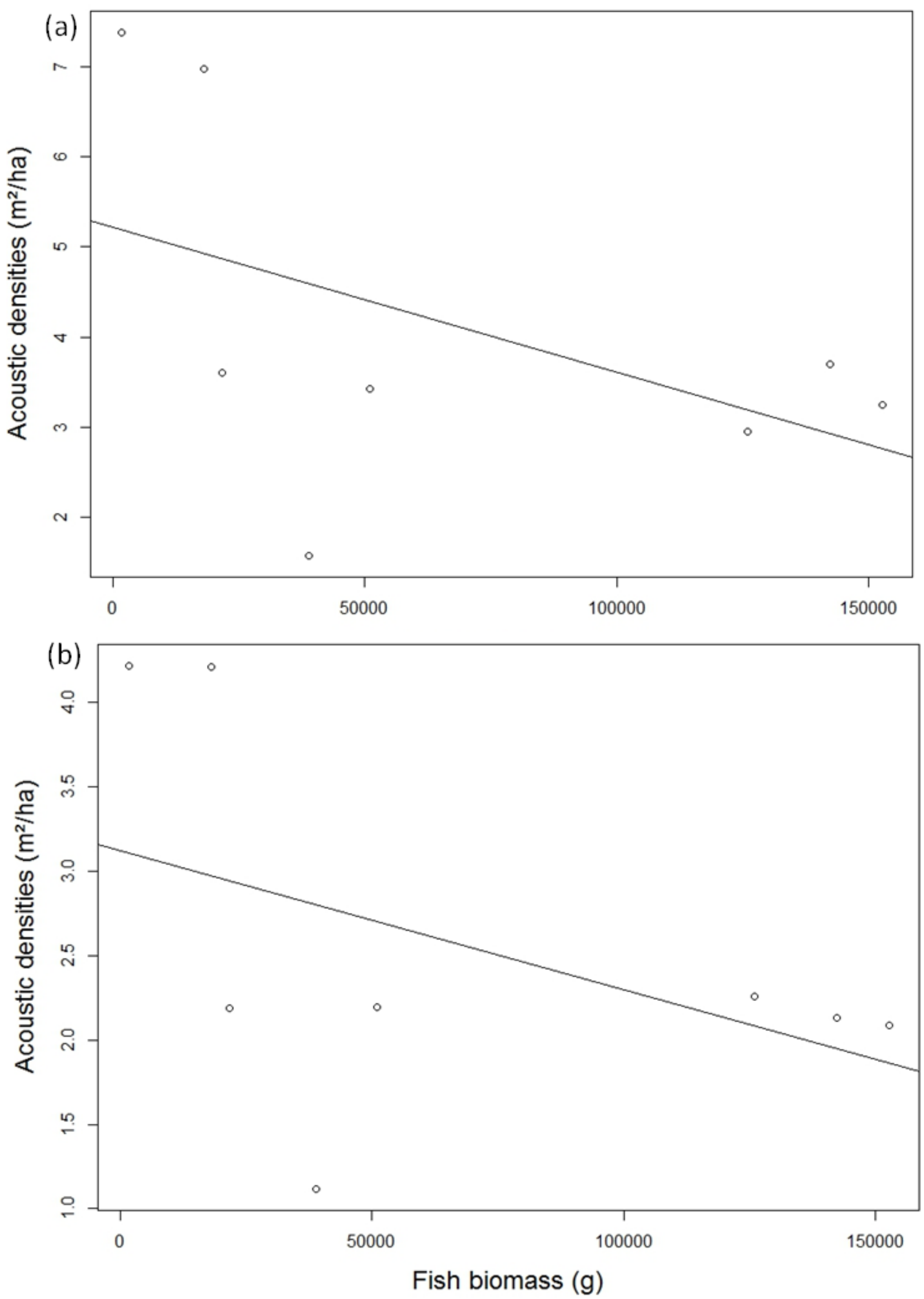


*Figure 19: Plot relation between acoustic and anchor net data in the Zeeschelde estuary (a) 70 kHz and (b) 120 kHz.*

### 3.2. In the Gironde estuary

#### *3.2.1. Evolution of the acoustic densities ($s_a$ and $S_v$)*

Overall, the fish densities in the Gironde estuary were very low, hardly reaching a density of $1m^2.ha^{-1}$ (Figure 20), in both sampling periods. The highest values were observed in Bordeaux station (a), located in the upper part of the Gironde estuary. Densities obtained from 70 kHz were slightly higher than densities obtained from 120 kHz. Acoustic values appeared relatively stable along the tidal cycle.

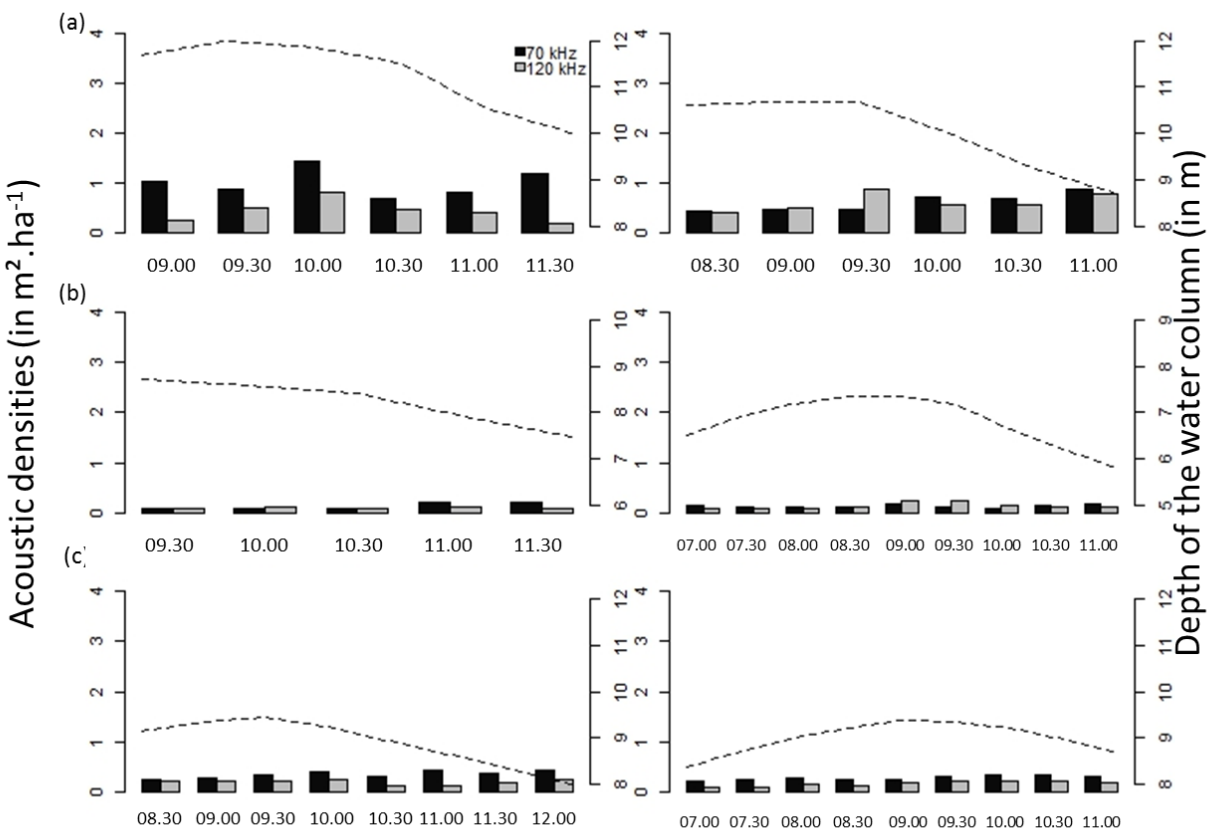


*Figure 20: Evolution of acoustic densities in $S_a$ ($m^2.ha^{-1}$), at 3 stations (a: Bordeaux, b: Ambes and c: Pauillac) in the Gironde estuary: on the left column in August 2013 and on the right column in October 2013.*

The analysis of densities by volume ($S_v$) also showed low acoustic densities, between -70 and -65 dB. The densities at frequency 70 kHz are slightly higher for Bordeaux and Pauillac in August, and for Pauillac in October. We can observe a relative stability in densities along the tidal cycle as the values do not vary significantly between the starting time and the end of the monitoring (Figure 21).

