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THESE

Présentée Par

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SPATIO-TEMPORAL DISTRIBUTION OF BENTHIC FORAMINIFERA IN INTERTIDAL AREAS OF HAUTS-DE-FRANCE: ENVIRONMENTAL APPLICATIONS AND IMPLICATIONS

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Extended abstract

Foraminifera are one of the most abundant and diversified groups of unicellular organisms, ubiquitous in all marine habitats. In modern environments only a limited number of species are planktonic, the rest are benthic. Benthic foraminifera are able to organise in specific assemblages, in response to different environmental conditions. Due to their high sensitivity to environmental parameter changes, they have been increasingly used as bio-indicators of natural changes and anthropogenic alterations, at global and local scale. Furthermore their excellent preservation in fossil sediments enables them to be a suitable tool for palaeoecological and palaeoenvironmental reconstructions.

The European Marine Strategic Framework Directive (MSFD, 2008/56/EC) requires the achievement of good ecological status for marine water bodies by 2020. Recently the consideration of benthic foraminifera for application under EU legislations has increased. The objective of the present PhD thesis is to study the response of benthic foraminifera to environmental changes in the Hauts-de-France region (Northern France). The coastal areas of the region extend about 240km along the southern part of the English Channel. The northern part of this area has experienced strong human modifications over the last 200 years, with the development of numerous anthropogenic activities. On the contrary the southwestern part includes mostly natural areas. In this context the thesis project focuses on 3 main targets: i) describe the living foraminiferal communities in natural and disturbed areas, ii) investigate the response of intertidal foraminifera to spatial and short-term environmental variations (seasons), iii) observe long term (hundred years) foraminiferal variations, across the intertidal areas of Hauts-de-France region. To achieve these objectives both living and fossil foraminiferal assemblages have been sampled in five sites along the intertidal areas of the region: from the most polluted Liane estuary with the harbour of Boulogne-sur-Mer, the Aa estuary, an embanked area of Grand-Fort-Philippe to the supposed less impacted one, the Bay of Somme passing by the Canche and Authie estuaries. A multiproxy approach based on foraminifera, environmental parameters and historical data, has been adopted to improve the interpretations.

Here a synthesis of the main contents of the present PhD thesis:

Chapter 1 introduces the study. It gives an overview on the world of foraminifers and places the thesis in its thematic and geographical context.

In Chapter 2, the ecology of living benthic foraminifera is investigated in three annual surveys from the salt marsh area along the Canche estuary. Two distinctive foraminiferal zones are identified along the vertical tidal gradient, a middle-high salt marsh assemblage dominated by agglutinated taxa and a low salt marsh assemblage by calcareous specimens. Hyper tidal exposure drives the foraminiferal vertical zonation in accordance with the tidal frame. The article is currently under review in *Estuarine, Coastal and Shelf Science*.

In Chapter 3, a seasonal survey is carried out from five intertidal areas. The objectives are to describe living benthic assemblages and to observe the influence of seasonality on driving foraminiferal distributions. The main outcome is that foraminiferal communities dominated by *Haynesina germanica,* vary consistently during the year. However they do not exhibit a clear and homogeneous trend across the 5 study sites. Benthic intertidal foraminifera show patchy distributions.

In Chapter 4 long-term variations of foraminiferal benthic communities in a polluted area (Boulogne-sur-Mer harbour), and a natural area (the Canche estuary), are investigated. The main objective is to monitor environmental changes over the last hundred years. In Boulogne-sur-Mer a pre-impacted period (reference conditions) is distinguished from an industrial period. The upper part of the core reflects better ecological conditions, indicating an environmental recovery. The article has been published in *Marine Environmental Research.* In the Canche estuary benthic foraminifera and historical aerial pictures, allow to reconstruct one hundred years of environmental transformations. A tidal flat has gradually replaced by a vegetated saltmarsh area. In the bottom part of the core, the sediment grain-size and the TOC are critical factors for the settlement and development of benthic foraminifera.

Chapter 5 provides a synthesis and discussion of the main results. General conclusion and future perspectives are as well included.

Résumé étendu

Les foraminifères sont un des groupes les plus abondants et diversifiés d'organismes unicellulaires ubiquistes dans des habitats marins. Dans les environnements modernes, seul un petit nombre d'espèces est planctonique, le reste étant benthique. Les foraminifères benthiques sont capables de s'organiser en assemblages spécifiques en réponse aux différentes conditions environnementales. À cause de leur haute sensibilité aux changements des paramètres environnementaux, les foraminifères ont été de plus en plus utilisés comme bioindicateurs de changements naturels et d'altérations anthropiques, aux échelles locales et globales. De plus, leur excellente préservation dans les sédiments fossiles leur permet d'être un outil approprié pour des reconstructions paléoécologiques et paléoenvironnementales.

La Directive-cadre Stratégie pour le milieu marin (DCSMM, 2008/56/CE) requiert que les masses d'eaux aient un bon statut écologique pour 2020. Récemment, l'utilisation des foraminifères benthiques pour l'application des lois européennes a augmenté. L'objectif de cette thèse de doctorat est d'étudier la réponse des foraminifères benthiques vivants aux changements environnementaux dans la région des Hauts-de-France. Les zones côtières des Hauts-de-France représentent environ 240 km le long de la rive Sud de la Manche. La partie nord de cette zone a subi de fortes modifications anthropiques durant les 200 dernières années, avec le développement des activités anthropiques. Au contraire, la partie sud-ouest inclut majoritairement des zones naturelles non contaminées. Dans ce contexte, le projet de thèse se focalise sur 3 objectifs principaux : i) décrire les communautés de foraminifères actuels vivants dans les zones naturelles contaminées et non contaminées, ii) décrypter la réponse des foraminifères intertidaux aux variations spatiales environnementales et à court-terme (à l'échelle des saisons), iii) observer les variations des foraminifères, sur le long-terme (centaines d'années), le long des environnements intertidaux des Hauts-de-France. Pour mener à bien ces objectifs, des assemblages de foraminifères fossiles et vivants ont été échantillonnés sur cinq sites le long des environnements intertidaux de la région : depuis l'estuaire de la Liane, le plus pollué, avec le port de Boulogne-sur-Mer, l'estuaire de l'Aa, à Grand-Fort-Philippe supposée être moins influencée par l'activité humaine, et la Baie de Somme en passant par les estuaires de la

Canche et de l'Authie beaucoup plus « naturels ». Une approche alliant plusieurs outils basés sur les foraminifères, les paramètres environnementaux et les données historiques, a été utilisée pour développer les interprétations.

Les principaux résultats de ce travail sont :

Le chapitre 1 introduit l'étude. Il donne une vue d'ensemble sur le monde des foraminifères et place la thèse dans le thème et dans le contexte géographique.

Dans le chapitre 2, l'écologie des foraminifères benthiques vivants est investiguée dans les marais salants de l'estuaire de la Canche à partir de 3 campagnes annuelles. Deux différentes zones de foraminifères sont identifiées le long du gradient tidal vertical. Un premier assemblage dominé par des taxons agglutinés se situe dans au milieu-haut du marais, alors que la partie basse est caractérisée par des spécimens carbonatés. L'exposition aux grandes marées engendre une zonation verticale des foraminifères, en fonction des amplitudes de marées. Ce chapitre soumis au journal *Estuarine, Coastal and Shelf Science* est actuellement en révision.

Dans le chapitre 3, une étude saisonnière a été menée dans 5 zones intertidales. Les objectifs de cette étude sont de décrire les assemblages des foraminifères benthiques vivants et d'observer l'influence des saisons sur leur distribution. Le résultat majeur est que les communautés de foraminifères dominées par *Haynesina germanica,* varient considérablement pendant l'année. Cependant ces communautés ne montrent pas une tendance claire et homogène dans les 5 zones d'étude. Les foraminifères benthiques intertidaux montrent une distribution en tache.

Le chapitre 4 présente l'étude des variations sur le long-terme des communautés de foraminifères benthiques dans des environnements pollués (port de Boulogne-sur-Mer) et non pollué (estuaire de la Canche). L'objectif principal est de mettre en évidence les changements environnementaux des cents dernières années. A Boulogne-sur-Mer, une période avant-pollution se distingue d'une période industrielle. La partie supérieure de la carotte reflète de meilleures conditions écologiques, indiquant un rétablissement de l'environnement après la période industrielle. Ces résultats sont publiés dans la revue *Marine Environmental Research*. Dans l'estuaire de la Canche, les foraminifères benthiques et des photographies aériennes historiques permettent de reconstruire cent ans de transformations environnementales. Une vasière a été graduellement remplacée par un marais végétalisé. Dans la partie basse de la carotte, la granulométrie et le Carbone Organique Total (TOC) sont des paramètres déterminants pour l'implantation et le développement des foraminifères benthiques.

Enfin, le chapitre 5 présente une synthèse et discussion des résultats majeurs. Les conclusions générales et des perspectives futures sont incluses.

Riassunto

I foraminiferi sono organismi unicellulari tra i piu' abbondanti e diversificati della biosfera terrestre. In prevalenza bentonici, sono diffusi in tutti gli ambienti marini odierni, dalle zone costiere ai remoti abissi. I foraminiferi bentonici sono capaci di organizzarsi in specifiche associazioni, in relazione alle differenti sollecitazioni ambientali. Altamente sensibili alle variazione dei parametri ecologici, sono sempre più frequentemente utilizzati come bioindicatori di cambiamenti di origine naturale ed antropogenica, a piccolo e lungo termine, sia a scala locale che globale. Inoltre, la loro eccellente capacità di fossilizzazione li rende uno strumento ideale per condurre ricostruzioni paleoambientali e paleoecologiche.

La direttiva europea "European Marine Framework Directive" (MSFD, 2008/56/EC) si è prefissa il raggiungimento di un buon stato ecologico per tutti gli specchi d'acqua per l'inizio del 2020. Così, di recente, è cresciuto l'interesse attorno all'applicazione dei foraminiferi bentonici nel quadro della suddetta legislazione europea. Il presente lavoro di tesi si inserisce in questo contesto con l'intento di studiare la risposta dei foraminiferi bentonici moderni ai recenti cambiamenti ambientali nella regione Hauts-de-France (Francia Settentrionale).

La linea di costa del Hauts-de-France si estende per circa 240 km bordando la parte meridionale del Canale della Manica. La parte nord della regione è stata soggetta ad una forte antropizzazione che, negli 200 anni, ha profondamente modificato l'ecosistema costiero. Al contrario, la parte più a sud, si costituisce, principalmente, di aree incontaminate.

Il presente progetto di dottorato ruota attorno a tre principali obiettivi: i) descrivere le comunità intertidali dei foraminiferi bentonici viventi in zone incontaminate ed antropizzate, ii) investigare la risposta di foraminiferi intertidali a variazioni spaziali e temporali (stagionali), iii) osservare le variazioni temporali a lungo termine (centinaia di anni) che hanno caratterizzato le zone intertidali del Hauts-de-France.

Con questi scopi, sono state analizzate le associazioni a foraminiferi, sia viventi che fossili, in cinque siti di studio localizzati nelle zone intertidali della regione: l' inquinato estuario della Liane con il porto di Boulogne-sur-Mer, le aree a flusso canalizzato di Grand-Fort-

Philippe, le zone incontaminate nella baia dell'Authie e baia la Somme, passando per l'estuario de la Canche. Per migliorare le interpretazioni, è stato utilizzato un approccio multidisciplinare basato sullo studio delle associazioni a foraminiferi, sui parametri ambientali e su dati storici.

I principali contenuti del lavoro di tesi sono:

Capitolo 1: introduzione di carattere generale sui foraminiferi e sulle tematiche della tesi nel contesto geografico in esame.

Capitolo 2: ecologia dei foraminiferi bentonici viventi delle aree salmastre nell'estuario della Canche. Sono state identificate due distinte associazioni lungo il gradiente tidale verticale, un'associazione di medio-alta salt marsh (zona salstrastra vegetata) dominata da specie agglutinanti ed un'associazione di bassa salt marsh, dominata da taxa calcarei. L'esposizione alla forte escursione di marea ha determinato la distribuzione verticale dei foraminiferi in relazione al gradiente tidale. Il presente studio è attualmente in revisione nella rivista *Estuarine Coastal and Shelf Science*.

Capitolo 3: studio di un sondaggio stagionale attraverso 5 siti di studio nella zone intertidali della regione. Gli obiettivi di questo capitolo sono stati quelli di descrivere le associazione a foraminiferi viventi e di osservare come la stagionalita' ha influenzato la distribuzione delle stesse. Il principale risultato è stato osservare che le popolazioni a foraminiferi, dominate da *Haynesina germanica,* variano consistentemente durante l'anno di osservazione. Tuttavia nei cinque siti studiati le associazioni non hanno mostrato lo stesso andamento, in termini di diversità e abbondanza. Inoltre le comunità a foraminiferi bentonici presentano elevate variazioni su scala centrimentrica.

Capitolo 4: sono state investigate le variazioni a lungo termine delle popolazioni bentoniche nelle zone inquanate del porto di Boulogne-sur-Mer e nelle aree poco contaminite lungo l'estuario della Canche, tramite l'utilizzo di carote sedimentarie. Gli obbiettvi sono stati quelli di monitorare i cambiamenti ambientali avvenuti nei secoli recenti. Nel aree portuali di Boulogne-sur-Mer le associazioni fossili permettono di distinguere chiaramente un periodo pre-industriale (definito come periodo di referenza per Boulogne-sur-Mer) da un periodo industriale. La parte alta della carota indica, nell'ultimo ventennio, un miglioramento delle condizioni ecologiche. Lo studio è stato pubblicato sulla rivista *Marine*

Environmental research. Nell'estuario della Canche, i foraminiferi bentonici e le immagini storiche, hanno permesso la riscotruzione di cento anni di modificazioni ambientali. I risultati evidenziano come un'iniziale piana tidale venga gradualmente rimpiazzata da un zona di saltmarsh. Nella parte bassa della carota, la granulometria e i contenuti in materia organica costituisco fattori critici per l'insediamento e sviluppo dei foraminiferi bentonici.

Capitolo 5: discussioni e conclusioni dei principali risultati, e delle prospettive future.

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Chapter 1 Introduction

1 Benthic Foraminifera

1.1 An overview

Foraminifera are marine protozoans (unicellular eukaryotes), belonging to the super-group of SAR (acronym of Stramenopiles, Alveolata, and Rhizaria), phylum Retaria (Cavalier-Smith and Chao, 2003), sub-phylum Foraminifera (d'Orbigny, 1826) (**Figure 1.1**) (For details on rRNA-based phylogenies look at Adl et al., 2012).

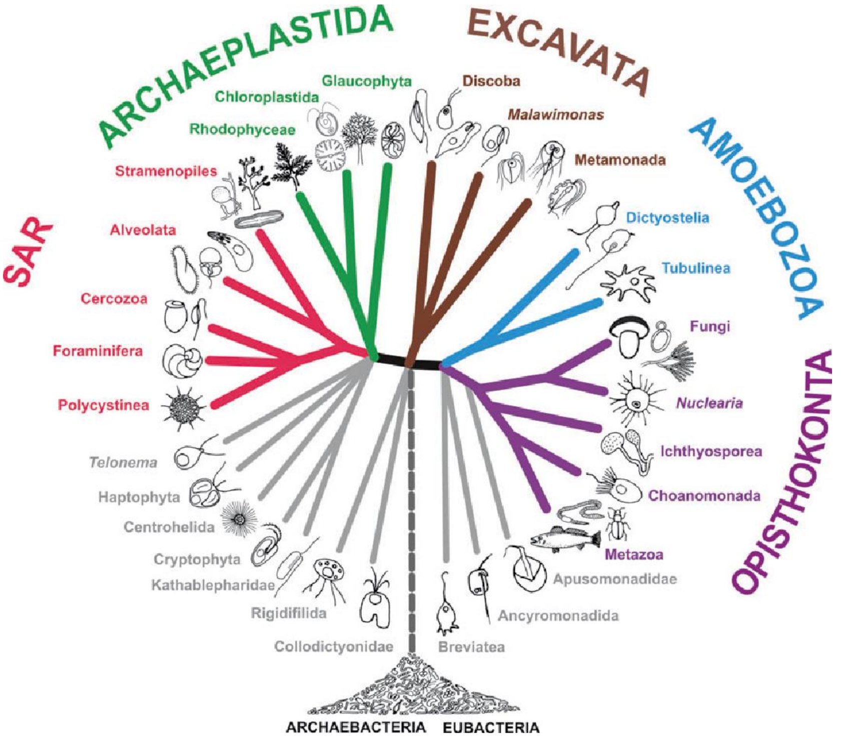


Figure 1-1 A view of eukaryote phylogeny reflecting the classification from (Adl et al., 2012).

Foraminifera have been the objects of a large number of classifications over the last two hundred years. Most of these were based on morphological criteria (amongst these d'Orbigny, 1826; Schultze, 1854; Carpenter et al., 1862; Loeblich jr and Tappan, 1964; Loeblich and Tappan, 1988; Sen Gupta, 1999; Mikhalevich, 2004; Kaminski, 2005). In the present work I used the most recent supra-ordinal classification of Pawlowski (2013), based on molecular phylogenetics, supplemented by morphological data. Foraminifera are considered as a phylum composed by three main groups: 1) the paraphyletic assemblage of "monothalamids"; 2) the class Globothalamea (**Figure 1.2 a**) and 3) the class Tubothalamea (**Figure 1.2 b**).

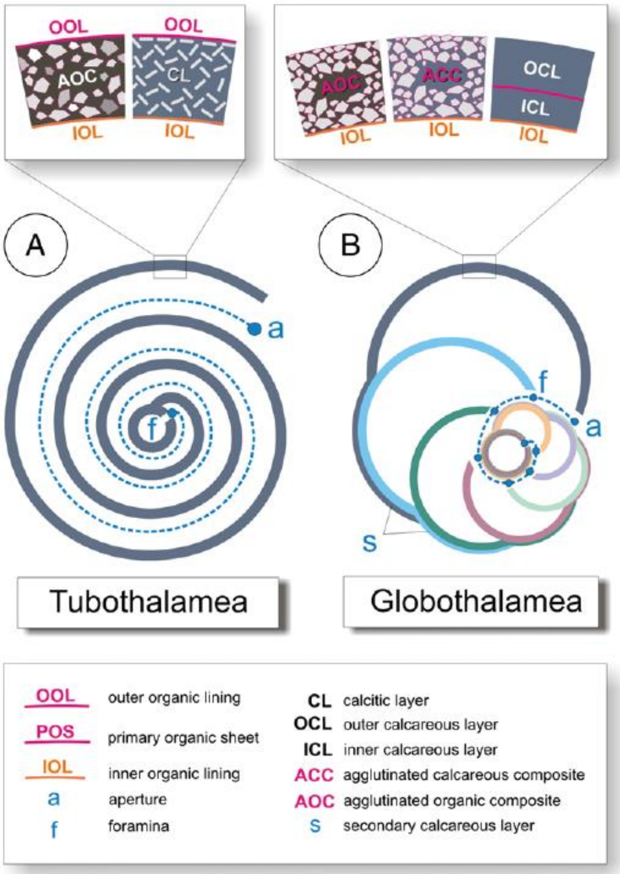
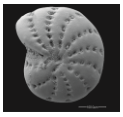


Figure 1-2 Principal morphological characteristics in Tubothalamea (a) and Globothalamea (b) after Pawlowski (2013).

Monothalamids have a single chamber test with an organic or agglutinated wall; the group comprises all genera included into the orders Allogromiida and Astrorhizida, as well as the deep-sea giant Xenophyophorea; it also includes freshwater and deep-sea species. Details can be obtained by reading the work of (Pawlowski et al., 2011). The class Tubothalamea has a bi- or multi-chambered test with tubular chamber at least in the juvenile stage; wall agglutinated or calcareous. In ancestral forms the test is composed of a spherical proloculus



(first chamber) followed by a spirally enrolled tubular chamber; more evolved forms have multi-chambered tests. Stratigraphic range is from Lower Cambrian to recent. It includes the Orders: Miliolida and Spirillinida. The class Globothalamea has multi-chambered test, typically trochospirally enrolled but may be triserial or uniserial. Chambers are globular or crescent-shaped in early stage; wall agglutinated or calcareous. Stratigraphic range is from Lower Cambrian to recent. It includes the Orders Rotaliida, Robertinida, Textulariida, and Carterinida. Incertae sedis orders consist of Lagenida, Fusulinida, and Involutinida.

Foraminiferal size ranges from tens of micron to centimetre dimensions. Even if unicellular organisms, they have the same functions of life performed by multicellular animals (such as eat, defecate, move, grow, reproduce, and respond to a variety of environmental stimuli) (Goldstein, 1999). They possess granular and reticulose pseudopodia that are essential for motility, attachment, for feeding, building and structuring tests, protection. Foraminifera feed on bacteria, diatoms and other protozoa, small crustaceans, molluscs, nematodes and invertebrate larvae (Armstrong and Brasier, 2013). The life cycle of foraminifera is characterized by an alternation between two generations: a gamont generation that reproduces sexually, and an agamont generation that reproduces asexually. Life cycle may be completed within a year in tropical latitudes and two or more years at higher latitudes.

Foraminifera have a large number of test typology which is thought to reduce biological, physical and chemical stress (Armstrong and Brasier, 2013). Foraminiferal tests vary on the base of wall structure and composition, growth, chamber architecture and shape, aperture, ornamentation. Detailed description of them can be found in many general works (Murray, 1991; Lipps, 1993; Sen Gupta, 1999; Scott et al., 2001; Armstrong and Brasier, 2013). Generally the most common classifications based on test characteristics divide foraminifera in three main groups: soft shell-organic wall (only Monothalamids), hard shell-calcareous and agglutinated specimens. In **Figure 1.3** the wall structures and the principal chamber arrangements are presented.

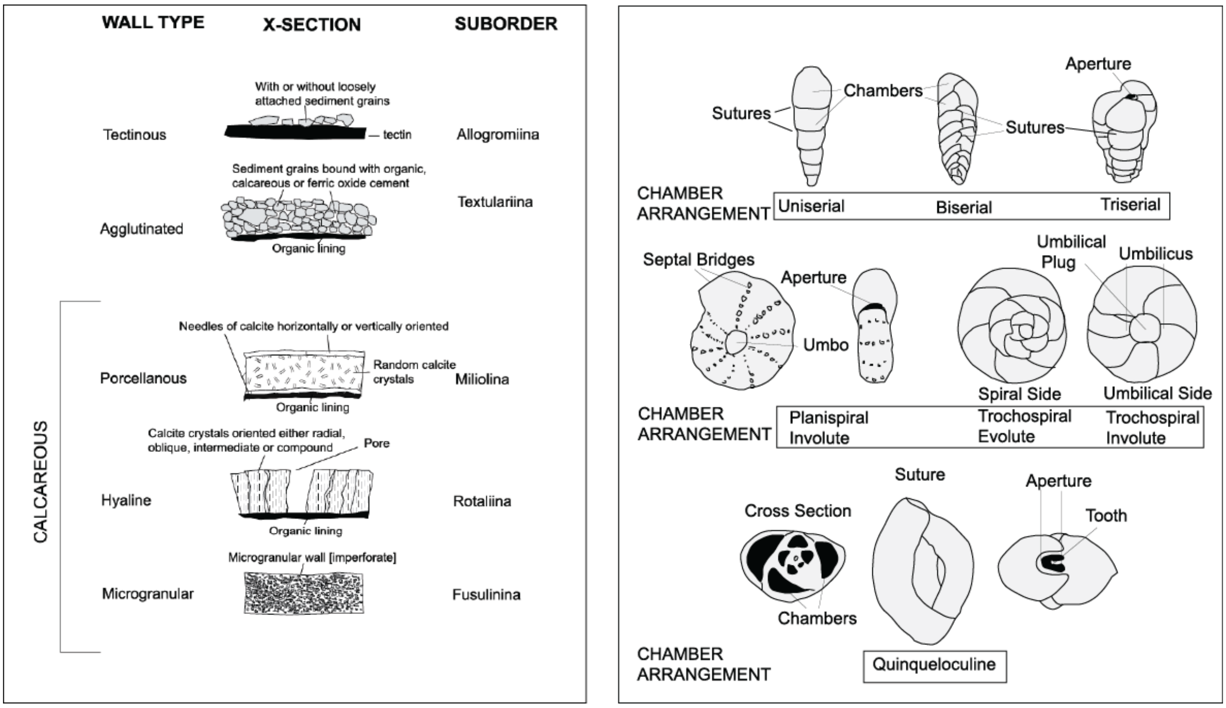
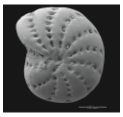


Figure 1-3 Wall structures and principal chamber arrangement in foraminifera after Culver (1993) in Scott et al. 2001.

Foraminifera constitute one of the most diverse and spread out group of organisms in marine environments (from coastal settings to deep sea) (Sen Gupta, 1999; Murray, 2001). Foraminiferal tests can be very abundant; in the modern ocean they comprise over 55% of Arctic biomass and over 90% of deep sea biomass (Armstrong and Brasier, 2013). The estimated number of modern foraminiferal species is between 10000 and 15000 (Adl et al., 2007). Of these 40-50 species are planktonic and the rest are benthic. All existent species of planktonic foraminifera (except for an Antarctic species, which overwinters in brine channels in sea ice species (Spindler and Dieckmann, 1986)) are holoplanktonic, spending their entire life freely floating in surface waters (Kucera, 2007). Benthic foraminifera can live actively (or inactively) at sediment surface (or in the proximity). They can be both epifaunal (on sediment surface) or infaunal (within the sediment). Epifaunal individuals live on sediment or firm substrates such as animals, shells, rocks and plants (called epiphytic in this case). Epifaunal taxa may be sessile (attached immobile), clinging (attached mobile), or free living (Murray, 2006). Infaunal taxa have been recorded living (stained) down to 30-60cm below the sediment surface (Goldstein et al., 1995; Bouchet et al., 2009) but in the majority of environments most live in the top surface (0–1 cm layer) (Alve and Murray,



2001; Milker et al., 2015a). Some motile benthic foraminifera can move vertically in the sediment column adapting to environmental change conditions (even though the knowledge of motion behaviour still need improvements (Seuront and Bouchet, 2015)).

Benthic fauna from Cambrian Period and planktonic fauna from Cretaceous have been used in many geological fields such as bio-stratigraphy, chrono-stratigraphy, palaeo-climatology, palaeo-bathymetry and bathymetry, palaeo-ecology and ecology.

My PhD work focuses on benthic foraminifera in intertidal environments.

1.2 Benthic foraminifera in intertidal areas

Intertidal areas are extremely dynamic environments characterized by high spatial and temporal variability in environmental parameters. The distribution and abundance of benthic foraminifera in these areas are linked to a large number of environmental factors, abiotic (such as salinity, temperature, dissolved oxygen, nutrients, tide, type of substrate, pollution, etc.), biotic (competition, food supply, etc.) and both biotic and abiotic (disturbance, control to anoxia) (Murray, 2006). For instance foraminifera can reflect different degree of salinity or confinement. These gradients may be horizontal and/or vertical depending on river flow and the tidal regime, ranging from brackish to hypersaline depending on climate (Scott et al., 2001). In estuarine areas where tides are intense, a vertical zonation of the intertidal zone may occur. Following the tidal gradient we may have: 1) salt marshes in temperate regions (and mangrove in tropical ones), which are vegetated areas cover by halophytic plants, completely flooded only during high astronomical tide and 2) tidal flats, un-vegetated wetland regularly and daily flooded by water.

A general overview of the dominant species in temperate Atlantic mesotidal to macrotidal environments have been documented in Debenay and Guillou (2002) (**Figure 1.4**). The foraminiferal assemblages of tidal flats are dominated by *Ammonia beccarii* on sandy substrate and by *Cribroelphidium excavatum* on muddy substrate. *Elphidium pulvereum*, *Haynesina depressula*, *C. excavatum*, and *A. beccarii* dominate in the lower estuary, passing upward to *A. tepida* and *C. gunteri*, and farther to *H. germanica*. Agglutinated forms, mainly *Miliammina fusca*, dominate the upper estuaries. The vertical zonation shows a progressive change from *A. tepida* dominant in the lower intertidal zone to *H. germanica* in the intermediate zone and *Ammotium salsum* in the upper intertidal zone. *Trochammina inflata* and *Entzia macrescens* dominate the supratidal marsh zone, rich in organic matter.

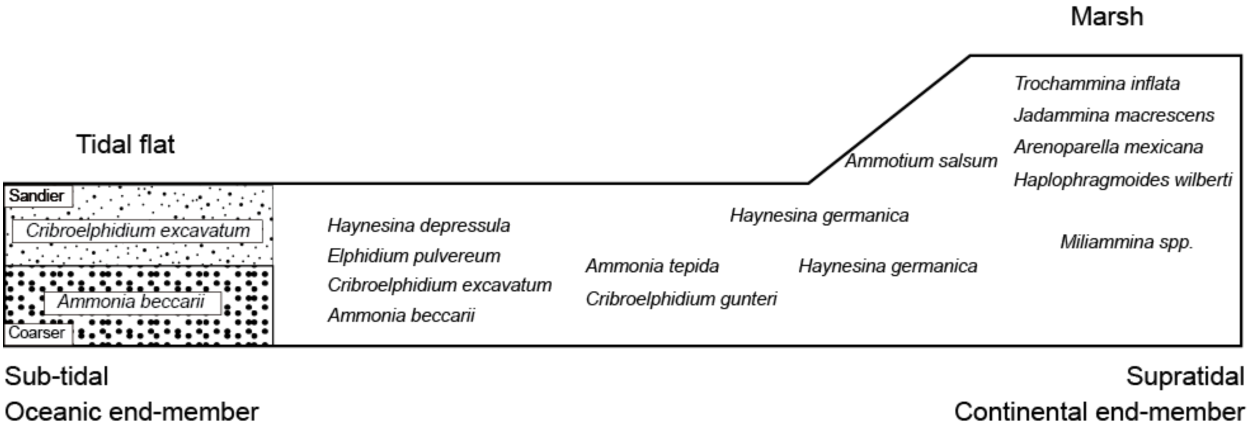


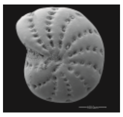
Figure 1-4 Species dominant distribution in meso-tidal to macro-tidal Atlantic environments along both a longitudinal marine-to-freshwater gradient and a vertical water-to-land gradient (after Debenay and Guillou, 2002).

Intertidal foraminifera, especially marsh foraminifera, have a precise vertical zonation constrain to the tidal gradient and they have been extensively as seas level indicators (Scott and Medioli, 1978; Scott and Medioli, 1980; Horton et al., 1999; Leorri et al., 2008b). These studies require that the elevation must be the primary control factor (Horton and Murray, 2007) or at least, other factors co-vary with it (Sen Gupta, 1999; Berkeley et al., 2007). However in marsh environments the relationships between benthic and other parameters like salinity or tidal exposure need further investigations, especially in areas where tidal range can increase parameter's variability. In addition the abundance and diversity of benthic intertidal foraminifera vary seasonally reflecting the fluctuation in biotic and abiotic environmental variables (Murray and Alve, 2000; Wilson and Dawe, 2006; Saad and Wade, 2016). This, added to their high small-scale variability (patchy distribution) (Buzas et al., 2002; Morvan et al., 2006; Buzas et al., 2015), makes more difficult the interpretations.

1.3 The use of benthic foraminifera

1.3.1 Ecological indicators and environmental monitoring

In the recent 200 years, coastal areas experienced intense and rapid degradations due to anthropic action. A large number of marine legislations worldwide have been recently

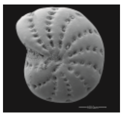


developed to remedy to the deleterious effects of human population (such as CleanWaterAct [CWA] and Oceans Act in USA, Australia and Canada; Water Framework Directive [WFD] and Marine Strategy Framework Directive [MSFD] in Europe, National Water Act in South Africa). Among these, the WFD defines a number of environmental indicators as good environmental quality. It states that relevant species and functional groups used, have to be characterized by fast turnover and specific habits. Remarkable efforts, made to develop new methodologies for biological monitoring, allowed the consideration of benthic foraminifera for application under EU legislation, especially after the standardization of sampling and analytical methods (Bouchet et al., 2012; Schönfeld et al., 2012; Barras et al., 2014; Alve et al., 2016; Dimiza et al., 2016; Dijkstra et al., 2017).

Benthic foraminifera result to be highly sensitive to environmental parameters variations (Yanko et al., 1999). They often show species-specific responses to ecological conditions (Fursenko, 1978). Several authors highlighted the advantage of "foraminiferal application" over chemical and biological techniques, and how their characteristics provide one of the most sensitive and inexpensive markers available for indicating changing of marine environments (Pati and Patra, 2012). The advantage of using benthic foraminifera, can be summarized as follows: 1) High biodiversity, benthic foraminifera contain more specialists, which are expected to be sensitive to environmental changes; 2) Small and abundant compared to other organisms commonly used in monitoring and impact assessment studies (e.g., molluscs, Nematodes, etc.); 3) Easy to collect and store, foraminiferal samples require no lengthy preparation, providing an especially highly reliable database for statistical analysis; 4) Short reproductive cycle and rapid growth, makes their community structure responsive to quick environmental change; 5) Rapidly preserved mineralized tests allow fossil foraminifers to be studied from sediment cores to assess decadal, century and millennial scale changes in assemblages at sites of interest. This provides historical baseline data even in the absence of background studies (Alve, 1991a; Hayward et al., 2004). For all these reasons benthic foraminifera can be used as potential ecological indicators (bioindicators) of global and local natural changes as well as human environmental alterations (Alve, 1991b; Yanko et al., 1999; Debenay et al., 2003; Armynot du Châtelet et al., 2004; Bouchet et al., 2007).

The ecology of benthic foraminifera has been intensively investigated over the past 40 years in modern environments (for general review look Murray, 1991; Sen Gupta, 1999; Scott et al., 2001; Murray, 2006). The principal scope that encouraged the researchers, was to provide reliable clues (analogues) for the understanding of marine environmental changes in the recent past (Sen Gupta, 1999) and the role of human being in these transformations. The first important studies, which focused on the potential use of benthic foraminifera as human stressed bioindicators, must be attributed to Resig (1960), Watkins (1961) and Zalesny (1961). Since then the number of papers published on environmental monitoring have increased exponentially. These studies have been carried out from different environments based on various sources of pollution such as outfall sewage, fertilizer, aquacultures, oil spills, heavy metals, pesticides among other (e. g. Alve, 1995; Armynot du Châtelet et al., 2004; Mojtahid et al., 2006; Bouchet et al., 2007; Coccioni et al., 2009; Frontalini et al., 2009; Vilela et al., 2011; Brunner et al., 2013; Martins et al., 2016a). Benthic foraminifera react to critical environmental parameter changes principally by the occurrence of local extinctions, dwarfism, morphological abnormalities, ratio as well as assemblage modifications (including density, diversity, assemblage structure) (Frontalini and Coccioni, 2008; Coccioni et al., 2009; Frontalini et al., 2009). For instance, in areas that undergone industrial pollution, the effect of trace metals increase (such as Cu, Cr, Pb, Hg, Zn, etc.) corresponds to a reduction in species diversity in foraminiferal assemblages (Frontalini and Coccioni, 2008; Coccioni et al., 2009; Martinez-Colon, 2009; Donnici et al., 2012). Experimentally it has been demonstrated that benthic foraminifera can incorporate trace elements in their shells (Nardelli et al., 2016) and cytological modifications (for example the increase of thickening of organic lining) can be related to the increase of Pb concentrations (Frontalini et al., 2015).

Although the majority of these studies showed the value of using benthic foraminifera in bio-monitoring programs, they also conceded, that distinguishing the human impact from natural stresses can be a difficult task and remains still controversial (Yanko et al., 1999; Armynot du Châtelet and Debenay, 2010).



1.3.2 Palaeoenvironmental and palaeoecological reconstructions

Holocene palaeoenvironmental changes have been interpreted on the basis of benthic foraminiferal in a lot of studies (Alve, 1991a; Gehrels, 1999; Hayward et al., 2010; Calvo-Marcilese et al., 2013; Delaine et al., 2014). Palaeoenvironmental reconstructions took place especially in coastal marginal areas, interesting for their high variability in term of physical-chemical-biological parameters, rapid landscape transformations, sea level changes, human interactions, accessibility, low cost of research. Foraminifera can serve as proxies, when used in conjunction to others, providing a first-order picture of past conditions in a variety of coastal settings (Scott et al., 2001). The use of a multiproxy approach, based on biological, physical/chemical parameters, historical data, etc., is essential to better detect environmental transformations along the sedimentary record (Leorri et al., 2008a).

Due to the recent coastal deterioration these aforementioned legislations aim at the assessment of the ecological quality status (EcoQS) defining the reference conditions in order to set up relevant comparisons with potential impacted sites. Several authors showed that the fossil record, and especially benthic foraminifera, has great potential to reconstruct palaeoecological quality status (PalaeoEcoQS) and thereby establish historical reference conditions from natural conditions (Alve, 1991b; Andersen et al., 2004; Alve et al., 2009). Lately, fossil benthic foraminifera have been used to define *in situ* reference conditions and to distinguish a pre-impacted stage from an impacted one (Alve et al., 2009; Dolven et al., 2013; Francescangeli et al., 2016).

2 Study area, sediment and former foraminiferal studies

The coastal areas of Hauts-de-France (northern France), subject of this PhD thesis, extend about 240km along the southern part of the English Channel (between the Belgium border to the Bay of Somme) (**Figure 1.5**). From a geological point of view the area is characterized by the presence of a large anticline (Weald–Artois anticline), running between the regions of the Weald in southern England and the Artois in north-eastern France. The fold formed during the Alpine orogeny, from the late Oligocene to middle Miocene. The folding resulted in the uplift of about 180 meters though concurrent erosion may have substantially reduced the actual height of the deposits.

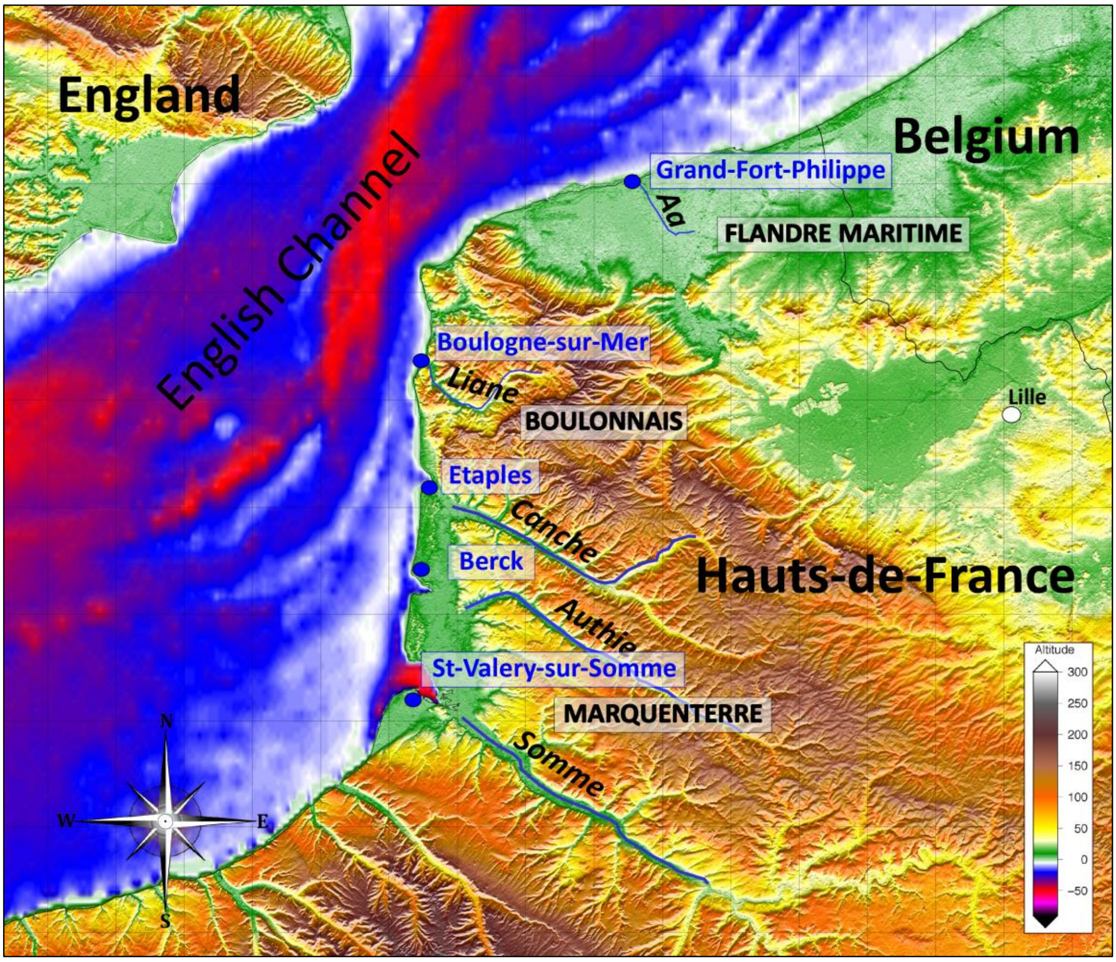


Figure 1-5 Topographic source SRTMV4 (NASA 2008), Bathymetric source ETOPO1 (NOAA, 2009); made by F. Graveleau, U.F.R. Sciences de la Terre and Géosystèmes, University of Lille 1 (2013). The picture allows seeing the hydrological pattern of the tree main rivers that mark the region. Five different study sites along the littoral on the Hauts-de-France region have been selected: Grand-Fort-Philippe (Aa river), Boulogne-sur-Mer's harbour (Liane river), Le Touquet (Canche Estuary), Berck (Authie Estuary), St–Valery-sur-Somme (Somme Estuary).

In the area, a wide geological record (Devonian-Present) is emerging with a complex structural setting (for details seeing (De Batist and Henriet, 1995; Gibbard and Lewin, 2003; Mansy et al., 2007). The Weald-Artois anticline divides the littoral area in three parts, from north to south: 1) "Flandre Maritime", the littoral zone of the Flemish coastal plain; 2) "Le Boulonnais", the topographic relief of Artois reaching the seashore; 3) "Le Marquenterre", the marine zone of the Picardie coastal plain. The "Flandre Maritime" is a coastal plain with almost a single row of coastal dunes (5-10 m high on average), protecting the countryside that could be below the sea level. The Boulonnais, located on the East side of the Calais-Dover Strait is characterized by rocky cliffs. Some formations of Cretaceous age are the French equivalents to the more famous White Cliffs of Dover while other cliffs, to the South, are made of Jurassic rocks. The Marquenterre is a coastal plain bordered by a series of coastal dunes lines and dissected by three estuaries (Canche, Authie and Somme), which follow an old tectonic pattern of Hercynian age, oriented northwest-southeast.

Sea bed associated to the coast of the region consists of a rocky substratum (Mesozoic and Cenozoic age) filled by gravel and sandy sediments (for details look at Crapoulet, 2015). The continental shelf corresponding to the current English Channel underwent to important erosion leading to the accumulation of fluvial and wind deposits along the coastal areas. The maxima sedimentation of continental deposits are in correspondence to estuarine areas. Estuaries, seaward portion of drowned valleys, are the results of complex interactions of river and marine (tidal and/or wave) processes (Dalrymple et al., 1992). The coasts of Hauts-de-France regions are formed by beach-dune systems, where onshore winds are prevailing (Battiau-Queney et al., 2001). The region is characterized by a semidiurnal (Chabert d'Hieres and Le Provost, 1978) macro tidal to hyper tidal regime (following the classification of McLusky and Elliott, 2004), variable during the year. Tidal range exceeds 10 m during highest astronomical tides to the south of the study area. The tidal range then decrease northward to the Belgian border where it reaches about 4 m. The combination between the dominant macrotidal conditions, compared with the relatively limited action of wave dynamics, leads to define this region as tide-dominated system (for more details on regional sedimentary processes look at Margotta, 2014). This determines the formations of large tidal flats and peculiar morphology characterized by the presence of ridge and runnel systems along the large beaches (for details Crapoulet, 2015). In the **Figure 1.6** is shown a schematic map of tide-dominated estuary in temperate areas. However, due to the strong anthropization, (in the northern part) some processes have been minimized and the current coastal dynamic has been modified.

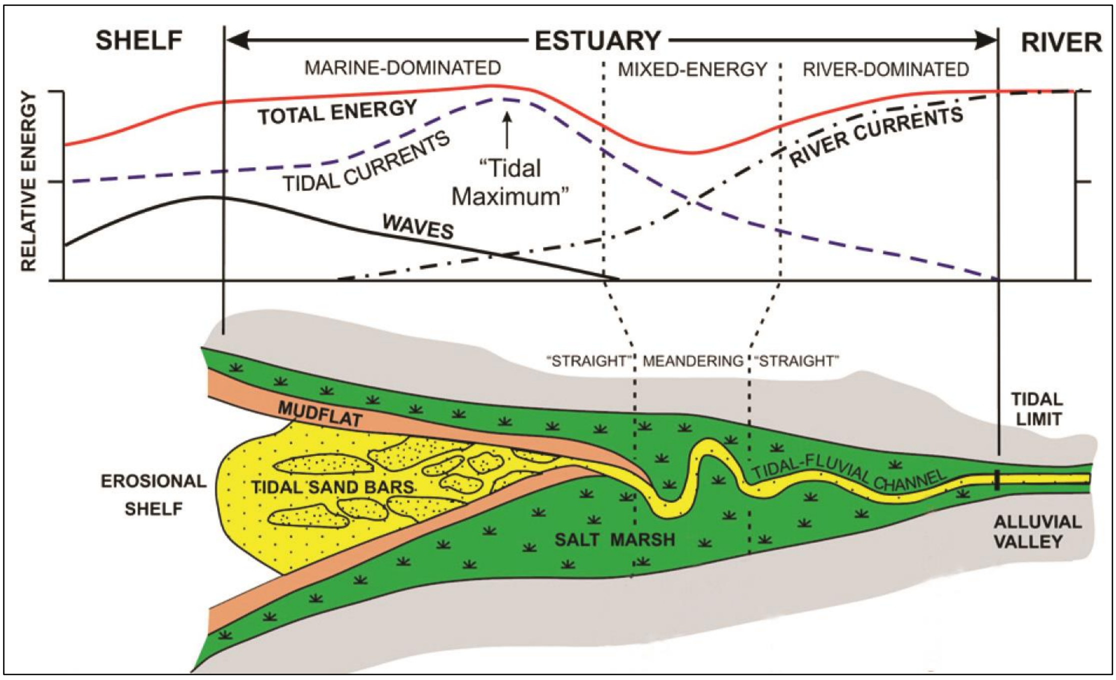


Figure 1-6 Schematic morphology of a tide-dominated estuary in temperate areas. In the upper part of the picture is represented the longitudinal variation of the intensity of the three main physical processes, river currents, tidal currents and waves, and the resulting directions of net sediment transport. At the lower side we can observe the funnel shape of the estuary, the systematic changes in channel geometry ("straight"-meandering-"straight"), the presence of elongate tidal bars in the seaward part, and the fringing muddy tidal flats and saltmarshes (from Dalrymple and Choi, 2007).

The dynamics of estuaries is a complex combination of forces having different origins: tides, waves, river stream. It results in a specific distribution of sedimentary facies (Dalrymple and Choi, 2007)and sedimentation rhythms (Deloffre et al., 2007). Our study will mainly focus on the central, mixed-energy dominated, part of estuaries. In this part of the estuaries, the energy is minimal that led the possibility for fine sediment to settle. These conditions are especially favourable for foraminifera.

The intertidal area is classically divided in belts ranging from the river stream to the uppermost areas. The river bed is often covered by the coarser sediments (coarse sands) and can be covered by sand dunes usually flood dominated (Ashley, 1990). The next belt corresponds to the slikke also called "tidal flat". This area is covered by fine sands while in some better sheltered estuaries it could consist in silts such as in the bay of Mont Saint-Michel (Tessier et al., 2010). The next belt is covered by vegetation and corresponds to the schorre of salt marshes. Distinctions can be done between lower- and higher-schorre depending on the elevation. Frequently, due to human occupation, the next belt corresponds to reclaimed surfaces also called polders. This is the case in some of the estuaries we worked on (**Figure 1-7**).

