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Marie Fouet

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L'UNIVERSITE D'ANGERS

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Sciences de la Mer et du littoral
Spécialité : Ecologie Marine

Par

Marie FOUET

Répartition des communautés de foraminifères dans les estuaires de la façade atlantique

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**Répartition des
communautés de
foraminifères dans les
estuaires de la façade
atlantique**

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« La douleur est éphémère, la fierté est éternelle. »

Maman

« If you can move, then you can stand, if you can stand, then you can fight. »

Hiro Mashima – Fairy Tail

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Chapitre 1

Introduction

1. Les milieux estuariens

1.1. Définition d'un estuaire

Les estuaires sont des environnements dynamiques, situés à l'interface entre le milieu fluvial et marin. Une définition des estuaires est proposée par Whitfield & Elliott (2011) : « une masse d'eau côtière semi-fermée qui est reliée à la mer de façon permanente ou périodique, dont la salinité diffère de celle de l'océan ouvert adjacent en raison de l'apport d'eau douce ou de l'évaporation et qui comprend un biote caractéristique ». Chaque estuaire est un environnement unique, résultant de sa situation géographique, de son histoire géologique, de son climat, et de son régime tidal. De par leur position à l'interface entre les milieux dulcicoles et marins, les écosystèmes estuariens sont sujets à des variations spatiales et temporelles considérables, des paramètres abiotiques (salinité, turbidité, courants, nutriments, etc.) (Day et al., 2012), et aussi des processus biogéochimiques (Kennish, 2019). Dans ces environnements, les paramètres environnementaux fluctuent constamment, à une échelle journalière selon le cycle des marées, mais aussi l'échelle saisonnière selon les périodes de crue et d'étiage du fleuve (Elliott & McLusky, 2002). Ainsi, ces écosystèmes complexes présentent des habitats variés et dynamiques abritant des organismes adaptées à ces perpétuels changements (Day et al., 2012; Taffs et al., 2017).

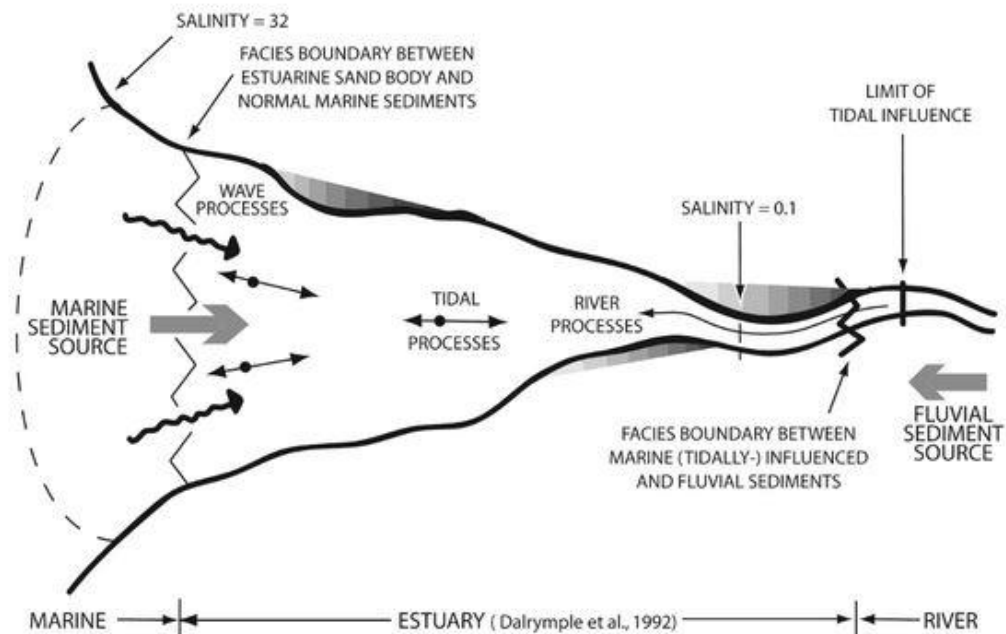


Figure 1.1 : Zonation estuarienne classique, depuis le milieu continental, où les processus fluviaux dominent, en passant par le milieu de l'estuaire, jusqu'à l'embouchure où les processus de marée et les vagues sont les forces physiques dominantes. Les différences d'intensité et de sources de forçage physique dans l'estuaire entraînent également la formation de faciès sédimentaires distincts (d'après Dalrymple et al., 1992).

1.2. Les paramètres contrôlant la répartition des organismes vivant dans les estuaires

a) Salinité

Dans la littérature scientifique, il y a un consensus pour dire que la salinité est le paramètre principal structurant la distribution des organismes dans les milieux estuariens (Scott et al., 1980; Cearreta, 1988; De Rijk, 1995; Ysebaert et al., 2003; Horton & Murray, 2007). Dans ces milieux, la salinité varie temporellement et spatialement selon les cycles de marée et les alternances d'étiage et de crue du fleuve. Ces variations de salinité sont les premiers facteurs de stress pour les organismes (Saunders, 2011; Whitfield et al., 2012). Les vasières intertidales sont des habitats spécifiques des estuaires qui sont émergés à marée basse et soumis aux conditions météorologiques. La salinité dans ces habitats peut fluctuer en fonction de l'évaporation et des précipitations lors de l'émergence à marée basse. Diz et al. (2009) ont mesuré la salinité sur une même station pendant un an. Ils ont montré que ce paramètre fluctuait énormément au cours de l'année mais aussi au cours du mois, avec des salinités de 18 à 34 pour une même station d'échantillonnage.

La relation entre les organismes et les changements de salinité le long du continuum marin-fluvial a été étudiée très tôt, par Remane (1934) qui propose un diagramme conceptuel (Figure I.2). Ce diagramme montre les changements progressifs des proportions relatives des espèces marines, saumâtres et dulçaquicoles le long du gradient de salinité. Ce modèle, qui initialement avait été développé pour la mer Baltique, a été adapté par Whitfield et al. (2012) pour les milieux estuariens (Figure I.2). Whitfield et al. (2012) ont montré que les espèces marines dominent fortement les parties polyhalines et mésohalines de l'estuaire alors que les espèces d'eau douce pénètrent à peine dans les eaux plus salines (limitées aux salinités inférieures à 4). Ainsi, les espèces retrouvées dans la zone estuarienne sont composées en grande partie d'espèces marines qui tolèrent les diminutions de salinité.

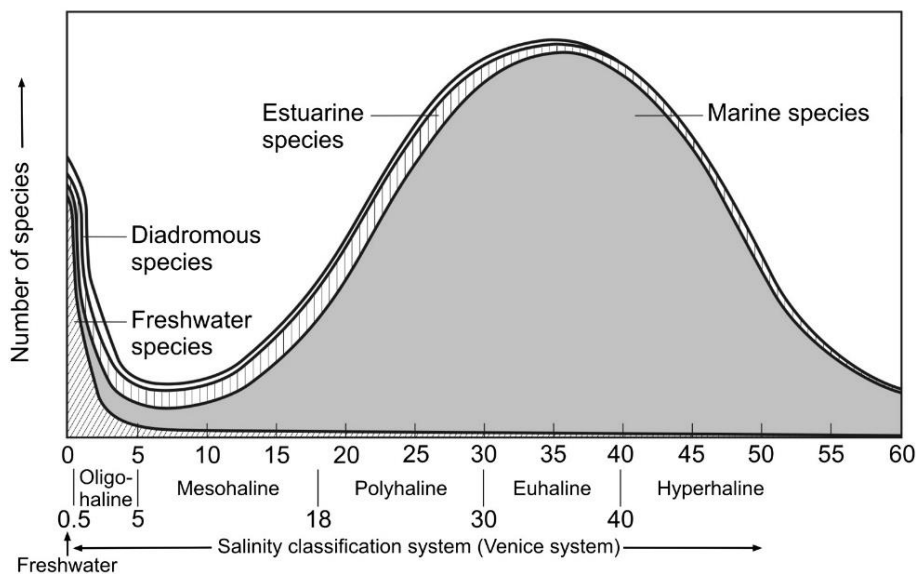


Figure I.2 : Modèle conceptuel de la biodiversité estuarienne (représentée par le nombre d'espèces par groupe) couvrant le continuum de salinité, de l'eau douce aux conditions hyperhalines (d'après Whitfield et al. 2012).

b) Emergence à marée basse

La majorité des estuaires sont soumis à des régimes tidaux plus ou moins importants. Sur les côtes européennes, beaucoup d'estuaires sont soumis à un régime de marée macrotidal avec des marnages de l'ordre de 4-6 mètres. Sur les portions de l'estuaire découvertes à marée basse ainsi qu'en milieu côtier, les organismes doivent faire face à plusieurs types de pression résultant de l'émergence. Les organismes doivent trouver un moyen de respirer (Nancollas & McGaw, 2021), de résister à la dessiccation (Hummel et al., 1994; Williams et al., 2011), aux changements de températures (Li et al., 2019; Deldicq et al., 2021), et doivent aussi se protéger de la prédation (Cheverie et al., 2014). Les organismes peuplant les vasières estuariennes vont ainsi s'étager le long du gradient du « haut de vasière » vers le « bas de vasière » selon leurs degrés de résistance à l'émergence. La durée d'émergence est donc un paramètre important contrôlant la distribution des organismes dans les vasières estuariennes (Horton & Murray, 2007; Camacho et al., 2015a; Mojtahid et al., 2016; Francescangeli et al., 2017).

c) Nutriments

Les milieux estuariens jouent un rôle crucial en influençant les flux de nutriments en provenance des eaux continentales vers la mer (Statham, 2012). La diversité des processus biologiques, physiques ou chimiques influençant les propriétés des nutriments confèrent aux estuaires un rôle de zone tampon, filtrant les nutriments inorganiques et organiques entre le milieu fluvial et marin (Humborg et al., 2003; Deborde et al., 2007). Ces apports de nutriments provenant des rivières vont avoir un rôle majeur dans le maintien d'une productivité élevée dans les écosystèmes estuariens et côtiers. La rétention en nutriments dans un estuaire est liée au temps de résidence de l'eau douce dans l'estuaire. Plus le temps de résidence de l'eau est long, plus les processus de transformation au sein de l'estuaire deviennent importants (Nedwell et al., 1999).

d) Matière organique

Les estuaires reçoivent des apports de matière organique provenant de sources différentes (Regnier et al., 2013). La matière organique dans les estuaires peut être subdivisée dans une partie autochtone (produite dans l'estuaire) et une partie allochtone (source externe, apportée du bassin versant ou par la mer) (Leng & Lewis, 2017). La matière organique autochtone résulte de la production *in situ*, dans la colonne d'eau (phytoplancton) ou au fond (microalgues et macroalgues benthiques, herbiers). Dans ce contexte, le microphytobenthos a un rôle clef dans la production de matière organique autochtone dans les estuaires (Ford et al., 2005). La matière organique allochtone peut provenir de la végétation des rives des estuaires, être apporté par le ruissèlement du bassin versant (Middelburg & Herman, 2007), mais aussi être importée de l'océan grâce aux marées (Bianchi, 2007). L'ensemble de la matière organique est modifié par les différents

processus géochimiques ainsi que par la dégradation par les organismes. Pour ces raisons aussi, on considère que le milieu estuarien agit comme une zone tampon, en transformant la matière organique d'origine fluviale avant son transfert vers la mer (Middelburg & Herman, 2007).

e) Turbidité

Les apports de matière en suspension par le fleuve, la remise en suspension des sédiments de fond et les épisodes d'efflorescence phytoplanctonique induisent des variations de turbidité. La turbidité atténue la pénétration de la lumière dans l'environnement aquatique. La lumière peut donc devenir un paramètre limitant pour les organismes phototrophes comme le microphytobenthos et le phytoplancton (Cloern, 1987; Scanes et al., 2017). Plusieurs facteurs peuvent influencer la turbidité. Il a été démontré que les estuaires longs et à forte marée présentaient une turbidité plus importante que les estuaires courts avec une faible marée (Uncles et al., 2002). La remise en suspension par les vagues va aussi générer localement une charge élevée de sédiment dans la colonne d'eau.

En résumé, à cause de la forte variabilité de tous ces paramètres environnementaux, les milieux estuariens présentent des conditions beaucoup plus variables que les autres milieux côtiers. Cette variabilité induit des stress naturels pour les organismes, qui se sont néanmoins adaptés à ces conditions de vie.

1.3. Les organismes peuplant les estuaires

Les estuaires comptent parmi les écosystèmes les plus productifs au monde (Day et al., 2012). De ce fait, ces milieux abritent un grand nombre d'organismes. Macro-, méio- et microfaune, macro- et micro-algues, poissons représentent un large éventail d'espèces qui se sont adaptées aux conditions de vie estuariennes et peuvent devenir abondantes dans ces milieux. Les estuaires sont aussi des habitats transitoires pour certaines espèces. Ils servent de nurserie pour de nombreuses espèces de poissons, mais représentent aussi une étape de migration pour certaines espèces d'oiseaux (Hill et al., 1993; Erwin, 1996) ou de poissons (Potter et al., 1990; Ray, 2005; Courrat et al., 2009; James et al., 2019).

Cependant, une autre espèce animale s'est implantée massivement sur les berges des estuaires : l'homme. Historiquement, les estuaires ont été des lieux d'installation privilégiés car ce sont des sites stratégiques pour le développement du commerce, mais aussi pour bénéficier des ressources abondantes (pêche et aquaculture). De plus, les industries ont su profiter pleinement de la localisation stratégique le long des estuaires. D'une part, l'accès au fleuve et à la mer facilitent le transport des matières premières et des produits finis. D'autre part, la proximité de l'eau est un avantage considérable pour les industries (p. ex., les centrales nucléaires, les papèteries). En outre, ces milieux abrités et productifs sont des lieux avantageux pour l'installation de structures aquacoles. Ces activités influencent localement les estuaires en transformant

les sédiments à proximité des structures (par biodéposition de matière organique et accumulation des coquilles) (Forrest et al., 2009). Aujourd'hui, la plupart des grands ports du monde sont construits autour des estuaires et deltas (p. ex., Shanghai, Calcutta, New-York, Rotterdam, Hambourg, Le Havre, Bristol). La présence humaine a donc radicalement transformé la morphologie de ces milieux. A titre d'exemple, l'estuaire de la Seine où se situe le port du Havre aurait perdu 90% de sa surface intertidale en 150 ans (Ducrotoy, 2010).

2. Qualité environnementale des milieux estuariens

2.1. Conséquences des actions anthropiques sur les estuaires

Beaucoup d'estuaires sont aujourd'hui largement impactés par les activités anthropiques, causant des problèmes environnementaux tels que la pollution (Birch, 2000), ou l'eutrophisation (Le Moal et al., 2019; Ratmaya et al., 2019). De plus, la réduction et la dégradation d'habitats importants provoque des perturbations des populations d'oiseaux migrateurs et un déclin des populations de poissons (McLusky et al., 1992). Dans certains sites, ces perturbations sont relativement modernes et remontent à ~200 ans (p.ex., Nouvelle-Zélande) (Taffs et al., 2008). Cependant, sur les côtes européennes, la plupart des estuaires (p.ex., la Tamise (Meddens, 1996; Waller & Grant, 2012), le Rhin (Nienhuis, 2008), la Loire (Tessier, 2001), la Gironde (Coquillas, 2001), la Charente (Billaud et al., 2001) et la Vilaine (Gauthier, 2006)) ont été modifiés par l'homme depuis des millénaires (par des activités tels que les modifications hydrologiques, les pâturages, les marais salants, les activités minières, etc.). Ceci rend encore plus difficile la différenciation entre les changements naturels et ceux induits par l'homme. Dans certains estuaires, les premiers procédés industriels susceptibles d'avoir entraîné une contamination métallique datent de l'Antiquité, voire même de l'âge du bronze (2700-900 av. J.-C.) (Chauris, 2002, 2011). En revanche, l'intensification des impacts anthropiques s'est accrue au cours des deux derniers siècles à cause de l'augmentation de la population et de la révolution industrielle (Freeman et al., 2019). Par conséquent, les estuaires comptent aujourd'hui parmi les écosystèmes les plus physiquement modifiés et les plus pollués (Taffs et al., 2017). Le degré de contamination d'un estuaire dépend donc de l'histoire de l'implantation humaine, du niveau et du type d'industries présentes sur ses rives et dans son bassin versant, ainsi que de la dynamique de l'environnement (p.ex., le temps de résidence des polluants et les processus de reminéralisation).

Les perturbations anthropiques peuvent être classées en quatre catégories (Day et al., 2012) : 1) l'eutrophisation 2) les altérations physiques ; 3) l'introduction de matières toxiques ; 4) les changements des communautés dus à la pêche ou à l'introduction d'espèces exotiques. Chacune de ces catégories est détaillée ci-dessous.

1) *L'eutrophisation*

L'eutrophisation résulte de l'apport important de nutriments inorganiques (composés azotés et phosphorés) et organiques (composés carbonés) (Cloern, 2001). De manière générale les nutriments sont des éléments clefs pour le développement de la production primaire. Cependant, l'augmentation des densités humaines et leurs activités à proximité des estuaires favorisent l'enrichissement des cours d'eau en nutriments, en composés azotés et phosphatés (Logan et al., 2011) ainsi qu'en matière organique labile (Mackenzie et al., 2011). Les sources des nutriments sont multiples. D'abord, les rejets urbains peuvent être à l'origine d'enrichissements (Paerl et al., 2004). Les industries (p.ex., industrie du papier), peuvent rejeter des composés organiques (Colodey & Wells, 1992). Aussi l'aquaculture est une source d'apports en matière organique (Bouchet & Sauriau, 2008). Finalement, l'agriculture intensive utilise massivement des nutriments (fumier, lisier ou engrais synthétiques) pour augmenter les productions agricoles (Dupas et al., 2018), et la déforestation aggrave le ruissellement et augmente ainsi l'apport de nutriments dans les estuaires. Les nutriments inorganiques sont absorbés par les producteurs primaires tels que les macro- et micro-algues et les cyanobactéries (Ratmaya et al., 2019). Cette prolifération des organismes photo-autotrophes entraîne une augmentation des bactéries aérobies les décomposent après leur mort. La dégradation bactérienne des fortes biomasses conduit à une augmentation de la demande en oxygène, nécessaire pour l'oxydation de la matière organique (Smith & Able, 2003). Ces apports importants créent des zones où la consommation d'oxygène excède sa production, ce qui induit un appauvrissement en oxygène du milieu, jusqu'à de possibles hypoxies (oxygène dissous <2.0mg/l) ou anoxies (oxygène dissous <0.1mg/l). Ces événements ont un impact important sur les communautés benthiques (Kennish, 2002), pouvant les déstabiliser et entraîner des changements dans la densité et la diversité (Alve, 1995; Mojtahid et al., 2008; Parent et al., 2021). En cas d'anoxie sévère et prolongée, les communautés benthiques seront éradiquées (Diaz & Rosenberg, 2008).

2) *Les altérations physiques*

Les altérations physiques sont des changements directs de la structure physique ou dynamique d'un estuaire. Les altérations physiques sont multiples, elles résultent des constructions de digues (Traini et al., 2015), barrages (Morais, 2008; Yang et al., 2011), chenaux, mais aussi du dragage et de la chenalisation pour la navigation (van Maren et al., 2015a) qui impactent l'équilibre hydro-sédimentaire naturel. Ces modifications hydro-morphologiques perturbent l'écoulement naturel (Jeuken & Wang, 2010; Monge-Ganuzas et al., 2013) et transforment ainsi la turbidité (van Maren et al., 2015b) ou le gradient de salinité naturel (Kerner, 2007; Winterwerp et al., 2013).

Au cours de ces siècles d'occupation humaine, les estuaires ont subi des pertes d'habitat par poldérisation. En général ces changements se sont produits de manière progressive avec des empiètements progressifs sur les zones humides pour répondre à des besoins agricoles (Eertman et al., 2002). Néanmoins, au cours du dernier siècle, pour répondre à des besoins portuaires, urbains et industriels, l'empiètement des structures

humaines sur les zones humides s'est intensifié (Probert, 2017). De plus, l'artificialisation des berges et la déméandrisation du fleuve a engendré des pertes d'habitats intertidaux. Ces espaces sont des zones clés pour les organismes qui y vivent de façon pérenne (McLusky et al., 1992) ou qui en ont besoin ponctuellement pour se nourrir ou se reproduire (p.ex., les oiseaux) (Burton et al., 2006)

3) *Les introductions de contaminants ou de polluants*

Les matières introduites dans l'environnement peuvent être des contaminants dont la concentration à l'état naturel est bien plus faible (p.ex., cuivre, zinc, fer), ou des polluants qui n'existent pas dans l'environnement à l'état naturel (p.ex., hydrocarbures, PCB, DDT, hormones de synthèse) (Kennish, 2002). Ces éléments peuvent présenter une toxicité aiguë ou, plus couramment, provoquer des effets chroniques ou sublétaux sur les organismes (Weis & Weis, 1994; Henriques et al., 2019). Les éléments toxiques peuvent également se bioaccumuler dans les chaînes alimentaires (Zhao et al., 2012; Yuan et al., 2020). Ces introductions de matières toxiques peuvent provenir de multiples sources. En effet, les pollutions peuvent se produire directement dans le milieu estuarien en tant que pollutions chroniques (p.ex., liées à la navigation, comme les rejets de carburant et les peintures antifouling (Dafforn et al., 2011) ou les rejets industriels et domestiques (Birch, 2000)) ou ponctuels (p.ex., les marées noires ou d'autres fuites accidentelles de polluants (Riaux-Gobin, 1985)). En outre, les estuaires sont aussi les réceptacles des ruissellements des pollutions occasionnées par les activités du bassin versant (Birch & McCready, 2009) (p.ex., aires urbaines, activités minières, pesticides). L'imperméabilisation des sols du bassin versant a augmenté ce ruissèlement et ainsi la quantité de polluants arrivant dans les eaux estuariennes.

4) *Les changements de communautés dus aux activités anthropiques*

a. Les activités de pêche

Pour l'ichtyofaune, comme pour les autres groupes d'organismes, les communautés estuariennes se caractérisent par une faible richesse spécifique comparée aux milieux marin et dulçaquicole (Whitfield, 2015; Whitfield & Harrison, 2021). Parmi les espèces de poissons importantes pour la pêche commerciale, peu vivent exclusivement dans les estuaires, mais ces milieux sont des habitats transitoires importants pour leurs cycles de vie (migrations de poissons diadromes comme le saumon ou l'esturgeon) (Probert, 2017). La surpêche est l'une des plus anciennes perturbations anthropiques occasionnées dans les estuaires. Cette perturbation conduit à une diminution de la biomasse et de la biodiversité estuariennes (Blaber, 2013). Si une espèce spécifique est exploitée de manière trop intensive, ses concurrents et ses proies peuvent augmenter, et des changements dans la composition de la biocénose estuarienne peuvent en résulter (Breitburg et al., 2009).

b. Les introductions d'espèces exotiques envahissantes

L'introduction de nouvelles espèces dans les écosystèmes côtiers a eu lieu pendant de nombreux siècles, à dessein ou par accident (Minchin et al., 2009). Cependant, ces invasions se sont multipliées avec l'augmentation des échanges maritimes (Cohen & Carlton, 1998). Beaucoup de ports se trouvant à l'embouchure des estuaires, ces milieux sont parmi les premières zones touchées par les introductions d'espèces non natives (Briggs, 2012). Parmi ces espèces dites exotiques, certaines se révèlent invasives et prolifèrent dans les milieux où elles ont été introduites. Ces espèces interagissent avec la biocénose en place ce qui peut conduire à des modifications (Ruiz et al., 1999). Par exemple, dans la baie de Chesapeake (U.S.A.), l'introduction de la balane *Loxothylacus panopaei* a affecté les communautés de crabes *Eurypanopeus depressus* en diminuant leur capacité reproductrice (O'Shaughnessy et al., 2014). Un autre exemple concerne le bivalve *Corbicula fluminea* dont l'introduction dans l'estuaire de Minho (Portugal) a provoqué des changements dans les communautés des espèces autochtones (Ilarri et al., 2014).

2.2. Besoin d'indicateurs écologique du milieu estuarien

Aujourd'hui, la plupart des milieux marins, dulçaquicoles, ou de transition, sont affectés de manière plus ou moins prononcée par les activités humaines. L'un des principaux défis du XXI^e siècle est donc de concevoir une occupation humaine dans le milieu naturel sans trop affecter la biodiversité et les processus écosystémiques. Dans ce contexte, des réglementations ont été adoptées pour protéger les milieux aquatiques. Depuis les années 2000, la directive-cadre européenne sur l'eau (Council of the European Communities, 2000) impose aux pays signataires de maintenir ou restaurer la bonne qualité écologique de toutes leurs masses d'eau continentales et côtières. Afin d'atteindre ces objectifs, la première étape a été de développer des indicateurs permettant d'évaluer l'état écologique des masses d'eau, pour pouvoir prendre des mesures afin de les restaurer et de les protéger. Par conséquent, la surveillance de la qualité écologique est devenue un enjeu majeur. Depuis une vingtaine d'années, une multitude d'indicateurs de qualité biologique ont été développés (Diaz et al., 2004). Certains indicateurs reposent sur des mesures des paramètres chimiques (Crane, 2003; Rodríguez et al., 2006; Tueros et al., 2008) ou physico-chimiques (Bald et al., 2005b; Best et al., 2007), reflétant souvent chacun un type de pollution. D'autres indicateurs sont basés sur les organismes vivant sur le site, ce qui permet d'évaluer l'impact cumulé de toutes les pressions occasionnées dans la zone d'étude. A cette fin, plusieurs organismes sont utilisés: les poissons (Delpech et al., 2010; Lepage et al., 2016), la macrofaune (Borja et al., 2009a; van Loon et al., 2015a), le phytoplancton (Devlin et al., 2007; Revilla et al., 2009), les macroalgues (Juanes et al., 2008) ou les angiospermes (Orfanidis et al., 2007). Parmi les groupes utilisés en bioindication, le phylum des foraminifères (Eukaryotes, Rhizaria) présente plusieurs avantages :

1. Ces organismes sont sédentaires, leur mobilité limitée les empêche de fuir les perturbations. De ce fait ils reflètent les conditions du site où ils se sont développés.
2. Ils ont un cycle de vie court (quelques mois à un ou deux ans (Boltovskoy, 1964)), la communauté réagit donc rapidement aux changements environnementaux (Alve, 1995; Murray, 2006; Albani et al., 2007).
3. Ce groupe est diversifié et présente une large gamme de stratégies écologiques (Schönfeld et al., 2012; Jones, 2013). Certaines espèces sont considérées comme sensibles et d'autres comme plus tolérantes (Jorissen et al., 2018).
4. Ces organismes sont généralement de petite taille (0.1-1mm). De ce fait, les foraminifères présentent généralement des abondances importantes dans de petits volumes de sédiment (Scott et al., 2007) (p.ex., dans cette thèse les densités moyennes étaient de ~500 ind/50cc). Ceci permet d'obtenir une base de données importante, permettant de faire des analyses statistiques robustes avec un effort d'échantillonnage réduit (Frontalini & Coccioni, 2011).
5. Ils sont présents dans presque toutes les eaux marines et les eaux de transition du globe, permettant leur utilisation comme bioindicateurs dans toutes les régions (Murray, 2006; Scott et al., 2007).
6. Ils sont sensibles à de multiples sources de pollution (p.ex., Alve, 1995; Burone et al., 2006; Frontalini & Coccioni, 2011; Dijkstra et al., 2017; Martínez-Colón et al., 2018).
7. Les foraminifères à test minéralisé peuvent fossiliser, créant ainsi des archives historiques qui peuvent fournir des conditions de référence d'avant les impacts anthropiques (p.ex., Scott et al., 2005; Alve et al., 2009; Dolven et al., 2013; Vidović et al., 2016).

3. Les foraminifères

3.1. Généralités sur les foraminifères

Les foraminifères sont étudiés dès le 19^e siècle par plusieurs naturalistes (p.ex., Alcide d'Orbigny, Henry Bowman Brady, Pierre Denys de Montfort, Christian Gottfried Ehrenberg). Initialement classés à tort parmi les céphalopodes (d'Orbigny, 1826), les foraminifères sont un phylum d'eucaryotes unicellulaires appartenant au groupe des Rhizaires. Les foraminifères sont constitués d'une seule cellule autour de laquelle la plupart d'entre eux construisent une coquille (appelée « test »), qui peut être organique (coquille protéique, non minéralisée), ou composée de carbonate de calcium ou de particules agglutinées (Kitazato & Bernhard, 2014). Ces tests comprennent une ou plusieurs chambres reliées entre elles par un petit orifice appelé « foramen ». Le nom de ce phylum vient de cette particularité morphologique, foraminifères signifiant « ceux qui portent un foramen » (D'Orbigny, 1839).

La morphologie et la composition du test constituent la base de la taxonomie du phylum. On peut diviser les foraminifères en quatre catégories informelles selon la construction de leur test : tests agglutinés, calcaires hyalins, calcaires, porcelanés et organiques (Figure I.3). Aujourd'hui, les analyses moléculaires ont montré que la séparation de ces groupes n'a plus de validité phylogénétique, mais ces groupes sont encore largement utilisés dans les études morphologiques. La coquille des espèces agglutinées est composée de grains de sédiment agrégés ensemble grâce à un ciment organique ou calcaire. Ces grains peuvent être constitués de quartz, mais aussi d'autres minéraux (p.ex de l'obsidienne) ainsi que de matériaux d'origine biologique comme des débris coquillers, des coccolithes ou des spicules d'oursins (Murray, 1979). Les espèces calcaires produisent leur tests de carbonate de calcium en changeant le pH autour de leur cytoplasme et en utilisant les ions Ca^{2+} et CO_3^{2-} contenus dans l'eau de mer (de Nooijer et al., 2009). Ce groupe est divisé entre les espèces hyalines et porcelanées. Les parois des tests des espèces hyalines sont composées de calcaire de calcite à basse teneur en magnésium, donnant un aspect vitreux à la coquille (Adl et al., 2019). Les parois des tests des espèces porcelanées sont composées de calcaire de calcite à haute teneur en magnésium. Les cristaux sont orientés au hasard et réfractent la lumière dans toutes les directions, donnant un aspect laiteux au test, rappelant la porcelaine (Adl et al., 2019). Enfin, certaines espèces de foraminifères sont dites à test organique car elles ne possèdent pas de coquille minérale, mais seulement une enveloppe protéique (Pawlowski, 2009).

La forme du test peut varier de formes très simples, avec des coquilles à une seule chambre à des formes plus complexes chez les espèces à plusieurs chambres (Adl et al., 2019). Après cette première distinction, les espèces sont ensuite divisées taxonomiquement en fonction de leurs traits morphologiques, comme l'arrangement des chambres, les ornements, la position de l'ouverture, etc. (Haynes, 1981). Ainsi, la taxonomie actuelle des foraminifères est essentiellement basée sur la morphologie du test. Depuis quelques décennies, les approches moléculaires ont permis de remettre en question certains éléments de classification. En effet, ces méthodes ont permis d'identifier des espèces cryptiques (Hayward et al., 2004; Darling & Wade, 2008; Deldicq et al., 2019) et d'améliorer la classification existante (Schweizer et al., 2008, 2009; Pawlowski et al., 2013) en précisant les limites interspécifiques (Tsuchiya et al., 2008; Pillet et al., 2013; Roberts, 2016).

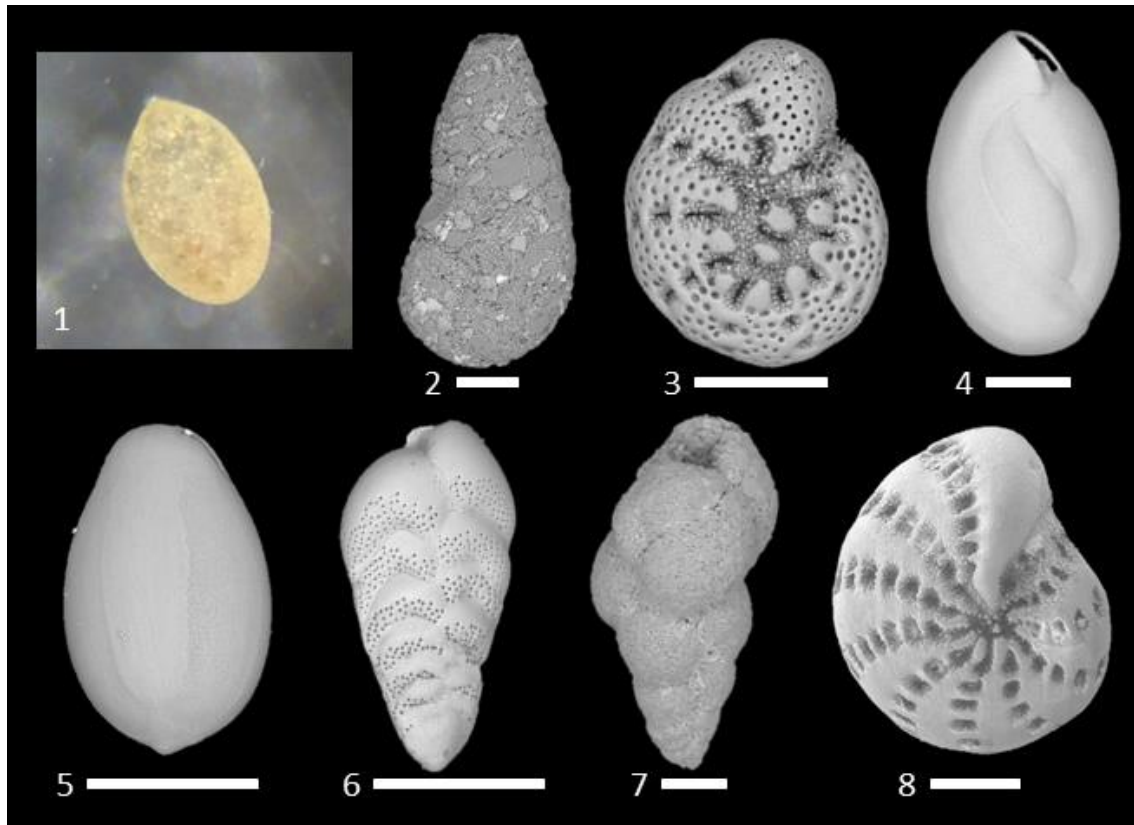


Figure I.3 :Exemples des quatre types de test : 1, test organique ; 2, agglutiné ; 3, hyalin ; 4, porcelané et de plusieurs types d'arrangement des loges : 5, une seule chambre ; 6, arrangement bisérié ; 7, trisérié ; 3, 8, planispiralé ; 4, miliole. Tous les spécimens proviennent d'estuaires de la façade Atlantique : 1, Belon ; 2,3,4,7 Auray ; 5,6,8 Vie. 1: Psammophaga sp. Arnold, 1982; 2: Ammotium salsum (Cushman and Brönnimann, 1948); 3: Elphidium oceanense (d'Orbigny in Fornasini, 1904); 4: Quinqueloculina oblonga (Montagu, 1803); 5: Fissurina sp. Reuss, 1850; 6: Bolivina sp. D'Orbigny, 1839; 7: Eggerelloides scaber (Williamson, 1858); 8: Elphidium williamsoni Haynes, 1973. Echelle : 100 μ m.

Toutes les espèces de foraminifères possèdent des longs filaments appelés granuloréticulopodes qui permettent à l'organisme d'interagir avec son environnement pour s'alimenter et se déplacer (Jones, 2013). Les différentes espèces utilisent une large gamme de stratégies trophiques qui incluent des modes de vie principalement hétérotrophes, mais aussi mixotrophes. Ils obtiennent leur nutriments par filtration (p.ex., Cedhagen, 1988), broutage (p.ex., Pascal et al., 2009), absorption de matière organique dissoute (p.ex., DeLaca et al., 1981), parasitisme (p.ex., Cedhagen, 1994; Nielsen, 2002), prédation (p.ex., Culver & Lipps, 2003; Dupuy et al., 2010), symbiose (p.ex., Bird et al., 2017; Takagi et al., 2019), et même par kleptoplastie (p.ex., Jauffrais et al., 2016; Jesus et al., 2022). Le développement d'un large éventail de stratégies et d'adaptations, a permis à ce groupe d'être présent dans la plupart des environnements et de survivre à des conditions adverses. A titre d'exemple, certaines espèces sont capables de survivre à des conditions anoxiques prolongées (Langlet et al., 2014) en entrant en dormance (LeKieffre et al., 2017), d'autres espèces sont capables de dénitrifier (Risgaard-Petersen et al., 2006). Les foraminifères vivent dans toutes les régions marines du globe, des côtes aux abysses, des tropiques aux pôles (Scott et al., 2007). Certaines espèces de foraminifères à test organique ont été détectées en eau douce et dans le sol (Holzmann et al., 2021). Les foraminifères constituent un élément important de la méiofaune (Pawlowski, 2009). Le nombre total

d'espèces actuelles de foraminifères a été estimé à 6000 (Jones, 2013). Quelques espèces de foraminifères sont planctoniques (environ 50 espèces morphologiques (Schiebel & Hemleben, 2017), incluant de nombreuses espèces cryptiques (Darling & Wade, 2008)) mais la grande majorité des espèces sont benthiques. Parmi les espèces benthiques, certaines sont épibenthiques, certaines vivent à la surface du sédiment, et d'autres sont endopéliques (Murray, 2006). Leur position verticale dans le sédiment est liée aux disponibilités de la nourriture et de l'oxygène (TROX model, Jorissen et al., 1995).

3.2. Un organisme modèle étudié par les géologues et les biologistes

Les premiers fossiles connus de foraminifères datent du Cambrien inférieur, il y a 540 millions d'années (Murray, 2006), mais ce groupe pourrait être apparu au Néoprotérozoïque, entre 690 et 1 150 millions d'années, d'après les données moléculaires (Pawlowski et al., 2003). Après la mort des organismes, les tests de certaines espèces de foraminifères peuvent être préservés dans le sédiment. Cette caractéristique permet aux géologues de les utiliser pour faire des reconstructions paléo-environnementales afin de retracer les changements climatiques et océanographiques passés.

A cette fin, deux types de méthodes sont utilisées : l'approche écologique et l'approche géochimique. La première est basée sur les connaissances des réponses des foraminifères aux différents paramètres environnementaux, elle permet de reconstruire les variations climatiques, les paléo-courants, la paléo-élévation du niveau marin, etc. (Schmiedl et al., 2010; Mary et al., 2017; Mojtahid et al., 2017; Rodriguez-Lazaro et al., 2017; Depuydt et al., 2022). La deuxième méthode se base sur les éléments incorporés pendant le processus de calcification de la coquille. Ces éléments servent de proxy des conditions physico-chimiques passées, et permettent de faire des reconstructions paléo-climatiques et paléo-océanographiques (Tzedakis et al., 2009; Koho et al., 2015; Mojtahid et al., 2019; Le Houedec et al., 2020). Il est donc fondamental d'étudier les assemblages vivants de foraminifère et leurs relations avec les paramètres environnementaux pour ainsi développer des clefs de compréhension pour l'étude des assemblages passés.

La préservation des coquilles de foraminifères dans le sédiment déposé actuellement (et dans un passé récent) permet d'obtenir des archives pour connaître l'état des communautés avant l'implantation des activités humaines (Francescangeli et al., 2016; Hess et al., 2020; de Jesus et al., 2020; Dolven & Alve, 2021). Cette caractéristique est un avantage considérable pour les biologistes, car elle peut fournir des connaissances sur les conditions de référence dans le cadre du biomonitoring.

L'étude des foraminifères par les biologistes permet aussi de suivre les changements dans un passé récent, comme l'évolution des glaciers marins (Fossile et al., 2020; Nardelli et al., 2022) ou la restauration des écosystèmes (Polovodova Asteman et al., 2015). Finalement, les foraminifères sont également des acteurs importants dans les cycles biogéochimiques, comme celui de l'azote (p.ex., dénitrification (Risgaard-Petersen et al., 2006; Piña-Ochoa et al., 2010; Choquel et al., 2021)) ou du carbone (Moodley et al., 2000; Cesbron et

al., 2016)). Leur étude peut ainsi contribuer à une meilleure compréhension du fonctionnement des milieux marins.

3.3. Indices biotiques basés sur les foraminifères

En utilisant l'abondance, la diversité et la composition des communautés ainsi que la sensibilité aux pollutions des différentes espèces de foraminifères, il est possible d'obtenir des indications sur l'état écologique de l'écosystème étudié. Les foraminifères ont montré des sensibilités face à différentes pressions : les pollutions en éléments métalliques (Alve, 1991; Frontalini & Coccioni, 2008; Cherchi et al., 2009; Coccioni et al., 2009; Caruso et al., 2011; Brouillette Price et al., 2019; Smith & Goldstein, 2019; Cong et al., 2021), les rejets des activités aquacoles (Oron et al., 2014; Pawlowski et al., 2016; He et al., 2019; Bouchet et al., 2020; Dubois et al., 2021), les rejets industriels (p.ex., rejets d'usine de pâte à papier (Nagy & Alve, 1987; Schafer et al., 1991)), les exutoires d'eaux usées (Burone et al., 2007; Parent et al., 2021), les marées noires (Morvan et al., 2004; Brunner et al., 2013; Young et al., 2021), etc. Ces sensibilités sont prometteuses pour l'utilisation des foraminifères comme bio-indicateurs des perturbations liées aux activités anthropiques dans les estuaires. Cependant, les indices biotiques existants ont été développés pour le suivi des milieux marins ouverts.

En milieu marin, la grande majorité des indices de qualité environnementale reposent sur la tolérance des espèces vis-à-vis de l'enrichissement en matière organique. Cet enrichissement entraîne des changements dans la composition des communautés et la dominance relative des différentes espèces. Ces indices reposent en grande partie sur les travaux de Pearson & Rosenberg (1978). Ces auteurs ont proposé une succession de la diversité, de l'abondance et de la biomasse des communautés le long d'un gradient d'enrichissement en matière organique. Cette succession est basée sur des observations de macrofaune benthique, mais peut aussi être utilisée pour l'étude de la méiofaune, telle que les foraminifères. Les courbes espèces/abondance/biomasse (SAB) (Figure I.4) résument les changements des paramètres faunistiques survenant le long d'un transect partant du point de rejet des effluents et aboutissant dans des zones situées au-delà de l'effet des matières rejetées. Les sédiments situés à proximité du point de rejet d'une forte pollution sont dépourvus de faune benthique. En s'éloignant de la zone de rejet, le nombre d'espèces s'accroît jusqu'à une valeur asymptotique. Les valeurs d'abondance augmentent rapidement entre le point de rejet et un pic d'espèces opportunistes. Cette augmentation est due à des populations extrêmement abondantes de seulement quelques espèces opportunistes. Puis l'abondance diminue tandis que la richesse spécifique augmente. Enfin les deux valeurs se stabilisent.

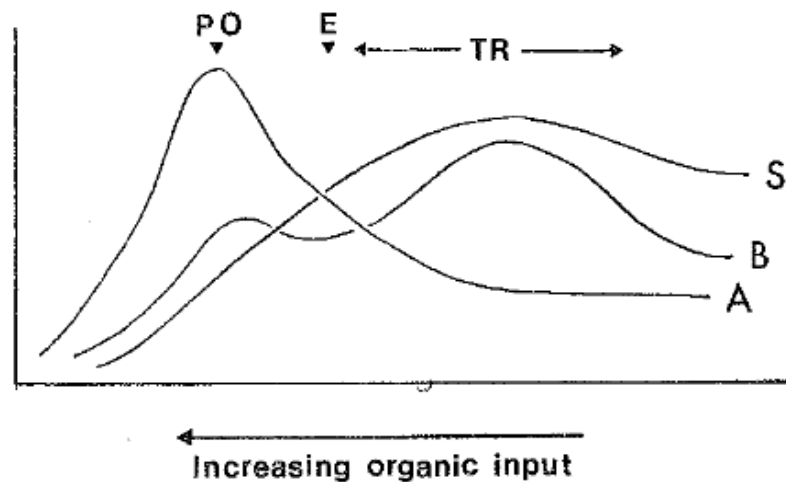


Figure I.4 : Modèle SAB basé sur les changements de S, le nombre d'espèces ; A, abondance totale ; B, biomasse totale, le long d'un gradient d'enrichissement organique. PO, pic d'opportunistes ; E, point d'écotone ; TR, zone de transition. D'après Pearson et Rosenberg, 1978.

Sur la base de ce concept, plusieurs indices basés sur les foraminifères ont été développés pour le milieu côtier :

1) L'indice foram-AMBI

L'indice AMBI (Borja et al., 2000) a initialement été conçu pour l'étude de la macrofaune benthique. Il est basé sur le concept de groupes écologiques de sensibilité/tolérance des espèces vis-à-vis des enrichissements en matière organique (Hily & Glemarec, 1990). Cet indice divise les espèces de macrofaune benthique en cinq classes appelées « groupes écologiques » selon leur sensibilité/tolérance face à un enrichissement en matière organique. Sur la base des pourcentages cumulatifs des cinq groupes, un indice de qualité environnemental est calculé. Récemment, Alve et al. (2016) puis Jorissen et al. (2018) ont transposé cet indicateur aux foraminifères benthiques en proposant une liste d'attribution des espèces aux groupes écologiques par rapport à leurs différentes réponses à l'enrichissement en TOC. Cette liste a été adaptée par Bouchet et al. (2021) aux écosystèmes de transition grâce aux données de 21 études testée dans 35 stations provenant de 5 sites.

2) Tolerant Species Index for the Mediterranean (TSI-Med)

La valeur de l'indice TSI-Med (Barras et al., 2014) est principalement basée sur des groupes de taxons tolérants au stress et/ou opportunistes. Le TSI-Med est basé sur un seul groupe de taxons tolérants au stress.

3) Foraminiferal Stress Index (FSI)

Le FSI (Dimiza et al., 2016) divise les individus en taxons « sensibles » et « tolérants », regroupés selon leur comportement face à l'enrichissement en matière organique et utilise les proportions relatives de ces deux groupes pour calculer la valeur de l'indice, en attribuant dix fois plus de poids au premier groupe. Cet indice a été testé sur les côtes grecques en Méditerranée.