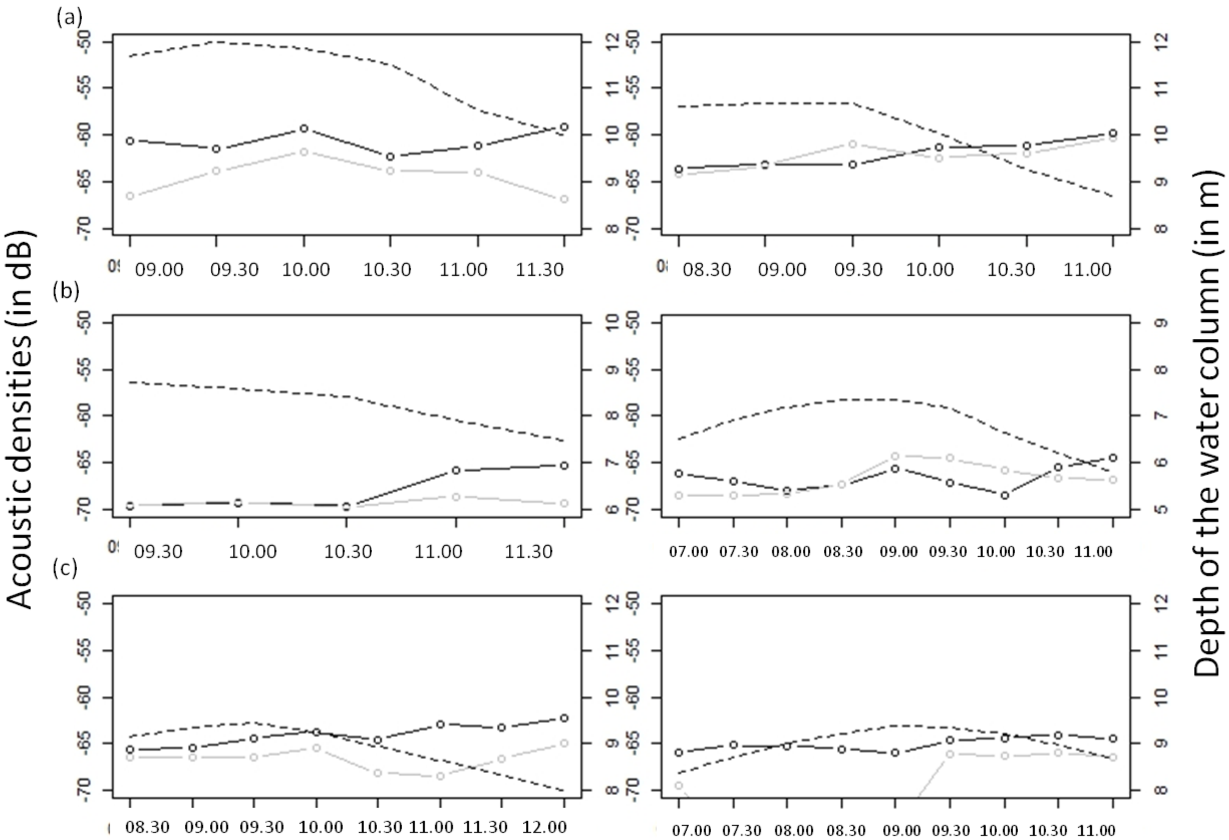


*Figure 21: Evolution of acoustic densities in $S_v$ (dB), at 3 stations (a: Bordeaux, b: Ambes and c: Pauillac) in the Gironde estuary at 70 kHz (in black) and 120 kHz (in grey) in function of the tidal rhythm: on the left column in August 2013 and on the right column in October 2013.*

### *3.2.2. Evolution of the size class*

When we observed the evolution of mean TS, results showed a stability of values along the tidal cycle, for both sampling periods and for all stations, with values varying between -65 and -60 dB (Figure 22). Comparing both frequencies, a difference of -2 dB between both frequencies was observed for most stations but the trend is the same.

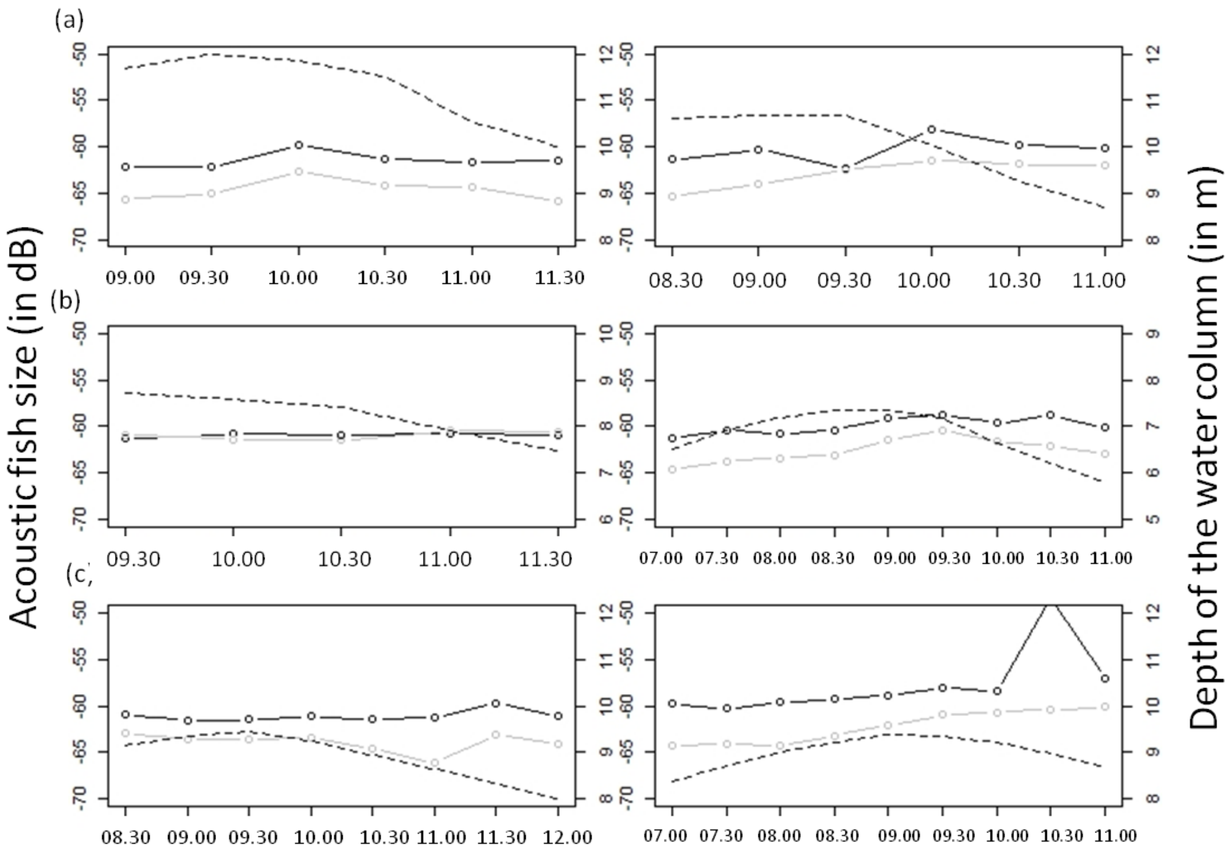


*Figure 22: Evolution of mean TS (in dB), at 3 stations (a: Bordeaux, b: Ambes and c: Pauillac) in the Gironde estuary at 70 kHz (in black) and 120 kHz (in grey) in function of the tidal rhythm: on the left column in August 2013 and on the right column in October 2013.*

The results obtained for size proportion (TS) in the assemblage in the Gironde estuary showed that the number of fish classified as "small" represents more than 80 % of the total population sampled, except for the last station (Pauillac) in September (Figure 23) where "small" and "medium" size are equivalent. Larger targets were not observed for all stations. At 70 kHz, the part of the "medium" targets seemed higher than the part of "medium" in 120 kHz (Figure 24). The "small" targets are largely dominating in the latter.