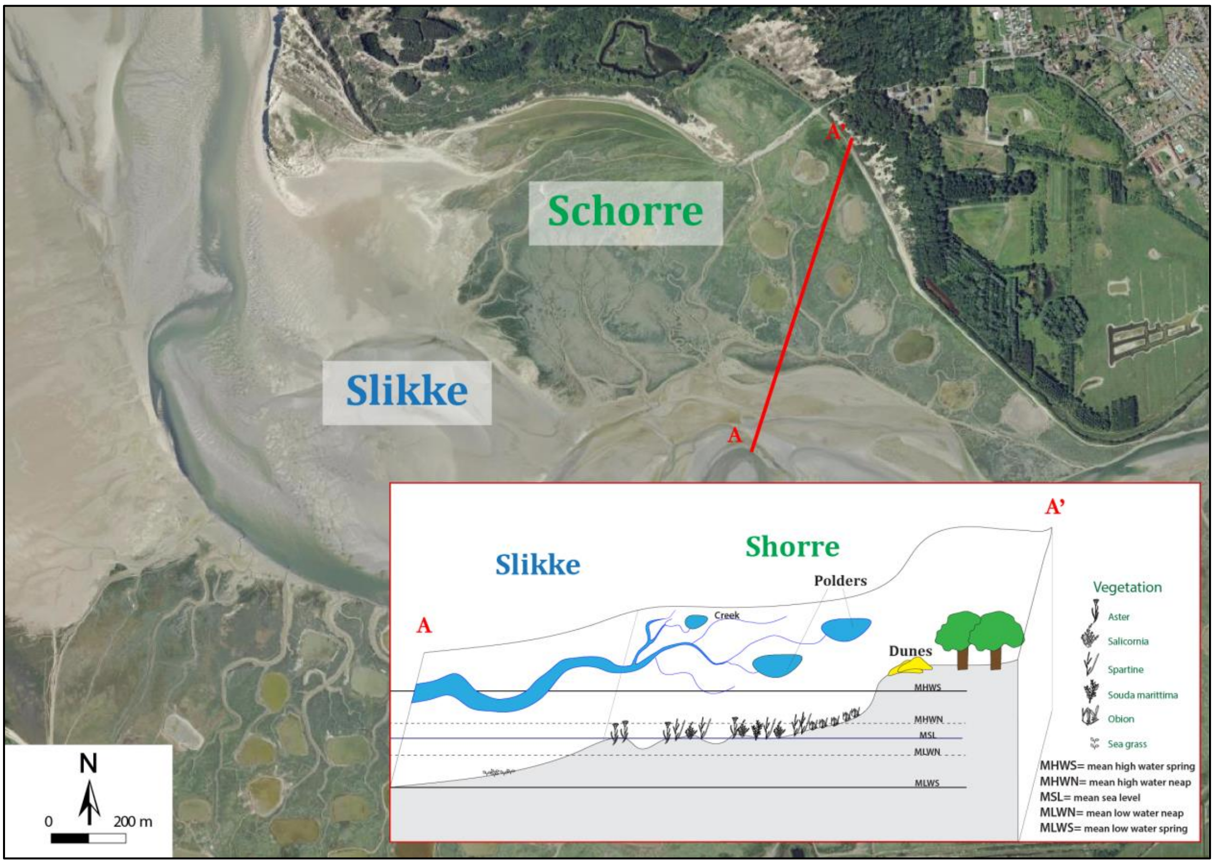


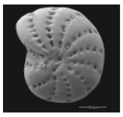
Figure 1-7 Distinction between schorre (marsh area) and slikke (tidal flat) in the bay of Authie, one of the selected studied estuary. In the profile typical herbs from these latitudes are represented.

In modern surface sample, an important fraction on the foraminiferal assemblages is made up by old reworked planktonic specimens (essentially belonging to Hedbergelidae, Globigerinoidae or Heterohelicidae), coming from the erosion of the continental shelf. In the studied area of my PhD thesis, benthic foraminiferal assemblages have been poorly studied. A comprehensive overview on the foraminiferal biocoenoses in the English Channel, was conducted by Rosset-Moulinier (1986). It concerned the study of infra-circalittoral (-20 to -40 m water depth) benthic foraminifera, focusing on substrates characteristics and annual temperature variations as drivers of their distribution. The author observed a low density of living fauna (<1 specimens for 50 cm^3 of wet sediment). For the rest before the beginning of the present PhD study, only two studies were published on intertidal foraminifera: Armynot du Châtelet et al. (2009a) in the Canche estuary (in the south-west of the region) and Armynot du Châtelet et al. (2011) in the Harbour of Boulogne-sur-Mer (in the northern part). The first was based on living fauna from a cross-shore transect across salt marsh area to the upper tidal flat. Agglutinants taxa (*Entzia macrescens* and *Trochammina inflata*) dominated the salt marsh area while *Haynesina germanica* and *Cribroelphidium williamsoni* dominated the upper tidal flat. In the latter, *H. germanica* and *Bolivina pseudoplicata* were considered as tolerant to heavy metals pollution and to high organic carbon contents. *Cribroelphidium excatum* and *C. magellanicum* were found tolerant to certain threshold of heave metals concentrations.

Although these studies bring important information about benthic foraminiferal distributions along the intertidal areas of northern France, they are not sufficient to have an overview on the main ecological processes/relationships occurring in the region. Furthermore all of these work did not consider that benthic foraminifera can have a high small-scale variability. At habitat scale the use of one single sampling (without replications), may bias the interpretations, especially if the scope is to investigate the benthic ecology (Seuront and Bouchet, 2015).

3 Objectives of this work

The present PhD project is born with the will of the region of Hauts-de-France (formerly Nord-Pas-de-Calais) to improve the ecological quality of its coastal territory, in line with the recent EU legislations. Over the last two centuries, the northern part of the region has experienced strong human modifications. Starting from the 2nd industrial revolution, especially coastal areas, have seen the development of numerous polluting activities (such as metallurgic, textile and chemical factories, harbours of Boulogne-sur-Mer, Calais and Dunkirk, and the nuclear power plant of Gravelines), leading to a large degradation of environmental quality. On the contrary the coast of Picardy includes mostly natural areas, protected by many natural parks. The differential degree of disturbance makes the coastlines of the region an excellent area to test the response of benthic foraminifera to environmental variations both in natural and anthopogenized zones. A second interesting aspect is the exceptional tidal range characterizing these coastal areas. It induces a high



variability in environmental parameters. A third element is that the diversity and ecology of benthic foraminifera along the transitional areas of Hauts-de-France has been poorly investigated.

In this context the objectives of this doctoral dissertation are 3-fold: i) to describe the living intertidal foraminiferal communities in natural and disturbed areas and associated environmental drivers, ii) to investigate the response of modern intertidal benthic foraminifera to short term (annual and seasonal) environmental variations, iii) to observe long term (hundred years) environment evolution based on foraminiferal assemblages under different degree of human pressure.

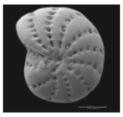
Both living and fossil foraminiferal assemblages have been sampled in five different study sites along the intertidal areas of the region: from the most polluted Liane estuary in the harbour of Boulogne-sur-Mer, the Aa estuary, in the embarked area of Grand-Fort-Philippe to the less supposed impacted one, Somme estuary, passing by the Canche and Authie estuaries. A multiproxy approach based on foraminifera, environmental parameters and historical data, has been adopted to improve the interpretations.

The present doctoral dissertation is an article thesis structured in 3 chapters based on the temporal and spatial scales considered:

In Chapter 2, living foraminifera are documented in three annual surveys from the salt marsh areas along the Canche estuary. Four transects were sampled to see the effects of maximal tidal constraints (shore transects), and minimal tidal constraints (alongshore transects). In the study we investigated the ecology of marsh foraminifera at local scale and we tested the effect of hyper tidal exposure on their distributions. The study is currently under review in Estuarine, Coastal and Shelf Science since September 2016.

In Chapter 3, I described the influence of short-term (seasonal) variations on foraminiferal distributions at a regional scale. To achieve these aims a seasonal survey was carried in the five selected intertidal areas across the coastlines of the region. Replicates for benthic fauna were collected to assess small-scale distributions.

In Chapter 4 the recent palaeoenvironmental and palaeoecological evolution of a polluted area i.e. Boulogne-sur-Mer harbour, and a natural area, i.e. the Canche estuary, are monitored. The purpose of this study is to describe the long-term variations of



foraminiferal communities. A multidisciplinary approach including major and trace metals, grain-size, total organic carbon and benthic fossil foraminifera, was performed on a 33-cm long core. The dating was carried out using the activity of ^{210}Pb and ^{137}Cs. The present study has been published in Marine Environmental Research (Francescangeli et al., 2016). Similarly, multidisciplinary analyses, based on sediment characteristics, geochemical parameters and benthic fossil foraminifera were carried out on an 80-cm long core in the Canche estuary. As it was not possible to obtain a reliable dating based on radiometric measurements, I proposed an alternative method based on the interpretations of historical maps.

Chapter 2 Does elevation matter? Living foraminiferal distribution in a hyper tidal salt marsh (Canche Estuary, Northern France)

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"Estuarine, Coastal and Shelf Science" (submitted 30/09/2016)

1 Abstract

In the present study we investigate the ecology and distribution of living benthic foraminifera to test the effect of hyper tidal exposure and their suitability as sea level indicators in three annual survey. Within a salt marsh area along the Canche Estuary (Northern France), four transects were sampled to see the effects of maximal tidal constraints (shore transects) and minimal tidal constraints (alongshore transects). Multivariate analyses have been performed to determine the correlations between biotic (foraminiferal absolute abundances) and abiotic factors (elevation, grain-size, TOC and total sulphur). For each of the principal benthic foraminiferal species the tolerance to tidal exposure have been as well estimated. Two distinctive foraminiferal zones have been identified along the vertical tidal gradient: a zone I in the higher part of the salt marsh dominated by agglutinated and porcelaneous taxa, and a zone II in the lower one dominated by hyaline specimens. Hyper tidal exposure constraints the foraminiferal vertical zonation in accordance with the tidal frame. However it does not constitute a threshold parameter able by itself to explain all the faunal variations in the Canche Estuary. For sea level indicators, foraminifera should be considered relative to tidal exposure rather than to absolute altitude.

2 Keywords

Hyper tidal estuary; Salt marsh; Benthic foraminifera; Living fauna; Elevation; La Canche Estuary

3 Introduction

Salt marshes are continent-marine transitional environments areas, covered chiefly by halophytic vegetation, and are regularly flooded by the sea (Allen, 2000). They have long been recognized as being among the most productive ecosystems in the world (McLusky and Elliott, 2004). They are the result of complex sedimentary processes, under physical and biological control (Pye and French, 1993). A major factor of the variability of these processes is the amplitude of the tide. In hyper tidal estuaries, tidal range (greater than 6m by definition, McLusky and Elliot, 2004) leads salt marshes to be extremely dynamic and instable areas. In north European estuaries, the salt marshes are not covered by the tide every day, but periodically by spring high tides. Flora and fauna that occur in these areas must be able to tolerate changes from emersion to immersion as well as changing-salinity waters, being covered occasionally by saline estuarine water (McLusky and Elliott, 2004).

Over the past decades the distribution and the ecology of salt marsh foraminifera have been largely investigated (among these Murray, 1971; Scott and Medioli, 1978; Hayward and Hollis, 1994; Alve and Murray, 1999). These studies point out that, although biogeography and local phenomena distributions might strongly influence the vertical zonation of marsh foraminifera, high-marsh and low-marsh fauna are marked by the same or similar groups of species in discontinuous geographic locations (Sen Gupta, 1999). It appears therefore necessary to understand which abiotic factor constrains benthic foraminiferal distribution in salt marsh areas. A lot of studies indicated that elevation is a primary factor constraining the colonization of intertidal areas by benthic living foraminifera (Scott and Medioli, 1980; Gehrels, 1994; Horton, 1999; Horton and Murray, 2007). Following the publication of Scott (1978), a new field of Quaternary research, based on benthic foraminifera as sea-level proxy, emerged (Hawkes and Lipps, 2014). Since then many works enhanced the relevance of salt marsh foraminifera for sea level reconstructions and palaeoenvironmental interpretations (Hayward et al., 1999; Edwards et al., 2004; Horton and Edwards, 2006; Leorri et al., 2010). However several authors pointed out the primary role of other environmental parameters such as salinity (Patterson, 1990; de Rijk, 1995; Fatela et al., 2007; Fatela et al., 2009) or grain-size (Armynot du Châtelet et al., 2009a) controlling the distribution of salt marsh foraminifera. In addition, taphonomic processes

and patchiness should be taken into account to have the best modern analogues for palaeoenvironmental reconstructions (Goldstein and Harben, 1993; Morvan et al., 2006; Berkeley et al., 2007; Kemp et al., 2011). Particularly, taphonomy can lead to the alteration of foraminiferal assemblages during the fossilization process. Small-scale spatial variations in living foraminiferal assemblages have been documented in many studies (Buzas, 1970; Murray and Alve, 2000; Swallow, 2000; Buzas et al., 2002). In some cases for all the species at neighbouring stations, differences exist between stations located in the same environment, even 10 m apart (Morvan et al., 2006). In other cases it has been observed a homogenous population of living foraminifera in salt marsh areas (Bouchet et al., 2007; Milker et al., 2015a). Despite the large number of studies on salt marsh foraminifera just a dozen have been carried in hyper tidal estuaries (among these Horton et al., 1999; Horton and Murray, 2006; Leorri and Martin, 2009; Mills et al., 2013). Some authors identified faunal zones along tidal levels (or tidal frame), in which the altitudinal range is the most indicative key factor at each zone (Haslett et al., 1997; Horton and Murray, 2006). Contemporary foraminiferal assemblages reflect as well the vertical floral zones based on vascular plants (Horton et al., 1999). However in these areas, the extreme tidal range leads to intensify the already highly instable conditions affecting the salt marsh. Consequently, tidal exposure, defined here as the time during which the marsh area is upon sub-areal conditions, appears a key-factor/stressor on driving foraminiferal distribution (Patterson et al., 2004). Even though a linear relationship exists between elevation and tidal exposure (Scott and Medioli, 1980), it seems interesting to observe the direct effect of the latter on benthic foraminifera. However, so far, the specific benthic foraminiferal tolerance to subaerial conditions has been scarcely quantified.

The first objective of our study is to investigate the distribution of living salt marsh foraminifera from a hyper tidal estuary, the Canche, in the northern France with the aim to contribute to the understanding of their ecology. The second objective is to investigate the effect of the tidal range (subaerial exposure) on benthic foraminifera in this hyper tidal salt marsh at annual scale. The third objective is to evaluate if living benthic foraminifera from the Canche tidal marsh could be used for sea level reconstructions.

4 Materials and methods

4.1 Study area

The study was carried out on the tidal marsh of the Canche Estuary (Hauts-de-France Region, Northern France) (**Figure 2.1**). The marsh area (ca. 7 km^2) is bordering an estuary ending an 88 km long river having a relatively small drainage basin of 1396km^2. This estuary is considered as an "estuarine back-barrier" type (Pye and French, 1993).

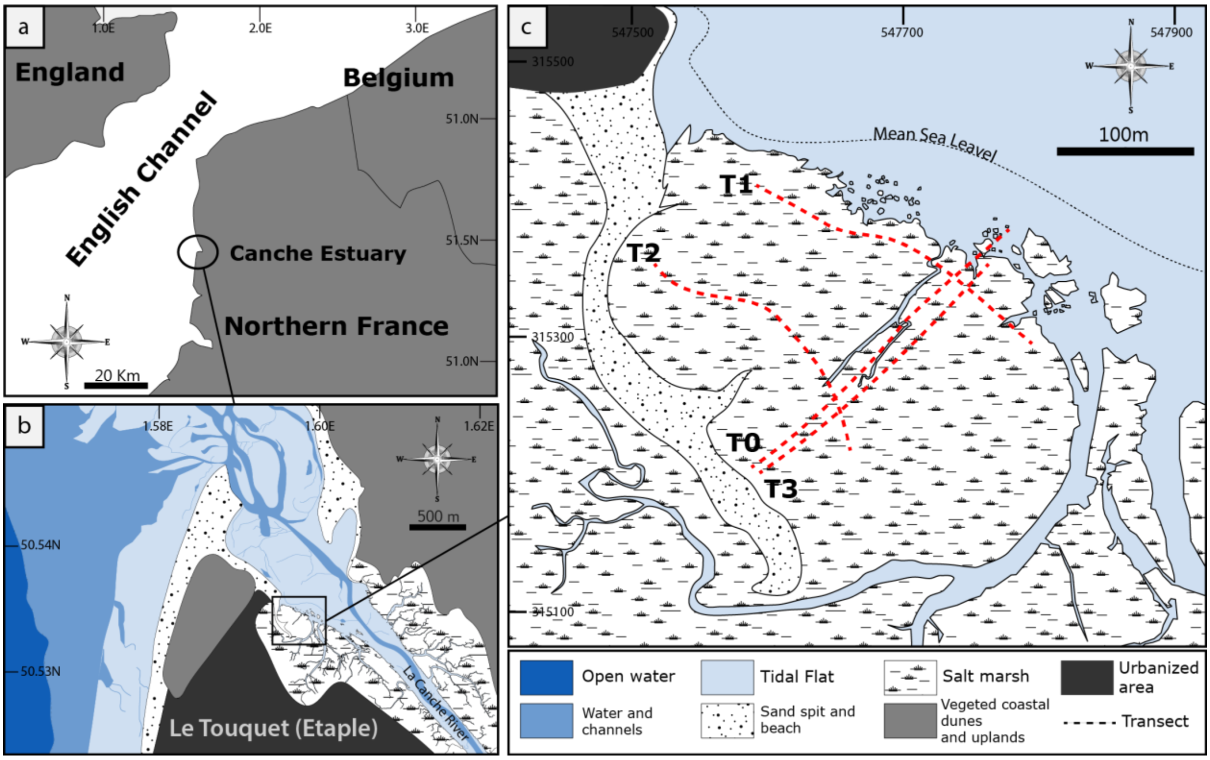


Figure 2-1 a) Study region, Northern France; b) Canche Estuary. Coordinates in WGS84 system; c) Sampling area with the localization of T0, T1, T2, T3 transects. Coordinates in metric UTM Zone 31N, WGS84. Limits between different surfaces has been drawn using the 20 aerial picture.

The morphology is marked by the presence of a ridge and runnel system on its marine side. Protected by a coastal barrier, the salt marsh is muddy. The marsh area is a planar vegetated platform regularly flooded by tides. As in the inner parts of the barrier system, it can show strong freshwater influences, arising from the lowered salinity and seasonal flooding (Allen, 2000) while undergoing high salinity after intense drying along summer season. The watershed freshwater input is of about 13m^3 s^{-1} (Selleslagh and Amara, 2008). The vegetation, marking the transition between the tidal flat and the salt marsh, is mostly dominated by halophytic grasses and herbs. Because of a tidal range exceeding 9m during highest astronomical tides (**Table 2-1**) and its relative protection from the coastal barrier, the morpho-sedimentary dynamic of the Canche Estuary is strongly influenced by the semidiurnal tide (**Figure 2.2**). Water temperature closely follows the sea temperature and that of the river. Values range between 9 °C in winter and 18 °C in summer (Armynot du Châtelet et al., 2009a).

Figure 2-2 Image of study area at high tide and low tide (photo credit Alain Trentesaux)

Chart Datum	MLWST	MLWNT	MSL	MHWNT	MHWST
IGN69	-3.245	-1.545	0.905	3.305	5.105

Table 2-1. Tidal elevation (m) for Canche estuary area (associated harbour: Etaples, modified after reference harbour Boulogne-sur-Mer), relative to French Geodetic Vertical Datum IGN69 from SHOM (2002). Mean low water spring tide (MLWST), mean low water neap tide (MLWNT), mean high water neap tide (MHWNT), mean high water spring tide (MHWST) and mean sea level (MSL) are provided.

4.2 Field sampling

Topographical surveys were carried out using a GPS Trimble GeoXT with a real time precision of 6.5m and maximum post treatment resolution of 1 cm in latitude and longitude and 2.5cm in altitude in optimal conditions. We used the French Spatial Reference System Lambert 93 to position the sampling stations. Elevations were referenced to French Geodetic Vertical Datum IGN69. A total of 38 surface samples were collected along 4 transects from the intertidal area (**Figure 2.3**): T0, a cross-shore transect in September 2012 through salt marsh (10 samples) and the very high part of the tidal flat (one sample T0-11); T1 (7 samples) and T2 (7 samples), two alongshore transects respectively from the lower and higher/middle salt marsh in September 2013, and T3 (14 samples) a cross-shore transect through the marsh area in September 2014.

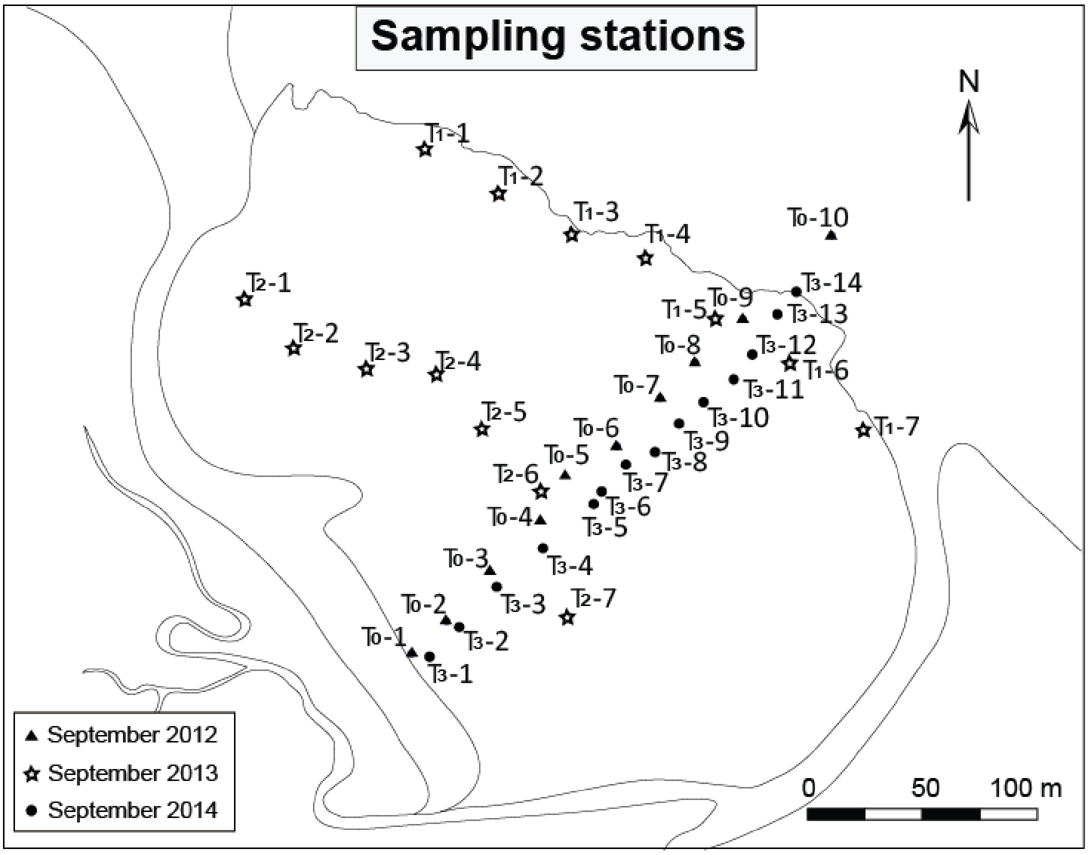


Figure 2-3 Sampling stations within the salt marsh of the Canche Estuary

The adopted sampling strategy was developed in order to assess faunal and environmental parameter variation both i) under altitude and tidal constraints (T0 and T3) and ii) under

minimal tidal constraints (T2 and T3). The cross-shore transects (T0 and T3) were sampled at different moment of the tidal cycle; during the T0 sampling the tidal range was lower.

In the study area, latitude and longitude were krigged at a 0.5m resolution using LiDAR surveys (see Crapoulet, 2015)

Considering the patchy distribution of benthic foraminifera in salt marshes (Buzas, 1970; Morvan et al., 2006; Barras et al., 2010; Buzas et al., 2015) three replicates per station were sampled(Bouchet et al., 2012; Schönfeld et al., 2012). The uppermost 1cm was scraped off for faunal analysis and stored in transparent graduated containers. A second set of samples was collected to analyse sediment characteristics (grain-size, total organic carbon, and total sulphur). The floral assemblages were described in an area of about 9 m^2 surrounding each sampling station. Coverage of the vegetation was qualitatively estimated.

4.3 Sediment analysis

Grain-size analyses were performed using the principle of diffraction and diffusion of a monochromatic laser beam on suspended particles (Malvern Mastersizer 2000, red He-Ne laser). The method is based on near-forward scattering of a laser beam by particles in suspension, as detailed previously (Trentesaux et al., 2001). Measurements can range from 0.02 to 2,000 μm with an obscuration ranging between 10 and 20%. Four grain-size fractions were considered: clays (< 4μm), fine silts (4-10μm), sortable silt (10-63μm) and sands (63 to 2000μm). Silt fraction and motility are discussed in McCave et al (1995).

A Flash EA 1112 Elemental Analyzer (Thermo) equipped with an auto-sampler was used for determining total contents of C and S. The analysis was performed on 1.5 to 2 mg of sample added to approximately 5mg of vanadium pentoxide, used as a combustion catalyst. 2.5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene (BBOT) was used as standard. The total organic carbon (TOC) was determined by subtracting carbonate carbon from total carbon concentration. Calcium carbonate content was determined using a Bernard calcimeter. Triplicated measurements were carried out for each sample using 0.5 g of finely crushed sediment.

4.4 Foraminiferal treatment and species identification

The foraminiferal samples were stained, on the field, with buffered Rose Bengal dye (2g of Rose Bengal in 1000ml of ethyl alcohol) to distinguish living tests (stained) from dead (unstained) at the time of collection (Walton, 1952; Lutze and Altenbach, 1991). The method gave reasonable result for salt marsh (Goldstein et al., 1995; Armynot du Châtelet et al., 2009a; Milker et al., 2015a). Although benthic foraminifera can live as deep as 30 cm in the sediment (Goldstein et al., 1995; Bouchet et al., 2009) this study was carried out on superficial samples because the highest numbers of living foraminifera are generally found in the surface 0–1 cm layer (Alve and Murray, 2001; Milker et al., 2015b). In the laboratory, wet samples (about 50 cm^3) were gently washed with tap water, through 315 and 63 µm mesh sieves, and dried at 40°C. Foraminiferal tests of the intermediate fraction were concentrated by flotation on trichloroethylene (Horton and Murray, 2006; Semensatto and Dias-Brito, 2007). Although heavy liquid separation should be avoided, in environments with low foraminiferal density due to a high sedimentation rate as in estuarine areas, it may be used (Schönfeld et al., 2012). Only specimens containing dense, brightly red-stained protoplasm were considered as alive (Alve and Olsgard, 1999; de Stigter et al., 1999). All living specimens were counted and identified, following Loeblich and Tappan (1988) for genera, and Debenay (2012) and Debenay et al. (2001) for species classification. The observations were carried out under a binocular microscope, model Olympus SZX16.

4.5 Data analysis

The absolute and the relative abundances of the taxa as well as the total faunal density (the number of specimens per cm^3 of sediment) were determined at each sampling station. The richness (S) and the effective number of species $\mathrm{Exp}(H')_{bc}$ (Bouchet et al., 2012), the most suitable measure of diversity (Beck and Schwanghart, 2010), were considered in the present study. Standard deviation was calculated on the absolute abundance of the main species to identify any patchiness effect within the replicates and a one way ANOVA was performed to evidence any difference between the foraminiferal densities within the transects. Then, due to the low faunal density, the counts for replicates were pooled per station to bypass the critical statistical threshold for reliable interpretation.

Multivariate statistical analyses were performed on samples containing more than 50 living individuals. Thirty-three sampling stations were taken into account. Station T1-1, T1-2, T1-3, T1-5 and T3-12 were excluded due to too low density. Only species with a relative abundance >5%, at least in one sample, were considered. As a consequence 12 main species were considered as explanatory variables. The main species represent from 92% to 100% of the total assemblage. A logarithmic transformation log (1 + x) was performed on raw data.

Pearson's correlation matrix has been calculated to determine the correlations between the biotic (species absolute abundances) and abiotic factors (elevation, grain-size, TOC and total sulphur). Multivariate regression trees (MRT) (De'Ath, 2002) was performed to evaluate the control of environmental constraints on explanatory species variables. Multivariate regression trees is a constrained clustering technique. The result is a tree whose "leaves" (terminal groups of sites) are composed of subsets of sites chosen to minimize the within-group sums of squares, but where each successive partition is defined by a threshold value (for details see Borcard et al., 2011). We selected the tree with the minimum cross-validated relative error. At each terminal group a small bar plot with species abundances is displayed. Species indicators are associated at each group (cluster) based on the indicator value (IndVal) index (Dufrene and Legendre, 1997). IndVal index combines species mean abundances and its frequencies of occurrence in the group. The indicator species are the species closely related to the ecological conditions of their group (Borcard et al., 2011). Statistical significance was assessed using $\alpha = 0.05$. Specimens with IndVal >0.60 were considered as good indicator species of the belonging clusters (Benito et al., 2016).

Tide water variations were estimated from Boulogne-sur-Mer Marel "buoy" (Lefebvre, 2015) that measures the sea elevation every 10 minutes. Altitudes were corrected for Canche Estuary after French Hydrographic Services. The time of inundation is the complementary value to 100% (of time) from subaerial exposure time. It is the percentage of time that a given area is covered by sea water. To evaluate the consequences of subaerial exposure for faunal content at each campaign, we calculated the tidal exposure prior the sampling day using available measured tidal curves. This has been done for 1, 2, 3, 4, 5, 7, 14, 21 and 42 days before each campaign, except for the T0 campaign were the tidal exposure for the 42 days prior sampling was not calculated due to a lack of data. At each sampling campaign the curves were fitted by a linear regression model. A weighted averaging regression was used to show the relationship between elevation and living foraminifera. It is based on weighted average optima and tolerance ranges from species absolute abundances and elevation (Simpson, 2007). Each optimal elevation was then correlated to the corresponding optimal specific subaerial exposure (for the main species), using the best fitting curve.

The R software was used for all the calculations, by using the following packages: Base (Team, 2016) (descriptive statistics and ANOVA), entropy (Hausser and Strimmer, 2014)(diversity calculation), ade4 (Dray et al., 2007), Hmisc (Harrell and Dupont, 2016)and corrplot (Wei and Simko, 2016) (Correlation matrix), mvpart (Therneau and Atkinson, 2014) and MVPARTwrap (Ouellette, 2013) (MRT and IndVal), analogue (Simpson and Oksanen, 2016) (optima and tolerance value, caterpillar plot).

5 Results

5.1 Elevation

The elevation of tidal marsh stations of the T0 and T3 transect ranges from 3.03m to 4.73m (relative to Vertical Datum IGN69). Along the parallel shore transects T1 and T2, the elevation varies between 3.03-3.52m and between 3.68-4.03m respectively. The only sampling station in the tidal flat (T0-10) is at 2.74m. The salt marsh-tidal flat transition is estimated about 3m. The comparison of the altitude measured with the LIDAR between 2011 and 2013 shows very limited variations of altitude within the marsh area, only due to vegetation growth differential (**Figure 2.4**). On the contrary we can notice the high dynamicity of the tidal flat due to combined processes of sedimentation.

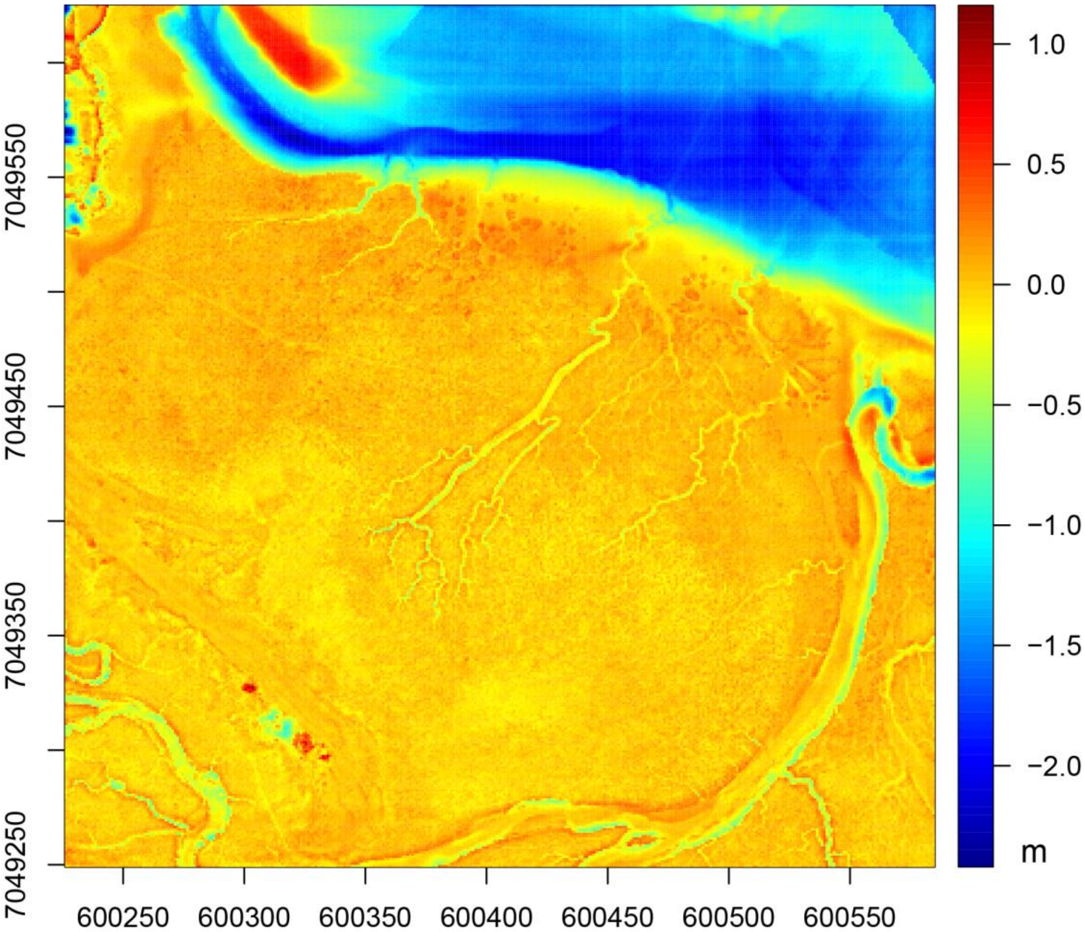


Figure 2-4 Map of the study area showing altitude variations between 2011 and 2013 (based on LIDAR acquisition). Positive values (towards red) indicate that sedimentation occurred, while negative values (towards blue) indicate that erosion occurred between 2011 and 2013.

5.2 Grain-size, TOC and sulphur

Grain-size (**Figure 2.5**) is quite homogeneous over the salt marsh. Silt is the most abundant fraction (average 80%). Sortable silt and fine silt vary respectively between 12-40% and 35-67%. Sand ranges between 8-33% (average 18%). Clay is the minor fraction, with values <1%. An increase of grain-size occurs towards the tidal flat and the main Canche stream (T0-10), where sand is the most abundant fraction (53%).

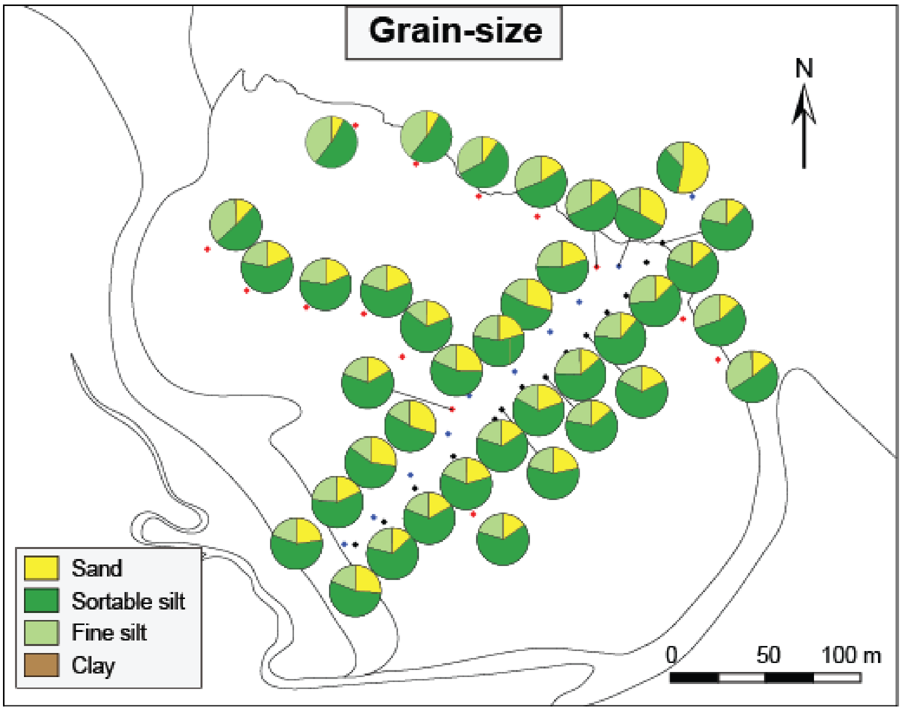


Figure 2-5 Surface distribution of sediment grain-size in the study

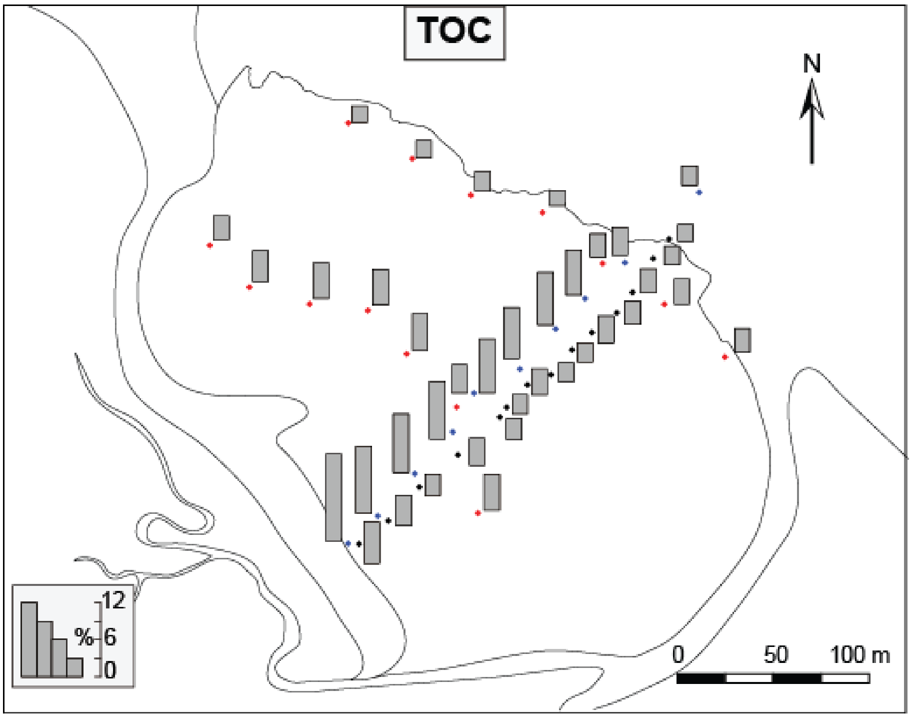


Figure 2-6 Surface distribution of TOC in the study area

Total organic carbon (**Figure 2.6**) ranges from 2.43% to 13.60%. Along T0, it shows high variations from 13.60% to 3.03% decreasing towards the tidal flat. The same trend occurs along T3 with values ranging from 6.47% to 2.58%. In T1 and T2, TOC is homogenous (StD T1=0.62, StD T2=0.76), with mean values respectively of 3.17% and 5.09%. Sulphur (**Figure 2.7**) varies between 0-0.7% and the highest values are located in the lower tidal marsh.

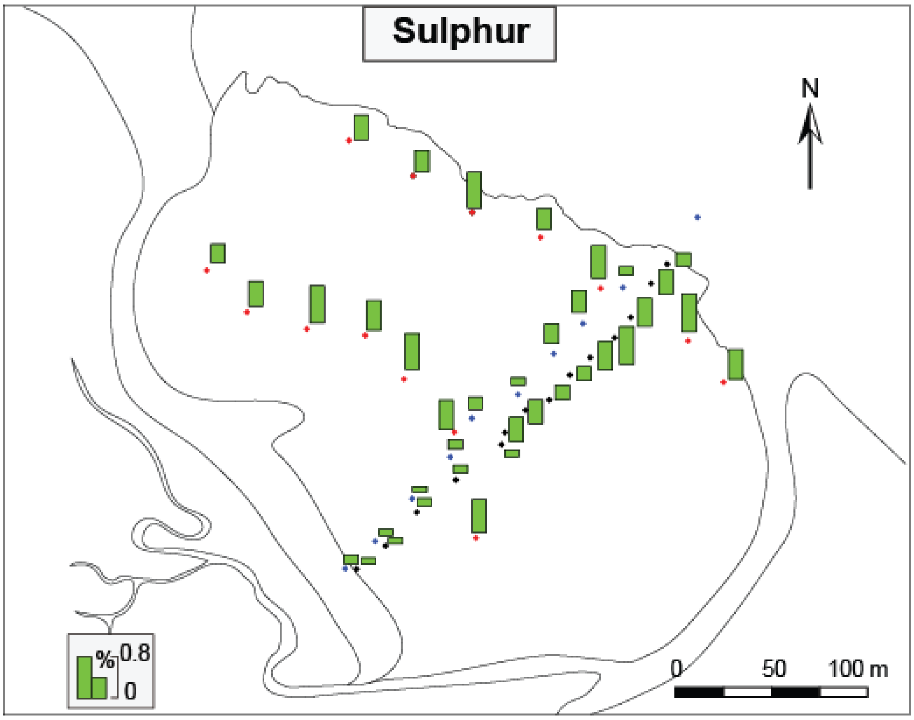


Figure 2-7 Surface distribution of sulphur in the study area

5.3 Vegetation

The most abundant halophytic plants are *Halimione portulacoides*, *Aster tripolium*, salicornia (*Salicornia europaea* and *herbacea*), *Spartina maritmima* and *Suaeda maritima* (supplementary materials). The low marsh is dominated by the occurrence of *Spartina maritima*, *Salicornia salicornia* and *Aster tripolium*. The mid marsh is covered with *Salicornia salicornia*, *Halimione portulacoides*, *Suaeda maritima* with sporadic occurrence of *S. maritima* and *A. tripolitum*. Finally the high marsh is principally covered with *H. portulacoides* with rare occurrence of *S. maritima* (**Figure 2.8**).

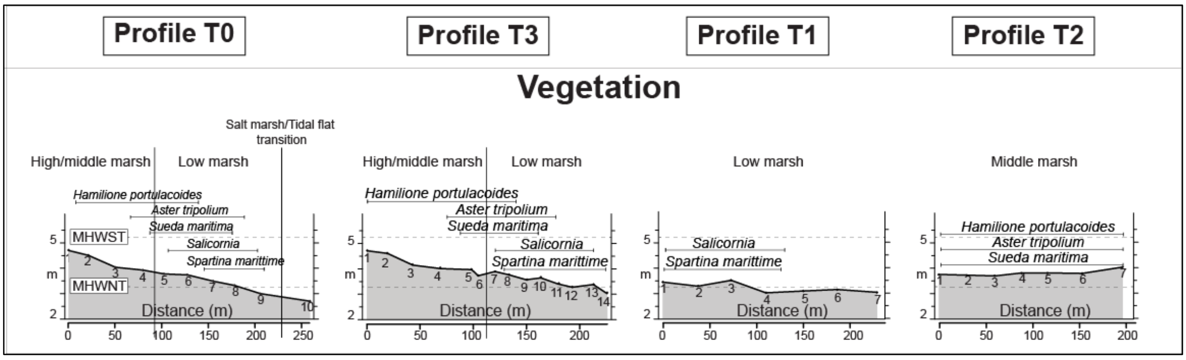


Figure 2-8 Elevation profile and floral distribution across the salt marsh of the Canche Estuary

5.4 Foraminiferal analysis

A total of 40 benthic living species have been found after the identification of ca. 28,000 specimens. Twenty-nine of these (50% of the specimens) are made of hyaline tests, 7 are agglutinated (31% of the specimens) and 4 porcelaneous (19% of the specimens) (**Figure 2.9**). Three stations (T1-1, T1-2 and T1-3) were azoic. Species diversity expressed as Exp(H')$_{bc}$ (**Figure 2.10**), do not show a clear trend within the marsh area; however it increases towards the tidal flat. Exp(H')$_{bc}$ varies from 2 to 10. Faunal density varies between 0.3 and 30 (forams/cm^3) (**Figure 2.11**). The highest densities are located in the high marsh and there is a gradual decrease towards the tidal flat.

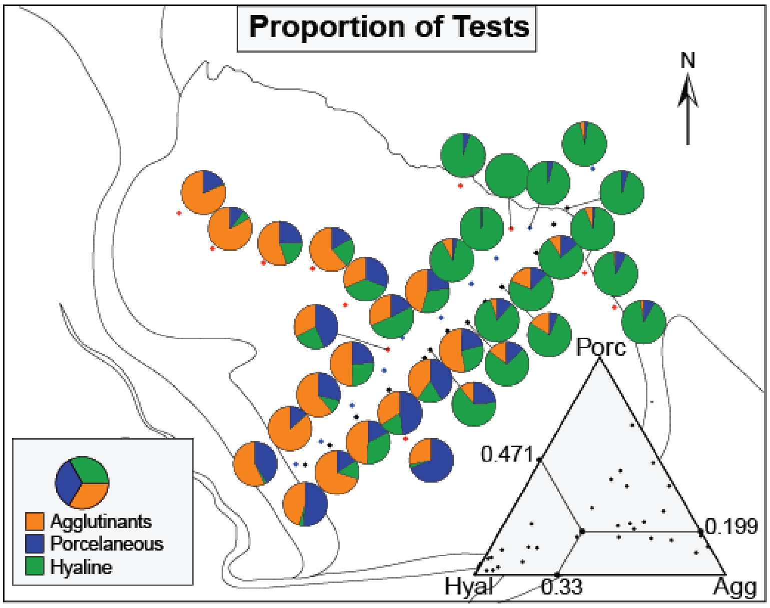


Figure 2-9 Proportion of tests of benthic living foraminiferal in the study area

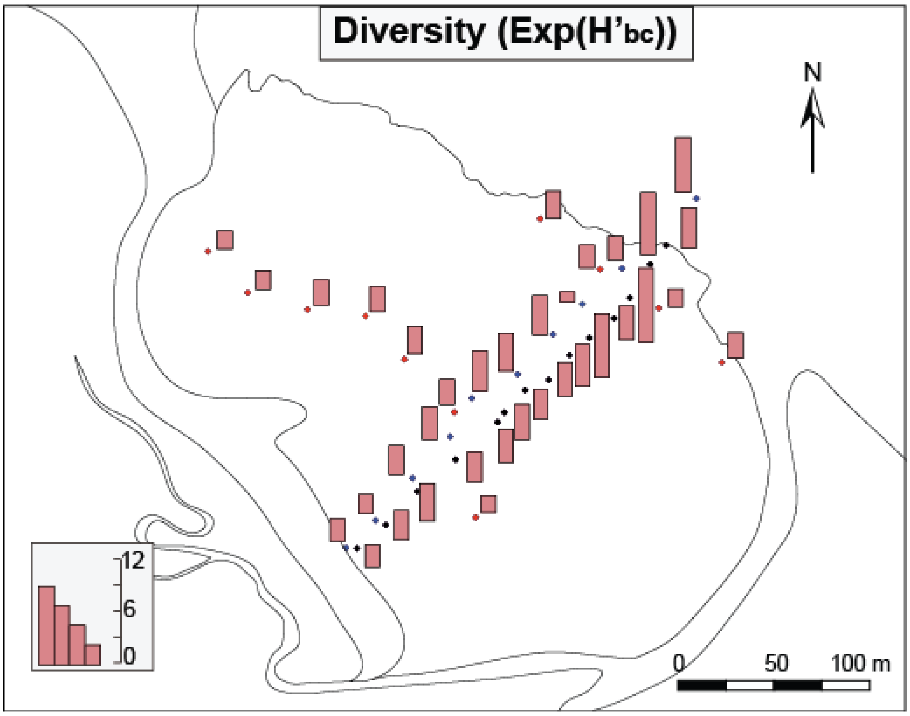


Figure 2-10 Diversity Exp(H')$_{bc}$ and g) of benthic living foraminifera in the study area

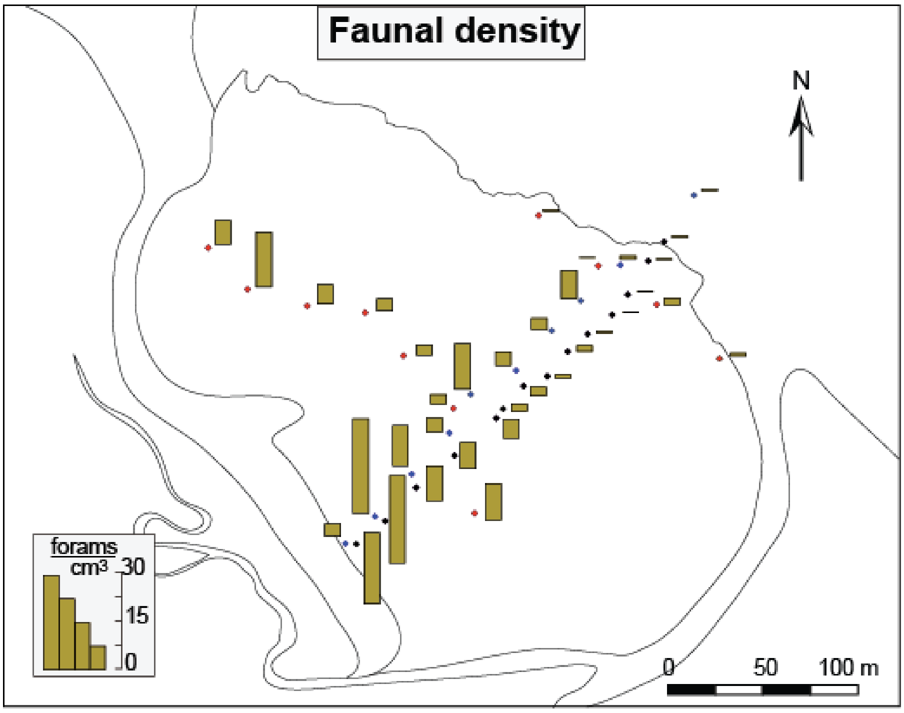


Figure 2-11 Faunal density (specimens/cm^3) of benthic living foraminifera in the study area

The densities of the main species (specimens with relative abundance >5% at least in one sample) varies significantly (one way ANOVA, *p*-Value <0.05) along the cross-shore transects T0 and T3 (supplementary materials 2). For the alongshore transects T1 and T2, the densities varies significantly only for a few species (*Entzia macrescens*, *Quinqueloculina seminula*, *Trochammina inflata, Bolivina variabilis*). Benthic foraminifera show variable patchy distributions within the study area (both low and high values of standard deviations). *Trochammina inflata*, *Quinqueloculina seminula* and *Cribroelphidium williamsoni* show patchier distributions than *Haynesina germanica*, *Entzia macrescens* (low standard deviations) (supplementary materials).