4) Le Norwegian Quality Index basé sur les foraminifères (NQI_f)

Initialement développé pour le suivi de la macrofaune (Rygg, 2006), le NQI (Norwegian Quality Index) a été adapté pour le suivi des foraminifères benthiques (Alve et al., 2019). Le NQI-f inclut une métrique basée sur la proportion d'espèces sensibles dans la communauté (foram-AMBI) et une métrique basée sur la diversité.

D'autre part, un indice de qualité environnementale basé sur la diversité a été proposé.

5) L'exponentiel de Shannon ($\text{ExpH}'bc$)

Bouchet et al. (2012, 2018) ont proposé d'utiliser le $\text{expH}'bc$, correspondant à l'indice de Shannon-Wiener (Shannon & Weaver, 1963) corrigé par Chao & Shen (2003). Cet indice a été utilisé dans les fjords norvégiens (Bouchet et al., 2012), puis dans les lagunes italiennes (Bouchet et al., 2018a) et les zones côtières tunisiennes (El Kateb et al., 2020).

4. Paradoxe estuarien

Les indices brièvement présentés précédemment sont déjà utilisés dans les milieux côtiers. Cependant leur application en milieu estuarien s'avère difficile. En effet, les organismes présents dans les estuaires doivent faire face aux multiples stress naturels induits par les variations des paramètres environnementaux liés aux apports variables d'eaux douces du fleuve. Par conséquent les estuaires présentent naturellement une faible diversité et sont habités par des espèces tolérantes à ces stress. Ces communautés présentent donc des caractéristiques biologiques similaires à celles observées dans des écosystèmes côtiers pollués. A cette variabilité naturelle s'ajoute la pression des impacts anthropiques, à la fois engendrée par les activités humaines dans l'estuaire mais aussi dans le bassin versant. Le fait que les communautés naturelles trouvées dans les estuaires présentent des caractéristiques similaires à celles d'environnements côtiers pollués est appelé « le paradoxe estuarien » (Dauvin, 2007; Elliott & Quintino, 2007). Pour cette raison, les indicateurs développés dans les milieux côtiers ne fonctionnent pas en milieu estuarien et doivent être adaptés à ce milieu (Dauvin & Ruellet, 2009; Blanchet et al., 2012; Bouchet et al., 2021). Afin d'adapter les indicateurs existants aux estuaires, il est d'abord nécessaire de parfaitement comprendre la répartition naturelle des espèces dans ces milieux. Cette compréhension est primordiale pour le développement d'indices biotiques adaptés aux estuaires. Une fois cette étape validée, il sera possible de détecter des déviations à cette répartition naturelle qui pourraient refléter des impacts anthropiques.

5. Contexte de la thèse

Le projet FORESTAT (FORaminifères des ESTuaires de la façade ATLantique), financé par l'Office Français pour la Biodiversité (OFB) et l'Université d'Angers, a pour objectif d'améliorer la compréhension de la répartition des espèces de foraminifères dans les milieux estuariens, avec comme finalité le développement un indice biotique adapté à ces milieux. Ce projet se focalise sur les estuaires de la façade

atlantique française. Les nouvelles connaissances acquises serviront à proposer des pistes pour l'élaboration d'un bio-indicateur de la qualité des vasières estuariennes. Ce projet combine deux approches pour l'étude des foraminifères : l'approche morphologique et l'approche du metabarcoding.

Afin de répondre aux besoins des gestionnaires des milieux estuariens, ce projet a pour objectif d'obtenir les connaissances écologiques nécessaires pour le futur développement d'un outil de biomonitoring basé sur les foraminifères. Ce projet contribuera notamment à l'amélioration de nos connaissances de la variabilité naturelle des communautés de foraminifères benthiques dans les estuaires. Ces acquis devraient non seulement nous fournir la compréhension nécessaire pour le développement d'un indice de qualité environnementale, mais aussi, de mieux concevoir le rôle des foraminifères dans ces écosystèmes complexes.

Dans ce contexte, le but de cette thèse est d'approfondir les connaissances de la distribution des communautés de foraminifères dans les milieux estuariens et d'identifier leurs relations avec les paramètres environnementaux, en utilisant une approche taxonomique traditionnelle. Les résultats obtenus permettront d'envisager des pistes pour le développement d'un indice biotique spécifiquement conçu pour les environnements estuariens.

6. Contenu de la thèse

Le **chapitre 2** se focalisera sur deux estuaires situés sur la côte atlantique française, la Vie et Auray. Le premier objectif de ce chapitre sera d'étudier la répartition des foraminifères et de connaître l'influence des paramètres environnementaux sur cette répartition. Le second objectif sera de tester l'indice d'influence marine (marine influence index, MII) sur ce jeu de données. Cet indice a été développé par Jorissen et al. (2022) (article disponible en annexe) et vise à quantifier l'influence des eaux marines à la station étudiée au moment de l'échantillonnage. L'indice se base sur trois paramètres : la distance de la station par rapport à l'embouchure de l'estuaire, le temps d'émergence, et la décharge fluviale dans les quatre semaines précédant l'échantillonnage. Le but de cet indice est de résumer plusieurs paramètres liés à l'influence marine (la salinité, l'apport de nutriments d'origine marine et l'hydrodynamisme) qui devraient structurer la répartition des communautés estuariennes.

Une partie importante de ce travail de thèse sera d'évaluer les niches écologiques d'espèces estuariennes nouvellement identifiées. En effet, la technique de barcoding de cellules individuelles a permis de révéler la présence d'un complexe d'espèces pseudo-cryptiques autrefois regroupées dans une même morpho-espèce. (Hayward et al., 2004; Schweizer et al., 2011; Darling et al., 2016; Richirt et al., 2019). En effet, l'espèce morphologique *Ammonia tepida* a longtemps été considéré comme une unique espèce possédant une grande plasticité phénotypique, jusqu'à ce que des analyses moléculaires révèlent la présence de plusieurs espèces pseudo-cryptiques (Hayward et al., 2004). Trois phylotypes T1, T2 et T6 ont d'abord été décrits. Puis des

analyses morphométriques ont permis de différencier morphologiquement ces trois phylotypes (Richirt et al., 2019). Finalement, les trois phylotypes T1, T2 et T6 ont été nommées *Ammonia veneta*, *Ammonia aberdoveyensis* et *Ammonia confertitesta*, respectivement (Hayward et al., 2021). Compte tenu de l'évolution récente de la taxonomie de ce complexe d'espèce, les chapitres de cette thèse sont donc parmi les premiers à décrire les préférences écologiques de ces espèces (avec Saad & Wade, 2016; Bird et al., 2020; Richirt et al., 2021). Au vu de la prédominance du morpho-groupe *Ammonia tepida* dans les écosystèmes estuariens (p.ex., Alve & Murray, 1994; Debenay et al., 2000; Camacho et al., 2015; Martins et al., 2016; Mojtahid et al., 2016; Saad & Wade, 2017; Francescangeli et al., 2021), il est important d'étudier leur écologie afin d'avoir une bonne compréhension de leur distribution. Pour cette raison, les **chapitres 3** et **4** de cette thèse se focalisent sur l'écologie de ces espèces.

Dans le **chapitre 3**, nous étudierons la distribution des trois espèces d'*Ammonia* dans sept estuaires, afin de déterminer si elles ont des préférences écologiques et une distribution géographique similaires ou différentes dans les milieux étudiés.

Parmi ces trois espèces, *Ammonia confertitesta* est considérée comme potentiellement invasive et supposée originaire des côtes est asiatiques (Pawlowski & Holzmann, 2007; Schweizer et al., 2011). Cette espèce est aujourd'hui massivement implantée le long des côtes européennes (Bird et al., 2020). Dans cette thèse, nous avons eu l'opportunité de réétudier deux carottes sédimentaires prélevées dans l'estuaire de la Loire en 2015. Ainsi, dans le **chapitre 4**, nous nous sommes concentrés sur l'étude des espèces d'*Ammonia* pour i) vérifier le caractère invasif d'*Ammonia confertitesta*, ii) donner une estimation de son époque d'introduction et des vecteurs possibles, iii) voir si l'arrivée de cette espèce a affecté l'espèce autochtone *Ammonia aberdoveyensis*.

Enfin, dans le **chapitre 5**, nous avons incrémenté notre jeu de données du chapitre 2 de sept estuaires supplémentaires. Les objectifs du chapitre 5 seront de 1) décrire les communautés estuariennes et leurs relations avec les paramètres environnementaux ; 2) synthétiser la répartition des espèces dans les estuaires par le biais d'un indice quantitatif ; 3) comparer l'indice d'influence marine (MII) avec l'indice de répartition des espèces afin d'évaluer la cohérence de ces indices ; 4) identifier les stations polluées et analyser les réponses éventuelles des communautés face à ce stress. Ce chapitre utilisera les connaissances apportées par les trois chapitres précédents pour proposer des pistes d'élaboration d'un outil de biomonitoring pour les estuaires.

Chapter 2

Foraminiferal distribution in two estuarine intertidal mudflats of the French Atlantic coast: testing the Marine Influence Index

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Abstract

This study focuses on the foraminiferal distribution on intertidal mudflats of two contrasted estuaries (Auray and Vie) along the French Atlantic coast. In both estuaries, the foraminiferal communities are dominated by *Haynesina germanica* and the *Ammonia tepida* group. Stations located near the outlets show a high diversity and abundance of species of the genus *Elphidium*. Stations in the inner estuary show a higher proportion of agglutinated species (*Ammotium salsum*, *Ammobaculites agglutinans*). Multivariate statistical analysis suggests that the distance to the sea and the percentage of fine sediment (<63µm) are the two main parameters explaining the foraminiferal distribution. Chemical analyses of the sediment show that the two studied estuaries are not affected by major anthropogenic pollution, so that the faunas should mainly reflect the natural controlling parameters. Three indices of environmental quality commonly used in coastal areas show counter-intuitive differences between stations, suggesting that these indices may be less reliable for use in intertidal estuarine mudflats. The newly developed Marine Influence Index (MII) integrates three major ecological factors: the position of the sampling point on the salinity gradient, the emergence time at low tide and the relative importance of fresh water discharge. In our dataset, MII shows significant correlations with the controlling environmental parameters (distance to the sea, percentage grains <63 µm), as well as with the foraminiferal patterns (PCA axis 1, species richness, percentage of *Elphidium* spp. and *Quinqueloculina* spp.). These results suggest that the MII explains a substantial part of the faunal variability on estuarine intertidal mudflats, and can be used to detect deviations from the natural distribution patterns in response to anthropogenic pollution.

Keywords: estuary; biomonitoring; foraminifera; environmental parameters

1. Introduction

Whitfield and Elliott (2011) defined estuaries as “*semi-enclosed coastal water bodies connected to the sea either permanently or periodically, having a different salinity compared to the adjacent open ocean due to freshwater inputs, and being inhabited by a characteristic biota*”. Estuaries are among the most dynamic ecosystems of the marine realm (Day et al., 2012). They host productive transitional water ecosystems of high ecological and economic interest (Cave et al., 2003). Estuaries are buffer zones protecting coastal plains against climatic events (i.e., storms, floods), and are important nursery habitats for fish and other migratory animals (Clark et al., 1994; Day et al., 2012). They are also suitable locations for human settlement due to their ideal geographical situation at the interface between continents and oceans. For centuries, they have been subjected to anthropogenic disturbances, stress and habitat modifications, because they host multiple economic activities (e.g., fishery, aquaculture, tourism, industry, urbanisation) (Kennish, 2002; Belzunce et al., 2004; Bald et al., 2005a). In this context, the European community has established the Water Framework Directive (Council of the European Communities, 2000), requesting member countries to monitor the environmental quality of various water bodies (including transitional systems such as estuaries), and when necessary, to take measures to return to a good water quality status.

Over the last few decades, the use of biotic indices to assess the environmental quality of coastal waters has expanded considerably. Several groups of organisms are used as bioindicators (e.g., fish, algae, benthic macrofauna and meiofauna) because of their sensitivity to natural and anthropogenic disturbance (Hummel et al., 2015). Among these, foraminifera (Eukaryotes, Rhizaria) have several advantages: (1) they have a sedentary lifestyle, (2) they have short life cycles (up to 2 years) that ensure a rapid response to environmental change (Murray, 2006; Albani et al., 2007), (3) they inhabit almost all marine regions and transitional waters (Murray, 2006), (4) they show high species diversity (about 9000 living species) with a large panel of functional traits (WoRMS, 2020), and (5) their fossil records can be analysed to gain insight into their community composition before recent anthropogenic impacts, and thereby can provide pristine reference conditions (Dolven et al., 2013; Alve et al., 2019).

Recently, several studies have developed foraminiferal biotic indices for coastal areas. Some of these indices are based on alpha diversity (e.g., Bouchet et al., 2012), whereas others apply an indicator species concept, using the relative proportion of stress-tolerant species (e.g., Barras et al., 2014; Alve et al., 2016, 2019; Dimiza et al., 2016; Jorissen et al., 2018). These species are often recognised as such by their response to organic matter enrichment in the sediment. However, in estuarine environments, both the spatial and the temporal variation in physical and chemical parameters are very high (e.g., salinity, temperature, water movement, turbidity, oxygen content, organic matter supplies, etc.). Such a strong variability induces natural stress that affects local biota. Consequently, estuaries show a low biodiversity, with a dominance of species adapted to adverse conditions. Even pristine estuarine communities are dominated by stress-tolerant species that are adapted to highly fluctuating environmental conditions, leading to low values for most indices of

environmental quality. This observation has been named the “estuarine paradox” (Dauvin, 2007; Elliott & Quintino, 2007). Consequently, the development of biotic indices for the highly complex and dynamic ecosystems found in estuaries is a real challenge (Elliott & Whitfield, 2011; Tweedley et al., 2015).

Due to the “estuarine paradox”, foraminiferal bio-indication indices developed in open marine environments cannot be applied directly to estuaries. Several strategies have been proposed to overcome this difficulty. Bouchet et al. (2018b) proposed to use the corrected Shannon diversity index $\text{Exp}(H'bc)$ in Mediterranean transitional zones and adapted the ecological class boundaries to this particular environment. More recently, Bouchet et al. (2021) proposed a list of stress-tolerant species, based on their response to organic matter gradients occurring in transitional waters.

In principle, the construction of a foraminiferal index of environmental quality in estuaries requires prior knowledge of their natural distribution. Only once this natural distribution, and the relation with the controlling parameters, is fully understood can it become possible to recognise distributional anomalies resulting from anthropogenic stress. However, at present, most estuaries are impacted by both natural and anthropogenic stresses. There is a general scientific consensus that the main natural parameter influencing the distribution of foraminifera in estuarine environments is salinity (e.g., Scott et al., 1980; Cearreta, 1988; De Rijk, 1995; Horton & Murray, 2007). Estuaries are characterised by strong daily and seasonal salinity variations. The daily aerial exposure due to the tidal cycle (twice a day in the studied area) is an additional natural stress factor for the foraminiferal communities on estuarine intertidal flats. This leads to high temperature and salinity variations due to evaporation/precipitation at low tide, and a mix of marine and fresh waters at high tide (Hayward & Hollis, 1994; Horton & Murray, 2007; Armynot du Châtelet et al., 2018). Other main controlling parameters are sediment grain size and natural organic matter quantity and quality (Armynot du Châtelet et al., 2009; Mojtahid et al., 2016).

When superposed on these natural parameters, anthropogenic stress can modify the distribution of benthic foraminifera. For instance, high concentrations in trace metals can influence the density, species richness and composition of communities, and can lead to an increase in abnormal tests (Alve, 1991; Armynot du Châtelet et al., 2004; Frontalini et al., 2009). An excess of anthropogenic organic matter (e.g., domestic sewage, or estuarine primary production resulting from fertiliser input) can also modify the community composition (Alve, 1991; Eichler et al., 2012). Moreover, inputs of large quantities of organic matter may induce hypoxic or anoxic zones, where oxygen consumption exceeds supply. Such events intensify during warm late summer and early autumn months (Tyson & Pearson, 1991). Other anthropogenic pollutants (e.g., hydrocarbons, hormones) and anthropogenic physical disturbance can impact living communities as well (Romano et al., 2009).

In an attempt to integrate the main natural factors affecting estuarine foraminifera in a single environmental index, Jorissen et al. (submitted this volume) proposed the Marine Influence Index (MII). The MII, calculated for each sampling station, is based on the position of the studied station along the salinity gradient,

the duration of tidal emergence and the relative importance of river runoff in the ambient water turnover. Therefore, this index reflects salinity, but implicitly also the hydrodynamic energy and fluvial and/or marine particle supplies.

The first aim of the present study is to assess the foraminiferal distribution (of the >125 µm fraction) living in the surficial sediment of intertidal mudflats along two contrasted estuaries of the French Atlantic coast. The main parameters controlling the foraminiferal communities in these environments will be identified. Next, we will investigate whether at one or more stations, the foraminiferal composition deviates from the composition expected under natural conditions, and whether this deviation is due to anthropogenic pollution. Some foraminiferal indices of environmental quality commonly used in transitional waters will be applied and assessed for their applicability to intertidal estuarine mudflats. Finally, we will investigate to what extent MII, which describes the marine influence in estuaries, is correlated with the characteristics of the foraminiferal communities, and can be used to detect distributional anomalies due to anthropogenic pollution.

2. Material and methods

2.1 Study area

In this study, the foraminiferal communities from intertidal mudflats of two small estuaries (~20 km long) on the French Atlantic coast, Auray and Vie, are investigated. Both estuaries are subject to a meso- to a low macrotidal regime, with a tidal range of about 4 m at the inlet (Office français de la biodiversité).

The Auray estuary (Figure II.1A and Supplementary Table II.S1), at about 47°35' N and 2°57' W, is a typical ria (drowned river valley) with its downstream part, or outer estuary, connected to the Morbihan Gulf, an enclosed marine bay. Two rivers flow out into the Auray estuary, the Loc'h and Bono, with a mean annual discharge of 2.71 and 1.52 m³/s, respectively (Banque Hydro). Together, they drain a catchment area of 324 km². Today, salt water penetrates up to the Tréauray Mill, 19.8 km from the mouth of the estuary at Kerpenhir Point (Office français de la biodiversité). During the 2019 environmental survey in the context of the Marine Framework Directive, based on data collected between 2012 and 2017, the environmental quality was described as “moderate” (Envlit). This evaluation was exclusively based on an indicator using fishes. Conversely, indicators based on opportunistic macro-algae, autotrophs other than phytoplankton, and hydromorphology all indicated a good environmental quality.

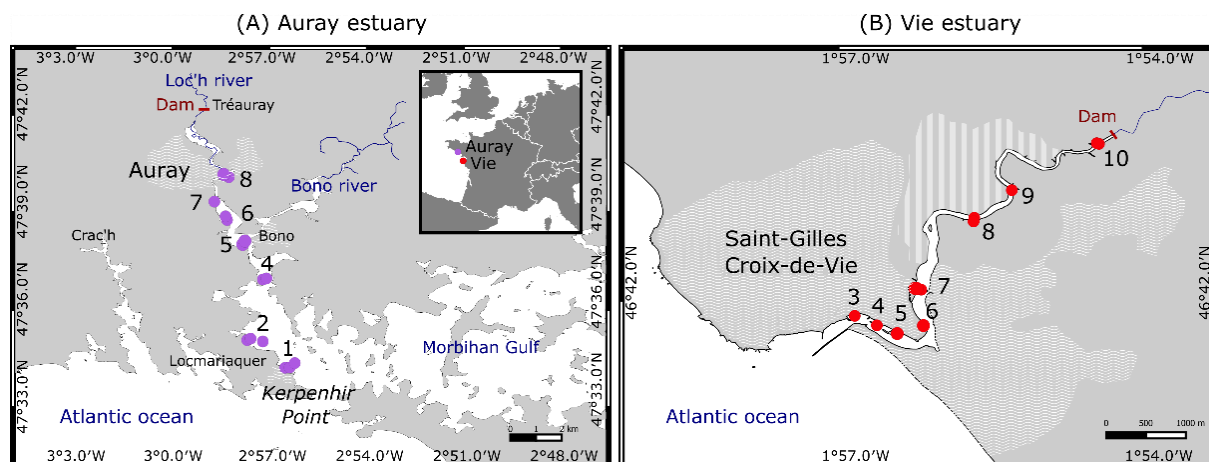


Figure II.1 : Location of sampling sites along the Auray (map A) and Vie (map B) estuaries. Sites Vie 1, Vie 2 and Auray 3 are not considered for this study. Hatched zones represent urbanised areas and stripes represent salt marshes.

The Vie estuary (Figure II.1B and Supplementary Table II.S1), situated at about 46°35' N and 1°56' W, can be characterised as a bar-built estuary. The river meanders through salt marshes before crossing the Saint-Gilles-Croix-de-Vie harbour to flow into the Atlantic Ocean. Its mouth is deflected northwards by a sandy spit. The Vie river drains a catchment basin of 84 km² and has a mean annual discharge of 1.17 m³/s (Banque Hydro; Office français de la biodiversité). At present, salt water penetrates up to 8 km landward where it is blocked by a dam. Based on the thalweg topography, Jorissen et al. (submitted this volume) deduced that before the construction of this dam, salt water penetrated up to the village of St. Maixent, 16.8 km from the mouth of the estuary. A 2019 environmental survey, performed in the context of the Marine Framework Directive, evaluated the environmental quality as “good”, on the basis of intertidal and opportunistic macroalgae and autotrophs other than phytoplankton (Envlit).

2.2. Sampling Procedure

In the Auray estuary, seven sites (Site 3 is not considered in this study), with a total of 15 stations, were sampled from the 10th to 12th of July 2019 (Figure II.1A). Samples of the surficial sediment (top centimetre) were collected at low tide on intertidal mudflats. At each site, two or three stations were sampled across the mudflat if it was large enough (A to C from upper mudflat to lower mudflat, Supplementary Table II.S2). In the Vie estuary, eight sites (Sites 1 and 2, located on sandy beaches outside the estuary, are not considered in this study), with a total of 12 stations, were sampled on the 23rd and 24th October 2018 (Figure II.1B). Most stations are located on intertidal mudflats, except for stations 4 and 5, which are positioned on mud-covered ramps in small semi-enclosed basins in the harbour of Saint-Gilles (Supplementary Table II.S2).

To study the distribution of foraminifera, three replicates were sampled for each station. Three tubes with an internal diameter of 9.6 cm were randomly placed and pushed by hand into the sediment. The top 1 cm of the sediment cores was sliced off. Samples for foraminiferal analyses were preserved in 96% ethanol and stained

with 2 g/L Rose Bengal, following the FOBIMO protocol (Schönfeld et al., 2012). Samples for grain size and organic matter analyses were taken between the cores by scraping off the surficial sediment layer.

For all sampling stations, the elevation was estimated with respect to French chart datum (lowest astronomical tide). For the stations sampled at low tide immediately above the water table, elevation was estimated using the tide tables published by the French Naval Hydrographic and Oceanographic Service (SHOM), which give the theoretical altitude of the water surface during the tidal cycles for all French harbours. For all stations higher on the mudflats, the altitude was estimated using an electronic theodolite (NA720[®] Leica; height accuracy <1 cm), and official benchmark altitude points. At stations located too far from referenced benchmarks, the lichen vertical zonation (Vail & Walker, 2021), developed on rocky outcrops and walls (quay, jetty, wharf...), was used. The conspicuous upper limit of the black lichen *Verrucaria maura* corresponds to the mean highest spring tide level, for which the altitude is given in the tide tables of the SHOM. Furthermore, this limit is highlighted by the lower limit of the orange lichen *Xanthoria parietina*, which marks the beginning of the supratidal zone. Taking into account the field constraints (and the uncertainty in the lichen vertical zonation) a range of uncertainty of +/- 10 cm was estimated for our elevation data. By combining elevation and tide tables from the SHOM (based on the TELEMAC-MASCARET modelling system), it was possible to evaluate for each station the duration of the tidal emergence. The theoretical emergence time was estimated using tidal data corresponding to mean spring tide conditions (i.e., the French tidal coefficient of 90). The “emergence time” was then expressed as a percentage of the duration of an entire mean spring tide cycle.

Salinity was measured in the tidal channel for the lowest stations, and in tidal pools for the stations higher on the mudflats. In view of the large temporal variability in salinity (Attrill, 2002; Diz et al., 2009), not only on the exposed mudflats (due to evaporation and precipitation) but also in the tidal channel (due to tide and river runoff), such one-off measurements are not representative for the foraminiferal habitat. Consequently, they are not further used in this study.

2.3. Laboratory Analyses

Sediment grain size was analysed with a Malvern 3000 Mastersizer laser diffraction particle size analyser. The sediment was not decarbonated before the analyses. To give an overall characterisation of the sediment, the percentage of grains <63 µm was used. The grain size distribution spectra were further analysed with the Peakfit[®] software (version 4.12), which allows for distinguishing and quantifying different sediment grain size populations. The classification proposed by Farrell et al. (2012) was used for the description of the sediment.

Trace metal concentrations were analysed with an Inductively Coupled Plasma Mass Spectrometer (Thermo Scientific® X-Series 2 ICP-MS) on freeze-dried sediment, after total acid digestion (HCl, HNO₃⁻ and HF) following the methodology described by Coynel et al. (2016). In all, seventeen chemical elements were measured: V, Cr, Co, Ni, Cu, Zn, As, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Pb, Th and U. All concentrations are expressed in mg/kg. The natural variability in trace metal concentrations depends on the grain size and mineralogical composition. To correct for grain size and mineralogical effects, trace metal concentrations need to be normalised by a reference (conservative) element. Following earlier studies (Coynel et al., 2007; Larrose et al., 2010), Thorium (Th) was used for this purpose. The Enrichment Factor (EF) quantifies anthropogenic metal enrichment in the sediment. The EF is obtained by dividing the Th-normalised concentration by the concentration found in an unpolluted reference sediment, representative of the regional geochemical background (Mason, 2013). Several studies have demonstrated that the regional geochemical backgrounds can be defined via the bottom/base of sediment cores in French continental systems, especially when the age model suggests that those deeper layers have been deposited before the onset of regional contamination sources (e.g., Audry et al., 2004; Grosbois et al., 2012; Dendievel et al., 2020; Gardes et al., 2020). To this end, values from the basis of a long sediment core sampled in the western part of the Morbihan Gulf (Perez-Belmonte, 2008) have been used. These samples date from about 5000 years ago (5182 cal BP), well before the major anthropogenic activities of today.

The enrichment factor is calculated as follows:

$$EF = \frac{\frac{M}{Th}^{obs}}{\frac{M}{Th}^{ref}}$$

Where M is the trace metal concentration, Th is the concentration of the conservative element, obs is the measured value and ref is the value of the geochemical background as observed in the sediment core samples. Following Larrose et al. (2010), enrichment classes are defined as follows:

- $EF < 1.5$: no enrichment;
- $1.5 < EF < 3$: minor enrichment;
- $3 < EF < 5$: moderate enrichment;
- $EF > 5$: strong enrichment.

Elemental carbon content (%C_{org}) and the carbon isotopic composition ($\delta^{13}C$) were measured on decarbonated freeze-dried sediment with a CHONS Elemental Analyser (EA Vario PYRO cube; Elementar®, Langensfeld, Germany) coupled to an isotope ratio mass spectrometer (IRMS; Isoprime precision, Elementar®, Langensfeld, Germany). Prior to EA-IRMS analysis, 300 mg of dried sediment was decarbonated with phosphoric acid to remove inorganic carbon (H₃PO₄, concentration 2 M). After

decarbonatation, samples were freeze-dried and ground. Three milligrams of samples were weighed in tin capsules. Possible offsets of raw IRMS values were corrected using both international IEAE (Vienna) and home-made standards (caffeine IAEA-600- and a sucrose-methionine-glycine mixture, referred to as SMG and cross-calibrated against caffeine IAEA-600 and Ag₂S IAEA-S-1, for C, N and S isotopes). Note that the linearity of the IRMS response was checked throughout to be less than 0.02‰ nA⁻¹, and furthermore, any possible size effect between samples was minimised by weighing the same amount for all samples. Standards (which had a %C_{org} different from that of samples) were weighed accordingly to have the same signal intensity. All isotopic data are expressed in the conventional delta notation versus V-PDB (‰): $\delta^{13}\text{C}_{\text{sample}} = (\text{R}_{\text{sample}}/\text{R}_{\text{V-PDB}} - 1) \times 1000$ with $\text{R} = {}^{13}\text{C}/{}^{12}\text{C}$ (Dubois et al., 2012).

Foraminiferal samples were sieved with 63, 125 and 500 μm meshes, and the fraction $>125 \mu\text{m}$ was used for foraminiferal analyses. Although the $>125 \mu\text{m}$ fraction contains only a part of the foraminiferal community, the study of this fraction has the advantage of being less time-consuming than that of the $>63 \mu\text{m}$ or $100 \mu\text{m}$ fraction. Therefore, the experimental design can include more samples. Moreover, since $>125 \mu\text{m}$ sufficiently reflects the foraminiferal community, the FOBIMO protocol proposes the study of this size fraction as an optimal compromise for biomonitoring studies (Schönfeld et al., 2012). When necessary, density separation using Ludox to remove organic material (Burgess, 2001; Somerfield & Warwick, 2013) or sodium polytungstate to remove sand (Parent et al., 2018) was applied. Samples with a high foraminiferal density were divided in aliquots using a wet splitter (Charrieau et al., 2018). Foraminifera were picked wet, using a Leica MZ16 stereomicroscope, and stored in Chapman slides. All stained individuals (all chambers except the last one or two brightly stained pink) were considered living. Complete aliquots were counted until a minimum of 300 specimens was obtained (Patterson & Fishbein, 1989; Fatela & Taborda, 2002). All hard-shelled foraminifera were identified to the species level (Figure II.2), using classical and modern reference publications on estuarine foraminifera (i.e., Murray, 1979; Hansen & Lykke-Andersen, 1976; Feyling-Hanssen, 1972; Scott & Medioli, 1980; Camacho et al., 2015), as well as the World Register of Marine Species (WoRMS, 2020). Studies combining genetic and morphological information were favoured to identify the *Ammonia* and elphidiid species (Schweizer et al., 2011; Pillet et al., 2013; Darling et al., 2016). The previously recognised morphospecies *Ammonia tepida* is composed of three different phlotypes in Europe (T1, T2 and T6, (Hayward et al., 2004)), which can be distinguished morphologically (Richirt et al., 2019). Following earlier studies (e.g., Richirt et al., 2019), we will refer to the *Ammonia tepida* group when speaking about this morphospecies, which includes phylogenetically unrelated phlotypes. With a stereomicroscope, it was mostly impossible to differentiate between these three phlotypes, particularly for small specimens. Consequently, in order to determine the presence/absence of the phlotypes' diversity in the *Ammonia tepida* group, 50 randomly sampled individuals per station were identified on the basis of SEM images, following Richirt et al. (2019).

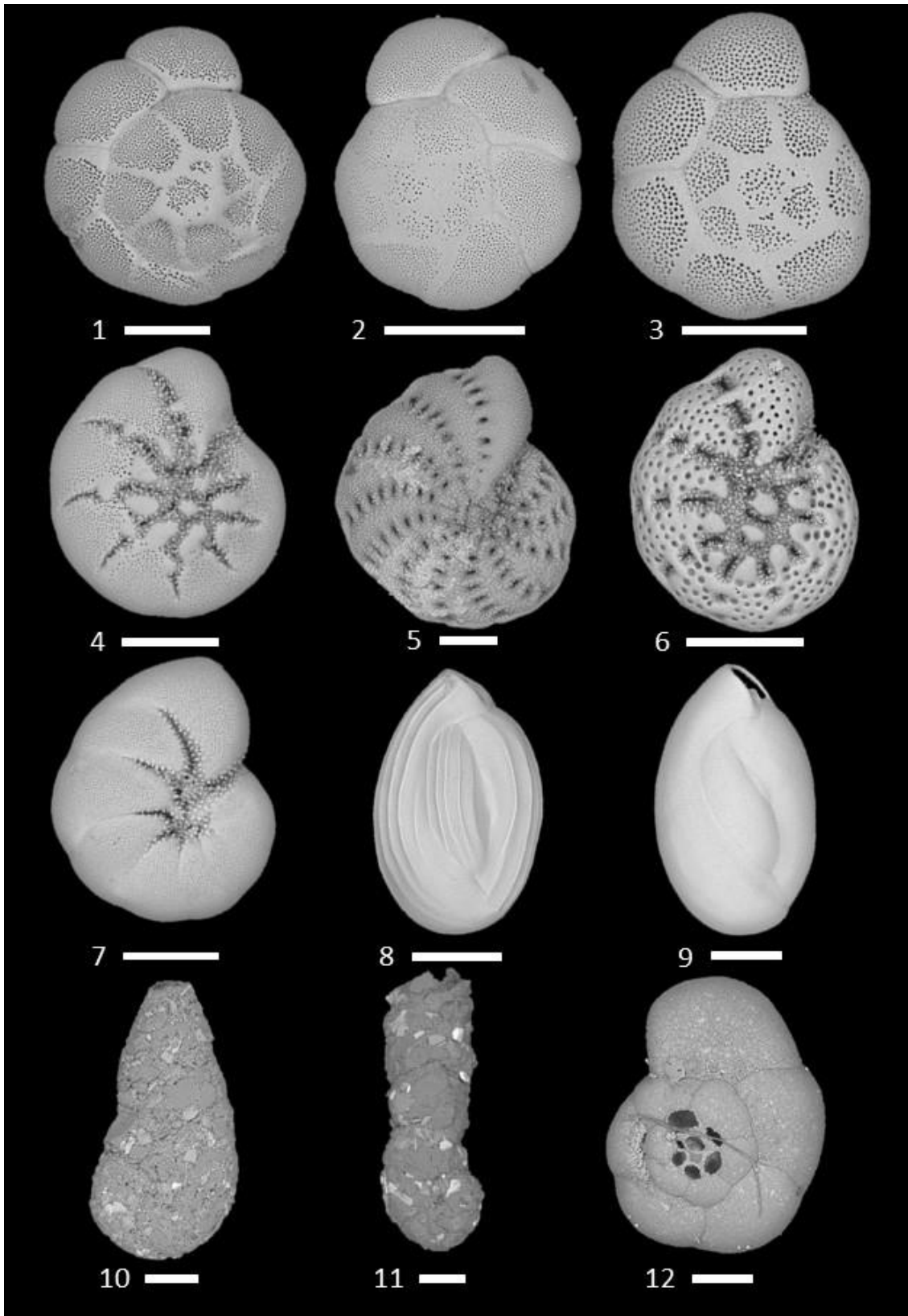


Figure II.2 : SEM images of the most abundant living benthic foraminiferal species in the study area. 1. *Ammonia* sp. T1.—Vie 4. 2. *Ammonia* sp. T2.—Vie 4. 3. *Ammonia* sp. T6.—Vie 4. 4. *Elphidium selseyense* (Heron-Allen and Earland, 1911)—Vie 3. 5. *Elphidium margaritaceum* Cushman, 1930—Auray 1C. 6. *Elphidium oceanense* (d'Orbigny in Fornasini, 1904)—Auray 1C. 7. *Haynesina germanica*. (Ehrenberg, 1840)—Vie 8B. 8. *Quinqueloculina jugosa* Cushman, 1944—Auray 1C. 9. *Quinqueloculina oblonga* (Montagu, 1803)—Auray 1C. 10. *Ammotium salsum* (Cushman and Brönnimann, 1948)—Auray 8B. 11. *Ammobaculites agglutinans* (d'Orbigny, 1846)—Auray 8B. 12. *Trochammina inflata* (Montagu, 1808)—Vie 7A. Scale bar = 100 μ m.

2.4. Marine Influence Index

The Marine Influence Index proposed by Jorissen et al. (submitted this volume) was tested in this study. The MII combines the position of the sampling site along the estuarine gradient, the duration of the tidal emergence, as well as the riverine fresh water input during the 30 days before sampling. The MII has been designed to give an integrated quantitative estimation of the influence of marine waters at the sampling stations. This index was calculated as follows:

$$\text{MII} = \left(1 - \frac{x}{s} * \alpha\right) * (1 - \text{ET} * \beta) * \left(1 - \sqrt{\frac{0.04 * \text{RD}}{\text{CS}}} * \gamma\right)$$

The first term of the equation is the normalised distance, in which x is the distance of the sampling site from the mouth of the estuary measured along the main estuarine channel, divided by s , the length of the salt intrusion. The second term refers to the normalised emergence time, in which ET, the duration of the tidal emergence at a sampling station, is expressed as a fraction of the tidal period, under mean spring tide conditions. The third term considers the relative river discharge, in which RD (m/s) is the river discharge in the 30 days before sampling, and CS is the cross section (m²) of the estuary at the study site, measured at mean high tide. α , β and γ are constants, used to weigh the three components of the equation. In this study, these constants are set to 1, giving equal weight to the three components. See Jorissen et al. (submitted this volume) for further explanations. The values of MII computed for this study are presented in Supplementary Table II.S10.

2.5. Statistical Analyses

All diversity indices (species richness, Shannon index, exp(H'bc index)) were based on raw data, using R software (version 4.0.4) (Team, 2013) and the “vegan” package (version 2.5–7, (Oksanen et al., 2020)). The calculation of the Foram-AMBI index was based on the species assignment list proposed by Bouchet et al. (2021).

A non-standardised Principal Components Analysis (PCA) was performed on non-transformed percentage data using the variance–covariance matrix. PCA reduces data matrices to a small number of factors explaining a maximum of the total variance. Only species exceeding 1% of the assemblage in at least one station were taken into consideration. Relevant variables were selected by forward selection based on PCA (*ordistep* function, R package “vegan”). Next, the environmental parameters were fitted to the PCA axes using the *envfit* function of the R package “vegan” (non-constrained version, the environmental parameters did not contribute to the ordination). All environmental data were standardised.

For all correlation tests, the normality of the data set was tested using the Shapiro–Wilk normality test, and the homogeneity of variance (homoscedasticity) with the Bartlett test. Finally, Pearson or Spearman

correlation tests were applied. A trend line was fitted to each scatter plot. For regression lines, the generalised linear model (“glm”) was used except when a nonlinear regression (“nls”) was found.

3. Results

3.1. Sediment Grain Size

All seven sites in the Auray estuary presented a silty–muddy sediment with $\geq 50\%$ fine ($< 63 \mu\text{m}$) grains (Figure II.3, Supplementary Table II.S3). A particle population with a mode of about $5 \mu\text{m}$ was dominant at almost all stations (e.g., Auray 2B, 5A, 6B shown on Figure II.3), while this $5 \mu\text{m}$ mode became secondary at stations 1B, 1C, 4A and 8B. A silt/very fine sand population, with a mode of 30 to $105 \mu\text{m}$, was dominant at all stations of sites 1 and 4. At sites where more than one station was sampled, the mode of the latter population increased towards the lower elevation. Station 8B was the only one with an important medium sand population, with a mode of $284 \mu\text{m}$.

In the Vie estuary, all sites had a silty–muddy sediment, with two dominant populations, a first one with a mode of 6 to $10 \mu\text{m}$, and a second one with a mode of 40 to $100 \mu\text{m}$ (Figure II.3, Supplementary Table II.S3). For both estuaries taken together, there is a positive correlation between elevation and the percentage of fine ($< 63 \mu\text{m}$) grains ($R^2 = 0.14$, $p = 0.056$).

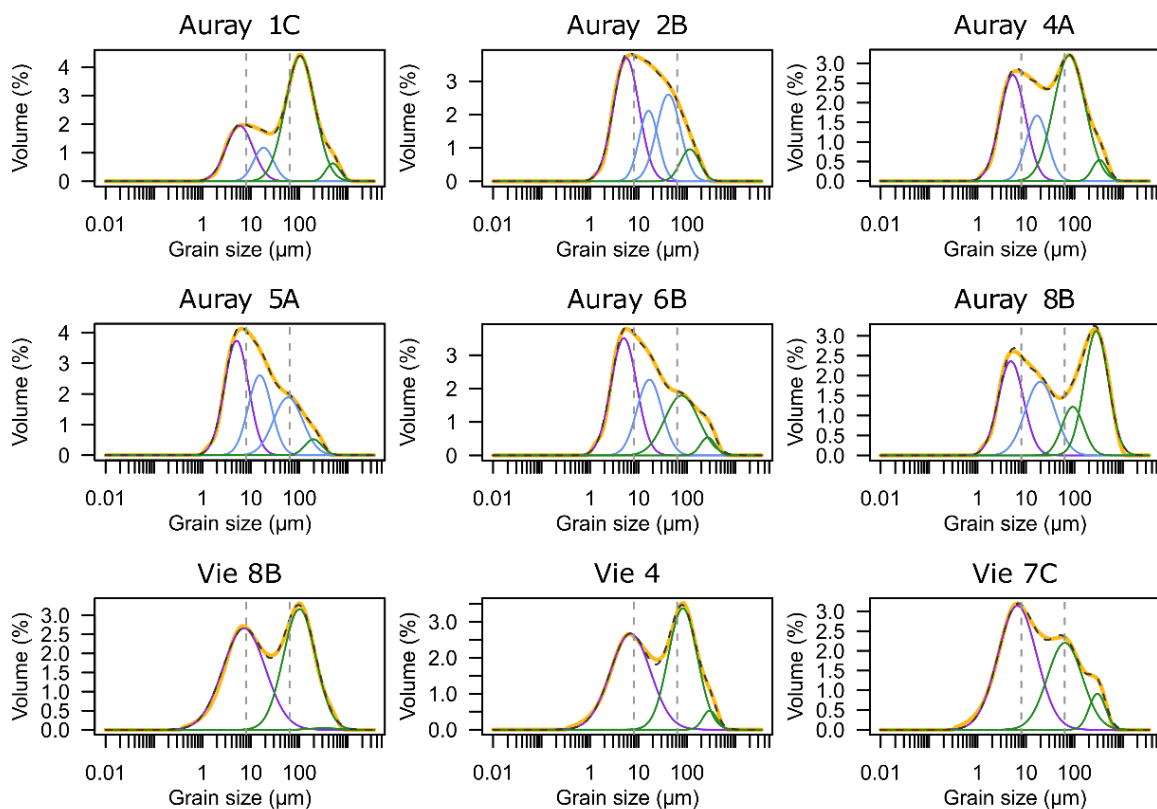


Figure II.3 : Sediment grain size distribution (yellow dashed curves), and grain size populations reconstructed with Peakfit® software version 4.12 (solid-coloured curves) for 10 typical stations of Auray and Vie estuaries (location on Figure II.S1). A, B, C: from high to low mudflat. See Supplementary Table II.S3 for numerical data and Supplementary Figure II.S1 for other stations.

3.2. Trace Metal Distribution

At the 27 stations, 17 trace metal elements were measured (Supplementary Table II.S4). The raw values for all trace metals were slightly higher in the Auray estuary compared to the Vie estuary, which is probably the result of a different geology of the catchment area (Supplementary Table II.S4). Enrichment factors (EFs) were computed for the six main trace elements (Cr, Ni, Cu, Zn, Cd, Pb) that could have an impact on community composition. In the Auray estuary, the EFs of trace metals were in the same range at all stations, except for Cu at station 1A (increased by a factor three), and station 8B, where Zn and Pb showed slightly raised values. Additionally, in the Vie estuary, the EFs were similar at all stations, except for Cu at stations 4, 5 and especially 6, where the EFs were 1.5 to 2.5 times higher than everywhere else (Figure II.4).

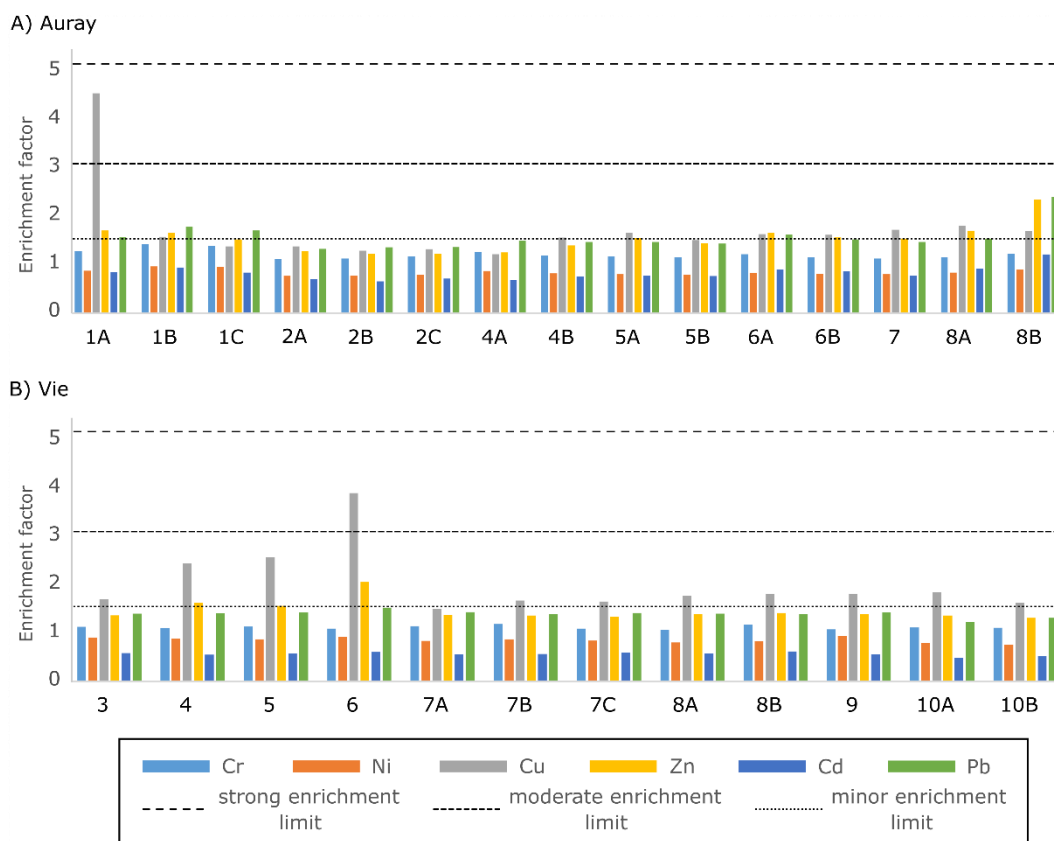


Figure II.4 : Enrichment factors calculated for Cr, Ni, Cu, Zn, Cd and Pb in stations of the Auray (A) and Vie (B) estuaries. Limits of strong, moderate and minor enrichment are according to Larrose et al., 2010. See Supplementary Table II.S4 for detailed data.