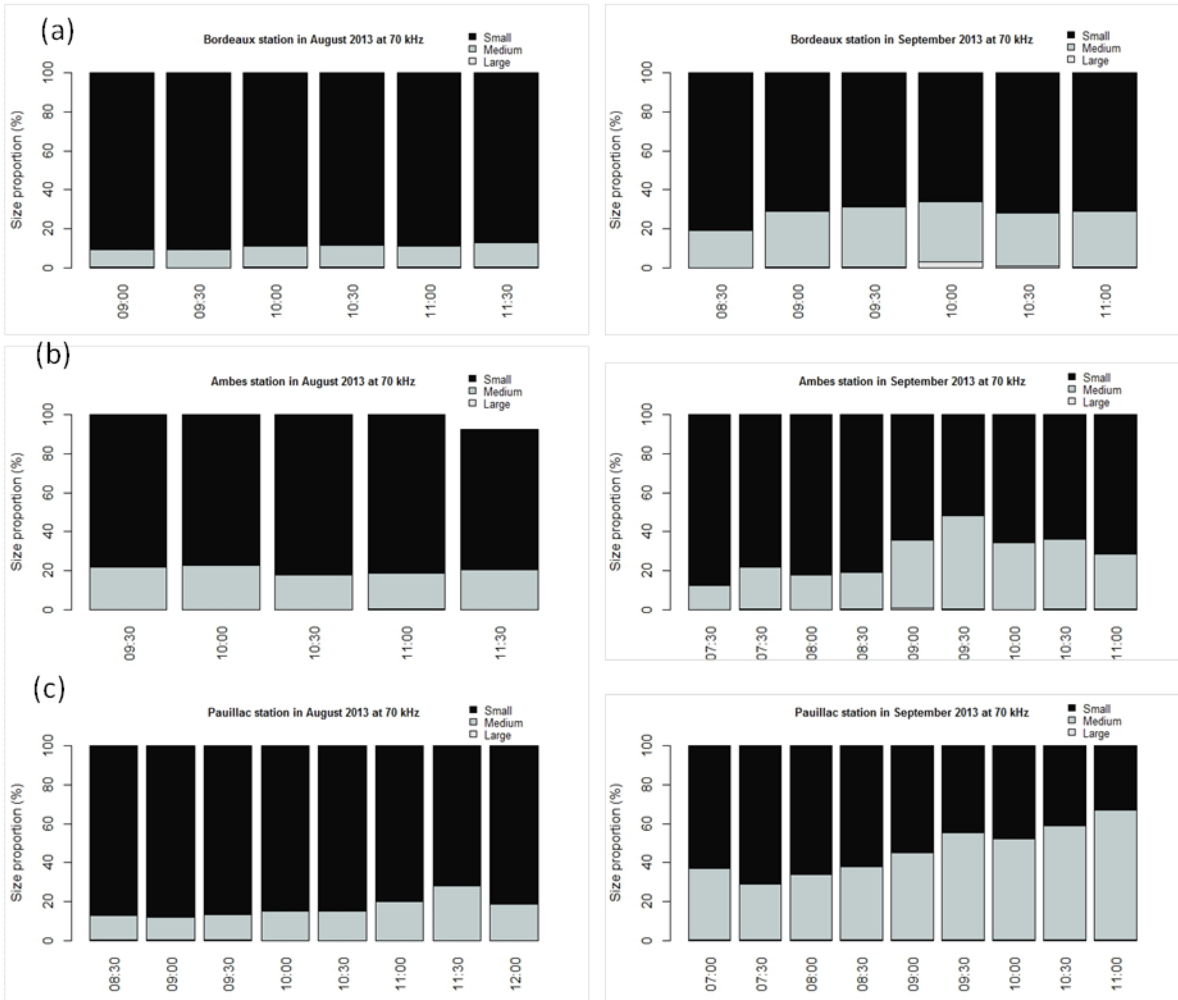


*Figure 23: Evolution of acoustic size class at 70 kHz, at 3 stations (a: Bordeaux, b: Ambes and c: Pauillac) in the Gironde estuary*

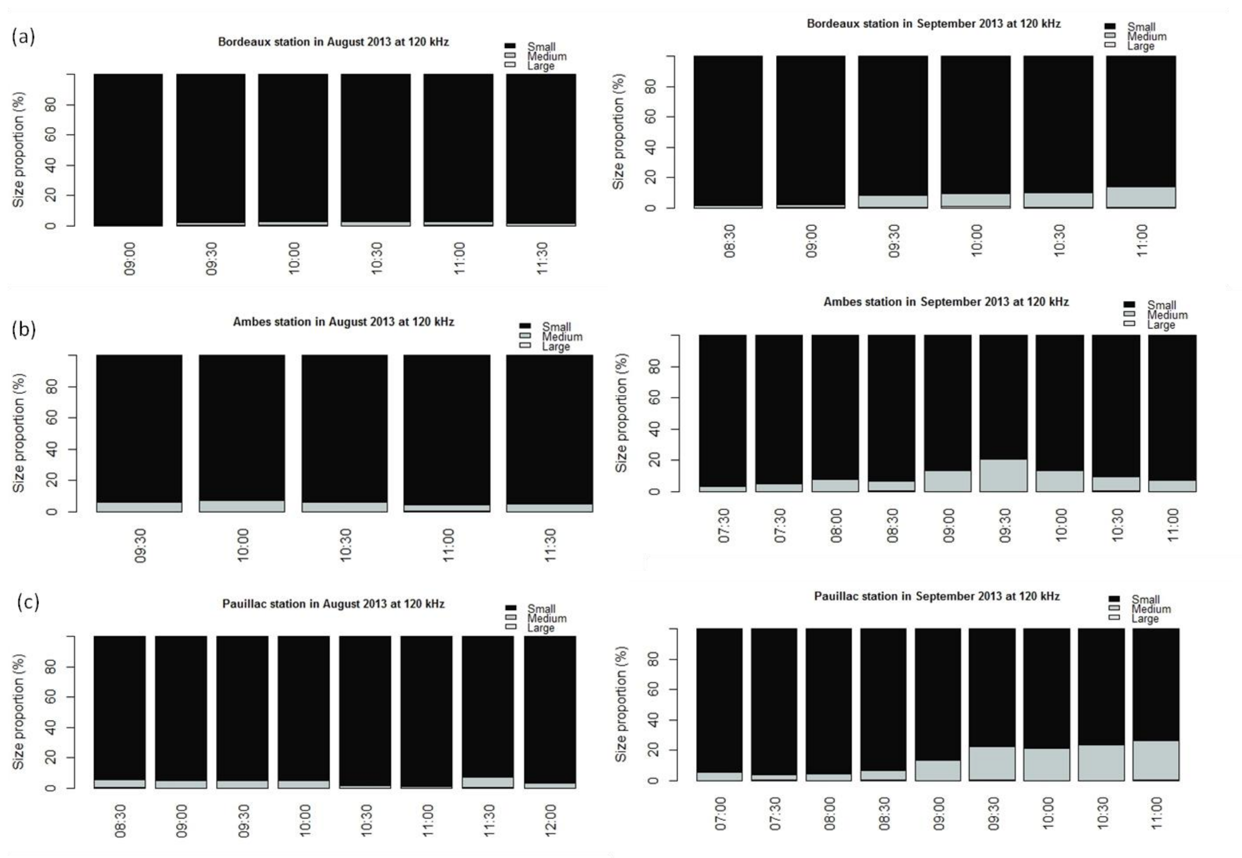


*Figure 24: Evolution of acoustic size class at 120 kHz, at 3 stations (a: Bordeaux, b: Ambes and c: Pauillac) in the Gironde estuary.*

### *3.2.3. Evolution of acoustic densities by stratum*

In the Gironde estuary, despite the low acoustic densities, the repartition of densities appeared uniform during the tidal cycle with a slight trend of higher densities in the upper half of the water column (Figure 25). Sometimes, we observed density peaks at different levels in the water column. This can be explained by the presence of a bigger target.