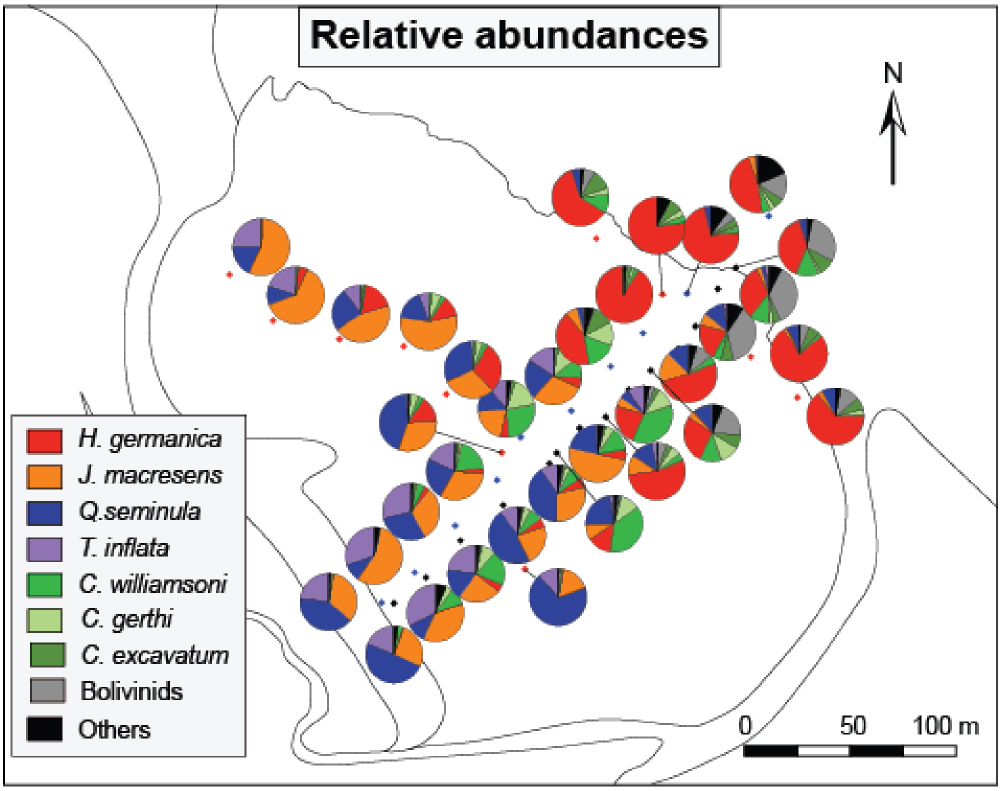


Figure 2-12 Relative abundance of the main benthic living foraminifera in the study area

The assemblages (after pooling replicates) are dominated by *Entzia macrescens*, *Quinqueloculina seminula*, *Trochammina inflata, Haynesina germanica* and *Cribroelphidium williamsoni* (respectively 2.21, 1.52, 1.22, 0.66 and 0.47 specimens/cm^3 on average) (**Figure 2.12**, **Figure 2.13**). Subordinary species (<5% on average on total assemblage) include *Cribroelphidium gerthi*, *Cribroelphidium excavatum, Cribroelphidium margaritaceum, Cornuspira* *involvens* and a few bolivinids (*Bolivina pseudoplicata, Bolivina variabilis* and *Buliminella elegantissima*). The results show two distinctive assemblages: a high/middle marsh assemblage dominated by agglutinated and porcelaneous specimens and a low marsh assemblage by hyaline taxa. Specifically in the high marsh *E. macrescens* (max=16.90), *Q. seminula* (max=11.26) and *T. inflata* (max=9.26) are the most abundant taxa associated to *Cornuspira involvens*. *Cribroelphidium williamsoni* (max=3.89), associated to *C. gerthi*, peaks in the transition middle/low marsh; they do not show a clear distribution within the marsh area. The low marsh is dominated by *H. germanica* (max=8.20). Bolivinids, *Cribroelphidium excavatum* and *Cribroelphidium margaritaceum* are the less abundant (max values <1); their absolute abundance increases towards the tidal flat. Note that the trend of the main species along T1 is constant, whereas it is more variable along T2.

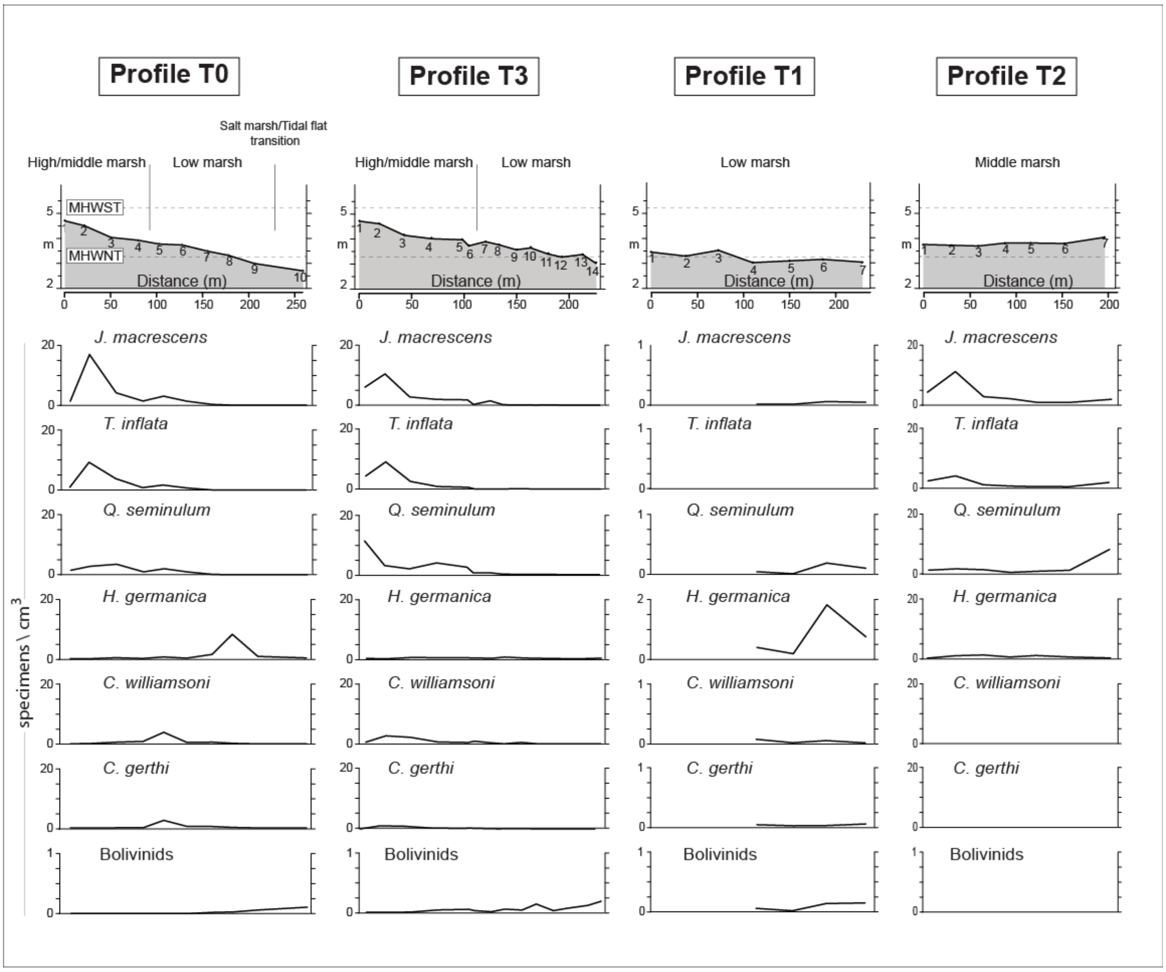


Figure 2-13 Trends of principal species (absolute abundances) across salt marsh profiles

5.5 Environmental constraints on foraminifera

In the correlation matrix (**Figure 2.14**) *Entzia macrescens*, *Quinqueloculina seminula*, *Trochammina inflata*, and *Cornuspira involvens* display a significant (*p*-Values <0.05) positive correlation to the elevation (respectively 0.73, 0.70, 0.71 strong correlation; 0.52 and 0.41 feeble correlation), *Bolivina variabilis, Buliminella elegantissima, Bolivina pseudoplicata,* and *Cribroelphidium margaritaceum* show significant negative correlation to the elevation (respectively 0.64, -0.63 strong correlation; -0.54, -0.49, feeble correlation). *Entzia macrescens*, *Quinqueloculina seminula*, and *Trocammina inflata* have a significant feeble positive correlation to TOC (0.6< Pearson's coefficient <0.4). Bolivinids have a significant feeble negative correlation (-0.6< Pearson's coefficient <-0.4) with the TOC. Only *Cribroelphidium margaritaceum* shows a significant strong positive correlation (0.7) to sand and a significant strong negative correlation to sortable silt (-0.74). Only *C. involvens*, *C. williamsoni* and *T. inflata* have significant feeble correlation to sulphur.

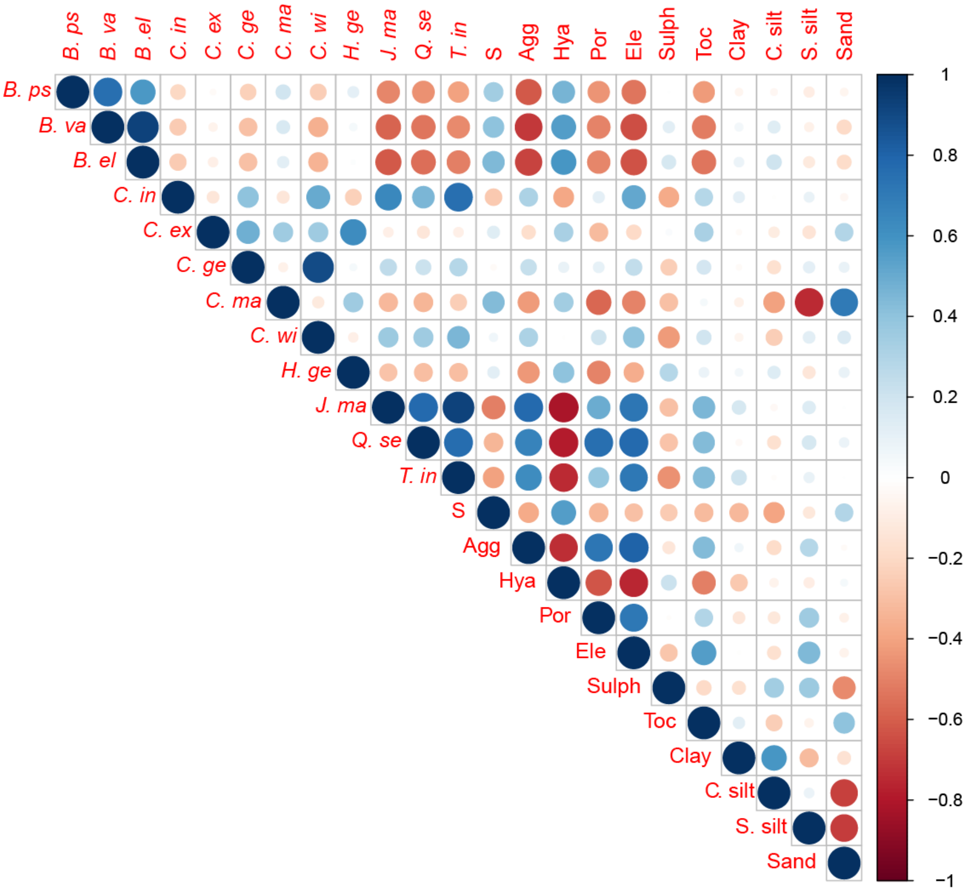


Figure 2-14 Correlation matrix for species absolute abundances and environmental parameters (correlation are given as colour: blue are positive and red negative). The p-values for significance are given as supplementary material.

In the MRT the 33 stations were split in 3 clusters (**Figure 2.15**). The elevation (Ele) is the only discriminant variable (threshold value) at each node. The clusters are structured, from right to left, along an elevation gradient. In the first node for Z=3.675m the sampling stations were split into 2 groups: 20 sites for Z≥3.68 and 13 for Z<3.68. They correspond to the sampling stations situated in the high/middle and low tidal marsh respectively (except for T3-6). At the final partition, sampling sites were split as follows: cluster 1 ≥4.05m (6 sites), 4.05m< cluster 2 ≤3.68m (14 sites), cluster 3 <3.68m (13 sites).

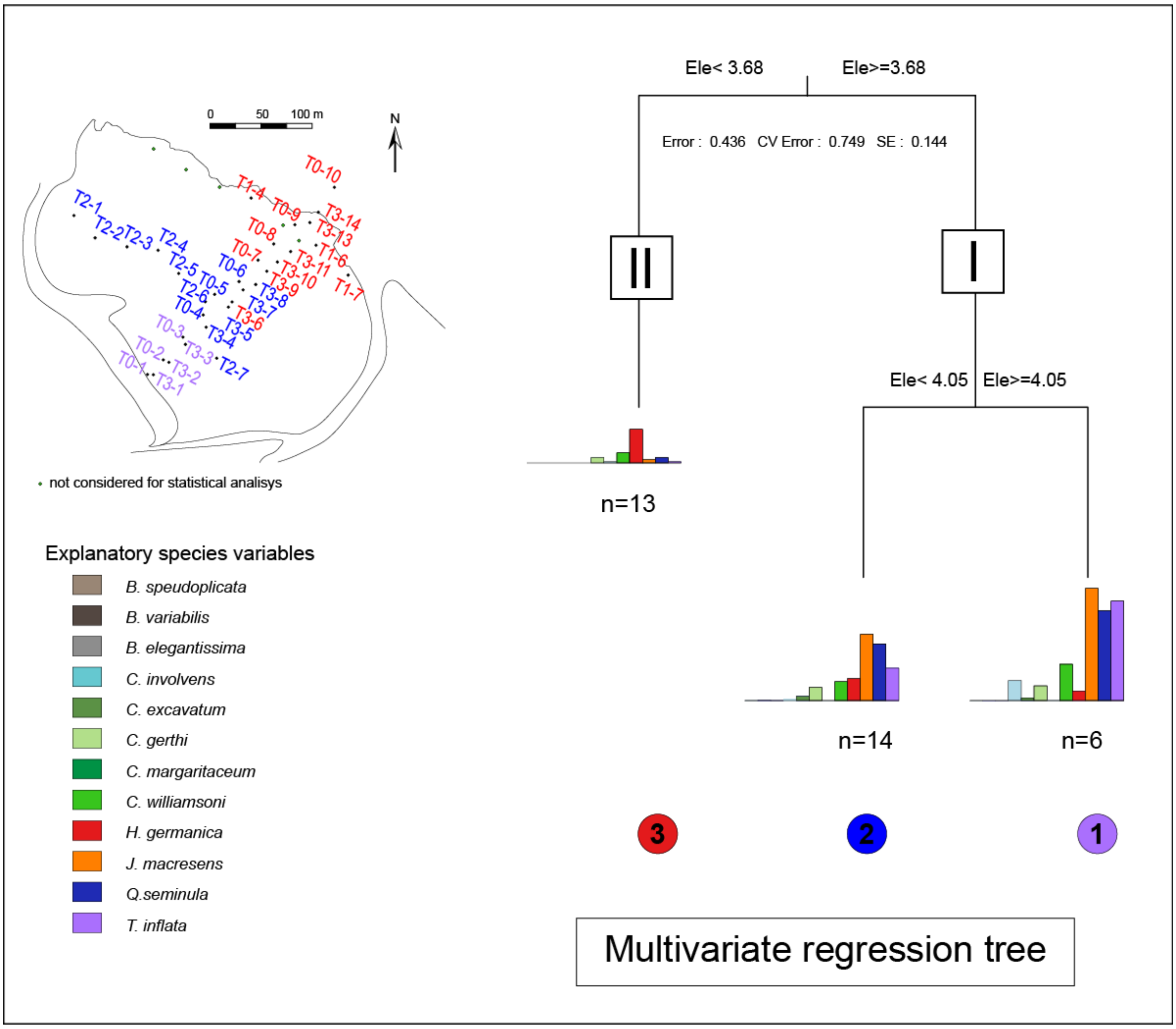


__Figure 2-15 Multivariate regression tree. At each node threshold values are indicated. The bar plots represent the species abundance at each leaf (cluster). In top-left sampling station positions within the study area coloured accordingly to cluster reference.__

The results of the IndVal analysis show that 8 species (on 12) have significant indicator values (IndVal) (Table **2-2**). Of these, six show a high IndVal (>0.6). *Cornuspira involvens*, *E.*

macrescens and *T. inflata* are good indicator species of the cluster 1 which groups the sites at the highest elevation in the middle/high salt marsh. Bolivinids are good indicator species of the cluster 3 which groups sites in the low salt marsh and tidal flat. None of them has a significant IndVal of the cluster 2.

Species	Cluster	IndVal	p-value
C. involvens	1	0.7703	0.001
T. inflata	1	0.7471	0.001
E. macrescens	1	0.6165	0.001
Q. seminula	1	0.5914	0.004
C. williamsoni	1	0.5583	0.059
C. gerthi	2	0.3865	0.721
B. variabilis	3	0.8739	0.001
B. elegantissima	3	0.8051	0.001
B. pseudoplicata	3	0.7599	0.002
C. margaritaceum	3	0.4612	0.017
H. germanica	3	0.5128	0.069
C. excavatum	3	0.7703	0.429

Table 2-2 Indicator values (IndVal) and p-value for the main species

Tidal elevation (related to IGN69) versus inundation time (%) (or subaerial exposure) is plotted in **Figure 2.16**. All curves show the same trend. There is a shift in altitude amplitudes of water level that is more marked during the period before T0 sampling (2012). In these considered intervals, the marsh area (above 3 m) is flooded by sea water from 0% to 31% of time. Species living at a higher elevation range have higher values of tidal exposure. This is the case of *Cornuspira involvens* which can undergo more than 90% (on average) of time under sub-areal conditions (**Table 2-3**). In **Figure 2.16** we took *T. inflata* as an example to underline how there are changes in the subaerial exposure considering different intervals and different sampling periods.

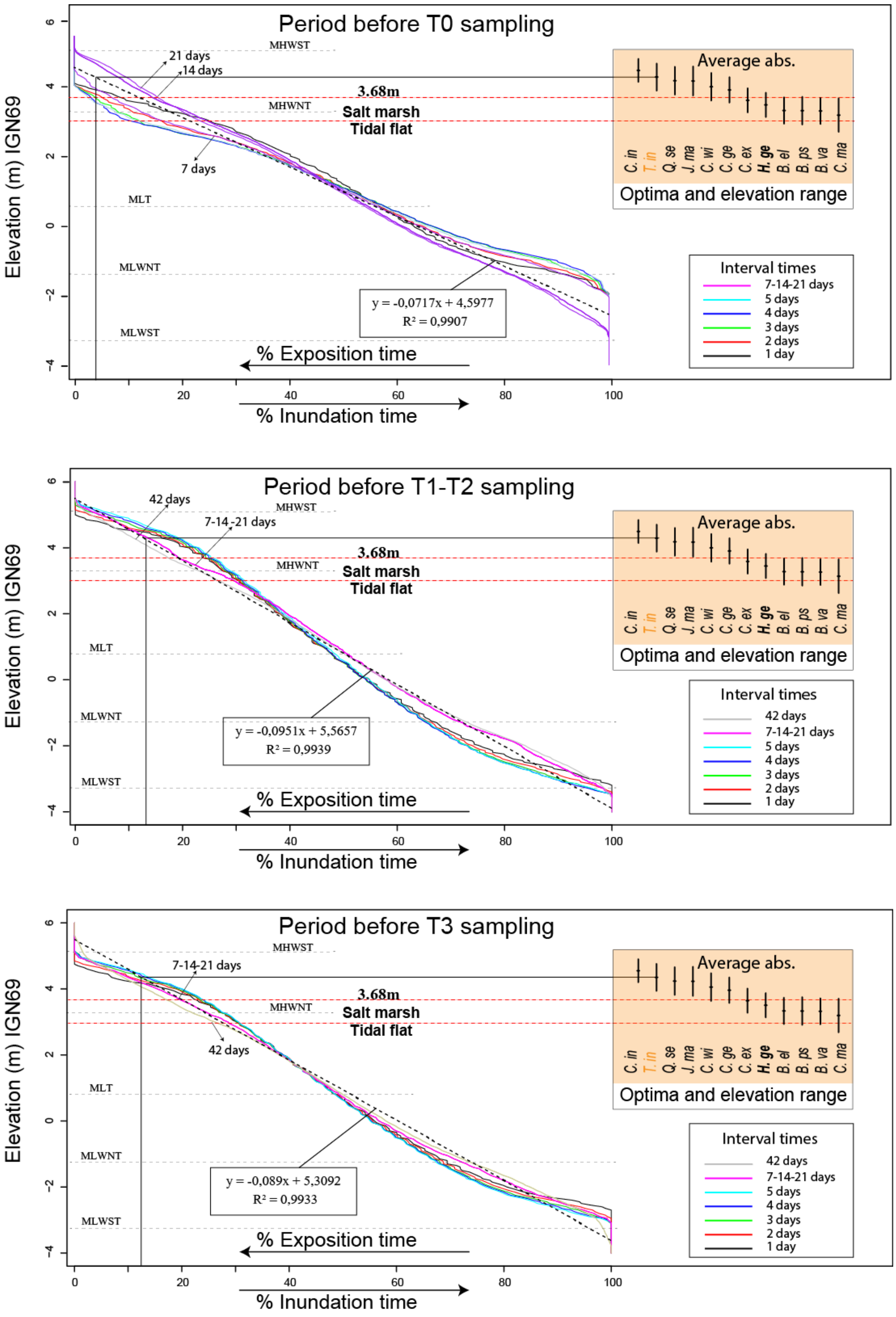


Figure 2-16 Elevation vs Inundation time (% of time whose station are covered by sea water) for each sampling compaing (T0, T1-T2 and T3). Tide variations were estimated from the Boulogne-sur-Mer Marel buoy that measures the elevation every 10 minutes. Curves are calculated for 1, 2, 3, 4, 7, 14, 21 and 42 (when data are available) days before the sampling date. The mean curve is indicated as regression line. Species elevation distribution for inundation time tolerance calculation are indicated. Species elevation distribution are based on absolute abundances of the main species. To show the differences between the variations in subaerial exposition times during different sampling periods we picked up T. inflata as example.

	2012		2013		2014		Average	
	Optima	Exposure	Optima	Exposure	Optima	Exposure	Optima	Exposure
C. involens	4.48	98.32	4.48	88.55	4.48	88.55	4.48	91.81
T. Inflata	4.29	95.68	4.29	86.57	4.29	86.57	4.29	89.61
Q. seminula	4.18	94.23	4.18	85.47	4.18	85.47	4.18	88.39
J. macrescens	4.18	94.11	4.18	85.38	4.18	85.38	4.18	88.29
C. williamsoni	4.01	91.84	4.01	83.67	4.01	83.67	4.01	86.39
C. gerthi	3.92	90.58	3.92	82.72	3.92	82.72	3.92	85.34
C. excavatum	3.62	86.43	3.62	79.59	3.62	79.59	3.62	81.87
H. germanica	3.50	84.64	3.50	78.24	3.50	78.24	3.50	80.37
B. elegantissima	3.34	82.40	3.34	76.55	3.34	76.55	3.34	78.50
B. pseudoplicata	3.33	82.33	3.33	76.50	3.33	76.50	3.33	78.45
B. variabilis	3.32	82.23	3.32	76.42	3.32	76.42	3.32	78.36
C. margaritaceum	3.20	80.57	3.20	75.17	3.20	75.17	3.20	76.97

Table 2-3 Subaerial tidal exposures (% of time not covered by sea water) of the main species ordered by tolerance to sub-areal conditions. Tolerance increases upward.

6 Discussions

6.1 Foraminiferal distribution and ecology

The living assemblage (hyaline 50%, agglutinated 31% and porcelaneous 19%) belongs to normal marine marshes (Murray, 1991; Fajemila et al., 2015). There is a rapid landward increase in agglutinated taxa replacing calcareous specimens, worldwide ascertained (e.g. Scott and Medioli, 1978; Scott and Medioli, 1980). In the Canche salt marsh the diversity and the faunal density are classically low like already observed in other restricted marginal environments (Murray, 2006). The dominant species *Entzia macrescens*, *Trochammina inflata*, *Quinqueloculina seminula*, *Haynesina germanica* and *Cribroelphidium williamsoni* are typical from assemblages in macrotidal marsh areas (Horton, 1999; Horton and Murray, 2007). Two main foraminiferal zones can be identified along the vertical tidal frame: i) zone I in the higher part of the salt marsh and ii) zone II in the lower one (synthesis in Fig. 8). In the middle-high part of the marsh area, the indicator species are *Trochammina inflata*, *Entzia macrescens* and *Cornuspira involvens*. The occurrence of the first two taxa in vegetated marsh is known worldwide and their ecology has been abundantly investigated (for a review look at Sen Gupta, 1999). *Cornuspira involvens* is an epifaunal species, widespread in marginal marine and shelf environments (Murray, 2006). Note that it is not an occasional taxon because, even though in low abundance, it occurs both in 2012 and 2014. In the Canche marsh, *C. involvens* shows the highest tolerance to extreme sub-aerial conditions. As observed in the Severn Estuary (Haslett et al., 1997) or in the Tees Estuary (Horton et al., 1999), *E. macrescens* and *T. inflata* occur close to the MHWST (Fig. 8). The presence of these two taxa as indicator species of the high vegetated marsh is characteristic in northern Europe region, from microtidal to macrotidal regimes (Murray, 1991). In the middle marsh *Quinqueloculina seminula* and *E. macrescens* are the most abundant. However they do not constitute good indicator species of this tidal portion. *Q. seminula* is an infaunal species (Severin, 1987) widespread in shallow environments (Debenay and Guillou, 2002). It classically dominates in the lower tidal marsh towards the tidal flat (Alve and Murray, 1999; Swallow, 2000), often related to muddy sediment (Lee et al., 1969; Müller-Navarra et al., 2016) (note that in most of the studies *Quinqueloculina* ssp. is indicated). However Franceschini (2005) reported the same unusual occurrence of *Quinqueloculina* ssp. in the higher part of the marsh area. Anomalously high abundances of the same species were as well observed at upper elevations in the Adriatic coast of Croatia (Shaw et al., 2016). The authors explained it by strong, regional winds enabling miliolid shells to occur in areas where agglutinated taxa typically dominate. However in macrotidal estuaries *Quinqueloculina* ssp. seems to be able to span from high tidal marsh to the low flat (Horton and Murray, 2007). The transition between the middle marsh and the low marsh (at 3.68m) is marked by a changing in vegetation, a decreasing of faunal densities and a rapid increasing of hyaline taxa. *Halimione portulacoides* dominant in the high/middle marsh is replaced by salicornia. The same distribution along the elevation gradient was been observed for these two macrophyte in Jennings (1992). The most abundant and frequent species is *Haynesina germanica*. It is an infaunal species (Bouchet et al., 2009; Seuront and Bouchet, 2015) and one of the most abundant hyaline taxon in all marginal environments (Murray, 2006; Bouchet et al., 2007; Francescangeli et al., 2016). However this large dominance of *H. germanica* in the lower vegetated marsh seems to be principally peculiar of North Europe tidal marshes (Horton et al., 1999; Swallow, 2000; Haslett et al., 2001). Despite this it does not statistically represent a good indicator species of the low marsh, showing a low correlation to the elevation. This is in accordance with the literature indicating *H. germanica* able to span in wide tidal range (Debenay and Guillou, 2002; Debenay et al., 2006; Armynot du Châtelet et al., 2016). Bolivinids are infaunal specimens, generally more abundant in open shelf environments than marginal ones (Murray, 2006). Their anomalous presence in the low marsh (especially along T3) could suggest the occurrence of low oxygen condition down layer, leading the moving up of these infaunal species (Jorissen et al., 1995b; Fontanier et al., 2002). Note that in other macrotidal estuaries at the same tidal level, different dominant taxa such as *Ammonia tepida* or *Miliammina fusca* are occurring (Haslett et al., 1997; Horton and Murray, 2006) (**Figure 2.17**). Ammonia group is generally composed by epifaunal species, widespread in marginal marine environments worldwide (Seuront and Bouchet, 2015); some taxa (such *Ammonia tepida* and *Ammonia beccarii*) can be dominant in low salt marsh areas (Swallow, 2000); *Miliammina fusca* is an euryhaline species commonly dominant in the lowest vegetated marsh elsewhere (Alve and Murray, 1999; Sen Gupta, 1999). Here in the Canche salt marsh *H. germanica* completely replaces these aforementioned taxa. It is interesting to note that Armynot du Châtelet (2009a) in the same estuary, but upstream, found *H. germanica* to be dominant in the tidal flat. Hence contrarily observed in high/middle salt marsh (zone I), in the low salt marsh (zone II, close to the MHWNT) foraminiferal species dominance seems to exhibit a higher variability. *Cribroelphidium williamsoni*, *Cribroelphidium gerthi* and *Cribroelphidium excavatum* have as well no correlation with the elevation. This could be liked the presence of a small tidal channel close to the sample that may locally increase the water dynamics. This distribution of these species linked to the presence of tidal creeks is in accordance with several studies in which the occurrence of *C. williamsoni* (Alve and Murray, 1999; Horton and Murray, 2007) and *C. excavatum* (Hayward et al., 1999) has been documented in salt marsh areas, sometimes in pounds or tidal channels (Debenay et al., 2000; Armynot du Châtelet et al., 2009a; Müller-Navarra et al., 2016). Bolivinids are the only indicator species of the lower part of the tidal marsh. *Cribroelphidium margaritaceum* exhibits the lowest tolerance to subaerial conditions being in fact a common species in the tidal flats towards more subtidal conditions (Armynot du Châtelet et al., 2011).

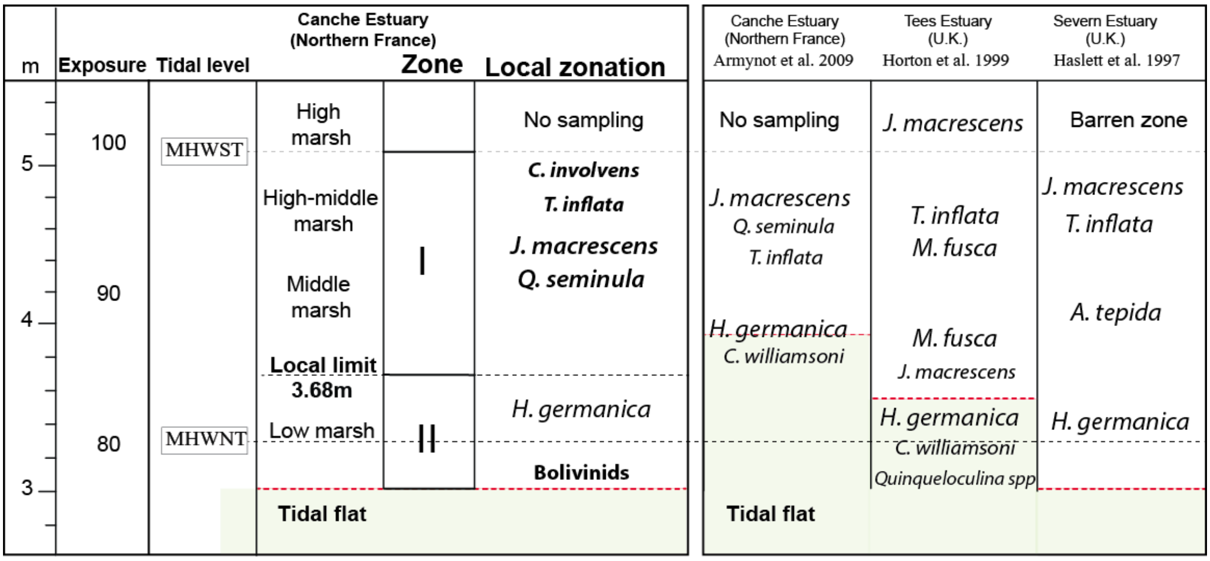


Figure 2-17 Foraminifera zonation in the Canche Tidal marsh and indicative comparison based on tidal frame about other studies in macrotidal estuaries in Europe, namely Canche Estuary (Northern France) (Armynot du Châtelet et al., 2009a), Tees Estuary (U.K.) (Horton et al., 1999) and Severn Estuary (U.K.) (Haslett et al., 1997). The colored area represents the tidal flat. In bold indicator tidal level species in the present paper.

6.2 Implication for sea level reconstruction

Foraminiferal living assemblages in the Canche tidal marsh are vertically constrained to the tidal frame. This suggests that foraminiferal species distribution is linked to elevation (Berkeley et al., 2007). Other parameters measured namely the TOC, sulphur contents and grain-size, do not primarily influence benthic foraminiferal distribution. Like the Bay of Fundy (Canada), which has the highest tides in the world, the Canche Estuary is a hyper-tidal environment, where tidal range has a significant impact on any organisms inhabiting the intertidal zone (Patterson et al., 2004). In this study, the specific tolerances to tidal exposure have been estimate for the main foraminiferal species. Variations in tidal elevation due to tidal cycle changes, correspond to different exposition times the area experienced. However no matter the tidal range the vertical zonation of benthic foraminifera is not altered. As a consequence at this temporal scale, the exposure time is not a critical threshold able to explain the transformation from zone I to zone II and vice versa. In such overstressed environment benthic foraminifera have developed a high tolerance to subaerial conditions. Anyhow it could be interesting, in further studies in other estuaries with various tide amplitude, to evaluate the specific tolerance of tidal exposure of the main species. By comparing world estuaries, it would be easier to use species distribution along subaerial tidal exposure gradient whether absolute altitude distribution. It is difficult to compare foraminiferal vertical zones when the reference to tide level is missing.

Recently tidal marsh foraminifera have been increasingly used for high-resolution sea level reconstructions (Edwards and Horton, 2000; Leorri et al., 2008b; Wright et al., 2011; Barnett et al., 2016). Given that the distribution of salt-marsh foraminifera is strongly influenced by surface elevation, foraminifera provide a suitable means of converting faunal data into environmental (i.e. elevation) (Horton and Edwards, 2006). In the Canche marsh we observed accordingly, that most of the foraminiferal variations are accounted by the tidal frame gradient. However if we consider the foraminiferal distribution along T2 there is a lateral variation of the indicator zonal species (*E. macrescens*, *Q. seminula* and *T. inflata*) as if they would follow an environmental gradient, which is not the elevation. Note that T2 is an alongshore transect and was sampled to minimize the tidal effect following a fixed elevation value. As already mentioned grain-size, TOC and Sulphur, scarcely influence the foraminiferal distribution in the Canche tidal marsh. This shows that assemblage variation along T2 may be driven by environmental variables that were not measured in the field (such as salinity, temperature, Chlorophyll *a*, etc.). Considering the results of Armynot du Châtelet (2009a), there is a migration upstream of the altitude of the transition salt marsh/tidal flat. This implies a shortening of zone I, and a seaward displacement of calcareous species. These observations point out that the vertical range of benthic living foraminiferal could vary within the same area. As a consequence it seems opportune to wonder how many transects or where in the estuary we should sample to have the best analogue of modern environment. The living fauna could be affected by monthly or seasonal variation (Camacho et al., 2015; Milker et al., 2015a) and may not be, as suggest in other papers (e.g. Horton and Murray, 2006), the best proxy to reconstruct past sea level change, especially in hyper-tidal environments. Foraminiferal distribution is an answer of a complex model whom elevation, in our case, is only one of the factors (Degré et al., 2006).

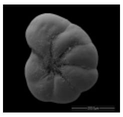
One of the limits of this study is the lack of measured environmental parameters (see aforementioned examples). Several studies underlined, for example, the primary role of the salinity on controlling the makeup of marsh foraminifera (Patterson, 1990; de Rijk and Troelstra, 1997; Fatela et al., 2007). The variations of *Q. seminula* could hypothetically suggest the occurrence of a sort of alongshore pore-water salinity gradient. Generally porcelaneous species can be evidence of slightly hypersaline, restricted environments (Murray, 1991). However the authors believe that instantaneous measurements of parameters, such as salinity, pH or water temperature, could be misleading in such high variable environments. As stated in Patterson (2004) these parameters vary considerably through the tidal cycle so no attempt is generally made to measure it. It might be more appropriate to carry out weekly or monthly survey to evaluate the impact of these environmental factors on the foraminiferal distribution (de Rijk, 1995; Fatela et al., 2016), but increasing costs and periods for implementing.

7 Conclusions

In the hyper tidal salt marsh of the Canche Estuary two main foraminiferal zones can be identified along the vertical tidal frame: i) zone I in the higher part of the salt marsh, dominated by agglutinated and porcelaneous taxa and ii) zone II in the lower marsh characterized by lower densities and hyaline specimens.

Subaerial tidal exposure, better than elevation, is an important parameter to be considered in salt marshes, which constraints the foraminiferal vertical zonation in accordance with the tidal frame. Anyhow at this temporal scale, it does not constitute a threshold parameter able by itself to explain all the faunal variations in the Canche Estuary.

By comparing world estuaries and finally use of foraminifera as sea level indicators, it would be easier to use species distribution along tidal exposure whether absolute altitude distribution. It could be that the salt-marsh benthic foraminifera, constrained by many parameters in these transitional environments, would not be as so good indicators for monitoring sea level changes as we could write.



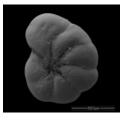
Chapter 3 Spatial and seasonal variability of benthic living foraminifera along intertidal areas of Hauts-de-France region (Northern France) under gradient of environmental stress

1 Introduction

The Hauts-de-France region is an area bordered on the north by the English Channel and the North Sea, on the northeast by Belgium, and on the south by Picardy. The northern France littoral extends along two segments, which are almost perpendicular. From the end of the XIXth century, in the northern part prominent commercial and industrial activities developed along the coastal areas (from Boulogne-sur-Mer and Dunking). In the southern part of the region (Bay of Somme, Bay of Authie, Canche estuary), the human pressure is less present, in fact there are many touristic attractions, natural reserves and protected areas (some of them being under the protection of the "Conservatoire du Littoral"). Benthic foraminifera from the Hauts-de-France region have been poorly studied. After a general work on benthic foraminiferal communities from the English Channel (Rosset-Moulinier, 1986), only two works focused on intertidal areas in Hauts-de-France region, namely Armynot du Châtelet (2009a) in the Canche estuary and Armynot du Châtelet (2011) in the harbour of Boulogne-sur-Mer. In the first study agglutinants species (*Entzia macrescens* and *Trochammina inflata*) dominated the salt marsh area while calcareous taxa (Haynesina *germanica* and *Cribroelphidium williamsoni*) dominated the upper tidal flat. In the harbour of Boulogne-sur-Mer, *H. germanica* and *Bolivina pseudoplicata* were tolerant to heavy metals pollution and to high organic carbon contents. *Cribroelphidium excatum* and *C. magellanicum* were found tolerant to certain threshold of heave metals concentrations. Actually Seuront and Bouchet (2015) investigated in laboratory the motion behaviour of *H. germanica* and *C. excavatum*, collected from the Authie bay in the southern part of the region.

Benthic intertidal foraminiferal ecology have been extensively investigated in natural habitats: salt marshes (Scott and Medioli, 1978; de Rijk, 1995; Horton, 1999; Berkeley et al., 2007) and tidal flats (Alve and Murray, 1999; Debenay et al., 2000; Murray and Alve, 2000; Alve and Murray, 2001; Debenay and Guillou, 2002); polluted areas (Alve, 1995; Armynot du Châtelet et al., 2004; Bouchet et al., 2007; Brunner et al., 2013) and in laboratory experiments (Denoyelle et al., 2012; Frontalini et al., 2015; Seuront and Bouchet, 2015). In temperate meso- to macro-tidal environments Debenay (2002) in a review paper, pointed out how *Cribroelphium excavatum* and *Ammonia beccarii* are dominant species in the lower part of the tidal flat, while *Haynesina germanica* and *A. tepida* dominate the upper part. The distribution of intertidal foraminifera is in most of the cases driven by the tidal gradient (Horton and Murray, 2007). However several other parameters can control the faunal distributions. For instance de Rijk (1995) observed a strong influence of the salinity in driving benthic foraminifera distributions, finding *Haplophragmoides manilaensi* as an important low salinity indicator. In tidal mudflats of the macrotidal Marennes-Oleron Bay (South western France) *A. tepida* was found to be the most tolerant taxa to temperature increase and hypoxic conditions whereas other species (*Brizalina variabilis* and *H. germanica)* were sensitive to organic degradation and hypoxia (Bouchet et al., 2007). Along the Skagerrak–Kattegat coast (eastern North Sea) *Entzia macrescens* was observed living epiphytically on decaying Carex leaf debris (Alve and Murray, 1999), testifying an influence of vegetation on driving the distribution of intertidal foraminifera. In the same areas the authors found the euryhaline species *Elphidium williamsoni* and *Miliammina fusca* common only in sediments with a mud content less than about 60%. In *Ammonia tepida* was observed to be typically favoured by an increase food resource, reflected by total organic carbon (Armynot du Châtelet et al., 2009b) and Chl *a* (Burone et al., 2007)

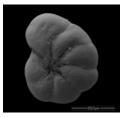
Temporal variations in foraminifera have been observed in many different environments from transitional to deep see zones (Gustafsson and Nordberg, 2000; Kawahata et al., 2002; Fontanier et al., 2003). In intertidal areas several temporal studies have been conducted from weeks (Boltovskoy and Lena, 1969) and months (Lutze, 1968; Murray and Alve, 2000; Debenay et al., 2006; Duchemin et al., 2008) over survey of more than a year (Swallow, 2000; Fatela et al., 2014). Fatela (2014) during an intra-decal study in the Caminha tidal marshes (North-western Portugal) observed a shift between brackish species (such as *Haplophragmoides manilaensis*, *H. wilberti, Trochamminita salsa/irregularis*) to *Entzia macrescens* and *Trochammina inflata*, as the result of 5 consecutive dry years and a corresponding salinity rise in sediment pore water. In the lower Guadiana estuary (South-eastern Portugal) during



winter, when fluvial discharge peaks, agglutinated species represented more than 80% of the total individuals (Camacho et al., 2015). In the same area, during summer, when marine conditions prevail, calcareous species became more dominant, their densities increase and they expand to higher marsh zones and estuarine upper reaches. Like other microorganisms, such as dinoflagellates (Fitt et al., 2000) or diatoms (Wang et al., 2015), the density and diversity of intertidal foraminifera can vary seasonally reflecting changes in biotic and abiotic environmental variables (such temperature, food supply, salinity) (Murray and Alve, 2000; Wilson and Dawe, 2006; Milker et al., 2015b).

Several works indicate that foraminifera can have one or more reproduction periods during the year (for a review look at Murray, 1991). For instance in intertidal areas of Puerto Desedao (Argentina) Boltovskoy (1964) observed that *Elphidium macellum* reproduced once a year (one peak par year of maxima abundance). On the contrary Murray (1983) reported that *Nonion depressulum* can even reproduce 8-9 times per year. Some studies documented seasonal reproduction cycles, mostly in spring and autumn (Saad and Wade, 2016). Accordingly Bouchet (2007) found a cyclic peak in the density of *C. excavatum* in Others showed that foraminiferal assemblages have peaks in maxima abundance which are not always controlled by a clear annual cyclicity (Murray and Alve, 2000; Morvan et al., 2006). However there are differences between years and seasons, and that the seasonality present in one year may not be present in another (Buzas et al., 2002).

Small-scale spatial distributions show the spatial arrangement among individuals within a habitat (Buzas et al., 2015). In marine soft sediments, benthic organisms often exhibit a patchy distribution (Morrisey et al., 1992; Seuront and Spilmont, 2002). In intertidal areas the sediment heterogeneity, e.g. food is not evenly distributed, leads to patchiness in the distribution of the meiobenthos, and particularly of foraminiferal assemblages, which complicates their observation (Boltovskoy and Lena, 1969; Debenay et al., 2015) Several studies investigated the small-scale spatial variability of living benthic foraminifera finding significant variations in foraminiferal assemblages (Buzas, 1970; Murray and Alve, 2000; Swallow, 2000, Hohenegger, 1989 #650). On the contrary, others documented a homogeneous population of benthic living foraminifera (Bouchet et al., 2007; Milker et al., 2015b). Sampling strategy has therefore to be adapted to make sure that results are well representative of the foraminiferal communities. Hence, a single sample may not be



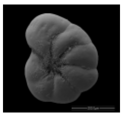
adequate to correctly investigate such patchy assemblages. One could wonder which would be the adequate number of replicates to represent all the variability in these environments. In foraminiferal studies two or three is the number of replicates recommended (Murray and Alve, 2000; Bouchet et al., 2012; Schönfeld et al., 2012). However this number would not be sufficient considering the large variability and adequate sampling design that would require a considerable number of samples (Spilmont et al., 2011)

Considering the poor knowledge on benthic foraminiferal communities in the Hauts de France area, the first objective of the present work is to describe benthic foraminiferal communities across five sites characteristics of the different type of intertidal environment occurring there. To explain the distribution of the different species, several environmental parameters were investigated: grain-size, total organic carbon, total sulphur, total nitrogen, major and trace elements. The second objective is to characterise the seasonal variations in abundance and diversity of benthic foraminifera over a one year survey. The third objective is to assess the small-scale (centimetre) foraminiferal distribution, to evaluate whether it is heterogeneous in the intertidal areas of the Hauts de France.

2 Materials and methods

2.1 Sampling strategy

Sixteen sampling stations were selected from five sampling areas along the intertidal zones of Hauts-de-France region (**Figure 3.1**): from the most disturbed Aa estuary, in the embarked area of Grand Fort-Philippe (with 3 stations) and Liane estuary in the harbour of Boulogne-sur-Mer (with 3 stations), to the less impacted Canche estuary (with 3 stations), and the natural areas of the Authie and Somme estuaries (with 3 and 4 stations respectively). At each of the four season (on April 2014, on July 2014, on October 2014, and on February 2015), four replicates were sampled at each station (the uppermost 1 cm in a surface of 1 m^2). Three replicates were used for the foraminiferal analysis and the fourth was used to measure sediment properties (grain-size, total organic carbon, C/N ratio, total sulphur). Only for the spring survey the major and trace elements concentrations were measured. In total 256 samples (192 for foraminifera and 64 for sediment properties) were collected at low tide. The 3 replicates for faunal analysis were stored in transparent



graduated containers with ethanol and rose Bengal (2g/L) (**Figure 3.2**). Sampling station positions and elevations were measured using a GPS Trimble GeoXT allowing a precision better than 1 m. We used the French Spatial Reference System Lambert 93 to position the sampling stations. Elevations were referenced to the mean sea level (MSL) (**Table 3-1**).