3.3. Organic Matter

The percentages of C_{org} and $\delta^{13}C$ measurements are presented in Supplementary Table II.S5. In the Auray estuary, the percentage of acid-resistant carbon in sediment samples ($\%C_{org}$) varied from 1.23 to 2.93% (Figure II.5; Supplementary Table II.S5). In general, $\%C_{org}$ was higher in the inner (2.15–2.93%) than in the outer estuary (1.43 to 2.33%), except for station 8B, with a low value of 1.23%. There was no systematic

trend between higher and lower stations on mudflats. The $\delta^{13}\text{C}$ (Figure II.5; Supplementary Table II.S5) varied from -17.71 to -23.86‰ , with a gradient from high values in the outer estuary (sites 1,2 and 4) to lower values in the inner estuary (sites 5, 6, 7 and 8).

In the Vie estuary, the $\%C_{\text{org}}$ varied from 1.16 to 2.27% (Figure II.5; Supplementary Table II.S5). Values were higher in outer estuary (1.96 to 2.27%) than in the inner estuary (1.16 to 1.96%). Similar to in the Auray estuary, there was a trend of decreasing $\delta^{13}\text{C}$ (Figure II.5; Supplementary Table II.S5), from -14.3‰ in the outer estuary (station 3) to -24.9‰ in the inner estuary (station 10A). However, this tendency was weakened at two stations (7C and 8A) with a high $\delta^{13}\text{C}$ compared to the other inner estuary stations.

The comparison of the $\%C_{\text{org}}$ with the $\delta^{13}\text{C}$ shows different patterns for the two estuaries (Figure II.5). In the Auray estuary, these parameters show a significant negative correlation ($R^2 = 0.41$, p -value = 0.01) when station 8B, with a very different sediment grain size (medium sand), is not included in the analysis. In the Vie estuary, the correlation is not significant.

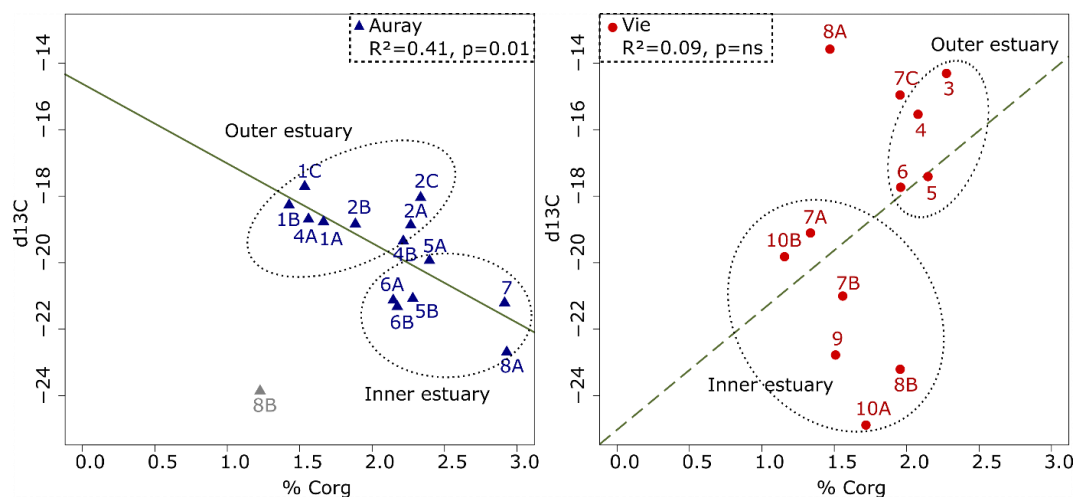


Figure II.5 : Relationship between the organic carbon content and $\delta^{13}\text{C}$ in the Auray (left) and Vie estuaries (right). In the Auray estuary, station 8B has not been taken into account.

3.4. Foraminiferal Distribution

3.4.1. Density

In the Auray estuary, the average foraminiferal density in the $>125\ \mu\text{m}$ fraction (based on the study of three replicates at each station) varied from ~ 25 to ~ 1700 individuals per $50\ \text{cm}^3$ (Figure II.6; Supplementary Table II.S6), without a clear spatial trend. Although the three replicates had comparable values for many stations, at stations 1B, 2A, 2B and 8B, the richest replicate was up to 10 times richer than the poorest one, suggesting considerable spatial patchiness at a decimetric scale. At several stations, foraminifera with a dissolved shell as well as organic linings were observed, but were not counted.

In the Vie estuary, the average foraminiferal density (for 3 replicates) varied from ~170 to ~1800 foraminifera per 50 cm³ (Supplementary Table II.S7), again without a clear spatial tendency (Figure II.6). The three replicates had comparable values. At all sites with a large mudflat (sampled on a cross-shore transect), the densities increased towards higher elevation.

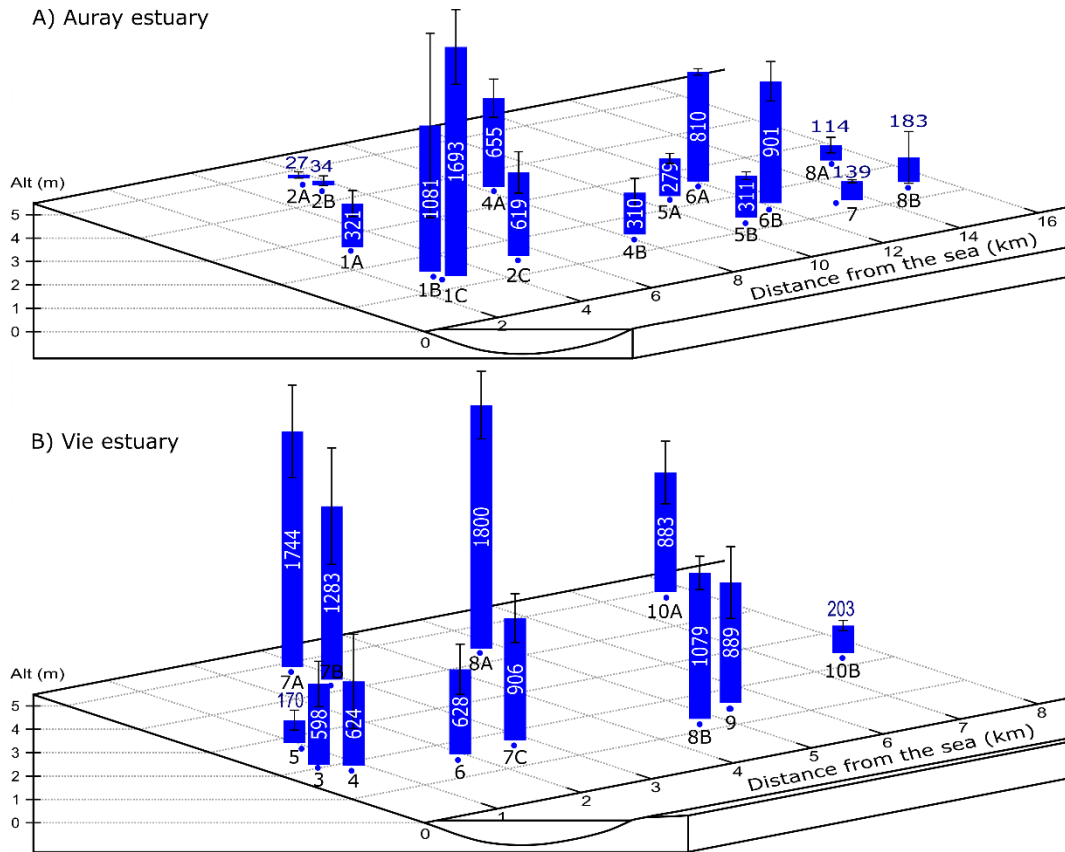


Figure II.6 : Total foraminiferal density in the Auray (A) and Vie (B) estuaries. The stations are ordered from the outer (left) to the inner estuary (right). The numbers correspond to the mean density of the three replicates and are expressed in individuals/50 cm³.

3.4.2. Foraminiferal Diversity

The total dataset contains 41 species (in the >125 µm fraction), of which 14 are common to both estuaries.

In the Auray estuary, 27 species were identified. Species richness (Table II.1) varied between 4 and 13, the Shannon index from 0.12 to 1.65 and Exp(H²_{BC}) from 1.14 to 5.23, without a clear tendency. However, at most sites with large mudflats (more than one station), there was a gradient from high values in the lower parts (stations B and C, lower elevation) to low values in upper parts (station A, higher elevation) of the mudflat.

In the Vie estuary, 28 species were recognised. Here, species richness varied between 7.3 and 14.3, the Shannon index from 0.18 to 0.63 and Exp(H²_{BC}) from 1.40 to 3.33 (Table II.1). Most indices had raised values at sites 3 and 4, in the outer estuary. At site 7, where three stations were sampled at different altitudes,

all diversity indices increased from the highest (7A) to the lowest station (7C). However, this tendency was not observed at sites 8 and 10, where two stations were sampled.

Table II.1 : Diversity indices in the Auray estuary. For every station the average of the three replicate samples is given.

Estuary	Station	Species Richness	Shannon	Exp(H' _{BC})
Auray	1A	8.0 (±2.0)	0.68 (±0.16)	2.01 (±0.31)
	1B	10.7 (±1.2)	1.44 (±0.08)	4.22 (±0.30)
	1C	12.7 (±2.5)	1.65 (±0.13)	5.23 (±0.72)
	2A	2.0 (±1.7)	0.15 (±0.28)	1.37 (±0.63)
	2B	4.3 (±4.9)	0.40 (±0.53)	1.80 (±1.02)
	2C	8.0 (±1.0)	0.80 (±0.16)	2.26 (±0.33)
	4A	8.7 (±1.5)	0.64 (±0.26)	1.95 (±0.48)
	4B	8.0 (±1.0)	0.62 (±0.17)	1.92 (±0.31)
	5A	5.0 (±1.7)	0.12 (±0.03)	1.14 (±0.05)
	5B	11.7 (±2.3)	0.86 (±0.24)	2.49 (±0.57)
	6A	7.3 (±0.6)	0.30 (±0.17)	1.37 (±0.24)
	6B	11.7 (±0.6)	0.73 (±0.26)	2.14 (±0.54)
	7	5.7 (±1.2)	0.69 (±0.04)	2.02 (±0.09)
	8A	8.3 (±1.2)	1.27 (±0.14)	3.73 (±0.34)
	8B	5.3 (±2.3)	0.68 (±0.29)	2.07 (±0.56)
	Vie	3	14.3 (±1.5)	1.14 (±0.20)
4		12.3 (±2.5)	1.09 (±0.35)	3.10 (±1.07)
5		9.3 (±2.3)	0.87 (±0.04)	2.45 (±0.30)
6		9.0 (±2.6)	0.68 (±0.17)	2.00 (±0.31)
7A		8.3 (±1.2)	0.33 (±0.01)	1.40 (±0.01)
7B		8.0 (±0.0)	0.40 (±0.11)	1.50 (±0.15)
7C		10.7 (±0.6)	0.99 (±0.16)	2.72 (±0.41)
8A		9.7 (±0.6)	1.19 (±0.07)	3.30 (±0.19)
8B		7.3 (±0.6)	0.65 (±0.11)	1.92 (±0.20)
9		9.7 (±0.6)	1.20 (±0.12)	3.33 (±0.06)
10A	8.7 (±0.6)	1.03 (±0.10)	2.84 (±0.34)	
10B	7.3 (±0.6)	0.71 (±0.22)	2.05 (±0.15)	

3.4.3. Foraminiferal Communities

In the Auray estuary, 16 species were found at least at one station with an average relative frequency higher than 1% for the three replicates (Plate 1, Supplementary Table II.S8). Eight of these species were rotaliids (calcareous perforate test), seven were textulariids (agglutinated test) and one was a miliolid (porcelaneous test). Rotaliids accounted for more than 88% of the foraminifera, except at stations 8A and 8B, in the inner estuary, where textulariids were abundant (Figure II.7). Miliolids were present at all stations but represented at most 6.5% of the relative abundance. At all stations except 8B, *Haynesina germanica* was dominant (50–96%). The *Ammonia tepida* group was present in the whole estuary (except at 8B), with values up to 18%. It showed much lower percentages at all stations at higher elevation (2A, 2B, 4A, 5A, 6A). Phylotype *Ammonia* sp. T1 was dominant (about ~75%), and was accompanied by *Ammonia* sp. T2 (~20%) and sp. T6

(~5%), without a clear spatial tendency. Elphidiids were mostly present in the outer estuary (site 1), at stations of lower elevation (1B, 1C, 2C), where they reached up to 30%, and were mainly represented by *Elphidium margaritaceum* and *Elphidium oceanense*. *Elphidium margaritaceum* was only observed at sites 1 and 2, while *E. oceanense* was also present in the inner estuary, but with lower densities. The agglutinated species, especially frequent at stations 8B (95%) and 8A (27%), were dominated by *Ammotium salsum*, with a minor contribution of *Trochammina inflata* and *Ammobaculites agglutinans*. Among the miliolids, *Quinqueloculina oblonga* occurred at almost all stations, with a maximum at 1B and 1C, whereas *Quinqueloculina jugosa* was rare, and never attained 1%.

In the Vie estuary, eight species were found at least at one station with an average relative frequency larger than 1% for the three replicates (Plate 1, Supplementary Table II.S9). Six of these species were rotaliids, whereas the other two were miliolids. Rotaliids always accounted for most of the foraminiferal community (89–99%). Miliolids (*Q. jugosa* and *Q. oblonga*) were present at all stations, with a maximum of 10.5%. The sampling sites can be divided into three zones (Figure II.7). At sites 3, 4, and 5, the assemblages were dominated by *Elphidium selseyense* (49–78%). The three sites in the middle estuary (6, 7 and 8) were dominated by *H. germanica* (56–92%), whereas *E. selseyense* dropped to low values (12.4% or less). *Elphidium selseyense* was virtually absent at the stations of highest elevation (7A, 8A and 10A). Towards the inner part of the estuary, the *Ammonia tepida* group increased, to become dominant (41–49%) at stations 9, 10A and 10B. At stations where the *Ammonia tepida* group was abundant, phylotypes T1 (~50%) and T6 (~40%) were dominant (~75%), accompanied by T2 (10%), without a clear spatial pattern. Miliolids (essentially *Q. jugosa* and *Q. oblonga*) showed slightly raised percentages at stations 4, 8B, 9 and 10B.

For all dominant species, correlations with the main environmental parameters and with MII were calculated (Supplementary Table II.S12). *Elphidium selseyense* and *Q. oblonga* are both negatively correlated with emergence time, whereas *H. germanica* shows a positive correlation with this factor in the Auray estuary. *Ammobaculites agglutinans* and *Ammotium salsum* show a negative correlation with the distance to the sea, as does the *A. tepida* morphogroup and *E. oceanense* in the Vie estuary. *Elphidium selseyense* is the only taxon showing a negative correlation with the sediment fine fraction. Finally, *E. selseyense* and *H. germanica* are positively correlated with %C_{org}, whereas *Q. oblonga* shows a negative correlation.

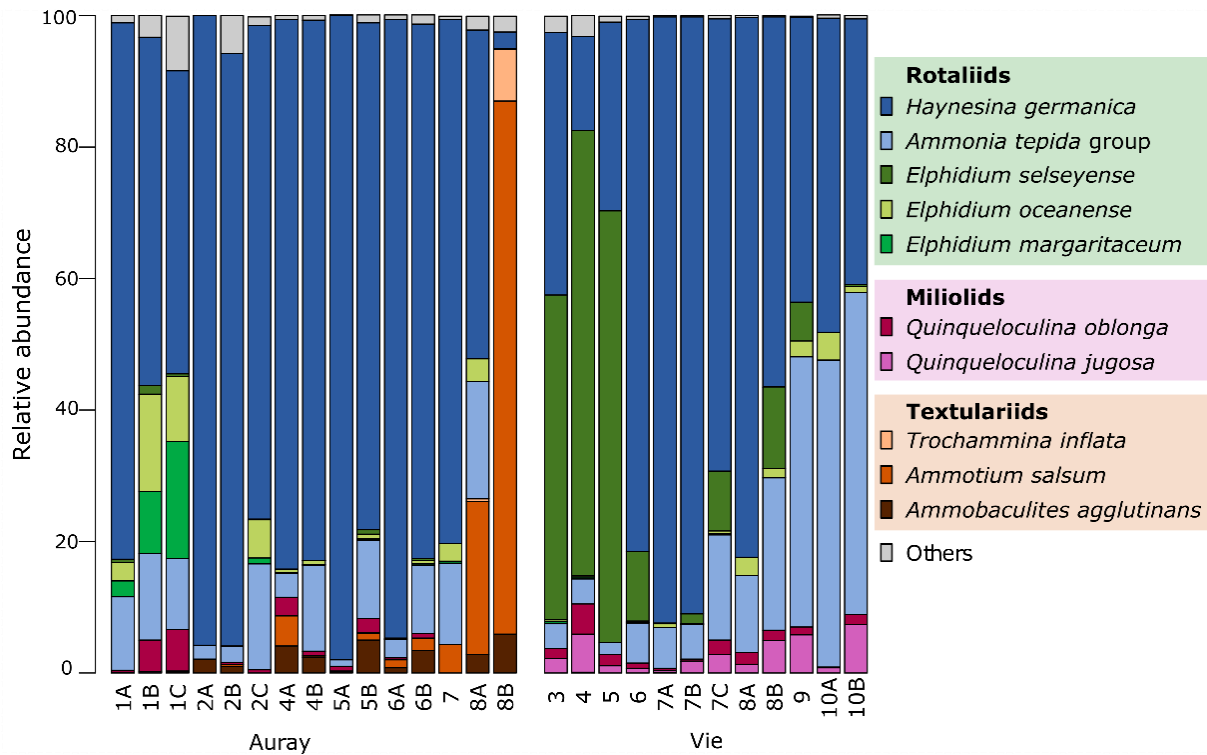


Figure II.7 : Composition of foraminiferal assemblages (>125 µm fraction) in the Vie and Auray estuaries.

The non-standardised PCA model based on percentage data of the dominant species yields three significant axes that explain, respectively, 58.2, 23.3 and 15.4% of the total variance (Figure II.8). The eigenvalues and species loadings on the three axes are listed in Supplementary Table II.S11.

The three first PCA axes are dominated by the four most common taxa. *Haynesina germanica* has a high positive loading on axis 1, whereas *E. selseyense* and *A. salsum* have a negative loading. The second PCA axis is positively loaded by *E. selseyense* and *H. germanica*, and negatively by *A. salsum* and the *A. tepida* group. Finally, the third axis is driven by the *A. tepida* group and *A. salsum*.

The ordination model is determined by a small number of stations with very distinct foraminiferal communities (Figure II.8). This concerns especially station 8B of Auray and stations 3, 4 and 5 of the Vie estuary. Station 8B of Auray is in the negative domain of both axis 1 and axis 2, because of the high percentage of *A. salsum*. The position of stations 3, 4 and 5 of the Vie estuary (negative on axis 1, positive on axis 2) is explained by their high percentage of *E. selseyense*.

For the remaining samples, the axial plot of PCA1 and PCA2 shows an overlap between the two estuaries, with samples being organised mainly along PCA1, largely as a function of the percentage of *H. germanica*. For most sites where several stations were sampled at different altitudes, the higher samples (with longer emergence times) have higher values on PCA1.

The axial plots of PCA1 and PCA3 show that PCA3 mainly distinguishes the inner estuary stations 9, 10A and 10B of the Vie estuary, which have high scores on PCA3, due to a high proportion of the *A. tepida* group.

Both the normalised distance to the sea ($R^2 = 0.46$, $p = 0.001$) and the $<63 \mu\text{m}$ grain size fraction ($R^2 = 0.30$, $p = 0.05$) show significant correlations with the PCA model. No significant correlations were found for the $\%C_{\text{org}}$ and the normalised emergence time (Table II.2).

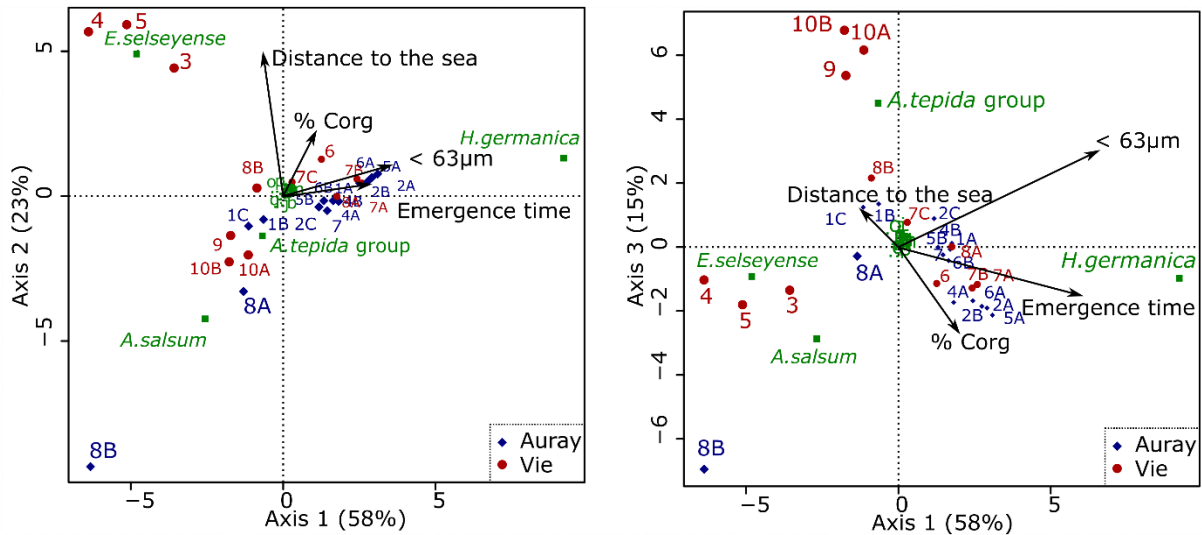


Figure II.8 : PCA ordination diagram based on foraminiferal data. **Left:** PCA1 vs. PCA2; **right:** PCA1 vs. PCA3. The environmental parameters were fitted to the PCA, and the length of the arrows is proportional to the strength of the fits.

Table II.2 : Envfit results: multiple regression of environmental variable with ordination axes. Asterisks indicate significant p -values.

	PCA1	PCA2	PCA3	R^2	p -value
$\%C_{\text{org}}$	0.37	0.78	-0.51	0.15	0.29
$<63 \mu\text{m}$ grain size	0.88	0.26	0.40	0.30	0.05 *
Distance to the sea	-0.13	0.98	0.12	0.46	0.001 *
Emergence time	-0.96	-0.15	0.24	0.16	0.24

3.4.4. Foraminiferal Indices of Environmental Quality

In the Auray estuary, at all stations except station 8B (Foram-AMBI: 1.41), the values of Foram-AMBI varied between 2.57 and 3.02, resulting in a good quality status ($1.2 < \text{Foram-AMBI} < 3.3$), although close to the upper limit of this category (Table II.3). In the Vie estuary, stations 3 to 5 had values between 0.95 and 1.5 (high to good ecological quality), whereas for all other sites, the values varied from 2.60 to 3.03 (good ecological quality).

Table II.3 : Results of the Foram-AMBI on Auray and Vie stations and associated ecological quality status (<1.2: High; 1.2-3.3: Good; 3.3-4.3: Moderate; 4.3-5.5: Poor; >5.5: Bad).

	Auray				Vie		
	Station	Foram-AMBI	Ecological Quality Status		Station	Foram-AMBI	Ecological Quality Status
	1A	2.92 ± 0.01	Good		3	1.49 ± 0.14	Good
	1B	2.83 ± 0.05	Good		4	0.95 ± 0.51	High
	1C	2.56 ± 0.10	Good		5	1.04 ± 0.04	High
	2A	2.97 ± 0.05	Good		6	2.68 ± 0.12	Good
	2B	2.90 ± 0.09	Good		7A	3.00 ± 0.01	Good
	2C	3.01 ± 0.06	Good		7B	2.94 ± 0.03	Good
	4A	2.95 ± 0.20	Good		7C	2.75 ± 0.16	Good
	4B	2.98 ± 0.00	Good		8A	2.60 ± 0.04	Good
	5A	3.02 ± 0.02	Good		8B	3.03 ± 0.03	Good
	5B	2.95 ± 0.06	Good		9	2.77 ± 0.08	Good
	6A	3.00 ± 0.03	Good		10A	2.93 ± 0.05	Good
	6B	2.94 ± 0.02	Good		10B	2.99 ± 0.00	Good
	7	2.93 ± 0.06	Good				
	8A	2.57 ± 0.16	Good				
	8B	1.41 ± 0.07	Good				

3.4.5. Relations between Foraminiferal Distribution and the Marine Influence Index (MII)

Jorissen et al. (submitted this volume) suggested that the Marine Influence Index (MII) should explain part of the foraminiferal distribution in estuaries. MII values are presented in Supplementary Table II.S10. MII values are higher (more marine influence) in stations located near the mouth than those located in the inner part of the estuary. MII values are also higher on the lower part of the mudflat compared to the upper part. In Figure II.9, the MII is compared with some major foraminiferal community characteristics. In Figure II.9a,b, the MII is compared with the sample scores on PCA axes 1 and 2. A weak but significant correlation is found for PCA1 ($R^2 = 0.19$, $p < 0.05$). The separation of the four stations on PCA2 (negative side: Auray 8B; positive side: Vie 3, 4 and 5) is reflected by their contrasting MIIs, but because most stations hardly load on PCA2, the overall correlation is not significant. In Figure II.9c, the MII is compared to species richness. A significant positive correlation is observed ($R^2 = 0.36$, $p < 0.01$) for the total dataset. The correlation is also significant for the Auray estuary alone ($R^2 = 0.36$, $p < 0.05$), but is not significant for the Vie estuary. Figure II.9d shows the relationship between the MII and the percentage of *Elphidium* spp. There is a similar tendency for both estuaries, with less than 5% *Elphidium* spp. at all stations where the MII is lower than 0.45. Elphidiids only become a dominant foraminiferal component when the MII is higher than 0.5. The transition between these two domains is abrupt. Figure II.9e shows a significant linear correlation ($R^2 = 0.42$, $p < 0.01$) between the MII and the percentage of *Quinqueloculina* spp. Finally, in Figure II.9f, the relationship between the MII and the percentage of the *Ammonia tepida* group is shown. The absence of a significant correlation is partly explained by the distribution in the Vie, where high percentages (>40%) of this taxon are observed at three stations in the innermost part of the estuary (9, 10A, 10B) with different MIIs, mainly due to differences in emergence time. Correlations between all dominant species and MII are

presented in Supplementary Table II.S12. *Quinqueloculina oblonga* (both estuaries), *Elphidium margaritaceum* (Auray), *E. oceanense* (Auray) and *E. selseyense* (Vie) are positively correlated with the MII, whereas *Haynesina germanica* shows a weak negative correlation with the MII.

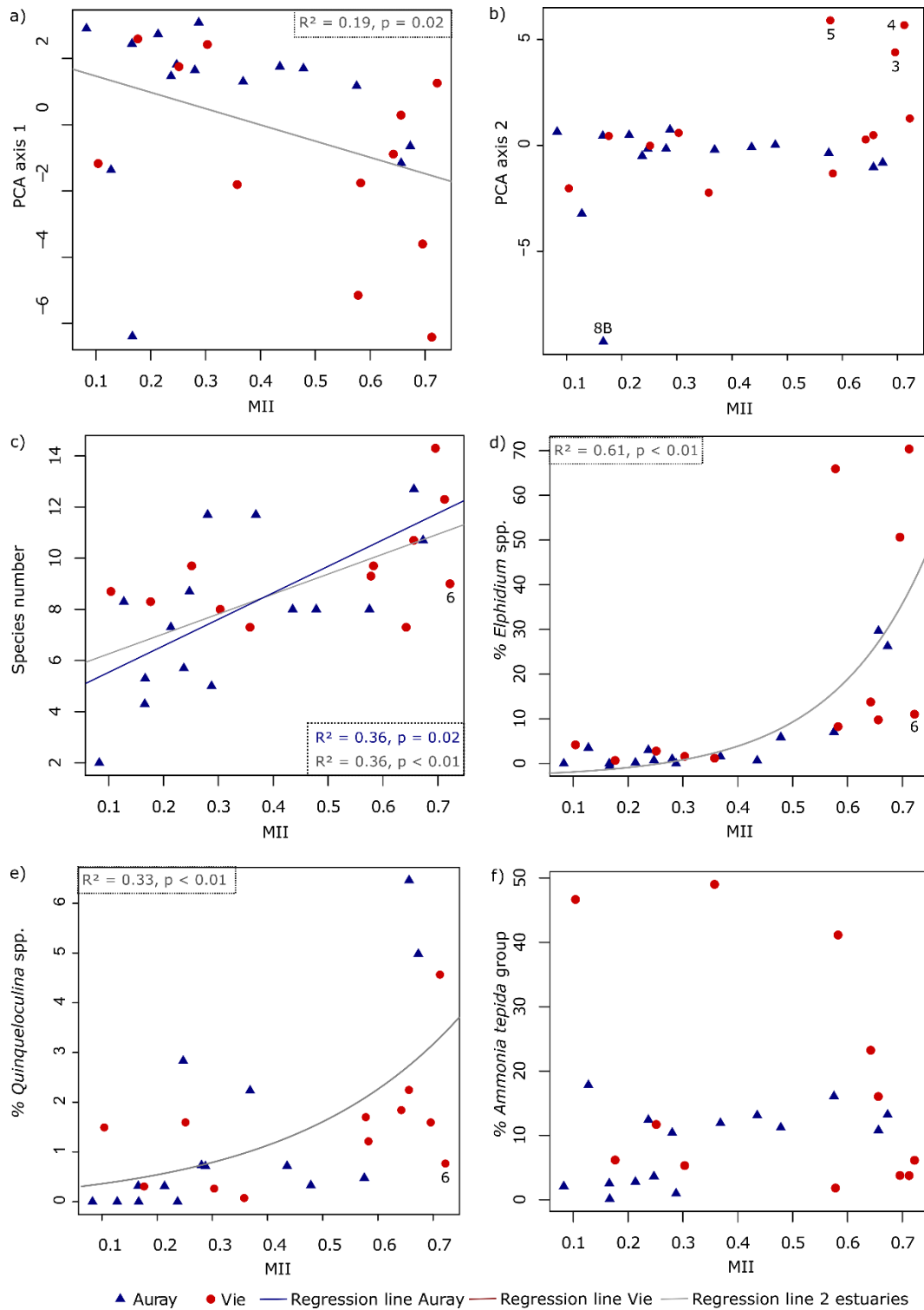


Figure II.9: Relationship between MII and (a) PCA axis 1, (b) PCA axis 2, (c) number of species, (d) % *Elphidium* spp., (e) % *Quinqueloculina* spp. and (f) % *Ammonia tepida* group. Auray estuary: blue triangles; Vie estuary: red dots. All correlations were performed with Spearman tests, solid lines correspond to significant correlations. Regression lines are based on generalised linear model (a,c) and non-linear regression (d,e). The MII tends to be maximal at lower stations in the outer estuary and minimal at higher stations in the inner estuary.

4. Discussion

This paper focusses on the foraminiferal assemblages of intertidal mudflats of the Auray and Vie estuaries. Both estuaries are short coastal waterways of the French Atlantic coast, with relatively low riverine water supply, and a mesotidal to low macrotidal regime, but they present contrasting geomorphological characteristics. The Auray estuary is a ria, with a relatively deep channel connecting the outer estuary to the marine Morbihan Gulf, and as such is a marine-influenced estuary. Conversely, the Vie estuary is a lowland meandering waterway, potentially with a stronger riverine influence (Figure II.1).

4.1. Environmental Characteristics

Besides the positions along the estuary and the altitudes of the individual sampling stations, three other environmental parameters were used to describe the foraminiferal habitat;

- (I). Sediment grain size—two dominant grain size populations coexist, which may correspond to a mix of sand deposits during high river discharge periods and the settling of clay-silt from riverine input during slack waters. There is a significant decrease in grain size towards stations higher on the mudflats where tidal currents are weaker;
- (II). Organic matter quantity and quality—different %C_{org} patterns underline the difference between the two estuaries. In the Auray estuary, the %C_{org} decreases seawards, whereas the opposite is observed in the Vie estuary. In fact, in the outer Auray estuary (sites 1 and 2), open to the sea, organically poor marine waters replace organically rich riverine waters. In both estuaries, $\delta^{13}\text{C}$ shows an overall seaward decrease, which corresponds to the natural gradual transition of continental to marine organic matter (Dubois et al., 2007; Dan et al., 2019). In the outer Auray estuary, $\delta^{13}\text{C}$ values vary from -18 to -20‰ , suggesting a major contribution of microphytobenthos (Dubois et al., 2012). In the Vie estuary, the low values observed at several stations (between -14 and -16‰) could be related to the presence of macro-algae (Riera et al., 1997; Dubois et al., 2012), which were observed during the sampling campaign. In summarising, it appears that both the %C_{org} and the $\delta^{13}\text{C}$ trends reflect natural patterns that seem not be biased by anthropogenic organic matter input;
- (III). Trace metal concentrations—in both estuaries, none of the six trace metals considered relevant for biota attains the strong enrichment limit (as defined by Larrose et al. 2010). Concerning Cu concentrations, the moderate enrichment at stations Auray 1A and Vie 6 and the minor enrichment at stations Vie 4 and 5 may be related to antifouling paint used in the nearby harbours. Only minor Pb and Zn enrichment was detected (mainly at station Auray 8B).

Low concentrations of trace metals and organic matter suggest that, except for stations Auray 1A and Vie 6 (close to harbours), the studied estuaries are not subjected to strong anthropogenic pollution. This confirms the overall evaluation of the 2019 survey (Envlit) of average (fish-based indicator) to good ecological quality

(opportunistic macro-algae, plants other than phytoplankton and hydromorphology) for the Auray estuary and good quality (intertidal and opportunistic macroalgae and plants other than phytoplankton) for the Vie estuary. Therefore, in both estuaries, the ecosystems should be mainly controlled by natural environmental parameters.

4.2. Foraminifera Communities

The foraminiferal faunas observed in the two estuaries show comparable density and diversity (Figures II.6–8, Tables II.1 and II.3). In the Auray estuary, *Haynesina germanica* and the *Ammonia tepida* group are dominant at almost all stations. In the outer estuary, open marine neritic species (e.g., *Elphidium margaritaceum*, *Elphidium oceanense*; see Supplementary Table II.S13 for an overview of the ecological characteristics of the dominant taxa) are more frequent. In the Vie estuary, an up- to downstream succession of the three main assemblages is observed, all dominated by taxa typical of estuarine ecosystems. *Elphidium selseyense* is dominant in the outer estuary, *H. germanica* in the middle estuary, and finally the *A. tepida* group is dominant in the inner estuary. This succession is in accordance with the literature, where *A. tepida* and *H. germanica* are commonly observed as dominant species in estuaries (Cearreta, 1988; Alve & Murray, 1994; Castignetti, 1996) where some *Elphidium* species may also be frequent (*E. williamsoni* (Cearreta, 1988; Castignetti, 1996); *E. oceanense* ((Cearreta, 1988); *E. margaritaceum* (Francescangeli et al., 2018); *E. selseyense* (Alve & Murray, 1994; Francescangeli et al., 2018)).

In view of the absence of strong pollution, the Auray and Vie estuaries can be considered as model ecosystems, representative of the environmental quality found today in most human-influenced, relatively weakly polluted estuaries. However, these two estuaries are obviously not pristine, but subjected to anthropogenic influences. The Vie estuary is closed upstream with a dam, whereas the outer estuary crosses an urbanised area (Saint-Gilles-Croix-de-Vie), with a marina and a fishing harbour (Figure II.1B). The Auray estuary is under high touristic pressure and is also an important centre for oyster farming. Even if metal enrichment is only minor or moderate, we cannot exclude the possibility that the combined effects of raised trace element concentrations and other stressors can affect the foraminiferal communities. In order to recognise the eventual impacts of multiple stresses, we will investigate (1) whether the moderate enrichment in Cu at two stations has affected the foraminiferal communities there, and (2) whether some deviating foraminiferal assemblage characteristics can eventually reflect localised pollution.

At the upper mudflat station Auray 1A, next to the harbour of Locmariaquer, moderate Cu enrichment was observed. However, the foraminiferal community was similar to that found at nearby (not Cu-enriched) stations (1B, 1C, 2C; Figure II.7). Similarly, at station Vie 6, a moderate Cu enrichment was also measured. The foraminiferal community at this station seemed to be intermediate between those found at the nearby sites 5 and 7 (Figure II.7). Apparently, there is no foraminiferal community response to the moderate Cu enrichment observed at these stations.

Two groups of stations stand out by their particular faunal composition; they are strongly opposed on PCA axis 2 (Figure II.8):

- (1). The foraminiferal assemblage at station Auray 8B is composed by more than 90% agglutinated species (*Ammotium salsum*, *Ammobaculites agglutinans*, *Trochammina inflata*). These agglutinated species, typical of oligohaline conditions, are commonly found in salt marshes (Bradshaw, 1968; Ellison & Murray, 1987; De Rijk, 1995) and may also occur in inner estuaries (Debenay & Guillou, 2002). This assemblage is compatible with the low salinity recorded (about 16 at high spring tide). Station 8B also stands out because of its much higher proportion of medium sand. Very close to station 8B, the estuary artificially narrows to a single channel to pass under a road bridge, leading to a higher current velocity (Figure II.3). This brackish and dynamic hydrological setting could explain the very different foraminiferal community observed at this station;
- (2). In the Vie estuary, stations 4 and 5 have been sampled on concrete shipping ramps, covered with several-decimetres-thick mud deposits, inside docks in the St-Gilles-Croix-de-Vie harbour, and undergo strong anthropogenic influence. This is partly evidenced by the slightly increased Cu concentrations at these two stations (Figure II.4), corresponding to minor enrichment (Coynel et al., 2007). At these stations, the foraminiferal community shows high abundances of *E. selseyense*. In view of the preference of *E. selseyense* for the outer estuary (Debenay & Guillou, 2002), and the occurrence of a similar community at station 3, where no increased Cu-content was observed, it seems unlikely that its dominance at these stations reflects strong pollution.

In conclusion, the foraminiferal patterns in both estuaries seem to be controlled by natural parameters. There is no indication that the different composition of the foraminiferal community at the sites discussed above is a response to anthropogenic pollution.

4.3. Comparison with Previous Studies

The foraminifera of the Auray and Vie estuaries have been extensively studied in the past (Redois et al., 1996; Debenay et al., 2003, 2006). Unfortunately, there are some important methodological differences between our study, using the recently proposed FOBIMO sampling protocol (Schönfeld et al., 2012), and these former studies, especially with respect to the determination of living specimens (by Rose Bengal staining), the preservation procedure and the studied size fractions. Although a detailed comparison is not possible, some observations can be made on the basis of these studies.

The Auray estuary was studied in 1994 by Redois (Redois et al., 1996). The areas corresponding to our sampling sites show the same main species: *Haynesina germanica*, *Ammonia tepida*, *Quinqueloculina oblonga* (determined as *Q. seminula* forma 2), *Elphidium oceanense* (determined as *Cribrorhynchium gunteri*), *E. margaritaceum* (determined as *E. pulvereum*) and *Ammonium salsum*. At our site 8, the only one

where *A. salsum* substantially contributes to the foraminiferal community (23% at 8B, 81% at 8A), Redois also observed this agglutinated species, at a maximum of 9%. In the middle estuary (stations 4 and 5), Redois (Redois et al., 1996) described *Q. oblonga* as a major species (up to 55%), while this is only a minor species (<6.3%) in our samples. This may be explained by the fact that Redois analysed the >50 µm size fraction, where the small-sized species *Q. oblonga* is logically more abundant than in the >125 µm fraction studied here. In Redois (1996), the study of the seasonal variability of the foraminiferal community reveals the maximum abundance during the summer months. The dominant species *H. germanica* and *A. tepida* show a second density increase in winter. Our sampling in July 2019 would therefore correspond to a maximum abundance period.

The Vie estuary was studied in the 2000s by Debenay et al. (2003, 2006). These authors described the same up–downstream biozonation as we found, with three dominant species. These observations confirm that the foraminiferal succession observed in the Vie estuary has lasted for at least 20 years. A difference with our study is the high abundance of *Bolivina striatula*, observed at many stations (Debenay et al., 2003, 2006). Since we observed this species in large numbers in the 63-125 µm size fraction, this is probably due to the use of a smaller size fraction (>63 µm) by Debenay et al. (Debenay et al., 2003). The study of the temporal variation in foraminiferal assemblages in the Vie estuary (Debenay et al., 2006) revealed two major reproduction periods, in spring and early autumn. If these reproduction periods are recurrent, our sampling in October 2018 would correspond to the end of the second reproduction period.

In summarising, the main conclusion of the comparison of our results with these earlier data is that the foraminiferal communities of both estuaries did not change substantially over the last 25 years.

4.4. Foraminiferal Indices of Environmental Quality

Since the faunal characteristics seem to be substantially affected by pollution (trace metal and organic matter pollution) at none of our stations, an index of environmental quality should give comparable values, indicative of a good environmental quality, for all stations. Here, three different approaches are tested to see whether this is indeed the case.

The first approach uses indicator species. The percentage of one or more species thought to be tolerant to pollution is used to estimate the extent of pollution. For instance, some authors considered *Haynesina germanica* as resistant to pollution, and suggested that its increasing dominance may be considered as an indicator of pollution impacts from organic matter, heavy metals and/or hydrocarbons (Armynot du Châtelet et al., 2004; Martins et al., 2013). However, many studies describe *H. germanica* as a dominant species in natural estuarine mudflats (Murray, 1983; Cearreta, 1988; Castignetti, 1996; Horton, 1999; Murray & Alve, 2000; Debenay et al., 2003; Horton & Murray, 2007; Martins et al., 2015; Mojtahid et al., 2016; Francescangeli et al., 2018; Buzas-Stephens et al., 2018). In the Auray and Vie estuaries studied here, *H.*

germanica was dominant (up to 98%) at most sites. Since there is no indication of major pollution impact in our estuaries, it appears that it is not possible to use the percentage of this taxon as an indicator of pollution in estuarine mudflats.

Following studies on the environmental quality in Norwegian fjords (Bouchet et al., 2012), Bouchet et al. (2018a) suggested that the diversity index $\exp(H'_{BC})$ calculated for living benthic foraminifera could be used to determine the Ecological Quality Status (EQS) in transitional waters. They applied this index in five coastal lagoons of the Mediterranean Sea, and showed that their index adequately assessed sites with a degraded environmental quality. According to the EQS ranges proposed by Bouchet et al. (2018a) for transitional water masses, the quality of all our samples should be classified as poor ($\exp(H'_{bc}) < 3$) or bad ($3 < \exp(H'_{bc}) < 7$) (Table II.1). In view of the absence of indications of strong pollution by metallic trace elements and organic matter enrichment, it appears that the EQS limits proposed by Bouchet et al. (2018a) are inappropriate to qualify our estuarine environments. In fact, the diversity index $\exp(H'_{BC})$ shows a weak positive correlation with marine influence (MII: $R^2 = 0.16$, $p < 0.01$), and a weak negative correlation with emergence time ($R^2 = 0.09$, $p < 0.01$) and with the percentage of grains $< 63 \mu\text{m}$ ($R^2 = 0.20$, $p < 0.01$). This suggests that in the Auray and Vie estuaries, foraminiferal diversity is at least partly controlled by these three natural parameters. The low environmental quality, as suggested by $\exp(H'_{BC})$, is not realistic, and illustrates the problem of the estuarine paradox (Dauvin, 2007).

In a recent article, Bouchet et al. (2021) investigated the possibility of applying Foram-AMBI (Alve et al., 2016) to intertidal and transitional environments. Similar to AMBI, originally developed for macrofauna (Borja et al., 2000), Foram-AMBI is calculated on the basis of the cumulative frequencies of five ecological groups (EGI to EGV). All foraminiferal species are attributed to one of these groups on the basis of the correlation of their percentage distribution with the percentage of organic carbon in the sediment (Alve et al., 2016; Jorissen et al., 2018). Bouchet et al. (2021) used the same strategy to attribute foraminiferal species from transitional water masses to these five ecological groups, based on 42 distributional studies from the Northeastern Atlantic and Mediterranean. They have then tested the Foram-AMBI method on data sets from intertidal areas along the French Atlantic coast and a harbour in Sardinia, Italy.

When the Foram-AMBI is applied to our dataset, all stations obtain a “high” to “good” quality status (Table II.3), which is in accordance with our expectations. However, when looking in more detail, it appears that stations 3, 4 and 5 of the Vie estuary, located in the strongly anthropogenised outer estuary, show lower Foram-AMBI values (indicative of a higher environmental quality) compared to stations found in the much more natural inner part of the estuary. This is the opposite of what could be expected.

In case of the low-diversity foraminiferal communities observed in estuaries, the Foram-AMBI values are almost entirely determined by the few dominant species. In the Vie estuary, *Elphidium selseyense* is dominant in the outer estuary, whereas *H. germanica* and the *Ammonia tepida* group prevail in the middle and the inner estuary, respectively. These species have been attributed to EGI (*E. selseyense*, as

Criboelphidium excavatum) and EGIII (*H. germanica* and *A. tepida* group), respectively. The lower Foram-AMBI values at stations 3, 4 and 5, in the outer part of the Vie estuary, are entirely due to the high percentage of *E. selseyense* at these stations.

Moreover, as Bouchet et al. (2021) correctly indicated, this method attains its limits of applicability when marsh environments are considered. Coastal and estuarine marshes are usually covered by dense vegetation, leading to raised organic carbon contents in the sediment. The foraminiferal communities in coastal marshes tend to be dominated by a low number of characteristic agglutinated species. Therefore, these species necessarily show a strong correlation with high C_{org} levels, even in completely natural conditions. If the Foram-AMBI classification could be rigorously applied, they should be placed in the EGV (first-order opportunistic species), indicative of strong pollution. For this reason, Bouchet et al. (2021) decided not to assign typical salt marsh species.

Although the Foram-AMBI method gives reliable answers for open marine waters and for other transitional water masses (such as marine intertidal areas or marine harbours), we think that in estuarine mudflats, this method should be applied with caution.