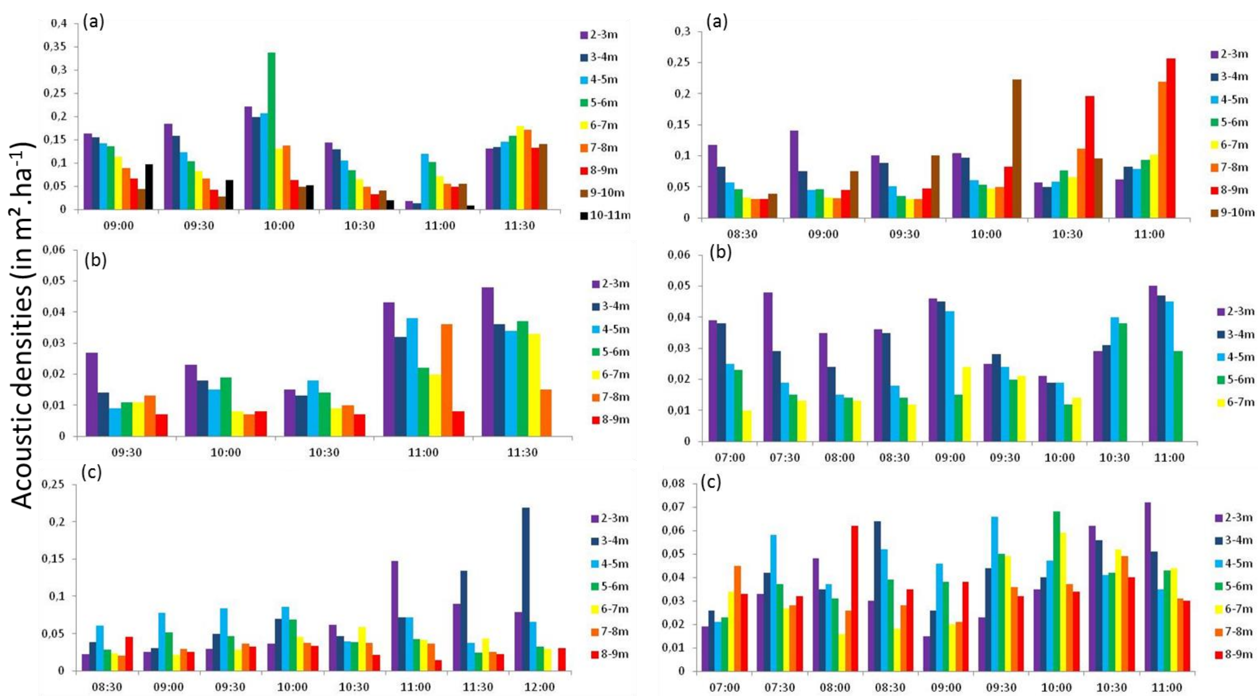


*Figure 25: Evolution of acoustic densities in $s_a$ (m².ha$^{-1}$) by strata (each meter), at 3 stations (a: Bordeaux, b: Ambes and c: Pauillac) in the Gironde estuary for 70 kHz: on the left column in August 2013 and on the right column in October 2013.*

## 4. Comparison between both estuaries

Natural ecological processes affect estuarine fish communities at different spatial scales. Large-scale but still intra-estuarine ecological patterns result from responses to dominant environmental gradients (Nicolas et al., 2010). With the same acoustic protocol applied in both estuaries (Gironde and Zeeschelde) i.e., around high tide, during daytime and at different fixed stations along the salinity gradient of estuaries, the comparative studies can be done to improve this knowledge on estuarine functioning. It could also support long-term acoustic approaches in estuarine ecosystems, especially as internationally accepted standards need to be created and outlined in order to ensure comparability of obtained results.

### 4.1. Descriptive approach

Our results show that densities are unevenly distributed in the investigated areas. Other studies have also noted this uneven spatial distribution (Lyons 1998). It is suggested that variations in fish distribution are associated with spatial and temporal changes (channel morphology, current velocity, water depth ...). Different ecological preferences of different taxa or functional groups can result in habitat portioning among fish assemblage (Lin et al., 2013). Whether in the Gironde estuary or Zeeschelde estuary, no school of fish was observed by the acoustic approach.

When we compared echograms, as in the fig. 27 example, for similar condition, equivalent salinity zone and time, the acoustic densities appear much more dense in the Zeeschelde estuary than in the Gironde estuary.

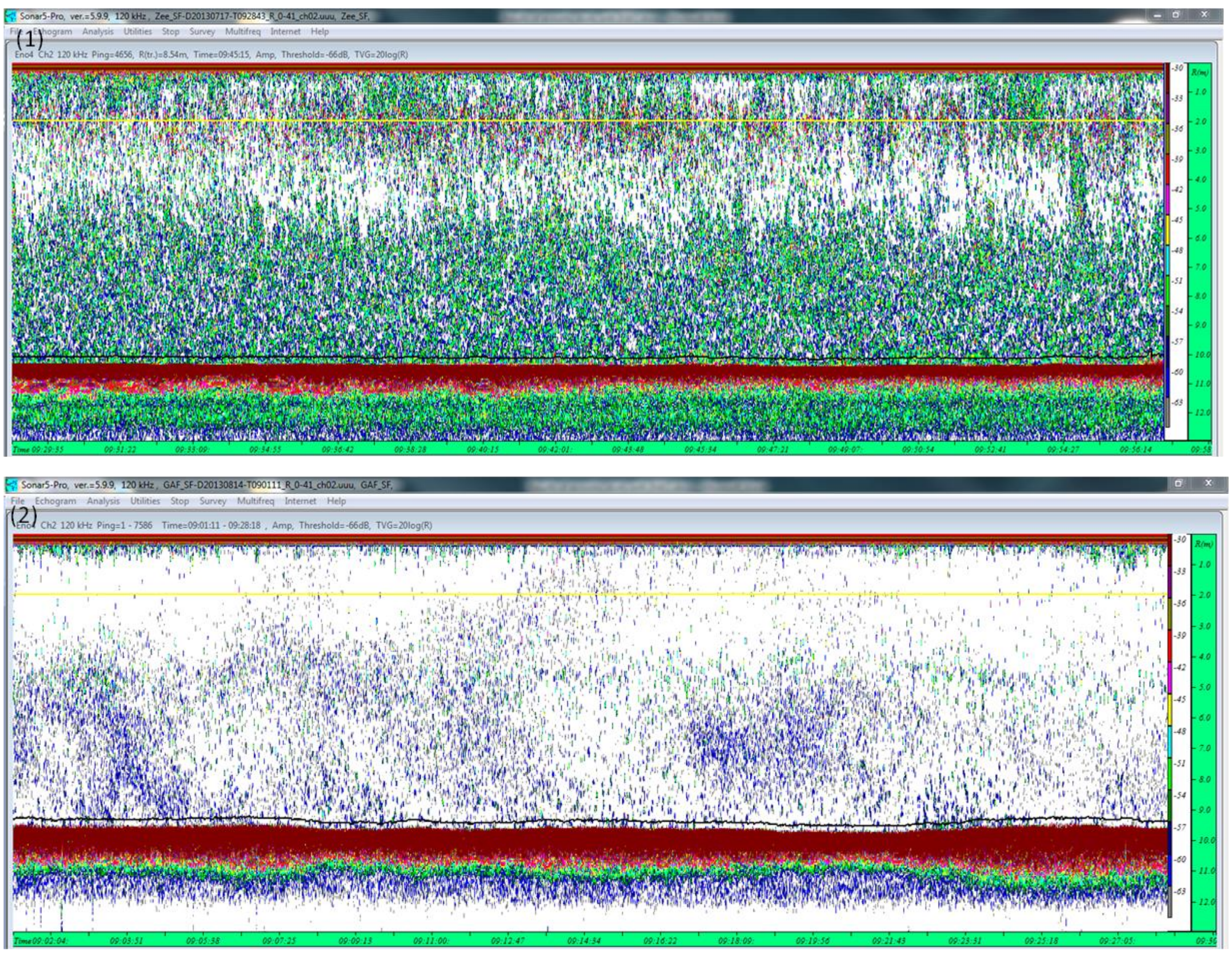


*Figure 26: Comparison between two echograms of 30 minutes for equivalent stations and time (in July/August) at 120 kHz: (1) in the Zeeschelde estuary (Kennedy) and (2) in the Gironde estuary (Pauillac).*