Stations	Elevation	East-X	North-Y	Site description	Human influence
FP1	-0.51	584091	367183	Tidal flat	**Antropogenized**
FP2	-1.10	583362	367802	Tidal flat	
FP3	1.74	583629	367581	Upper tidal flat	
BL1	-0.51	547945	336396	Harbour	**Antropogenized**
BL2	1.15	547408	336708	Harbour	
BL3	-1.15	546159	335739	Harbour	
CA1	3.09	547697	315306	Upper tidal flat	**Slightly impacted**
CA2	2.73	547740	315310	Upper tidal flat	
CA3	1.87	547715	315372	Tidal flat	
AU1	3.49	547262	297412	Upper tidal flat	**Pristine**
AU2	2.85	547319	297291	Tidal flat	
AU3	3.17	547368	297240	Tidal flat	
SO1	1.96	544874	279694	Tidal flat	**Pristine**
SO2	1.39	544816	279633	Tidal flat	
SO3	3.25	545068	279873	Upper tidal flat	
SO4	1.88	545114	279833	Tidal flat	

Table 3-1 Elevation (mean sea level), geographic coordinates (Lambert 93), station description of the 16 sampling stations within the 5 samplings sites. AU refers to bay of Authie, BL to Boulogne-sur-Mer, CA to the Canche estuary, FP to Grand-Fort-Philippe, and SO to the Bay of Somme. Information on regional pollution can be found in Amara et al. (2007), Henry et al. (2004), Berthet et al. (2003), Kerambrun (2012a), Billon (2001).

Figure 3-1 Position of the 16 sampling stations within the 5 selected sampling areas in the Hauts-de-France region: Grand-Fort-Philippe (Aa river), Boulogne-sur-Mer harbour (Liane river), Le Touquet (Canche Estuary), Berck (Authie Estuary), Saint Valery-sur-Somme (Somme Estuary). All the surface samples were collected form intertidal areas.

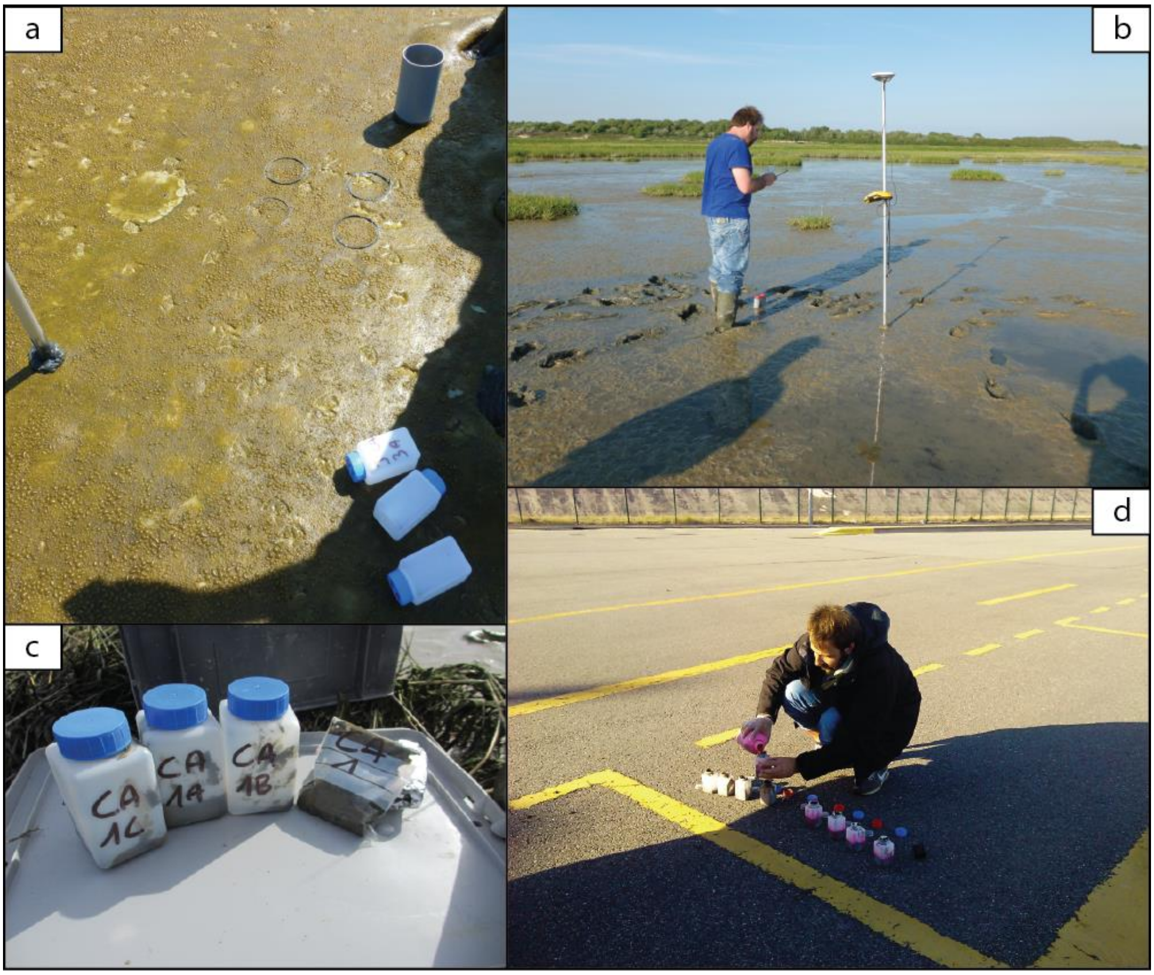
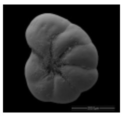


Figure 3-2 Different phases of field sampling: a) selection of the 4 replicates in 1m², b) topographical survey using a GPS Trimble GeoXT, 3) samples storing in graduated containers (foraminiferal samples) and plastic bag (environmental variables) and 4) faunal samples treatment with buffered Rose Bengal dye.

2.2 Sediment analysis

Grain-size analyses were performed using the principle of diffraction and diffusion of a monochromatic laser beam on suspended particles (Malvern Mastersizer 2000, red He-Ne laser). The method is based on near-forward scattering of a laser beam by particles in suspension, as detailed previously (Trentesaux et al., 2001). Measurements can range from 0.02 to 2,000 µm with an obscuration ranging between 10 and 20%. Three grain-size fractions were considered: clays (< 4µm), silt (4-63µm) and sands (63 to 2000µm).

Calcium carbonate content was determined using a Bernard calcimeter. Triplicated measurements were carried out for each sample using 0.5 g of finely crushed dry sediment (Armynot du Châtelet et al., 2009a; Armynot du Châtelet et al., 2016). A Flash EA 1112



Elemental Analyzer (Thermo) equipped with an auto-sampler was used for determining total contents of C, H, N and S. The analysis was performed on 1.5 to 2 mg of sample added to approximately 5mg of vanadium pentoxide, used as a combustion catalyst. 2.5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene (BBOT) was used as standard. The total organic carbon (TOC) was determined by subtracting carbonate carbon from total carbon concentration. The C/N ratios were used to distinguish between algal and land-plant origins of sedimentary organic matter (for detailsMeyers, 1994). Algae typically have atomic C/N ratios between 4 and 10, whereas vascular land plants have C/N ratios of ≥20. This distinction arises from the absence of cellulose in algae and its abundance in vascular plants.

2.3 Major and trace elements concentrations (total mineralization)

The total concentrations of Al, Co, Cr, Cu, Fe, Mn, V and Zn were analysed at each sampling station only for the spring season. After drying the sediment particles at room temperature, the samples were sieved at 63 μm and gently crushed. Then, 200 mg of the fine fraction were attacked first with a mixture of 5 mL of suprapur nitric acid and 10 mL of a concentrated HF solution at ebullition over 48 hours. After evaporation of these acids, 10 mL of a freshly prepared HNO_3/HCl mixture (1/2 v:v) was added in order to eliminate the remaining solid grains. The recovered solutions were subsequently diluted in a known volume of ultrapure water and analysed using ICP-AES (inductively coupled plasma – atomic emission spectroscopy; Varian Vista-PRO, axial view) and ICP-MS (inductively coupled plasma – mass spectroscopy; Thermo Elemental X Series). This attack procedure was validated and the accuracy of the analytical procedure was checked by means of the following sediment standard reference materials (Canadian International Standards): HISS-1, MESS-3 and PACS-2. It was found that standard materials certified and measured results were in good agreement.

In order to assess sediment contamination and to evaluate anthropogenic influences, the enrichment factor (EF) (Woitke et al., 2003; Hasan et al., 2013; Martins et al., 2013; Duan et al., 2014) was calculated for each element, as follows (Eq. 1):

$$EF = ([X_i]/[Al_i])/([\,X_0]/[Al_0]) \qquad \text{(Eq. 1)}$$

Where $[X_i]$ and $[Al_i]$ are respectively the concentrations of the element X and Al in the sample I; $[X_0]$ and $[Al_0]$ are the local geochemical background concentrations of the element X and Al, respectively. Note that in this relation, the enrichment factor is normalized against aluminium to take into account the grain-size influence, especially concerning the fine particles (silts and clays), overcoming partly the bias bound to the nature of the sediment. The calculation of EFs were possible only for elements and sampling areas where reference values were estimated before. We used Francescangeli (2016) for Boulogne-sur-Mer, Francescangeli et al. (submitted) for the Canche estuary and Billon (2001) for Authie estuary. The following criteria, estimated by previous studies (Birth, 2003; Kerambrun et al., 2012a), are used to evaluate the degrees of pollution according to the values of EF: $EF<3$: no relevant enrichment; $3<EF<10$: moderate contamination; $EF>10$ strong contamination.

2.4 Foraminiferal treatment and species identification

The 3 foraminiferal replicates were stained, on the field, with buffered Rose Bengal dye (2g of Rose Bengal in 1000ml of ethyl alcohol) to distinguish living tests (stained) from dead (unstained) at the time of collection (Walton, 1952; Lutze and Altenbach, 1991). The method gives good results for intertidal areas (Goldstein et al., 1995; Armynot du Châtelet et al., 2009a; Milker et al., 2015a). Although benthic foraminifera can live as deep as 30 cm in the sediment (Goldstein et al., 1995; Bouchet et al., 2009) this study was carried out on superficial samples because the highest numbers of living foraminifera are generally found in the surface 0–1 cm layer (Alve and Murray, 2001; Milker et al., 2015b). In the laboratory, wet samples (about 50 cm^3) were gently washed with tap water, through 315 and 63 μm mesh sieves, and dried at 40°C. Foraminiferal tests of the intermediate fraction were concentrated by flotation on trichloroethylene (Horton and Murray, 2006; Semensatto and Dias-Brito, 2007). Although heavy liquid separation should be avoided, in environments with low foraminiferal density due to a high sedimentation rate as in estuarine areas, it may be used (Schönfeld et al., 2012). Only specimens containing dense, brightly red-stained protoplasm were considered as alive (Alve and Murray, 1999; de Stigter et al., 1999). All living specimens were counted and identified, following Loeblich and Tappan (2007) for genera, and Debenay (2012), Debenay et al. (2001) for species classification. The observations were carried out under a binocular microscope, model Olympus SZX16. The total faunal density (FD) (the total number of specimens per cm^3 of sediment) was determined at each sampling station. The effective number of species Exp(H')$_{bc}$ (Bouchet et al., 2012), the most suitable measure of diversity (Beck and Schwanghart, 2010), were considered in the present study. The absolute and the relative abundances of living taxa were as well calculated.

2.5 Data analysis

A one-way ANOVA (two factors: sampling areas=5 and seasons=4) was performed to evidence any significant variations (p-value <0.05) of the environmental variables within the study area.

Standard deviation was calculated on the absolute abundance of the main species as well as homogeneity variance tests (Bartlett test and non-parametric Kruskal-Wallis test) (Bartlett, 1937; Kruskal and Wallis, 1952) (p-value < 0.05) to identify any patchiness effect within the replicates. Due to the low faunal density, the counts for replicates were pooled per station to bypass the critical statistical threshold for reliable interpretation. A one-way ANOVA (two factors: sampling areas=5 and seasons=4) was then performed on pool data to evidence any significant variations (p-value < 0.05) of FD, diversity and the main foraminiferal species within the study area (to test for instance the null hypothesis that seasonal changes did not affect the absolute abundance of benthic foraminifera) (Zar, 1984). Species abundances were square-root transformed to improve the equality of variance and normality.

Canonical Correspondence Analysis (CCA) was used to assess the statistical significance of environmental variables (sediment grain-size, major and trace elements, S, TOC and C/N ratio) on all the spatial and temporal variations of foraminiferal assemblages (ter Braak, 1986; ter Braak and Verdonschot, 1995). Major and trace elements were considered unvaried in one year interval. Species abundances were log-transformed. Canonical analysis is a direct gradient analysis, which allows to perform a direct comparison between species composition and environmental descriptors at the same location (Legendre and Legendre, 2012).

The R software was used for all the calculations, by using the following packages: Base (descriptive statistics, ANOVA and Bartlett test) (Team, 2016), lawstat (Krustal-Wallis) (Gastwirth et al., 2015), entropy (diversity calculation) (Hausser and Strimmer, 2014), vegan (CCA) (Oksanen et al., 2016).

3 Results

3.1 Sediment characteristics

Silt results to be the most abundant fraction in all of the sampling sites (on average 71%) (**Figure 3.3**). It contributes to 26% to 86% of the sediment. Sand is the second most abundant sediment faction (on average 24.5%) (**Figure 3.3**). It varies between 9-71%. Clay is the minor faction (on average 4.5%), ranging from 1.5 to 8% (**Figure 3.3**). The highest contents of mud (silt+clay) are found in Grand-Fort-Philippe (on average 86%) while the sampling stations in the Somme estuary have the highest contents of sand (on average 34%). All the sediment grain-size fractions vary significantly among the sampling areas (ANOVA two factors, *p*-value <0.05). For silt and sand seasonal differences were not statistically significant (*p*-value >0.05). Clay varies significantly during the seasons (*p*-value <0.05) (This shows a non-seasonal influence on the sediment grain-size distribution).

The TOC % varies between 0.02 and 3.8% (**Figure 3.4**). The highest values were recorded in the Canche Estuary (2.37% on average). The TOC % varies significantly among the sampling areas (ANOVA two factors, *p*-value <0.05). For TOC seasonal differences were not statistically significant. The seasonal variations of TOC % do not show a homogenous trend (each sites seems to have a random influence of the seasons) within the sampling areas. We recorded indeed different picks of TOC % in different areas and for different seasons. For instance in the Canche estuary we observed picks on spring (CA1) and autumn (CA2 and CA3), while in the harbour of Boulogne-sur-Mer on summer. The C/N ratio ranges from 0.38 and 19.45 (6 on average) (**Figure 3.5**). The highest values were recorded in the Somme Estuary. C/N ratio does not vary within the sampling areas. On the contrary it shows significant seasonal variations (*p*-value <0.05). Even though its seasonal trend is not perfectly clear, the C/N ratio seems to exhibit the highest values on Spring 2014 and Summer 2014. Sulphur ranges from 0.05 to 0.685% (**Figure 3.6**). The highest values were recorded in the Authie estuary. It shows significant variations within the sampling sites and during the seasons (ANOVA two factors, *p*-value <0.05). Globally sulphur exhibits the highest values on autumn 2014 and winter 2014/2015 and the lowest on spring 2014.

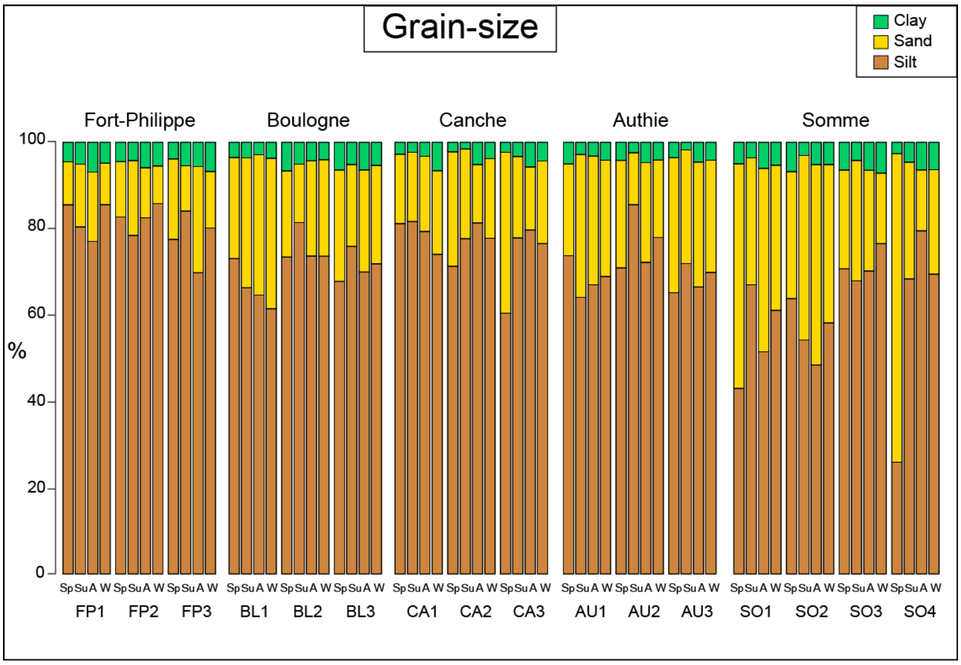


Figure 3-3 Seasonal sediment grain-size variations in the 16 sampling stations within the 5 sampling areas

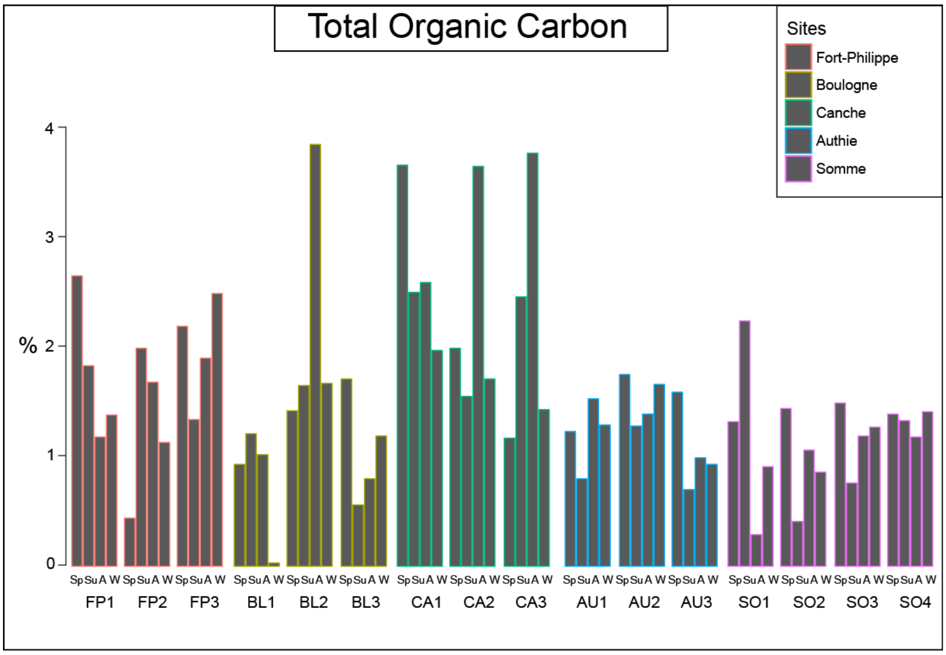


Figure 3-4 Seasonal Total Organic Carbon variations in the 16 sampling stations within the 5 sampling areas.

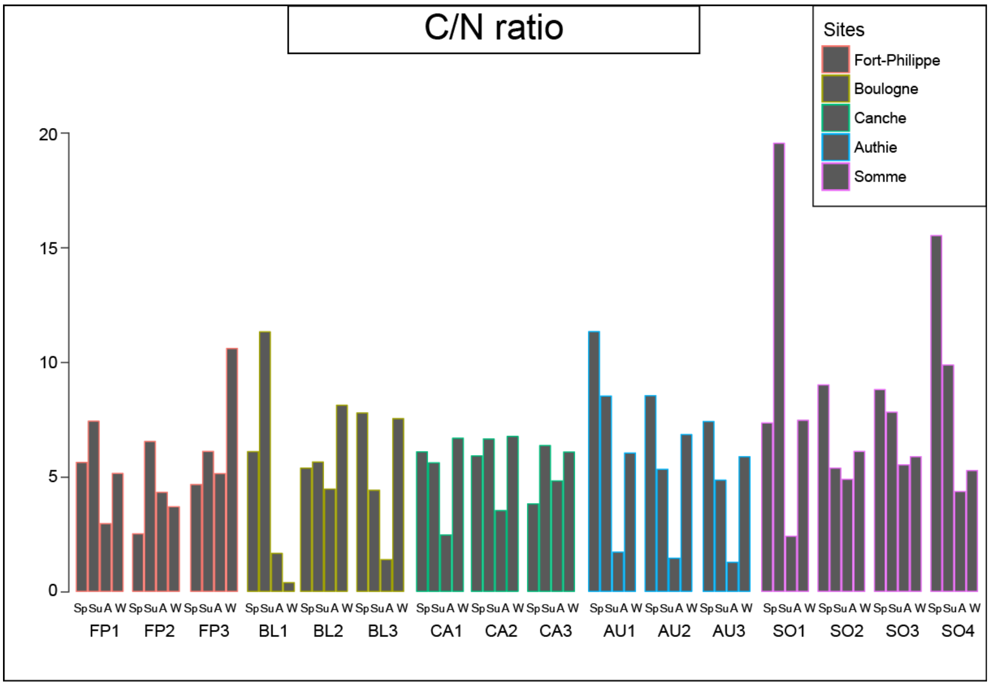


Figure 3-5 Seasonal C/N ration variations in the 16 sampling stations within the 5 sampling areas

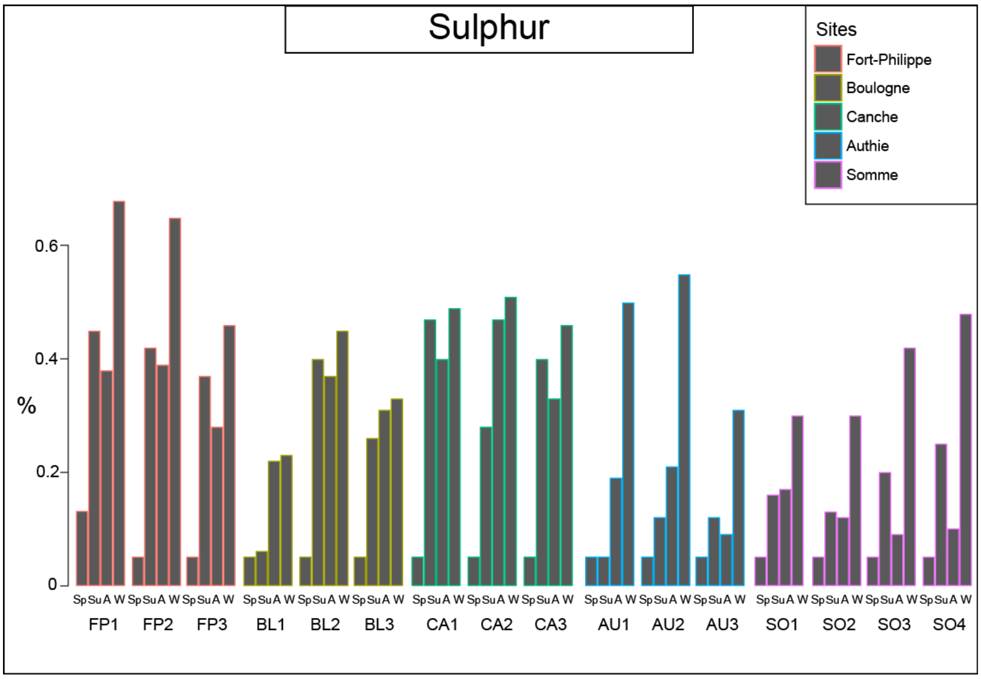
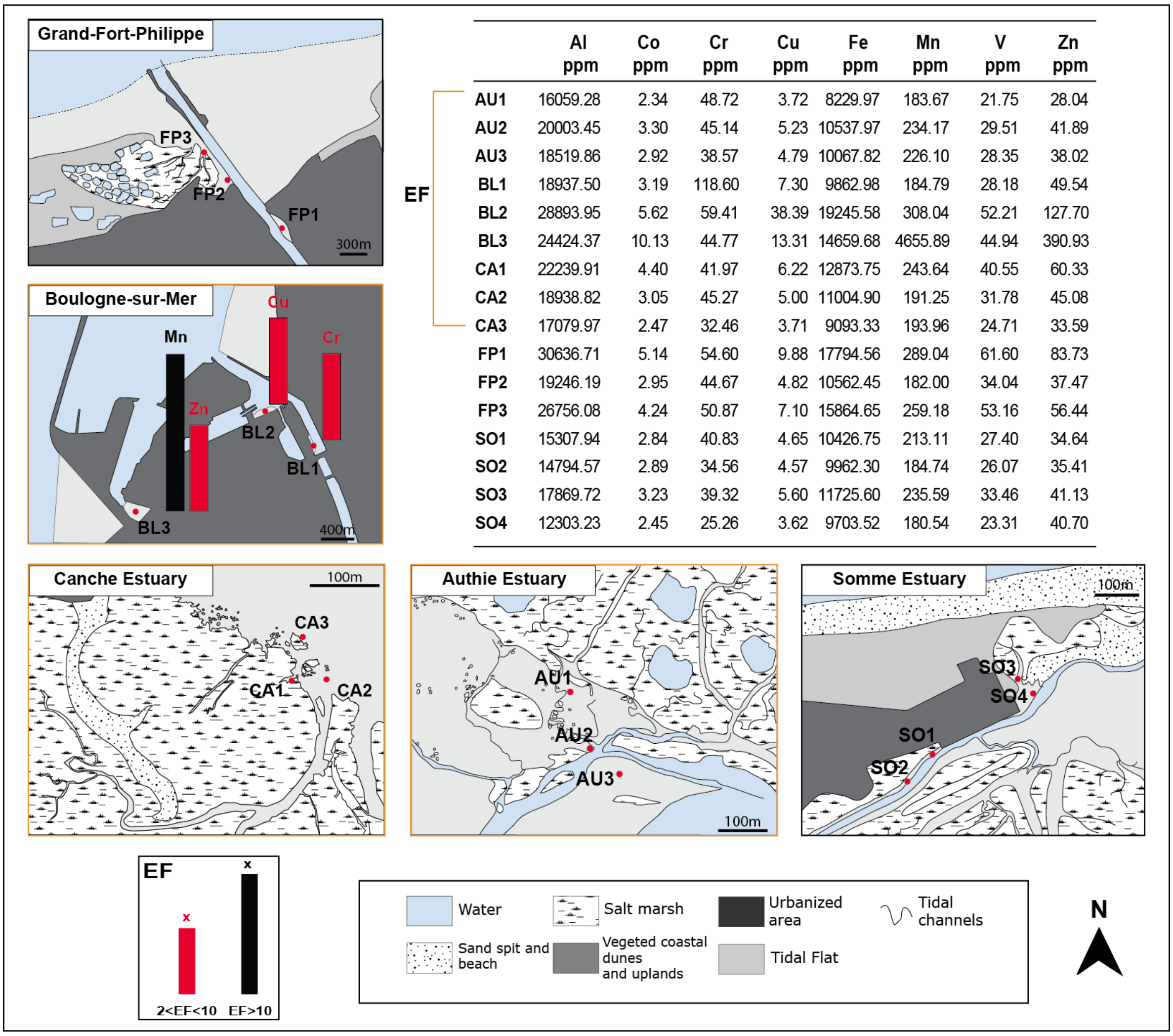


Figure 3-6 Seasonal sulphur variations in the 16 sampling stations within the 5 sampling areas

3.2 Major and trace elements (only for spring 2014 survey)

The concentrations of analysed elements (Al, Co, Cr, Cu, Fe, Mn, V, and Zn) are presented in

Figure 3.7. The enrichment factors (EFs) were calculated for the sampling stations in areas where local background values were available (Boulogne-sur-Mer, Canche estuary and Authie estuary). The highest EFs occur in the harbour of Boulogne-sur-Mer, with moderate enrichments for Cr, Cu and Zn (2<EFs<10) and strong enrichment for Mn (EF>10), specifically in the Petit-Port (BL3). In the Canche estuary and in the Authie estuary no significant enrichment has been recorded.



	Al ppm	Co ppm	Cr ppm	Cu ppm	Fe ppm	Mn ppm	V ppm	Zn ppm
AU1	16059.28	2.34	48.72	3.72	8229.97	183.67	21.75	28.04
AU2	20003.45	3.30	45.14	5.23	10537.97	234.17	29.51	41.89
AU3	18519.86	2.92	38.57	4.79	10067.82	226.10	28.35	38.02
BL1	18937.50	3.19	118.60	7.30	9862.98	184.79	28.18	49.54
BL2	28893.95	5.62	59.41	38.39	19245.58	308.04	52.21	127.70
BL3	24424.37	10.13	44.77	13.31	14659.68	4655.89	44.94	390.93
CA1	22239.91	4.40	41.97	6.22	12873.75	243.64	40.55	60.33
CA2	18938.82	3.05	45.27	5.00	11004.90	191.25	31.78	45.08
CA3	17079.97	2.47	32.46	3.71	9093.33	193.96	24.71	33.59
FP1	30636.71	5.14	54.60	9.88	17794.56	289.04	61.60	83.73
FP2	19246.19	2.95	44.67	4.82	10562.45	182.00	34.04	37.47
FP3	26756.08	4.24	50.87	7.10	15864.65	259.18	53.16	56.44
SO1	15307.94	2.84	40.83	4.65	10426.75	213.11	27.40	34.64
SO2	14794.57	2.89	34.56	4.57	9962.30	184.74	26.07	35.41
SO3	17869.72	3.23	39.32	5.60	11725.60	235.59	33.46	41.13
SO4	12303.23	2.45	25.26	3.62	9703.52	180.54	23.31	40.70

Figure 3-7 Concentration of major and trace elements in the sampling areas. Enrichment factors (EFs) were calculated only for the sampling areas (Boulogne-sur-Mer, Canche estuary and Authie estuary) where backgrounds values were available.

The correlation coefficients calculated between the concentrations of elements and Al, display high significant values for Fe and V (0.92 and 0.96 respectively; *p*-value <0.01) (**Figure 3.8** and supplementary materials). Conversely for Cr, Mn and Zn, the correlations with Al are the low with less significance (*p*-value <0.5). In the scatter (XY) plot (**Figure 3.8**) it can be observed how the most enriched elements exhibit the lowest significant correlations with Al, due to the occurrence of a few outlier values. These values correspond to the sampling stations of Boulogne-sur-Mer's harbour.

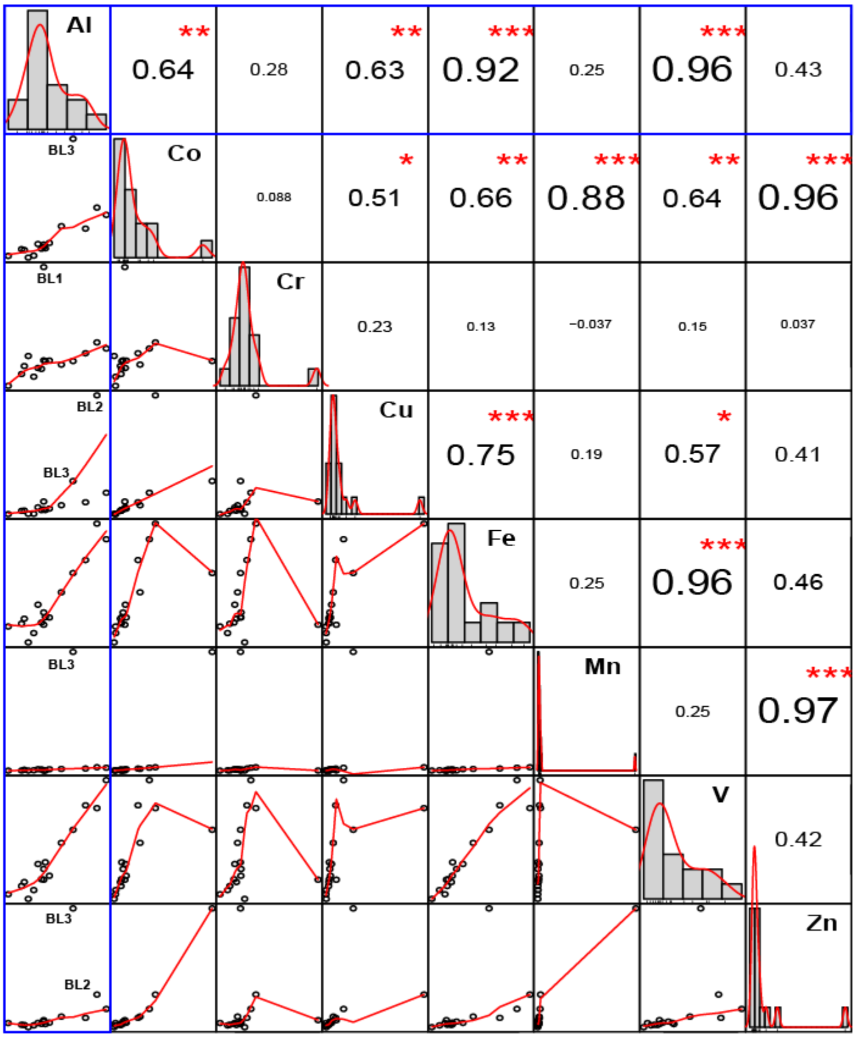
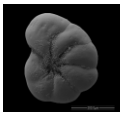


Figure 3-8 Correlations matrix with relative significance (p<0.1*, p<0.05**, p<0.001) and scatter (XY) plots for the analysed elements ((Al, Co, Cr, Cu, Fe, Mn, V, and Zn). In diagonal matrix, histograms of the selected variables, showing a non-normal distribution of enriched elements.***



3.3 Living foraminiferal analysis

3.3.1 Diversity and faunal density

A total of 48 benthic living species have been found after the identification of ca. 46,000 specimens (counting table in supplementary materials). Thirty-five of these are made of hyaline tests, 8 are agglutinated and 5 porcelaneous. Five samples on 192 replicates were azoic. Diversity ($\exp(H'_{bc})$ varies between 1.73 and 20.71 (**Figure 3.9**). Diversity varies significantly among sampling areas (ANOVA two factors p-value <0.001). The highest values of diversity are found in the Authie estuary and the lowest ones in the Boulogne-sur-Mer harbour (9.65 and 3.41 on average, respectively). Diversity varies significantly during sampling periods (ANOVA two factors p-value <0.001). Seasonal variations of diversity and density do not follow a clear trend, however globally the highest values are found during spring 2014 and summer 2014. FD ranges between 0 and 32.14 (specimens/cm^3) (**Figure 3.10**). FD varies significantly among sampling areas (ANOVA two factors p-value <0.001). The highest values are revealed in the Authie estuary and the lowest ones in Grand-Fort-Philippe (6.75 and 1.34 on average, respectively). FD varies significantly during the sampling periods (ANOVA two factors p <0.01). Seasonal variations do not follow a clear trend (as previously for diversity), however in most of the sampling stations the highest foraminiferal abundance are registered in spring 2014 and winter 2014/2015.

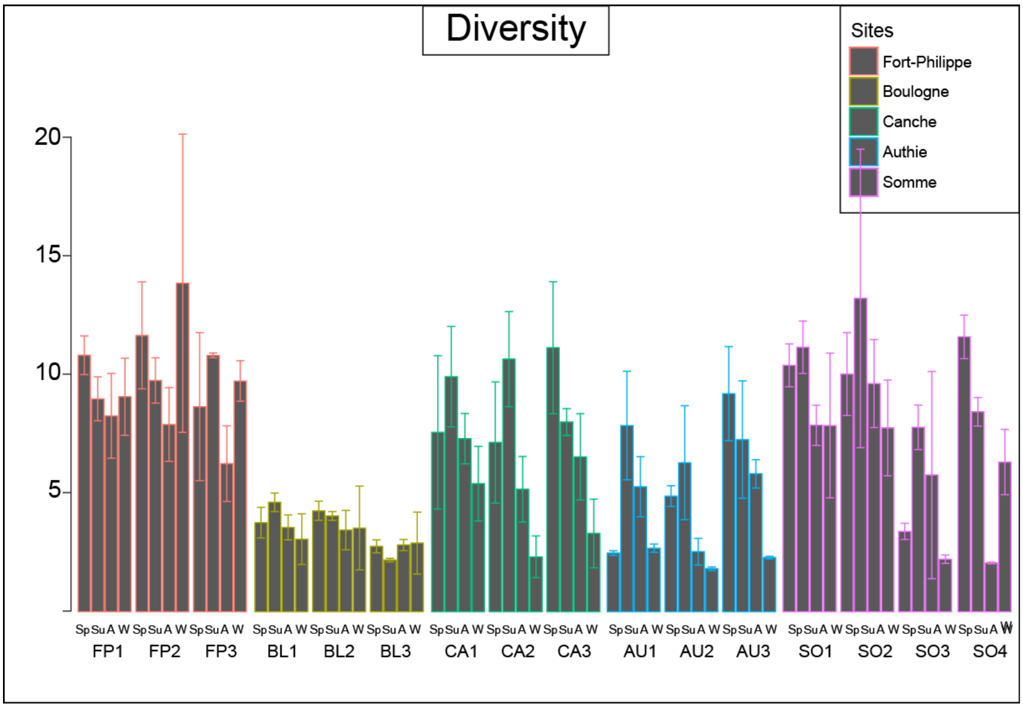


Figure 3-9 Seasonal variations of diversity (exp(H'$_{bc}$)) in the 16 sampling stations within the 5 sampling areas.

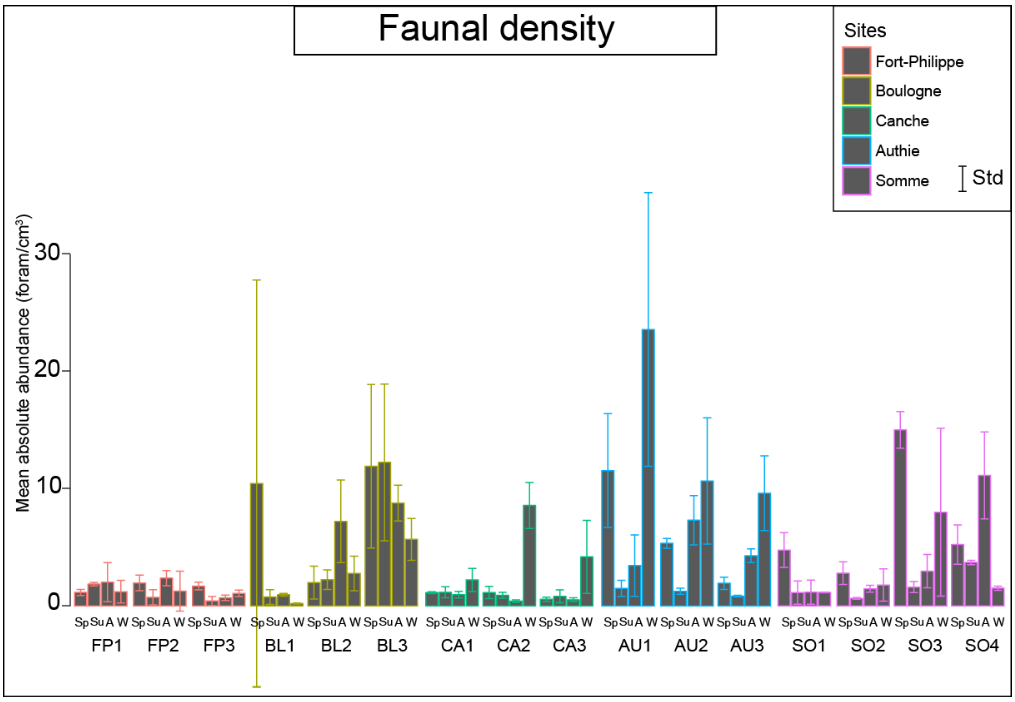


Figure 3-10 Seasonal variations of faunal density in the 16 sampling stations within the 5 sampling areas.

3.3.2 Distribution and abundance of benthic foraminifera

Globally the assemblages are dominated by calcareous taxa. Agglutinated specimens (always in low percentages) occur only in areas adjacent to tidal marshes. *Haynesina germanica* represents the 60% of the total living assemblages associated to a ten of recurring species (i.e. *Cribroelphidium excavatum, Cribroelphidium williamsoni, Bolivina variabilis, Cribroelphidium margaritaceum, Bolivina pseudoplicata, Cribroelphidium gerthi, Quinqueloculina seminula, Ammonia tepida, Buliminella elegantissima, Entzia macrescens, Trochammina inflata*). The seasonal variations of the main benthic foraminifera are shown in **Figure 3.11** and **Figure 3.12**.

Haynesina germanica is the most abundant taxon (2.32 specimens/cm^3 on average). Its abundance varies significantly among the sampling areas and the highest abundances are found in the Authie estuary (4.72 specimens/cm^3 on average). It varies significantly during the sampling periods (ANOVA two factors p-value <0.01) and seems to peak principally during the spring 2014 and the winter 2014/2015 (especially in Canche estuary and Authie estuary). *Cribroelphidium excavatum* is the second most abundant specimens (0.38 specimens/cm^3 on average). Its abundance varies as well significantly among the sampling areas and the highest densities are found in the harbour of Boulogne-sur-Mer (1.03 specimens/cm^3 on average). It varies significantly during the sampling periods (ANOVA two factors p-value <0.001) with maximum abundance during the springs 2014. The abundance of *C. williamsoni*, *C. gerthi* and *C. margaritaceum* vary significantly among the sampling areas (ANOVA two factors p-value <0.001); in the Authie estuary and the Somme estuary are registered the highest values (10.16, 6.07 and 1.38 specimens/cm^3 on average respectively). There is a seasonal influence on their density (ANOVA two factors p-value <0.001) reaching the highest values in spring 2014 and winter 2014/2015. *Bolivina variabilis* and *B. pseudoplicata* are the dominant species in Grand-Fort-Philippe (0.35 and 0.28 specimens/cm^3 on average, respectively). Their absolute abundance is not affected by a seasonal influence. *Quinqueloculina seminula* occurs principally as dominant species in the Canche estuary (CA3) during the summer 2014. *Ammonia tepida* is present in low abundance in the harbour of Boulogne-sur-Mer and in the Authie estuary (0.12 and 0.10 specimens/cm^3 respectively). Its abundance varies significantly during the sampling periods (ANOVA two factors p <0.01) and the highest values are registered principally in winter 2014/2015. *Buliminella elegantissima*, *E. macrescens* and *T. inflata* are the less abundant among the most frequent species. *Buliminella elegantissima* is associated to the *B. variabilis* while *E. macrescens* and *T. inflata* are present only in upper tidal flat areas close to tidal marsh.

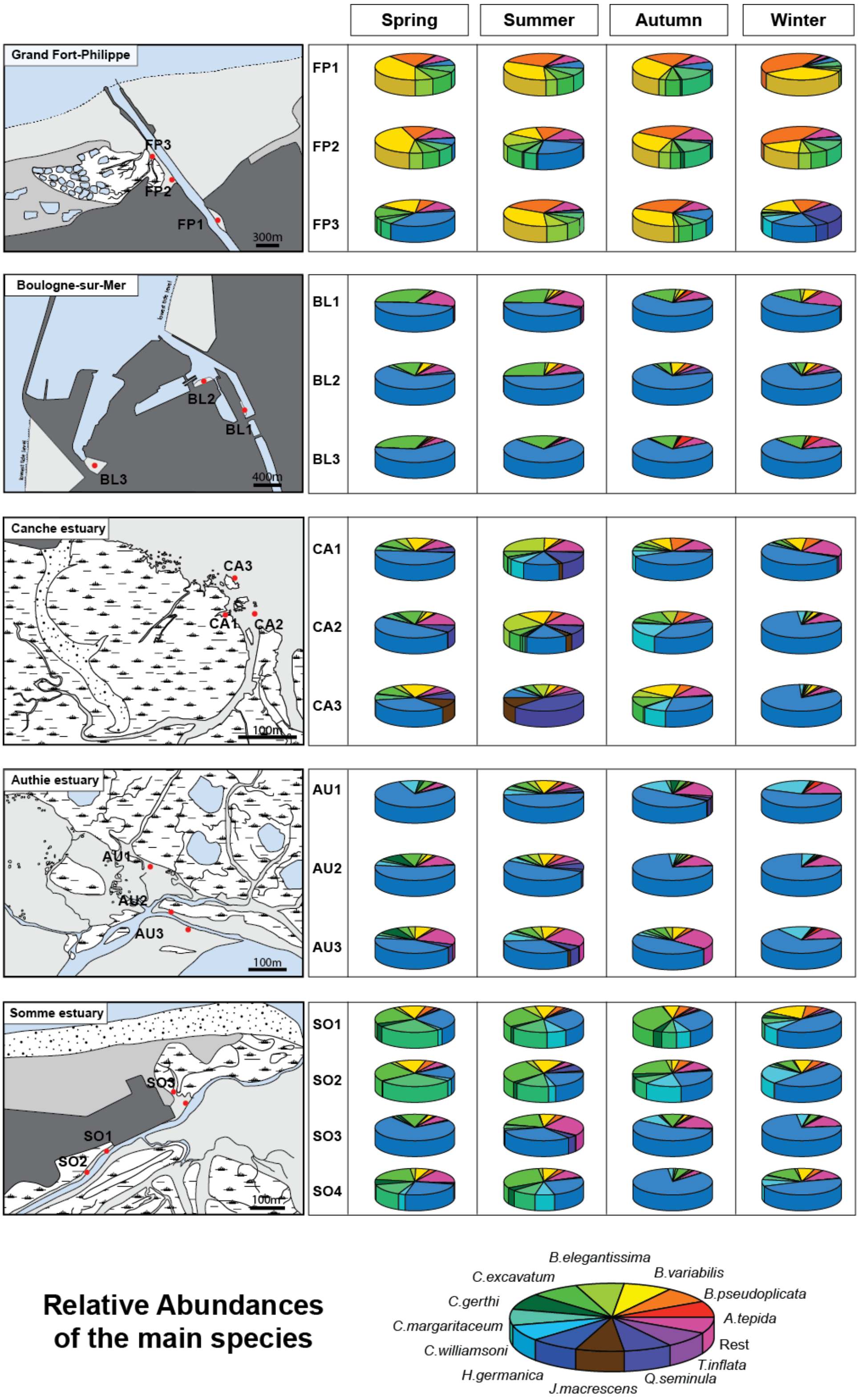


Figure 3-11 Pie-charts of the seasonal variations of relative abundances of the main species in the 16 sampling stations within the five sampling stations

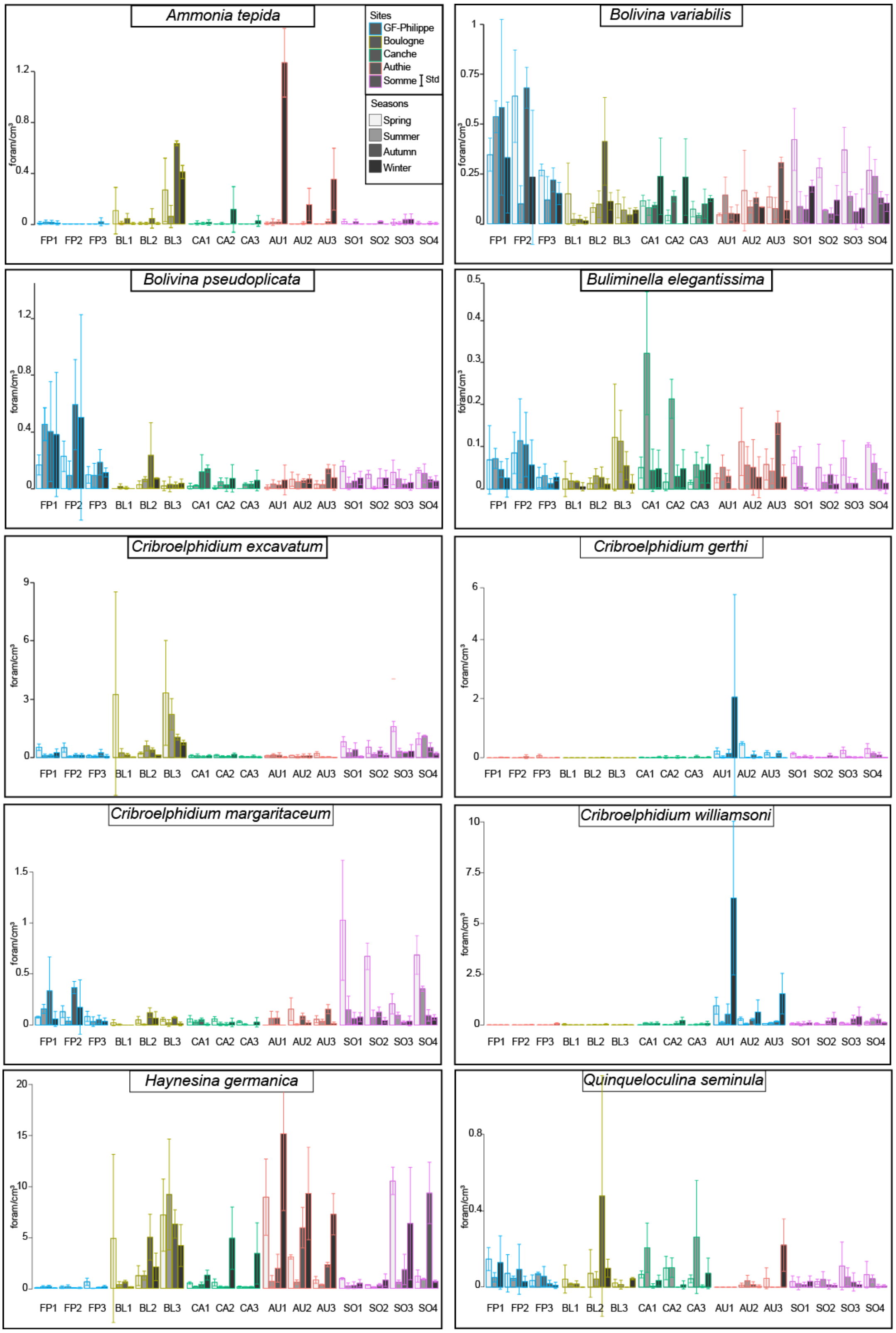


Figure 3-12 Seasonal variations of relative abundances of the main species in the 16 sampling stations within the five sampling stations.