It appears that none of the three above mentioned approaches, which have been successfully applied in coastal waters as well as in other transitional water masses, give fully satisfactory results in estuarine intertidal mudflats. This is mainly due to the fact that the foraminiferal assemblages there are naturally dominated by a small number of stress-tolerant and/or opportunistic taxa. In this specific context, a prerequisite for a successful index of environmental quality is to know the theoretical composition of the assemblage that responds to natural environmental characteristics. Only once this is achieved can major deviations from the natural distribution be recognised and attributed to anthropogenic pollution. In the next section, the Marine Influence Index, proposed by Jorissen et al. (submitted this volume) as an integrative descriptor of the main controlling parameters in estuaries, will be investigated to see if it can help to assess the natural foraminiferal composition, and thereby detect distributional anomalies due to pollution.

4.5. Foraminiferal Community and the Marine Influence Index (MII)

The Marine Influence Index (MII) has been proposed as an integrative measure of several factors controlling the foraminiferal community's characteristics. If correctly defined, the MII should show significant relationships with the main foraminiferal parameters in estuaries not substantially affected by pollution.

The rationale for the MII is the fact that, although salinity (specifically its variation) is probably the major factor influencing community distribution in estuaries, this parameter is very difficult to measure, especially on intertidal mudflats at low tide. Since salinity may show large changes over tidal and weekly to monthly periods (Diz et al., 2009), a punctual measurement is not representative of all conditions to which the foraminiferal community adapts (Attrill, 2002). For this reason, we prefer to not use the on-off salinity

values measured during sampling, but instead use the MII index, which is supposed to estimate the degree of influence of marine waters at a sampling point.

In our study, the MII shows a weak positive correlation with axis 1 ($R^2 = 0.19$, $p < 0.05$) of the Principal Component Analysis. Although the relationship with PCA axis 2 is not significant, the four stations that are separated on this axis (Vie 3, 4 and 5 versus Auray 8B) have very different MII values. Figure II.8 shows that PCA axis 1 is positively correlated with emergence time, whereas axis 2 is correlated with distance to the sea (Table II.2), two parameters integrated into MII. PCA axis 1 also shows a strong positive correlation with the percentage of grains $<63 \mu\text{m}$, which increases towards more elevated stations with longer emergence times.

A relationship between the MII and several individual foraminiferal parameters (Figure II.9) is also observed: (1) a significant positive correlation with species diversity, (2) a significant nonlinear relationship with the percentage of *Elphidium* spp., which only rises above 10% when the MII is larger than 0.5, and (3) a positive correlation with the percentage of *Quinqueloculina* spp. This suggests that the MII includes some of the main environmental parameters controlling the foraminiferal community, and that different MII scores correspond to different community characteristics.

Elphidium spp. and *Quinqueloculina* spp. show an increase in their relative frequencies as they move towards higher MIIs, and both have a preference for more marine conditions (Cearreta, 1988; Debenay & Guillou, 2002). These species are indeed less tolerant to the stressful natural conditions characterising estuarine mudflats, as suggested by their increasing frequency towards the outer estuary and the lower part of the mudflats. When strong anthropogenic pollution is superposed on natural stress, this should lead to a further decrease in their frequency. In this context, it is interesting to observe that the percentages of both taxa are unexpectedly low (compared to the observed regression) at station 6 in the Vie estuary (Figure II.9). In view of the moderate Cu enrichment observed at this site, it cannot be excluded that the lower percentage of these two groups is due to anthropogenic pollution. This example illustrates how the use of the MII can help us to detect stations with anomalous community characteristics due to pollution. However, individual cases should be treated with care. In the case of station Vie 6, the unexpectedly low percentages of *Elphidium* spp. and *Quinqueloculina* spp. could also be explained by an over-estimation of the MII. There may be several reasons for this:

- (1) The uncertainty about the distance to the sea, especially in cases of man-made structures, which artificially block salt intrusion;
- (2) The difficulty of measuring altitude (used to calculate emergence time). As explained in the methods section, different approaches were used for the stations at higher and lower altitudes. This may have led to errors, especially for the higher stations;
- (3) Both estuaries have been sampled after dry periods, with exceptionally low runoff volumes in the 30 days before sampling. Consequently, our RD/CS values (river discharge divided by the estuarine

channel cross-area) are almost zero, and do not play a role in the calculation of our MII. As shown by Jorissen et al. (submitted this volume), this will be different in different seasons;

- (4) The scaling factors α , β and γ , contributing the three factors making up the MII (distance along the salinity gradient, emergence time, relative importance of river discharge), have not been determined yet. A much larger data set is needed to define these constants.

In spite of these uncertainties concerning the calculation of the MII for individual stations, our first results are promising. The MII is significantly correlated both with the main environmental parameters controlling the foraminiferal community and with several foraminiferal characteristics.

5. Conclusions

Estuarine ecosystems are controlled by an intricate complex of environmental parameters. In this context, the relationship between foraminiferal communities and environmental drivers is not yet fully understood.

Today, all estuaries on the French Atlantic coast are to some extent influenced by human activities, and none of them can be considered as pristine. However, on the basis of organic matter and trace metal analyses, we suggest that the Auray and Vie estuaries are not subjected to strong anthropogenic pollution. Thus, these two estuaries can be used to better understand the foraminiferal distribution in estuarine environments affected by low to moderate anthropogenic influence.

In both the Auray and Vie estuaries, the foraminiferal communities are dominated by *Haynesina germanica* and the *Ammonia tepida* group. Stations located near the mouth of the estuary show a higher diversity, with an increasing contribution of *Elphidium* and *Quinqueloculina* species. Stations located in the inner estuary, with a stronger river influence, show a higher proportion of agglutinated species (*Ammotium salsum*, *Ammobaculites agglutinans*). Multivariate statistical analyses suggest that (1) the distance to the sea and (2) the percentage of fine sediment (<63 μm) are the main parameters explaining the foraminiferal distribution.

Several biotic indices of environmental quality have been tested on our estuarine dataset. When using the Ecological Quality Status (EQS) ranges based on the diversity index $\text{Exp}(H'_{\text{BC}})$, all our stations were classified as “poor” or “bad”. In view of the absence of indications of severe pollution, the limits between EQS classes appear unsuitable for estuarine environments characterised by low-diversity communities. In Forum-AMBI, the classification of species in ecological groups is based on their position on a theoretical C_{org} gradient. In our estuarine intertidal mudflats, where a significant correlation between foraminiferal distributions and $\%C_{\text{org}}$ was not observed, and where the foraminiferal assemblages are dominated by a low number of species, this method should be applied with caution.

For the 27 stations of our two estuaries, the Marine Influence Index (MII) is significantly correlated with the first axis of our PCA model. MII also shows a significant correlation with several individual foraminiferal

parameters, such as the species number and the percentages of *Elphidium* spp. and *Quinqueloculina* spp. This suggests that MII indeed integrates the main environmental parameters controlling the foraminiferal community's characteristics. At station 6 in the Vie estuary, where moderate copper enrichment was observed, the values of the three faunal parameters related to MII were lower than expected on the basis of MII, possibly due to the effect of pollution. This example shows how MII can be used to detect deviations from the natural distribution patterns. However, before applying this concept routinely, the values of the MII weighing constants α , β and γ , which were all set at 1.0 here, have to be calibrated in a much larger data set. Once the calibration of MII is fully achieved, this index can be used in polluted estuaries to recognise major deviations from the natural distribution due to anthropogenic pollution.

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Supplementary material

Supplementary Table II.S1 : Overall characterisation of the Auray and Vie estuaries.

	Auray	Vie
Estuary type	Ria	Lowland estuary
Limit of tidal influence and saltwater intrusion	19.8 km	8 km (estimated 16.87 km before the construction of a dam at 8 km from the mouth)
Width at the mouth	950m	200m
Catchment area	324km ²	84km ²
Average water flow per year (last 49 years) (Banque HYDRO)	4.53 m ³ .s ⁻¹	1.17 m ³ .s ⁻¹
Average yearly rainfall	951mm	901mm
Overall Ecological status (2019 MFD)	Average	Good

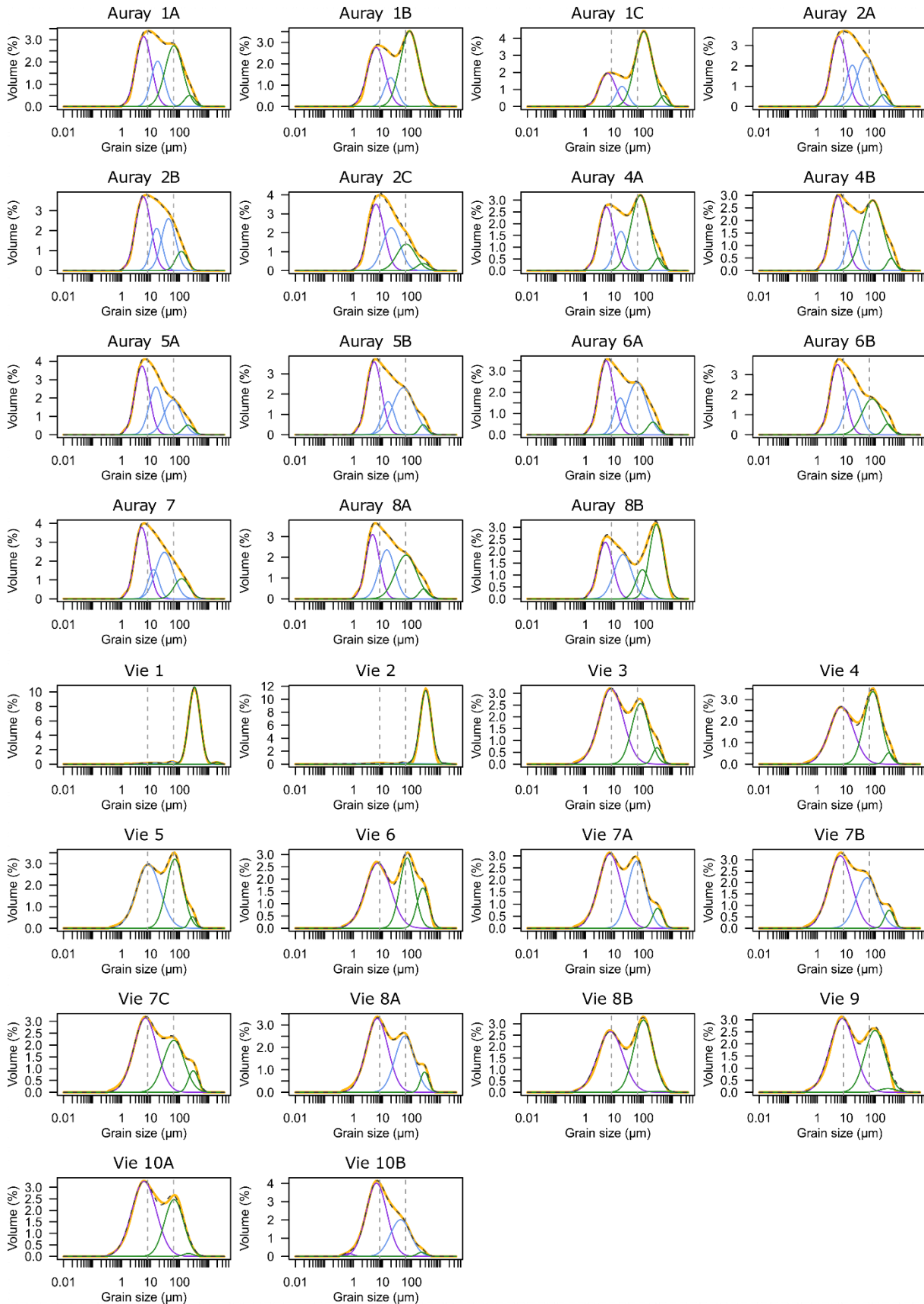
Supplementary Table II.S2 : Description of the sampling stations in Auray and Vie estuaries.

Auray estuary			
Site n°	Station n°	Altitude	Localisation within the estuary
Site 1	Station 1A: Upper mudflat, near the slipway	2.52m	Outer estuary
	Station 1B : Center of the mudflat, near the slipway	1.29m	
	Station 1C : Lower mudflat, near the main channel	1.38m	
Site 2	Station 2A: Upper mudflat	4.50m	Large middle estuarine mudflat, near oyster farming areas.
	Station 2B : Center of the mudflat	4.20m	
	Station 2C : Lower mudflat	1.38m	
Site 4	Station 4A: Upper mudflat	3.30m	Large middle estuarine mudflat
	Station 4B : Lower mudflat	1.37m	
Site 5	Station 5A: Upper mudflat	2.20m	Large middle estuarine mudflat
	Station 5B: Lower mudflat	1.21m	
Site 6	Station 6A: Upper mudflat	2.50m	Large middle estuarine mudflat
	Station 6B: Lower mudflat	1.49m	
Site 7	Mudflat along small auxiliary creak	1.31m	
Site 8	Mudflat in the city of Auray	2.30m	Inner estuary
	Station 8A: Upper mudflat	1.29m	
	Station 8B: Lower mudflat	1.29m	
Vie estuary			
Site 1	Beach in the mouth immediately outside the estuary	-	Outside the estuary
Site 2	Beach just before the entrance of the estuary	3.35m	
Site 3	Dock for ferry boat	2.00m	Outer estuary, urban area
Site 4	Slipway for pleasure ships	1.82m	
Site 5	Slipway in fishing harbour	2.67m	
Site 6	Dock for touristic boat	1.44m	
Site 7	Large intertidal mudflat	4.82m	Middle estuary
	Station 7A : Upper mudflat	4.18m	
	Station 7B : Center of the mudflat	1.61m	
Site 8	Station 7C : Lower mudflat, near the main channel	1.61m	Inner estuary
	Station 8A: Upper mudflat	4.21m	
Site 8	Station 8B: Lower mudflat, near the main channel	1.15m	Inner estuary
	Lower mudflat, near main channel	1.22m	
Site 9	Mudflat 200 m before the sluice	4.86m	Inner estuary
	Station 10A: Upper mudflat	2.24m	
Site 10	Station 10B: Lower mudflat, near the main channel	2.24m	

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Supplementary Table II.S3 : Grain size data.

Station	Population 1		Population 2		Population 3		Population 4		% grains < 63 μm	% grains < 8 μm
	(very fine silt)		(medium silt)		(coarse silt – very fine sand)		(medium sand)			
	mode (μm)	Population %	mode (μm)	Population %	mode (μm)	Population %	mode (μm)	Population %		
Auray										
1A	5.8	42.4	17.6	14.8	64.1	40.2	224.4	2.6	76.6	27.8
1B	6.1	40.0	19.2	9.1	86.3	50.9	256.2	0.1	66.9	24.6
1C	5.8	27.3	18.3	7.3	104.2	60.2	492.6	5.2	49.4	17.2
2A	5.6	47.2	16.3	14.2	48.9	36.0	187.0	2.6	83.0	32.0
2B	5.6	49.6	16.2	12.5	41.7	29.9	115.9	7.9	85.5	33.1
2C	6.0	49.5	20.3	26.4	66.2	21.5	252.7	2.7	83.4	33.1
4A	5.4	36.0	16.9	12.2	79.1	48.6	332.0	3.1	67.0	25.5
4B	5.2	39.5	16.9	11.0	80.7	46.6	353.0	2.9	68.3	28.1
5A	5.1	47.5	15.3	22.3	58.8	27.7	196.2	2.5	83.9	36.9
5B	5.2	46.8	15.7	11.7	53.0	39.4	254.0	2.1	80.4	34.4
6A	5.3	46.2	16.1	11.7	60.1	39.1	213.8	3.0	77.2	32.8
6B	5.0	47.0	16.9	21.1	78.5	29.2	270.3	2.7	79.0	35.6
7	5.0	46.7	12.7	10.7	30.1	30.9	118.1	11.6	84.7	36.3
8A	4.6	36.7	14.1	25.4	65.1	35.5	265.8	2.4	78.5	33.9
8B	4.9	32.0	19.7	23.1	92.9	14.3	284.2	30.7	54.9	24.7
Vie										
3	7.8	59.2			79.3	37.0	296.9	3.7	72.4	31.7
4	6.9	49.2			82.4	48.2	296.8	2.6	66.0	29.0
5	8.4	52.6			68.6	45.4	276.5	2.0	72.9	27.1
6	7.1	51.2			71.5	38.0	247.8	10.8	65.0	29.2
7A	7.0	53.5			59.3	41.3	310.3	5.2	75.0	31.5
7B	6.2	58.0			52.1	37.5	301.7	4.6	79.0	36.4
7C	6.6	57.1			63.6	37.2	297.2	5.6	74.7	34.3
8A	6.6	56.1			58.9	39.7	283.2	4.2	76.3	34.2
8B	7.3	52.0			100.8	47.5	321.1	0.5	63.8	27.8
9	7.3	57.9			98.1	40.2	274.6	1.9	70.2	31.5
10 A	6.0	61.1			64.8	38.3	202.9	0.6	79.4	38.2
10 B	6.2	66.1			42.2	31.4	224.2	0.9	88.5	43.1



Supplementary Figure II.S1 : Overall grainsize distribution (yellow dashes curves), and reconstructed grain size populations (solid coloured curves).

Supplementary Table II.S4 : Measured values of metallic trace elements (in mg/kg of dry sediment).

	V	Cr	Co	Ni	Cu	Zn	As	Sr	Mo	Ag	Cd	Sn	Sb	Ba	Pb	Th	U	% grains < 63 μ m	
Auray (July-2019)	1A	97.7	76.4	9.0	28.0	61.5	168	16.2	121	2.0	0.3	0.6	6.3	1.0	341	41.8	7.7	3.0	76.6
	1B	82.5	63.9	7.8	23.4	16.2	123	11.4	146	2.0	0.2	0.5	5.2	0.9	412	35.9	5.8	2.7	66.9
	1C	77.1	59.0	7.4	21.8	13.4	107	11.1	159	3.0	0.2	0.4	4.8	0.9	369	32.4	5.5	2.8	49.4
	2A	104	81.8	9.1	30.3	23.1	154	17.1	123	2.7	0.3	0.6	6.5	1.0	295	43.4	9.4	3.3	83.0
	2B	98.7	77.0	8.6	28.5	20.2	139	15.8	137	2.9	0.3	0.5	6.1	1.0	287	41.5	8.8	3.5	85.5
	2C	89.9	71.8	8.0	26.2	18.6	125	16.4	125	2.2	0.3	0.5	5.7	0.9	289	37.4	7.9	3.0	83.4
	4A	86.3	66.0	7.9	24.5	14.6	108	13.3	142	4.4	0.2	0.4	5.2	0.9	358	35.0	6.7	3.3	67.0
	4B	97.9	75.5	8.9	28.1	22.6	146	14.6	122	2.7	0.3	0.5	6.1	1.0	334	41.6	8.2	3.4	68.3
	5A	98.3	79.2	9.1	29.2	25.7	173	18.0	161	1.5	0.4	0.6	7.1	1.0	297	44.4	8.7	3.0	83.9
	5B	103	82.5	9.5	30.7	24.7	170	18.0	133	1.2	0.4	0.6	7.2	1.0	308	46.0	9.2	2.9	80.4
	6A	87.7	71.4	8.4	26.2	21.8	160	17.8	137	1.4	0.4	0.6	6.2	1.0	313	42.6	7.5	2.9	77.2
	6B	89.7	72.6	8.6	27.5	23.3	162	18.2	118	1.3	0.4	0.6	6.2	0.9	277	42.8	8.1	2.8	79.0
	7	106	87.0	10.1	33.4	30.4	196	21.1	120	1.2	0.5	0.7	7.5	1.1	294	50.7	9.9	3.3	84.7
	8A	85.9	71.1	8.4	27.8	25.5	173	19.2	103	1.6	0.4	0.6	6.3	0.9	272	42.5	7.9	3.0	78.5
	8B	48.9	40.3	5.5	15.9	12.7	126	12.2	139	0.6	0.3	0.4	4.6	0.7	470	35.2	4.2	1.9	54.9
	Vie (Oct-2018)	1	33.3	18.1	4.0	12.0	9.4	40	8.4	501	0.3	0.2	0.1	1.6	1.2	293	10.8	2.6	1.1
2		22.1	11.6	3.0	7.9	5.0	27	7.4	419	0.9	0.1	0.1	1.0	0.8	241	12.7	1.9	0.8	4.1
3		70.7	51.7	8.3	22.3	17.8	104	15.5	463	0.8	0.4	0.3	3.8	1.0	213	28.7	5.9	1.7	72.4
4		73.4	52.5	8.9	22.6	26.5	128	16.7	446	2.4	0.4	0.3	4.0	1.0	218	30.0	6.2	1.7	66.0
5		82.2	60.3	9.8	24.9	31.4	138	19.3	369	0.9	0.4	0.3	4.7	1.2	245	33.9	6.9	1.9	72.9
6		84.4	62.8	10.6	28.5	51.3	195	16.4	376	1.3	0.4	0.4	5.8	1.3	287	39.2	7.5	2.2	65.0
7A		81.7	63.9	10.2	25.1	19.3	127	14.5	324	2.5	0.5	0.3	4.8	1.2	268	35.9	7.3	2.3	75.0
7B		94.8	68.6	12.6	27.1	22.2	130	16.6	306	1.0	0.5	0.4	4.9	1.3	266	36.1	7.5	2.2	79.0
7C		79.9	59.8	10.0	24.7	20.8	122	17.5	412	1.0	0.4	0.4	4.7	1.2	266	34.8	7.1	2.0	74.7
8A		69.0	53.0	9.3	21.3	20.2	114	15.6	321	1.6	0.4	0.3	4.0	1.1	280	31.1	6.4	2.0	76.3
8B		81.9	64.5	10.2	24.7	22.9	129	17.8	251	1.4	0.5	0.4	5.5	1.3	307	34.4	7.2	2.2	63.8
9		64.1	50.6	9.1	23.7	19.6	108	14.9	247	0.7	0.9	0.3	3.8	1.1	293	30.1	6.1	1.8	70.2
10A		102.3	80.1	12.3	30.7	30.5	162	24.0	234	1.0	0.5	0.4	6.1	1.5	314	39.8	9.3	2.3	79.4
10B		76.7	62.1	9.5	22.8	20.9	121	13.2	175	0.9	0.4	0.3	4.5	1.2	332	32.9	7.2	2.3	88.5
Reference VIVAN 8	119.5	81.9	14.2	43.9	18.8	135	23.4	163	30.2	0.5	0.9	4.5	1.5	310	36.7	10.3	13.4		

Supplementary Table II.S5 : %C and $\delta^{13}C$ values in decarbonated samples from Auray and Vie estuaries

Auray			Vie		
Station	Corg (%)	$\delta^{13}C$ (‰)	Station	Corg (%)	$\delta^{13}C$ (‰)
1A	1.66	-18.77	3	2.27	-14.31
1B	1.43	-18.27	4	2.08	-15.54
1C	1.53	-17.71	5	2.15	-17.41
2A	2.27	-18.87	6	1.96	-17.73
2B	1.88	-18.84	7A	1.34	-19.11
2C	2.33	-18.04	7B	1.56	-21.00
4A	1.56	-18.69	7C	1.95	-14.96
4B	2.22	-19.35	8A	1.47	-13.58
5A	2.40	-19.93	8B	1.96	-23.20
5B	2.28	-21.08	9	1.51	-22.77
6A	2.15	-21.13	10A	1.72	-24.88
6B	2.17	-21.32	10B	1.16	-19.82
7	2.91	-21.21			
8A	2.93	-22.69			
8B	1.23	-23.86			

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Supplementary Table II.S6 : Auray estuary. Foraminiferal density standardised for 50 cm³. For all stations, data are presented for the three replicates and for the two depth levels (0-1.5 and 0.5-1 cm).

Site	Station	Replicate	0-0.5cm total ind / 50 cm ³	0.5-1 cm total ind / 50 cm ³	0-1 cm total ind / 50 cm ³	0-1 cm average / 50 cm ³	standard deviation	0-1 cm average / 50 cm ³	standard deviation
1	1A	A	821.1	27.6	424.4	320.9	96.1	75.9	11.1
		B	464.4	4.1	234.3				
		C	530.8	77.4	304.1				
	1B	A	1835.6	232.2	1033.9	1081.3	681.3	242.2	252.6
		B	747.5	102.3	424.9				
		C	3140.5	429.4	1785.0				
	1C	A	2123.1	1675.3	1899.2	1693.2	275.2	331.2	181.7
		B	2139.7	621.5	1380.6				
		C	2172.9	1426.5	1799.7				
2	2A	A	27.6	19.4	23.5	26.5	23.3	24.0	11.0
		B	6.9	2.8	4.8				
		C	85.7	16.6	51.1				
	2B	A	116.1	34.6	75.3	33.9	35.9	35.9	35.5
		B	19.4	4.1	11.7				
		C	29.0	0.0	14.5				
	2C	A	1537.1	20.7	778.9	618.8	153.0	62.2	76.8
		B	948.2	0.0	474.1				
		C	1197.0	9.7	603.4				
4	4A	A	888.3	513.1	700.7	655.1	141.0	116.3	66.1
		B	821.1	172.8	496.9				
		C	1120.5	414.7	767.6				
	4B	A	840.4	15.2	427.8	309.9	104.4	102.0	61.6
		B	456.1	2.8	229.5				
		C	541.8	2.8	272.3				
5	5A	A	349.7	125.8	237.7	279.0	36.2	9.9	6.0
		B	454.8	156.2	305.5				
		C	275.1	312.4	293.7				
	5B	A	562.6	116.1	339.3	310.8	28.7	50.0	15.5
		B	436.8	127.2	282.0				
		C	479.6	142.4	311.0				
6	6A	A	1135.3	497.6	816.5	810.0	23.3	14.9	12.9
		B	1318.7	340.0	829.4				
		C	995.2	573.2	784.2				
	6B	A	1360.1	774.1	1067.1	901.2	145.6	16.2	13.1
		B	989.7	599.9	794.8				
		C	1103.0	580.5	841.8				
7	7	A	194.9	56.7	125.8	138.7	11.4	2.3	2.0
		B	150.7	143.8	147.2				
		C	124.4	161.7	143.1				
8	8A	A	294.4	66.3	180.4	114.3	57.3	0.7	0.7
		B	156.2	4.1	80.2				
		C	164.5	0.0	82.2				
	8B	A	432.8	363.5	398.2	183.2	191.0	0.5	0.4
		B	58.1	8.3	33.2				
		C	233.6	2.8	118.2				

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Supplementary Table II.S7 : Vie estuary. Foraminiferal density standardised for 50 cm³. For all stations, data are presented for the three replicates.

Station	Replicate	0-1 cm total ind / 50 cm ³	0-1 cm average / 50 cm ³	standard deviation	0-1 cm average / 50 cm ³	standard deviation
3	A	434.0	598.1	167.3	41.0	44.1
	B	591.6				
	C	768.5				
4	A	431.3	623.9	348.0	36.9	37.6
	B	1025.6				
	C	414.7				
5	A	184.5	169.6	73.4	34.8	26.1
	B	89.8				
	C	234.3				
6	A	427.1	628.3	184.9	23.3	15.5
	B	667.2				
	C	790.6				
7A	A	1721.6	1744.2	340.6	0.0	0.0
	B	2095.5				
	C	1415.4				
7B	A	1152.1	1283.4	430.0	3.7	3.2
	B	934.4				
	C	1763.8				
7C	A	981.4	906.1	180.4	38.1	22.9
	B	1036.7				
	C	700.3				
8B	A	937.2	1079.1	122.9	84.8	37.7
	B	1150.0				
	C	1150.0				
8A	A	1981.5	1799.5	249.6	0.0	0.0
	B	1515.0				
	C	1902.0				
9	A	591.6	889.3	265.2	37.8	28.0
	B	975.9				
	C	1100.3				
10B	A	239.8	202.5	38.4	9.7	6.0
	B	163.1				
	C	204.6				
10A	A	1094.7	883.3	231.6	0.2	0.4
	B	919.2				
	C	635.8				

Supplementary Table II.S8 : Auray estuary. Relative frequencies of all major species (at least 1.0% for the average of the three replicates at one of the stations). For all taxa. Average values are given for the relative frequencies in the three replicates.

Station	<i>Acostata mariei</i>	<i>Ammobaculites agglutinans</i>	<i>Ammotium salsum</i>	<i>Eggerelloides scaber</i>	<i>Morulaeplecta bulbosa</i>	<i>Trochammina inflata</i>	<i>Miliammina fusca</i>	<i>Quinqueloculina oblonga</i>	<i>Ammonia tepida</i> group	<i>Aubignyna perlucida</i>	<i>Elphidium decipiens</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium selseyense</i>	<i>Haynesina depressula</i>	<i>Haynesina germanica</i>	Others
1A	0.0	0.0	0.0	0.7	0.0	0.1	0.0	0.3	11.2	0.0	0.2	2.4	2.8	0.5	0.0	81.6	0.2
1B	0.0	0.0	0.0	2.0	0.0	0.0	0.0	4.8	13.2	0.0	0.6	9.4	14.8	1.3	0.5	53.0	0.4
1C	0.0	0.2	0.0	3.0	0.1	0.0	0.2	6.3	10.8	0.0	1.0	17.8	9.9	0.4	3.3	46.1	1.0
2A	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	95.8	0.0
2B	2.0	1.0	0.3	0.0	1.6	0.0	0.0	0.3	2.5	1.3	0.0	0.0	0.0	0.0	0.3	90.1	0.6
2C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	16.1	1.1	0.2	0.9	5.8	0.1	0.0	75.1	0.1
4A	0.0	4.1	4.6	0.4	0.0	0.0	0.0	2.8	3.6	0.0	0.1	0.2	0.5	0.0	0.0	83.6	0.1
4B	0.1	2.4	0.2	0.4	0.0	0.0	0.1	0.7	13.1	0.0	0.0	0.1	0.6	0.0	0.0	82.2	0.1
5A	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.7	1.0	0.0	0.0	0.0	0.0	0.0	0.0	98.0	0.0
5B	0.0	5.0	1.0	0.5	0.0	0.1	0.0	2.2	11.9	0.0	0.0	0.2	0.7	0.7	0.0	77.1	0.6
6A	0.0	0.8	1.2	0.0	0.0	0.0	0.0	0.3	2.8	0.7	0.0	0.0	0.1	0.1	0.0	94.1	0.0
6B	0.0	3.4	1.9	0.7	0.0	0.0	0.0	0.7	10.4	0.2	0.0	0.2	0.5	0.3	0.1	81.3	0.3
7	0.0	0.0	4.3	0.0	0.0	0.0	0.0	0.0	12.4	0.0	0.0	0.3	2.7	0.0	0.0	79.7	0.5
8A	0.1	2.8	23.3	0.0	0.0	0.4	1.1	0.0	17.8	0.0	0.0	0.0	3.5	0.0	0.0	50.0	0.9
8B	0.3	5.9	81.1	0.0	0.0	7.9	1.9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.2

Supplementary Table II.S9 : Vie estuary. Relative frequencies of all major species (at least 1.0% for the average of the three replicates at one of the stations). For all taxa, average values are given for the relative frequencies in the three replicates.

Station	<i>Quinqueloculina jugosa</i>	<i>Quinqueloculina oblonga</i>	<i>Ammonia tepida</i> group	<i>Elphidium magellanicum</i>	<i>Elphidium oceanense</i>	<i>Elphidium selseyense</i>	<i>Elphidium willtamsoni</i>	<i>Haynesina germanica</i>	Others
3	2.3	1.5	4.0	0.6	0.3	50.2	1.2	38.7	1.2
4	5.7	4.9	3.7	1.7	0.3	68.5	0.2	14.1	1.2
5	1.2	1.8	1.5	0.3	0.0	66.2	0.1	28.4	0.6
6	0.8	0.7	6.2	0.1	0.1	9.9	0.1	81.7	0.5
7A	0.3	0.3	6.2	0.0	0.7	0.1	0.2	92.2	0.1
7B	1.8	0.3	5.7	0.0	0.1	1.7	0.1	90.0	0.0
7C	2.6	2.1	16.3	0.1	0.5	8.5	0.3	69.5	0.3
8B	5.1	1.6	23.0	0.0	1.5	12.2	0.0	56.4	0.0
8A	1.2	1.8	11.7	0.0	2.7	0.0	0.3	82.3	0.2
9	5.8	1.3	42.4	0.0	2.5	5.5	0.2	42.3	0.0
10B	7.3	1.6	49.8	0.0	0.9	0.2	0.2	39.7	0.0
10A	0.8	0.2	35.2	0.0	3.2	0.0	0.2	60.4	0.3

Supplementary Table II.S10 : Marine Influence Index (MII) values for the studied stations.

		Station	MII			Station	MII
		Auray				1A	0.48
	1B		0.67			4	0.71
	1C		0.65			5	0.58
	2A		0.08			6	0.72
	2B		0.17			7A	0.19
	2C		0.57			7B	0.31
	4A		0.25	Vie		7C	0.65
	4B		0.43			8A	0.26
	5A		0.28			8B	0.64
	5B		0.37			9	0.58
	6A		0.21			10A	0.11
	6B		0.28			10B	0.36
	7		0.24				
	8A		0.13				
	8B		0.17				

Supplementary Table II.S11: Eigen values, % of variance and species scores of the PCA based on relative frequencies of all major species (at least 1.0% for the average of the three replicates at one of the stations).

Axis	PCA1	PCA2	PCA3
Eigenvalue	890.4	356.2	236.0
% of variance explained	58.2%	23.3%	15.4%
<i>Acostata mariei</i>	0.02	-0.01	-0.03
<i>Ammobaculites agglutinans</i>	-0.03	-0.32	-0.29
<i>Ammonia tepida</i> group	-0.63	-1.38	4.44
<i>Ammotium salsum</i>	-2.64	-4.16	-2.92
<i>Aubignyna perlucida</i>	0.03	0.00	-0.01
<i>Eggerelloides scaber</i>	0.00	-0.02	0.03
<i>Elphidium decipiens</i>	0.00	-0.01	0.01
<i>Elphidium magellanicum</i>	-0.08	0.08	-0.02
<i>Elphidium margaritaceum</i>	-0.11	-0.12	0.17
<i>Elphidium oceanense</i>	-0.07	-0.24	0.41
<i>Elphidium selseyense</i>	-4.77	5.02	-0.97
<i>Elphidium williamsoni</i>	-0.03	0.02	0.01
<i>Haynesina depressula</i>	-0.02	-0.02	0.02
<i>Haynesina germanica</i>	9.26	1.30	-1.01
<i>Miliammina fusca</i>	-0.07	-0.11	-0.07
<i>Morulaeplecta bulbosa</i>	0.02	0.00	-0.01
<i>Quinqueloculina jugosa</i>	-0.35	0.14	0.43
<i>Quinqueloculina oblonga</i>	-0.20	0.15	0.09
<i>Trochammina inflata</i>	-0.26	-0.37	-0.28

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Supplementary Table II.S12 : Results of Spearman correlation test between all dominant species (>5%) and environmental parameters. Values correspond to R, ns means that the relation is not significant, * that the p.value is < 0.05 and ** that the p.value is < 0.01. Blue indicates negative correlations, red positive correlations and grey non-significant correlations.

		MII	Emergence time	Normalised distance to sea	< 63um	C _{org} (%)
<i>Ammobaculites agglutinans</i>	2 estuaries	x	x	x	x	x
	Auray	-0.47 ns	0.01 ns	-0.53 *	-0.21 ns	-0.11 ns
	Vie	x	x	x	x	x
<i>Ammonia tepida</i> group	2 estuaries	0.17 ns	-0.32 ns	-0.15 ns	-0.1 ns	0.09 ns
	Auray	0.45 ns	-0.39 ns	0.12 ns	-0.05 ns	0.34 ns
	Vie	-0.38 ns	-0.05 ns	-0.93 **	0.35 ns	-0.68 *
<i>Ammotium salsum</i>	2 estuaries	x	x	x	x	x
	Auray	-0.59 *	-0.06 ns	-0.88 **	-0.03 ns	0.1 ns
	Vie	x	x	x	x	x
<i>Elphidium margaritaceum</i>	2 estuaries	x	x	x	x	x
	Auray	0.83 **	-0.44 ns	0.58 *	-0.33 ns	-0.27 ns
	Vie	x	x	x	x	x
<i>Elphidium oceanense</i>	2 estuaries	0.16 ns	-0.21 ns	-0.05 ns	-0.13 ns	-0.06 ns
	Auray	0.68 **	-0.46 ns	0.36 ns	-0.33 ns	0.01 ns
	Vie	-0.47 ns	0.12 ns	-0.79 **	0.25 ns	-0.55 ns
<i>Elphidium selseyense</i>	2 estuaries	x	x	x	x	x
	Auray	x	x	x	x	x
	Vie	0.81 **	-0.61 *	0.75 *	-0.74 *	0.84 **
<i>Haynesina germanica</i>	2 estuaries	-0.43 *	0.47 *	-0.17 ns	0.17 ns	0.40 *
	Auray	-0.31 ns	0.68 *	0.1 ns	0.49 ns	0.23 ns
	Vie	-0.41 ns	0.27 ns	-0.25 ns	0.24 ns	-0.54 ns
<i>Quinqueloculina jugosa</i>	2 estuaries	x	x	x	x	x
	Auray	x	x	x	x	x
	Vie	0.34 ns	-0.54 ns	-0.19 ns	-0.11 ns	-0.06 ns
<i>Quinqueloculina oblonga</i>	2 estuaries	0.73 **	-0.38 *	0.47 *	-0.23 ns	-0.47 *
	Auray	0.75 **	-0.3 ns	0.5 ns	-0.4 ns	-0.36 ns
	Vie	0.48 ns	-0.38 ns	0.34 ns	-0.33 ns	0.33 ns

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Supplementary Table II.S13 : Ecological characteristics of the dominant species of our dataset.

<i>Ammonia tepida</i>	<i>Ammonia tepida</i> is considered as one of the dominant species in intertidal sediments (Debenay et al., 2000). It is characteristic of lagoons, estuaries and inner shelves (Murray, 1979) and known to be tolerant to all kinds of stressed conditions (e.g. low salinity, organic input, hypoxic conditions) (Bradshaw, 1968; Bouchet et al., 2007). <i>A. tepida</i> is considered as 2 nd order opportunist by Jorissen et al. 2018, and as a 3 rd order opportunist by Bouchet et al. 2021.
<i>Haynesina germanica</i>	<i>Haynesina germanica</i> is a major species in estuarine areas and is considered as extremely euryhaline (Murray, 1979; Alve and Murray, 1994). It is present in polluted (Armynot du Châtelet et al., 2004 ; Martins et al., 2013) as well as unpolluted estuaries (Cearretta et al., 1988 ; Castignetti et al., 1996). <i>H. germanica</i> is considered as a 3 rd order opportunist by Jorissen et al. 2018, and Bouchet et al. 2021.
<i>Elphidium margaritaceum</i>	<i>Elphidium margaritaceum</i> is dominant in estuary mouths, nearshore areas and marine areas (Murray, 1979; Martins et al., 2015; Franscscangeli et al., 2018). It is considered as a sensitive species by Bouchet et al., 2021.
<i>Elphidium oceanense</i>	<i>Elphidium oceanense</i> is a brackish to marine species, commonly found in estuaries, estuary mouths and nearshore areas (Murray, 1979). This species is considered as a 3 rd order opportunist by Jorissen et al. 2018 and Bouchet et al. 2021.
<i>Elphidium selseyense</i>	<i>Elphidium selseyense</i> is an inner shelf species, tolerant of brackish conditions (Murray, 1979; Alve et al., 1994; Mojtahid et al., 2016). This species is considered as a sensitive species by Bouchet et al., 2021.
<i>Quinqueloculina oblonga</i>	<i>Quinqueloculina oblonga</i> is commonly found in estuary mouths and nearshore areas (Murray, 1979 ; Cearretta et al., 1988). This species is considered as a 3 rd order opportunist by Jorissen et al 2018 and as a 1 st order opportunist by Bouchet et al 2021.
<i>Quinqueloculina jugosa</i>	<i>Q. jugosa</i> is considered as a 1 st order opportunist by Bouchet et al 2021.
<i>Ammotium salsum</i>	<i>Ammotium salsum</i> is widespread in brackish environments (Akers, 1971; Poag, 1978) and has a preference for environments with a salinity comprised between 10 and 20 (Ellison et al., 1987). It is especially common in salt marshes (Scott et al., 1980; De Rijk et al., 1995). <i>A. salsum</i> was found in environments subjected to stressful conditions (e.g., high temperature variations, low oxygen in sediment, elevated trace metal) and considered as a very robust species (Buzas-Stephens et al., 2018). It favours substrates rich in organic matter (Ellison et al., 1987).
<i>Ammobaculites agglutinans</i>	<i>Ammobaculites agglutinans</i> is commonly found in inner part of estuaries and salt marshes (Ellison et al., 1972) and tolerates a wide range of temperature and salinity (Ellison et al., 1987). It favours substrates rich in organic matter (Ellison et al., 1987).
<i>Trochammina inflata</i>	<i>Trochammina inflata</i> is predominant in brackish tidal marshes (Murray, 1979; Armynot du Châtelet et al., 2018; Francscangeli et al., 2018). It tolerates a high environmental variability, including periods of low oxygen availability (Murray, 1979). <i>T. inflata</i> , like others salt marsh species (e.g., <i>A. salsum</i> and <i>A. agglutinans</i>), shows a strong correlation with high organic carbon levels, even in completely natural conditions (Armynot du Châtelet et al., 2009). For this reason, this species is not assigned to an ecological group by Bouchet et al. 2021.

Chapter 3

Biogeographic distribution of three *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*; Foraminifera, Rhizaria) in estuaries of the French Atlantic coast.

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Abstract

Three pseudocryptic species formerly mixed together in the morphospecies *Ammonia tepida* occur on estuarine mudflats along the European coasts: *Ammonia veneta* (T1), *A. aberdoveyensis* (T2), *A. confertitesta* (T6). Since the distinction of these species has only become possible recently, the eventual differences in ecological characteristics are poorly known. In order to fill in this gap, we study here the distribution of these three species at two different scales, by comparing 64 sites within and between seven estuaries along the French North Atlantic coast. The presence of the three species was revealed in all estuaries by morphological and/or molecular methods. No clear distributional differences were found within the seven estuaries, although *A. veneta* and *A. aberdoveyensis* showed a slight preference for stations lower on the mudflats. The high variability in absolute and relative densities of *A. confertitesta* suggests a more opportunistic behaviour for this species. In fact, despite the presence of *A. confertitesta* in eDNA data from all estuaries, this species was rare in the morphological inventory of the northern estuaries (Elorn, Aulne, Odet, Crac'h and Auray), whereas further to the south, it was common in the Vie, and dominant in the Vilaine estuary. We hypothesise that this geographical pattern can be explained by the supposedly invasive character of this species. In spite of the widespread presence of genetic material (including adults, juveniles and propagules), the *A. confertitesta* population has not yet fully developed everywhere. The seven investigated estuaries could represent different stages of replacement of the autochthonous species *A. veneta* and *A. aberdoveyensis* by *A. confertitesta*. We hypothesise that favourable conditions for such a replacement could be created by frequent major river floods, creating temporarily vacant ecosystems. These would be preferentially recolonised by *A. confertitesta*, because of its higher opportunistic propensity and perhaps a better tolerance to low salinity.

1. Introduction

Foraminifera (Eukaryota, Rhizaria) are distributed worldwide in all marine environments, from estuaries and coastal areas to the deep sea. This clade is highly diverse with about 9000 described species (WoRMS - World Register of Marine Species, 2021).

Ammonia was one of the first foraminiferal genera to be described (Brünnich, 1771). The taxon is found in coastal open marine as well as estuarine habitats. In total, 69 morphospecies have been described in the genus *Ammonia* (Hayward et al., 2022). This genus is characterised by a hyaline wall and trochospirally coiled chambers. For decades, different morphotypes of *Ammonia* were considered as ecophenotypes, often as variants of a single species, e.g., *A. beccarii* var. *tepida*, *A. beccarii* var. *parkinsoniana* (e.g., Schnitker, 1974; Jorissen, 1988; Walton & Sloan, 1990). Today, the combination of DNA barcoding and precise morphological studies has allowed the validation and the redescription of 26 different distinct species (Hayward et al., 2004; Bird et al., 2020), which recently have been reviewed by Hayward et al., 2021.

Ammonia tepida was initially considered as a cosmopolitan species, dominant in intertidal environments (Debenay et al., 2000). In the last decade, a large range of different phylotypes have been sequenced, especially morphotypes previously considered as *A. tepida*. At least three of these phylotypes (i.e. T1, T2, T6) have been found along the European Atlantic coasts (Hayward et al., 2021). Recently, Richirt et al., (2019) have proposed a method to distinguish phylotypes T1, T2 and T6 morphologically. These authors demonstrated that the examination of two morphological characters (pore diameter and flushed or raised sutures on the spiral side) under a scanning electron microscope (SEM) is sufficient to discriminate morphologically these three phylotypes with a success rate of > 90%. These species have been renamed as *A. veneta* for T1, *A. aberdoveyensis* for T2 and *A. confertitesta* for T6 (Hayward et al., 2021). Regarding their biogeography, *A. veneta* presents a cosmopolitan distribution (Hayward et al., 2004, 2021). *A. aberdoveyensis* is extensively present along European coasts and is also observed along the Atlantic coast of North America (Hayward et al., 2004, 2021). Finally, *A. confertitesta* shows two disjunct distributional areas, the eastern coasts of Asia (i.e., Japan, Toyofuku et al., 2005; China, Hayward et al., 2004), and the European coasts (Schweizer et al., 2011; Richirt et al., 2020). The disjunct distribution of *A. confertitesta*, and its present occurrence in areas where no representatives of *Ammonia* had been observed in a historical past has led to the hypothesis that *A. confertitesta* could be a recently introduced species in Europe, originating from eastern Asia (Pawlowski & Holzmann, 2007; Schweizer et al., 2011).