### 4.2. Acoustic densities

Based on the previous example (Figure 27), i.e. for one period of 30 minutes in the middle of estuaries, we want to assess if there is a difference in terms of acoustic densities and fish sizes. Results showed that the acoustic density is much higher in the Zeeschelde estuary than in the Gironde estuary and the targets size are much larger in the Zeeschelde estuary (Table 2).

*Table 2: Values of acoustic results ($s_a$, $S_v$ and TS) for echograms (figure 26) in Summer 2013 at 120 kHz: (1) in the Zeeschelde estuary (Kennedy) and (2) in the Gironde estuary (Pauillac).*

| | $s_A$ | $s_v$ | TS |
|---|---|---|---|
| 1) Kennedy | 4,828 | -53,185 | -57,97 |
| 2) Pauillac | 0,212 | -66,384 | -63,52 |

When we compare acoustic densities in both estuaries, overall results showed the same, i.e., acoustic densities in the Zeeschelde estuary were around five times higher than in the Gironde estuary (Figure 26). However, for each estuary, the highest acoustic densities were observed for the most upstream stations of estuary for both sampling periods, respectively Branst for the Zeeschelde estuary and Bordeaux for the Gironde estuary.

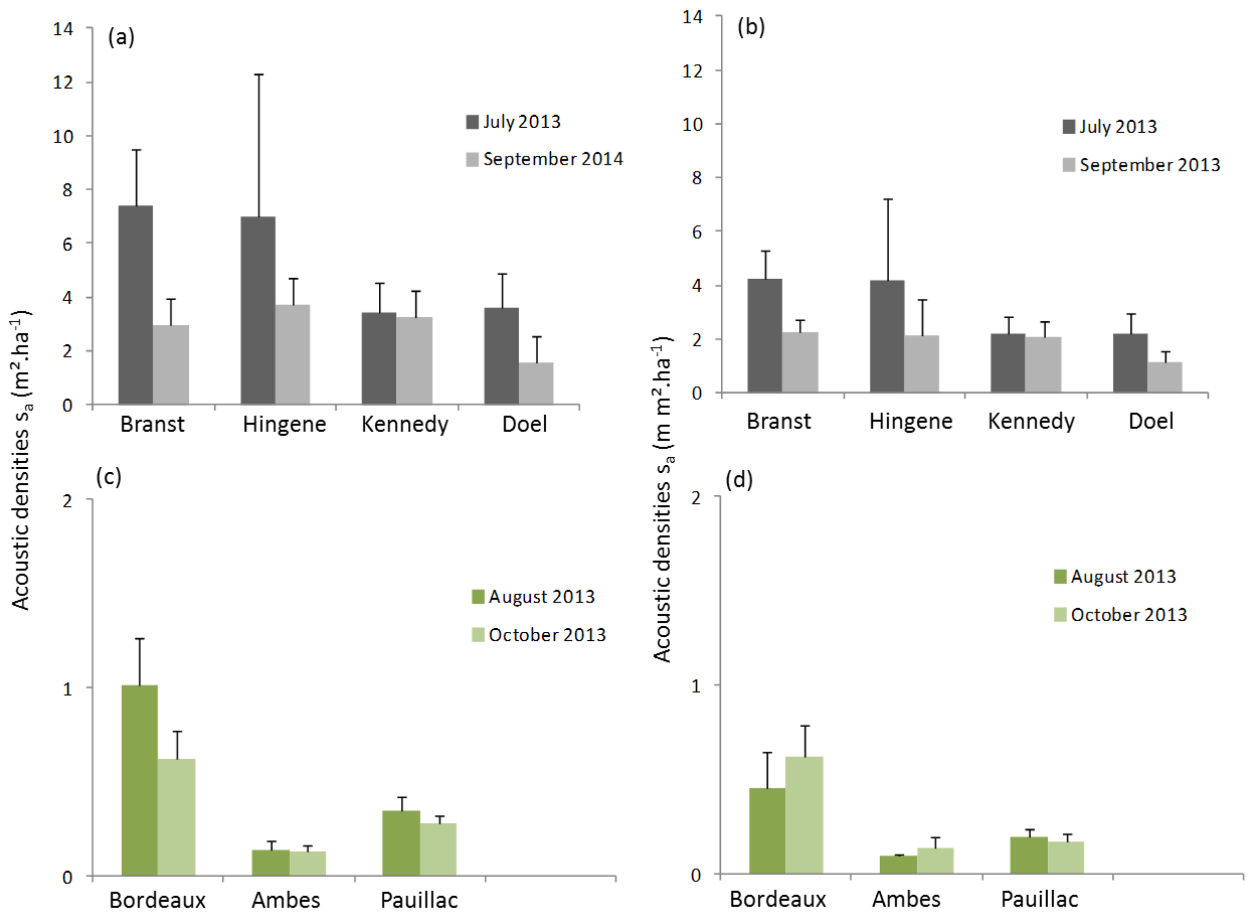


*Figure 27: Evolution of $s_a$ (m².ha$^{-1}$) in the Zeeschelde estuary at 70 kHz (a) and at 120 kHz (b), in the Gironde estuary at 70 kHz (c) and at 120 kHz (d).*

### 4.3. Acoustic fish size

As TS values consider the surface of the target fish in the detection cone, the size of targets in the Zeeschelde estuary was much greater than these of the Gironde estuary. Indeed, two groups were present in the Zeeschelde estuary: small and medium targets (Figures 14 and 15) whereas in the Gironde estuary, the group “small targets” dominated largely (Figures 23 and 24).

### 4.4. Multi-frequency approach

The intensity of echoes from targets depends on the targets acoustic density relative to the water, size, orientation, and echo-sounder frequency. Studying the frequency response from different targets can give important information about what the targets are. Sonar5 can give visual and quantitative insight in the frequency response. Multiple echograms can be set up to show the echoes from the different frequencies simultaneously. All echograms can be synchronized. Individual echograms can also be released from the synchronizing. This allows the operator to study and compare different echograms at the same time and different events at different times.

As it is common practice in fishery acoustics (Godlewska et al., 2009), measurements were performed at 70 and 120 kHz. Although echo-sounders can operate simultaneously, they do not detect organisms in the same way at different frequencies (Simmonds and MacLennan 2005).

In hydroacoustics, different studies conducted in estuaries did not show difference between frequencies (Godlewska et al., 2009; Samedy et al., Submit). In this study, it seemed interesting to check this information. In this example (Figure 28), a very small visual difference was observed and this is also confirmed for the whole data set. It tends to show a better detection of the very small individuals fraction on the echogram reflecting a slight increase in sensitivity of the 120kHz compared to 70 kHz.