3.3.1 Micro-distribution

The analyses of replicates show for diversity, FD and for the main species both low and high values of standard deviation. This variability seems not to be linked to any seasonal or depending site influence. Standard deviations seem to be higher for density than diversity. All benthic foraminifera exhibit randomly high micro-distribution variability (patchiness) within the study area. For all the homogeneity tests for diversity, FD and main species absolute abundance, the null hypothesis (*p*-value< 0.05) was always refused. It means that the replicated variances between the study sites are non-homogenous (supplementary materials).

3.4 Benthic foraminiferal assemblages

From the CCA (Figure **3.13**) the plane delimited by the two first axis, displays 36% of total inertia. The eigenvalues of axis 1 and axis 2 are respectively 0.37 and 0.31. The first two axes explain the 68% of the total variance.

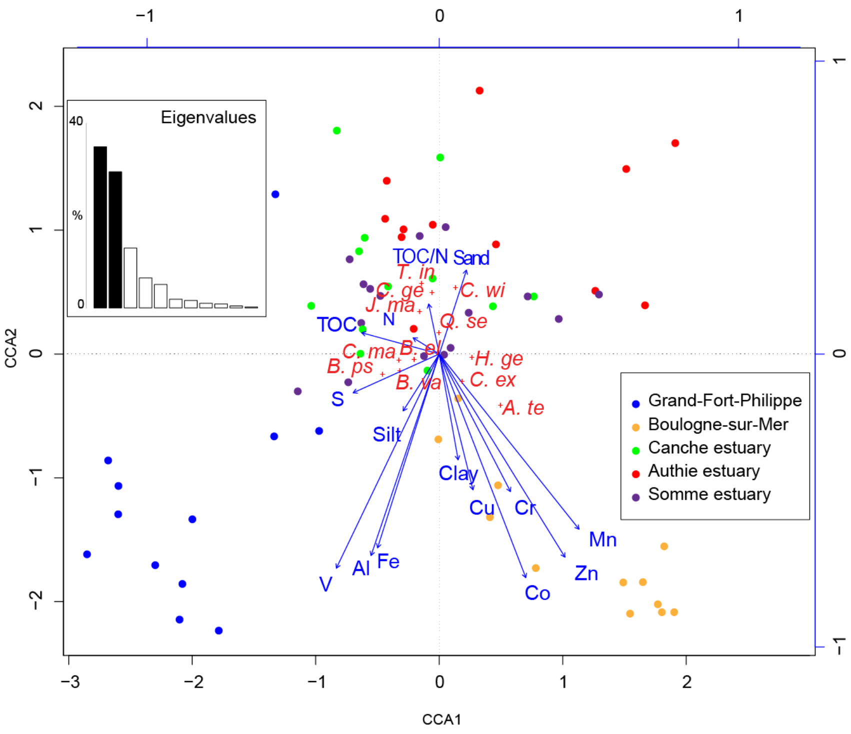


Figure 3-13 Species-conditional triplot based on canonical correspondence analysis (CCA). The arrows represent the environmental constraints. Coloured dots are the sampling sites. Small red crosses are the main species: A. tepida (A. te) B. pseudoplicata (B. ps), B. variabilis (B. va), B. elegantissima (B. el), C. excavatum (C. ex), C. gerthi (C. ge), C. margaritaceum (C. ma), C. williamsoni (C. wi), H. germanica (H. ge), E. macrescens (J. ma), Q. seminula (Q. se), T. inflata (T. in). In top-left histogram of eigenvalues.

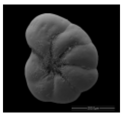
In three main zones can be distinguished: 1) Boulogne-sur-Mer sampling stations at positive of the first axis and at negative of the second one, correlated to: clay, enriched elements (Co, Cr, Cu, Mn and Zn) and to *A. tepida, C. excavatum* and *H. germanica;* 2) Grand-Fort-Philippe sampling stations at negative of the first axis, correlated to: sulphur, TOC, silt, Al, Fe, V and to *B. variabilis, B. elegantissima, B. pseudoplicata and C. margaritaceum*; 3) the sampling stations of the estuarine areas (Canche, Authie and Somme) at positive of the second axis, correlated to sand, TOC/N and to *C. gerthi, C. williamsoni, E. macrescens, Q. seminula* and *T. inflata*. Species score and sites scores are shown in supplementary materials.

4 Discussion

4.1 Foraminiferal communities in the Intertidal areas of Hauts-de-France

4.1.1 The Harbour of Boulogne-sur-Mer

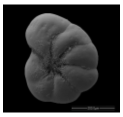
Within the five selected sampling areas only the harbour of Boulogne-sur-Mer results to show moderate and strong enrichments in metal concentrations ($\text{EFs}>3$ for Cr, Cu, Zn and Mn). Our results are quite in line with the findings of other previous studies along the Hauts-de-France coasts (Berthet et al., 2003; Henry et al., 2004; Amara et al., 2007; Kerambrun et al., 2012a). The harbour of Boulogne-sur-Mer is impacted by municipal and industrial discharges, fishing and shipping activities. It has been well observed that the increase of industrial pollution leads to a reduction in the benthic foraminiferal diversity (Alve, 1995; Armynot du Châtelet et al., 2004; Frontalini et al., 2009; Francescangeli et al., 2016; Romano et al., 2016). In the harbour of Boulogne-sur-Mer the diversity has the lowest values ($\exp(H'_{bc})<5$) amongst the five study areas. Considering criteria developed by Bouchet et al (2012), the study site could be considered to have a bad ecological quality status. However in Petit-Port (south-western zone of Boulogne-sur-Mer), Francescangeli (2016) suggested a tendency towards an environmental recovery. In the CCA, it is possible to observe how all sampling stations (in all the sampling periods) of Boulogne-sur-Mer harbour are grouped and positively correlate to the enriched elements. Assemblages are largely dominated by *Haynesina germanica* associated to *Cribroelphidium excavatum*. These species are common taxa in intertidal environments (Debenay and Guillou, 2002; Murray,



2006; Seuront and Bouchet, 2015). Both taxa have been considered as tolerant to trace metal pollution (Debenay, 2001; Romano et al., 2008; Armynot du Châtelet and Debenay, 2010; Armynot du Châtelet et al., 2011). The occurrence of *Ammonia tepida* could indicate as well the presence of polluting activities. This species has been reported as dominant close to outfalls discharging heavy metals (for a review Alve, 1995). Hence in the harbour of Boulogne-sur-Mer the low diversity of the living assemblages and largely dominance of opportunist species, is the response of the biota to human stress conditions.

4.1.2 The embankment area of Grand-Fort-Philippe

The Aa estuary in Grand Fort-Philippe is an embarked area, where the natural flowing of the river channel has been modified. These artificial levees shores up flood waters, probably contributing to the accumulation of terrigenous inputs (such as silt, Al). Foraminiferal assemblages exhibit the lowest values of the faunal density of all the studied sites and they are dominated by *Bolivina variabilis* and *B. pseudoplicata*. Bolivinids include infaunal species, common in intertidal environments (Francescangeli et al., submitted, Murray, 2006); some of them are tolerant to anoxic conditions (Alve, 1990; Debenay, 2001). They have an elongated and flattened morphology which seems to be an adaptation to a lack of oxygenation (Bernhard, 1986). A first hypothesis would be that the higher contents of sulphur associated to anoxic tolerant species, suggest the occurrence of lower oxygen conditions in the sediment of Grand Fort-Philippe. However Bouchet et al (2007) found that *B. variabilis* was sensitive to hypoxia and to organic matter degradation. Hence another hypothesis could be that Bolivinids moved up from the deep sediments layer due to the low oxygen conditions, as mentioned in de Stigter (1996) and Jorissen (1995a). The low total foraminiferal density, added to the fact we sampled the first cm of sediment, could have had an effect on the increase of infaunal species proportion, which live in normal oxygen conditions, deeper in the sediment. In any case the high terrigenous input in Grand Fort-Philippe favoured *B. variabilis* and *B. pseudoplicata* rather than *H. germanica,* dominant in the other study sites. The development of low oxygen conditions in the sediments or organic matter degradations are hypothesis which need a deeper investigation.



4.1.3 Natural and slightly impacted estuaries

The Authie estuary and the Somme estuary are considered as natural environments (Henry et al., 2004). Conversely, the Canche estuary is slightly polluted by the urban development of the city of Etaples and Le Touquet (Amara et al., 2007). Anyhow our data do not evidence any significant enrichments of trace metals. The macrotidal areas of these tidal flats are characterised by a higher sand content than Grand-Fort-Philippe and Boulogne-sur-Mer. In the Canche and Authie estuaries the assemblages are quite similar. They are dominated by *H. germanica* associated to Elphididae (*Cribroelphidium excavatum, C. gerthi, C. margaritaceum* and C. *williamsoni*), *Q. seminula* and *B. variabilis*. In the Somme estuary *H. germanica* is the dominant sp. associated to a high percentage of Elphididae. Within the Elphididae family the most abundant species are *C. excavatum* and *C. margaritaceum* in the lower tidal flat. Both taxa are common in marginal environments (Thomas and Schafer, 1982; Alve and Murray, 1994; Bouchet et al., 2007). Along the coasts of eastern North Sea these specimens have been mainly found in non-marsh intertidal and subtidal environments (Alve and Murray, 1999). *Cribroelphidium excavatum* prefers the lowest part of the intertidal gradient (Armynot du Châtelet et al., 2005), influenced by more marine conditions (Debenay and Guillou, 2002). This taxon seems to be influenced by temperature and salinity (for details on different morpho-types look at Goubert, 1997). *Cribroelphidium excavatum* and *C. margaritaceum* occur principally in the Somme estuary in tidal flat areas close to the main channel, where there are the highest contents of sand, reflecting a higher hydrodynamic. This was found as well by Francescangeli et al. (submitted) in the Canche estuary revealing the presence of *C. margaritaceum* in the sandy tidal flat area. This could suggest an influence of grain-size in driving the distribution of these taxa. In a recent study Arslan et al. (2016) reported that species belonging to this genus can better survive in quartz sandy substrates. *Cribroelphidium gerthi* and *C. williamsoni* have been documented in salt marsh areas and tidal flat areas (Debenay et al., 2000; Armynot du Châtelet et al., 2009a). In accordance with Francescangeli et al. (submitted) they could indicate the occurrence of tidal channels (and/or small pounds) with a local fluctuation of water dynamics (Alve and Murray, 1999; Müller-Navarra et al., 2016). *Entzia macrescens* and *Trochammina inflata* are found in very low abundance only in upper-tidal areas close to the limit to the salt marsh. These agglutinated taxa are the most spread and abundant salt marsh foraminifera elsewhere (Scott and Medioli, 1978; Scott and Leckie, 1990; Scott et al., 2001). Hence it is normal to find them close to the salt marsh/tidal flat limit. To conclude as in the most of intertidal areas in temperate European tidal flats, *H. germanica* results to be the dominant species of benthic foraminiferal assemblages. However the hydrodynamic variability of the estuarine system seems to favour the Elphididae family (such as *C. excavatum*, *C. williamsoni* and *C. margaritaceum*).

4.2 Seasonal variability

Along the intertidal areas of the studied area three main foraminiferal assemblages can be identified which reflects different environmental characteristics at a regional scale. Foraminiferal living assemblages show a significant seasonal variability across the five study sites, as already observed in other studies (Gustafsson and Nordberg, 2000; Murray and Alve, 2000; Kawahata et al., 2002; Fontanier et al., 2003; Fontanier et al., 2006; Horton and Murray, 2006; Duchemin et al., 2008; Buzas et al., 2015; Milker et al., 2015b). Conversely Milker (2015b) documented a lack of significant inter-annual variations in the live populations. In the 5 intertidal areas benthic foraminiferal assemblages show different maxima abundances in different periods of the years. On the contrary Saad (2016) in a recent study carried out along the intertidal zone of North Norfolk (UK), found peaks in total foraminiferal abundances in autumn, between September and October, and in late spring, and a decline during the summer and winter. The same decline was observed in the summer by (Swallow, 2000). Effectively for some study areas (Authie and Somme estuaries) this tendency was confirmed. Murray (2000) found in the intertidal area of Hamble estuary (England), a not clear annual cyclicity for total foraminiferal abundances but a more evident cyclicity for species diversity. In our study areas species diversity as well does not exhibit any clear seasonal trend.

A seasonal variability of species dominance can be observed in some sampling stations. For example in CA3 we can notice how the dominance of *H. germanica* is replaced by *Quinqueloculina seminula* during the summer 2014. *Quinqueloculina seminula* is widespread taxon in shallow environments (Debenay and Guillou, 2002). As other porcelaneous taxa can testify the occurrence of slightly hypersaline (Murray, 1991). Hence in the Canche estuary the summer drier conditions could have induced in the upper part of the tidal flat a local increase of the salinity. This could have favoured the settlement of *Q. seminula*. *Ammonia tepida* occurs in the Authie estuary only during the Winter 2014/2015. It is a common species in transitional areas (Bouchet et al., 2007; Frontalini et al., 2013). This species is not correlated (apart for metals) to any environmental parameter. Hence one can only speculate that a not-measured parameter influence the seasonal distribution of this taxon (such as oxygen, organic fresh matter, temperature). For instance Bouchet (2007) observed a positive correlation between *A. tepida* and the increase of temperature and hypoxic conditions. If the first hypothesis seems to be quite improbable (the peaks are in winter), the second could be more reasonable. Peaks of sulphur, occurring in the Authie estuary during the winter, could suggest the development of low-oxygen conditions. Note that in this case Bolivinids are not favoured. Another influencing parameter could be the salinity. *Ammonia tepida* is dominant in brackish and river-dominated environments (Debenay et al., 1998, Goineau, 2011 #99). In the Vie estuary (West France) were observed oscillations between brackish and marine species dominance due to annual salinity changings (Debenay et al., 2006). Hence the higher rainfall during the winter could have led to a decrease of the salinity favouring the settlement of *A. tepida* in the Authie estuary.

A general observed tendency is that benthic foraminifera seem to be reproducing rapidly in the spring and autumn months, with the general increase in abundance, while the occurrence of stressed environmental conditions, during the summer and winter months, reduce number of individuals (Saad and Wade, 2016). However in our study region we do not observe the same clear cyclicity. This is could be attributed to three main reasons:

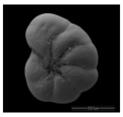
1) Frequency of the sampling. Considering that some species can reproduce from once to about ten times during the year (Murray, 1991), probably a seasonal survey is not enough to observe all the variability. A higher frequency survey (weekly or monthly) would be more efficient. However considering the surface of the study (we remind to the reader that the main aim was to have a global overview on the benthic assemblages at regional scale), this would have enormously increased time and costs of analyses.

2) Sampling period. Foraminifera may reflect seasonal oscillations in environmental parameters, but to observe an eventual phenomena of cyclicity in benthic assemblages, we should extend the observation periods to more than one year.

3) Spatial distance. In most of the cases the reproduction period, namely the maxima abundance of the standing crop, is related to Seasonal fluctuations in food supply (Murray, 2000a). For instance Myers (1943) observed that peaks in abundance of *Ephidium crispum* occurred in spring corresponding the phytoplankton boom. The distance between our sampling areas is about 30-40 km each one. Hence one can speculate that we had blooms (such as diatoms) in different periods. Coastal benthic foraminifera may not have reproducible annual life cycles and living assemblages may provide different or even contradictory results, depending if the sampling is done during the bloom of foraminiferal nutrients or not (Morvan et al., 2006).

4.3 Small-scale variability

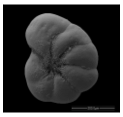
Benthic foraminifera across the northern France show a high small-scale variability. In an intertidal pool of the North Adriatic Sea patchy distributions at the same centimetre scale of benthic foraminifera were also been found by Hohenegger (1989). They consider that the micro-distributional patterns of foraminifera were related to those of algae, but that, as well as food resources, competition between foraminiferal species could control foraminiferal distribution. Similarly to our results, the use of replicates in the study of Swallow (2000) clearly demonstrate the patchy nature of the living foraminiferal distribution in intertidal environments (tidal marsh in that case). In benthic ecological studies the replication is largely adopted but not necessarily in foraminiferal studies. For instance in micro-phytobenthic studies from 3 to 5 replicate samples are typically taken within randomly located 1 m^2 quadrats in a given environment (Seuront and Spilmont, 2002). However this high small scale spatial variability can only be assessed with adequate sampling design that would require a considerable number of samples (Spilmont et al., 2011). In the present study we used three replicates. However in benthic foraminiferal literature there are no specific recommendations for replication techniques. In a study conducted in sub-tidal areas Bouchet (2012) suggested that three replicates is an adequate number to represent this small scale variability. In intertidal areas Armynot, et al. (Com. Pers.) (Accordingly to Spilmont et al., 2011) found that all the replicates (in a total surface of 0.5 m^2) should be used to model the real benthic foraminiferal density's variations/patchiness.



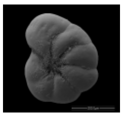
In foraminiferal several studies to limit the bias due to small-scale patchiness (centimetre to decimetre scale) the authors performed pseudo-replications (Morvan et al., 2006), which consists in sub-sampling randomly and mixing the sub-samples together (Hurlbert, 1984). This method is suggested by Debenay (2015) in mangrove areas where the high heterogeneity can influenced the choice of replicate place. Collecting replicates in such an environment is likely to introduce substantial bias. This pooling method may be sufficient, when targeting the most abundant taxa. However this method can be inconsistent if the objective is to analyse the small-scale patchiness. On the contrary the use of separate replicates allows directly evaluating this small scale variability. To do that different procedures have been adopted. Some authors used what can be called the 'expert judgement' (without any statistical approach) (Swallow, 2000) or the affinity index of Rogers (1976) (Saad and Wade, 2016). Other researchers used statistical approaches such as Student t-test (Bouchet et al., 2007), Analysis Of Variance (ANOVA) (Buzas et al., 2002), Non-Parametric Multivariate ANalysis Of VAriance (NPMANOVA) (Milker et al., 2015a). In the present study we used homogeneity tests (Bartlett and Kruskal-Wallis tests). So far in foraminiferal literature any article documents which is the best technique to evaluate small-scale variability. In this work we used the homogeneity test because is a quick method, with statistical significance, to evaluate eventual patchiness in foraminiferal distributions.

5 Conclusions

- Along the intertidal areas of the northern France three main foraminiferal assemblages can be identified which reflects different environmental characteristics at regional scale: 1) the embankment area of Grand-Fort-Philippe dominated *B. variabilis* and *B. pseudoplicata* favoured by the high terrigenous input; 2) the metal enriched harbour of Boulogne-sur-Mer characterized by low diversity of the living assemblages and dominance of opportunist species (*H. germanica, C. excavatum* and *A. tepida*); 3) natural or little impacted estuarine areas under macro-tidal regime, largely dominated by *Haynesina germanica* is associated to Elphidiae (*C. excavatum, C. williamsoni* and *C. margaritaceum*) favoured by higher sand contents.



- In the intertidal benthic foraminiferal of the Hauts de France living assemblages show a significant seasonal variability. However there is not a homogeneous trend identifiable at regional scale. The different peaks in total foraminiferal abundance could be linked to different blooms of food availability across the study region.
- The analysis of replicates based on statistical homogeneity tests, clearly show a heterogeneous (though patchy) distribution of benthic intertidal foraminifera. This confirms the need of using replication methods, to un-bias interpretations.



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Chapter 4 Palaeo-Ecological Quality Status based on foraminifera of Boulogne-sur-Mer harbour over the last 200 years

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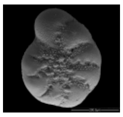


1 Abstract

Over the last centuries, coastal areas have experienced dramatic degradations of their environmental quality, which has led to a huge reduction of marine biodiversity. The objective of the present study was to use geochemical parameters and benthic fossil foraminifera to assess environmental changes that have occurred over the last 200 years in a harbour area (Boulogne-sur-Mer, Northern France) heavily modified by human activities. A multidisciplinary approach including major and trace metals, grain-size, total organic carbon and benthic fossil foraminifera, has been performed on a 33-cm long core. The dating was carried out using the activity of ^{210}Pb and ^{137}Cs. Embayment of the area and increase of trace metals concentrations induced a shift in benthic communities. Human activities modified a sandy nearshore bank, colonized by typical marine foraminiferal species, such as *Cribroelphidium excavatum*, into a sheltered environment, dominated by brackish end-members, such as *Haynesina germanica.* Along the sedimentary record, the interaction between meiofaunal and geochemical elements made it possible to distinguish between a pre-impacted period and an industrial period. The upper part of the core reflects better ecological conditions, indicating an environmental recovery. Our results provide baselines for future environmental bio-monitoring in the area.

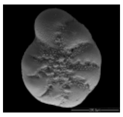
2 Keywords

Harbour area; pollution; multiproxy approach; sediment grain size; metals; benthic fossil foraminifera; palaeo-ecological quality status;

3 Introduction

Human population growth and associated activities over the last centuries has led to a dramatic degradation of the quality of water and of sediment run off that increasingly discharges into coastal marine systems (Diaz and Rosenberg, 2008). Over the last 150 years, drastic spreading of dead zones in coastal marine systems has led to a massive loss of species affecting ecosystem functioning (Solan et al., 2004). The damaging effects of human population growth are to be addressed by the implementation of numerous marine legislations worldwide (CleanWaterAct [CWA] and Oceans Act in USA, Australia and Canada; Water Framework Directive [WFD] and Marine Strategy Framework Directive [MSFD] in Europe, National Water Act in South Africa) (Stelzenmüller et al., 2010; Borja et al., 2012). These legislations define the environmental quality not only as an assessment of the biodiversity status but also as an integration of the functioning of the ecosystem, suggesting the use of multidisciplinary approaches (Borja et al., 2008a). For instance, Leorri et al. (2008a) pointed out that combining geochemical and meiofaunal monitoring approaches would be useful as it would reflect the sediment quality in heavily polluted environments. The MSFD (Directive 2008/56/EC) aims at achieving good environmental status for all marine water bodies by 2020. The assessment of the ecological quality status (EcoQS) needs to define the reference conditions in order to set up relevant comparisons with potential impacted sites. In the WFD, reference conditions have been defined as "… the biological, chemical and morphological conditions associated with no or very low human pressure" (L 327/38). In the legislation, several approaches have been suggested to define reference conditions, such as using natural areas, hindcasting (historical reference conditions), modelling and expert judgment (Bald et al., 2005; Borja et al., 2012). Definition of reference conditions using natural areas is, in most cases, difficult because most of the marine systems have been modified by human activities or by climatic changes (Hinz et al., 2011).

For approximately 15 years, different biological indicators have been used to determine the EcoQS of surface water bodies (Simboura and Zenetos, 2002; Rosenberg et al., 2004; Devlin et al., 2007). Benthic macrofauna is the most widely biological group used to monitor the EcoQS of coastal and transitional marine environments (Borja et al., 2000;



Blanchet et al., 2008; Bouchet and Sauriau, 2008). Finding natural areas and their associated benthic communities is rarely possible (see afore-mentioned references). In addition, hindcasting is not possible with soft-bottom sediment macrofauna because they do not leave many fossil records; in fact, for most species, they do not leave any fossil records (Elliott and Quintino, 2007; Bigot et al., 2008; Blanchet et al., 2008). Hence, using benthic macrofauna, stakeholders can only determine reference conditions using expert judgment. Other biological groups, with fair fossilisable capacities, could be used in hindcasting (such as foraminifera). Several authors showed that the fossil record has great potential to reconstruct palaeoecological quality status (PalaeoEcoQS) and thereby establish *in situ* historical reference conditions from pre-impact times (Alve, 1991b; Andersen et al., 2004; Borja et al., 2008b; Alve et al., 2009). Recently, the development of new methodologies for biological monitoring has led to benthic foraminifera to be considered for application under EU legislation (Bouchet et al., 2012; Barras et al., 2014; Alve et al., 2016; Dimiza et al., 2016).

Benthic foraminifera constitute one of the most diverse and spread out groups of unicellular organisms in modern oceans (Murray, 1991; Sen Gupta, 1999; Murray, 2007). They are ubiquitous in marine ecosystems (Todo et al., 2005), and play a key role in the functioning of the environment (Groß, 2002; Degré et al., 2006). They are increasingly used as bio-indicators of global changes, due to both natural and anthropogenic alterations (Armynot du Châtelet et al., 2004; Mojtahid et al., 2008; Frontalini et al., 2013; Frontalini et al., 2014). Preservation of foraminiferal tests in fossil sediments enables them to be a suitable tool for palaeoecological and palaeoenvironmental reconstructions (Alve, 1991a; Hayward et al., 2004; Alve et al., 2009; Dolven et al., 2013). Lately, fossil benthic foraminifera have been used to define *in situ* reference conditions and to distinguish a pre-impacted stage from an impacted one (Alve et al., 2009; Dolven et al., 2013).

The Nord-Pas-de-Calais (Northern France) is an area that has been highly impacted by polluting activities (e.g., coal and textile productions, metallurgical factories, fishing and agriculture activities, food, chemical and automobile industries); most of them are located close to or in harbour areas (Boulogne-sur-Mer, Calais, Dunkerque). Hence, upper sediments in this part of France allow for the monitoring of anthropogenic activities of anthropogenic activities from the beginning of the second industrial revolution. Note that there are few studies in this area characterizing environmental conditions using benthic foraminifera (Armynot du Châtelet et al., 2009a; Armynot du Châtelet et al., 2011). None of these studies have been based on palaeoecological or palaeoenvironmental reconstructions; therefore, the fossil historical background is to be assessed.

In the context of using benthic foraminifera to assess the palaeoenvironmental evolution in a harbour area heavily modified by human activities over the last 200 years, the aims of this study are two-fold: i) to show how changes in foraminifera are supported by environmental data; and ii) to evaluate the PalaeoEcoQS of the area.

3.1 Study area

The present study is based on a sediment core collected from an intertidal area called "Le Petit Port" in the harbour of Boulogne-sur-Mer (hereafter called Boulogne) (**Figure 4.1**,**Figure 4.2**) This area is particularly interesting since it has been successively modified by anthropogenic alteration, which has transformed the natural environment over the last 200 years (**Figure 4.3a**) in a highly industrialized harbour (Kerambrun et al., 2012a) (**Figure 4.3c**). From the middle of the 19thcentury, the development of the port area led to the construction of the Napoleon Basin (1853-1867), the Carnot dike (1878-1887), and subsequently, the Loubet Basin, the marina of 'Le Petit Port' (1990-1912)(Karl, 1910) (**Figure 4.3b**). After a pause during the two world wars, the construction of the Sarraz Bournet docks (1967) resulted in a rapid increase of traffic. Coincidently, many industrial activities developed, opening an intense industrialization phase during the 1970-1980s (e.g., handcrafted and industrial fishing; activities connected to freight transport; metallurgic and chemical factories). Among these, a ferromanganese plant, with significant sewage discharge (Wartel et al., 1990), played a pivotal role in the pollution of the harbour (1967-2003). Following the closing of the factory, environmental remediation works have been performed in order to enhance recovering processes (collecting and sorting waste).

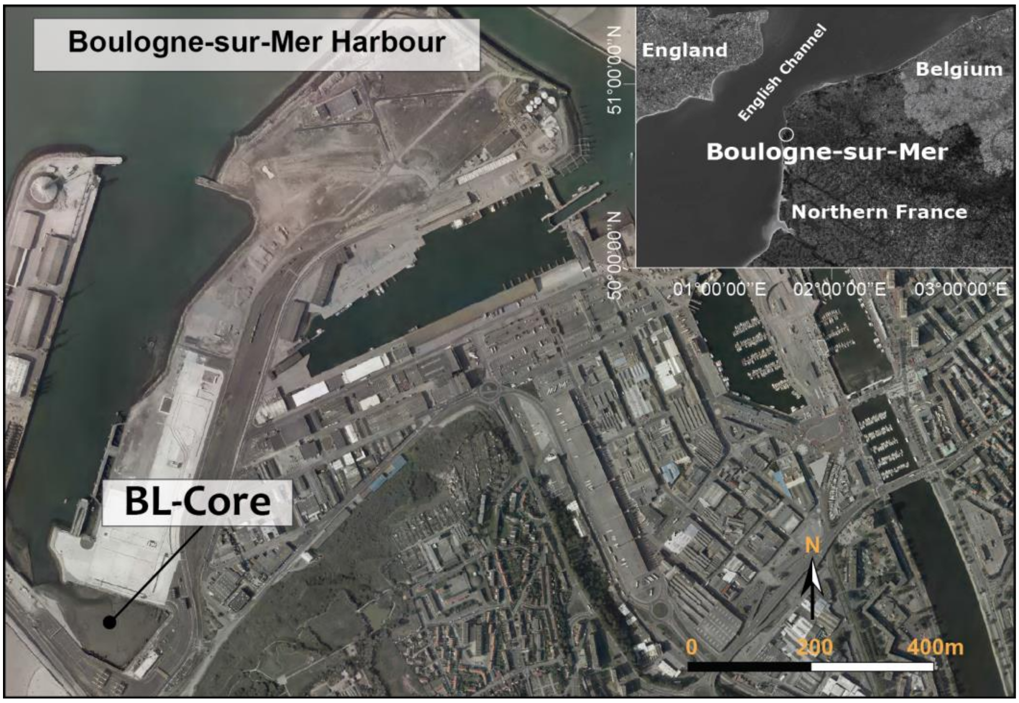


Figure 4-1 Position of BL-Core within the study area

Figure 4-2 Image of Petit Port at high tide

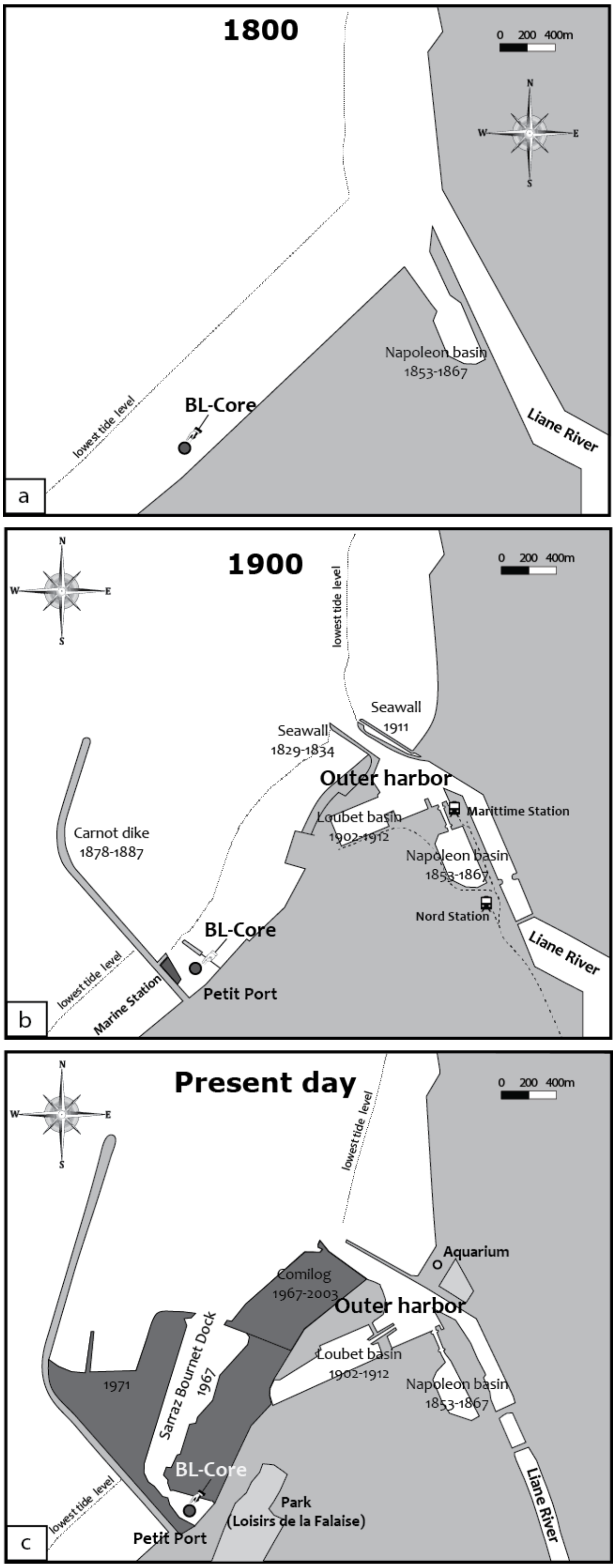


Figure 4-3 Reconstruction of the harbour's physiography during the 1800 (a), 1900 (b), and present (C), extracted from Geoportail (http://www.geoportail.gouv.fr) and Karl (1910).

4 Materials and methods

A 33-cm long core (1° 34’ 28” E 50° 43’ 4” N; -0.68 m water depth), sampled on May 2013, was sectioned every 1 cm down to 20 cm and 2 cm till the bottom of the core; this resulted in the collection of 26 samples. The first aliquot was used to measure environmental variables (sediment grain-size, carbonate content, major and trace elements concentrations, total organic carbon) and dating. The second aliquot was freeze-dried and used for foraminiferal analysis.

4.1 Grain-size and total organic carbon

Grain-size analyses were performed using the principle of diffraction and diffusion of a monochromatic laser beam on suspended particles (Malvern Mastersizer 2000, red He-Ne laser). The method is based on near-forward scattering of a laser beam by particles in suspension, as previously detailed (Trentesaux et al., 2001). Measurements can range from 0.02 to 2,000 µm with an obscuration ranging between 10% and 20%. Three grain-size fractions: clays (< 4 µm), silts (4 to 63 µm), and sands (63 to 2000 µm) were considered.

A Flash EA 1112 Elemental Analyser (Thermo) equipped with an auto-sampler was used for determining the total organic carbon content (TOC). The analysis was performed on 1.5 mg to 2 mg of sample added to approximately 5mg of vanadium pentoxide, used as a combustion catalyst. A 2.5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene (BBOT) was used as standard. The TOC was then determined by subtracting carbonate carbon from total carbon concentrations. The calcium carbonate content was determined using a Bernard calcimeter and expressed as dry sediment weight percentage. Triplicated measurements were carried out for each sample using 0.5 g of finely crushed sediment.

4.2 Major and trace elements concentrations (total mineralization)

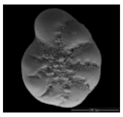
The total concentration of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn were analysed for each level. After drying the sediment particles at room temperature, the samples were sieved at 63 µm and gently crushed. Then, 200 mg of the fine fraction were attacked first with a mixture of 5 mL of suprapur nitric acid and 10 mL of a concentrated HF solution at ebullition over 48 hours. After evaporation of these acids, 10 mL of a freshly prepared HNO_3/HCl mixture (1/2 v:v) was added in order to eliminate the remaining solid grains. The recovered solutions were subsequently diluted in a known volume of ultrapure water and analysed using ICP-AES (inductively coupled plasma – atomic emission spectroscopy; varian vista pro, axial view) and ICP-MS (inductively coupled plasma – mass spectroscopy; thermo elemental X series). This attack procedure was validated and the accuracy of the analytical procedure was checked by means of the following sediment standard reference materials (Canadian International Standards): HISS-1, MESS-3 and PACS-2. It was found that standard materials certified and measured results were in good agreement.

In order to assess sediment contamination and to evaluate anthropogenic influences, the enrichment factor (EF) (Woitke et al., 2003; Kerambrun et al., 2012b; Hasan et al., 2013; Duan et al., 2014) was calculated for each element, as follows (Eq. 1):

$$EF = ([X_i]/[Al_i])/([X_0]/[Al_0]) \qquad \text{(Eq. 1)}$$

Where $[X_i]$ and $[Al_i]$ are respectively the concentrations of the element *X* and Al in the sample I; $[X_0]$ and $[Al_0]$ are the local geochemical background concentrations of the element X and Al, respectively. Note that in this relation, the enrichment factor is normalized against aluminium to take into account the evolution of the grain-size, especially concerning the fine particles (silts and clays), overcoming partly the bias bound to the nature of the sediment. For the calculation of EFs, reference values were taken from the very bottom of the core, as there are no previous studies in the area. The following criteria, estimated by previous studies (Birth, 2003; Kerambrun et al., 2012a), are used to evaluate the degrees of pollution according to the values of EF: EF < 2: no relevant enrichment; 2<EF<10: moderate contamination; EF>10 strong contamination. The Tomlinson's pollution load index (PLI) (Tomlinson et al., 1980; Martins et al., 2013) was calculated as well (Eq. 2). The PLI is obtained as a concentration factor (Eq. 3) of each trace metal with respect to the background values; it aims at easily highlighting anthropogenic contributions of trace elements along the core. The fingerprint of the global enrichment of several chemical elements X (in this study: Cd, Co, Cr, Cu, Pb and Zn), whose enrichment in the sediment may be toxic, has been calculated as follows:

$$PLI = \sqrt[n]{(CF_{Cd}xCF_{Co}x \dots xCF_n)} \qquad \text{(Eq. 2)}$$



Where $CF_X = [X_i]/[X_0]$ (Eq. 3)

4.3 Chronology

The samples have been analysed for the activity of ^{210}Pb, ^{226}Ra and ^{137}Cs via gamma spectrometry at the Gamma Dating Centre, Department of Geosciences and Natural Resource Management, University of Copenhagen. The measurements were carried out on a Canberra ultra-low background Ge-detector. ^{210}Pb was measured via its gamma-peak at 46.5 keV, ^{226}Ra via the grand daughter^{214}Pb (peaks at 295 and 352 keV) and ^{137}Cs via its peak at 661 keV. The concentration of unsupported ^{210}Pb was found by subtracting the supported ^{210}Pb (measured as ^{214}Pb) from the total ^{210}Pb concentration. To build the age model, a modified version of the constant rate of supply (CRS) model was applied to the profiles (Appleby, 2001). The measured age model did not cover the whole sedimentary record. A linear extrapolation, assuming constant sedimentation rates, was therefore performed to obtain an indication of ages below the oldest radiometric point (Dolven et al., 2013).

4.4 Foraminiferal analysis

Freeze-dried samples were weighted, gently washed through a 63 μm mesh sieve, and oven dried at 40 °C. The investigations were carried out on the> 63 μm size fraction. Approximately 300 tests were picked in each sample. This number is recommended to consider a species proportion of more than 1% associated to a probability of failure to detect of 1% (Fatela and Taborda, 2002); this value is realistic for such a study. All the picked specimens were mounted on faunal micro-slides, identified and counted, following Loeblich and Tappan (1988) for genera, Debenay (2012) and Debenay et al. (2001) for species classification. The observations were carried out under a binocular microscope, model Olympus SZX16, with a maximal. The relative abundances of the taxa and the benthic foraminiferal accumulation rate (BFAR) (number of tests/cm^2/year) (Herguera, 1992; Naidu and Malmgren, 1995) were determined for each sample. The effective number of species Exp(H')$_{bc}$, (for details see the work of Bouchet et al., 2012), which is the most suitable measure of diversity (Beck and Schwanghart, 2010), was considered in the present study. It is based on the nonparametric estimation of Shannon's index of diversity to avoid the problem of unobserved species (Chao and Shen, 2003).

In order to point out ecological changes through time, foraminiferal species were assigned to ecological groups according to ecological criteria. The four ecological groups are: A) Marine, B) Brackish, C) Widespread, D) Marine dysoxic/anoxic tolerant. According to Debenay and Guillot (2002) the Marine group and the Brackish group represent the opposite end-member of our intertidal system along a salinity gradient. The "Marine" group includes intertidal (to subtidal) species living in open systems (such as a tidal flat); they are under marine influence more than the "Brackish" group. The "Brackish" group includes specimens living in more restricted conditions (such as tidal marshes or sheltered areas). The ecological attributions of the main species (Annex xx) are based principally on the works of Murray (2006), Debenay and Guillot (2002), Scott et al. (2001) and ecological groups discussed in Delaine et al. (2014). The ecology of benthic foraminiferal species is always a matter of debate. As an example, Debenay and Guillot (2002) mentioned *Cribroelphidium excavatum* as typical in tidal flat zones in temperate Atlantic macrotidal environments. However, there are several references considering the same taxon as a "brackish species" (Polovodova et al., 2009). Other authors (Murray and Alve, 2000; Papaspyrou et al., 2013) stated that it occurs in intertidal environments often related to *Ammonia tepida* and *Haynesina germanica* which are considered in this study as "brackish" specimens. That suggests that local environmental factors should be considered to characterize the distribution of species along a coastal gradient. Here after local study on both living and dead fauna (Armynot du Châtelet et al., 2009a; Armynot du Châtelet et al., 2011) *C. excavatum* and *H. germanica* have been considered as two opposite end-members along a marine water gradient, from lower to higher vertical positions.

To avoid the effects of possible taphonomic processes, only the species with a minimum of f (frequency) ≥ 20 and/or an average relative abundance ≥ 0.5, were considered (an average of 88% of the total assemblage). Specimens having an uncertain ecological attribution were not taken in account into the ecological model; they were left in an unassigned category.

In the study to evaluate the PalaeoEcoQS, we used $\mathrm{Exp}(H')_{bc}$ as biotic index (BI), following Bouchet et al. (2012). Ecological status with class boundaries was determined using local references following recommendations from Krause-Jensen et al. (2005). The ecological status classes were defined based on ecological quality ratios, representing the deviation of actual levels of a quality element from reference conditions. Local-specific classes for Exp(H')$_{bc}$ have been defined as follows: Exp(H')$_{bc}$<7 "Bad", 7-12 "Poor", 12-16 "Moderate", 16-18 "Good" and >18 "High" EcoQS (Fig. 4a).

The relation between PLI and Exp(H')$_{bc}$ was used to trace the PaleaoEcoQS through time.

4.5 Statistical analysis

The main species were grouped using a constrained hierarchical clustering analysis (HCA). A similarity tree was produced using the Euclidian distance. Coniss (Grimm, 1987) was used as the clustering method.

A detrended correspondence analysis (DCA), an ordination method that arranges samples and species along gradients, was carried out on the species' relative abundances. It arranges samples that are more similar in species composition closer together. DCA uses the variation in species composition between the samples to determine the underlying gradients influencing the data. The basic assumption of this method is that the most important environmental gradient causes the largest variation in the species composition. By means of a two way weighted averaging algorithm, the direction of this variation was calculated and represented as the first DCA axis (Hill and Gauch, 1980; Versteegh and Zonneveld, 1994).

The R software (version 3.2.2) (R-Core-Team, 2014) was used for the calculations, by using the following packages: Base (descriptive statistics), Entropy (diversity calculation), Rioja (construction of diagrams with timescale), Vegan, Hmisc, ade4 and corrgram (Correlation matrix, CHA and DCA).

5 Results

5.1 Chronology

The core showed relatively uniform concentration of unsupported ^{210}Pb in the upper 12 cm and a decrease with depth below this level. Unsupported ^{210}Pb was absent from a depth of approximately 18 cm, indicating that sediment below this level was deposited at least

~120 years ago. Cesium-137 was only present at very low concentrations close to or below the detection limit of approximately 2 Bq kg^{-1}, and showed no distinct trend or peaks. A ^{210}Pb-based chronology was calculated for the upper 18.5 cm using a modified CRS model of Appleby (2001) in which the inventory below 18.5 cm was calculated on the basis of a regression of unsupported ^{210}Pb vs. cumulated mass depth in the interval 12 cm to 18.5 cm. Below this depth, absence of unsupported ^{210}Pb precludes dating by this isotope. Instead, an indicative extrapolated chronology was calculated on the basis of the down-core accumulated mass depth using the calculated average accumulation rate between 14.5 cm and 18.5 cm (**Figure 4.4**).

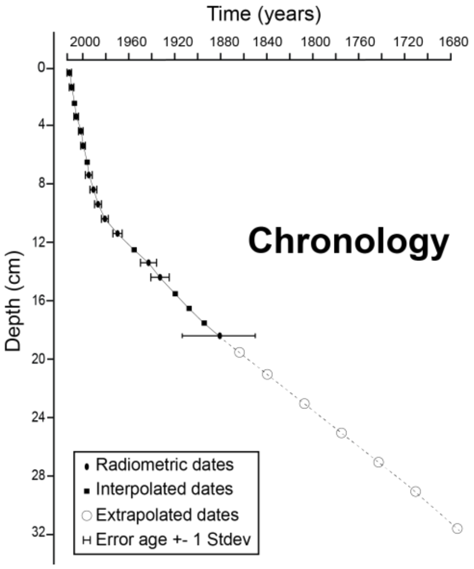


Figure 4-4 Chronology based on the activities of ^{210}Pb, ^{226}Ra and ^{137}Cs. Below 18.5 cm depth an indicative extrapolated chronology was calculated.

5.2 Environmental variables

The sediment grain-size (**Figure 4.5**) shows a clear decrease of sandy fraction (from 63% to 26%) as well as a gradual increase of silt (from 32% to 70%) moving upward. Clay fraction increases from 4.9% to 11.4% and then decreases to 2.9% in the upper part of the core. The TOC contents range between 0.2% and 1.9% (**Figure 4.5**). The TOC shows an increase at beginning of the 1900s, reaching the highest values after the 1980s. None of the analysed elements (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn) show any significant variations until approximately the middle of 19th century (**Figure 4.5**). Later metals can be divided in two groups according to their evolutions: group 1 (Al, Cr, Fe, and V) characterized by a gradual increase of the concentrations through time, and group 2 (Cd, Co, Cu, Mn, Ni, Pb and Zn), whose concentrations first increase to reach a peak during the 1970/80s, and subsequently decrease to the present day.

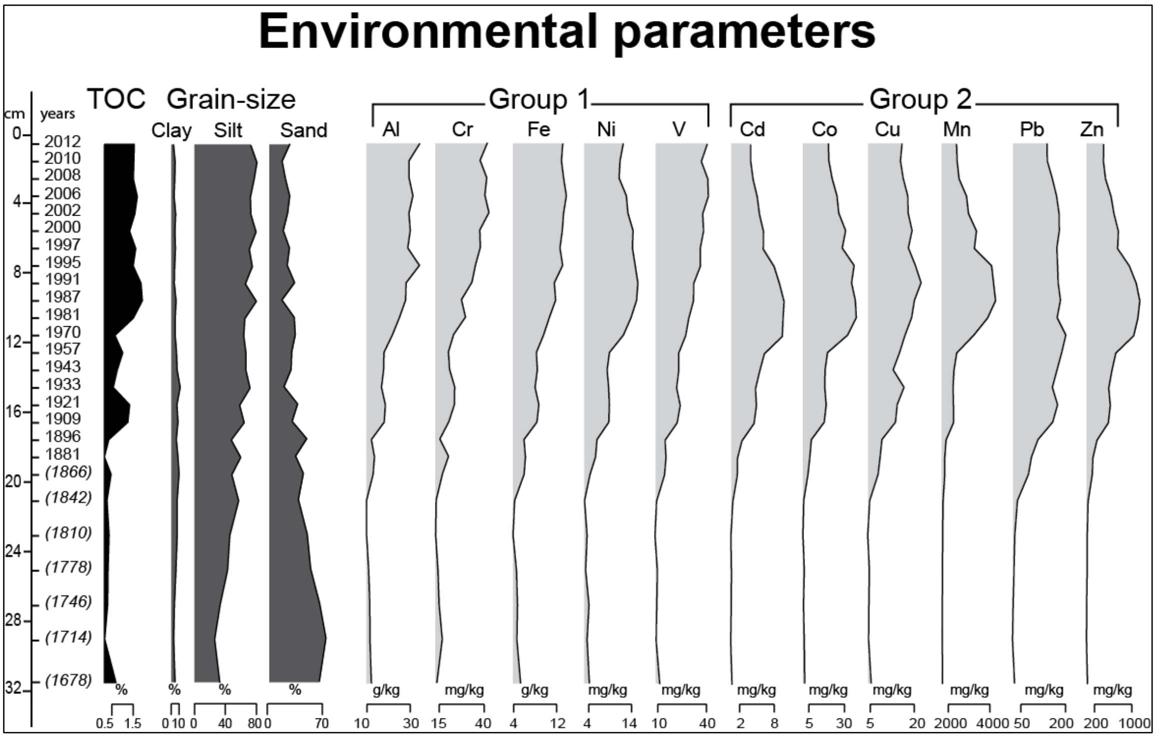


Figure 4-5 Trends of environmental parameters considered in the study: TOC, grain-size (Clay, Silt and Sand), geochemical variables (group 1: Al, Cr, Fe, and V; group 2: Cd, Co, Cu, Mn, Ni, Pb and Zn).