The sensitivity to ecological gradients and anthropogenic disturbance of *Ammonia* spp. is used both in environmental quality assessments (e.g., Armynot du Châtelet et al., 2004; Coccioni et al., 2009; Ferraro et al., 2006; Frontalini et al., 2009; Frontalini and Coccioni, 2008; Morvan et al., 2004; Samir, 2000) and in palaeoenvironmental reconstructions (Debenay, 1995; Horton & Edwards, 2006; Dissard et al., 2010). *Ammonia* spp. are also regularly used as a model species in laboratory experiments studying the biological response of foraminifera to stressed conditions (e.g., resistance to anoxia, acidification, tolerance to

pollutants (Morvan et al., 2004; Le Cadre & Debenay, 2006; Ernst et al., 2006; Keul et al., 2013; LeKieffre et al., 2017) or for a better understanding of the role and function of these organisms in benthic ecosystems (e.g., de Nooijer et al., 2009; Diz et al., 2012; Dupuy et al., 2010; Jauffrais et al., 2016; Toyofuku et al., 2017). Because it has only recently become possible to distinguish *A. veneta*, *A. aberdoveyensis* and *A. confertitesta* morphologically, (Richirt et al., 2019; Hayward et al., 2021) earlier studies considered these taxa as a single species. Today, a more deep-going understanding of the ecology of these different species is needed, so that they can be used more efficiently in environmental studies.

Estuaries are good model habitat to define the ecological preferences of these three *Ammonia* species, because of their strong environmental gradients (e.g., salinity, emersion time, sediment grain size...). Moreover, *Ammonia* is one of the dominant genera in estuarine ecosystems (Cearreta, 1988; Alve & Murray, 1994; Castignetti, 1996). Different hypotheses have been proposed regarding the distribution of these *Ammonia* species. Some authors have proposed *A. confertitesta* as a euryhaline species able to live in a wide salinity range (Richirt et al., 2019; Bird et al., 2020; Hayward et al., 2021). Higher abundances of *A. aberdoveyensis* at higher elevation on the mudflats (Bird et al., 2020) were also observed. However, these observations concern only a few stations (e.g., three stations on a mudflat in the Dart estuary (Bird et al., 2020)). To our knowledge, the spatial distribution of these *Ammonia* species within estuaries has not been studied extensively yet. This study will therefore investigate the distribution of the three *Ammonia* species in seven estuaries along the French Atlantic coast.

The first objective of this study is to investigate whether the three species have a different distribution within the estuaries, that could be indicative of different ecological preferences. In seven estuaries, the species distribution will therefore be investigated along an inner-outer estuary gradient and along transects perpendicular to the mudflats. The second objective is to analyse the biogeographic distribution patterns of *A. veneta*, *A. aberdoveyensis* and *A. confertitesta* in estuaries along the north French Atlantic coast, and to see whether eventual differences corroborate the hypothesis that *A. confertitesta* is an invasive species. These results should help to improve the interpretations of future environmental and biomonitoring studies of intertidal estuarine habitats.

2. Material and method

2.1. Study area

This study focuses on seven estuaries located along the north French Atlantic coast: Elorn, Aulne, Odet, Crac'h, Auray, Vilaine and Vie (Figure III.1). The Elorn and Aulne estuaries are both rias (drowned river valleys) located in the inner part of the roadstead of Brest, an enclosed marine bay. The Odet, Crac'h and Auray estuaries are also rias; the former two are directly connected to the Atlantic Ocean, whereas the latter

flows out into the Morbihan Gulf. The Vilaine estuary is a typical lowland estuary, open to the Atlantic Ocean, whereas the Vie estuary can be characterised as a lowland bar-built estuary. Its mouth is deflected northwards by a sandy spit. All studied estuaries are subjected to a meso- to low macrotidal regime with a tidal range of 4 to 5.5 m at the inlet, except for the Vilaine estuary, where the tidal range is higher (about 7.5 m at the inlet).

Table III.1 : Overall characteristics of the seven studied estuaries. The values marked with ¹ come from Office français de la biodiversité and marked with ² from Banque Hydro.

Estuary	Elorn	Aulne	Odet	Crac'h	Auray		Vilaine	Vie
Number of sampling stations	5	3	4	4	15	15	6	14
Sampling campaign	October 2020	October 2020	October 2020	October 2020	July 2019	September 2020	May 2019	October 2018
Estuary type	Ria	Ria	Ria	Ria	Ria		Lowland estuary	Lowland estuary closed by a bar
¹ Salt water penetration	15 km	28.8 km	20 km	13 km	19.8 km		12 km	8.25 km
¹ Catchment area	385 km ²	1797 km ²	715 km ²	64 km ²	324 km ²		10536 km ²	751 km ²
¹ Width at the mouth	570 m	1460 m	1000 m	1016 m	950 m		4400 m	200 m
² Flood discharge	54 m ³ /sec	330 m ³ /sec	76 m ³ /sec	-	31 m ³ /sec		810 m ³ /sec	20 – 25 m ³ /sec
² Low flow discharge	1.1 m ³ /sec	1.5 m ³ /sec	0.79 m ³ /sec	-	0.18 m ³ /sec		5.50 m ³ /sec	0.01 m ³ /sec
² Mean annual discharge volume	5.59 m ³ /sec	25.00 m ³ /sec	7.45 m ³ /sec	-	2.72 m ³ /sec		74.00 m ³ /sec	1.18 m ³ /sec

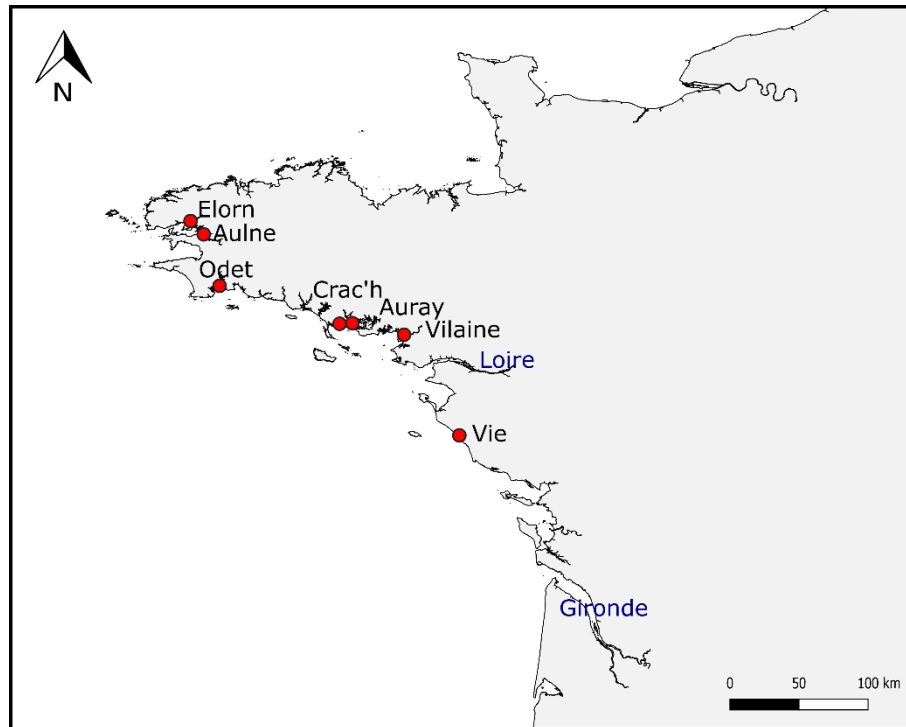


Figure III.1 : Location of the studied estuaries along the French Atlantic coast. Studied estuaries are indicated with a red dot, the Loire and Gironde estuaries are added because they are treated in the discussion.

2.2. Sampling

All samples were collected at low tide. For all sampling stations, the altitude was estimated using two methods. For the stations sampled immediately above the water (at low tide), the altitude was estimated using the tide tables published by the French Naval Hydrographic and Oceanographic Service (SHOM), which give the theoretical altitude of the water surface during the tidal cycles for all French harbours. With the exact sampling hour, the absolute altitude of the sampling point can be obtained. For all stations higher on the mudflats, the altitude was estimated using an electronic theodolite (NA720 ® Leica; height accuracy <1 cm) in combination with official benchmark altitude points, or the vertical lichen zonation (see Fouet et al., 2022 for more detailed information). A range of uncertainty of ± 10 cm was estimated for elevation data.

For foraminiferal morphological analyses, at each station, three tubes with an internal diameter of 9.6 cm were randomly placed and pushed into the sediment. The top 1 cm of the sediment cores was sliced off and preserved in 96% ethanol and stained with 2 g/l Rose Bengal, following the FOBIMO protocol (Schönfeld et al., 2012). In addition, at each station, replicates sediment samples were taken for eDNA analysis and stored rapidly at -20°C prior to DNA extraction (details below).

2.3. Morphological analysis

For the morphological analyses, samples were sieved on 125 μ m. The choice of the >125 μ m mesh size is motivated because the distinction between the three *Ammonia* species is very difficult for juveniles. Foraminifera were picked wet, using a Leica MZ16 stereomicroscope. For each station, about 40 *Ammonia* specimens were selected randomly. When the total number of *Ammonia* specimens was under 40, we selected all available specimens.

We acquired Scanning Electron Microscopy (SEM) overview images (using a Hitashi TM4000) of the spiral side for all 2188 selected individuals. For 581 individuals of the Vie estuary, detailed images of the penultimate chamber (at 1000x magnification) were acquired. The mean pore diameter of these individuals was calculated following the method of Petersen et al. (2016). Based on these images, specimens were determined using the criteria of Richirt et al., 2019. In some cases, species assignment was not possible. This was especially the case for specimens with a damaged test, or very small individuals.

Since we analysed only part of the *Ammonia* specimens of the total assemblages (i.e. ~ 40 individuals or less per station), we estimated the proportion of the three *Ammonia* species in the total foraminiferal assemblage by multiplying the relative proportion of *Ammonia* spp. in the total foraminiferal assemblage with the relative frequency of each species (i.e., *A. veneta*, *A. aberdoveyensis* and *A. confertitesta*) in the 40 (or less) specimens analysed morphologically. For this calculation, specimens that could not be assigned confidently to one of the three species were not taken into account.

2.4. DNA extraction, amplification, sequencing

Environmental DNA (eDNA) was extracted from the sediment using the DNeasy PowerMax Soil, the DNeasy PowerSoil Pro kits (Qiagen) (Vie, Vilaine, Auray 2019) and the FastDNA Spin Kit for Soil (MP Biomedicals) (Elorn, Aulne, Odet, Crac'h, Auray 2020) according to the manufacturers' instructions. PCR was carried out with AccuPrime™ Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific). The 37f hypervariable region (Pawlowski et al., 2014b) (amplicon size: 135-190) was amplified using the specific foraminiferal primers s14F1 (Pawlowski, 2000) and s15r (Lejzerowicz et al., 2014). For each DNA extraction, three PCR replicates were done. The PCR conditions consist in an initial denaturation of 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 45 s and extension at 68 °C for 90 s plus a final extension at 68 °C for 10 min. PCR product replicates were pooled and then quantified using the QuBit HS dsDNA (Invitrogen). Each sample was then pooled with the same amount of DNA and purified using Sera-Mag™ Magnetic carboxylate modified particles (GE Healthcare). Library preparation and MiSeq (paired-end, 2x250bp) sequencing were performed at the ANAN platform (SFR QUASAV, INRAE, Beaucauzé, France) for the Vie and Vilaine and at ID-Gene Ecodiagnosics (Geneva, Switzerland) for the Elorn, Aulne, Odet, Crac'h and Auray samples from 2020.

2.5. Bioinformatics and taxonomic assignment

Tag and primers were removed from the sequences using cutadapt v. 3.4. Clustering of the reads were done using the R package DADA2 (v. 1.16; (Callahan et al., 2016)). Raw reads were quality controlled by truncating the reads (forward and reverse length of 120 bp) and filtering to a maximum number of ‘expected errors’ of two. Amplicon sequence variants (ASV) were dereplicated if identical, clustered and pair-end reads merged using a minimum overlap of 12 bp and maximum mismatch of 0 bp. Chimeras were removed using the ‘pooled’ method. The ASVs were roughly taxonomically assigned using VSEARCH v. 2.18.0 (Rognes et al., 2016) using our custom foraminifera reference database based on NCBI. Then, all ASVs affiliated to the genus *Ammonia* were verified manually by comparison with the GenBank database using BLAST and quick neighbour joining tree analyses were performed to place phylogenetically ambiguous ASVs to *A. veneta*, *A. aberdoveyensis* and *A. confertitesta*. The reads of the ASVs were finally merged according to each species.

In order to use a semi-quantitative approach of the eDNA results, a transformation (i.e., log transformed) was performed on sequence abundance data as recommended by Pochon et al. (2015).

3. Results

3.1. Morphological identification

In total, among the 2188 analysed individuals, 1247 (57%) specimens were determined as *Ammonia veneta*, 296 (13%) as *A. aberdoveyensis*, 454 (21%) as *A. confertitesta* and the remaining 191 specimens (9%) could not be identified with sufficient reliability.

The total count for the three *Ammonia* species are presented in Table III.2. Of the 51 investigated stations, four did not contain *A. veneta*, *A. aberdoveyensis* or *A. confertitesta* (Vie-1, Vie-2; Elorn-1; Crac’h-4). At the 47 remaining stations, *A. veneta*, *A. aberdoveyensis* and *A. confertitesta* together accounted for 0% (when *Ammonia* species were only observed during one of the two sampling campaigns) to 96.7%. *A. veneta* occurred at 42 stations, *Ammonia* sp. *A. aberdoveyensis* was observed at 34 stations and *A. confertitesta* at 28 stations. To estimate the relative frequency of the three *Ammonia* species in the foraminiferal assemblage, we only considered samples with more than 20 individuals of *Ammonia* spp., considering that with less specimens, it is not possible to obtain a reliable estimate. This reduced the data set to 41 stations (Aulne-3; Auray 2020-2A, 2B; Vilaine-3; Vie-5 and Elorn-4 were eliminated). At 25 of the 41 stations, all three *Ammonia* species were observed, whereas at 12 stations only two species were found (nine stations with *A. veneta* and *A. aberdoveyensis* and three stations with *A. veneta* and *A. confertitesta*). Finally, at four stations in the Vilaine estuary (1B, 1C, 2A, 2B) only *A. confertitesta* was observed (Table III.2). For all stations, the

relative frequencies of the three species in the total foraminiferal assemblage were estimated by multiplying the relative proportion of *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta* undifferentiated), with the relative frequency of each of the three individual species (as determined in the selection analysed under the SEM, considering only specimens that could be assigned to one of the three species).

Considering all the 41 stations, on average, *A. veneta* accounted for 10.2% ($\pm 8.3\%$) of the total foraminiferal assemblage, with a maximum frequency of 29% (Vie-10B). *Ammonia* sp. *A. aberdoveyensis* was much less frequent, with an average percentage of 2.2 % ($\pm 2.6\%$), and a maximum of about 10% (Elorn-2, Vie-10B). Finally, *A. confertitesta* was generally rare (0-2%), but was observed in large numbers in the Vie and Vilaine estuaries and at a single station in the Crac'h estuary. At these sites, its relative abundance increased substantially (Vilaine: 60.4 $\pm 26.6\%$; Vie: 6.2 $\pm 6.3\%$), attaining maximum values of 97% (Vilaine 2A), 23% (Vie-10A) and 7% (Crac'h-1), respectively.

Figure III.2 shows the distribution of the three species, according to the distance from the sea and the altitude of the sampling station. Stations with less than twenty individuals are not represented on this figure. On an inter-estuary scale (all stations in an estuary considered together), all species were observed in all estuaries, except in the Vilaine estuary, where *A. aberdoveyensis* was not observed, and in the Elorn estuary where no *A. confertitesta* was found (Figure III.1, Table III.2). The seven estuaries can be divided into three groups. The Aulne, Elorn, Odet, Crac'h and Auray estuaries showed a majority of *A. veneta* (Figure III.1), and very low numbers of *A. confertitesta*. The Vie estuary showed comparable frequencies of *A. veneta* and *A. confertitesta*. Finally, the Vilaine estuary stood out by the dominance of *A. confertitesta*, whereas *A. veneta* was only observed in very low numbers at a single station (Vilaine-1A) and *A. aberdoveyensis* was not found.

Regarding the upstream-downstream gradient, no clear trends were visible, except in the Vie estuary, where all three *Ammonia* species were more abundant in the inner part of the estuary. In terms of absolute elevation (on the mudflats), in the Vie and Auray estuaries, *A. aberdoveyensis* (and to a lesser degree also *A. veneta*) showed a higher relative abundance at stations lower on the mudflat. In the Auray estuary, this tendency was clearer in 2019 than in 2020. In fact, the relative densities of *A. veneta* and *A. aberdoveyensis* show a significant negative correlation with the absolute elevation (Spearman correlation test, p-value: 0.019 and 0.007 respectively), while this is not the case for *A. confertitesta* (Supplementary Figure III.2). No preference for a specific part of the mudflats was observed for *A. confertitesta*.

The Auray estuary has been sampled twice, in July 2019 and September 2020. The overall patterns were comparable for the two sampling campaigns, except that *A. veneta* was more frequent in 2020 (13.6 \pm 7.4% versus 8.1 \pm 4.8% in 2019).

Chapter 3: Biogeographic distribution of three *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*; Foraminifera, Rhizaria) in estuaries of the French Atlantic coast.

Table III.2 : Estuary, sampling station, sampling month/year, total abundance of foraminifera, relative frequency of *Ammonia* species in the total foraminiferal assemblage, total number of individuals analysed, numbers assigned to each of the three *Ammonia* species and the number of non assigned specimens.

Estuary		Station	Sampling period (month/year)	Abundance of foraminifera (ind/50 cm ³)	% of the three <i>Ammonia</i> spp. within the total assemblage	Number of individuals	<i>Ammonia veneta</i>	<i>Ammonia aberdoveyensis</i>	<i>Ammonia confertitesta</i>	Undetermined	Estuary																					
Station	Sampling period										Abundance of foraminifera (ind/50 cm ³)	% of the three <i>Ammonia</i> spp. within the total assemblage	Number of individuals	<i>Ammonia veneta</i>	<i>Ammonia aberdoveyensis</i>	<i>Ammonia confertitesta</i>	Undetermined															
Elorn	1	10/20	319	0.0	0	0	0	0	0	0	Vilaine	1A	5/19	2954	53.6	39	2	0	35	2												
	2		446	13.2	40	8	27	0	5	1B		1257		33.5	40	0	0	39	1													
	3A		709	24.9	40	26	8	0	6	1C		285		51.1	40	0	0	39	1													
	3B		71	20.4	22	14	8	0	0	2A		231		96.7	40	0	0	39	1													
	4		62	8.8	8	1	5	0	2	2B		652		90.3	40	0	0	40	0													
Aulne	1	10/20	649	32.8	40	30	5	1	4	3	Vic	1	10/18	7	40	2	0	0	2	0												
	2		361	19.1	40	32	4	1	3	2		50		0.0	0	0	0	0	0													
	3		70	5.9	6	5	0	0	1	3		220		0.0	0	0	0	0	0													
Odet	1	10/20	504	10.1	40	29	5	1	5	4	Vic	3	10/18	597	3.8	63	34	4	25	0												
	2		773	15.7	40	32	2	2	4	4		623		3.8	33	15	2	16	0													
	3		1334	29.8	40	28	4	0	8	5		169		1.8	9	2	0	7	0													
	4		866	9.5	40	35	1	1	3	6		627		6.1	57	18	21	17	1													
Crac'h	1	10/20	499	14.9	40	18	0	15	7	7A	10/18	7A	10/18	1742	6.2	48	36	3	8	1												
	2		209	25.3	40	22	0	1	17	7B		1282		5.3	59	28	3	28	0													
	3		861	12.8	40	29	4	0	7	7C		905		16.0	68	18	14	36	0													
	4		0	0	0	0	0	0	0	8A		1798		11.7	55	24	5	26	0													
Auray	1A	7/19	318	11.2	40	31	6	2	1	8B	10/18	8B	10/18	1078	23.2	44	27	5	12	0												
		9/20	1178	15.8	40	30	6	2	2	9		888		41.1	52	26	11	14	1													
	1B	7/19	1061	13.2	40	25	12	2	1	10A		10/18		10A	10/18	882	46.7	51	25	1	25	0										
		9/20	665	13.1	40	28	4	0	8	10B				202		49.0	42	25	9	8	0											
	1C	7/19	1683	10.8	40	31	6	3	0					10/18			10/18															
		9/20	259	27.9	40	31	8	0	1																							
	2A	7/19	22	0.0	0	0	0	0	0							10/18			10/18													
		9/20	242	0.3	1	1	0	0	0																							
	2B	7/19	32	0.0	0	0	0	0	0									10/18			10/18											
		9/20	89	1.6	1	1	0	0	0																							
	2C	7/19	607	16.1	40	25	3	0	12											10/18			10/18									
		9/20	524	13.7	40	25	5	1	9																							
	4A	7/19	652	3.6	40	29	3	0	8													10/18			10/18							
		9/20	37	10.9	6	4	1	0	1																							
4B	7/19	295	13.1	37	20	8	0	9		10/18			10/18																			
	9/20	1363	30.4	40	27	5	0	8																								

Chapter 3: Biogeographic distribution of three *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*; Foraminifera, Rhizaria) in estuaries of the French Atlantic coast.

5A	7/19	278	1.0	12	8	2	1	1	
	9/20	842	23.6	40	34	6	0	0	
5B	7/19	310	11.9	40	27	12	0	1	
	9/20	166	19.9	40	27	11	0	2	
6A	7/19	809	2.8	40	17	20	0	3	
	9/20	204	16.6	40	35	3	0	2	
6B	7/19	900	10.4	40	21	15	0	4	
	9/20	136	10.2	40	31	5	1	3	
7	7/19	138	12.4	40	30	2	1	7	
	9/20	501	18.3	40	29	1	0	10	
8A	7/19	113	17.8	40	31	1	1	7	
	9/20	184	11.2	33	29	0	0	4	
8B	7/19	173	0.0	0	0	0	0	0	
	9/20	2560	25.1	40	31	0	2	7	

Chapter 3: Biogeographic distribution of three *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*; Foraminifera, Rhizaria) in estuaries of the French Atlantic coast.

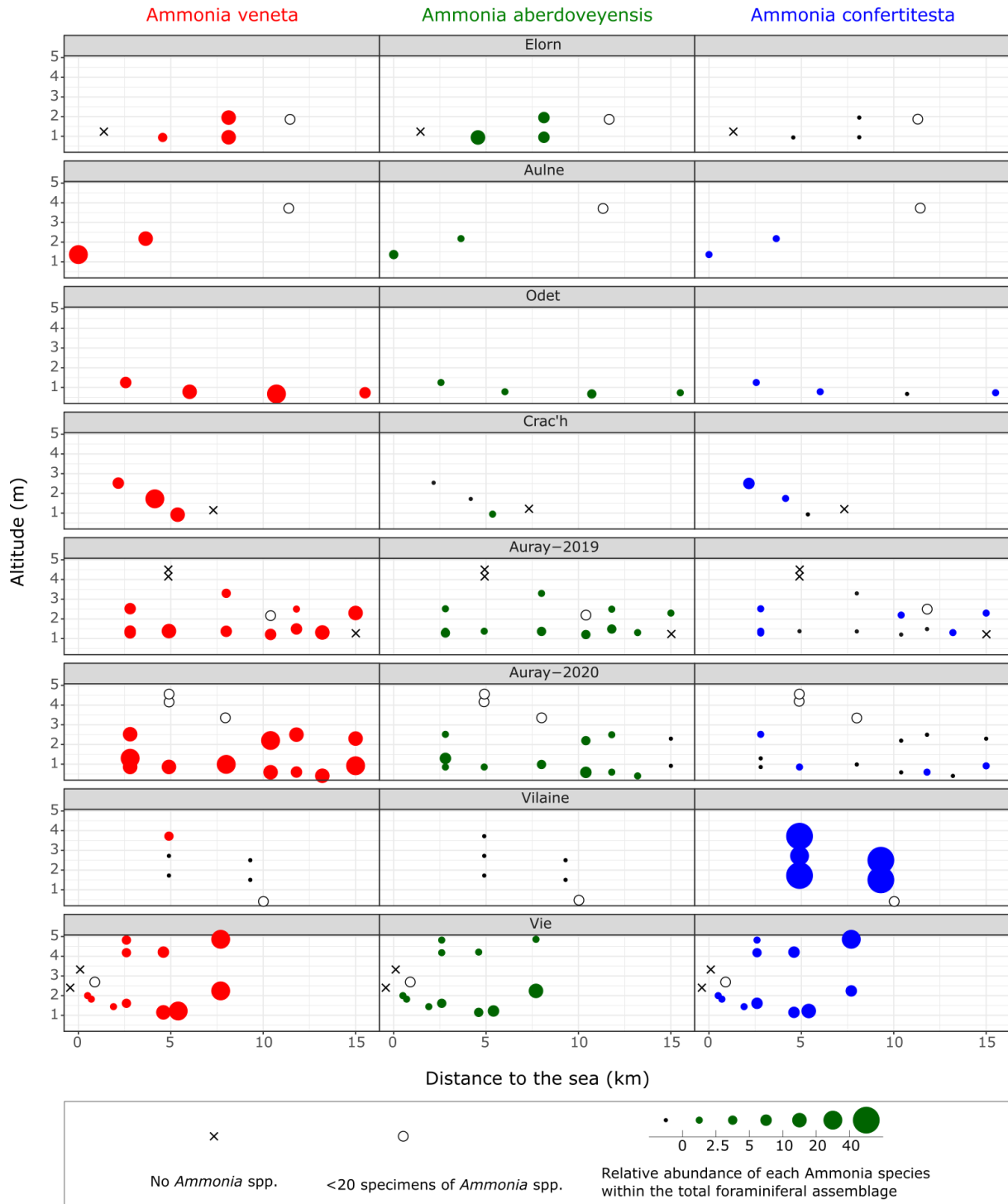


Figure III.2 : Distribution of the three *Ammonia* species (*A. veneta*, *A. aberdoveyensis*, *A. confertitesta*) at all stations. The X axis represents the distance of the sampling point to the mouth of the estuary. The Y axis indicates the absolute altitude of the sampling station. Stations with less than twenty individuals are represented by an empty dot. The size the circles varies in function of the relative abundance of the taxon in the total foraminiferal assemblage.

3.2. eDNA analysis

A table with the number of reads per species and per station is provided in the supplementary material (Table III.S2). *A. veneta* was detected at 46 of the 48 stations investigated for eDNA. Reads of *A. confertitesta* and *A. aberdoveyensis* were found at 38 and 22 of the 48 studied stations, respectively.

Figure III.3 shows the distribution of the *A. veneta*, *A. aberdoveyensis* and *A. confertitesta* in the seven studied estuaries as determined by environmental DNA sequencing. Data presented in this figure were calculated by a log transformation of the number of reads of the three *Ammonia* species.

The co-existence of several *Ammonia* species is common in our samples. All three species of *Ammonia* were detected at 20 of the 48 stations, whereas at 18 stations only two species were found. When two species were observed, in most cases (16 stations) these were *A. veneta* and *A. confertitesta*, whereas at two stations *A. veneta* and *A. aberdoveyensis* were present. At the remaining 10 stations a single species was observed, which was *A. veneta* at eight stations and *A. confertitesta* at the remaining two stations. *A. aberdoveyensis* was never observed alone (Figure III.3).

The seven studied estuaries can be divided into three groups:

- 1) In the Elorn, Aulne and Auray estuaries, only a few reads were assigned to *A. aberdoveyensis*. In the Aulne estuary, it was not observed at all, whereas it was only found in the innermost part of the other two estuaries. Otherwise, *A. veneta* and *A. confertitesta* were observed at most stations, with a few exceptions in the downstream part of Auray estuary, where only *A. veneta* was observed.
- 2) In the Crac'h, Odet and Vie estuaries, reads corresponding to *A. aberdoveyensis* were detected at most of the stations, whereas the other two species were generally well represented (except Crac'h 3 and 4 where *A. confertitesta* was not present).
- 3) Finally, in the Vilaine estuary, the results showed a large number of reads for *A. confertitesta* at all stations, whereas only a few reads were assigned to the other two species.

Considering the seven estuaries as a whole (i.e., all stations in an estuary considered together), all three species were detected in all estuaries, except in the Aulne estuary where no reads were assigned to *A. aberdoveyensis* in the samples.

Concerning the upstream-downstream gradient in the seven estuaries, the three *Ammonia* species did not show a clear and systematic preference for specific parts of the estuary. However, in the Elorn, Auray and Odet estuaries, the data showed reads of *A. aberdoveyensis* only in stations located in the innermost parts.

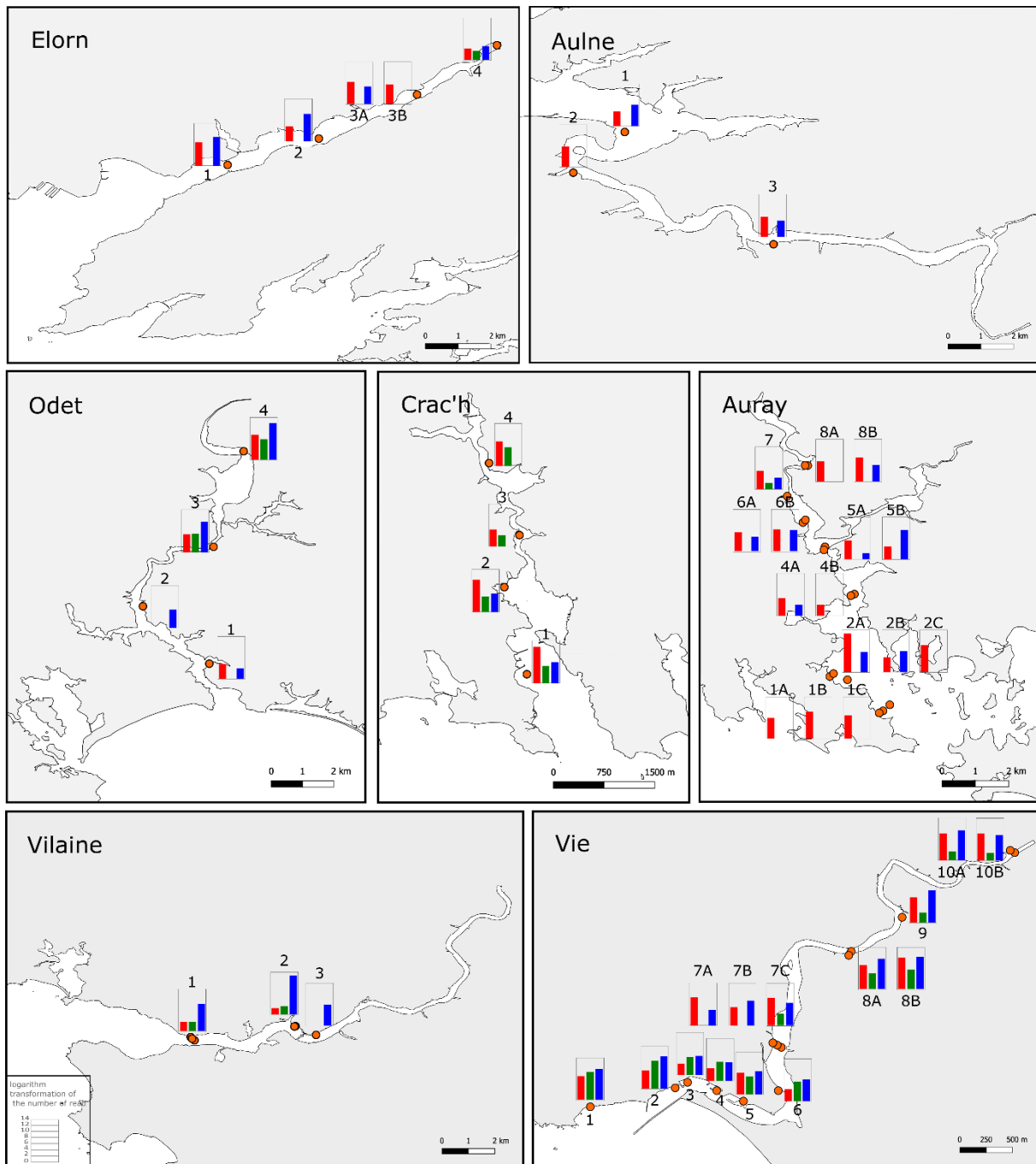


Figure III.3 : Distribution of the three *Ammonia* species (*A. veneta* in red, *A. aberdoveyensis* in green, *A. confertitesta* in blue) based on eDNA sequencing analysis (percentage data based on a log transformation of the number of reads). The localisation of the different estuaries is presented in Figure III.1.

4. Discussion

4.1. Limitations of the present study

4.1.1. Morphometric and genetic discrimination of the three *Ammonia* species.

On the 2188 analysed specimens, 191 (i.e., 8.7%) could not be determined. The impossibility to identify these specimens, that came from all estuaries, was due to *i*) their small size (12 specimens), *ii*) the presence of deformation and/or dissolution (24 specimens), or, *iii*) indecisive morphological characteristics (155 specimens). The latter 155 specimens concerned uniquely the distinction between *A. veneta* and *A. confertitesta*.

Such assignation difficulties are mentioned in previous studies (e.g., for damaged individuals; Richirt et al., 2019; Pavard et al., 2021). Nevertheless, for their respective datasets, Richirt et al. (2019; 2021) estimated an accuracy of $\geq 90\%$ and 95%, respectively, for the morphological determination method. However, the specific problem of distinguishing between *A. veneta* and *A. confertitesta* encountered in our material is not mentioned in these earlier studies.

Richirt et al. (2021) present a dichotomous determination procedure (Figure III.4). The average pore diameter is the primary criterion to distinguish *A. aberdoveyensis* from the two other species, with a threshold value of 1.4 μm .

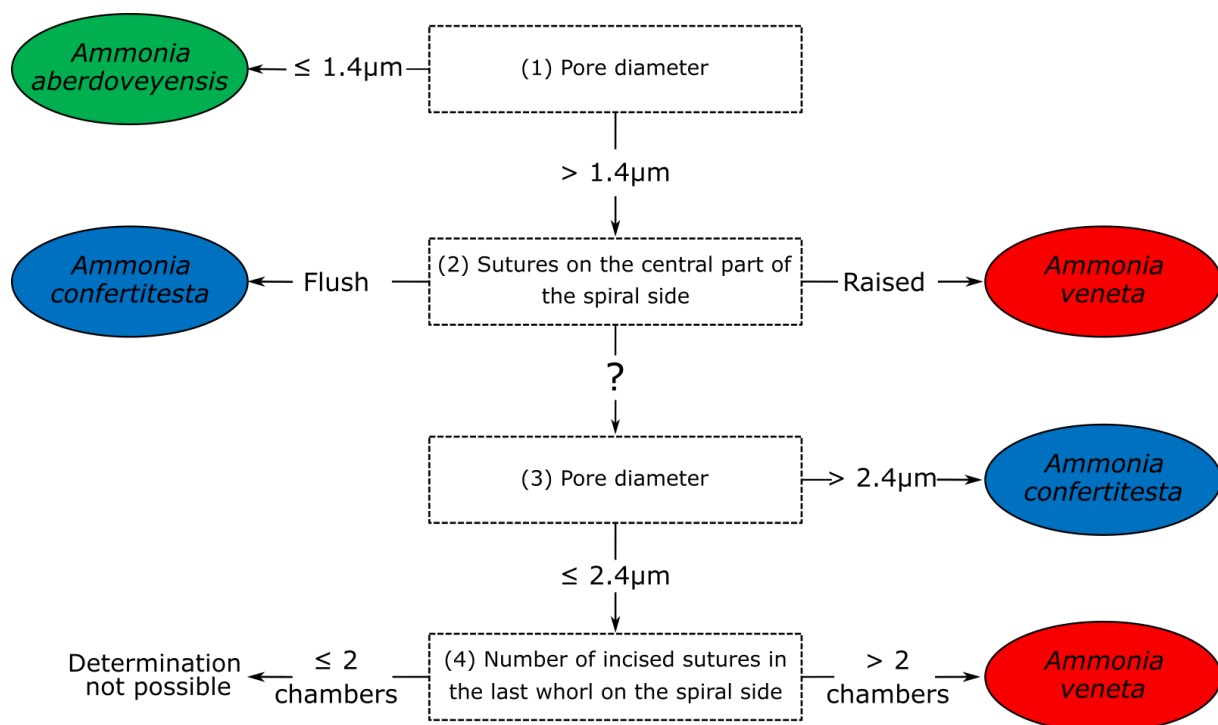


Figure III.4 : Dichotomous procedure to discriminate *A. veneta*, *A. aberdoveyensis* and *A. confertitesta* (from Richirt et al., 2021).

Next, the main criterion to distinguish *A. veneta* and *A. confertitesta* is the shape of the spiral sutures on the central part of the dorsal side, flush in *A. confertitesta* versus raised in *A. veneta*. In our material, this difference was evident in typical representatives of both species, but there were also numerous specimens with an intermediate morphology, with slightly raised sutures.

Richirt et al. (2019; 2021) proposed the average pore diameter as a secondary criterion to distinct *A. veneta* from *A. confertitesta*, with a threshold value of 2.4 μm . All specimens with an average pore diameter larger than 2.4 μm should be *A. confertitesta*, whereas specimens with a smaller pore diameter could belong to either of the two species. In our study, the pore diameter was measured for 578 specimens from the Vie estuary. The range of the mean pore diameter for *A. aberdoveyensis* was 0.59-1.40 μm (n=78), similar to the literature data (Table III.3). The range of the mean pore diameter for *A. confertitesta* was 1.42-3.14 μm (n=222). This range is somewhat lower than the one given by Richirt et al. (2019; 2021), but is identical to that found by Pavard et al. (2021). Finally, the range of the mean pore diameter we observed for *A. veneta* was 1.40-3.15 μm (n=278). The lower part of this range is similar as in previous studies, but very surprisingly, many typical *A. veneta* specimens (with clearly raised sutures on the dorsal side) had a pore diameter well above the threshold value of 2.4 μm , until a maximum of 3.15 μm , much higher than the maximum of 2.62 μm observed previously. In fact, in our dataset, the range of the mean pore diameter for *A. veneta* was not statistically different from that of *A. confertitesta* (t-test, p-value: 0.72).

Consequently, in our study, the criterion “average pore diameter” was efficient to distinguish *A. aberdoveyensis* from *A. veneta* and *A. confertitesta*, but inadequate to discriminate between *A. veneta* and *A. confertitesta*.

Table III.3: Range of measured average pore diameters for the three *Ammonia* species in our study and in the literature. All measurements were made according to the method proposed by Richirt et al. (2019) except for Hayward et al. (2004), who measured only 10 pores per individual, compared to 20-80 in the method of Richirt et al. (2019).

	Number of individuals	<i>Ammonia aberdoveyensis</i>	<i>Ammonia veneta</i>	<i>Ammonia confertitesta</i>
Hayward et al., 2004	20	0.70-1.00 μm	1.30-2.20 μm	1.40-2.00 μm
Richirt et al., 2019	94	0.77-1.32 μm	1.63-2.49 μm	1.92-3.55 μm
Richirt et al., 2021		0.76-1.30 μm	1.51-2.62 μm	1.46-3.84 μm
Pavard et al., 2021	444	0.66-1.40 μm	-	1.42-3.14 μm
This study	578	0.59-1.40 μm	1.40-3.15 μm	1.42-3.14 μm

The third criterion to distinguish *A. veneta* and *A. confertitesta* proposed by Richirt et al. (2021) is the number of incised sutures (between successive chambers) in the last whorl of the spiral side (Figure III.4). *A. confertitesta* shows less than two incised chambers, whereas specimens with more than two incised chamber sutures only occurred in *A. veneta*.

Summarising, the pore diameter criterion could not be used to discriminate *A. veneta* and *A. confertitesta* in our material because of the overlap of values (Table III.3). Therefore we based our determination exclusively on the two remaining criteria, flush or raised sutures in the centre and the number of incised sutures in the last whorl (Figure III.4). However, because of the presence of numerous specimens with

intermediate dorsal suture characteristics (slightly raised), the distinction between *A. veneta* and *A. confertitesta* was very challenging and numerous adult specimens, could not be assigned to a species with sufficient reliability (155 of the 1856 specimens, or 8.4%). These specimens were all considered as “undetermined”. Pictures of some examples are presented as supplementary material (Supplementary Figure III.1).

In view of the impossibility to use pore diameter for the distinction of *A. veneta* and *A. confertitesta*, and the presence of numerous individuals with intermediate suture morphology, even for the specimens that we finally assigned to either of the two species, our decision was sometimes somewhat subjective. Consequently, all occurrences of either of these species with a very small number of individuals should be considered with caution.

4.1.2. eDNA species assignation

Assignation of the ASVs to a known species was made on the basis of existing reference sequences of *A. veneta*, *A. aberdoveyensis* and *A. confertitesta*, present in the NCBI database GenBank (Hayward et al., 2004, 2021; Schweizer et al., 2011; Saad & Wade, 2016; Richirt et al., 2019; Bird et al., 2020). However, there are potentially more variants than the ones deposited in GenBank. Therefore, the absence of a species in the dataset is not a proof of its non existence at a certain sampling site, as it could be represented by non sequenced variants. Therefore the eDNA dataset should be carefully interpreted.

4.1.3. Seasonality

Another potential limitation of this study concerns the different sampling seasons and periods (May to October) of the investigated estuaries. The different *Ammonia* species could have different reproduction time and the different occurrence patterns observed for the seven studied estuaries could be partly explained by this factor.

In fact, various studies have shown that foraminiferal communities on estuarine mudflats may show large seasonal and inter-annual variability (e.g., Murray, 1983; Cearreta, 1988; Buzas & Hayek, 2000; Debenay et al., 2006; Morvan et al., 2006; Choquel, 2021), and that different species can have different reproduction and growth periods. For instance, in the Bay of Bourgneuf, Choquel (2021) showed that *Elphidium oceanense* (d’Orbigny, 1904) attains high densities in autumn whereas *Elphidium selseyense* (Heron-Allen and Earland, 1911) shows high densities in spring and summer. Concerning the recent distinction of the three species of the *Ammonia tepida* morphogroup, there is no information about species-specific reproduction periods. However, our two sampling campaigns in Auray (in July 2019 and September 2020) did not show major changes in the distribution of the three *Ammonia* species. For the time being, we have no indications for seasonal or inter-annual differences between these three species, but this question should be investigated in the future.

4.2. Distribution of the three *Ammonia* species according to the morphological analysis.

The objective of this study was to determine the distribution of the three studied *Ammonia* species at several scales, in order to investigate whether there are indications for different ecological characteristics and/or requirements.

At an intra-estuary scale, along the upstream-downstream gradient, although the three species were not found at all sites, none of them showed a clear preference for a specific part of the estuary. Next, the distribution according to absolute elevation was examined. The absolute elevation determines the emersion time at low tide, when the organisms are exposed to potentially harsh conditions, such as elevated temperature, low or high salinity and predation. Consequently, several authors have suggested that elevation is a primary control of the distribution of foraminifera in estuarine environments (e.g., Horton & Murray, 2007; Francescangeli, 2017; Armynot du Châtelet et al., 2018; Jorissen et al., 2022). In our study, *A. veneta* and *A. aberdoveyensis* showed a slight preference for stations located lower on the mudflats. This observation (contrary to that of Bird et al., 2020) confirms the importance of this parameter, which should absolutely be measured when *Ammonia* spp. (and other intertidal species) are compared between different locations.

Summarising, it appears that the three *Ammonia* spp. in the investigated estuaries do not show clear distributional differences reflecting different ecological characteristics. However, in all estuaries, the absolute and relative densities of the three *Ammonia* species showed some recurrent particularities. At the 51 studied stations, *A. veneta* was most common, and was often the dominant *Ammonia* species (10% on average of the total foraminiferal assemblage). *A. aberdoveyensis* was mostly present as a minority species, that accounted for about 2% of the total foraminiferal assemblage and never exceeded 10%. Finally, the density of *A. confertitesta* was much more variable than that of the other two species. All northern estuaries (Elorn, Aulne, Odet, Crac'h and Auray) showed low proportions of *A. confertitesta* (at most 1% of the foraminiferal assemblage, except in station Crac'h-1). Conversely, much higher proportions of *A. confertitesta* were encountered in the two southern estuaries, up to 23% of the total foraminiferal community in the Vie estuary, and up to 97% in the Vilaine estuary.

These differences in absolute and relative densities between the three *Ammonia* species must reflect differences in their ecological characteristics. *A. veneta* and *A. confertitesta* can both appear with high densities (for example: *A. veneta* attains 300 specimens per 50cm³ in the Odet and Auray estuaries; *A. confertitesta* attains more than 500 specimens per 50cm³ in the Vilaine estuary), whereas this is not the case for *A. aberdoveyensis* (maximum absolute density 80 specimens per 50cm³ in the Vie estuary). Furthermore, *A. confertitesta* dominated the foraminiferal community in the Vilaine estuary; such a dominance was never observed for *A. veneta*. These observations suggest that the three *Ammonia* species have various degrees of opportunistic behaviour, with *A. confertitesta* being most and *A. aberdoveyensis* least opportunistic.

When considering the distribution of the three *Ammonia* species at an inter-estuary scale (all stations in an estuary considered together), other distributional differences occur. All three *Ammonia* species were present in all the estuaries, except in the Elorn estuary, where *A. confertitesta* was absent. However, in the Aulne estuary, the presence of *A. confertitesta* is only based on two not very typical specimens. This scarcity of *A. confertitesta* in the northern estuaries could again reflect slightly different ecological requirements, or, eventually a different distributional history (see section 4.4)

The fact that in most of our estuaries the three *Ammonia* species occur together at many different sampling stations contrasts with most of the previous studies. Both Saad and Wade (2016) and Bird et al (2020) observed that *A. confertitesta* only rarely co-exist with the other two species. Also Richirt et al. (2021) suggested that the co-existence of different *Ammonia* species at the same station is rare. However, most of these observations are based on small numbers of sequenced specimens (10 or less), which could be insufficient to detect the co-existence of the three *Ammonia* species at single sampling stations. Conversely, our results showed a co-occurrence of the three *Ammonia* species in half of the stations (25 out of 41), whereas at only four stations, a single species was found (always *A. confertitesta*). At the 12 stations where two species were present, in nine cases *A. veneta* co-occurred with *A. aberdoveyensis* and in three cases with *A. confertitesta*. *A. aberdoveyensis* was never observed without *A. veneta*. The combination of *A. aberdoveyensis* and *A. confertitesta* (without *A. veneta*) was not observed in our study, but was described in the Gironde estuary (Pavard et al., 2021).