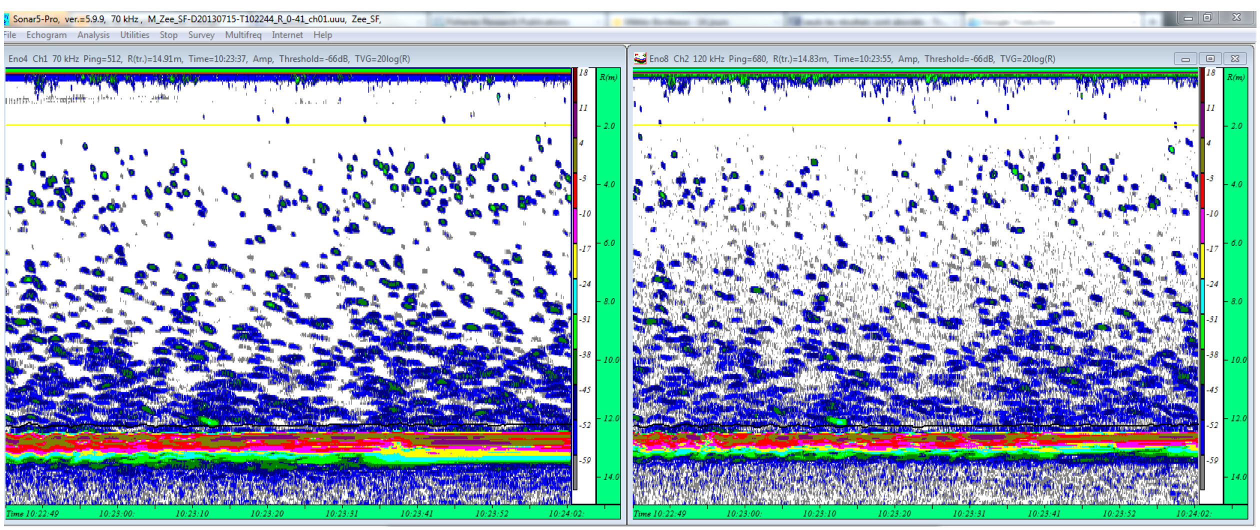

*Figure 28: Echogram view at two frequencies (70 kHz on the left and 120 kHz on the right).*

Results revealed a maximal difference of ±2 dB between both frequencies for fish densities (Figures 29 and 30), suggesting that both frequencies can be equally effective in an estuarine setting (Guillard et al., 2004; Coll et al., 2007; Godlewska et al., 2009; Guillard et al., 2012).

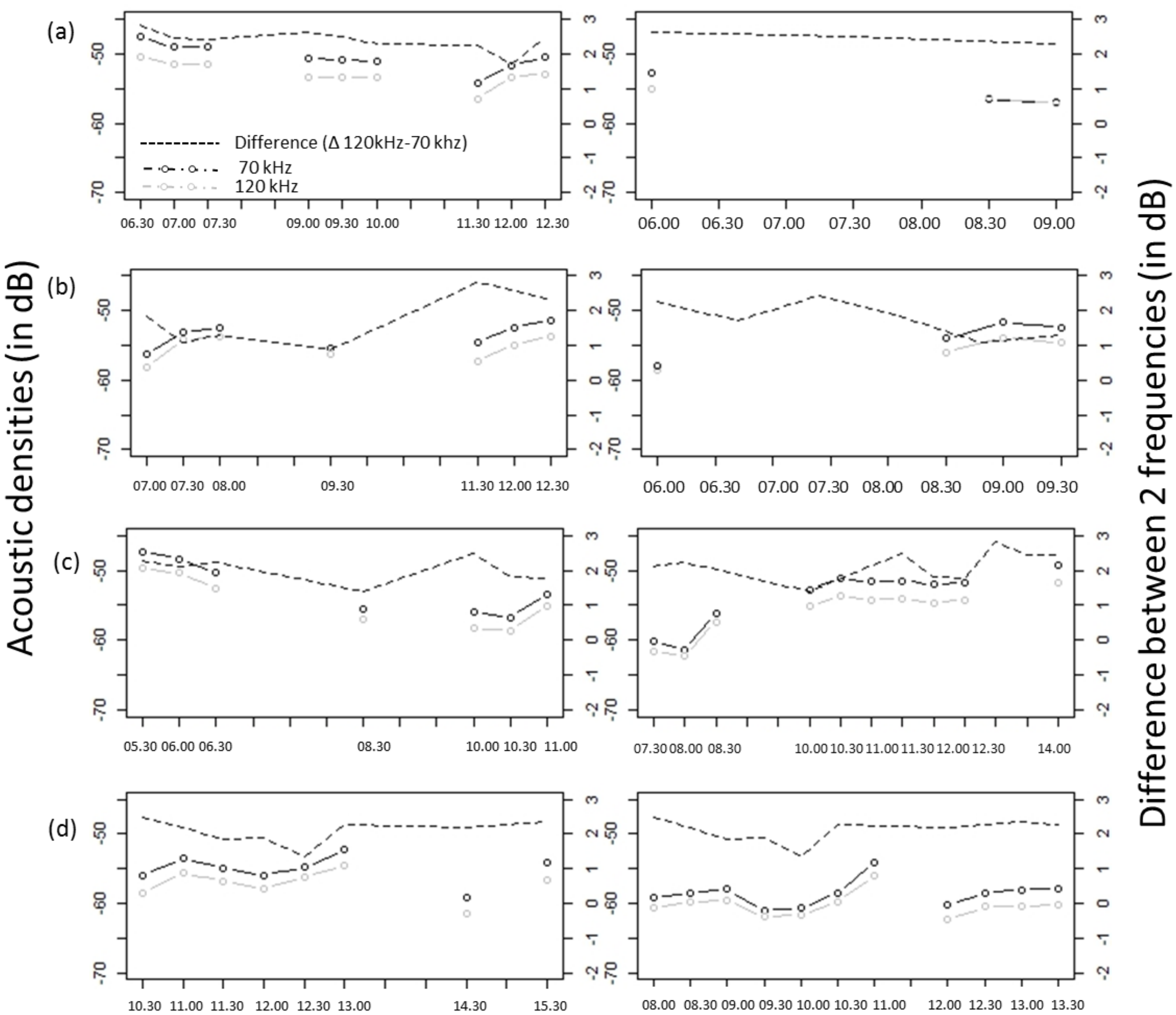


*Figure 29: Evolution of acoustic densities in $S_v$ (in dB) in function of the difference between both frequencies (70 and 120 kHz) at 4 stations (a: Branst, Kennedy, c: Hingene and d: Doel) in the Zeeschelde estuary, on the left column in July 2013 and on the right column in September 2013.*