There are no relevant enrichments for the first group ($EF_{max}<2$). Conversely, metals belonging to the second group show strong enrichments, especially for Mn and Zn, with maximal values of EF during 1960s/1980s, reaching 35 and 14, respectively (**Figure 4.6**). Sediments are moderately enriched in Cd, Co, Cu and Pb ($EF_{max}<8$), showing also a recovery in the upper part of the sediment core. PLI (**Figure 4.6**) profile exhibits the same trend than those observed for metals belonging to group 2: an increase during the middle of 19th century, a maxima during 1970s/1980s and then a decrease close to the surface sediments. The correlation coefficients calculated between the concentrations of elements of group 1 and Al, display higher values than 0.92 (p <0.01). Conversely, for metals belonging to group 2, the correlations with Al are lower than 0.86 (p<0.01) (**Figure 4.7**).

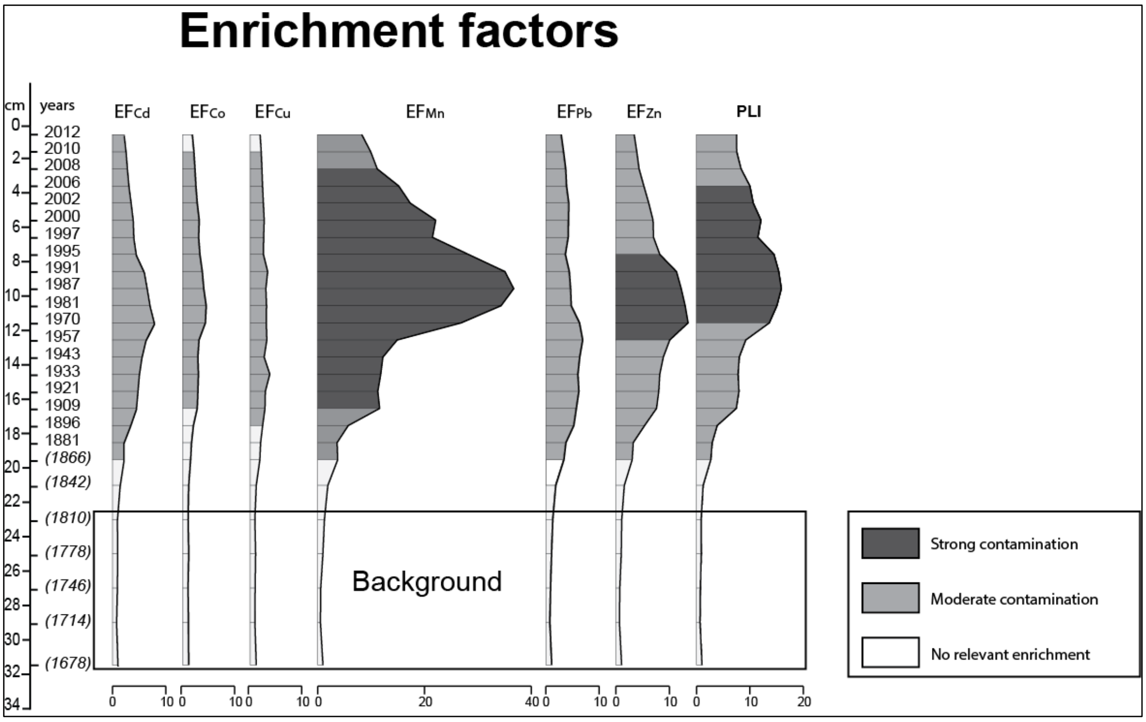


Figure 4-6 Enrichment factors (EFs) for metals belonging to group 2 and pollution load index (PLI) profiles.

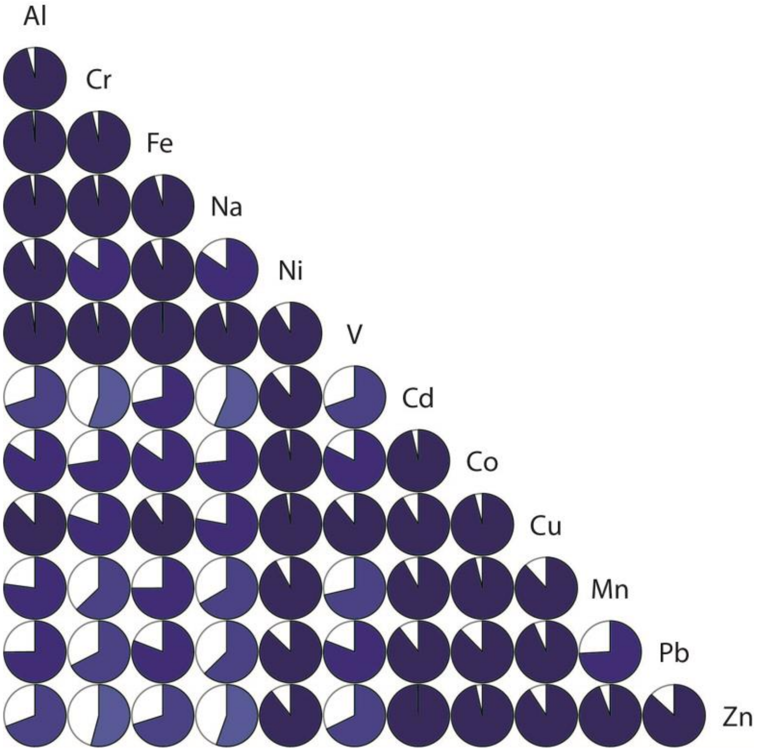


Figure 4-7 Correlation matrix for geochemical variables of group 1-2. All the coefficients have p<0.01.

5.3 Foraminiferal assemblages and ecological groups

A total of 95 species were observed along the core. Eighty species of these (85%) are made of calcareous tests (counting table in supplementary material). Two species dominate (between 31% and 74%; 56% on average) the assemblage: *Cribroelphidium excavatum* and *Haynesina germanica*; five species are subsidiary (between 6% and 36%; 15% on average): *Quinqueloculina seminula, Cribroelphidium gunteri*, *Ammonia beccarii, Lobatula lobatula*, and *Miliolinella subrotunda* (**Figure 4.8a**). *Cribroelphidium excavatum* (33% on average) is the most common in the bottom part of the core. Its relative abundance starts to decline after the end of the 19th century (1881), reaching the lowest values (12%) in the very upper part of the core, after 2006. In contrast, *H. germanica'* s relative abundance (23% on average) increases gradually after the end of the 19th century (1881), and it ranges from 3% at the bottom of the core to 39% close to the sediment surface. *Quinqueloculina seminula* displays the highest values of relative abundance (12%), among the subsidiary species. This mostly occurs in the upper part. The proportion of *C. gunteri* starts to be relevant at the beginning of the 20th century; a maximal relative abundance (11%) is observed during the 1970s/1980s, followed by a significant decrease in the upper part. The presence of *A. beccarii* occurs at the very bottom part with a maximal relative abundance of 10% and then it disappears almost totally after the beginning of the 1900s. Note that the density of *L. lobatula* also shows the highest values in the bottom part of the core.

BFAR (**Figure 4.8a**) ranges from <1 to 19 (tests/cm^2/year), showing the lowest values at the bottom and a rapid increase starting at the beginning of the 1980s. $\text{Exp}(H')_{bc}$ (**Figure 4.8**a) varies between 6.5 and 24. The highest values are located at the bottom. Starting from the middle of the 19th century, diversity decreases with the lowest values principally occurring during the 1960s/1980s; a rapid diversity recovery occurs from 2006 onwards ($\text{Exp}(H')_{bc\ Max}=16$). The core is therefore characterized by 3 zones: "High" or "Good" EcoQS in the bottom part, principally "Poor" in the interval between the middle of the 19th century and 2006, and "Moderate" and "Good", and over the last 10 years.

Among the 95 observed species in the assemblage, 19 (88% of the total abundance), were assigned to an ecological group (**Figure 4.8b**). The Marine group (group A) is the most abundant. It is dominant in the bottom of the core (maximum of 70%), and after 1896 starts to gradually decrease (with minimal values of 30%) up to the present day. An opposite pattern is evident in the Brackish group (group B): the lowest percentages at the bottom (3%) followed by an increase after 1896 and a dominance in the upper part (maximum of 47%). The Widespread group (group C) does not show an evident trend along the core. The Marine dysoxic/anoxic tolerant group (group D) is the less abundant (maximum 8%) and does not display any clear trend. However, it seems to have the highest values in the bottom and upper part of the core.

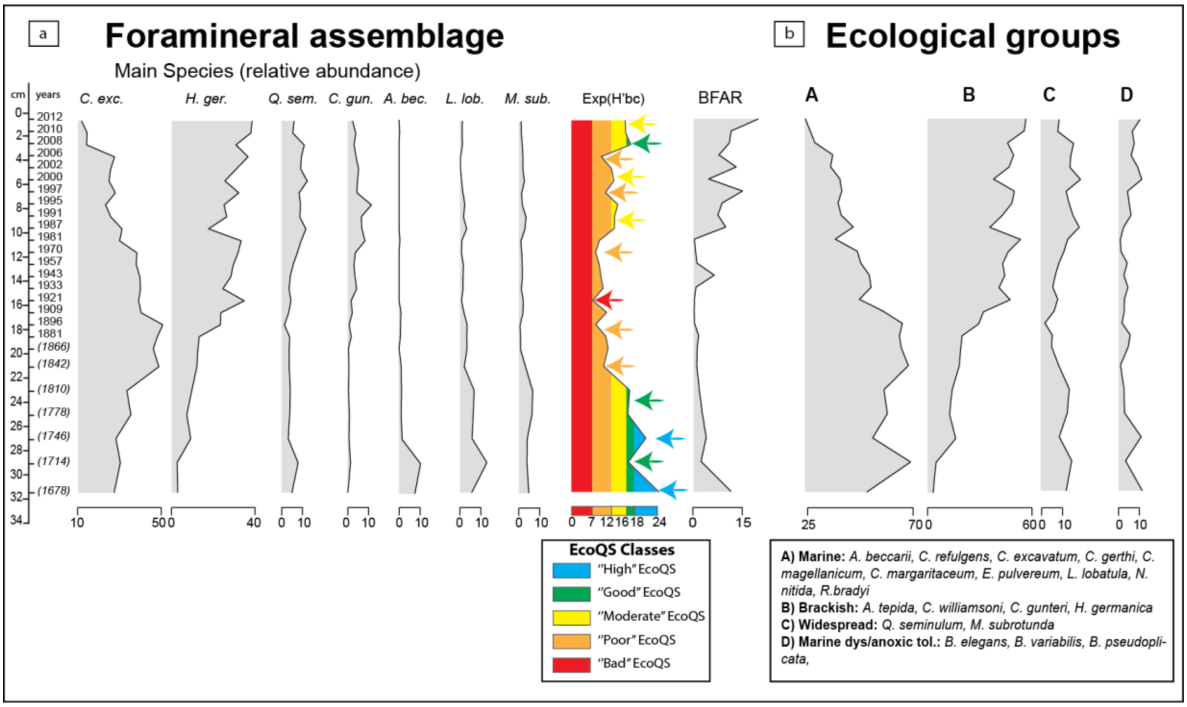


Figure 4-8 a) Trends of relative abundance of the main species: C. exc = Cribroelphidium excavatum, H. ger = Haynesina germanica, Q. sem. = Quinqueloculina seminula, C. gun. = Cribroelphidium gunteri, A. tep. = Ammonia beccarii, L. lob. = Lobatula lobatula, M. sub. = Miliolinella subrotunda. Trend of diversity (Exp(H')bc) and related ecological quality status (EcoQS): Exp(H')bc <7 "Bad" ecological status, 7-12 "Poor", 12-16 "Moderate", 16-18 "Good" and >18 "High" status. Trend of benthic foraminiferal accumulation rate (BFAR: tests/cm2/year). b) Trends of 4 ecological groups. Each group represents the percentage of specimens with the same ecological affinity.

5.4 Foraminifera assemblages' evolution through time

The HCA analysis classified the samples along the core into 2 main clusters I and II (**Figure 4.9**). Cluster I and Cluster II were divided into sub-clusters. As the analysis was only based on the relative abundance of benthic foraminifera in each sample, the resultant faunal groups reflect the evolution of ecological features through time. Each cluster (and sub-cluster) represents a time interval with different ecological characteristics. Cluster I includes all the samples from the bottom of the core up to 1909; Cluster II embraces the rest of the samples from 1921 to the top of the core.

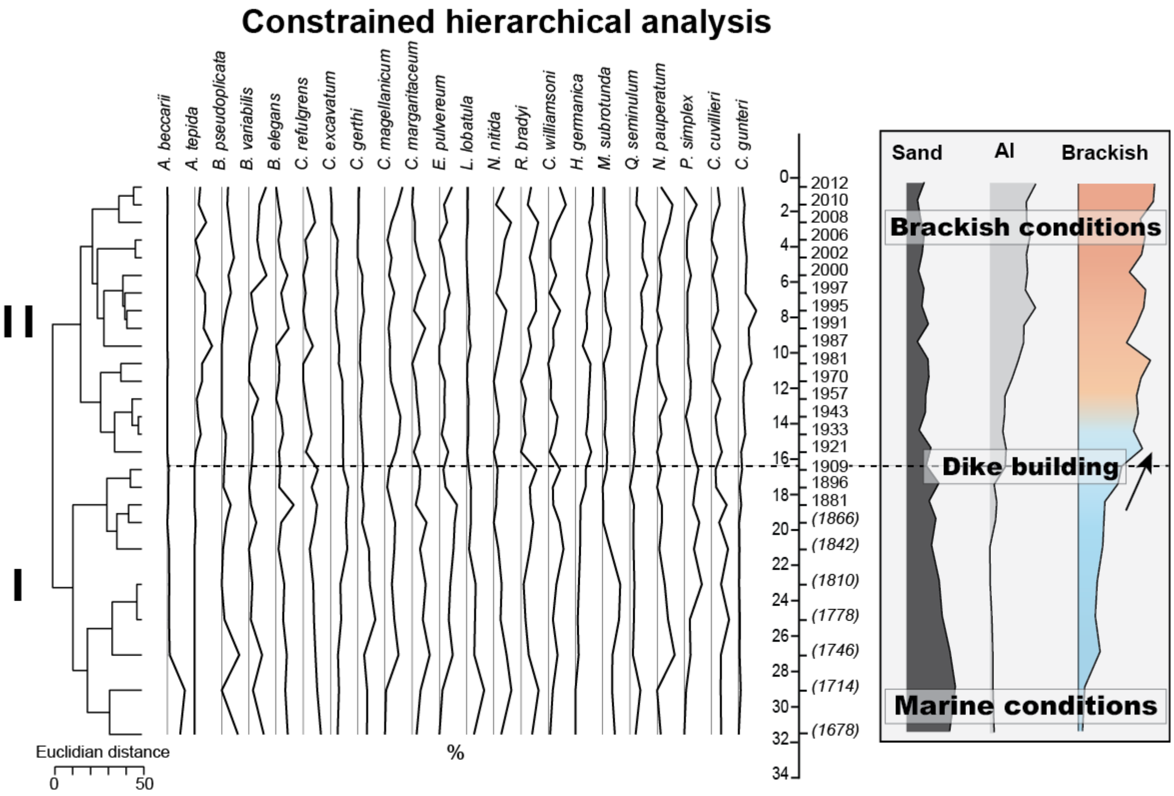


Figure 4-9 Constrained Hierarchical Clustering analysis (HCA) based on species abundances. The two Clusters identified, I and II, reflect the different environmental conditions occurring before and after the end of the 19th century, corresponding to the period of the building of the Carnot dike and the marina of Petit Port.

In the DCA, the two first axes explain about 20% of the total variation (eigenvalues of 14% and 6%, respectively) (**Figure 4.10**). Samples belonging to Cluster I (blue dots in the plot) are placed at the positive position of the first DCA axis. Most of the foraminiferal species belonging to ecological group A (marine specimens) are located at the positive position of the first axis as well. On the contrary, the samples of Cluster II (red dots in the plot) and the foraminiferal species belonging to ecological group B (brackish specimens) are located on the negative part of the first axis. Group D species are located on the positive part of the second axis.

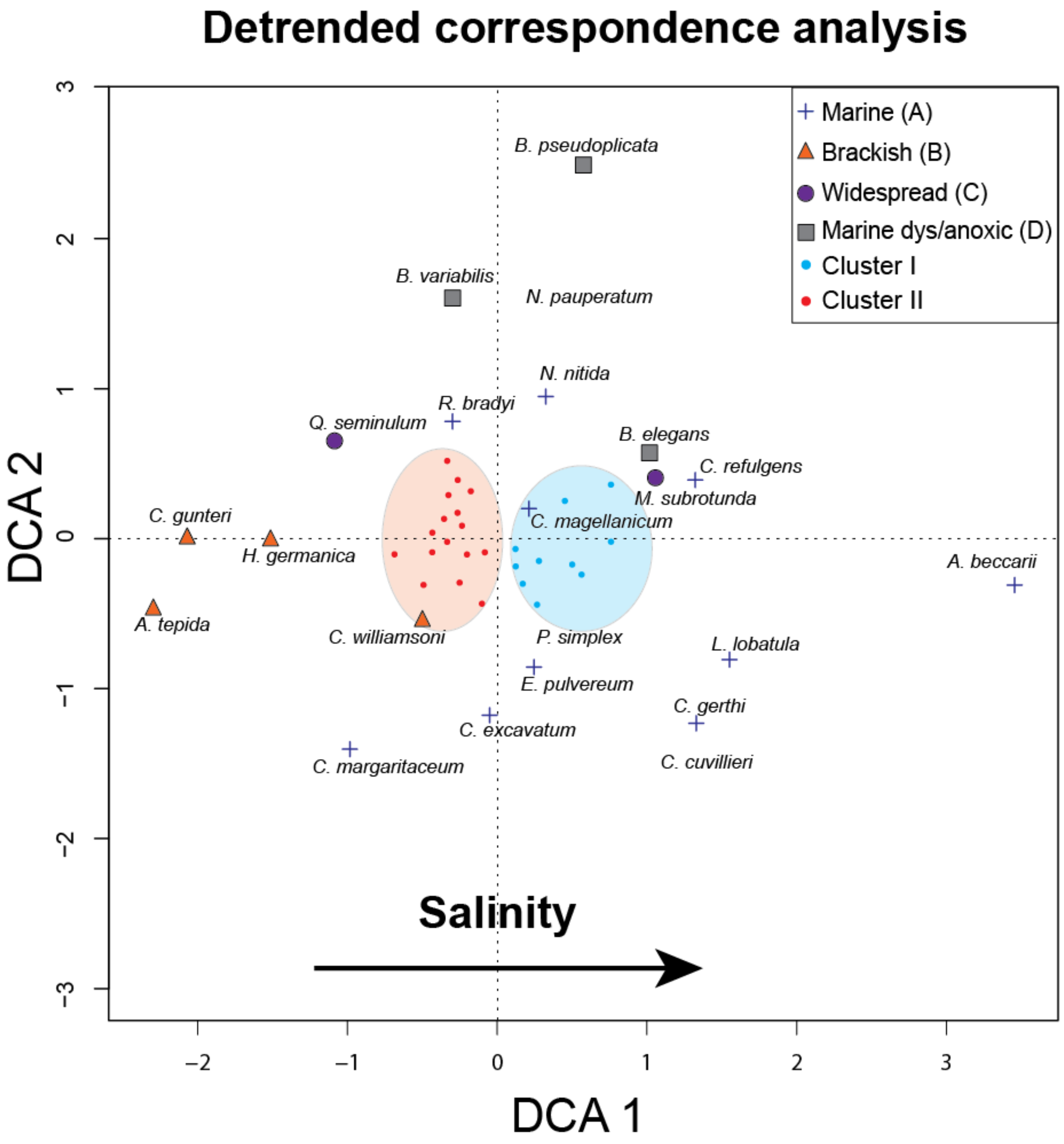
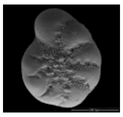


Figure 4-10 Detrended correspondence analysis (DCA) based on species' abundances. After the ecological assignment of the species, the first axis represents a salinity gradient. Blue dots = samples of Cluster I (i.e., before the end of the 19th century); red dots= samples of Cluster II (i.e., after the end of 19th century).

6 Discussion

6.1 Pre-industrial period

At the bottom part of the core up to the first years of the 19th century, all geochemical variables show the lowest values below toxic levels (Bakke et al., 2010) without significant



increases of their concentrations. In the area of Petit-Port, there was no industry at that time, and human pressure was only related to fishing activities (Schmitt, 2012). This interval represents the conditions occurring during the pre-industrial period. Hence, it can be considered as the reference conditions of Boulogne's harbour for both environmental parameters (geochemical background) and foraminiferal assemblages. The substrate is mostly constituted by sand in accordance with a dynamic environment open to the sea. During this period, there is a gradual decrease of the sediment grain-size, following the natural evolution of an intertidal area in coastal/estuarine systems in Northwest Europe (Allen, 2003). Foraminiferal diversity shows the highest values over the last 200 years. The assemblage is dominated by marine species belonging to ecological group A. *Cribroelphidium excavatum* was the most abundant species. The presence of epiphytic specimens living on seagrass meadows (Debenay and Payri, 2010; Mateu-Vicens et al., 2010; Sadri et al., 2011), such as *Lobatula lobatula*, associated with sandy sediment show a substrate covered by seagrasses and macroalgae. Disappearance of *Ammonia beccarii* in the upper layers confirms the aforementioned switch from sandy to sandy-muddy sediments. Debenay and Guillou (2002) found that *A. beccarii* prefers coarser sandy sediments rather than sediments containing silt and clay. The presence of tolerant species to anoxia belonging to group D (*i.e., Bolivina variabilis*, *Bolivina pseudoloplicata*, *Bulimina elegans)*, would indicate occurrences, maybe seasonally, of low oxygen conditions. Bolivinids and Brizalinids are species tolerant to anoxia (Alve, 1990; Debenay, 2001). Observations of seasonal hypoxia during the spring season along the western Atlantic coast of France would support this hypothesis (Bouchet et al., 2007).

During the pre-industrial period (reference conditions), Petit-Port area was presumably a sandy nearshore bank enriched with silt. It was colonized by typical foraminiferal species from environments under open marine influence.

6.2 Petit-Port's embayment

Starting in 1829 with a seawall building, the Boulogne's harbour was strongly modified by human activities. A partial enclosure after the construction of Petit Port marina, from 1878 to 1909, modified the hydrodynamic characteristics of the study site. The enclosure ended in 1967 with the construction of the Sarraz Bournet docks. The reduction of hydrodynamic energy enhanced fine particles settling within a more sheltered system. The gradual enrichments in Al corresponds to major terrigenous inputs. Enclosure may have limited deposition of marine sediment and increased deposition of continental sediment. Hence, starting from the end of the 1800, the grain-size profile shifted from coarse to finer sediments, with silt dominating over sand.

These environmental shifts induced strong changes in the foraminiferal assemblages. There was a rapid increase in brackish species from ecological group B (*Haynesina germanica*, *Cribroelphiudium gunteri* and *Cribroelphidium williamsoni*) associated with a decrease of marine species from ecological group A. These species are observed in sheltered systems (such as tidal marshes, or lagoons), under freshwater influence (Debenay and Guillou, 2002; Debenay et al., 2005; Debenay et al., 2006). Therefore, the replacement of *C. excavatum* by *H. germanica*, during the last years, indicates the settlement of more brackish conditions (Martins et al., 2015b). These changes are clearly shown in the DCA and the CHA. After the ecological assignment of the species, this first axis of DCA represents a salinity gradient. Subsequently, Cluster I embraces a time interval under marine conditions and Cluster II under brackish ones. The limit between the two clusters corresponds, in fact, to the period when the Carnot dike and the marina of Petit Port were built. One can also notice that the harbour is located along the sides of the Liane River, formerly an estuarine setting. It is reasonable to assume that the freshwater plume might be less diluted in this lower hydrodynamic environment.

The enclosure of Petit-Port most likely induced the infilling of the area. During the last decades, *Cribroelphidium excavatum* is definitively replaced by *Haynesina germanica*. Salinity, vertical elevation and subaerial exposure are important parameters driving the vertical distribution of benthic foraminifera along the intertidal zone (Armynot du Châtelet et al., 2005). Hence, the shift from *C. excavatum* to *H. germanica* dominance would suggest not only more brackish conditions, but an increase, as well, of the elevation of the study site due to silting up.

6.3 Industrial period

From the end of the 19th century, a growing number of commercial and industrial metallurgical activities took place throughout the harbour. The first signal of human industrial activity is shown by the enrichment of several trace metals in the sediments: $EF_{Zn} = 7$, $EF_{Pb} = 5$, etc. Due to the enclosure of the area, pollutants associated with industrial activities became trapped in the sediments of the area. The EF values of trace metals increased, and reach the maxima during the 1970/1980s, surpassing the geochemical background values. The pollution acme during the 1970s is concomitant with the construction of the ferromanganese factory, underlined by the high EF values of Mn, 35 times higher than background values. The effect of high concentration of Mn on benthic foraminifera has yet to be clearly assessed. Langer and Gehring (1994) found that Mn could be associated with organic shell deposits in foraminiferal tests but it is not genetically controlled. However, the effect of trace metals pollution increase corresponds to a reduction in species diversity in foraminiferal assemblages (Coccioni et al., 2009; Martinez-Colon, 2009; Frontalini and Coccioni, 2011; Donnici et al., 2012). For example, experiments on benthic foraminiferal colonization of Cu-contaminated sediments showed that the lowest number of species was associated with the highest Cu concentrations (Alve and Olsgard, 1999). Our results are perfectly in accordance with the aforementioned references: the observed increase in pollutant concentrations at the beginning of the industrial period induced a drop of foraminiferal diversity. However, note that the total concentrations do not offer an accurate assessment of the toxicity of the sediments; therefore, sequential and/or partial extractions need to be performed in the future to gain more insight on the real bioavailable fraction. Nevertheless, it has been pointed out in previous studies (see for instancePandey et al., 2014) that inputs of metals from anthropogenic origin increase the lability percentage of the metals. As a consequence, when metal pollution is detected, as is the case in these sediments, it can be assumed that the fraction of bioavailable metal (and so its concentration) increases as well (for more details see Deckere et al., 2011).

Foraminiferal assemblages during this period are dominated by three species: *Cribroelphidium excavatum*, *Haynesina germanica* and *C. gunteri*. At the beginning of the industrial period, a first drop in *C. excavatum's* relative abundance to 40% is observed. Our results shows that *C. excavatum* cannot tolerate high level of trace metals concentrations. *Haynesina germanica* is considered a tolerant species to trace metal pollution (Samir and El-Din, 2001; Romano et al., 2008; Armynot du Châtelet et al., 2011). In this study, *H. germanica* resisted at the beginning of the industrial period, but at the maximum of the pollution peak during the 1980s, the relative abundance of *H. germanica* dropped. It shows that this species has limited tolerance capacity to heavy metals pollution. *Cribroelphidium gunteri* relative abundances are significantly correlated with trace metals increases. It is a pioneer species with a better colonization capacity and better dispersal ability compared to other species (Debenay et al., 2003; Bouchet et al., 2007). Hence, *C. gunteri* clearly benefited from the heavily degraded conditions. It appears to be as an opportunistic species to trace metals pollution. Decrease in diversity and increase in opportunistic species reflects the increasing footprint of human activities in the Boulogne's harbour and the associated environmental degradation (Leorri et al., 2006; Albani et al., 2007; Alve et al., 2009).

6.4 Recovery period

Over the last 2 decades, the Port of Boulogne has taken measures to protect the environment around its various activities. Hence, sediments deposited after 2000s, are characterized by a gradual decrease of metal concentrations, as shown by the low/moderate values of EF for metals of Group 1. Co, Cu and Cd levels recovered almost completely, with values close to reference conditions. The closing of the ferromanganese factory in 2003 helped these measures. This is evident when looking at EF_{Mn} whose values decreased from 17 in 2002 to 8 in 2012. However, it cannot completely exclude the presence of some processes bound to diagenetic translocation, as has been evidenced in previous studies (see for instance Swennen and Van der Sluys, 2002; Fekiacova et al., 2013; Zhao and Marriott, 2013; Martins et al., 2015a). Considering that Al is not able to migrate easily as dissolved species, this element can be considered as a tracer of fine particles. Fe and Al profiles are very similar, suggesting that Fe is not translocated during possible oxic/anoxic alternations. The possible translocation may occur slightly from 1980 up to now because the maximal concentrations of Cd, Co, Ni, Mn and Zn are not located exactly at the same depth. In a similar way, the decreases of concentrations in the upper layers do not occur identically for all metals. However, it is necessary to suppose that the source of pollution is the same for Ni, Cd, Co, Cu, Mn and Zn, assumption we cannot in reality fully sustain. Consequently, we cannot rule out the possibility that slight translocations of several metals, especially for Cd, Co, Ni, Cu and Zn, took place over the last 20-30 years.

Recovery from chemical disturbance is not only related to the exposure pattern and the mode of action of the toxicant (Barnthouse, 2004). It also depends on ecological factors, environmental variables and the connectivity of the contaminated site to its surrounding environment, which control the speed of recovery of populations exposed to chemical stress (Gabsi and Preuss, 2014). That is why to evaluate the recovery of the site as a whole, it is important to consider the action of the biota in addition to geochemical indices. Several studies have shown the usefulness of benthic foraminifera to assess recovering processes following human-induced disturbances (Leorri et al., 2008a; Hess et al., 2014; Polovodova Asteman et al., 2015). Our results show that benthic foraminiferal diversity increased rapidly after 2006. We observed a change, over a few years, from a "Poor" EcoQ to "Moderate/Good" EcoQ. The same trend was also reported by Stott et al. (1996), who reported the same increase in foraminiferal diversity in response to a decrease in metal contaminations.

6.5 PLI versus diversity index (Exp(H')$_{bc}$)

In recent years, many new methods have been developed to define EcoQS in an integrative way, by using several biotic indices (BIs), together with physico-chemical and pollution elements (to a review look at Blanchet et al., 2008; Borja et al., 2008a). According to Borja et al. (2012) assessing the EcoQ of marine habitats requires: (i) tools (i.e., indices) to assess the relative quality of the considered habitat, and (ii) reference conditions to infer the absolute EcoQ of the considered habitat.

In our study we used Exp(H')$_{bc}$ and geochemical elements to set reference conditions. Bouchet et al. (2012) defines criteria based on foraminiferal diversity to assess EcoQS. Following the same approach, it is possible to assess Petit-Port Palaeo-EcoQs. The EcoQ of a study site is the measurement of the deviation from the reference conditions. Defining local reference conditions allows a more precise assessment of EcoQS, rather than the use of an absolute scale (Krause-Jensen et al., 2005). Note that the reference conditions for Petit-Port corresponds to a maximal diversity of 24 (corresponding to the period roughly before the 19th century). During the industrial period, a drastic drop of the diversity to 7 is observed, which means there was a rapid environmental degradation from excellent to poor EcoQS. In this work, reference conditions were based on historical data, i.e., fossil data, through hindcasting. Although there are few studies questioning the use of palaeoecology to define reference conditions (Borja et al., 2012), this study demonstrated clearly that the hindcasting method can be applied to define reliable reference conditions. This supports other works (Alve, 1991b; Andersen et al., 2004; Alve et al., 2009; Dolven et al., 2013) promoting fossil assemblages as a suitable method for defining reference conditions.

Based on this, to show in a synthetic way the evolution of the PalaeoEcoQS through time, we proposed the use of the plot Exp(H')$_{bc}$-PLI (**Figure 4.11**). PLI represents the environmental conditions and Exp(H')$_{bc}$ the state of the foraminiferal assemblages. Along the trend line, which follows the temporal displacement of the points, three areas can be distinguished. They correspond exactly to the periods identified in the study: i) pre-industrial period (reference condition): no anthropogenic impact, "High" and "Good" EcoQS; ii) industrial period: high anthropogenic impact, principally "Poor" EcoQS; and iii) recovery period: decrease of anthropogenic impact, "Moderate" and "Good" EcoQS. In this plot a large deviation is evidenced during the industrial period, compared to the reference conditions. It is also undoubtedly visible that even though concentrations of pollutants do not recover to reference conditions (as the pre-industrial period), the increase of the diversity, during the recovery period, follows the tendency towards a resilience of the environment (Elmqvist et al., 2003).

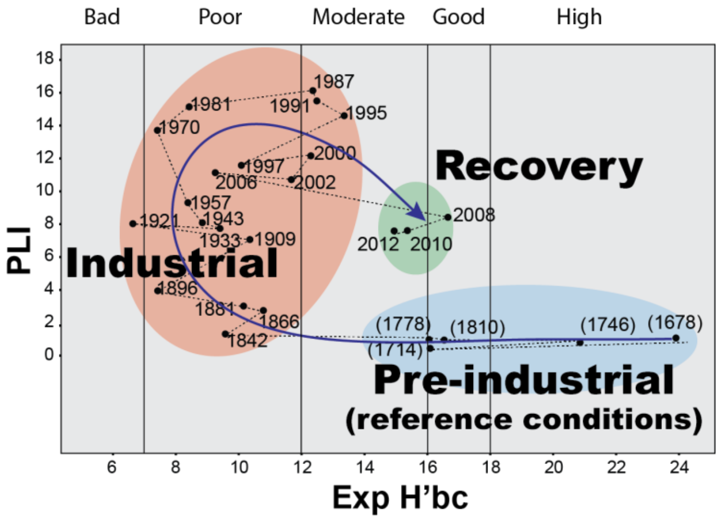
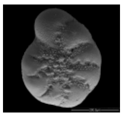


Figure 4-11 PLI vs. Diversity: Relation between Tomlinson's pollution load index (PLI) and diversity Exp(H')$_{bc}$. Three areas can be identified along the trend line corresponding to: i) pre-impacted period, ii) industrial period, and iii) recovery period.



7 Conclusion

The current study is a good example of the strong potential of palaeoecology to answer questions about the ecological quality status of modern environments. It points out the efficiency of the foraminiferal method to define temporal *in situ* changes in ecological quality status. It also shows that hindcasting, using fossil benthic foraminifera, is an effective and relevant option to define *in situ* reference conditions within the WFD, and to set up targets within the MSFD. In coastal settings, especially harbour areas, the interpretation of fossil evolution is often problematic, because natural and human-induced stresses are simultaneous. In our study, the use of the multiproxy approach has allowed us to better understand the role of human impact on the transformation of the study areas, and to assess the variations of the PalaeoEcoQS. A pre-impacted phase has been distinguished from an impacted one. In more recent years, better ecological conditions suggest a tendency towards an environmental recovery. Our results provide a baseline for future environmental bio-monitoring in coastal areas of Northern France.

Chapter 5 One hundred years of environmental transformations in the Canche estuary: historical pictures vs benthic foraminifera

1 Introduction

Benthic foraminifera have fast turnover rates and quick responses to environmental parameters changing (Boltovskoy et al., 1991; Murray, 2006). They are able to organise in specific assemblages depending on different environmental conditions (Bouchet et al., 2007; Armynot du Châtelet et al., 2009b; Frontalini et al., 2014; Seuront and Bouchet, 2015). Their high abundance and biodiversity in marine sediments allows a good preservation potential along the sedimentary record (Alve, 1991a; Murray, 2007). For these reasons, they have been largely used in palaeoenvironmental reconstructions and to monitor local and global changes in marine habitats (Alve, 1991a; Hayward et al., 2004; Leorri et al., 2008a). In modern tidal areas, salt marsh foraminifera have been shown to occur within narrow vertical zones, constrained by the tidal frame (Scott and Medioli, 1978; Scott and Medioli, 1980; Horton and Murray, 2007). As a consequence they are considered as extremely efficient in sea level reconstructions (Gehrels, 1999; Horton and Murray, 2007; Callard et al., 2011). However Berkeley et al. (2007) highlight that the use of benthic foraminifera as sea level tracers is accurate only when all environmental variables exhibit a strong degree of covariance with tidal elevation. Depending on the study site, salinity, grain-size or food supply can play a primary role on the faunal distributions (de Rijk, 1995; Fatela et al., 2007; Armynot du Châtelet et al., 2009a; Fatela et al., 2009).

Taphonomic processes involve post-mortem modifications of the assemblages during the burial. For example, in areas with high variability of water pH, like salt marshes, the dissolution of calcareous test can alter the fossil assemblages (Goldstein and Watkins, 1999). At the salt marsh/tidal flat transition in the Canche Estuary Armynot et al. (comm. Pers.) observed differences between the dead fauna and the living one. The dead fauna resulted as a mix of autochthonous and more marine specimens. As a consequence, comparison between modern and past assemblages in salt marshes (Jonasson and

Patterson, 1992; Patterson et al., 1999) make palaeoenvironmental reconstruction based only on fauna controversial (for a review look atBerkeley et al., 2007). To bypass taphonomic problems, the use of multidisciplinary approaches allows a better interpretation of the modern environments (Patterson et al., 2005; Grauel et al., 2013; Delaine et al., 2014). To combine geochemical and meiofaunal monitoring programs is recommended to reflect the palaeoenvironmental quality in estuarine systems (Leorri et al., 2008a; Francescangeli et al., 2016).

For paleo-environmental reconstruction, the estimation of the sedimentation rate and the relative age of the correspondent sediments appears essential. Over the past 40 years, the ^{210}Pb dating (associated to ^{137}Cs) constitutes one of the most common means for dating the most recent sediment (about 150-200 years) for historical reconstructions (Appleby and Oldfield, 1978; Appleby, 2001). However in areas where there is large scale disturbance of sediments (such as erosional episodes) the vertical profiles of excess ^{210}Pb ($^{210}\mathrm{Pb}_{xs}$) could provide erroneous or less reliable information on sediment accumulation rates (Baskaran et al., 2014). As a method of dating, the use of historical maps and pictures can provide as well important information on the environmental variations (Lukas, 2014), such as costal evolutions (Crowell et al., 1991) or salt marsh losses (Bromberg and Bertness, 2005). Note that this method should be use with cautious considering issues related to both the survey moment (high tide vs low tide) and image quality (Stäuble et al., 2008).

The present work is part of comprehensive study on the recent evolution of coastal intertidal environments in Hauts-de-France region (Northern France). Its objective is to assess environmental transformations within the salt marsh of the Canche estuary (Hauts-de-France, Northern France), considered as under low human pressure. Thanks to a multiproxy approach (based on benthic foraminifera and environmental parameters) we test the value of historical pictures to support the abiotic and biotic data, and finally reconstruct the evolution of the coastal area landscape.

2 Materials and methods

2.1 Study area

The study was carried out on the tidal marsh of the Canche estuary (**Figure 4.12**) (Nord-Pas-de-Calais currently Hauts-de-France, Northern France). The marsh area (ca. 7 km^2) is ending an 88 km long river having a relatively small drainage basin of 1396 km^2. It could be considered as an "estuarine back-barrier" marsh type (Pye and French, 1993). The morphology is marked by the presence of a ridge and runnel system on its marine side. This type, protected by a coastal barrier, is usually muddy when watered by a major river. The Canche estuary displays globally muddy sediments (silt dominated), which became very fine (clayish mud) in the innermost parts of the estuary. The marsh area is a planar vegetated platform regularly flooded by tides. The vegetation, marking the transition between the tidal flat and the salt marsh, is mostly dominated by halophytic grasses and herbs. Because of a tidal range > 6 m (hyper-tidal estuary following the classification of McLusky (2004), exceeding 9 m during highest astronomical tides, the morpho-sedimentary dynamic of the Canche estuary is strongly influenced by the tide. The water circulation as well, is mainly dependent on the tides and on a small freshwater input of about 13 m^3 s^{-1} (Selleslagh and Amara, 2008). The tidal regime is semidiurnal under bi-monthly periodicity, responsible to cycles of minimal and maximal tidal range. In spring tide conditions, the seawater broadly enters the estuary. During neap tide, salt marshes are free of marine water and mixed flat is only influenced by river water (even though the river flux is not sufficient to fill all the estuary). Water temperature closely follows the sea temperature and that of the river. Values range between 9 °C in winter and 18 °C in summer (Armynot du Châtelet et al., 2009a). The Canche estuary can be considered as a little impacted environment, linked only to urban development (Amara et al., 2007).

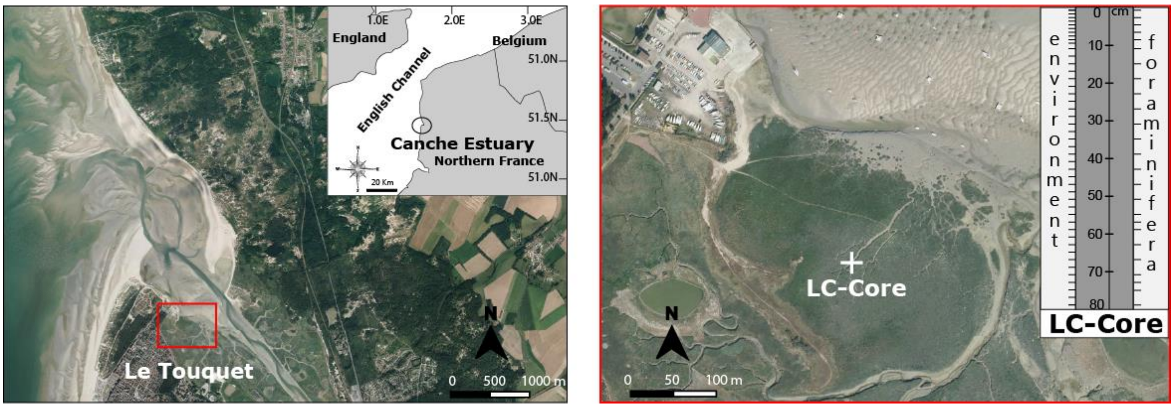


Figure 5-1 Location of the study area in the Canche estuary, Northern France. At right position of LC-Core in the middle salt marsh, with a scheme of the sample frequency for environmental and micro-faunal analyses. (From Aerodata France, PPIGE 2009, Hauts-de-France Regional Council).

2.2 Core sampling

An 80-cm long core (1.59° E 50.53° N; 4.3 m above MSL), sampled on July 2013 from the middle marsh area. It was sectioned every 1 cm down to 10 cm and every 2 cm till the bottom of the core; this resulted in the collection of 46 samples.). A first aliquot was used to measure environmental variables (sediment grain-size, carbonate content, major and trace elements concentrations, total organic carbon and Sulphur) and dating (^{210}Pb and ^{137}Cs activity). A second aliquot, with a different sampling interval (25 samples were selected, one in two adding samples 56-58 and 68-70) (**Figure 4.12**) was used for micro-faunal analysis.

2.3 Grain-size and total organic carbon

Grain-size analyses were performed using the principle of diffraction and diffusion of a monochromatic laser beam on suspended particles (Malvern Mastersizer 2000, red He-Ne laser). The method is based on near-forward scattering of a laser beam by particles in suspension, as detailed previously (Trentesaux et al., 2001). Measurements can range from 0.02 to 2,000 µm with an obscuration ranging between 10 and 20%. Four grain-size fractions were considered: clays (< 4 µm), fine silts (4-10 µm), cohesive sortable silt (10-63 µm) and sands (63 to 2000 µm). Silt fraction and motility are discussed in McCave et al (1995). The sorting as descripted parameter of the grain-size was calculates following Folk (1957); better is sorted lower are the values.

A Flash EA 1112 Elemental Analyzer (Thermo) equipped with an auto-sampler was used for determining total contents of C and S. The analysis was performed on 1.5 to 2 mg of sample added to approximately 5 mg of vanadium pentoxide, used as a combustion catalyst. 2.5-Bis (5-tert-butyl-benzoxazol-2-yl) thiophene (BBOT) was used as standard. The Total Organic Carbon (TOC) was determined by subtracting carbonate carbon from total carbon concentrations. This calcium carbonate content was determined using a Bernard calcimeter. Triplicated measurements were carried out for each sample using 0.5 g of finely crushed sediment.

2.4 Major and trace element concentrations (total mineralization)

The total concentration of Al, Cd, Cu, Fe, Pb, and Zn were analysed for each selected level). After drying the sediment particles at room temperature, samples were sieved at 63 μm and gently crushed. Then, 200 mg of the fine fraction were attacked first with a mixture of 5 mL of suprapur nitric acid and 10 mL of a concentrated HF solution at ebullition over 48 hours. After evaporation of these acids, 10 mL of a freshly prepared HNO_3/HCl mixture (1/2 v:v) was added in order to eliminate the remaining solid grains. The recovered solutions were subsequently diluted in a known volume of ultrapure water and analysed using ICP-AES (inductively coupled plasma – atomic emission spectroscopy; varian vista pro, axial view) and ICP-MS (inductively coupled plasma – mass spectroscopy; thermo elemental X series). This attack procedure was validated and the accuracy of the analytical procedure was checked by means of the following sediment standard reference materials (Canadian International Standards): HISS-1, MESS-3 and PACS-2. It was found that standard materials certified and measured results were in good agreement.

In order to assess sediment contamination and to evaluate possible anthropogenic influences, the enrichment factor (EF) (Woitke et al., 2003; Duan et al., 2014) was calculated for each element (for details look at Francescangeli et al., 2016). The enrichment factor is normalized against aluminium to take into account the evolution of the grain-size, especially concerning the fine particles (silts and clays), overcoming partly the bias bound to the nature of the sediment. For the calculation of EFs, reference values were taken from the very bottom of the core and compare to a previous study in the same regional area (Francescangeli et al., 2016). The following criteria are used to evaluate the degrees of pollution according to the values of EF: EF<2: no relevant enrichment; 2<EF<10: moderate contamination; EF>10 strong contamination (Birth, 2003; Kerambrun et al., 2012b).

2.5 Chronology

The samples have been analysed for the activity of ^{210}Pb, ^{226}Ra and ^{137}Cs via gamma spectrometry at the Gamma Dating Centre, Department of Geosciences and Natural Resource Management, University of Copenhagen. The measurements were carried out on a Canberra ultra-low background Ge-detector. ^{210}Pb was measured via its gamma-peak at 46.5 keV, ^{226}Ra via the grand daughter^{214}Pb (peaks at 295 and 352 keV) and ^{137}Cs via its peak at 661 keV. The concentration of unsupported ^{210}Pb was found by subtracting the supported ^{210}Pb (measured as ^{214}Pb) from the total ^{210}Pb concentration.

2.6 Aerial pictures

Several historical aerial pictures were used to observe the environmental variations through time (from 1935 to 2009). The pictures were selected from http://www.geoportail.gouv.fr. Each picture was integrated in QGIS (version 2.14.5) using at least four ground control points. The coordinates were integrated using a reference map already present in the GIS with a coordinate system (Aerodata France, PPIGE Nord-Pas-de-Calais currently Hauts-de-France). The reference coordinate system is Lambert 93 (RGF93). Stable territorial features (such as buildings, crossroads) were used as ground control points. In each pictures the exact location of the LC-CORE has been plotted. In order to allow temporal image comparisons, it was adopted the same areal extend and all the pictures were converted to grayscale.