The recurrent co-existence of the three species at many stations shows that their ecological niches must be close, although the differences in density and in geographical distribution seem to point to different degrees of opportunism.

4.3. Comparing morphological and eDNA data

In Table III.4, we compare the presence-absence data for our morphological observations and environmental DNA sequencing. The results show some large differences. In fact, the two methods only fully agree for 14 of the 48 stations.

Several types of observations can be made:

- 1) eDNA revealed the presence of *Ammonia* spp. at the four stations where they were not observed in the morphological study (Elorn 1, Crac'h 4 and Vie 1 and 2).
- 2) *A. veneta* systematically showed a good match between morphological and eDNA data. At 41 of the 48 stations, it was detected by both methods.
- 3) *A. aberdoveyensis* was often not detected with the eDNA approach, while it was present in the morphological inventory (Elorn, Aulne and Auray). However, at some stations in the Crac'h and Vilaine estuaries, the reverse was observed. At the 48 stations, *A. aberdoveyensis* was more represented in the morphological dataset (31 stations) than in the eDNA dataset (22 stations).

- 4) Finally, a major difference between the two data-sets concerns *A. confertitesta*, which was more frequently detected with the eDNA data (38 stations) than in the morphological data (25 stations). This difference concerns especially the northern estuaries (Elorn, Aulne and Auray).

These important differences can be explained by the different nature of the two methods, that give an entirely different picture of the available biota. First, the results of morphological analyses are exclusively based on adult specimens, larger than $>125\mu\text{m}$. Conversely, the eDNA sequencing is based on the total sediment, without size selection. The result of eDNA sequencing therefore includes adult specimens, but also juveniles (Pawlowski et al., 2014b) and even propagules. Both approaches give therefore different, but complementary results (Lejzerowicz et al., 2013; Pitsch et al., 2019, Schweizer et al., in prep). Our morphological analysis indicates the presence of a population of active adult specimens, whereas the eDNA analysis describes the presence of genetic material of the investigated species. In fact, eDNA analysis does not allow the distinction between an active population, including mature individuals, interacting with the environment, or a stock of propagules awaiting the appropriate conditions to develop. As such, the eDNA inventory describes the presence of a taxon (the potential niche), whereas the morphological analysis shows an active population (the realised niche).

With this important difference in mind, we can now interpret the observed differences between eDNA and morphological data. Essentially, we observed three different cases.

- 1) *A. veneta* was detected by both approaches at 41 of the 48 stations. It appears therefore that the realised niche of this species is very close to its potential niche. This suggests that the environmental conditions were generally favourable for the development of this taxon.
- 2) *A. aberdoveyensis* was frequently detected with the morphological method but not by the eDNA method. This difference can not be explained by a difference between realised and potential niches. We think that this discrepancy is related to the sampling procedure. The morphological analysis showed that *A. aberdoveyensis* was widely present at the sampled stations but always in low numbers. In fact, the volume of sediment analysed is an order of magnitude higher for morphological analysis (three replicates of ~80g each) than for eDNA analysis (two or three replicates with a total of 10-12g). It is possible that the absence of *A. aberdoveyensis* in many eDNA samples is the consequence of its relative scarcity, eventually in combination with a patchy distribution. With other words, we hypothesise that the volume of sediment analysed for eDNA was not enough to systematically detect this scarce species. This issue concerning the quantity of sampled material was earlier mentioned by Pawlowski et al., 2014 as a potential bias.
- 3) The presence of *A. confertitesta* in the eDNA data often contrasted with an absence in the morphological analysis. In this case, it appears that the eDNA results revealed the presence of juvenile specimens and/or propagules, whereas an active mature population has not developed. Consequently, the combination of morphological and eDNA data shows that at these stations, the

species is present, but that its niche has not yet been realised, most probably because the environmental conditions were not appropriate yet. This situation could also be explained by a different seasonality of the three *Ammonia* species, where our sampling periods may not coincide with the optimal growth period of *A. confertitesta*.

An alternative explanation could be found in the distributional history of the three species. Several studies considered that *A. veneta* and *A. aberdoveyensis* are autochthonous species, whereas *A. confertitesta* would be an exotic, invasive species (Pawlowski & Holzmann, 2007; Schweizer et al., 2011). If this hypothesis is correct, the difference between morphological and eDNA data for this taxon could be explained by the fact that genetic material of this taxon is present in all estuaries, but that the species has not yet established active populations in all these estuaries. This possibility will be further discussed in the next section.

Table III.4: Presence-absence matrix for three *Ammonia* species with two methods: morphological observations and eDNA analysis.

		<i>Ammonia veneta</i>		<i>Ammonia aberdoveyensis</i>		<i>Ammonia confertitesta</i>	
		Morphology	eDNA	Morphology	eDNA	Morphology	eDNA
Elorn 2020	Elorn-1		*				*
	Elorn-2	*	*	*			*
	Elorn-3A	*	*	*			*
	Elorn-3B	*	*	*			
	Elorn-4	*	*	*	*		*
Aulne 2020	Aulne-1	*	*	*		*	*
	Aulne-2	*	*	*		*	
	Aulne-3	*	*				*
Odet 2020	Odet-1	*	*	*		*	*
	Odet-2	*		*		*	*
	Odet-3	*	*	*	*		*
	Odet-4	*	*	*	*	*	*
Crac'h 2020	Crac'h -1	*	*		*	*	*
	Crac'h -2	*	*		*	*	*
	Crac'h -3	*	*	*	*		
	Crac'h -4		*		*		
Auray-2020	Auray-1A	*	*	*		*	
	Auray-1B	*	*	*			
	Auray-1C	*	*	*			
	Auray-2A	*	*				*
	Auray-2B	*	*				*
	Auray-2C	*	*	*		*	
	Auray-4A	*	*	*			*
	Auray-4B	*	*	*			

	Auray-5A	*	*	*			*
	Auray-5B	*	*	*			*
	Auray-6A	*	*	*			*
	Auray-6B	*	*	*		*	*
	Auray-7	*	*	*	*		*
	Auray-8A	*	*				
	Auray-8B	*	*			*	*
Vilaine 2019	Vilaine-1A	*	*		*	*	*
	Vilaine-2A		*		*	*	*
	Vilaine-3					*	*
Vie-2018	Vie-1		*		*		*
	Vie-2		*		*		*
	Vie-3	*	*	*	*	*	*
	Vie-4	*	*	*	*	*	*
	Vie-5	*	*		*	*	*
	Vie-6	*	*	*	*	*	*
	Vie-7A	*	*	*		*	*
	Vie-7B	*	*	*		*	*
	Vie-7C	*	*	*	*	*	*
	Vie-8A	*	*	*	*	*	*
	Vie-8B	*	*	*	*	*	*
	Vie-9	*	*	*	*	*	*
	Vie-10A	*	*	*	*	*	*
Vie-10B	*	*	*	*	*	*	

4.4. Presumed invasive behaviour of *Ammonia confertitesta*

Several authors have suggested that *A. confertitesta* is an invasive species originating from eastern Asia (e.g., Pawlowski & Holzmann, 2007; Schweizer et al., 2011; Richirt et al., 2021). The two main lines of evidence supporting this hypothesis are 1) the disjoint geographical distribution; the species appears both in Eastern Asia (Toyofuku et al., 2005) and on the European coasts (Schweizer et al., 2011) and 2) the recent appearance of *A. confertitesta* at sites in Europe where it has never been identified in the past (e.g., the Baltic Sea (Flensburg Fjord in Polovodova et al., 2009; Kiel Bay in Schweizer et al., 2011) or the North Sea (Grevelingenmeer in Petersen et al., 2016; Elbe estuary in Francescangeli et al., 2021). Some authors have proposed that this species could have been transported from Asia through ballast waters (Pawlowski & Holzmann, 2007). This species is now widely present along the European coasts, from the Baltic Sea to France (Bird et al., 2020).

Recently, Richirt et al. (2021) studied the distribution of the three *Ammonia* species along the English Channel and Great Britain coasts. They hypothesised that marine currents could be the main vector of transport of foraminiferal propagules away from their source population (important harbours). The authors noticed that *A. confertitesta* was still absent from the south British coast and suggested that this could perhaps be explained by the dominant W-E current in the English Channel, that would hamper the westward spreading of this species. They also hypothesised that there are some refuge zones, mainly in high marshes, where *A. veneta* and *A. aberdoveyensis* persisted, and were not replaced by *A. confertitesta* yet. These authors predicted that ultimately, *A. confertitesta* would largely replace the two other species.

In our study, the morphological observations of the distribution of the three *Ammonia* species showed a clear difference between the Vilaine estuary, with a very strong dominance of *A. confertitesta*, The Vie estuary, where *A. confertitesta* co-occurred with the other two species (with comparable frequencies of *A. confertitesta* and *A. veneta*), and the other five estuaries (Elorn, Aulne, Odet, Crac'h and Auray) where this species was rare or absent.

Together with the greater opportunistic potential of *A. confertitesta*, deduced from the quantitative distribution patterns in our study, these observations seem to corroborate the “invasive species hypothesis”. Our data suggest that *A. confertitesta* has fully colonised the Vilaine estuary, that the colonisation of the Vie estuary is in progress, whereas the colonisation of the other five estuaries is still at an early stage.

If true, this pattern could be explained by two complementary features: 1) the proximity of the source area(s), and 2) the easiness of access of the various estuaries.

Richirt et al. (2021) considered especially major harbours (e.g., Cardiff, Le Havre, Rotterdam) as source areas, related to intense international maritime traffic and the introduction of exotic ballast waters. In our study area, historically, the two major commercial harbours are Nantes-St. Nazaire and Bordeaux, on the Loire and Gironde estuaries, respectively (Figure III.1). The *Ammonia* assemblages of both estuaries are indeed largely dominated by *A. confertitesta* (Loire (Thibault de Chanvalon et al., 2022) ; Gironde (Pavard et al., 2021)).

The complete colonisation of the Vilaine estuary, immediately northward of the Loire, and the ongoing colonisation of the Vie estuary, immediately south of the Loire estuary, would be logical if the Loire estuary is indeed the source area of *A. confertitesta* in this region. As would be the fact that the colonisation of all more northern areas, farther away from the Loire estuary, is still in an early stage.

The second parameter, the easiness of access of the various estuaries, is related to their morphology. The two southern estuaries, Vilaine and Vie, are both lowland estuaries. The Vilaine estuary shows a wide mouth, the Vie estuary is partly closed with a sand spit. Conversely, all five northern estuaries are rias, flooded river valleys with a steep relief, and often with sills at the entrance. It appears therefore that the estuaries already inhabited by large populations of *A. confertitesta* could have an easier access than those in which this taxon is still at an early stage of colonisation. The importance of the form of the estuaries as a factor facilitating or

hampering the introduction of *A. confertitesta* seems to be confirmed by literature evidence. A closer inspection of the sites studied by Saad and Wade 2016, Bird et al., 2020, and Richirt et al., 2021 shows that *Ammonia* assemblages dominated by *A. confertitesta* are mainly found in open estuaries, whereas ria-type estuaries are dominated by *A. veneta* or *A. aberdoveyensis* (Supplementary Table III.S2).

However, our eDNA data detected the presence of *A. confertitesta* in these northern estuaries. This observation suggests that the colonisation of this taxon not only depends on the spreading mechanisms (away from its source area and into the individual estuaries). Once potentially present, the species needs appropriate conditions for its development, allowing it to replace the presumed autochthonous taxa *A. veneta* and *A. aberdoveyensis*.

In view of the apparently similar ecological requirements of the three species, but the potential higher degree of opportunism of *A. confertitesta*, the ideal conditions for a replacement by this species would be created by conditions that would oblige the foraminiferal community to repeatedly recolonise the estuarine mudflats. In estuaries, such conditions are especially created by river floods, which can completely annihilate the foraminiferal community.

The importance of major river floods as a factor causing the colonisation by highly opportunistic species was earlier shown by Goineau et al. (2012). At a station located in front of the Rhone Delta (Mediterranean Sea), the foraminiferal assemblage sampled two days after a major river flood, contained a very dense, almost monospecific population of *Leptohalysis scottii* (Chaster, 1892). The authors concluded that *L. scottii* is a pioneer species that could colonise the newly formed empty habitat first due to its greater reproduction and/or dispersal rates.

This means that after each major river flood, the foraminifera have to recolonise the intertidal mudflats. If *A. confertitesta* is indeed a more opportunistic species, with a higher reproduction rate, it would ultimately replace the other two species. A better tolerance to low salinity could be an additional factor favouring *A. confertitesta*. Although our distributional data do not show a significant difference between the three species, the recent appearance of *A. confertitesta* at numerous sites with brackish waters, where no *Ammonia* species were reported before (Polovodova et al., 2009; Schweizer et al., 2011) could indicate a higher brackish water tolerance for this species.

The relative intensity of river floods largely depends on the extent of the catchment basin and the riverine discharge (Table III.1). Along the French Atlantic coast, the Gironde and Loire estuaries (Figure III.1) have by far the largest average and maximal discharge volumes (mean annual discharge volume of 854 m³/sec for the Loire estuary and 846 m³/sec for the Gironde estuary (Banque Hydro)), followed by the Vilaine estuary (74 m³/sec), whereas the other estuaries have more than ten times smaller average and maximal discharge values (Table III.1). In the Vie estuary, although the river flow is small (1.18 m³/sec), the repetitive openings of a sluice (at 8 km from the mouth) create fresh water floods that are considerable on the scale of this small estuary. These observations seem to corroborate our hypothesis that major river floods could be a main factor

allowing the progressive replacement of the autochthonous *Ammonia* species by *A. confertitesta*. In fact, the Gironde (Pavard et al., 2021), Loire (Thibault de Chanvalon et al., 2022) and Vilaine estuaries are all dominated by this species.

5. Conclusion

This study investigates the distribution of *A. veneta*, *A. aberdoveyensis* and *A. confertitesta* on intertidal mudflats in seven estuaries along the north French Atlantic coast. None of the species showed a clear preference for specific parts of the estuaries, although *A. veneta* and *A. aberdoveyensis* were slightly more frequent in the lower parts of the mudflats. This suggests that the three species have similar ecological preferences. However, whereas *A. veneta* and *A. confertitesta* can be dominant species in the foraminiferal community, *A. aberdoveyensis* was always a minor species. This difference could be indicative of a more opportunistic life strategy for the former two species.

The combination of morphological and molecular methods confirmed the presence of the three species in all seven estuaries. However, these two methods do not give the same information. The detection of these taxa in eDNA can be due to the presence of adults, juveniles and/or propagules. Conversely, their detection in the living (Rose Bengal stained) assemblage of the >125 µm fraction certifies the presence of an active, mature population.

In this study, there were some important differences between the eDNA and morphological data. *A. aberdoveyensis*, for which adult specimens were observed at many stations, in all estuaries, was often absent in the eDNA inventories. This could be explained by the relative scarcity of this taxon, which may have been missed because of an insufficient sample volume for eDNA. Conversely, *A. confertitesta* was well represented in the eDNA data set, at most sites in all estuaries, whereas this taxon showed a large differences between estuaries in the morphological inventory. In the five northern estuaries (Elorn, Aulne, Odet, Crac'h and Auray), *A. confertitesta* was rare, or even absent (Elorn), whereas it was common in the Vie estuary, and attained very high densities at all stations in the Vilaine estuary. This observation corroborates the hypothesis of the invasive nature of *A. confertitesta*. The systematic presence of this taxon in eDNA, that contrasts with an absence, or scarcity, in the morphological data, suggest that although genetic material is present, the species has not everywhere replaced the autochthonous species *A. veneta* and *A. aberdoveyensis* yet. If true, the seven estuaries could present different stages, with a completely replacement in the Vilaine, an ongoing replacement in the Vie, and an early stage of ecological replacement in the other five estuaries.

These different stages of colonisation by *A. confertitesta* could be explained by: 1) the relative distance to the potential source area, 2) the facility of access in each of the estuaries, and 3) the presence of favourable conditions for the development of *A. confertitesta*. We hypothesise that such favourable conditions could be brought about by major flooding events, creating empty ecological niches. *A. confertitesta* could be more

Chapter 3: Biogeographic distribution of three *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*; Foraminifera, Rhizaria) in estuaries of the French Atlantic coast.

successful in recolonising such empty habitats than the other two taxa, because of its more opportunistic lifestyle, and maybe, because of its higher tolerance of low salinity conditions.

Finally, this study underlines the strength of the combination of morphological and eDNA metabarcoding approaches to assess the distribution of closely related species. This combination allows a better understanding of complex distributional patterns, by distinguishing between potential and realised assemblages.

Supplementary Material

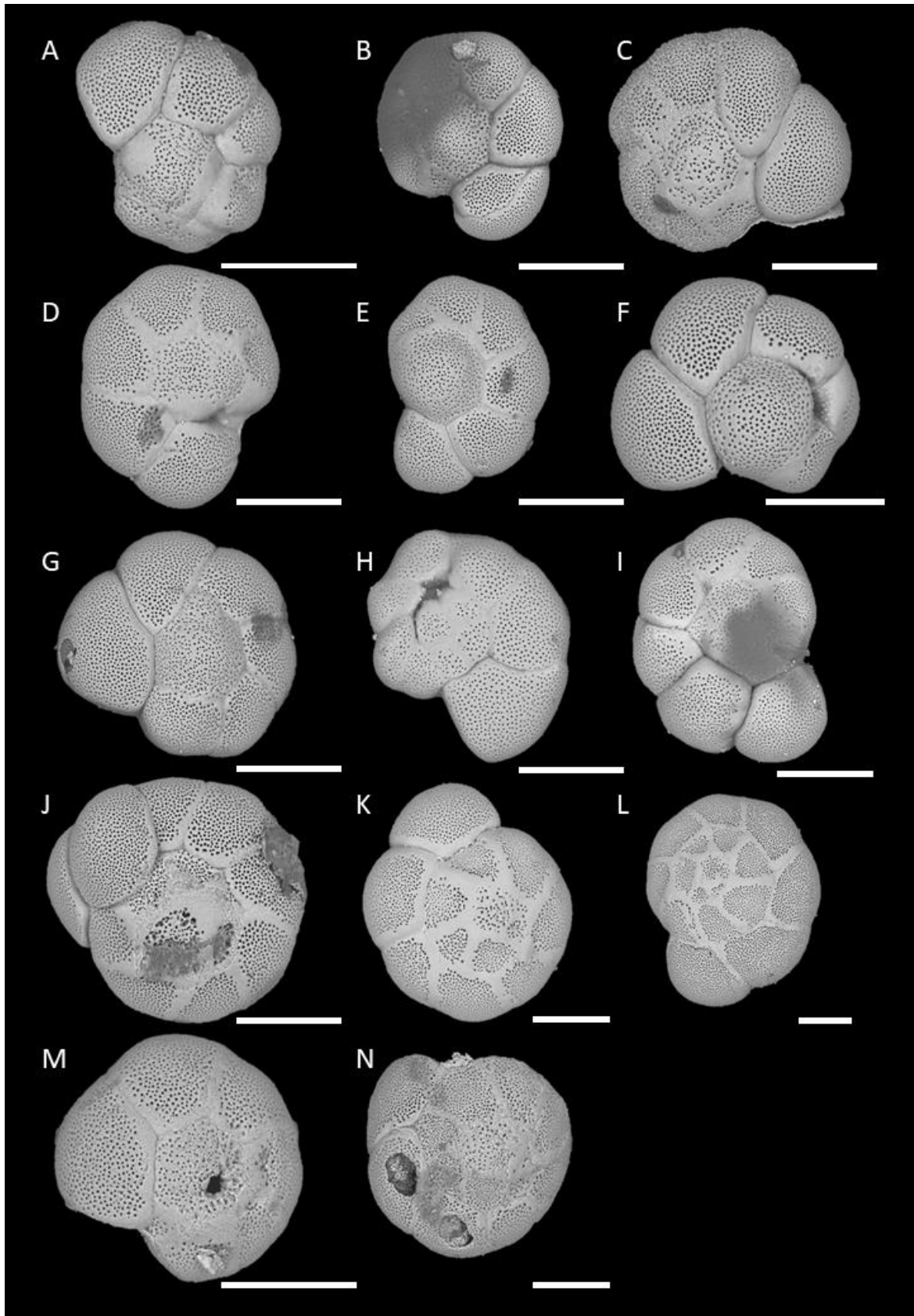
Supplementary Table III.S1 : Number of read per sample

Estuary	Station	<i>Ammonia aberdoveyensis</i>	<i>Ammonia confertitesta</i>	<i>Ammonia veneta</i>
Elorn	1	0	396	117
	2	0	265	16
	3A	0	28	79
	3B	0	0	43
	4	4	12	7
Aulne	1	0	63	14
	2	0	0	50
	3	0	19	48
Odet	1	0	5	14
	2	0	33	0
	3	34	562	31
	4	53	2090	147
Crac'h	1	27	64	2122
	2	18	35	837
	3	7	0	25
	4	38	0	151
Auray (2020)	1A	0	0	59
	1B	0	0	247
	1C	0	0	111
	2A	0	50	3509
	2B	0	64	15
	2C	0	0	241
	4A	0	6	27
	4B	0	0	6
	5A	0	2	36
	5B	0	405	9
	6A	0	14	40
	6B	0	62	72
	7	2	7	35
	8A	0	0	49
8B	0	21	121	
Vilaine	1A	4	264	4
	2A	3	3747	2
	3	0	59	0
Vie	1	305	604	117
	2	288	795	34
	3	32	41	7
	4	38	36	9
	5	31	102	68
	6	42	74	8
	7A	0	18	314
	7B	0	148	32
	7C	7	87	279
	8A	20	539	123
8B	43	867	664	
9	5	819	161	
10A	4	529	260	
10B	3	165	243	

Chapter 3: Biogeographic distribution of three *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*; Foraminifera, Rhizaria) in estuaries of the French Atlantic coast.

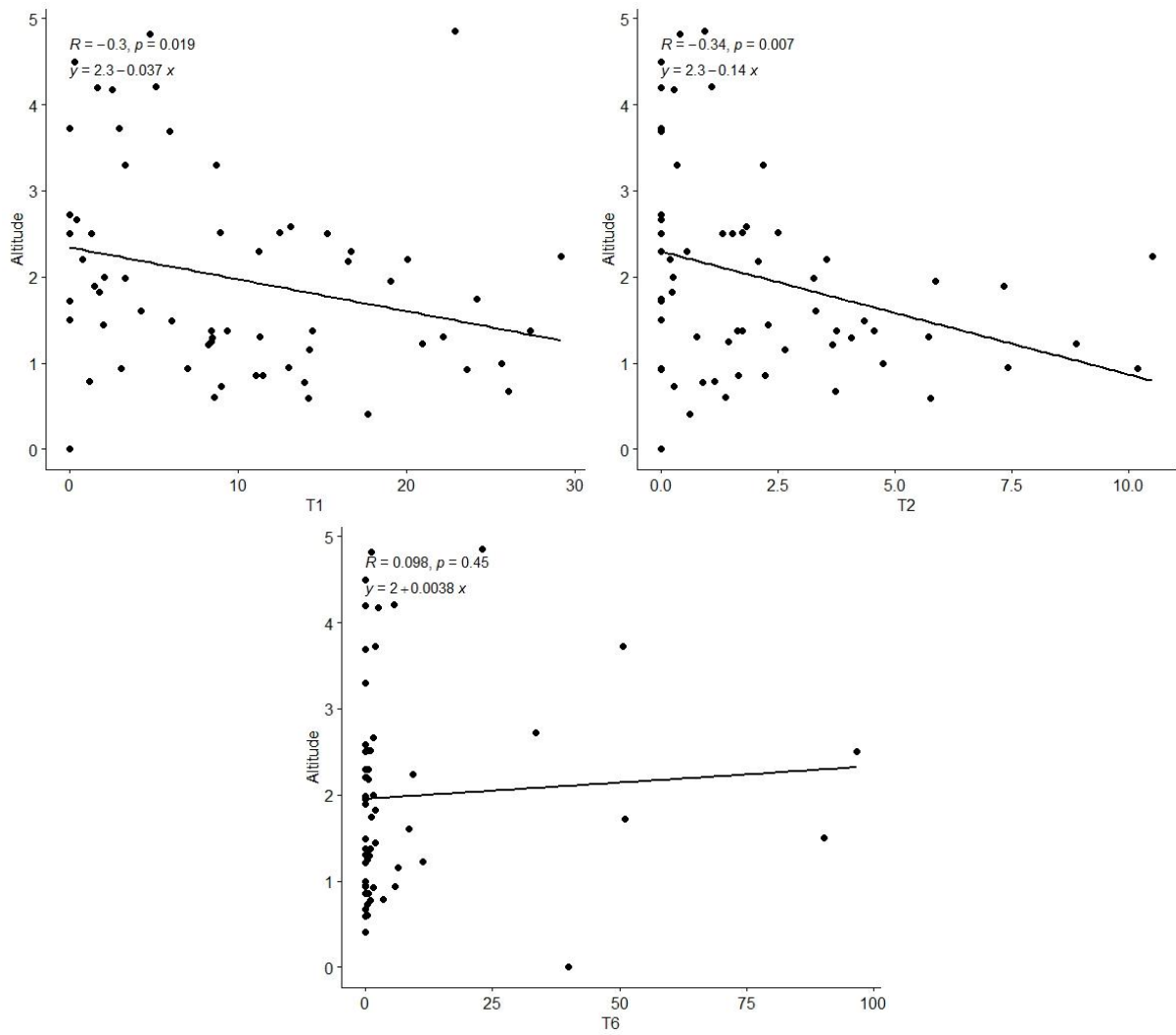
Supplementary Table III.S2: Geographic distribution of the three *Ammonia* spp. in the literature. The locations are classified in 5 types: saltmarsh, open marsh, lowland estuary (e.g., Vilaine), lowland estuary semi-enclosed (e.g., Vie), ria/fjord (e.g. Elorn).

	Site	Latitude	Longitude	Type	<i>Ammonia</i> spp.
Richirt et al., 2021	Authie	50°22'23.80"N	1°35'44.00"E	Lowland estuary	T6 (n=4)
	Biezelingse Ham	51°26'53.40"N	3°55'49.79"E	Lowland estuary	T6 (n=51)
	Ouireham	49°16'16.40"N	0°14'12.20"W	Lowland estuary semi-enclosed	T1 (n=1) T2 (n=1) T6 (n=5)
	Rade de Brest	48°24'13.10"N	4°21'16.00"W	Ria/fjord	T2 (n=2)
	Seine estuary	49°26'31.30"N	0°16'25.20"E	Lowland estuary	T6 (n=32)
	St. Vaast-La-Hougue	49°34'38.60"N	1°16'38.80"W	Open marsh	T1 (n=1) T2 (n=3)
	Veerse Meer	51°33'12.24"N	3°52'25.34"E	Lowland estuary	T2 (n=5) T6 (n=4)
Bird et al., 2020	Cromarty	57°40'45.59"N	04°02'28.12"W	Ria/fjord	T2 (n=1)
	Torry Bay	56°03'28.3"N	03°35'02.5"W	Lowland estuary	T6 (n=8)
	Cramond	55°58'54.2"N	03°17'56.5"W	Lowland estuary	T6 (n=52)
	Loch na Cille	55°57'36.00"N	05°41'24.00"W	Ria/fjord	T2 (n=13)
	Whiterock	54°29'05.42"N	05°39'12.58"W	Ria/fjord	T2 (n=18)
	Den Oever	52°56'24.8"N	05°01'30.6"E	Lowland estuary	T6 (n=1)
	Norfolk	52°49'02.41"N	00°21'46.16"E	Lowland estuary	T6 (n=30) T2 (n=1)
	Laugharne Castle	51°46'12.00"N	04°27'00.00"W	Lowland estuary	T6 (n=2)
	Cork	51°38'29.40"N	08°45'44.50"W	Lowland estuary	T1 (n=2) T2 (n=28)
	Cardiff	51°29'25.40"N	03°07'19.50"W	Lowland estuary	T6 (n=20)
	Dartmouth	50°21'04.84"N	03°34'11.33"W	Lowland estuary	Upper shore T2 (n=6)
					Mid shore T1 (n=2) T2 (n=12)
					Lower shore T1 (n=2) T2 (n=49)
Baie de l'Aiguillon	46°15'17.00"N	01°08'27.00"W	Lowland estuary	T6 (n=2)	
Bangor	53°14'02.41"N	04°07'04.26"W	Open marsh	T1 (n=5)	
Saad and Wade, 2016 – modified by Richirt et al., 2021	Barmouth	52°43'17.26"N	04°02'27.43"W	Lowland estuary	T1 (n=9) T6 (n=1)
	Barrow-in-Furness	54°05'24.16"N	03°14'29.61"W	Open marsh	T6 (n=9)
	Barton-upon-Humber	53°41'50.86"N	00°26'40.08"W	Lowland estuary	T6 (n=9)
	Brancaster Staithe	52°58'11.78"N	00°40'05.05"E	Saltmarsh	T2 (n=7)
	Braunton	51°05'55.09"N	04°09'52.15"W	Lowland estuary	T6 (n=10)
	Burnham Overy Staithe	52°58'06.76"N	00°40'05.08"E	Saltmarsh	T6 (n=10)
	Galmpton	50°23'31.53"N	03°34'31.15"W	Lowland estuary	T2 (n=4)
	Hambleton	53°52'40.15"N	02°57'52.46"W	Lowland estuary	T6 (n=2)
	Lymington	50°45'16.36"N	01°31'39.34"W	Lowland estuary semi-enclosed	T2 (n=8)
	Pembroke Dock	51°41'59.66"N	04°55'14.72"W	Lowland estuary	T6 (n=8)
	Pen Clawdd	51°38'36.28"N	04°06'20.18"W	Lowland estuary	T6 (n=10)
	Queenborough	51°25'01.47"N	00°44'21.15"W	Lowland estuary	T6 (n=11)
	Severn Beach	51°33'17.99"N	02°40'11.37"W	Lowland estuary	T6 (n=6)
	Shoreham-By-Sea	50°49'49.04"N	00°16'30.79"W	Lowland estuary semi-enclosed	T1 (n=1)
					T2 (n=7)
					T6 (n=2)
South Queensferry	55°59'34.28"N	03°24'38.18"W	Lowland estuary	T6 (n=6)	
St Osyth	51°47'54.83"N	01°03'50.32"W	Lowland estuary	T6 (n=9)	
Thornham	50°57'59.35"N	00°34'20.09"E	Open marsh	T6 (n=6)	



Supplementary Figure III.1 : Examples of individuals classified as undetermined. A: Auray-2019_2C(28); B: Auray-219_7(20); C: Auray-2020_1A(40); D: Auray-2020_1B(32); E: Auray-2020_6B(23); F: Auray-2020_6B(25); G: Auray-2020_7(30); H: Elorn_4(01); I: Vie-2018_7A(87); J: Vie-2018_9(09); K: Vilaine_1A(04); L: Vilaine_1A(12); M: Aulne_1(37); N: Aulne_1(02). Scale bar: 100µm

Chapter 3: Biogeographic distribution of three *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*; Foraminifera, Rhizaria) in estuaries of the French Atlantic coast.



Supplementary Figure III.2 : Relation between absolute elevation and the percentage of *Ammonia* spp. in the total assemblage.

Chapter 3: Biogeographic distribution of three *Ammonia* spp. (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*; Foraminifera, Rhizaria) in estuaries of the French Atlantic coast.

Chapter 4

Historical reconstruction of the arrival of the invasive species *Ammonia confertitesta* (Foraminifera) in the Loire estuary.

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Abstract

Recent molecular and morphological analyses have enabled to distinguish *Ammonia veneta*, *Ammonia aberdoveyensis* and *Ammonia confertitesta* within the former morphospecies *Ammonia tepida* (Eukaryotes, Rhizaria, Foraminifera). Several studies have suggested that among these species, *A. confertitesta* could be invasive on the European coasts, originating from East Asian coasts. In order to test this hypothesis, *Ammonia* spp. specimens from 57 samples in two long cores taken from the Brillantes mudflat (Loire estuary, France) were investigated. In both cores, only *A. aberdoveyensis* were observed in the oldest samples. The first record of *A. confertitesta* was observed in samples located at depth in sediments dating back several centuries (PV1: 2.50m; PV2: 1.50m). Upper in the core, many samples contained specimens of both species. Finally, the surface samples contained only *A. confertitesta*. These results firstly confirm the invasive character of the species *A. confertitesta*. Secondly, our data show that the introduction of this species into the Loire estuary took place several centuries ago, demonstrating that its introduction in North East Atlantic Ocean is much older than previously suggested. Finally, our data show that the gradual establishment of *A. confertitesta* in this area has gone with a decrease or even disappearance of the autochthonous species *A. aberdoveyensis*. This result suggests that these two species may have occupied the same ecological niche. It is possible that the intensification of long-distance shipping from the 17th century onwards caused the arrival of the exotic species *A. confertitesta*.

1. Introduction

For hundreds of years, humans have crossed the oceans for commercial, migratory or warlike purposes. In the course of these journeys, biological species have been transported voluntarily or not (Gollasch & Leppäkoski, 1999). Thus, when species develop outside their original distributional range due to direct or indirect anthropic activity, they are called introduced species. Some of these introduced species can proliferate and disrupt the functioning of ecosystems by causing the disappearance of native species (Grosholz, 2002), they are called invasive species. The introduction of certain species has had disastrous high consequences for ecosystems (e.g., *Caulerpa taxifolia* (Ceccherelli & Cinelli, 1999), *Crepidula fornicata* (Blanchard, 1995), *Sargassum muticum* (Belsher & Pommellec, 1988), *Codium fragile* (Provan et al., 2005)). Many vectors of introduction can be responsible for marine species invasions. Maritime transport has favoured the introduction of invasive species, particularly through fouling of ships (Godwin, 2003). Over the last two centuries, biological introductions due to shipping have increased for several reasons: shipping technologies have developed significantly, increasing the number of inter-continental exchanges, shortening the crossing time (and thus increasing the chances of survival of organisms during the journey), and the means of ballasting have changed (Mills et al., 1993). Indeed, the advent of metal-hulled ships and the use of ballast water to replace solid ballast have favoured the transport of species (e.g. the Japanese crab *Hemigrapsus sanguineus* (Breton et al., 2002), the razor clam *Ensis directus* (Tulp et al., 2010), the ctenophore *Mnemiopsis leidyi* (Costello et al., 2012)). In addition, digging canals at strategic locations (e.g. Suez Canal, Panama Canal) has allowed many species to move from their native range to colonise new area (Hewitt et al., 2006). In addition, aquaculture is also a significant vector of introduction. There are cases of voluntary introductions for aquaculture purposes, such as that of the Pacific oyster (*Crassostrea gigas*), which originated in Japan and was introduced to the European coast to replace the endemic European flat oyster (*Ostrea edulis*). But there are also non-voluntary introductions, by accompanying species, epibionts or endobionts (e.g., the algae *Sargassum muticum*, (Belsher & Pommellec, 1988) and *Undaria pinnatifida* (Peteiro, 2008); the mollusc *Crepidula fornicata* (Montaudouin & Sauriau, 1999)). Finally, aquariology can also be a vector of introduction. This was the case for *Caulerpa taxifolia*, an ornamental alga for aquaria, which was mistakenly released into the environment, with high consequences in the Mediterranean (Boudouresque et al., 1995). Thus, the vectors and consequences of introducing organisms such as fish, macro-algae and macrofauna are fairly well known (Gollasch & Leppäkoski, 1999; Arenas et al., 2006). However, introductions do not only concern these macro-organisms. Although meiofaunal cases of introduction are less well known, because their installation is more discreet due to their size (Blanchard, 1995), the meiofauna also undergoes species transport. In foraminifera (unicellular protists), several cases of introduction and invasion have been reported. This is for example the case of *Nonionella* sp. T1 along the Skagerrak coast (Polovodova-Asteman & Schönfeld, 2015), *Trochammina hadai* (McGann et al., 2012) on the American west coast and *Amphistegina* spp. (Langer & Mouanga, 2016) in the Mediterranean. Another example could be the species *A. confertitesta*. In the past, this pseudocryptic species was not recognised

separately as it was included in the morphological species *Ammonia tepida*. However, recently, molecular (Hayward et al., 2004) and then morphometric (Richirt et al., 2019) analyses have allowed the distinction of this species from the other morphologically related species of the same morphogroup. Among the different species recognised, *A. confertitesta* shows a distributional gap between its supposed area of origin (i.e. Asian coasts (Toyofuku et al., 2005; Hayward et al., 2004), and European coasts (Schweizer et al., 2011; Bird et al., 2020; Richirt et al., 2020)). For this reason, several authors have suggested that *A. confertitesta* is an invasive species (Pawlowski & Holzmann, 2007; Schweizer et al., 2011). Foraminifera have the advantage that their tests are preserved in the sediment. The study of long cores allows historical reconstruction and thus to know which species were present in the past (Barmawidjaja et al., 1995; Cearreta et al., 2013; Dolven et al., 2013; Francescangeli et al., 2016; Daniel et al., 2019). In the case of *A. confertitesta* putative invasion, historical reconstructions could be used first to verify the invasive nature of this species, then to define when this species appeared on the European coasts, and what the possible introduction vectors might have been. On the Atlantic coast, *A. confertitesta* is now dominant in several estuaries (e.g. the Loire (Thibault de Chanvalon et al., 2022), the Gironde (Pavard et al., 2021), the Vilaine (chapter 3)). The present study focuses on the Loire estuary. Located on the French coast, the Loire is one of the largest estuaries on the European Atlantic coast and it hosts a historically important harbour (Nantes). In this estuary, two long cores were collected in March 2015 from the Brillantes mudflat, located on the left bank in the middle part of the estuary. The foraminifera from these two cores were partly studied by Durand, 2017, but at that time the morphological distinction of the different *Ammonia* species was not yet possible. Thus, the objective of this chapter is to study the succession of the different *Ammonia* species (*A. veneta*, *A. aberdoveyensis* and *A. confertitesta*) in these two cores. This study will allow to verify whether *A. confertitesta* has indeed arrived in the Loire in the recent past and, if so, to determine the time of its appearance. Finally, in the case of a recent introduction, this study should provide information on its impact on the autochthonous species of the genus *Ammonia*.

2. Material and Methodes

2.1. The Brillantes mudflat

The Loire estuary is one of the largest estuaries on the Atlantic coast. Its catchment area covers 7470 km². The average annual flow of the river is 840 m³/s (Banque Hydro), varying from 140 m³/s during low water periods to 5,200 m³/s during flood periods. The estuary is about 60km long, from the mouth to the limit of saline intrusion (Office français de la biodiversité).

The Brillantes mudflat is located on the left bank of the estuary, near Paimboeuf. It is the largest mudflat in the estuary, covering 1350 hectares. The tidal range of this area is 6m (SHOM).

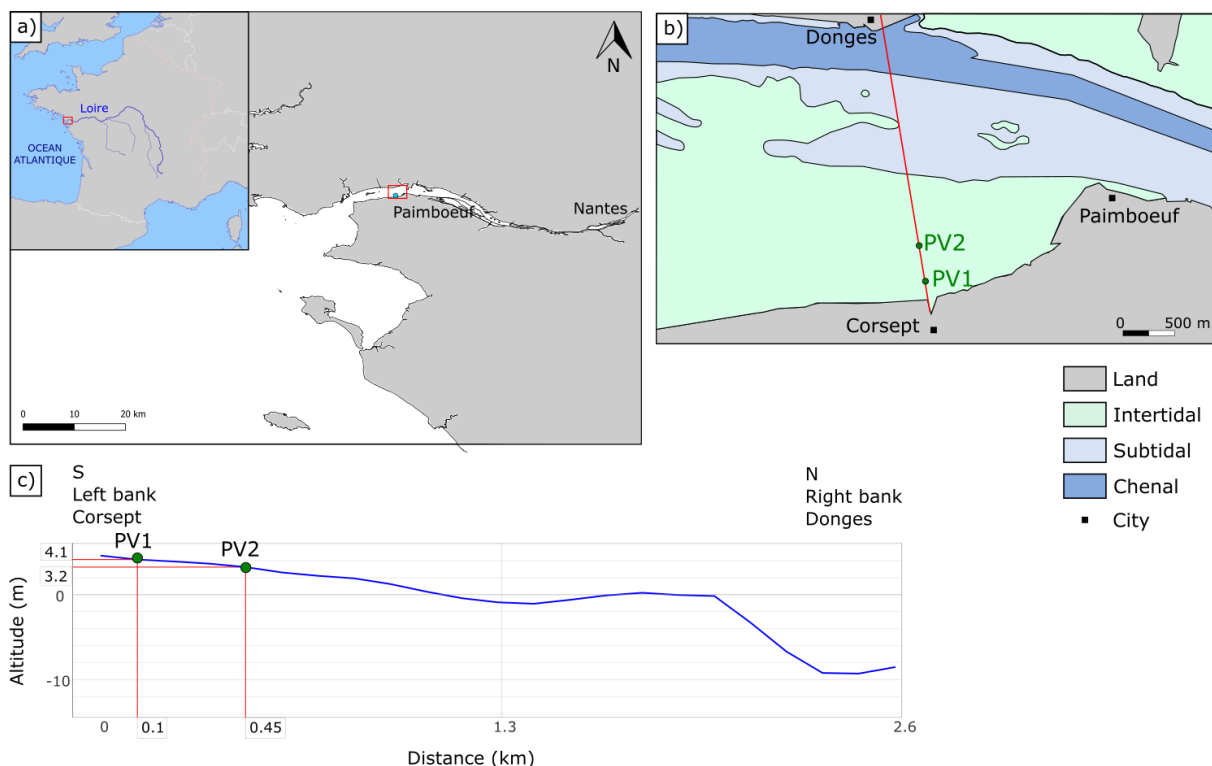


Figure IV.1 : a) Location of the sampling site. b) Location of the two sampled stations. c) Topographical profile linking the two sampled stations, to the left bank opposite. The blue line indicates the topographic profile.

2.2. Sampling

Sampling methods, grain size analyses and $^{210}\text{Pb}_{\text{xs}}$ analyses of the two cores are detailed in Durand (2017). Briefly, cores PV1 and PV2 (measuring 5.39m and 5.42m long respectively) were collected from the Brillantes mudflat in March 2015. Core PV1 ($47^{\circ}16'59.05''\text{N}$; $2^{\circ}3'47.63''\text{W}$) was located 150m and PV2 ($47^{\circ}17'10.07''\text{N}$; $2^{\circ}3'50.26''\text{W}$) 450m from the shore (Figure IV.1). They were collected using a percussion corer. In addition, two 40cm deep interface cores were taken by hand in the vicinity of each of the long cores for analysis of the $^{210}\text{Pb}_{\text{xs}}$ profiles. The long cores were cut into 1m sections and placed in cold storage ($\sim 4^{\circ}\text{C}$).

2.3. Sedimentological analyses

Durand (2017) produced high resolution photographs to describe the sedimentology of the cores studied. These descriptions are accompanied by high-definition X-ray images, allowing the internal organisation of the sediments to be visualised. In addition, grain size analysis by laser diffraction was carried out on sediment that had been decarbonated (with a 30% hydrochloric acid solution) using a Malvern Mastersizer 3000 laser diffractometer. The analytical techniques used are described in more detail in Durand (2017). These results will be used to relate the *Ammonia* species found to their sedimentary environment.

2.4. Reconstruction of the chronological framework

Durand, 2017 developed a chronological framework by combining measurements of ^{210}Pb activity in the interface cores and some ^{14}C dating in the long cores. The first method is based on the measurement of excess ^{210}Pb (noted $^{210}\text{Pb}_{\text{xs}}$). The ^{210}Pb isotope, with a half-life of 22.3 years, is used to determine the sedimentation rate for the most recent part of the core, over a period of about 100 years. The steps for normalising the $^{210}\text{Pb}_{\text{xs}}$ data are detailed in Durand, 2017. Following these analyses on the interface cores, a sediment accumulation rate (SAR) was calculated for each core. These sedimentation rates were then extrapolated over the entire length of the long cores, to obtain an estimate of the age of the deeper sedimentary levels. Since such an extrapolation carries significant risks (of changes in sedimentation rates, or sedimentary gaps), it is recommended to validate the extrapolation of the age model obtained from the $^{210}\text{Pb}_{\text{xs}}$ analysis with independent chronological measurements (Appleby, 2001). Thus, five ^{14}C dates were measured for core PV2 and only one for PV1. Dated were performed on plant debris and total organic carbon.

2.5. *Ammonia* spp. morphometry

For this study, 31 samples of 1cm thickness were taken in core PV1, distributed between the surface and 4.00m depth. For core PV2, 21 samples, distributed between the surface and 2.33m depth, were analysed and complement the work of Durand 2017. Each sample was carefully washed on 125 μm mesh sieves. The choice to use only the >125 μm fraction is motivated by the impossibility to identify *Ammonia* individuals to species level when they are too small. The fraction >125 μm was dried and analysed under a Leica MZ16 stereomicroscope. Individuals belonging to the genus *Ammonia* were collected and placed in Chapman cells. These individuals were then arranged on stubs and all were analysed with a scanning electron microscope (Hitashi TM4000). For each individual, an image of the whole specimen on the dorsal side and a detailed image of the penultimate chamber (at x1000 magnification) were acquired. On the basis of these images, specimens were determined down to species level using the criteria of Richirt et al., 2019 (Figure IV.2). Indeed, *A. aberdoveyensis* can be distinguished from *A. veneta* and *A. confertitesta* by their smaller pore diameter (<1.4 μm). Secondly, *A. veneta* have raised sutures, whereas the sutures are rather smooth in *A. confertitesta* (see chapter 3 for further explanation). To get an idea of the contribution of the *Ammonia* spp. group within the total assemblage, for some levels (13 for PV1 and 7 for PV2) all foraminifera were picked, identified and counted.