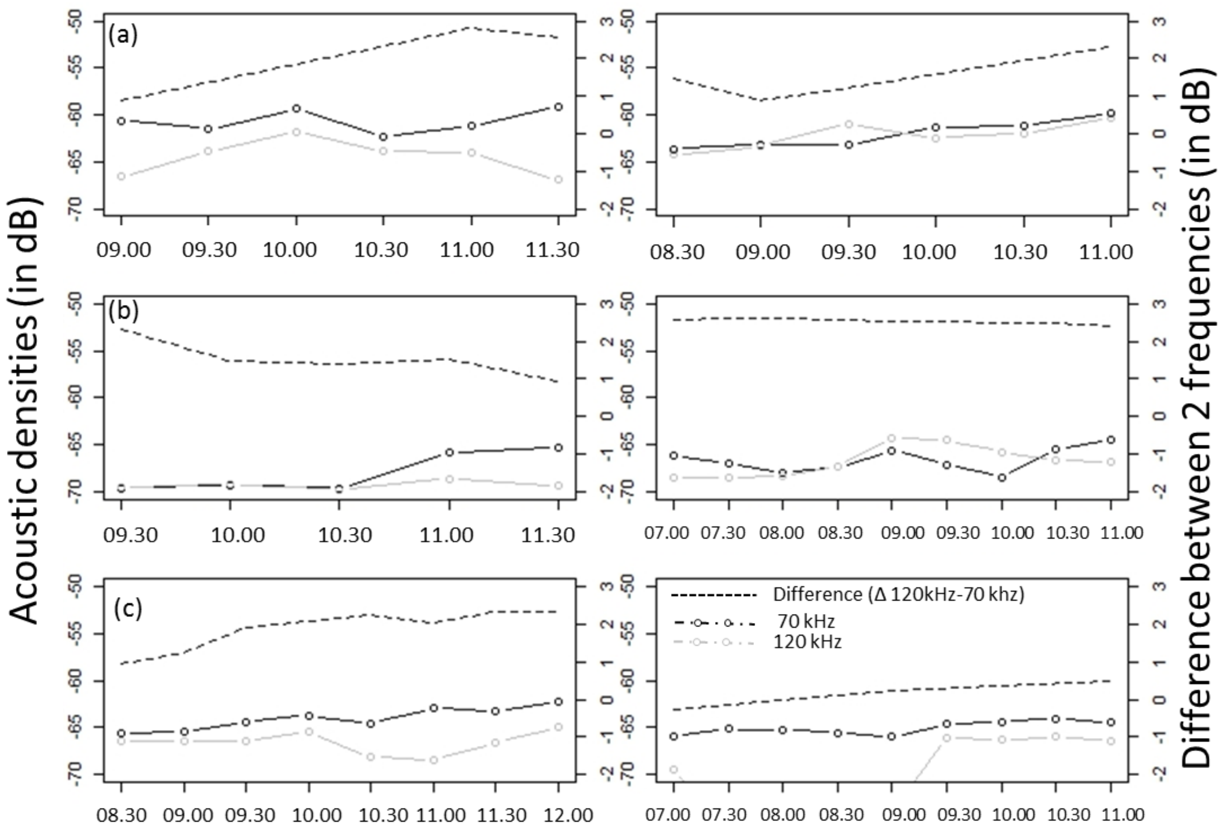


*Figure 30: Evolution of acoustic densities in $S_v$ (in dB) in function of the difference between both frequencies (70 and 120 kHz) at 3 stations (a: Bordeaux, b: Ambes and c: Pauillac) in the Gironde estuary: on the left column in August 2013 and on the right column in October 2013.*

However, the evolution of densities ($S_a$ and $S_v$) or the mean TS compared with a Mann-Whitney (U) tests showed a significant difference between both frequencies in July for most results, but no significant difference for most results in September (Table 3) for $S_a$ and $S_v$ only. Thus, in studies where a finer description of fish assemblages is necessary, the multi-frequential approach becomes, at least partially, a relevant alternative to cope with the problems of fish identification (Jech and Michaels 2007; Korneliussen et al., 2008). In certain cases, it allows different species of fish to be identified, fish to be distinguished from other organisms (Horne 2000; Knudsen and Sægrov 2002; Korneliussen et al., 2009; Stanton 2012) or to classify different groups (Fernandes et al., 2006; Fernandes 2009).

*Table 3: Results of the Mann-Whitney (U) test applied to compare the difference between both frequencies (70 and 120 kHz) in the Zeeschelde estuary (a: $S_a$, b: $S_v$ and c: TS).*

(a)

| 70 vs 120 | Branst | Kennedy | Hingene | Doel |
|---|---|---|---|---|
| **N obs** | 9 | 7 | 7 | 8 |
| **U** | 71 | 41 | 34 | 54 |
| **July 2013** | *0,005636 (*)* | *0,03788 (*)* | 0,2593 (NS) | *0,02067 (*)* |
| **N obs** | 3 | 4 | 10 | 11 |
| **September 2013** | 5 | 13 | 72 | 81 |
| **U** | 1 (NS) | 0,2 (NS) | 0,1051 (NS) | 0,1932 (NS) |

**(b)**

| 70 vs 120 | Branst | Kennedy | Hingene | Doel |
|---|---|---|---|---|
| **N obs** | 9 | 7 | 7 | 8 |
| **U** | 66 | 38 | 33 | 51 |
| **July 2013** | *0,02443(*)* | 0,09732 (NS) | 0,3176 (NS) | *0,04988 (*)* |
| **N obs** | 3 | 4 | 10 | 11 |
| **September 2013** | 5 | 12 | 74 | 94 |
| **U** | 1 (NS) | 0,3429 (NS) | 0,07526 (NS) | *0,02807 (*)* |

**( c)**

| 70 vs 120 | Branst | Kennedy | Hingene | Doel |
|---|---|---|---|---|
| **N obs** | 9 | 7 | 7 | 8 |
| **U** | 75,5 | 44 | 37 | 58 |
| **July 2013** | *0,002304 (*)* | *0,01107 (*)* | 0,1282 (NS) | *0,004662 (*)* |
| **N obs** | 3 | 4 | 10 | 11 |
| **September 2013** | 8 | 15 | 95 | 117 |
| **U** | 0,2 (NS) | 0,05907 (NS) | *0,000765 (*)* | *0,0002348 (*)* |

## 5. CONCLUSIONS

Hydroacoustics is rarely used to analyse fish populations in large estuaries, even though such approaches in the past have proven to be effective in providing information on fish distribution, abundance and size-structure in other aquatic systems. The significant seasonal and spatial fluctuations within estuaries (McLusky and Elliott 2004; Selleslagh et al., 2012) present a challenge when assessing the dynamics of biotic communities, as sampling needs to take into account these frequent changes (Mason and Brandt 1999). Monitoring medium and long-term ecosystem evolution in estuaries calls for innovative methods (Borja et al., 2010; Lepage 2013).

Standard fishing techniques mainly provide information on the composition and size of fish (Godø 1998) and have been widely used in fish monitoring over the last few decades due to their versatility. However, these methods have a negative effect on natural environments and can also be expensive. They are also largely unsuitable for use in some areas, such as marine protected area or navigation channels. With some traditional sampling tools, fish may not behave naturally (e.g. avoidance) (Chopin and Arimoto 1995; Godø 2003) making it impossible to obtain a clear picture of their density in a given area. In addition to this, in a highly fluctuating habitat, it can become necessary to conduct sampling many times over (Rotherham et al., 2007). Another issue is that the majority of sampling equipment is geared toward a particular range of fish sizes, and targets a specific section of the water column.

Acoustic surveys allow fish populations to be sampled in areas where standard tools would be ineffective, while using a minimum of time and manpower (Mehner and Schulz 2002). One of the main arguments in favor of using hydroacoustics is that it minimizes any potential impact on the ecosystem, and fish are not harmed. In addition, acoustic surveys have become well established as a useful technique in fishery research (Simmonds and MacLennan 2005) and are increasingly recommended (Koslow 2009; Trenkel et al., 2011) in fish population monitoring. While a long time series of experimental fishing and/or direct observations on fish biology is often necessary to interpret ecological data (Coll et al., 2007), acoustic technique allows estimates of spatio-temporal distribution and size frequency to be made almost instantly.

While acoustic methods have been used in various aquatic systems (Simmonds and MacLennan 2005; Rudstam et al., 2013) and are increasingly implemented in shallow waters (Krumme and Saint-Paul 2003; Guillard et al., 2004; Boswell et al., 2007), they do lack effective protocols for sampling fish populations in large estuaries. Before being incorporated into standard procedures, these new methods should be field-tested to ensure their accuracy and precision (Bonar and Hubert 2002; Rudstam et al., 2009). Using the large macrotidal estuaries as case studies, this work demonstrated that using an acoustic spatial sampling strategy adapted to estuaries is possible and is an effective way to study fish populations. Building on previous recommendations made by Samedy et al. (2013) this study also brought up a range of technical and methodological issues relating to sampling methods and estuarine fish dynamics. Firstly, while hydroacoustics is effective in estimating biomass and fish size, we still used $S_a$ and TS metrics as density and size proxies, in order to avoid any uncertainty stemming from conversion methods to fish weight or fish length (Rose et al., 2000; Godlewska et al., 2009).