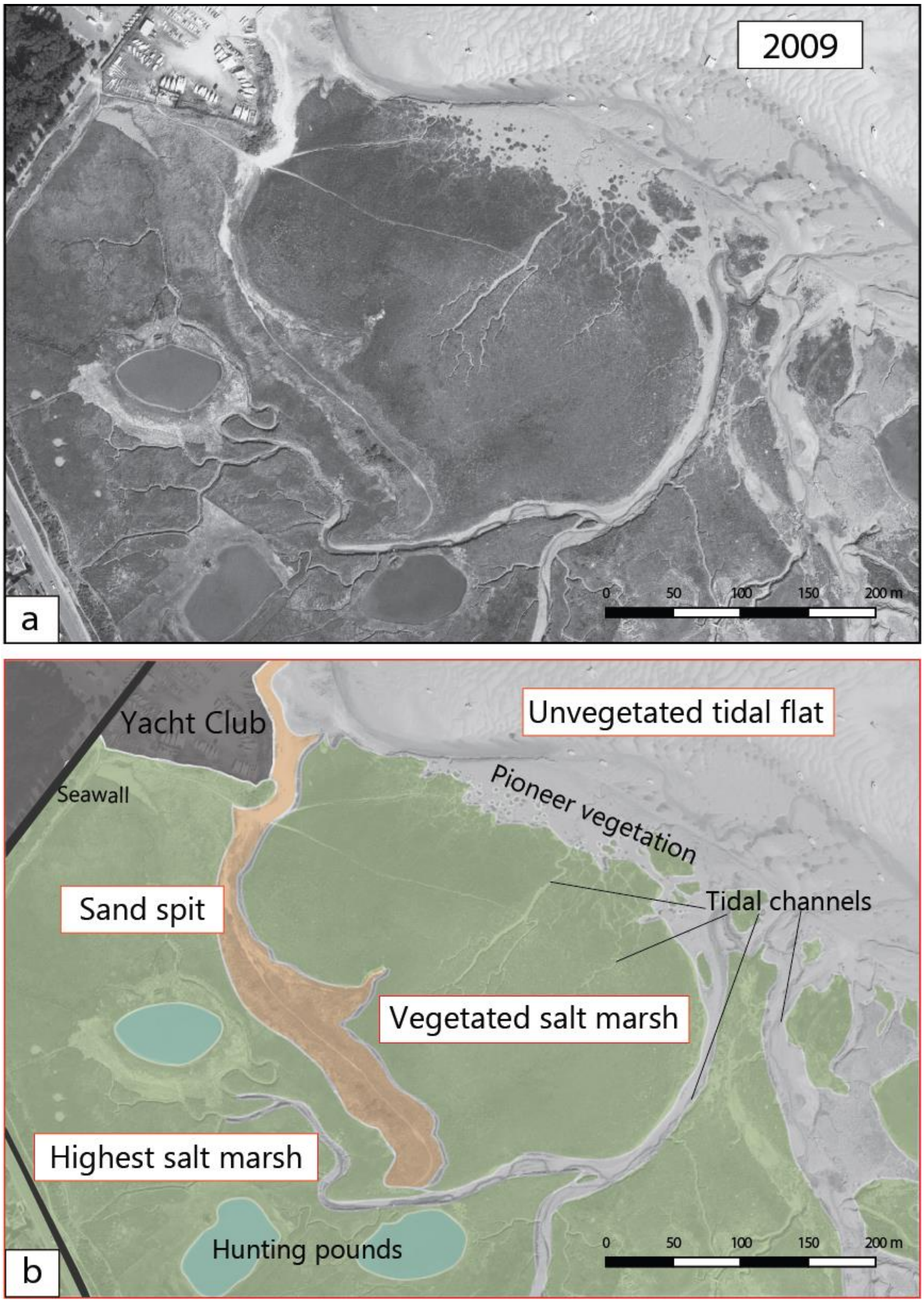


Figure 5-2 a) Aerial picture of study area in 2009 from PPIGE 2009 (Aereodata France); b) Interpretation of the principal sedimentary objects. The vegetated marsh area is darker than the un-vegetated tidal flat. Sand bodies (such as sand spit) have generally a lighter color than mudflat sediments. In the highest marshes hunting pounds can be identified. Tidal channels are draining the salt marsh area

The reference map PPIGE 2009 **(Figure 4.13)** displays the way the pictures have been interpreted. Based on many field campaigns, we were able to associate each colour (or shades of grey) with a given surface sediment and coverage. The studied, natural, intertidal surface is separated from the supratidal zone by two rectilinear seawalls. In front of them, the vegetated salt marshes are darker than the un-vegetated tidal flats. In this area, an elongated sand body corresponding to a sandspit extending from the Yacht Club parking appears in lighter colour than muddier tidal flat sediments. Sand spits usually develop parallel to the coast according to the longshore drift direction. This is the case here where, due to a man-shaped coast (embankments), the spit has a bowed shape. At the origin, the sand spit separated two domains. One in front with quite exposed conditions corresponding to a sandy surface tidal flat or internal beach and, behind it, a protected area prone to mud sedimentation where salt marshes developed. This is no longer the case as salt marshes also developed in front of the 'abandoned' spit that transformed in a narrow eolian dune field. The lowest part of the tidal flat is sandier and is covered by hydraulic sand dunes, especially in the deepest parts of the main Canche channel. Tidal channels are draining the salt marsh area. In the highest salt marshes hunting pounds can be identified.

2.7 Foraminiferal analysis

The foraminiferal samples were weighted, washed through a 63 μm mesh sieve, and then oven dried at 40 °C. The investigations were carried out on the >63 μm size fraction. Approximately 300 tests were picked in each sample. This number is recommended to consider a species proportion of more than 1% associated to a probability of failure to detect of 1% (Fatela and Taborda, 2002); this value is realistic for such a study. All the picked specimens were mounted on faunal micro-slides, identified following Loeblich and Tappan (1988) for genera, Debenay (2012) and Debenay et al. (2001) for species classification. A binocular microscope, model Olympus SZX16, was used for the observations. The relative and the absolute abundance of the taxa as well as the faunal density (Tests/g) were determined for each sample. The effective number of species $\mathrm{Exp}(H')_{bc}$, (for details see the work of Bouchet et al., 2012), which is a most suitable measure of diversity (Beck and Schwanghart, 2010), was considered in the present study.

Groups	Species
Middle/High Salt marsh	*Entzia macrescens* (elsewhere)
	Quinqueloculina seminula (Francescangeli et. sub.) (Shaw et al., 2016) (Franceschini et al., 2005)
	Trochammina inflata (elsewhere)
Low Salt marsh	*Bolivina pseudoplicata (Francescangeli et. sub.)*
	Brizalina variabilis (Francescangeli et. sub.)
	Buliminella elegantissima (Francescangeli et. sub.)
	Cribroelphidium gerthi (Francescangeli et. sub.)
	Cribroelphidium williamsoni (Francescangeli et. sub.) (Debenay and Guillou, 2002; Armynot du Châtelet et al., 2009a) (Berkeley et al., 2007)
	Haynesina germanica (Debenay and Guillou, 2002) (Francescangeli et al. sub.) (Horton and Murray, 2007) (Haslett et al., 2001)
Tidal channel/tidal flat	*Cribroelphidium excavatum elsewhere (Alve and Murray, 1999; Horton and Murray, 2007; Müller-Navarra et al., 2016)*
	Cribroelphidium magellanicum (Francescangeli et al. sub.)
	Cribroelphidium margqtaceum (Francescangeli et al. sub.)
	Cribrononion gerthi
Seaward	*Rosalina sp. (Horton and Murray, 2006)*
	Lobatula lobatula (Alve and Murray, 1999)
	Hopkinsina atlantica
	Stainforthia fusiformis (Alve, 1994)
	Bulimina elegans (Murray, 1991)
	Bolivinita quadrilatera ??

Table 1. Main species grouped along the tidal gradient and associated references

To evaluate palaeoenvironmental variations, foraminiferal species were assigned to 4 groups according to the tidal vertical gradient (based on previous works, look forward). The four groups are: A) Middle/high salt marsh, B) Low salt marsh, C) Tidal flat/tidal channel, D) Seaward. It needs to point out that in the Seaward group were included species which have never (or rarely) been found as living within the salt marsh/upper tidal flat of Canche estuary (Francescangeli et 2016, submitted). These allochthonous specimens generally live in more subtidal condition, probably transported upstream during more energetic sedimentation processes (It is what Eric observes in the paper the 1 m^2 as well). As pointed out in Francescangeli (2016), local environmental factors should be considered to characterize the distribution of species along a coastal gradient. Hence the attributions of the main species (Tab. 1) are principally based on the local works of Francescangeli et al. (Submitted) and Armynot du Châtelet (2009b) in the Canche estuary. Species

assignments were then compared and implemented to the wider works of Debenay and Guillou (2002), Scott et al. (2001) and Murray (2006) and to other foraminiferal studies in macrotidal/hypertidal estuaries (such as Haslett et al., 2001; Horton and Murray, 2007). In order to avoid possible taphonomic effects only the main species with a minimum of f (frequency) ≥ 10 samples and an average relative abundance $\geq 3\%$, were considered. The loss assemblage (percentage of specimens not considered in the grouping) was indicated as well at each level.

2.8 Data analysis

The main species were grouped using a constrained hierarchical clustering analysis (HCA). A similarity tree was produced using the Euclidian distance. Coniss (Grimm, 1987) was used as the clustering method.

A detrended correspondence analysis (DCA), an ordination method that arranges samples and species along gradients, was carried out on the species' relative abundances. It arranges samples that are more similar in species composition closer together. DCA uses the variation in species composition between the samples to determine the underlying gradients influencing the data. The basic assumption of this method is that the most important environmental gradient causes the largest variation in the species composition. By means of a two way weighted averaging algorithm, the direction of this variation was calculated and represented as the first DCA axis (Hill and Gauch, 1980; Versteegh and Zonneveld, 1994).

The R software (version 3.2.2) (R-Core-Team, 2014) was used for the calculations, by using the following packages: Base (descriptive statistics), Entropy (diversity calculation), Rioja (construction of diagrams with timescale), Vegan, Hmisc, ade4 (HCA and DCA), ggplot2 (histograms of foraminiferal relative abundances).

3 Results

3.1 Chronology

Analysis of ^{210}Pb and ^{137}Cs do not allow having a reliable dating of the core (**Figure 4.14**). We can just notice a peak of ^{137}Cs at 24cm which could correspond to the 1984. It could be connected to the accumulation of nuclear reprocessing activities at COGEMA-La Hague in France in that period (Cearreta et al., 2002), already observed in Marion (2014) in the Authie estuary (Northern France). Hence the few points, above the detection limit, could allow only approximate estimation of the sedimentation rate about 0.84 cm/yr for the first 24 cm.

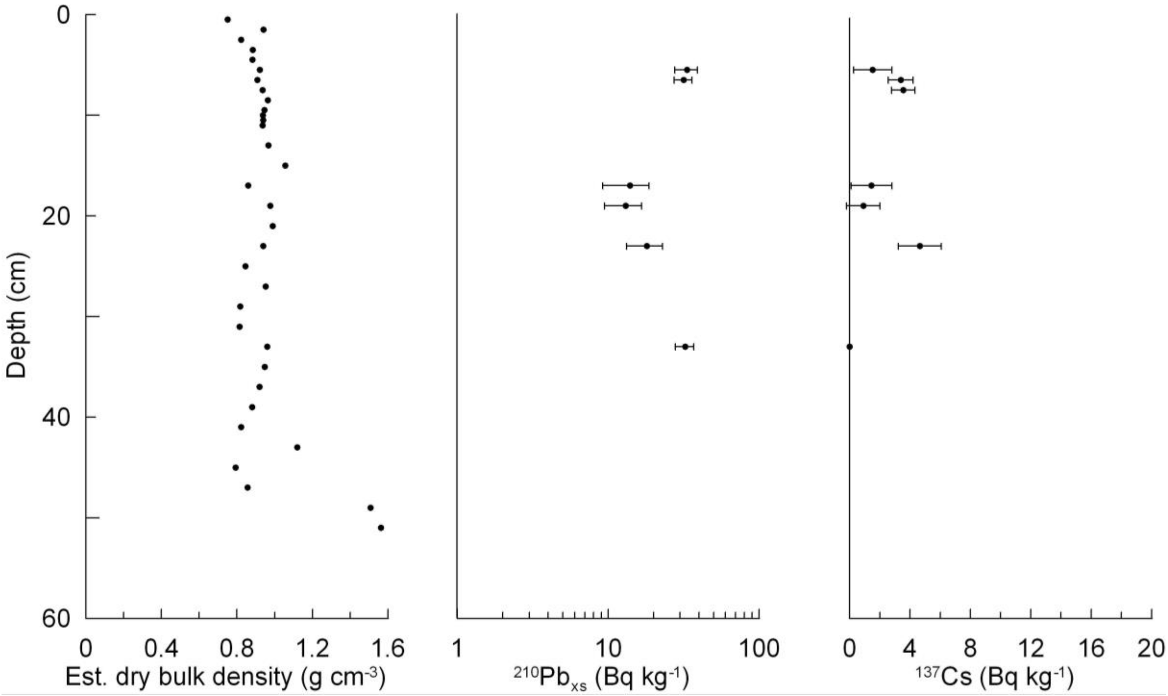


Figure 5-3 Dry bulk density and profiles of ^{210}pb and ^{137}Cs activity

3.2 Environmental variables

The core starts from its base with sediments composed by about 40% of sand and 60% of silt (**Figure 4.15a**). At 72 cm, there is a sudden increase in sand content, reaching 93%. The sand fraction gradually decreases from 58 to 48 cm to reach values close to 20%, replaced by silts and clay. Sortable silt dominates the silty fraction. Clay is the less abundant grain-size fraction (max 5%) showing the same trend of fine and sortable silts. Sediment is poorly sorted, showing the lowest values (i.e. better sorted) close to 1 in the 67-61 cm sandiest interval.

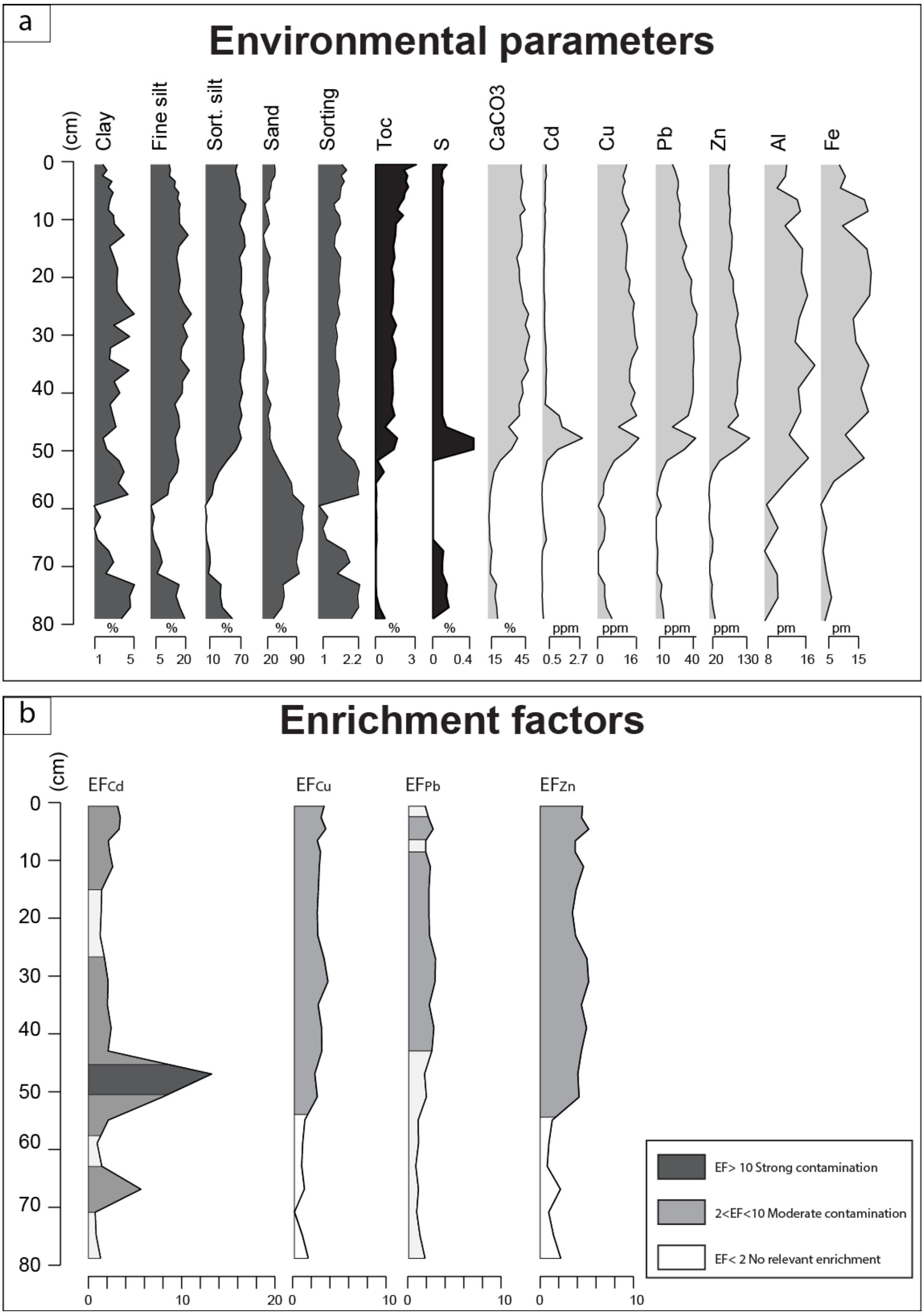


Figure 5-4. a) Trends of environmental parameters along the CL-Core: grain-size (Clay, Fine silt, Sortable silt, Sand, sorting), TOC, Sulphur (S), CaCo$_3$, trace metals (Cd, Cu, Pb and Zn), Al, Fe. b) Enrichment factors (EFs) for trace metals (Cd, Cu, Pb and Zn) along the CL-Core.

The TOC content (**Figure 4.15a**) ranges between 0% and 3.05%. It shows the lowest values in the bottom part (close to 0%) and increases from 55 cm upward. Sulphur (**Figure 4.15a**) shows low values (<0.1% on average) throughout the core, but a peak (0.44%) at 51-49 cm. The $CaCO_3$ trend (**Figure 4.15a**) is similar to the one of the TOC, with the highest values (48%) from 55 cm upward.

All the analysed elements (Cd, Cu, Pb, Zn, Al and Fe) show the lowest values in the bottom of the core (**Figure 4.15b**). From 55 cm upward there is a rapid increase of them, reaching stable values. Only Cd peaks at 51-45 cm (coinciding to the peak of sulphur). Enrichment factors (EFs) of Cu, Pb and Zn show the same trends (**Figure 4.15b**): there is no relevant enrichment in the bottom of the core ($EF_{max}<2$) and just moderate contamination starting from 55 cm ($EFZn_{max}= 5$). Cadmium presents globally the same trend of trace metals, however a strong contamination peak occurs at 47 cm ($EF_{Cd}= 13$).

3.3 Foraminiferal assemblages

A total of 78 species were identified along the core. Sixty-eight of these (87%) are hyaline, six are porcelaneous and four species are made of agglutinant test (Counting table in supplementary materials). A barren zone occurs at the sandy 61-67 cm interval. The assemblage (**Figure 4.16** relative abundance; **Figure 4.17** absolute abundance) is dominated by *Haynesina germanica* associated to *Cribroelphidium margaritaceum*, *Quinqueloculina seminula*, *Entzia macrescens,* and *C. excavatum*. *Cribroelphidium margaritaceum* (2-38%; 10% on average) and *C. excavatum* (0-41%, 8.8% on average) occur principally in the bottom part of the core. *Haynesina germanica* (4-71%; 29% on average) is largely dominant in the middle part of the core; the lowest values of its relative abundance (and absolute) are within the intervals and 71-51cm and 8.5-0.5cm. *Quinqueloculina seminula* (0-39%; 10% on average) and *E. macrescens* (0-47%, 9% on average) are the most common in the upper part of the core. Within the minor species (<5% on average) bolivinids (*Bolivina quadrilatera, B. pseudoplicata, B. variabilis, Bulimina elegans* and *Buliminella elegantissima*) occur irregularly along the core, (the lowest values in the upper part), while *Trochammina inflata* is present only in the upper part.

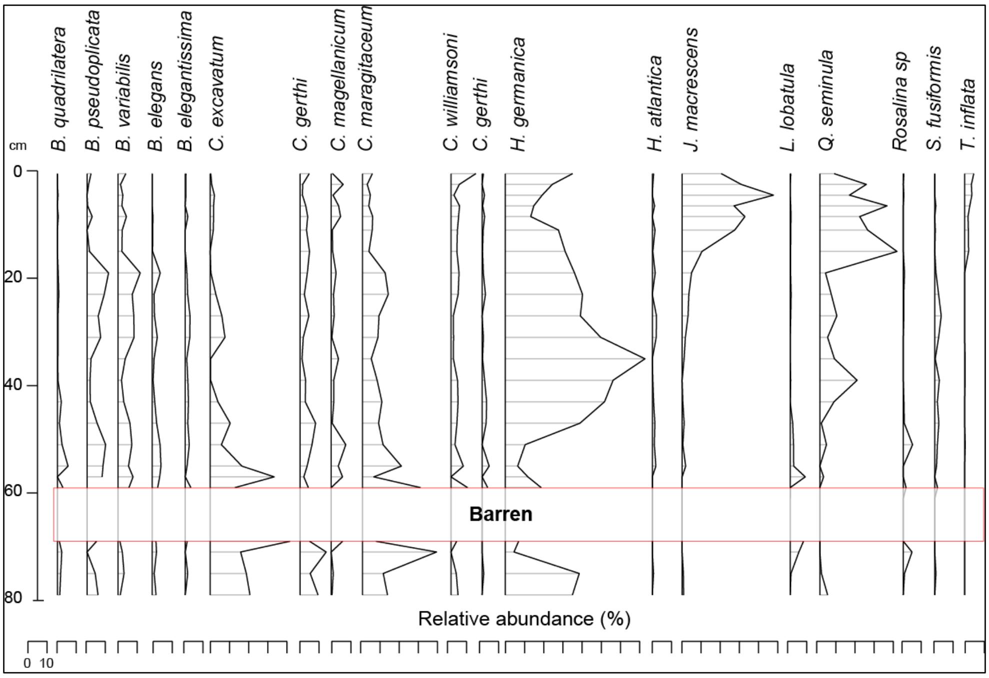


Figure 5-5. Trends of the relative abundance of the main species

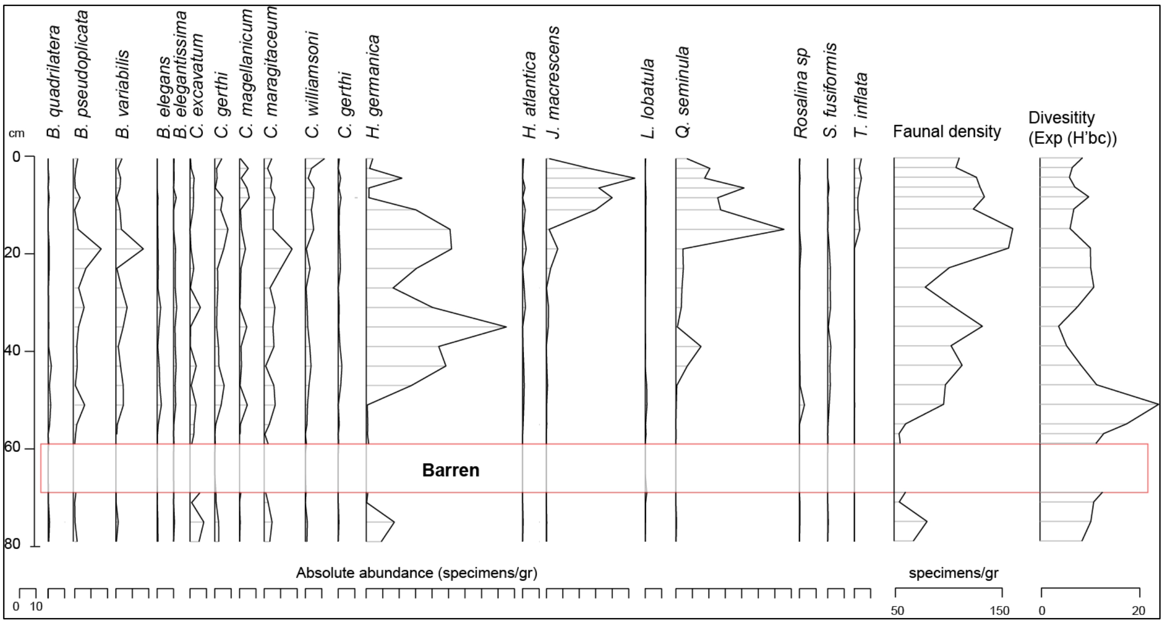


Figure 5-6. Trends of the absolute abundance of the main species (specimens/gr), faunal density ((specimens/gr) and diversity (Exp(H')$_{bc}$) along LC-Core

The faunal density (**Figure 4.17**) varies between 6 and 166 (tests/gr). Lowest values are found at the base of the core and rapidly increase from 55 cm upward. The diversity

(Exp(H')$_{bc}$) (**Figure 4.17**) ranges from 4 to 24. It does not show a clear trend however on average the highest values are located in the bottom of the core, below 45 cm.

3.4 Ecological groups

Nineteen species were assigned (according to selected criteria) to 4 ecological groups and considered forward for statistical analysis (**Figure 4.18**; **Figure 4.19**). The average of loss assemblage due to the grouping is 5.86%. In the very bottom part of the core Low salt marsh is the dominant group (> 50%). In the 71-55 cm interval, Tidal flat/Tidal channel group dominates and decreases upward. The Low salt marsh group largely dominates in the middle part of the core (47-19 cm) with proportions on average greater than 60%. In the upper part of the core Middle/high salt marsh group takes over becoming the dominant one.

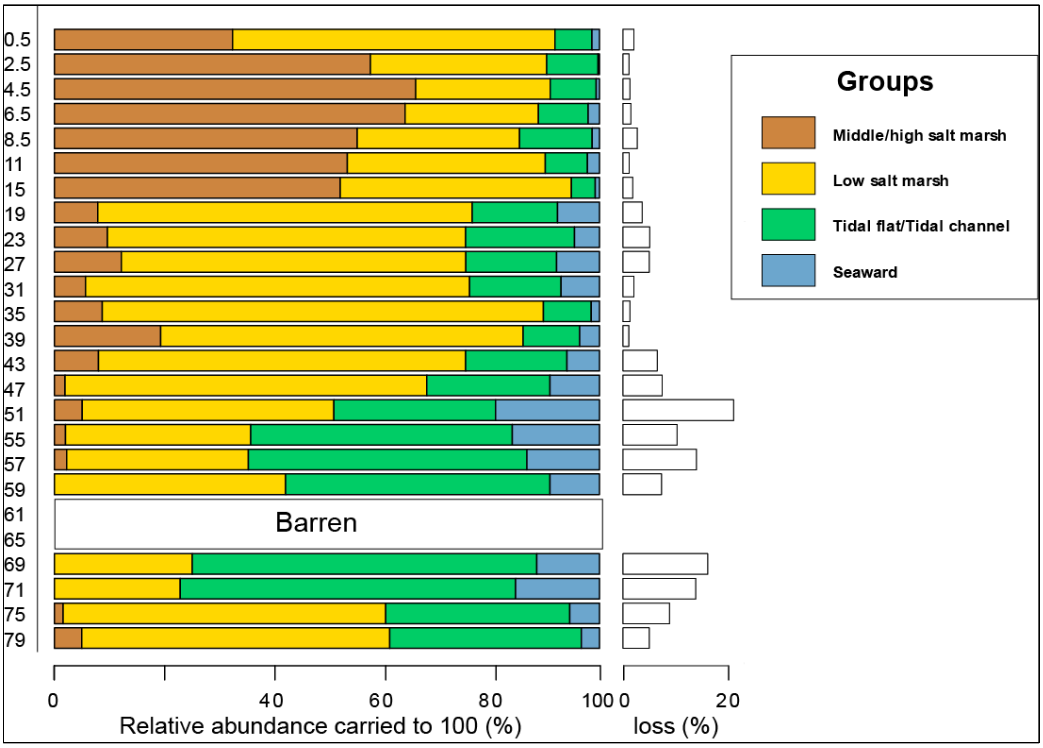


Figure 5-7. Histograms of the temporal evolution of the ecological groups carried to 100 (%). The grouping is based on the relative abundances of the assigned species. White bars represent the assemblage loss (%) at each level.

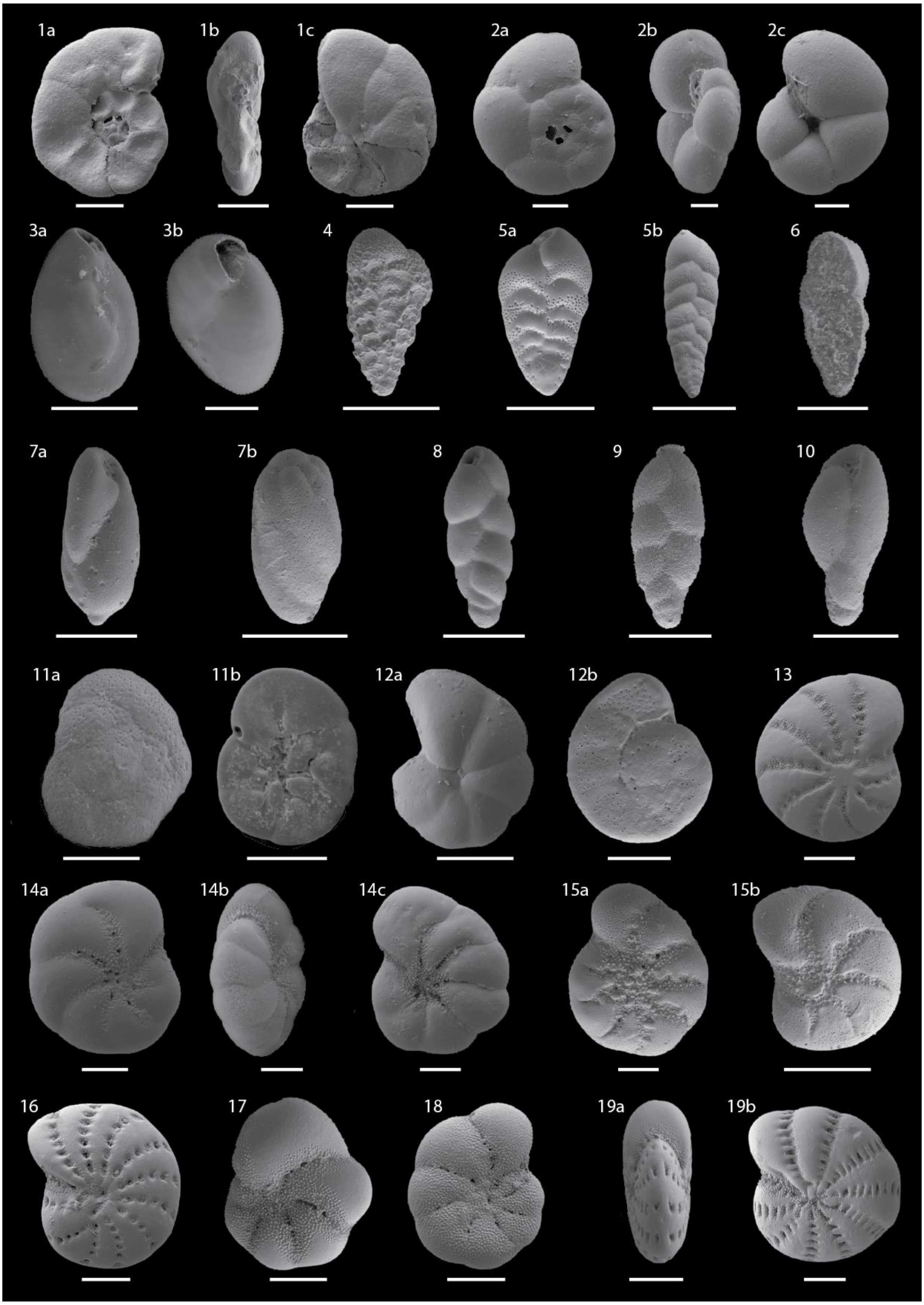


Figure 5-8. Scanning Electron Microscope (SEM) photographs of the main species of benthic foraminifera in the Canche core. Entzia macrescens (a) spiral side with secondary apertures, (b) apertural view, (c) umbilical side; (2) Trocammina inflata (a) spiral side, (b) apertural view, (c) umbilical side; (3) Quinqueloculina seminula (a) side view, (b)

apertural view ; (4) Bolivina pseudoplicata; (5) Brizalina variabilis (a) side view (b) side view; (6) Bolivina quadrilatera; (7) Buliminella elegantissima (a) apertural view (b) side view; (8) Bulimina elegans; (9) Hopkinsina atlantica; (10) Stainforthia fusiformis; (11) Rosalina sp. (a) spiral side, (b) umbilical side; (12) Lobatula lobatula (a) spiral side, (b) umbilical side; (13) Cribrononion gerthi; (14) Haynesina germanica (a) side view (b) apertural view (b) side view; (15) Cribroelphidium excavatum (a) side view (b) side view; (16) Cribroelphidium gerthi; (17) Cribroelphidium magellanicum; (18) Cribroelphidium margaritaceum; (19) Cribroelphidium williamsoni (a) apertural view, (b) side view.

3.4.1 Sample grouping and vertical structure of the core

In the HCA analysis, three clusters I, II, III are identified along the CL-Core (**Figure 4.20**). Cluster III includes all samples from the bottom of the core up to 43 cm. Cluster II includes samples from 39 to 19 cm. Cluster I contains all samples from the upper part of the core. The analysis is only based on foraminiferal relative abundances, hence each cluster represents the variation of different environmental conditions through time.

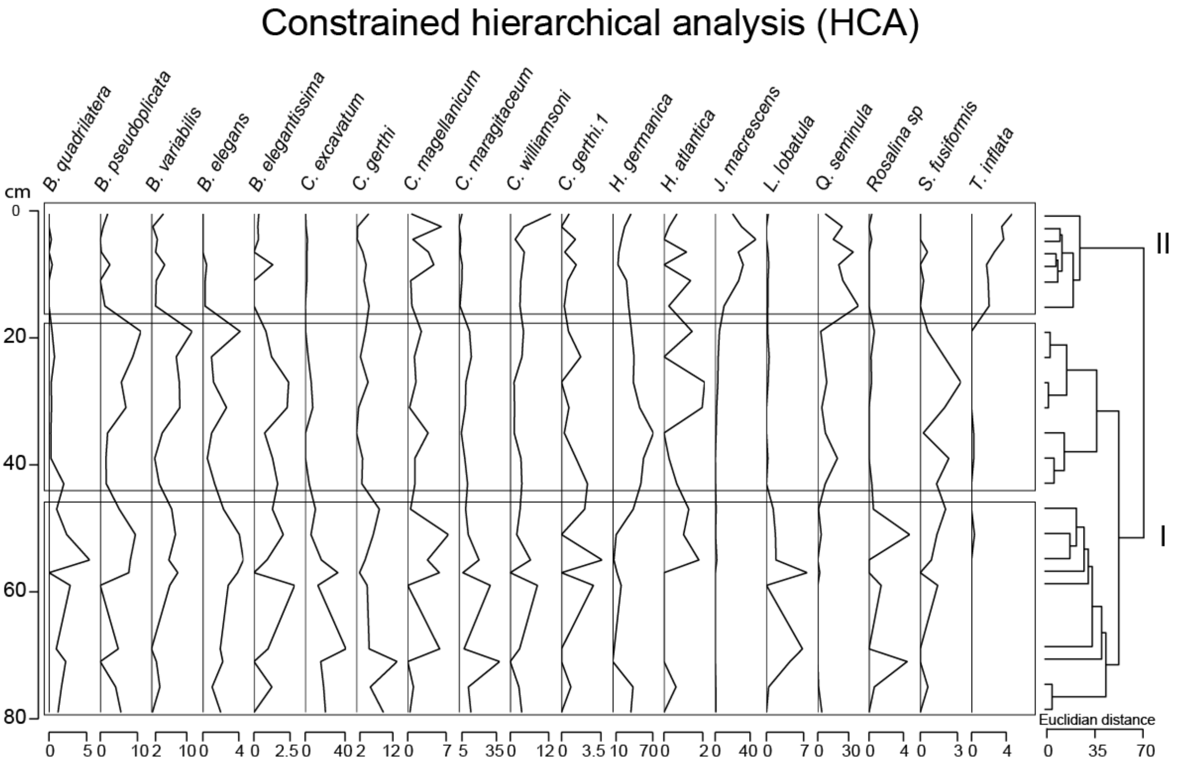


Figure 5-9. Hierarchical cluster analysis (HCA) based on the relative abundance of the main species. Two Clusters can be identified along the LC-Core reflecting different environmental conditions.

In the DCA, the two first axes explain about 25% of the total variation (eigenvalues of 20% and 5%, respectively) (**Figure 4.21**). Samples are chronologically placed along the first axis. Samples belonging to Cluster I are placed at the positive and samples belonging to Cluster III at negative position of the first DCA axis, respectively. Foraminiferal species belonging to the Middle/High salt marsh group are located at the positive position of the first axis. Specimens of the Low salt marsh group are mainly places at positive of the first axis. On the contrary species belonging to the Tidal flat/Tidal channel and Marine infralittoral are principally located at the negative of the first axis.

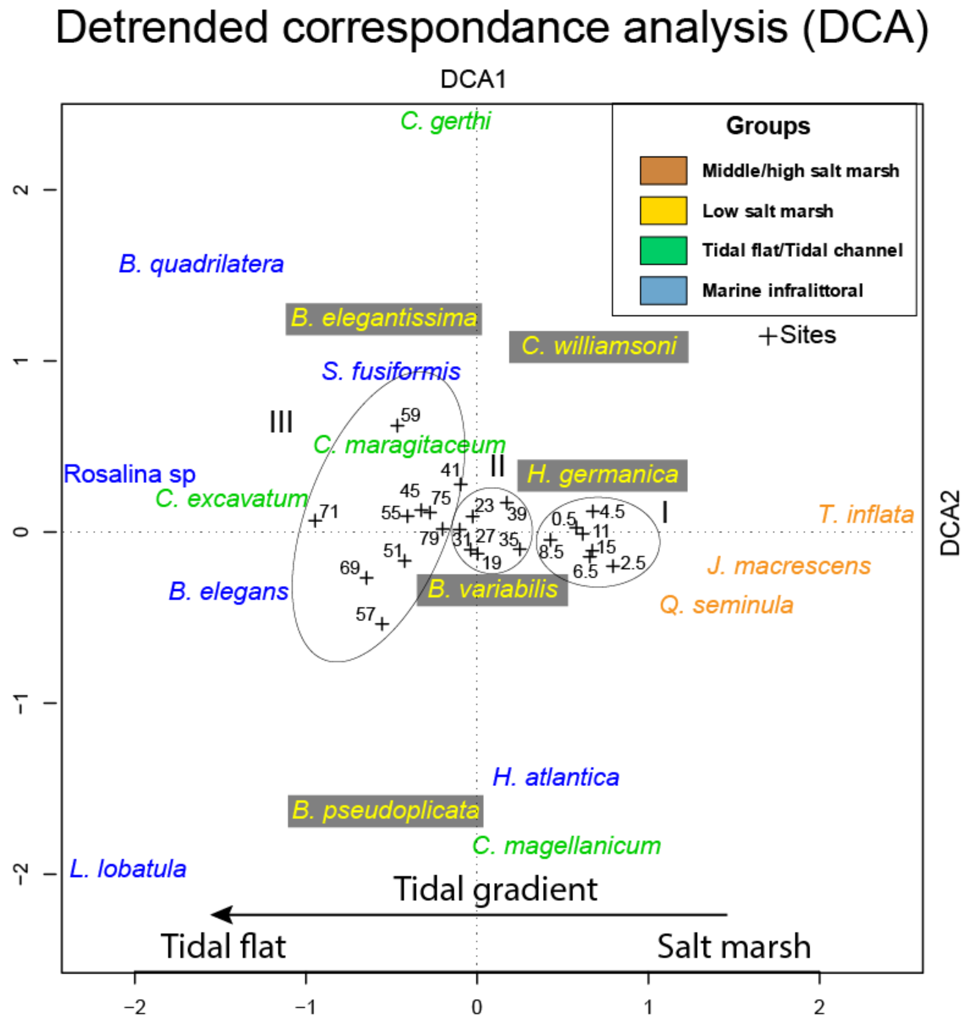


Figure 5-10. Detrended correspondence analysis (DCA) based on species' relative abundance. After the ecological assignment of the species, the first axis represents a tidal/vertical gradient. Black crosses represent the samples.

3.5 Salt marsh landscape evolution

Between 1935 and 2009, a lot of changes in the area landscape can be observed. (**Figure 4.22**). In 1935, the LC-Core is located within the upper part of the tidal flat area close to the limit between vegetated salt marshes and tidal flats. Further north, an elongated sandspit develops, but does not reach LC-core position. The 1947 picture displays a high number of bombing impacts and the development of the sandspit reaching the LC-Core. From 1947 to 1963 the sandpit develops and starts to be eroded on the 1963 picture. At that time, LC-Core is facing a large intertidal area. Subsequently (1969 picture) the sandspit is eroded, with retreating shoreline as outlined by the limit between the salt marsh and tidal flat. From 1969 to 1977 LC-Core stays in the tidal flat area. In 1983 dark patchy objects start to develop, corresponding to the occurrence of pioneering plant patches. After this period the vegetation rapidly develops. From 1988 onward, the vegetation continues its development and invades most of the study area, except the Canche channel. (Note that 1995 picture was taken during the high tide concealing the tidal flat/salt marsh limit). From 2005 and 2009 (reference map PPIGE 2009 interpreted previously **Figure 4.12**) there are no relevant changings on the landscape configuration.

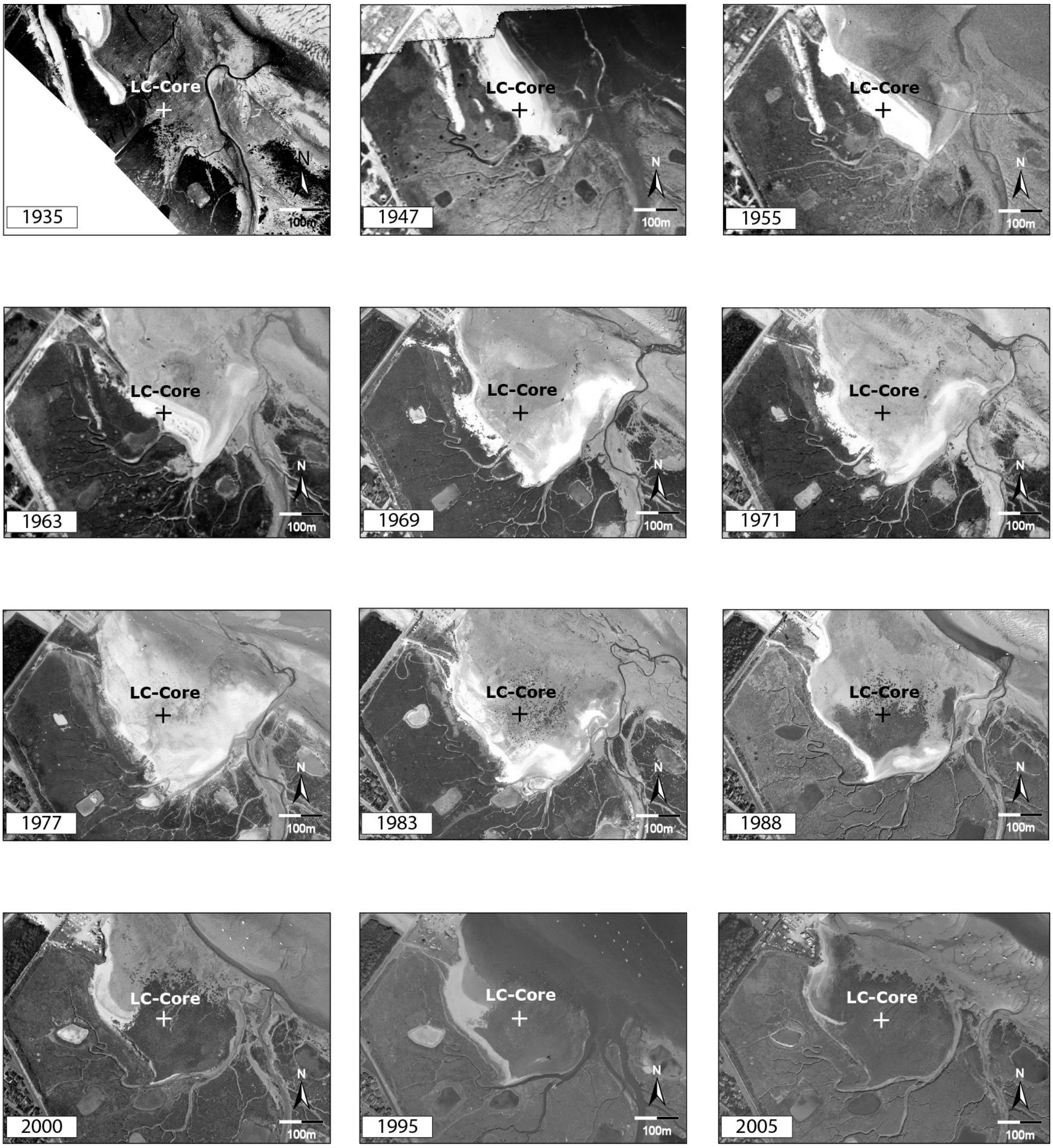


Figure 5-11. Historical aerial pictures of the Canche estuary, at the marsh area scale, from 1935 to 2005. In the GIS at each pictures the location of the LC-CORE has been plotted (cross point).

4 Discussion

4.1 Temporal scale of environmental evolution

In agreement with Amara (2007) the Canche estuary can be considered as slightly impacted by human activities. Hence the evolution of the area is mostly due to its natural feature i.e. a sheltered bay with low energy. The LC-Core represents a typical fining-upward succession in a tide-dominated estuary filled by progradation (for details look at Dalrymple et al., 1991; Dalrymple et al., 1992). The same situation was modelled in (Haslett et al., 2001) in the Severn estuary (U.K.). The authors described an emergence sequence where sea-level rise is less than accretion introducing progressively higher foraminiferal zones at the site. Accordingly in the Canche core we observe a shift from tidal flat to salt marsh foraminiferal assemblages, highlighting an increase in the elevation at the study site (already observed in Boulogne Harbour in Francescangeli et al. (2016). As observed in other estuaries (such as Haslett et al., 1997) an increasing sea level slower than the sedimentation rate has led to a general silting up of the site and to a progressive "continentalisation" of this part of the estuary.

Although radionuclides analyses do not allow to any reliable dating, the sedimentation rate was estimated to about 0.8 cm/yr in the first 24cm of the LC-core. This sedimentation rate can be compared with the values obtained by Marion (2007), in the Authie estuary, a very similar estuary 30 km southward of the study area (supplementary materials). Considering an approximate constant sedimentation rate all along the LC-Core (which is not due to the erosional episodes during the sandspit development), we could estimate that the sediments were accumulated during a period of tens of years within a global range inferior to 100 years. Another element to be considered to define the temporal scale is the strong enrichment of Cd recorded in the middle of the LC-Core. In this little impacted environment it could connected to the use of cadmium-containing fertilizers in agricultural treatments. The use of these have been often quoted as the primary reason for the increase in the cadmium content of soils over the last 20 to 30 years in Europe (Jensen and Bro-Rasmussen, 1992). As a consequence, the use of historical pictures that cover the last 80 years coupled with faunal record, appear a reasonable tool to estimate an age model for the LC-Core.

4.2 Tidal flat vs salt marsh

Grain-size and benthic foraminifera constitute excellent tools to investigate environmental changes in estuarine areas (Alve and Murray, 1999; Allen et al., 2006; Mojtahid et al., 2009). In the Canche estuary these proxies coupled with historical pictures allow to identify a chronological vertical succession from an upper tidal flat to a salt marsh area, spaced out by the sedimentation of a sandspit (**Figure 4.23**).

The bottom part of the core (up to 50 cm) is characterized by the highest contents of sand, similar of sedimentary bodies like sand bar (sandspit) or sand/mixed flat (Saïdi et al., 2014). In the 61-67 cm interval the sediment is better sorted than in the rest of core, indicating a more energy of the depositional environment. It could correspond to the setting of an elongated sandspit. The assemblage is dominated by species belonging to the tidal flat/tidal group. *Cribroelphium excatum, C. margaritaceum* and *Haynesina germanica* are the most abundant taxa. They are common in marginal environments (Thomas and Schafer, 1982; Alve and Murray, 1994; Bouchet et al., 2007; Seuront and Bouchet, 2015). Whilst *H. germanica* is quite abundant everywhere along the core, *C. excavatum* and *C. margaritaceum* are peculiar of this interval. *Cribroelphidium excavatum* prefers the lowest part of the intertidal gradient (Armynot du Châtelet et al., 2005). According to Murray (2006) this taxon would be able to span within the continental shelf down to several hundred meters. Along the coasts of eastern North Sea *C. excavatum* and *C. margaritaceum* have been mainly found in non-marsh intertidal and subtidal environments (Alve and Murray, 1999). In the Canche estuary *C. margaritaceum* was only found as living in the upper part of the tidal flat (Francescangeli et al., submitted). Hence the occurrence of these two taxa associated to sandy substrate could suggest a lower tidal flat, close to a main river channel, often immersed. This hypothesis is also confirmed by the highest proportions of the seaward group along the same temporal interval. Therefore the bottom part of the core up to about 50 cm could be related to the historical maps of the Canche estuary from 1935 to 1969. They show indeed that the core was located in an un-vegetated tidal flat close to the salt marsh transition, space out by the deposition of the sandspit.