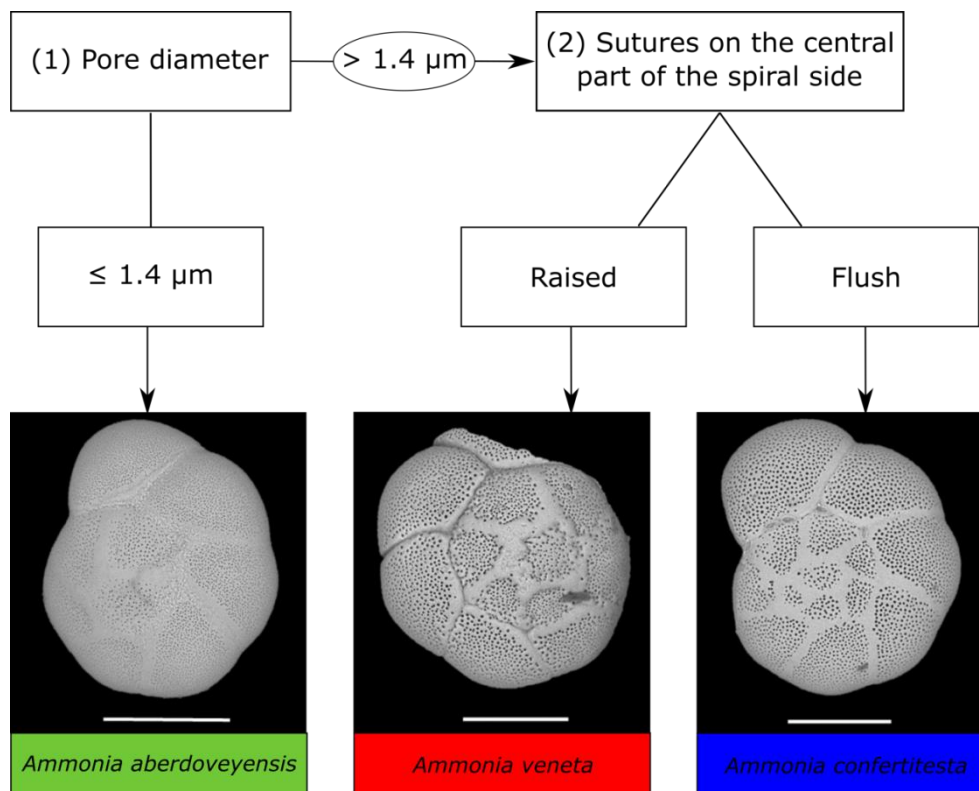


Figure IV.2 : Determination of the three *Ammonia* species according to Richirt et al., 2019. The specimens shown in this figure are from samples analysed in this study. The scale corresponds to 100μm.

3. Results

3.1 Sedimentary analysis

The sedimentary study of the cores was carried out by Durand 2017. Its results are summarised and partially reinterpreted here.

In core PV1 (Figure IV.3), taken from the upper part of the mudflat, the base of the core (540-360 cm) showed an alternation of sands and mud/silt (Fig. 3: facies Ff). The absence of meio- and macrobenthos shells (including benthic foraminifera) suggests a marsh depositional environment, while the sandy levels are interpreted as flood deposits. Between 360 and 100 cm, there was an alternation of two facies: 1) fine sands, containing shell debris and sea urchin spicules (facies Fd), and 2) black muds, dominated by silts, containing shell debris and burrows (facies Fe). This alternation has been interpreted as the deposition of the lower to middle part of a mudflat (near the channel). Finally, the upper metre consisted of silty clays (facies Fe). This part of the core should correspond to a deposit in the middle and upper part of the mudflat. The levels sampled in our study were mainly located in the upper 3.6 m of the core (facies Fd and Fe), corresponding to a deposit on an mudflat. Only two samples were taken in the marsh level (facies Ff).

Core PV2 (Figure IV.3), taken 350 m further down the channel (about 0.9 m lower), also contained a marsh facies at its base (540-330 cm) (facies Ff), interspersed with sandy levels, indicative of flooding events, both

without marine fossils. Then there was a succession of gravel facies (Fa, between 330 and 270 cm) and a facies of fine sands (Fc, between 270 and 230 cm). This succession has been interpreted as the establishment of a tidal channel. Between 230 and 40cm, the sediment was composed of an alternation of a facies of fine sands with shell debris (facies Fd), and black silty muds with shell debris (facies Fe). This alternation is interpreted as the lower to middle part of a mudflat. Finally, the last 40 cm was made up of silty clays (facies Fe), revealing an environment of the middle to upper part of the intertidal mudflat (Fe). The levels sampled in our study were in the upper 2.35 m, on facies Fd.

Both cores were marked by an erosion surface, located at 360 cm in core PV1 and 330 cm in core PV2 (Figure IV.3). This surface appears to represent the transition from a marsh environment to a tidal channel environment (core PV2), or even to a mudflat environment (core PV1).

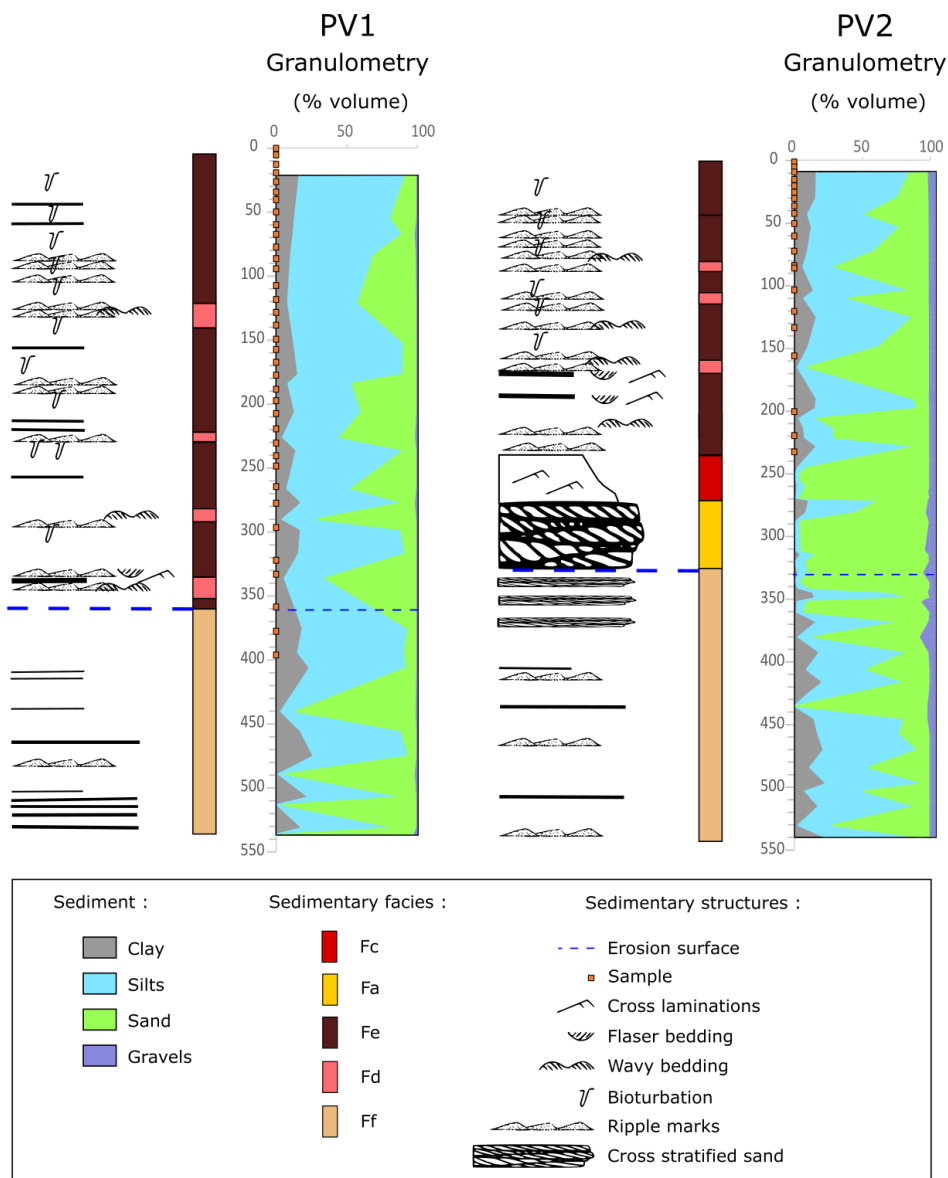


Figure IV.3 : Grain size distribution according to clay (0-4 μ m), silt (4-63 μ m), sand (63 μ m - 2mm), gravel (>2mm). The orange squares represent the levels sampled for our study.

3.1 Chronology

Profiles of $^{210}\text{Pb}_{\text{xs}}$ were obtained from the first 30-35 cm of sediment in the interface cores.

In core PV1, after relatively stable $^{210}\text{Pb}_{\text{xs}}$ values in the upper 3 cm, a regular decrease from 100 to 50 mBq g^{-1} was observed down to 20 cm depth. In the deeper sediments (20 to 35 cm) the $^{210}\text{Pb}_{\text{xs}}$ value remained around 50 mBq g^{-1} .

In core PV2, a linear increase in $^{210}\text{Pb}_{\text{xs}}$ was observed in the first 10 cm, from 100 to 50 mBq g^{-1} . Beyond that, values varied from 40 to 60 mBq g^{-1} , with no significant trend.

Based on the decreasing profiles, between 2 and 20 cm for core PV1 and between 0 and 10 cm for core PV2, sedimentation rates (SAR) of ~ 0.7 cm/year for PV1 and ~ 0.3 cm/year for PV2 can be estimated (Figure IV.3).

For core PV1, a single ^{14}C dating was carried out, on plant debris taken from the 538-539 cm level. The result of this dating, 250 ± 30 cal BP (Table IV.1), would correspond to a sedimentation rate of ~ 2.2 cm/year, three times higher than that calculated from the $^{210}\text{Pb}_{\text{xs}}$ analyses. For this reason, Durand (2017) considered this dating to be unreliable, and discarded it.

For core PV2, five ^{14}C dates were performed (Table IV.1, Figure IV.4), on total organic carbon and plant samples, taken at 109-110, 189-190, 330.5-331.5, 424.5-425.5 and 538.5-539.5 cm depth. These dates gave ages of 890 ± 30 , 126 ± 30 , 1295 ± 30 , 75 ± 30 and 2880 ± 30 cal BP, respectively.

Two of these dates (126 ± 30 and 75 ± 30) gave anomalously young ages and sedimentation rates (of 1.5 and 5.6 cm/year, respectively) that do not correspond at all to the sedimentation rates determined based on $^{210}\text{Pb}_{\text{xs}}$ profiles. Durand (2017) suspected possible contamination and therefore discarded these dates. The three remaining dates appeared consistent with the sedimentation rate calculated from $^{210}\text{Pb}_{\text{xs}}$ (0.7 cm/year), while resulting in lower sedimentation rates of 0.11, 0.55, and 0.12 cm/year, respectively.

For core PV2, the combination of sedimentation rates calculated on the basis of the $^{210}\text{Pb}_{\text{xs}}$ data (0.3 cm/year) and the three retained radiocarbon dates allowed estimating fairly wide age ranges for the different levels of the core (Figure IV.5). It seems that the boundary between facies Ff (marsh environment) and Fa (tidal channel), at a depth of 3.30 m, is between 1150 and 1300 years old. The transition between facies Fc (tidal channel) and Fd/Fe (mudflat), at 2.30 m, would be between 850 and 1100 years old. For core PV1, higher up on the mudflat, where reliable radiocarbon dates were not available, extrapolating the sedimentation rate determined for the upper 35 cm based on $^{210}\text{Pb}_{\text{xs}}$ would give an age of 500 years for the facies transition from Ff (swamp) to Fd/Fe (mudflat), at 360 cm depth.

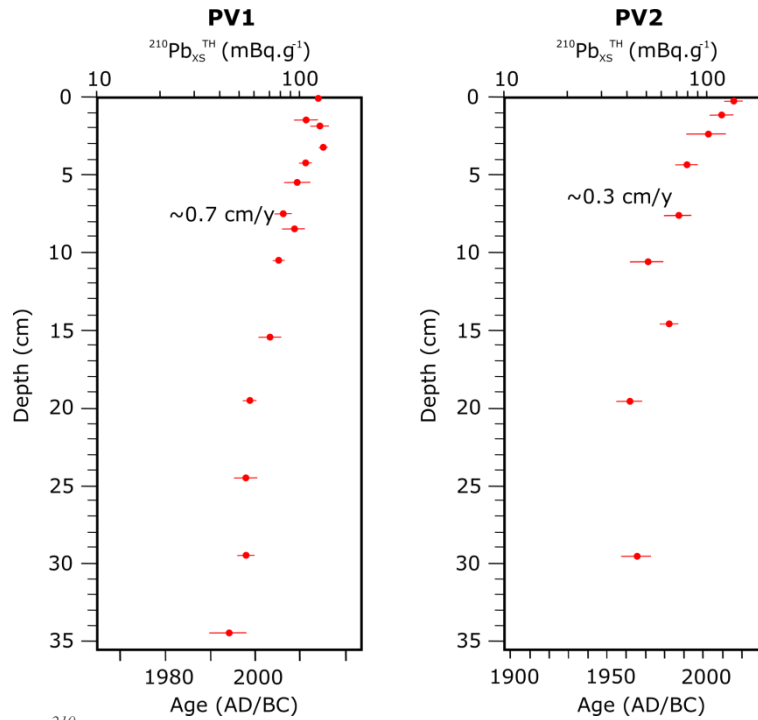


Figure IV.4 : Decay profile of $^{210}\text{Pb}_{\text{xs}}$ in the upper 30-35 cm of interface cores taken from the same location as PV1 and PV2 long cores. From Durand 2017.

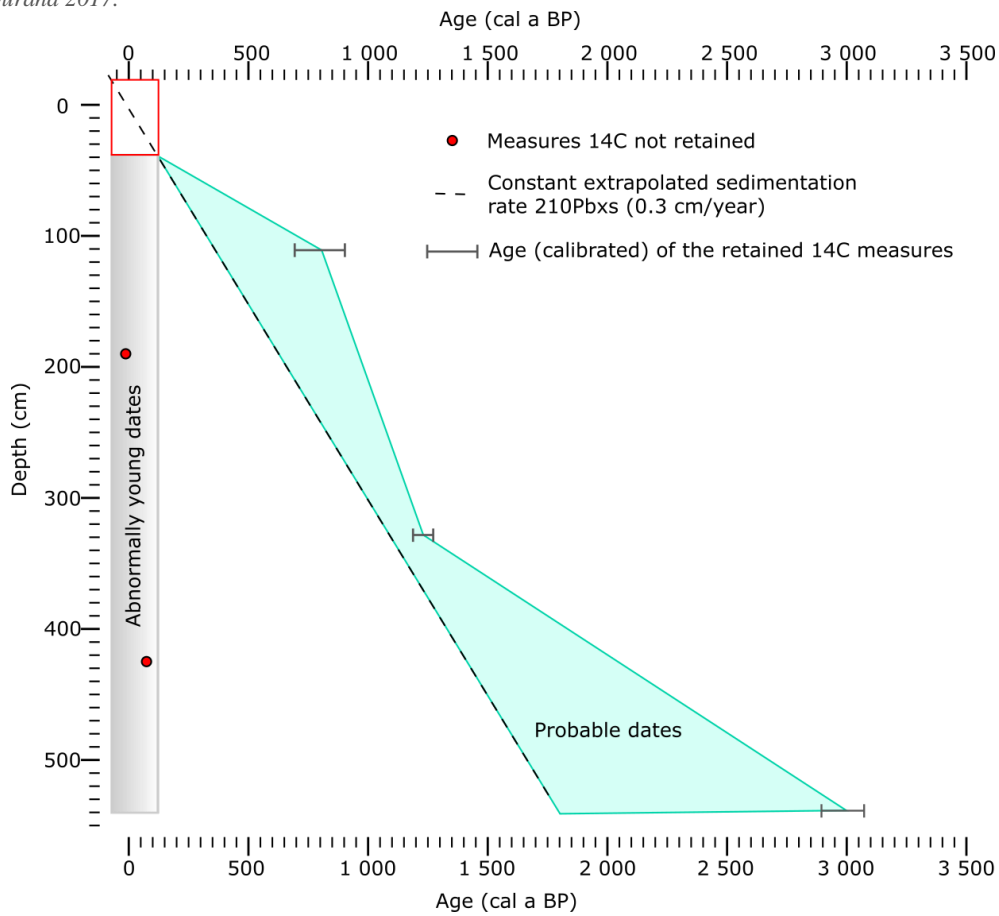


Figure IV.5 : Radiocarbon dates measured on the retained (grey bar) and non-retained (red dot) PV2 core as well as the sedimentation rate from the $^{210}\text{Pb}_{\text{xs}}$ decay profile (dotted line) (after Durand, 2017).

Table IV.1: Radiocarbon dates, after Durand (2017). Selected dates are shown in black and those discarded in grey. The table gives both radiocarbon ages (^{14}C AMS), expressed in BP (Before Present) radiocarbon years, and calibrated ages, in BP calendar years. TOC: Total Organic Carbon. The 2σ probability of the calibrated ages is specified in brackets.

Core	Depth of the sample (cm)	Age ^{14}C AMS (BP radiocarbon years)	Calibrated ages (2σ) (cal BP)	Dated material
PV1	538 - 539	250 \pm 30		Plant debris
PV2	109 - 110	890 \pm 30	834 - 733 (0.626) 909 - 843 (0.374)	Plant debris
PV2	189 - 190	126,26 \pm 0,36 pMC		Plant debris
PV2	330,5 - 331,5	1 295 \pm 30	1 287 - 1 218 (0.660) 1 215 - 1 181 (0.340)	TOC
PV2	424,5 - 425	75 \pm 30		Plant debris
PV2	538,5 - 539,5	2 880 \pm 30	3 079 - 2 922 (>0.900)	TOC

3.3 Morphological analysis of *Ammonia aberdoveyensis* and *Ammonia confertitesta*.

For a dozen levels per core (13 for PV1 and 7 for PV2), all foraminifera in the sample were counted (Table IV.2). These counts give an idea of the contribution of *Ammonia* spp. to the total foraminiferal assemblage. For core PV1, *Ammonia* spp. constituted 3-30% of the community in the deepest levels (between 189 and 94 cm), and contributed 25-70% from 90cm to the surface. For core PV2, *Ammonia* spp. made up 10-30% of the community in 103-9 cm, and 39-67% in the top five centimetres.

For each level studied, all *Ammonia* spp. individuals were extracted. A maximum of 50 specimens were analysed under SEM if the numbers of cells were sufficient. The number of *A. veneta*, *A. aberdoveyensis* and *A. confertitesta* individuals identified for each studied level is presented in Table IV.2 and Figure IV.6.

In core PV1, between 0 and 173 individuals of *Ammonia* spp. were found per studied level. In several levels, no *Ammonia* spp. were observed. The five deepest levels (264-333 cm) contained only *A. aberdoveyensis*. At levels 248-249cm and 207-208cm, a single individual of *A. confertitesta* was observed in an assemblage dominated by *A. aberdoveyensis*. From level 166-167cm the number of *A. confertitesta* increased to 10-20 individuals per sample. From level 149-150cm, *A. confertitesta* were systematically more numerous than *A. aberdoveyensis*. From the 77-78cm level onwards, only *A. confertitesta* were present. Concerning *A. veneta*, only two typical individuals were observed, at levels 219-220cm and 128-129cm.

In core PV2, between 1 and 106 *Ammonia* spp. were found per studied level. The three deepest levels (233-200 cm) contained only *A. aberdoveyensis*. Then, from level 155.5-156.5cm, the samples contained a mixture of both species, first (156.5-42 cm) with a strong dominance of *A. aberdoveyensis*, then (37-9 cm) with a dominance of *A. confertitesta*. In the last two samples (5-6 cm and 1-2 cm) only *A. confertitesta* was found. Concerning *A. veneta*, a typical individual was observed, in the 133-134 cm level.

Thus, a very similar trend was observed in both cores. The deepest samples had only *A. aberdoveyensis* and then the samples contained a mixture of both species. The proportion of *A. aberdoveyensis* decreased until only *A. confertitesta* was present in the surface samples (surface-6 cm).

In terms of chronology, the first *A. confertitesta* (levels 248-249cm and 207-208cm) for core PV1 were observed in samples with an estimated age of 300 to 350 years. The last individuals of *A. aberdoveyensis* were observed in the 86-87 cm level, with an estimated age of about 125 years. In core PV2, the first sample containing *A. confertitesta* (155.5-156.5 cm) has an estimated age of 450-850 years. *A. aberdoveyensis* were present down to the 12.5-13.5cm level, with an estimated age of about 40 years.

Table IV.2: List of the different levels analysed and number of individuals identified.

Depth (cm)	PV1							PV2							
	Total number of foraminifera in the sample	Number of <i>Ammonia</i> spp. in the sample	% of <i>Ammonia</i> spp. in the sample	Number of <i>Ammonia</i> spp. identified	<i>Ammonia veneta</i>	<i>Ammonia aberdoveyensis</i>	<i>Ammonia confertitesta</i>	Depth (cm)	Total number of foraminifera in the sample	Number of <i>Ammonia</i> spp. in the sample	% of <i>Ammonia</i> spp. in the sample	Number of <i>Ammonia</i> spp. identified	<i>Ammonia veneta</i>	<i>Ammonia aberdoveyensis</i>	<i>Ammonia confertitesta</i>
0-1	245	173	71	18	0	0	18	1-2	31	12	39	12	0	0	12
12.5-13.5	57	20	35	6	0	0	6	5-6	6	4	67	4	0	0	4
26-27	28	7	25	6	0	0	6	9-10	64	17	27	14	0	6	8
34-35	12	6	50	6	0	0	6	15-16	15	2	13	2	0	0	2
49.5-50.5	39	18	46	13	0	0	13	20-21	50	2	4	2	0	2	0
77-78		0		0	0	0	0	25-26	46	5	11	5	0	1	4
86-87		15		15	0	4	11	30-31	103	7	7	6	0	3	3
94-95	607	17	3	14	0	2	12	36-37	38	12	32	7	0	0	7
107-108		23		23	0	0	23	42-43	201	38	19	26	0	19	7
118-119		5		5	0	0	5	50-51	238	26	11	23	0	20	3
128-129		30		30	1	13	16	60-61	494	106	21	51	0	40	11
138-139	45	13	29	11	0	1	10	72-73	157	27	17	20	0	16	4
149-150		5		5	0	1	4	83,5-84,5	437	75	8	35	0	32	3
157-158		0		0	0	0	0	85,5-86,5	207	40	19	22	0	16	6
167-168		14		14	0	8	6	103-104	50	10	14	7	0	7	0
177-178		0		0	0	0	0	120-121		37		37	0	32	5
188-189	270	24	9	9	0	9	0	133-134		36		36	1	32	3
197-198		0		0	0	0	0	155.5-156.5		47		47	0	44	3
207-208		31		31	0	30	1	200-201		1		1	0	1	0
219-220		1		1	1	0	0	219-220		32		32	0	32	0
230-231		1		1	0	1	0	232-233		15		15	0	15	0
240-241		0		0	0	0	0								
248-249		5		5	0	4	1								
264-265		8		8	0	8	0								
277-278		7		7	0	7	0								
296-297		6		6	0	6	0								
321.5-322.5		2		2	0	2	0								

Chapter 4: Historical reconstruction of the arrival of the invasive species *Ammonia confertitesta* (Foraminifera) in the Loire estuary.

332.5-333.5	2		2	0	2	0								
358-359	0		0	0	0	0								
377-378	0		0	0	0	0								
395.5-396.5	0		0	0	0	0								

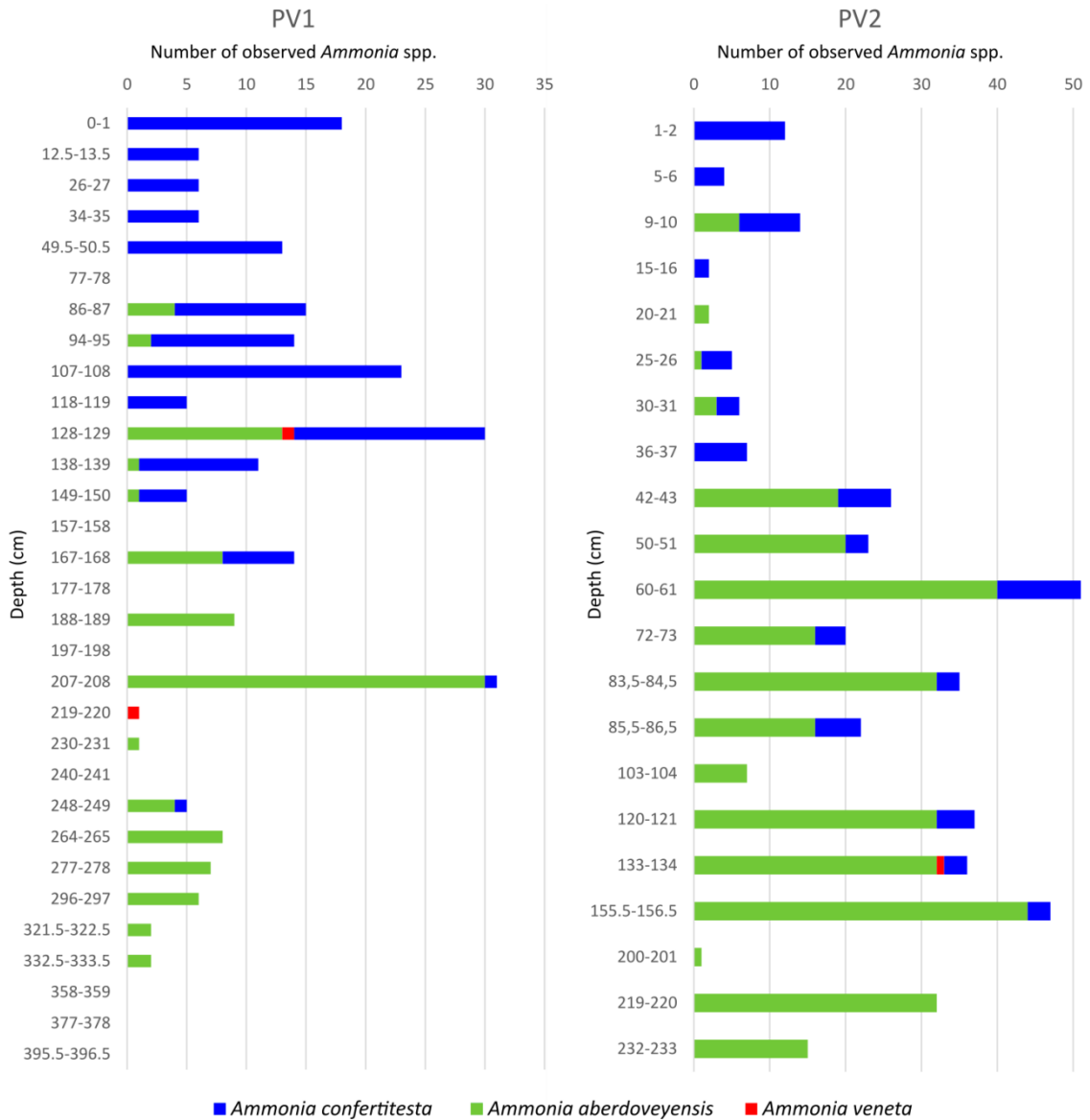


Figure IV.6 : Number of observations of the three *Ammonia* species under SEM, as a function of depth in the cores.

4. Discussion

4.1. Brief history of harbour activities in the study area

Before the 14th century, the harbour of Nantes was open to a limited numbers of international trade. However, from this period onwards, harbour activities increased, particularly towards Spain, England and Ireland (Le Maho, 2001; Bouédec, 2009). From the 17th century onwards, trade across the Atlantic developed, towards Newfoundland and the West Indies. From 1665 the French East India Company developed trade to China and India (Martin, 1926). In the 18th century, maritime traffic intensified further and Nantes became one of the most important harbours in Europe (Bouille, 1972). At this time, the harbour of Paimboeuf, which served as an fore-port for Nantes, reached its peak. Indeed, large ships could not sail up the Loire to Nantes, so they stopped at Paimboeuf to load and unload goods which were then transported to Nantes on barges (Bouédec, 2009). This harbour activity declined from the middle of the 19th century, after the development of the harbour of Saint Nazaire located 25km downstream.

4.2. Brief history of hydromorphological changes in the Loire Estuary

For centuries, numerous structures have been built on the banks of the Loire estuary to regulate its course. As early as the 9th century, dykes were built to protect the banks from flooding (Dupont & Moatar, 2016). In the 19th and 20th centuries, major development work was undertaken, which considerably modified the morphology of the estuary, to meet the growing needs of navigation (Sanchez et al., 2000). During this period, the size of ships increased considerably, so that the water depth had to be sufficient to allow the passage of large commercial ships. The river bed was narrowed by filling in the side arms (Bona & Prud'homme Lacroix, 2009, Bona et al., 2010), and a channel was dug (in 1930, Dupain, 1981), thus eliminating many of the islands that had been scattered along the estuary. Figure IV.7 illustrates the above-mentioned changes in hydromorphology between the 19th century and today.

All these works have strongly modified the hydro-sedimentary dynamics of the estuary in general, and the Brillantes mudflat has also been influenced by these modifications. Indeed, Durand 2017 argued that the implementation of the channel on the right bank concentrated the tidal energy along this bank and favoured the fattening of the Brillantes mudflat. As a result of these developments, the dynamic tide penetration increased from 63km from the mouth in 1903 to 100km in 1992 and the salt tide from 38km in 1948 to 70km in 1991 (Sanchez et al., 2000). This marination of the estuary has been most probably accompanied by changes in fauna, allowing fully marine species to establish themselves further upstream in the estuary.

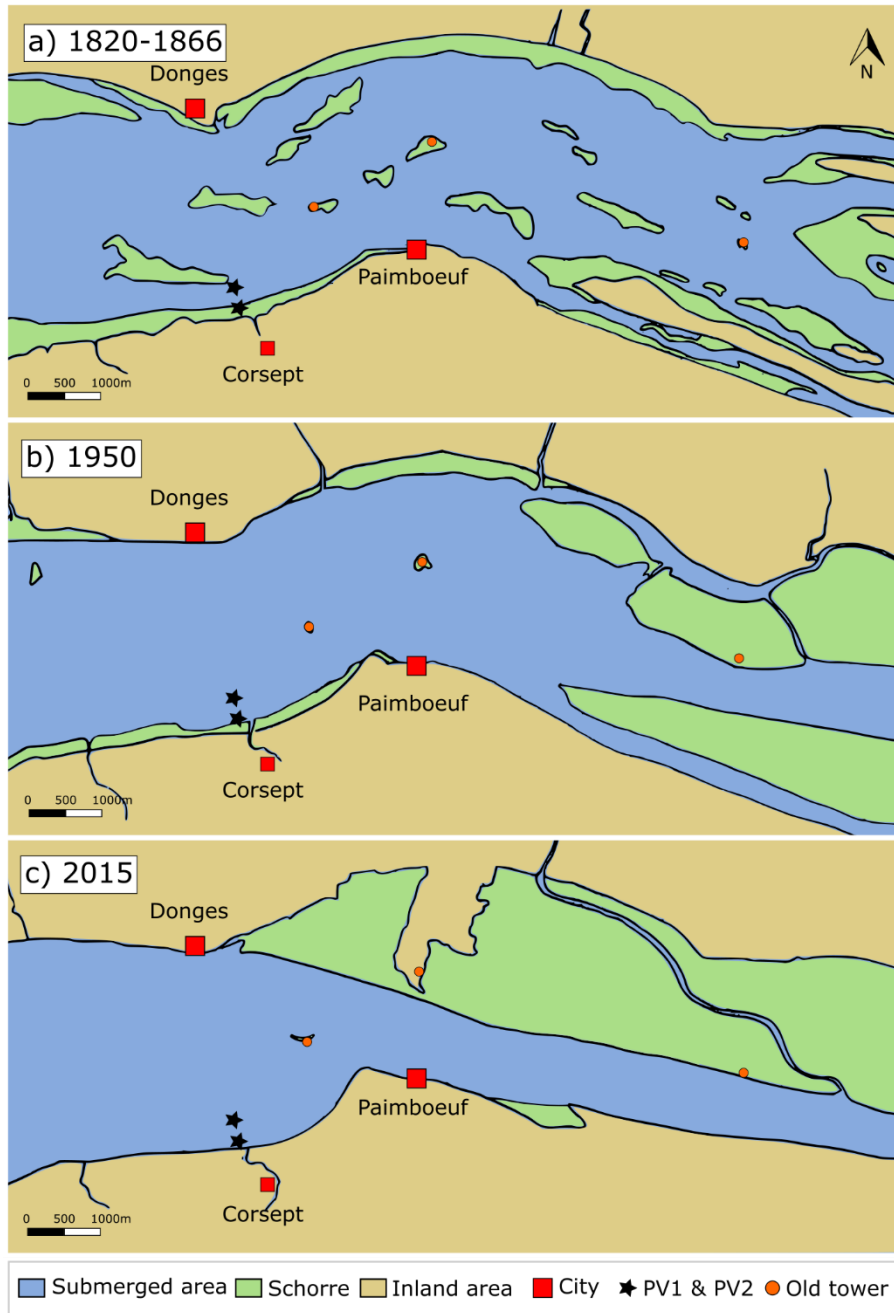


Figure IV.7 : Evolution of the morphology of the study area between the 19th and 21st centuries. The representations are based on maps from a) 1820-1866; b) 1950; c) current map (source: IGN). The towns are indicated by a red square, the study sites by a black star and the orange dots indicate former lighthouses which serve as geographical references.

4.3. Limitations of the age model

Our age estimates are based on a combination of sedimentation rates determined from $^{210}\text{Pb}_{\text{xs}}$ profiles (for the upper 35 cm of each of the two sites) and six radiocarbon dates, half of which were considered unreliable. For core PV2, the three radiocarbon dates considered reliable give ages greater than those determined by extrapolation of the $^{210}\text{Pb}_{\text{xs}}$ data, although of the same order of magnitude. In core PV2, this allows us to give

a range for the probable age of each level (Figure IV.4). For core PV1, for which no reliable radiocarbon dates are available, our age estimates are necessarily based only on the $^{210}\text{Pb}_{\text{xs}}$ profiles in the upper 35 cm.

Few studies based on the dating of recent ^{210}Pb sediments have been carried out in estuarine environments (Andersen, 2017). This is because these environments are very dynamic and the sedimentary deposits are often heavily reworked. In addition, mudflats often exhibit strong bioturbation activity which can cause non-linearities in the record (Benninger et al., 1979). It is therefore difficult to obtain continuous sediment records in such environments. Ways to prevent these difficulties are detailed in Andersen, 2017. Briefly, certain conditions must be considered: fine grain size and limited bioturbation. In our case, the upper 40 cm of the cores show fine grain size with sediments consisting of more than 50% silt. Analysis of the Durand 2017 X-ray images reveals traces of limited bioturbation.

All our age estimates for the deeper parts of the cores are largely based on extrapolation of the sedimentation rates obtained for the upper 35 cm. Given the morphological and sedimentary changes that have taken place over the last two centuries at the study site, the rate of sediment accumulation is likely to have varied substantially over time, potentially leading to significant over- or underestimates of the true age of the sediment. Sedimentary gaps, caused by erosive events, would lead to significant underestimations of the age of deeper levels. However, HD photographs and rX images from Durand, 2017 do not show significant erosive contacts in the study area (Figure iv-3, Durand, 2017). Despite this, we cannot completely eliminate the presence of sedimentary gaps, leading to an underestimation of the age of the deeper environments.

In general, it can be expected that on the lower part of the mudflat, where the hydrodynamic energy is still high (at least temporarily during flooding, as shown by the periodic deposition of the facies Fd sand layer, see Figure IV.2), the rate of sedimentation is lower than at the top of the mudflat, where the hydrodynamic energy is less important. This difference is corroborated by the sedimentation rates obtained for the upper 35 cm of our two cores, 0.7 cm/year for core PV1, at the top of the mudflat, and 0.3 cm/year for core PV2, 0.9 m lower down the mudflat.

As the depositional environment at our two sites has become progressively shallower over time (in both cores there is an evolution from a 'low mudflat' environment to an 'intermediate/upper mudflat' environment), an increase in sedimentation rate over time can be expected. The sedimentation rates obtained for the upper 35 cm of both cores should therefore correspond to a maximum rate. If sedimentation rates have been lower in the past, as might be expected, this would mean that our age estimates for the deeper levels are underestimated.

In summary, the age model of this study does have several limitations: (1) bioturbation activity in the upper part of the core, (2) a sedimentation rate that may have increased during core deposition, (3) potential erosive episodes, (4) several inconsistent ^{14}C measurements attributed to contamination. Thus, the age model in this study must be taken with great caution. However, limitations (2) and (3) would lead to an underestimation of

the age of the deeper core levels. Our age estimates should therefore correspond to the minimum age of the levels studied.

4.4. Temporal evolution of *Ammonia aberdoveyensis* and *Ammonia confertitesta* in the Brillantes mudflat

Currently, the living foraminiferal community of the Loire mudflats are mainly composed of three taxa: *Ammonia* spp., *Haynesina germanica* and *Cribrorhynchium excavatum* (called *Elphidium selseyense* in this thesis) (Mojtahid et al., 2016). Of these three species, *Ammonia* spp. largely dominated the communities (83% for our station PV1 and 84% for our station PV2) in the >150 μ m fraction in surface sediment. In view of recent taxonomic advances, these *Ammonia* spp. individuals were re-identified and it appears that in the formerly identified *A. tepida*, *A. confertitesta* was largely dominant (>95% of *Ammonia* spp.) accompanied by a few specimens of *A. veneta* (Richirt, personal communication). No specimens of *A. aberdoveyensis* were identified. Since then, Thibault de Chanvalon et al., 2022 have confirmed that *A. confertitesta* is the predominant *Ammonia* species in the Brillantes mudflat today. These data therefore provide information on the current conditions of the mudflat: *A. confertitesta* is the dominant foraminifera species, with highly variable densities and *A. aberdoveyensis* is not observed.

In our samples, the contribution of *Ammonia* spp. to the total assemblage was lower than in the surface samples of Mojtahid et al. (2016). However, this contribution increased towards the top of the core (~70% for PV1 and ~40% for PV2). Regarding the specific analysis of *Ammonia* spp. several trends could be observed in both cores. Firstly, both cores show an arrival of *A. confertitesta*, which was not observed in the deeper levels. Secondly, there is a progressive decrease in the proportion of *A. aberdoveyensis*, until they disappear in the levels closer to the surface. This decrease is accompanied by a progressive and proportional increase of *A. confertitesta*. In both cores, the first observations of *A. confertitesta* are from sediments with age estimates of at least several centuries.

However, the estimated dates of the first observations of *A. confertitesta* show substantial differences between the two cores. In core PV1, the first *A. confertitesta* was observed in the 248-249 cm level, with an estimated minimum age of 300-350 years. In core PV2, it is in the 155.5-156.5 cm level, with a minimum age estimated to be 600-900 years. In addition, the disappearance period of *A. aberdoveyensis* individuals, which is much more recent (and therefore with a potentially lower age estimation error), differs between the two cores: it is dated at about 125 years in PV1 against 40 years in PV2.

The most likely explanation for these differences is given by the reliability limitations of the age model, which tends to underestimate the age of lower levels in the core, apparently differently for the two cores.

However, the spatial difference between these two cores could also play a role in explaining these differences. Indeed, these two cores are located 350 m apart along a transect on the mudflat. Core PV2 is

located 0.9 m lower on the mudflat than PV1 (estimated current elevations 3.2m and 4.1m respectively, see Figure IV.1). Organisms at the PV1 core site are presently subjected to a longer emersion time than those at PV2. Several studies have suggested that this factor is important in the distribution of foraminifera (e.g., Francescangeli et al., 2017; Fouet et al., 2022; Jorissen et al., 2022). In a study of different *Ammonia* species in surface sediments of estuarine mudflats in the same geographical area (French Atlantic coast) it was indeed observed a slight preference of *A. veneta* and *A. aberdoveyensis* for the lowest part of the mudflat (chapter 3). It is conceivable that the top of the mudflat is more favourable to *A. confertitesta*, while the bottom of the mudflat could be more favourable to *A. aberdoveyensis*. It therefore appears that the ecological preferences of the species could partially explain the chronological differences observed between the two cores. This hypothesis is illustrated in Figure IV.8. The diagram shows the state of the mudflat before and after fattening (explained in section 4.2.). During this change, core PV1 changes from a low mudflat environment to a high mudflat environment and core PV2 changes from a subtidal environment to a low mudflat environment. Thus, the optimal area for *A. aberdoveyensis* would be concentrated on core PV1 before the mudflat is fattened, and then on PV2. This would explain a later arrival of *A. confertitesta* and a later disappearance of *A. aberdoveyensis* in core PV2, lower on the mudflat.

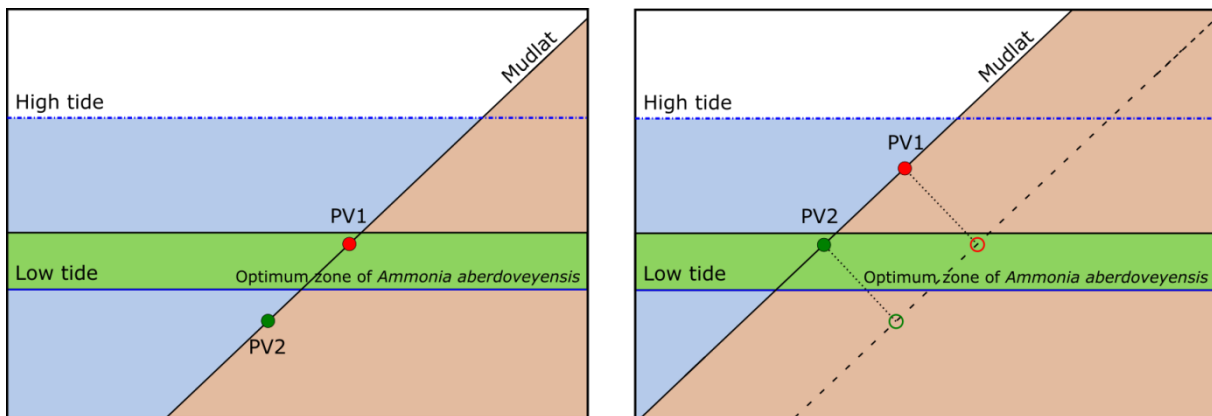


Figure IV.8 : Diagram explaining the change in position of stations PV1 and PV2 in relation to the optimal zone of *Ammonia aberdoveyensis* before (left side) and after (right side) the growth of the mudflat.

4.5. Hypotheses concerning the introduction of *Ammonia confertitesta* in the Loire estuary

A. aberdoveyensis has a restricted range in the North Atlantic (Hayward et al., 2021) and is considered indigenous to Europe. In contrast, many authors have suggested that *A. confertitesta* may be an invasive alien species introduced to European coasts from the East Asian coasts (Pawłowski & Holzmann, 2007; Schweizer et al., 2011; Bird et al., 2020; Richirt et al., 2021). This assumption is based on the distance between its two major ranges (Figure IV.9) and the fact that it has recently been observed in places where it was absent from past surveys (e.g. Baltic Sea (Schweizer et al., 2011); Grevelingenmeer (Richirt et al., 2022)).

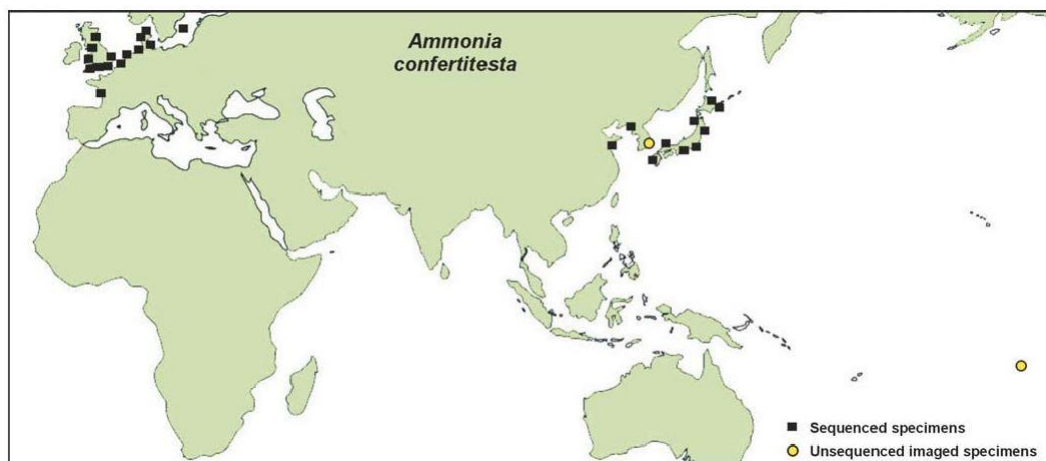


Figure IV.9 : Geographical distribution of *Ammonia confertitesta* based on sequenced specimens and images analysed by Hayward et al., 2021. Map from Hayward et al. 2021.

Several hypotheses concerning the vectors and the time of introduction have been proposed.

Massive imports of Japanese oysters between the 1960s and 1980s represent a potential introduction vector for *A. confertitesta* as it has been the case for many species (Wolff & Reise, 2002). However, the absence of *A. confertitesta* in estuaries where oyster farming is massively established (e.g., Auray (Richirt 2020; chapter 3)) has already raised some doubts about this hypothesis.

On the other hand, ballast water could represent a potential vector for the introduction of these species. Indeed, Richirt et al., 2021 showed that in areas near large harbours (e.g. Bristol, Immingham, Le Havre, Liverpool, London, Milford Haven, Rotterdam), *A. confertitesta* is dominant compared to the other two *Ammonia* spp.

Most authors supposed that *A. confertitesta* may have arrived in Europe in the 20th century (Pawlowski & Holzmann, 2007; Schweizer et al., 2011; Richirt et al., 2021, 2022). The results of the present study disagree with this hypothesis. Despite an imperfect age model, it appears that the arrival of *A. confertitesta* in the Loire Estuary dates back several centuries. As detailed above, sedimentary processes may have led to an underestimation of the age, while a major overestimation seems very unlikely. The proposed date of introduction is therefore a minimum estimate of the arrival of *A. confertitesta* in this mudflat.

Despite the number of exotic species invasions has increased in recent decades, introductions of alien species have already taken place in more distant past. For example, the bivalve *Mya arenaria* is thought to have been introduced from America to Europe several centuries ago by shipping. Authors date its introduction between the 13th and 17th centuries (Petersen et al., 1992; Daniel et al., 2019)

Although at that time ships were ballasted with stones instead of water, organisms could have been transported by fouling. Indeed, in a historical reconstruction of a 16th century ship, Carlton & Hodder, 1995 showed that species present in the natural fouling of the hull could be transported and survive several days at high sea without significant loss of abundance or diversity.