The presented study is important to convince more traditional fisheries scientist of the utility of hydroacoustics. While each method has its own bias and element of uncertainty, the results of our study and other previously-documented experiments would tend to inspire greater confidence in them. The relevance of using hydroacoustics to monitor fish density and dynamics in large estuarine ecosystems was demonstrated in these estuaries. In particular, the results of our study highlighted that both traditional monitoring systems and hydroacoustic approaches indicated consistent fish density patterns. As such does the evaluation of the Zeeschelde-ecosystem using fish not only give information about the ecological functioning of the estuary but it provides information about the ecological quality of the water bodies in the Zeeschelde river basin.

The benefits of combining various different techniques have been highlighted (Godlewska et al., 2009; Boswell et al., 2010). Indeed, methods represent two "true" but different pictures of the target stock or ecosystem, like pictures of an object from different angles (Kubečka et al., 2009). By combining biological information from standard methods with the results of acoustic scans at the same temporal and spatial scales, it is possible to obtain reliable data on the distribution and organisation of large estuarine fish communities (Samedy et al., Submitted). In particular, our results highlight that both traditional monitoring systems and hydroacoustic approaches indicated consistent fish density patterns.

It is therefore advisable to use a combination of different sampling techniques, particularly to ensure the relevance of using acoustic equipment alongside traditional approaches to fish sampling, as internationally-accepted standards need to be created and outlined in order to ensure comparability of results.

## 6. Recommendations

By defining a standard and robust protocol (Achleitner et al., 2012; CEN 2009), hydroacoustic methods could be extended to finer scales, and may lead to the definition of acoustic-based metrics. These would complement fish-based indicators, such as those laid down in the European Water Framework Directive (e.g. the Estuarine and Lagoon Fish Index already established for assessing the ecological status of French estuaries (Delpech et al., 2010).

While hydroacoustics has the obvious disadvantage of being unable to distinguish different types of species, its benefits when combined with other sampling techniques have been extensively documented (Godlewska et al., 2009; Boswell et al., 2010). As in the Zeeschelde estuary, the fish surveys was already established, this combination of techniques is possible. The acoustic system can be rapidly installed and integrated abundance over a larger area, using continuously recorded data. The key to success is the best compromise in the choice of sampling areas, periods of observations,...

### 6.1. Acoustic materials

In hydroacoustics, one of the major problems is to determine the species and size of detected targets. Species identification could result from the objective interpretation of acoustic data combined with biological sampling. However, species identification could be based on multi-frequency approach that can solve the problem of classification. Given the type of fish populations (small fish), it seems interesting to ask if a mono-, bi- or multi-frequency approach is necessary. Acoustic discrimination between fish, plankton and other elements of the environment is not always easy because the real composition of the aquatic living resources is often not well known. In our study, we used this facility to compare two SIMRAD echo-sounders (Godlewska et al., 2009). Measurements were performed at 70 and 120 kHz. Although echosounders can operate simultaneously, they do not detect organisms in the same way at different frequencies (Simmonds and MacLennan 2005). Nevertheless, in studies where a finer description of fish assemblages is necessary, the multi-frequency approach becomes, at least in part, a relevant alternative to cope with the problems of fish identification (Jech and Michaels 2007; Korneliussen et al., 2008). In certain cases, it allows different fish species to be identified, or can help in distinguishing fish from other organisms (Horne 2000; Knudsen and Sægrov 2002; Korneliussen et al., 2009; Ballón et al., 2011; Stanton 2012).

In heterogeneous environments, the choice of a sampling strategy determines the nature and quality of the data. In this work, a deliberate choice was made for the use of data acquired by vertical emissions to the detriment of those recorded in the horizontal emissions. Only extremely calm weather conditions can provide good quality data from horizontal emissions. Traffic in the navigation channel and weather conditions generated layer of bubbles near the surface and prevented the analysis of the horizontal acoustic survey during this study. However, as its use is widely advocated in these shallow ecosystems (Boswell et al., 2007), this horizontal approach could be considered again in a different form, taking into account the constraints of the ecosystem. Indeed, the movements of the ship during mobile surveys do not allow stabilizing emissions-receptions echoes. However, if the echosounders are used in fixed stations, data should be better. The horizontal acoustic approach is justified by the fact that the sub-surface layer cannot be sampled by vertical sonar emissions, which can sometimes be proportionally important in terms of targets detected in these shallow ecosystems. In addition, this approach could be used to explore some areas where information is sorely lacking.

In a very turbid environment, the use of acoustic camera is not recommended.

### 6.2. Spatial-temporal conditions

In this study, acoustic data were mainly analysed at the level of populations. Acoustic approach can also access the information at very small spatial and/or temporal scales, offering the opportunity to make observations at the level of individuals. The observation of individual behaviour characteristics of fish, estimates of swimming speed or direction of motion, are difficult to achieve from direct measurements fishing, especially in turbid ecosystems. However, the split-beam transducers, such as those used in this study, can provide access to such information. Moreover, when a target is identified as the individual target, based on different criteria (size of the echo, ...), and can be followed over several successive emissions, it is possible to reconstruct the path of the target and deduce its speed and direction of travel (Ehrenberg and Torkelson 1996). Data from fixed monitoring stations could be used to study the behaviour of fish swimming in terms of speed and or direction of the current (flood or ebb tides) although data recorded at stationary hydroacoustics give information from a relatively restricted spatial area.

The limitation in our study was that surveys were conducted over two periods of the year only. As such different fish distribution and activity patterns may have been missed. Therefore, a long-term observation should be made to understand better the spatial distribution and behavioural rhythms of fish. Knowledge of short-term (hours to days) fish densities in estuarine systems is relatively lacking and may be strongly structured by different cycles (tidal, seasonal, diurnal ...). Fish species distribution differs with environmental conditions such as time-of-day and tidal conditions, and is exploited when planning the timing and design of surveys. The interpretation of acoustic data can be further complicated by diel variation in the behaviour of fish. In daytime pelagic fish are often found close to the bottom, thus potentially in the dead zone, which leads to variability in the results. In a future protocol, a day/night cycle approach should be considered.

### 6.3. Data analysis

The acoustic data must be converted to be analysed (conversion, definition of bottom, elimination of noises ...). Corrections are not made according to criteria of simple choices, but involve a (subjective) human decision-making. A large local knowledge and good experience in the interpretation of acoustic data is very important. The quality of data depends on these choices.

In this study, only a comprehensive view of the estuarine population was discussed. With the acoustic approach, it is possible to go much further in the data analysis and related issues, if the number of data and time data processing are sufficient.

Using the information from different types of sampling, the acoustic approach is capable of evaluating the fish biomass. Moreover, as the ecosystem is multispecies and sizes-index reflection relationship has not been established, the equation of Love (1977) can be used to classify targets encountered in different categories (Sow and Guillard 2010). However, there are several unresolved issues in particular the fact that the acoustic values highly vary in function of the angle of inclination (Koslow 2009), with the depth of the presence or absence of the air bladder, e.g. the ontogenetic complicating the TS-size relationship (Gorska and Ona 2003) .The values of acoustic densities are more complex and the interpretation is more questionable as it depends on many factors, especially the number of individual targets detected, individual fish behaviour and physiology (Ona 1999). The intensity of echoes from targets depends on the targets acoustic density relative to the water, size, orientation, and echosounder frequency. Studying the frequency response from different targets can give important information about what the targets are. Sonar5 can give visual and quantitative insight in the frequency response. Multiple echograms can be set up to show the echoes from the different frequencies simultaneously. All echograms can be synchronized. Individual echograms can also be released from the synchronizing. This allows the operator to study and compare different echograms at the same time and different events at different times.

Hydroacoustics must extend the limits to discuss the multidisciplinary issues such as exploitation, conservation, sustainability of living resources for all aquatic ecosystems (Demer and Hewitt 1995). Sustainable management depends on a high level of knowledge and, most importantly, reliable monitoring of fish populations through scientific surveys (Godø 1998). Therefore, ecosystem-based fisheries management has been widely promoted (Trenkel et al., 2011). Hydroacoustics allow the monitoring of entire communities, and leans towards a more ecosystemic approach (Demer et al., 2009).

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