In the middle part of the core (50-19 cm) sand content decreases, being gradually replaced by silt. The sediment grain-size along this core interval (silt dominated) is similar to the contents measured in Francescangeli et al. (submitted) within the salt marsh area of the Canche estuary. In this interval the assemblage is dominated by species belonging to the low marsh foraminiferal group whose species *Haynesina germanica* is largely the most abundant taxon. Although it is able to span in wide tidal range (Debenay and Guillou, 2002; Debenay et al., 2006; Armynot du Châtelet et al., 2016), in the Canche salt marsh it has only been found dominant in the lower vegetated marsh (Francescangeli et al., submitted). This tendency has been reported as well in other North Europe salt marshes (Haslett et al., 1997; Horton et al., 1999; Swallow, 2000). Hence this interval could be related to the historical areal pictures from 1969 to 1988. They point out an environmental shift from an upper tidal to a lower marsh gradually colonized by vegetation.

The upper part of the core is characterized by benthic foraminifera belonging to the middle-high marsh group are the most abundant: *Haynesina germanica* is replaced by *Entzia macrescens* and *Quinqueloculina seminula*. *Entzia macrescens* is one of the most abundant salt marsh foraminifera worldwide (Scott and Medioli, 1980; Scott et al., 2001). It is dominant in the middle/high part of vegetated marshes, often associated to *Trochammina inflata* (Sen Gupta, 1999). In the LC-Core *T. inflata*, even in low abundance, only occurs in this interval. The presence of these two taxa is as well characteristic of high vegetated marshes in northern Europe region, from microtidal to macrotidal regimes (Murray, 1991). *Quinqueloculina seminula* is widespread in marginal environments (Debenay and Guillou, 2002; Horton and Murray, 2007) and dominant in the lower tidal marsh/ tidal flat (Alve and Murray, 1999; Swallow, 2000). However as it has been discussed in Francescangeli et al. (submitted) *Q. seminula* could be able to move up along the tidal gradient venturing into the middle marsh (Franceschini et al., 2005; Horton and Murray, 2007; Shaw et al., 2016). Therefore the upper interval of the core can be related to the historical pictures from 1988 onward. They show how, initial patchy vegetation, rapidly developed, covering the whole tidal area and reaching the present landscape configuration.

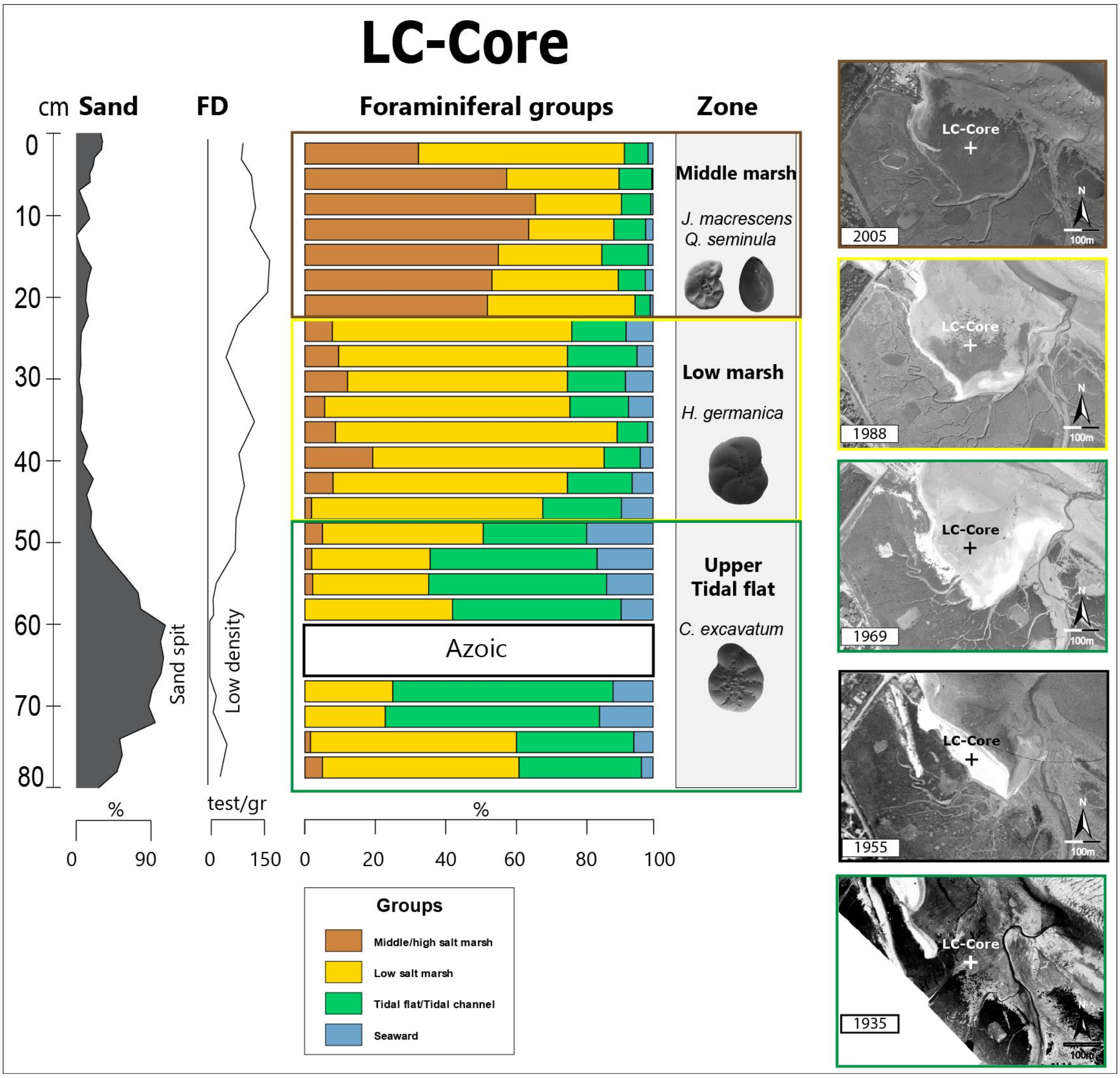


Figure 5-12 Interpretation of environmental changes along LC-Core. Chronologically three sub-environments can be identified: upper tidal flat, low salt marsh and middle marsh. From left to right the parameters used for the interpretation: Sand, Faunal Density (FD), foraminiferal groups, historical areal pictures.

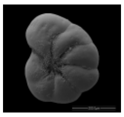
4.3 Environmental parameters constraints (sediment grain size and TOC)

Along the LC-Core sediment grain-size decreases and TOC increases upward, moving from tidal flat to salt marsh, parallel to an increase of foraminiferal densities. Accordingly, in the same area Francescangeli et al. (submitted) and Armynot du Châtelet (2009b) observe a very low densities of living species in sandier substrate towards the tidal flat. On the contrary, Diz (2004) found a relatively high abundance in sandy sediments and suggested that such substrates may provide a favourable settlement for benthic foraminifera. However they explained that only epiphytic specimens dominated the assemblage. In such kind of substrates bacterial biofilms can develop on sandy particles as a trophic resource for epifaunal feeding (Bernhard and Bowser, 1993). In the LC-Core the low TOC contents in the lower part could explain the relative low density of benthic foraminifera, but it is not necessary a bi-univocal reason. For instance in the Danish slope (Northern Sea) sand with low contents of TOC had a high benthic fertility due to high food availability linked to particulate organic matter (and the associated bacteria) (Alve and Murray, 1997). Hence as pointed out in Murray (2006) the general assumption "more organic matter in fine sediment than in sand", is not necessarily a reliable clue of food availability.

In addition the sediment becomes azoic when sand contents is higher than 88% and TOC is close to 0%, during the development of the sandspit. When the sandspit expanded, the vertical sedimentation rate was extremely high, one or two meters of sediment could be deposited in one stormy event. This prevented any faunal settlement. Then the sandspit was partly eroded, but the low-faunal interval lasted sandy and azoic. In the Canche estuary the high energy of the tidal flat area (sandspit or not) inhibits particulate sedimentation (Armynot du Châtelet et al., 2009a). The sediment could be washed out, preventing any development of bacterial biofilm or algal mats. Therefore such environmental conditions in the tidal flat make difficult benthic foraminifera to feed, settle and develop. It means that both sediment grain-size and total organic contents are limiting factors when they overstep their critical thresholds (Murray, 2001). In a recent study Arslan (2016b) pointed out how in silicoclastic sandy sediments the mechanical agitation of water waves have a significant effect on the benthic fauna, namely the destruction of small and more fragile specimens. They found that *Ephidium* are more resistant to action of the waves compare to other specimens (like *Ammonia*). This could mean that the occurrence of *C. excavatum* and *C. margaritaceum* in the bottom part is not only a matter of different tidal level. In Francescangeli et al. (submitted) we principally focused on the salt marsh area of the Canche estuary, asserting that grain-size and TOC do not primarily influence benthic foraminiferal distributions. Here we can remark that extending faunal investigations to a wider tidal range, the abiotic-biotic interactions increase, making more and more difficult ecological and palaeo-ecological interpretations.

5 Conclusion

- Sediment grain-size and benthic foraminifera, supported by historical pictures, have efficiently been used as proxy to reconstruct past environments in coastal environments.
- Historical pictures constitute a valuable option to date recent sediments (<100 years), where radionuclides measurement results are inadequate.
- An upper tidal flat (spaced out by a sandspit development) has been gradually replaced by a saltmarsh sediment.
- The core stratigraphy represents a typical fining-upward succession in a tide-dominated estuary filled by the progradation of the sediment.
- In the bottom part of the core, the sediment grain-size and the TOC were critical factors for the settlement and development of benthic foraminifera.



Chapter 6 General discussion and perspectives

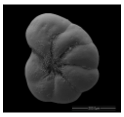
In the introduction of this PhD thesis (see chapter 1), I pointed out a series of objectives/questions that were discusses either on a single chapter, or along some of them. However during the realization of this work, other questions about the role benthic foraminifera in modern environments leaped out and merged with the original questions. This chapter aims to synthetize and discussed the principal outcomes around four arisen questions: 1) In estuarine areas, not only natural parameters govern the foraminiferal distribution, but also human-induced perturbations. Is it possible to propose a conceptual model of the interactions between all parameters? 2) As taphonomical processes alter the original assemblages, is it reasonable to study the present-day living faunas to understand the environmental evolution based on fossil (dead) assemblages? And 3) One may think that there are too many bioindicators to monitor the environment. Are foraminifera appropriate to complete that list?

1 Species dominant distribution and main drivers

The investigation of living foraminifera allowed for the first time to describe the foraminiferal distribution along the coastal areas of northern France (**Figure 5.1**):

1) <u>Natural and low impacted areas</u>: Tidal flat: the assemblages are largely dominated by *Haynesina germanica*, especially in the upper part, passing downward to an increase of *C. excavatum* and *C. margaritaceum*. Salt marsh: agglutinated taxa, *Entzia macrescens* and *Trochammina inflata*, dominate the high/middle marsh associated to porcelaneous taxa such as *Quinqueloculina seminula*; hyaline specimens, *Haynesina germanica* and *Cribroelphidium williamsoni*, are dominant in the low salt marsh.

2) <u>Disturbed areas</u>: Embanked estuary: the tidal flat is dominated by *Bolivina variabilis* and *Bolivina pseudoplicata,* associated to *Haynesina germanica.* Harbour: the metal-enriched intertidal area is largely dominated by *Haynesina germanica* associated to *Cribroelphidium excavatum* and *Ammonia tepida.*



My results are in agreement with the literature on foraminiferal distributions in Atlantic meso-macro intertidal environments (Horton, 1999; Horton et al., 1999; Murray and Alve, 2000; Debenay and Guillou, 2002). Agglutinated taxa (*E. macrescens* and *T. inflata*) dominate marsh assemblages while hyaline specimens mostly colonize tidal flat areas. In these macrotidal environments *H. germanica* thrives under highly variable conditions (Murray, 2006). However it is interesting to note how the abundance of *Miliammina fusca* and *A. tepida* is quite low, considering they are generally dominant in salt marshes and tidal flats respectively. In the first case we can speculate that *Q. seminula* takes the ecological niche of *M. fusca*, hypothesis which could make sense if we consider this taxon "genetically" more closely related to porcelaneous than agglutinated specimens (Fahrni et al., 1997). Indeed *M. fusca* is a more brackish species which dominates higher estuarine areas with lower salinity (Camacho et al., 2015), like other brackish agglutinants (Fatela et al., 2014). This hypothesis is also confirmed in the study of Armynot du Châtelet et al. (2009a), where *M. fusca* has been found in the upper part of the tidal marsh, in a position where probably the salinity is lower than the marsh areas investigated in the present work. Referring to the second case, in the model of Debenay (2002) (presented in Chapter 1) *C. excavatum* dominates in the lower estuary, passing upward to *A. tepida* and *C. gunteri*, and farther to *Haynesina germanica*. This principally means that we have a sort of cut of the "*A. tepida* zone" as demonstrated both along the cores of Boulogne-sur-Mer (Francescangeli et al., 2016) and Canche estuary. Reason for this cut could be found either in the salinity or in the occurrence of low-oxygen conditions as discussed in chapter 3. However in both cases we do not have enough information to formulate concrete statements.

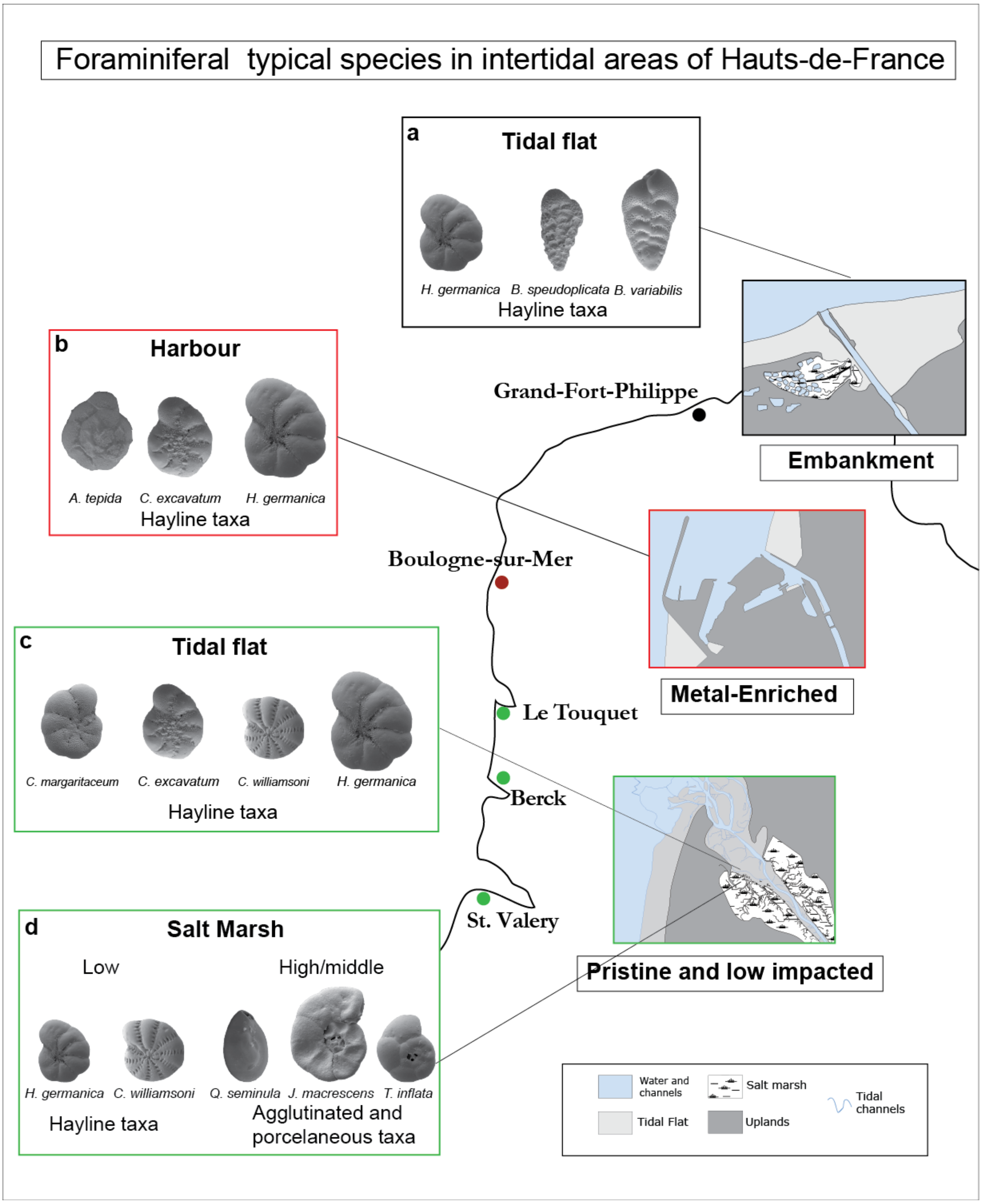


Figure 6-1 Main foraminiferal species along the intertidal areas of the northern France. Bigger size indicates the most abundant species.

1.1 Natural and low impacted areas

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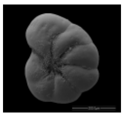


northern France (some parameters are only indirectly deduced). In natural and low impacted areas, we can consider that, globally, the natural morphology of investigated sites has been not altered. In the marsh areas, colonized by vegetation, foraminiferal assemblages are driven by the tidal exposure. Based on this, a vertical foraminiferal zonation can be observed. The TOC decreases gradually towards the upper tidal flat corresponding to a general decrease of the total foraminiferal density. The sediment grain-size does not constitute an influencing parameter. In the tidal flat areas the assemblages seem to be less driven to the vertical tidal gradient. However a vertical zonation can be as well observed. Elphididae increase downwards. In these areas the sediment grain-size and TOC can constitute limiting factors. When the sediment is too sandy (larger than 70-80%), TOC concentrations are very low. The combination of these two factors, which are linked to the high hydrodynamic conditions, prevent the feeding, the settlement and the development of benthic foraminifera.

Although it is true that we have a vertical foraminiferal zonation, the ecological transitions between the assemblages may not occur always at the same elevation. Some species can move along-shore influenced by other parameters such as salinity. In fact, as mentioned by Berkeley (2007), foraminiferal species are only indirectly related to the elevation. Elevation is not a stand-alone parameter. It is only the measure of a geographic point in a given coordinates system. This could re-open the question in literature about the use of coastal foraminifera as sea level indicators. The "take home message" is that to use foraminifera as see level indicators we should be sure that all the environmental parameters co-vary with elevation, and that the along-shore gradient is minimal.

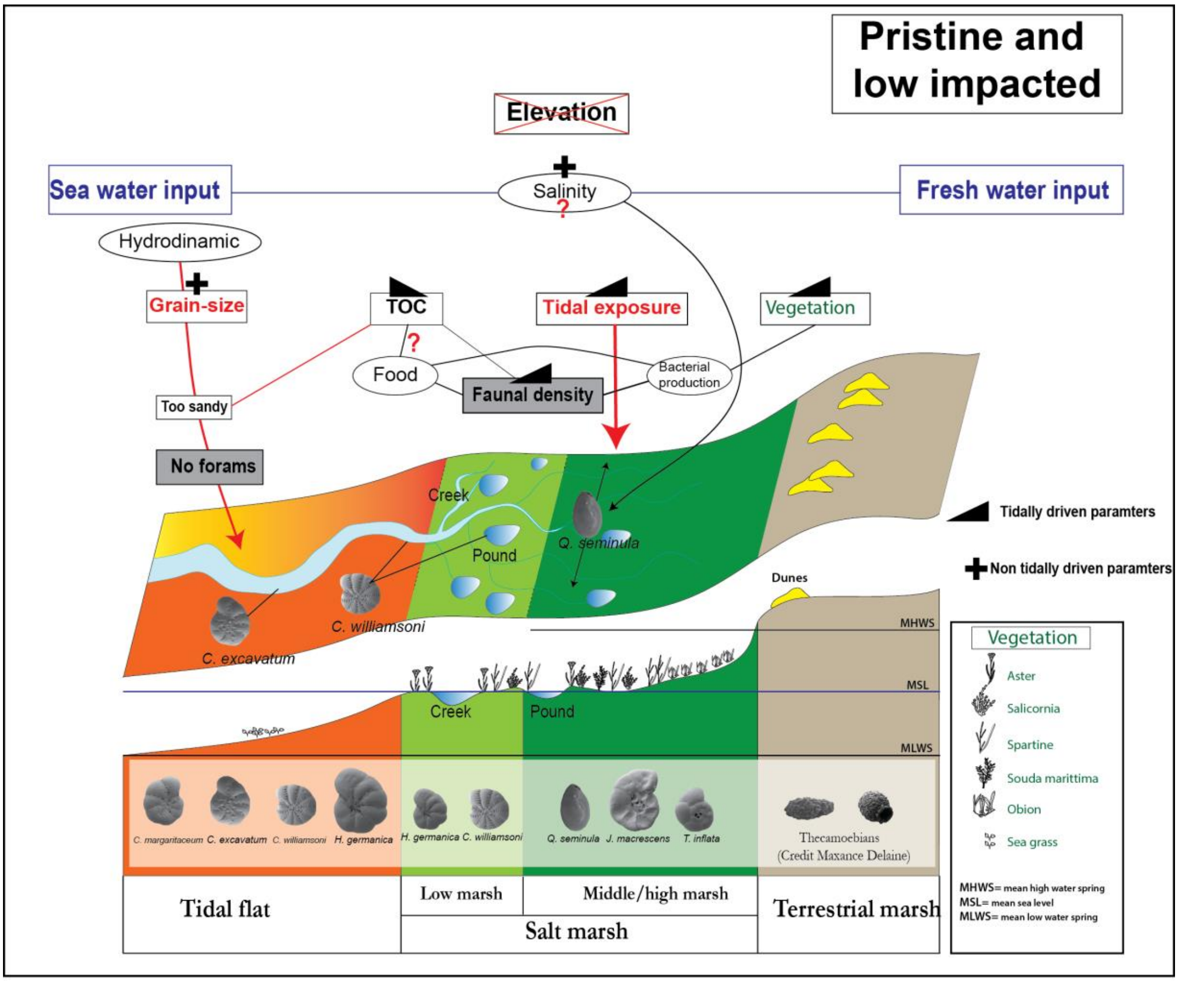


Figure 6-2 Scheme showing the main foraminiferal drivers in natural intertidal areas of the study region. In circles environmental parameters not directly measured. Red "question marks" indicate the relations to be better understood. A vertical profile with the dominant species and vegetation is as well proposed. The vertical scale is exaggerated to evidence ecological transitions between the intertidal areas.

1.2 Disturbed areas

In disturbed areas, human action may alter directly or indirectly the original environment. For instance, chemical pollution is a direct modification of the natural (background) concentration of a given element. The building of a dike cannot be considered as a pollutant activity *sensu stricto*, but it constitutes anyhow a perturbation of the original environmental setting. In embanked estuarine tidal flats of Hauts-de-France, the altered-human morphology can lead to the high accumulation of terrigenous input. This fact favours the dominance of Bolivinids (*B. variabilis* and *B. pseudoplicata*). These infaunal taxa are commonly considered in most of works as tolerant to low oxygen conditions. One can speculate that high terrigenous accumulation could have contributed to the development, down layer, of reduced-oxygen conditions. The harbour areas, are metal-enriched by industrial pollution. We can observe that the enrichment in metal concentrations have a negative effect on the assemblages. Opportunistic species like *H. germanica, C. excavatum* and *A. tepida,* dominate the assemblages. During a year of observations in these areas, species diversity was always low. However in some natural areas we can find the same low values of species diversity. In chapter 3 we observed that this is due to variations of benthic foraminifera, in the response to seasonal environmental parameter variations. That is why the choice of a small-scale temporal observation was essential to un-bias foraminiferal behaviour interpretations in intertidal environments.

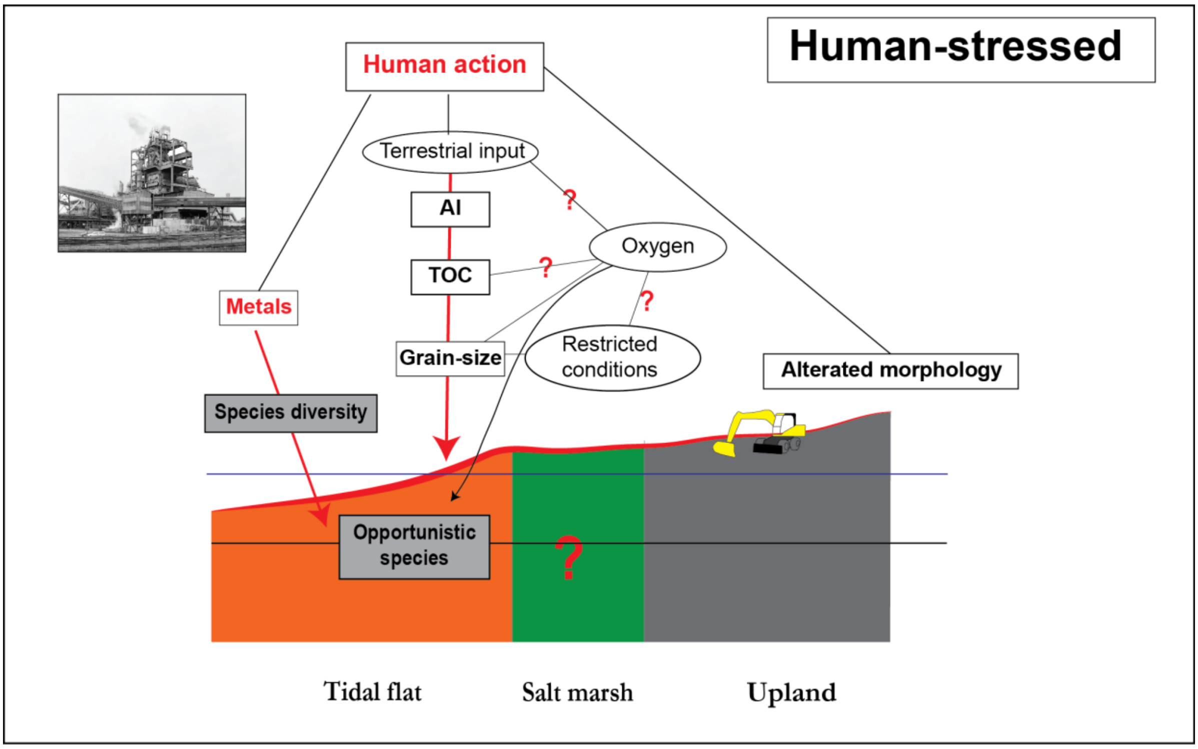
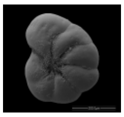


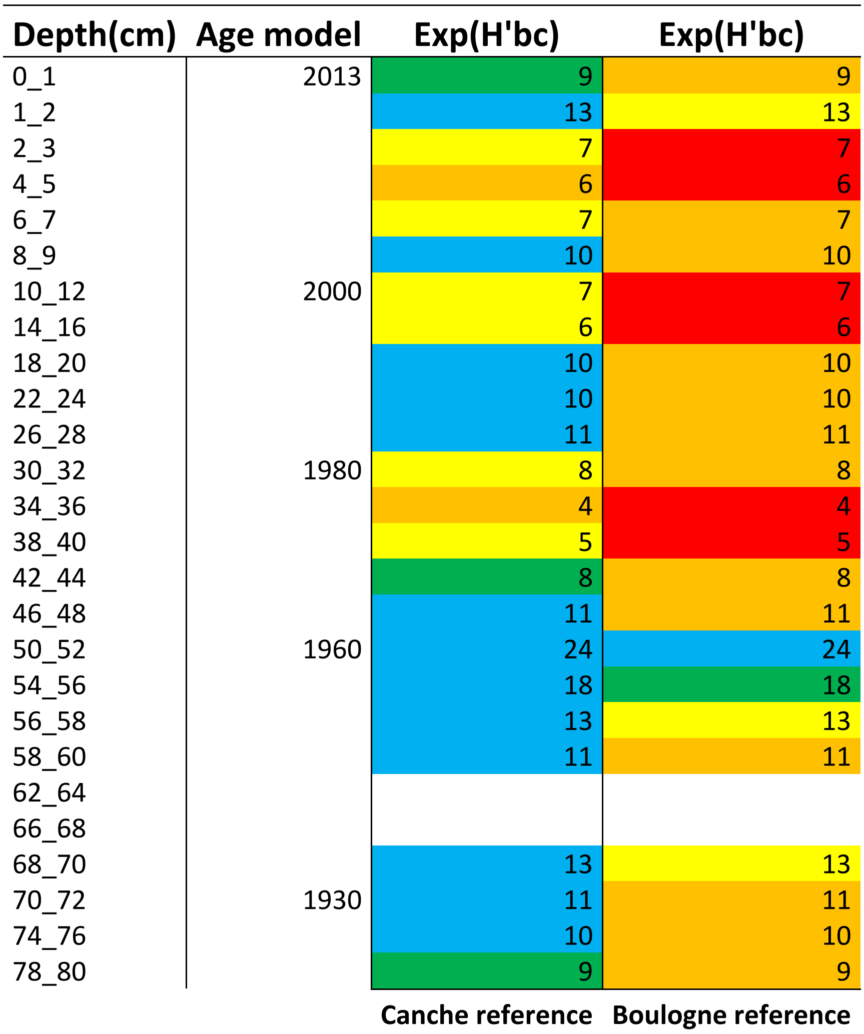
Figure 6-3 Scheme showing the main foraminiferal drivers in disturbed intertidal areas of the study region. In circles environmental parameters not directly measured. Red "question marks" indicate the relations to be better understood. Due to the altered morphology only a vertical profile has proposed.



1.3 Recent foraminiferal evolutions

The investigation of the living intertidal foraminifera has allowed to better understand their response to environmental parameter changes in the study region. In Boulogne-sur-Mer harbour and the Canche estuary, foraminiferal variations on a long-term century scale, I have been able to monitor and distinguish natural and human-induced transformations.

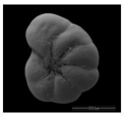
The European Water Framework Directive (WFD) (2008/56/EC, 2008) requires the definition of reference conditions, namely pre-impacted conditions to distinguishing the effects of pollution from the natural "background (Alve, 1991a; Alve et al., 2009). Since the northern part of Hauts-de-France cannot be referred as natural, reference conditions have been assessed by the analysis of historical data (like for example in Danish coastal areas by Nielsen (2003)). Coupling geochemical and micro-faunal approaches, reference conditions from the harbour of Boulogne-sur-Mer have been defined. Based on ecological quality ratios (EQR), (representing the deviation of actual levels of a quality element from reference conditions, WFD) ecological status classes have been assessed. I showed that after the degradation of the EcoQS during the industrial period, a partial environmental recovery occurred in the recent decades. However, in an attempt to apply the Boulogne's criteria to the LC-Core (Canche estuary), I noticed that we have globally "Poor and bad" EcoQS (**Table 5-1**). Geochemical analyses showed that the Canche estuary is barely impacted, in accordance with Amara et al. (2007) and Henry et al. (2004). It appears evident that using Boulogne's reference conditions in La Canche estuary gives unreliable results. It shows that to assess the EcoQS, the use of sites-specific reference conditions would be more appropriate as observed by Krause Jensen in Danish estuaries for eelgrasses (Krause-Jensen et al., 2005). In marine coastal zones, most severe impact occurred after the late 1800s. Hence, to set up reference conditions we should use the environmental indicators in sediments deposited before the mid-1800s (Alve et al., 2009). This was not possible in the LC-Core because its base was estimated to date back to the beginning of the 1900s. However if we consider that at that period, the study area was a rural place without any significant human activities, foraminifera from the core bottom may be used as fossil background assemblages. Applying these local criteria, the evaluation of EcoQS appears more reliable. Globally, I observed "moderate to high" EcoQS along the LC-Core. This example suggests that only the use of local reference conditions gives an accurate assessment of the ecological quality.



Depth(cm)	Age model	Exp(H'bc)	Exp(H'bc)
0_1	2013	9	9
1_2		13	13
2_3		7	7
4_5		6	6
6_7		7	7
8_9		10	10
10_12	2000	7	7
14_16		6	6
18_20		10	10
22_24		10	10
26_28		11	11
30_32	1980	8	8
34_36		4	4
38_40		5	5
42_44		8	8
46_48		11	11
50_52	1960	24	24
54_56		18	18
56_58		13	13
58_60		11	11
62_64			
66_68			
68_70		13	13
70_72	1930	11	11
74_76		10	10
78_80		9	9
		Canche reference	**Boulogne reference**

Table 5-1 Attempt to PalaeoEcoQS assessment of the LC-Core based on diversity indices (exp(H'$_{bc}$) Bouchet (2012)), using site-specific criteria (Canche estuary) and Boulogne-sur-Mer reference conditions. In the table it has been proposed an age model based on the interpretations of historical maps.

Although my work confirms the potential of benthic fossil assemblages for palaeoecological reconstructions, some reflections can be carried out comparing living and dead faunas. During optical observations I noticed a large difference between living stained benthic foraminifera and dead ones. Armynot et al. (comm. Pers.), at the transition between the tidal flat/salt marsh in the Canche estuary, observed as well that the dead fauna is by far more diverse than the living one and results from a mix of autochthonous and marine



subtidal fauna. The dead assemblage corresponds to time-averaged over several to many years living assemblages and modified to a lesser or greater extent by taphonomic processes (such as transport or test dissolutions) (Murray, 2000b) The high hydrodynamic leads to the transport and re-sedimentation of benthic foraminifera altering strongly the original composition of the assemblages. In this context is it appropriate to study the living faunas to understand the environmental evolution based on fossil assemblages?

2 Environmental monitoring

The international FOBIMO working group (FOraminiferal BIo-Monitoring) is aiming at improving the use of benthic foraminifera in biomonitoring studies. Several works have been recently published in order to enhance benthic foraminifera for European program monitoring (Bouchet et al., 2012; Schönfeld et al., 2012; Barras et al., 2014; Alve et al., 2016; Dimiza et al., 2016; Dijkstra et al., 2017). At the beginning of my PhD, I took part at the FOBIMO workshop (in Texel, The Netherland) where we discussed about the possible adaptation of A Marine Biotic Index (AMBI) (Borja et al., 2000), for use on benthic foraminifera. This well-established index is applied to assess the ecological-quality status in marine environments. For the calculation of what has been called "Foram-AMBI", the first step is to classify benthic foraminiferal in ecological groups, according to their sensitivity/tolerance to increasing stress gradient. Specific assignments are based on the foraminiferal relative abundances associated to TOC gradients. The TOC has been selected because it is one of the most frequently measured parameters in foraminiferal studies. Benthic specimens can be classified in 5 ecological groups (**Figure 5.4**): "Sensitive species" are sensitive to organic matter enrichment (Group I); "Indifferent species" are indifferent to organic matter enrichment (Group II); "Tolerant species" are tolerant to excessive organic matter enrichment (Group III); "2nd-order opportunistic species" show a clear positive response to organic matter enrichment (Group IV); "1st-order opportunistic species" benefit from higher stress level induced by organic matter enrichment (Group V). To achieve this aim, groups of foraminiferal researchers, started to select and analysis a large number of dataset. A first outcome is the recent work of Alve (2016), where 128 benthic foraminiferal species from continental shelves and slopes, in North-East Atlantic and Arctic fjords, have been assigned.

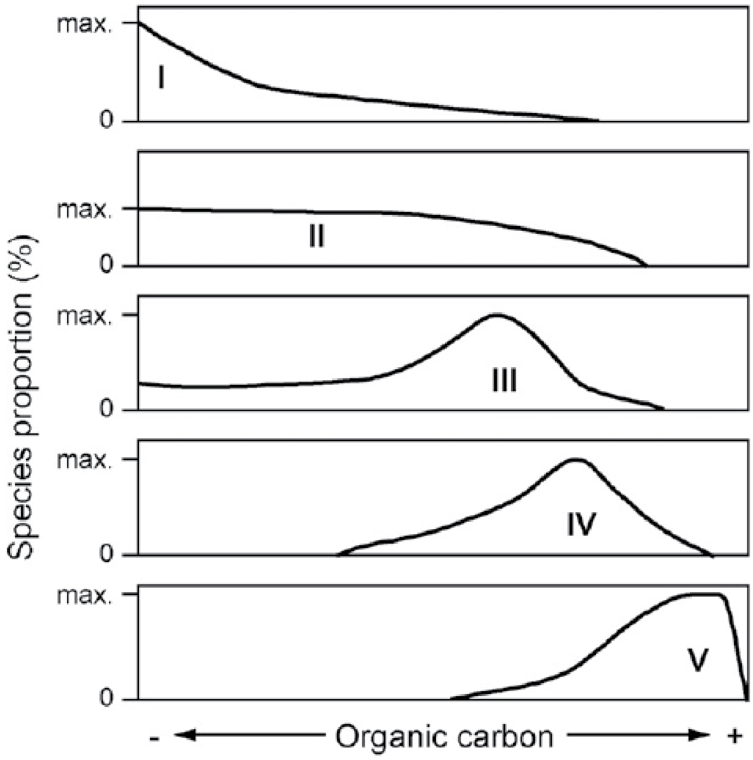


Figure 6-4 Relative abundance pattern related to the environmental stress gradient (TOC). Benthic foraminiferal species are assigned to the five ecological groups according to these five different patterns (from (Alve et al., 2016), based on Grall (1997) and Borja (2000))

In transitional environments, marine scientists (headed by Eric Armynot du Châtelet and Vincent M.P. Bouchet) started as well to work along this direction. In order to contribute to this project, benthic foraminiferal species from the Hauts-de-France region, have been classified following the criteria described by Alve (2016). Two datasets (seasonal survey and annual survey) were merged together in order to analysis the relationships between living foraminifera and TOC gradients. As the TOC concentrations were much higher in one of the two dataset, as preliminary analysis, only the most abundant species have been considered. Totally 12 species were selected (**Figure 5.5**). The XY plots have been visually compared with patterns in (**Figure 5.4)**.

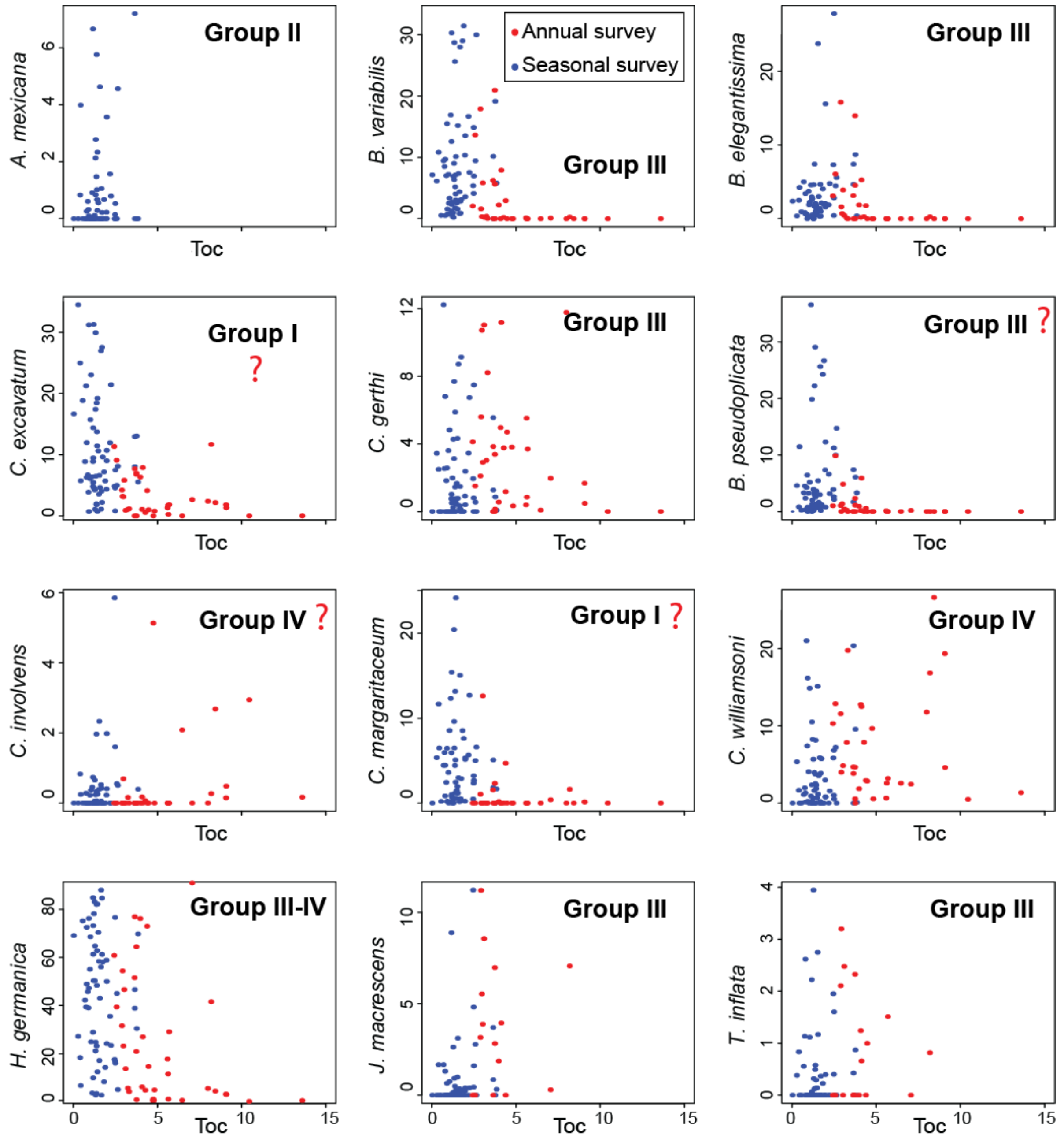
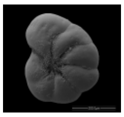


Figure 6-5 Assessment of benthic foraminiferal ecological groups based on the patters above shown.

As shown in the **Figure 5.5** some interpretations are “easy”, others (red question marks) need more opinions. For instance it seems clear that *Arenoparella mexicana* belongs to the second group, or *Buliminella elegantissima* to the third one. On the contrary it seems more difficult to assign a group to *Cribroelphidium excavatum*. My results indicate that this species belong to the first group, sensible to TOC increase. This is contrasting with literature data where this opportunist taxon is mostly considered as tolerant to high contents of TOC



(Murray, 2006). More reasonable the assignment to group III-IV to *Haynesina germanica* able to span in all the intertidal domain. My interpretations allowed hence the preliminary assignments of 12 intertidal species. Obviously this needs to be compared and discussed to other datasets in transitional marine environments. This is only a first step which can support anyhow the development of this project. More species need to be assigned to obtain a more efficient Foram-AMBI in coastal areas.

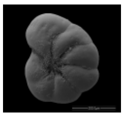
Hence the method could be applied in transitional areas and constitutes another example of the reason why foraminifera should be considered in worldwide marine legislations as bio-indicators of environmental quality.

3 Conclusions and perspectives

Short-term scale observations at regional scale, give for the first time a global overview of the foraminiferal distribution along the intertidal areas of Hauts-de-France region. The investigation of the living intertidal foraminifera has allowed identifying the main environmental drivers in the study region. Through long-term scale observations natural and human-induced environmental transformations have been distinguished by monitoring changings in benthic foraminiferal communities.

The main outcomes can be summarized as follows:

- In natural estuarine areas a vertical zonation of benthic foraminifera occurs along the tidal gradient. In the upper marsh areas the assemblages, dominated by agglutinated taxa, are driven by the tidal exposure. In tidal flat areas the dominant species is *Haynesina germanica*. The grain-size constitutes a critical factor when reaching a certain threshold value.
- In embanked estuary the accumulation of terrigenous input favours Bolivinids. In metal-enriched harbours areas a few opportunistic taxa dominant low diversified assemblages.
- Fossil benthic foraminifera constitute an excellent tool for palaeoenvironmental and palaeoecological reconstructions. Foraminifera can be used to define site-specific reference conditions, distinguishing pre-impacted periods from polluted ones. This



testifies how they should be considered for applications in biomonitoring programs under worldwide marine legislations.

3.1 Perspectives

1) At the end of the second paragraph in the present chapter, we let open the question of the possible use of dead assemblages for palaeoenvironmental reconstructions when taphonomic processes modify the original living assemblages. In literature there is still some discussions to define what would be the best assemblage (living, dead or total assemblage) to be considered as the best analogue of modern environments. For example (Murray, 2000b) suggested that the use of total assemblage in ecological studies should be avoided. In sea level reconstructions some authors recommend the use of dead assemblages, when dead fauna is in equilibrium with the depositional environments (Horton, 1999). Living foraminifera indeed can be use as sea-level indicators, but with caution where post mortem processes are intense (Armynot du Châtelet et al., 2005). Despite the discussion lasts for a long time, a way to quantify and reproduce these phenomena has not been modelled yet. A perspective could be to perform lab experiments (and/or numerical models?), with the aim to reproduce the stream action and quantify the effects of these processes on perturbing living assemblages. The idea could be to measure hydrodynamic parameters on the field and recreate natural environmental depositional setting (morphology, sediment, living and dead specimens) in a mesocosm. Then we can simulate the process starting from a simple model as shown in **Figure 5.6**. We observe at t1 the assemblage 1 along a section A, in down-stream areas, and the assemble 2 along section B upper-stream. Then we can simulate a currency event and observe the effect of transport and re-deposition at t2 on the two assemblages.

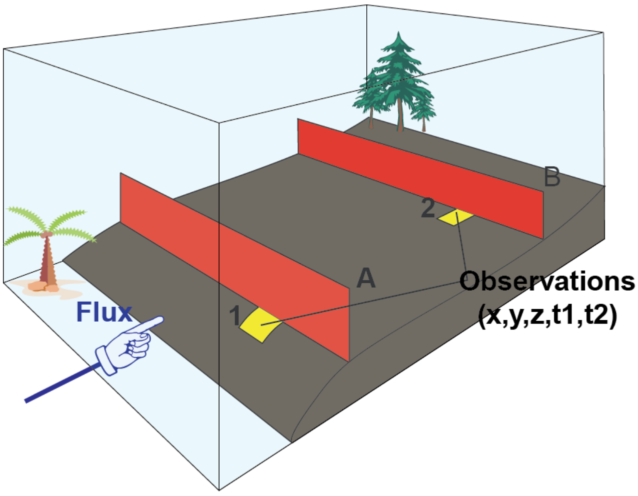


Figure 6-6 Shema showing the principal elements to model transportation and re-deposition of benthic foraminifera.

2) Along the manuscript I mentioned several times that salinity is an important parameter driving foraminiferal distributions in intertidal areas. As in these environments there is a big tidal range, the measurement of the salinity is not an easy task. First of all, which salinity need to be measured? Water salinity? Pore water salinity? As benthic foraminifera live on the sediment surface, I believe that it is more appropriate to measure the pore-water salinity (even if most studies are based on water salinity). Second, the salinity can vary largely during a day or a week, e.g. as a function of tidal range or precipitation level. This means that a single measure only represents an instant in changing conditions. Hence a perspective (to better understand for instance the results) from chapter 2 could be to monitor the salinity variations in the tidal marsh of the Canche estuary at high frequency both along-shore and cross-shore. This needs to be done in different periods of the year, to control the effect of the temperature, the precipitation, and the different tidal ranges.

3) Although many studies regarding the relationship between benthic foraminifera and organic matter have been carried out, the precise role of this parameter has not been followed out. The organic matter is a complex compound and the simple analysis of the TOC and C/N (historically quantitative and qualitative indexes) is not accurate enough to understand actually its control upon benthic foraminifera and a more detailed "investigation" is required. A high content of organic matter in the sediment in fact, does not give any information about the quality of organic matter and does not necessary imply

high foraminifera standing stocks (Licari et al., 2003). Following this direction over the last decade, several authors have been focused on the possible ways benthic foraminifera are using the organic matter as food source. Sediment phytopigment analysis (*Chl-a* and *phaeo*) called CPE are used to evaluate the fresh organic matter availability for the benthos. Contreras-Rosales et al. (2012) documented, in living-deep sea benthic foraminiferal communities, a significant correlation between foraminiferal abundance and CPE, indicating the OM as corresponding to phytodetritus. Moreover analysis of lipids constitutes another remarkable tool for improving the knowledge. The lipid composition of an organism is related to its taxonomy and as such, they are used as biomarkers to study trophic relationships (Pond and Sargent, 1998). For instance sterols, a group of lipids, can be used to trace sewage matters. Other molecules, such as coprostal (sterols) are able to reveal human and terrestrial mammalian faeces contribution. Fatty acids can be used as tracer for bacteria and diatoms (Alfaro et al., 2006). Even though the efficiency of biomarkers techniques, they have been poorly used in foraminiferal studies (Suhr et al., 2003; Ward et al., 2003; Topping et al., 2006). Hence an interesting prospective could be to develop these techniques in foraminiferal research.

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Supplementary materials

Following the ecological way of the thesis, all the data called as "supplementary materials" in text, are not provided in the printed version of the present manuscript, to save paper. In case of need, one can directly send an email to the author of the PhD thesis to: fabio.francescangeli85@gmail.com.