It therefore appears that the species *A. confertitesta* was introduced into Europe well before the 20th century. Its introduction into the Loire estuary dates back at least to the 18th century. At that time the most likely vector of introduction was shipping. *A. confertitesta* could therefore have been introduced into the Loire estuary via the increase of trade with China and India from the 16th and 17th centuries.

However, if this species has indeed been present for several centuries on the European coasts, it is surprising that *A. confertitesta* is not yet dominant in all estuaries of these coasts. It is also surprising that the replacement of *A. aberdoveyensis* by *A. confertitesta* in the Loire, as observed in our results, has taken place over a prolonged period. Here we will present several hypotheses to explain these observations.

- 1) The establishment of *A. confertitesta* would depend on decadal hydrosedimentary events.

Previously, we have shown that although *A. confertitesta* has been genetically detected in seven estuaries of the Atlantic coast, this species is only present, or even dominant, morphologically in a few sites (chapter 3). We hypothesised that the presence of propagules or juveniles is not the only condition for the definitive establishment of *A. confertitesta*, but that this species depends on important hydrosedimentary events that would eradicate the community in place (e.g. decadal floods) allowing the exotic species to supplant the autochthonous *Ammonia* species and to colonise the environment. As these events were punctual, the replacement of the native species by the invasive one occurred erratically over a long period.

- 2) Principle of competitive exclusion.

In chapter 3 we showed that *A. aberdoveyensis* and *A. confertitesta* have very similar ecological niches. However, the "principle of competitive exclusion", proposed by Gause (1934), states that two species with similar ecological niches cannot coexist indefinitely (Holt, 2017; Kneitel, 2019). Examples of species replacement show that the time scales of replacement are highly variable. An example in crabs shows that the invasive species *Hemigrapsus sanguineus* replaced the previous invasive species *Carcinus maenas* very quickly in some areas (2-3 years), whereas this replacement was much longer in environments where refuge areas were available (Griffen et al., 2011). In foraminifera, the species *Trochammina hadai* has shown a relatively rapid colonisation of San Francisco Bay (~20 years), and has caused the decline of the species *Elphidium excavatum* in this area (McGann et al., 2000). In contrast, the replacement process can take much longer and require many generations, resulting in a state of "transient coexistence" (Holt, 2001). In the case of *A. aberdoveyensis* and *A. confertitesta* living on mudflats, it is very likely that these two species occupy very similar ecological niches, but estuaries are areas of high productivity (Day et al., 2012), so their resources are not limited. There is therefore little competition between these two species, leading to a very gradual replacement.

- 3) Replacement may have been favoured by changes in environmental conditions due to human activities.

Finally, a last hypothesis could be linked to the morphological changes in the Loire estuary over the last two centuries. These changes have led to an increase in tidal influence (dynamic and saline) in the estuary. These changes in salinity and currents could potentially have caused changes in the foraminiferal community that would have favoured the increase of *A. confertitesta* or the disappearance of *A. aberdoveyensis*. Subsequently, the intensification of marine currents could have favoured the spread of *A. confertitesta* outside the estuary.

5. Conclusion

The study of *Ammonia* specimens from two long cores taken from the Brillantes mudflat showed that the deepest samples contained only *A. aberdoveyensis*. This was followed by a phase where both species were present. Finally, in the surface samples only *A. confertitesta* was present. Age models show that the arrival of *A. confertitesta* in the mudflat dates back several centuries and probably took place in the 17th-18th centuries. At that time, the most likely vector of introduction was shipping. Future investigations on historical reconstructions would allow a better dating of the introduction of this invasive species across Europe. As a first step, a re-investigation of *Ammonia* specimens from already published historical reconstructions (e.g., (Cearreta et al., 2000, 2002, 2013; Francescangeli et al., 2016, 2018; Daniel et al., 2019)) would strengthen this hypothesis.

The results show that both species of *Ammonia* have cohabited on the same station for several centuries. Biological factors (competitive exclusion principle), natural environmental factors (repetition of decadal flood events) or perhaps anthropogenic factors (significant hydro-morphological modifications) may have eventually caused the decline of *A. aberdoveyensis* in the studied mudflat. These observations could help to better understand the mechanisms and speed of colonisation of this species, as well as the response of native species to the invasion of an exotic species.

Chapitre 5

Répartition des foraminifères dans les vasières de neuf estuaires de la façade atlantique française

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Résumé

Cette étude se focalise sur la répartition des foraminifères de neuf estuaires situés sur la côte atlantique française (du nord vers le sud : Elorn, Aulne, Odet, Belon, Laïta, Crac'h, Auray, Vilaine et Vie) et sa relation avec différents paramètres environnementaux. Les paramètres environnementaux mesurés sont la granulométrie, la matière organique et les éléments traces métalliques ainsi que l'indice d'influence marine (MII). Cet indice estime la part d'influence des eaux marines par rapport aux eaux fluviales. Il intègre plusieurs paramètres liés à l'intrusion d'eau marine dans les estuaires, notamment la salinité, mais aussi l'apport de nutriments d'origine marine et l'hydrodynamisme. Les résultats des analyses des éléments traces métalliques et de la matière organique montrent qu'à l'exception de l'Aulne, les estuaires étudiés ne sont pas soumis à des enrichissements majeurs d'origine anthropique. L'estuaire de l'Aulne présente des concentrations élevées en plomb dans le sédiment, mais les communautés de foraminifères ne semblent pas perturbées par cet enrichissement. Les résultats montrent que la diversité spécifique est corrélée avec la granulométrie et le MII. En outre, plusieurs taxa individuels montrent une corrélation positive (*Elphidium margaritaceum*, *Elphidium selseyense*, *Quinqueloculina oblonga*, *Quinqueloculina jugosa*) ou négative (*Ammotium salsum* et *Miliammina fusca*) avec le MII. Ces résultats suggèrent que le MII permet de savoir si la répartition des foraminifères est en accord avec les paramètres naturels caractérisant les vasières estuariennes. Dans le cas contraire, une déviation des communautés pourrait être une réponse à une pollution anthropique.

1. Introduction

Les estuaires sont des zones de transition entre le milieu marin et fluvial. Malgré des contraintes environnementales importantes (p. ex. de fortes variations de salinité dues au cycle des marées et aux crues du fleuve), la productivité élevée de ces écosystèmes en font des lieux d'installation privilégiés pour beaucoup d'organismes (McLusky & Elliott, 2004). Les estuaires représentent aussi des zones clefs dans le cycle de vie de certaines espèces migratrices (p. ex., poissons (Ray, 2005), oiseaux (Hill et al., 1993)). Mais depuis des temps millénaires, les estuaires représentent aussi des lieux de vie stratégiques pour l'homme (Bell & Neumann, 1997; López-Romero et al., 2021). Cette interface entre le milieu marin et fluvial représente un atout majeur pour le commerce, et la forte productivité en ressources halieutiques de ces zones a incité les hommes à s'implanter massivement le long des zones estuariennes (Ducrotoy & Dauvin, 2008). Villes, ports, chenaux, canaux, digues, infrastructures industrielles, barrages, champs, ont fleuri le long des berges pour répondre à des besoins commerciaux, urbains, agricoles ou halieutiques. Ces aménagements ont non seulement modifié l'hydrologie des estuaires, mais les activités liées à ces structures sont aussi devenues des sources de pollutions chimiques ou biologiques (Kennish, 2002; Ducrotoy, 2010).

Dans ce contexte, le biomonitoring est devenu un enjeu primordial pour la bonne gestion des zones estuariennes. En Europe, la directive cadre sur l'eau (DCE) demande à ses états membres de surveiller, et si besoin, de restaurer la qualité écologique de toutes les masses d'eau situées sur leurs territoires (Council of the European Communities, 2000). Pour cette raison, les chercheurs sont encouragés à développer des indicateurs afin d'évaluer l'état écologique des masses d'eau. Depuis une vingtaine d'années, de nombreux outils ont été développés. Plusieurs indicateurs reposent sur la détection et la quantification de certains polluants (métaux lourds, HAP, TBT) (Borja et al., 2009b; Dafforn et al., 2012). D'autres reposent sur les organismes vivant dans ces milieux, qui reflèteraient l'ensemble des perturbations occasionnées dans ce milieu. Plusieurs groupes d'organismes sont utilisés pour la bio-indication (p. ex., poissons, macro-algues, macrofaune), chaque groupe présentant des avantages spécifiques pour évaluer au mieux l'état de la masse d'eau (Diaz et al., 2004). Parmi ces organismes, l'embranchement des foraminifères (Eucaryota, Rhizaria) sont de plus en plus utilisés pour le biomonitoring des zones côtières car ils présentent plusieurs avantages:

- 1) Ces organismes sont sédentaires, leur mobilité limitée les empêche d'éviter les perturbations. Ils permettent de donner une illustration fiable des conditions environnementales du site où ils se sont développés.
- 2) Ils ont un cycle de vie court (3 mois à 2 ans). La communauté réagit donc rapidement aux changements environnementaux (Murray, 2006; Albani et al., 2007).
- 3) Ils vivent dans presque toutes les eaux marines et les eaux de transition du globe, permettant d'utiliser ces indicateurs dans toutes les régions (Murray, 2006).

- 4) Les foraminifères à test fossilisent dans le sédiment, créant ainsi des archives historiques pouvant fournir des informations concernant les conditions de référence, présentes avant les impacts anthropiques (Alve et al., 2009; Dolven et al., 2013).

Cependant, le biomonitoring des écosystèmes estuariens présente une difficulté majeure, qui s'applique à tous les organismes vivants dans ces milieux : le « paradoxe estuarien ». Ce phénomène a déjà été détaillé dans le chapitre précédent, ainsi que par de nombreux auteurs (Dauvin, 2007; Elliott & Quintino, 2007; Elliott & Whitfield, 2011; Tweedley et al., 2015). Pour résumer, dans les milieux estuariens, les variations naturelles d'une multitude de paramètres entraînent des stress naturels agissant sur les organismes. Par conséquent, la diversité spécifique de ces milieux est limitée et inclut surtout les espèces dites « tolérantes », capables de s'adapter à ces conditions adverses. La difficulté dans les estuaires de séparer les stress anthropiques et naturels, a été appelée « Estuarine Quality Paradox » (Dauvin, 2007; Elliott & Quintino, 2007). Ce terme reflète donc la contradiction entre un bon état écologique du milieu et une mauvaise évaluation résultant de l'étude des bio-indicateurs. En effet, puisque la communauté estuarienne est adaptée à des stress naturels dus aux fortes variabilités spatiales et temporelles, cette communauté estuarienne présente des similitudes avec les communautés trouvées dans les écosystèmes marins côtiers fortement pollués. Ces caractéristiques des communautés estuariennes mènent systématiquement à une mauvaise évaluation de la qualité environnementale, même dans des milieux peu anthropisés. La conséquence du paradoxe estuarien est qu'il est très difficile de détecter les stress dus aux pressions anthropiques sur la base des communautés benthiques estuariennes.

Afin de pallier à cette difficulté, il est indispensable de bien connaître la répartition naturelle des organismes en milieu estuarien, et de comprendre comment cette répartition est façonnée par les paramètres naturels du milieu. C'est seulement si cette compréhension est acquise qu'il sera possible de détecter une déviation à cette répartition naturelle, qui pourrait être liée à des impacts anthropiques. Récemment, Jorissen et al., 2022 ont proposé un Indice d'Influence Marine (MII), visant à quantifier la part d'influence marine par rapport à l'influence fluviale. Cet indice, qui inclut la distance à la mer, le temps d'émergence et le débit de la décharge fluviale (au moment de l'échantillonnage et par rapport à la taille de l'estuaire au site échantillonné), ne se contente pas de décrire la variabilité spatiale et temporelle de la salinité, mais aussi l'équilibre entre d'autres paramètres et processus d'origine marine et/ou continentale, tels que les sels nutritifs, la matière organique, l'apport sédimentaire ou l'hydrodynamisme. L'objectif de cet indice est d'avoir une seule métrique qui caractérise les conditions naturelles du milieu estuarien. La comparaison entre le MII et la répartition observée des organismes devrait ensuite permettre de conclure si cette répartition est plutôt naturelle, ou affectée par une pollution anthropique.

Plusieurs études se sont penchées sur la répartition des foraminifères en milieu estuarien, mais souvent sur des jeux de données focalisés sur un nombre assez restreint d'estuaires et de stations (p.ex., Debenay et al., 2000 : deux estuaires ; Francescangeli et al., 2020 : cinq estuaires regroupant 16 stations ; Fouet et al., 2022 :

deux estuaires regroupant 27 stations). La présente étude comprend 90 stations provenant de neuf estuaires situés sur la façade atlantique française. Pour chaque station, des mesures de matière organique et de métaux lourds ont été effectuées afin de caractériser le degré de pollution. Ceci permettra de vérifier si les assemblages observés montrent une répartition naturelle, ou sont potentiellement affectés par un stress anthropique superposé. Pour déterminer s'il y a une déviation de la répartition observée par rapport à la répartition naturelle, nous utiliserons entre autres le MII (Indice d'Influence Marine). Dans une première étude (Fouet et al., 2022 ; chapitre 2) cet indice a été testé sur un jeu de données restreint (27 stations) provenant de deux estuaires relativement peu affectés par les activités anthropiques. Dans cette étude, le MII a montré des corrélations avec plusieurs paramètres biotiques. Nous avons donc fait l'hypothèse qu'un écart de la répartition naturelle des foraminifères pourrait être repéré, par une déviation d'un ou plusieurs paramètres faunistiques par rapport à la relation établie de ces paramètres avec la MII.

Ainsi, le premier objectif de cette étude est de décrire la répartition des foraminifères dans les neuf estuaires étudiés, et d'établir à quel degré cette répartition peut être expliquée par les données environnementales naturelles. Ensuite, nous rechercherons s'il y a des sites avec des enrichissements d'origine anthropique de métaux et de matière organique, et si les communautés de foraminifères de ces sites sont affectées par ces pollutions. Pour déceler des anomalies de répartition dues à la pollution, le MII sera utilisé. Le deuxième objectif est donc de tester cet indice et d'évaluer son efficacité à relever des anomalies de répartition en l'appliquant à un plus grand nombre de stations que dans la première étude. Le but final est de développer une méthode qui permette de relever les anomalies de distribution dues à des pollutions anthropiques, et si possible, de développer un indice de bio-indication capable de reconnaître des impacts anthropiques.

2. Matériel et méthodes

2.1. Zone d'étude

L'étude se concentre sur neuf estuaires situés dans une zone géographique restreinte (Figure V.1), au nord-ouest de la façade atlantique française. Le choix d'une zone géographique restreinte est motivé par la nécessité de limiter les variations environnementales liées à la distribution géographique. Ainsi, les neuf estuaires de cette étude sont soumis à un régime méso-macrotidal (marnage ~ 4 - 6 m) et un climat relativement similaire (océanique tempéré).

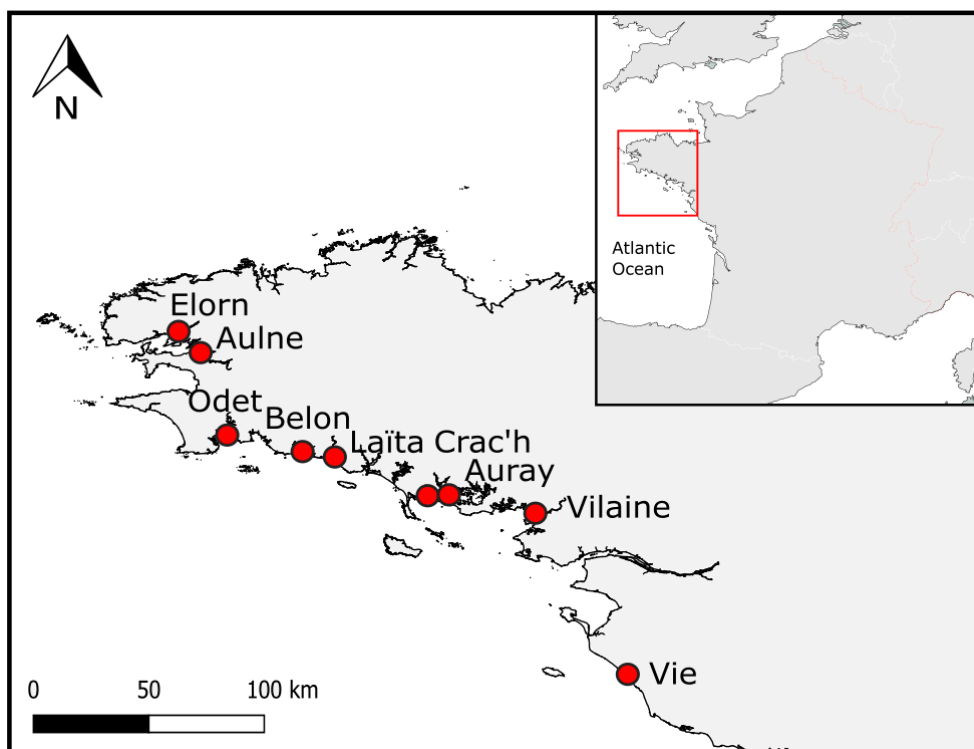


Figure V.1 : Localisation des sites d'étude

Tableau V.1: Caractéristiques générales des neuf estuaires étudiés. Les valeurs marquées d'un ¹ proviennent de l' Office français de la biodiversité et celles marquées d'un ² de la Banque Hydro.

Estuaire	Elorn	Aulne	Odet	Belon	Laïta	Crac'h	Auray		Vilaine	Vie	
Nombre de stations échantillonnées	5	3	4	9	3	4	15	15	6	14	12
Campagne d'échantillonnage	10/2020	10/2020	10/2020	5/2019	10/2020	10/2020	7/2019	9/2020	5/2019	10/2018	6/2020
Type d'estuaire	Ria	Ria	Ria	Ria	Ria	Ria	Ria		Estuaire de plaine	Estuaire de plaine fermé par une barre sableuse	
¹ Limite de pénétration saline	15 km	28.8 km	20 km	6.8 km	6.5 km	13 km	19.8 km		12 km	8.25 km	
¹ Superficie du bassin versant	385 km ²	1797 km ²	715 km ²	95 km ²	88.5 km ²	64 km ²	324 km ²		10536 km ²	751 km ²	
¹ Largeur de l'embouchure	570 m	1460 m	1000 m		1800 m	1016 m	950 m		4400 m	200 m	
² Débit de crue	54 m ³ /sec	330 m ³ /sec	76 m ³ /sec	-	-	-	31 m ³ /sec		810 m ³ /sec	20 – 25 m ³ /sec	
² Débit d'étiage	1.1 m ³ /sec	1.5 m ³ /sec	0.79 m ³ /sec	-	-	-	0.18 m ³ /sec		5.50 m ³ /sec	0.01 m ³ /sec	
² Débit moyen annuel	5.6 m ³ /sec	25.0 m ³ /sec	7.5 m ³ /sec	-	-	-	2.7 m ³ /sec		74.0 m ³ /sec	1.2 m ³ /sec	

2.2. Échantillonnage

Pour chaque station, un à trois répliqués ont été échantillonnés à marée basse. Selon la taille de la vasière, à chaque site, une ou plusieurs stations ont été échantillonnées sur un transect perpendiculaire au chenal principal. La lettre A correspond à une station située en haut de la vasière, B et C à des stations situées au milieu ou en bas de la vasière. Le premier centimètre de sédiment a été récolté à l'aide d'un carottier de

96mm de diamètre interne. Les échantillons ont été préservés dans de l'éthanol à 96% et colorés avec 2g/L de Rose Bengal. De plus, des échantillons de sédiment de surface ont été récoltés pour analyser la granulométrie, les éléments trace métalliques et la matière organique. Pour chacune des stations, l'élévation a été mesurée à l'aide d'un théodolite. Les mesures de l'élévation sont expliquées en détail dans le chapitre 2.

2.3. Analyses en laboratoire

Les échantillons destinés à l'analyse des foraminifères ont été lavés sur des tamis de 125 μ m. Quand cela était nécessaire, des séparations par densité ont été utilisées pour enlever le surplus de débris organiques (utilisation du Ludox (Burgess, 2001; Somerfield & Warwick, 2013)) ou le sable (utilisation du Sodium PolyTungstate (Parent et al., 2018)). Les échantillons avec une forte densité de foraminifères ont été divisés en aliquots en utilisant un splitter humide (Charrieau et al., 2018). Un ou plusieurs de ces aliquots ont été analysés entièrement jusqu'à obtenir 300 individus. Les échantillons ont été analysés avec une loupe binoculaire Leica MZ16. L'analyse sous loupe binoculaire permet de distinguer la grande majorité des espèces, à une exception importante près : les différentes espèces du genre *Ammonia*. En effet, il a été découvert récemment que l'espèce morphologique *Ammonia tepida* est composée de trois espèces distinctes génétiquement (Hayward et al., 2004) et morphologiquement (Richirt et al., 2019) : *Ammonia veneta*, *A. aberdoveyensis* et *A. confertitesta*. Dans les estuaires étudiés, ces trois espèces sont présentes. Avec un stéréomicroscope, il est possible de distinguer *A. aberdoveyensis* de *A. veneta* et *A. confertitesta* pour des exemplaires adultes (Pavard et al., 2021). En revanche, la distinction entre ces deux dernières espèces n'est réalisable que dans le cas de spécimens suffisamment représentatifs (individus relativement gros, ne présentant pas de déformation) ; pour les individus petits ou déformés, cette distinction se révèle impossible. Pour cette raison, nous nous référerons au taxon *Ammonia* spp. dans ce chapitre. Une investigation plus détaillée de la répartition de ces trois espèces, utilisant des analyses moléculaires ainsi que des images MEB est présentée dans le chapitre 3.

La granulométrie a été mesurée à l'aide d'un granulomètre à diffraction laser Malvern 3000 Mastersizer sur du sédiment non décarbonaté afin de caractériser les pourcentages d'argile (0-4 μ m), de silt (>4-62.5 μ m) et de sable (>62.5 μ m).

Les concentrations des éléments traces métalliques ont été mesurées à l'aide d'un spectromètre de masse à plasma à couplage inductif (Thermo Scientific® X-Series 2 ICP-MS) sur du sédiment lyophilisé après l'ajout d'acide (HCl, HNO₃⁻ et HF) en suivant la méthodologie proposée par Coynel et al., 2010. En tout, 16 éléments chimiques ont été mesurés : Ag, As, Ba, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sb, Sn, Th, V, U, Zn. Leurs concentrations sont exprimées en mg/kg.

Afin de quantifier l'apport anthropique en métaux dans les sédiments, les facteurs d'enrichissement (EF) ont été calculés en suivant la méthode proposée par Larrose et al. (2010). Ces calculs sont détaillés dans le

chapitre 2. En résumé, dans un premier temps les concentrations des métaux traces sont normalisées par un élément conservatif, afin de corriger pour l'effet de la taille des grains de sédiment. A cette fin, le Th a été choisi, car cet élément a déjà été utilisé pour l'étude de sédiments estuariens (Larrose et al., 2010). Ensuite, l'EF est obtenu en divisant la concentration en élément trace métallique (normalisée par le Th) par la concentration trouvée dans un sédiment de référence non pollué, représentatif du fond géochimique régional. La formule est la suivante :

$$EF = \frac{\frac{X}{Th}^{obs}}{\frac{X}{Th}^{ref}}$$

Le sédiment de référence provient d'une longue carotte échantillonnée dans la partie ouest du Golfe du Morbihan. Ce sédiment est daté d'il y a environ 5000 ans. Des valeurs supérieures à 5 correspondent à des enrichissements forts, des valeurs comprises entre 3 et 5 à des enrichissements modérés et des valeurs comprises entre 1,5 et 3 correspondent à des enrichissements mineurs.

Les teneurs en carbone organique total (TOC), en azote total (TN) et la composition isotopique du carbone ($\delta^{13}C$) ont été mesurées sur des sédiments décarbonatés et lyophilisés à l'aide d'un analyseur élémentaire (EA Vario PYRO cube ; Elementar®) couplé à un spectromètre de masse à rapport isotopique (IRMS ; Isoprime precision, Elementar®). Avant l'analyse EA-IRMS, 300 mg de sédiments séchés ont été décarbonatés avec de l'acide phosphorique pour éliminer le carbone inorganique (H_3PO_4 , concentration 2 M). Après décarbonatation, les échantillons ont été lyophilisés et broyés. Trois milligrammes d'échantillon ont été pesés dans des capsules d'étain. De plus amples explications concernant la correction des valeurs brutes sont données dans le chapitre 2.

2.4. Analyses statistiques

L'indice d'influence marine a été calculé pour chacune des stations en suivant la formule suivante :

$$MII = \left(1 - \frac{x}{s}\right) * (1 - ET) * \left(1 - \sqrt{\frac{0.04 * RD}{CS}}\right)$$

Des explications approfondies du calcul du MII sont données dans Jorissen et al., 2022 et Fouet et al., 2022 (chapitre 2). Pour résumer, le premier terme de l'équation est la distance à la mer normalisée, dans laquelle x est la distance du site d'échantillonnage jusqu'à l'embouchure de l'estuaire (mesurée en suivant le talweg) divisée par s, la longueur de l'intrusion saline. Le second terme est la durée d'émergence de la station à marée basse (ET). Le troisième terme considère le débit de la rivière (RD) dans les 30 jours avant l'échantillonnage, divisé par la section transversale de l'estuaire au niveau de la station échantillonnée. Chacun de ces trois

termes est normalisé pour obtenir un score où la valeur maximale (1) correspond systématiquement à une influence marine maximale.

Les indices de diversité (richesse spécifique et indice de Shannon) ont été calculés grâce au package vegan (version 2.5-7) (Oksanen et al., 2020) du logiciel R (version 4.0.4) à partir des données brutes.

Deux analyses en composantes principales (ACP) ont été réalisées dans cette étude. La première est basée sur les valeurs des teneurs en métaux normalisées, la deuxième sur les données d'abondance relative non standardisées. Les variables environnementales normalisées ont été ajustées à posteriori à l'ordination à l'aide de la fonction envfit du package vegan (version non contrainte, les paramètres environnementaux ne contribuent pas à l'ordination).

Enfin, pour la grande majorité des tests de corrélation réalisés dans cette étude (à l'exception des Figures V.28 et V.29 qui utilisent des tests de Pearson), la méthode de Spearman a été utilisée car elle permet également de détecter des corrélations non linéaires. Pour les droites de régression, un modèle linéaire ("lm") ou exponentiel a été utilisé selon la nature de la tendance observée.

3. Résultats

3.1. Paramètres abiotiques

3.1.1. Granulométrie

La majorité des stations (77 sur 90 stations) présentent une granulométrie fine, composée de 45 à 90% par des argiles et des silts (Figure V.2). Les 13 stations restantes présentent une granulométrie plus grossière. Les stations Crac'h 1, Vie₂₀₁₈ 1 et 2, Vie₂₀₂₀ 2, Elorn 1 et Laïta 1 présentent un sédiment essentiellement composé de sables fins (27 à 87%) et moyen (9 à 68%). La fraction fine (grains <63µm) représente au maximum 17% du sédiment. Toutes ces stations sont situées à l'embouchure des estuaires. Les stations Belon 3B et 4B présentent une granulométrie similaire, mais diffèrent par leur position, situées dans la partie intermédiaire de l'estuaire. Enfin, les stations Laïta 2 et 3, Belon 1, 4A et 6A présentent une granulométrie intermédiaire composée de 30 à 38% de silts et argiles. Les 77 stations restantes sont caractérisées par un sédiment vaseux composé principalement de silts (de 40 à 76%). Les stations situées dans l'estuaire de la Vilaine présentent les plus fortes teneurs en silt (71-76%).

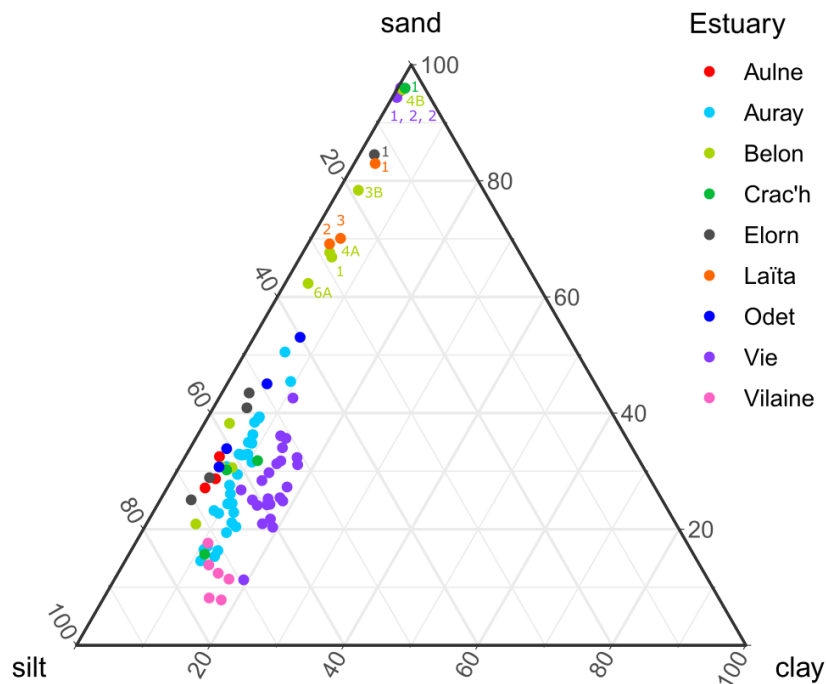


Figure V.2 : Distribution des différentes classes granulométriques (argile 0-4 μ m ; silt : 4-62.5 μ m ; sables : >62.5 μ m) dans les différentes stations.

3.1.2. Métaux traces

Pour chacune des stations, 15 métaux traces ont été mesurés (Tableau supplémentaire V.S1). Afin de présenter les résultats de façon plus synthétique, une ACP a été réalisée sur la base des concentrations normalisées (Figure V.3). Au vu de leurs eigenvalues, les deux premiers axes de l'ACP sont significatifs ; ils expliquent 68.2% de la variance totale (Tableau supplémentaire V.S3). L'ensemble des métaux sont situés sur la partie positive de l'axe 1. Concernant l'axe 2, le Cd, Pb, Ag, Zn et Sb sont situés sur la partie positive tandis que le Ba, Mo, Sn, Cu, U, Co, As, Ni, V, Cr sont situés sur la partie négative. Les 13 stations caractérisées comme plus sableuses (Figure V.2) se situent ensemble sur la partie gauche de la figure. Cela reflète une plus faible concentration en éléments traces métalliques à ces stations où la granulométrie est plus grossière. Ensuite, les trois stations de l'estuaire de l'Aulne sortent du groupe principal et sont situées dans la partie en haut à droite. En effet, ces stations présentaient des valeurs très élevées en plomb (Pb), cadmium (Cd), argent (Ag) et zinc (Zn) par rapport aux autres stations (Tableau V.2). Dans une moindre mesure, les stations Elorn 3A et 4 présentaient aussi des valeurs plus élevées pour ces trois métaux. La station Vilaine 3 est aussi positionnée du côté droit de l'ACP. Cette station présentait des valeurs élevées de cobalt (Co), nickel (Ni) et zinc (Zn) (Tableau supplémentaire V.S1). Sur la Figure V.3, on remarque également que, sur l'ACP, les stations des différents estuaires sont plutôt regroupées. Ainsi, cette analyse montre une forte spécificité des différents estuaires et met en évidence l'importance du bruit de fond géochimique, dû à des socles géologiques différents dans les bassins versant des fleuves s'écoulant dans les estuaires.

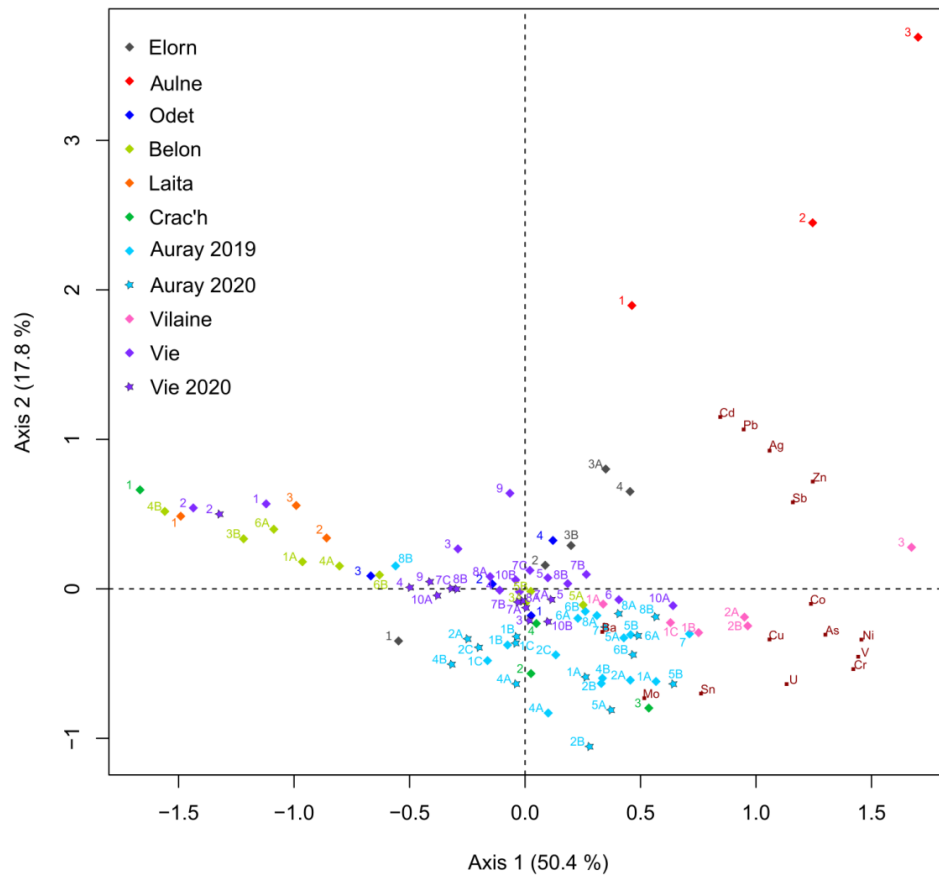


Figure V.3 : ACP réalisée à partir des mesures en métaux traces normalisées.

Le facteur d'enrichissement permet de mesurer le degré de contamination du sédiment par rapport au bruit de fond géochimique régional (Larrose et al., 2010). L'intégralité des valeurs d'enrichissement est présentée dans le Tableau supplémentaire V.S2. Six métaux pour lesquels un enrichissement conséquent (au-dessus de l'enrichissement mineur) a été observé sont présentés dans la Figure V.4.

Les stations de l'estuaire de l'Aulne montraient des facteurs d'enrichissement élevés pour plusieurs métaux : Ag, Cd, Pb et Zn. En effet, on peut observer un gradient d'enrichissement d'aval en amont. La station 3, en amont, présentait un enrichissement fort en Cd et Pb et modéré en Ag et Zn. La station 2, au milieu de l'estuaire, présentait un enrichissement fort en Pb, modéré en Cd et Zn et mineur en Ag. Enfin, la station 1, à l'embouchure, présentait un enrichissement fort en Pb, modéré en Zn et mineur en Ag et Cd.

Plusieurs autres estuaires montraient des stations avec des facteurs d'enrichissement modérés à mineurs pour l'Ag (Belon 4B ; Vie₂₀₁₈ 9 ; Odet 4), le Cd (Elorn 3A, 3B, 4, Belon 4B, 6B, Laita 2,3, Vilaine 3), le Co (Belon 1, 4B, Laita 3, Vilaine 3) et le Pb (Belon 4B, Crac'h 1). Dans certains cas (Belon 4B, 3B, Laita 2, 3 et Crac'h 1) il s'agit de stations à sédiment sableux, et les facteurs élevés semblent être des artefacts résultant de la normalisation. Pour les autres stations, l'enrichissement pourrait refléter une véritable contamination métallique.

Pour le Cu et le Zn, un grand nombre de stations montraient des facteurs d'enrichissement modérés à mineurs. Puisque tous les estuaires sont concernés, il semble que les concentrations élevées soient plutôt dues à des plus faibles concentrations de ces éléments dans la carotte de référence, prélevée dans le Golfe de Morbihan. Néanmoins, pour les estuaires de Crac'h, Auray et Vie, on observe une forte variation au sein de l'estuaire, avec quelques stations avec des facteurs d'enrichissement plus élevés. C'est notamment le cas pour le Cu aux stations Auray₂₀₁₉ 1A et Vie₂₀₁₈ 6 et le Zn à la station Vilaine 3. Pour ces trois cas, il s'agit de stations situées dans des zones portuaires.

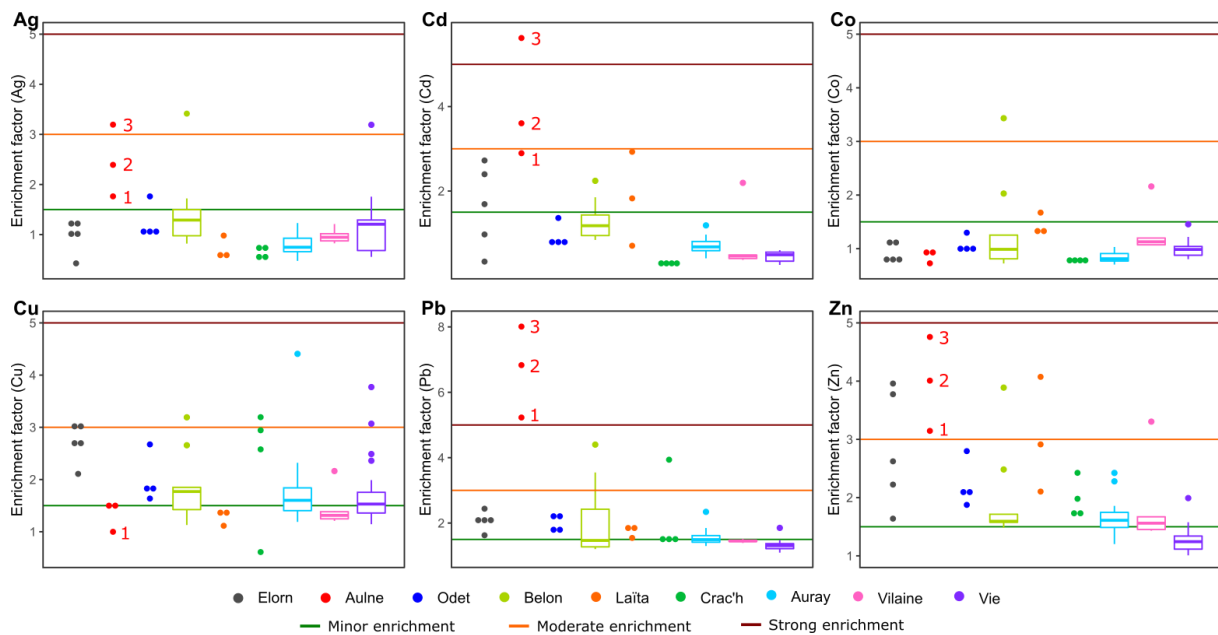


Figure V.4 : Boîte à moustaches indiquant les facteurs d'enrichissements pour les métaux montrant des dépassements. Pour les estuaires où moins de cinq stations ont été échantillonnées, seules les valeurs sont représentées. Les numéros de stations sont indiqués pour l'estuaire de l'Aulne où de forts enrichissements ont été détectés. Les facteurs d'enrichissements sont calculés avec la méthode proposée par Larrose et al., 2010.

3.1.3. Matière organique

Les valeurs de TOC variaient de 0.1 à 3.6% (1.8% en moyenne) (Figure V.5 ; Tableau supplémentaire V.S4). Les valeurs les plus élevées ont été trouvées dans l'estuaire du Belon (stations 5A et 5B), les valeurs les plus basses dans les estuaires de la Vie (stations 1 et 2) et de l'Elorn (station 1). La Figure V.6 montre la distribution des valeurs des rapports C/N et $\delta^{13}\text{C}$ pour chacune des stations. En arrière-plan de la figure sont indiquées ces mêmes rapports pour les différentes sources de matière organique dans les environnements côtiers, d'après Lamb et al., 2006. Cette figure montre que la plupart des stations forment un groupe homogène avec le rapport C/N compris entre 5 et 11 et les valeurs du $\delta^{13}\text{C}$ comprises entre -28‰ et -17‰. Au sein de ce groupe, les valeurs les plus élevées de $\delta^{13}\text{C}$ (-17 à -24‰) correspondent à une signature de carbone organique marin particulière et d'algues marines. Les valeurs les plus basses (-24 à -28‰) correspondent plutôt à la signature de carbone organique particulière et d'algues d'eau douce. Le

gradient au sein de ce grand groupe traduit donc la transition de sites à dominance marine vers des sites à dominance fluviatile. Une dizaine de stations se situent en dehors de ce groupe. Il s'agit des stations Vie₂₀₁₈ 3, 4, 7C, 8A et Belon 4B, qui présentaient des valeurs de $\delta^{13}\text{C}$ et de C/N bien plus élevées que le groupe principal. Les stations Vie₂₀₂₀ 4 et Vie₂₀₁₈ 9 présentaient des valeurs de rapport C/N légèrement au-dessus de celles du groupe principal. La station Vilaine 3 était la seule à présenter une valeur de $\delta^{13}\text{C}$ très faible (-29‰) et un rapport C/N faible (5.8). Ces valeurs peuvent s'expliquer par sa localisation dans la partie amont de l'estuaire, au-delà qu'un barrage.

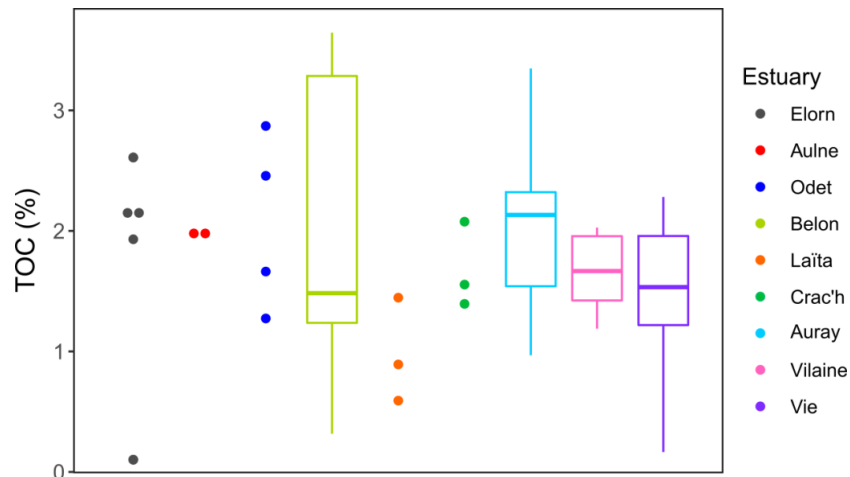


Figure V.5 : Boxplot indiquant les valeurs de TOC (%) dans les différents estuaires. Pour les estuaires où moins de cinq stations ont été échantillonnées, seuls les valeurs sont représentées.

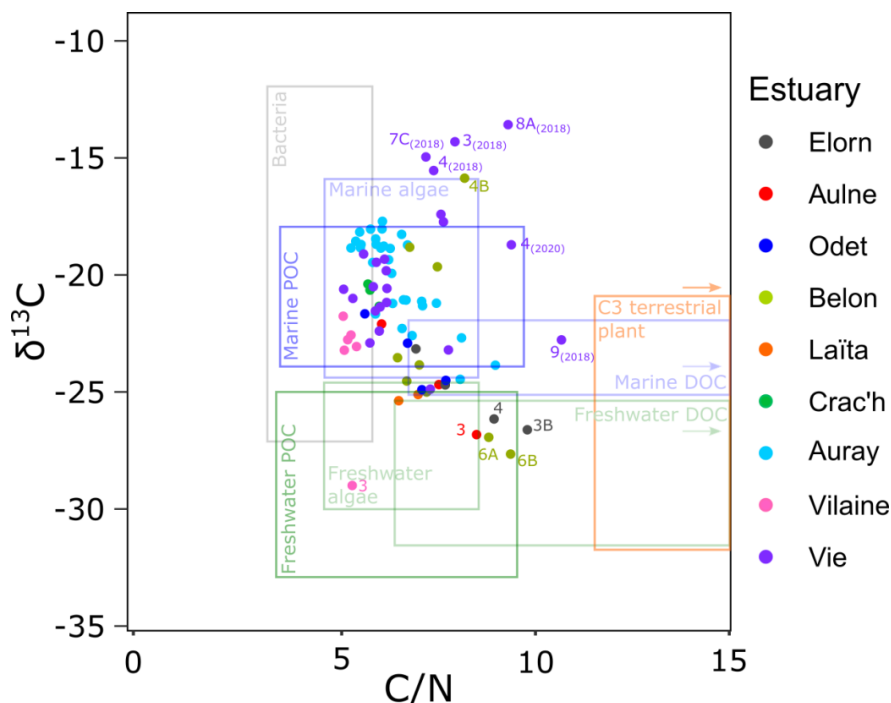


Figure V.6 : Distribution des valeurs des rapports C/N et $\delta^{13}\text{C}$ pour chacune des stations. Les zones indiquées en arrière-plan correspondent aux gammes de valeurs typiques pour les différentes sources de matière organique dans les environnements côtiers, d'après Lamb et al., 2006.

3.1.4. Relation entre les paramètres environnementaux

La Figure V.7 et le Tableau V.2 illustrent les relations entre les différents paramètres environnementaux mesurés dans cette étude. Dans ces figures, la distance à la mer, le temps d'émergence et la décharge fluviale sur la surface de la coupe transversale (RD/CS) ont été normalisés, de sorte que la valeur maximale (1.0) corresponde à une influence marine maximale pour chaque paramètre. Sur la Figure V.7, la distance normalisée montre une corrélation positive avec la décharge fluviale normalisée (1-(RD/CS)). Ceci s'explique par le fait que quand l'estuaire s'élargit vers l'aval, le facteur 1-(RD/CS) augmente. Ensuite, la distance à la mer est fortement corrélée au $\delta^{13}\text{C}$, reflétant le mélange entre matières organiques marine et continentale le long de l'estuaire. Finalement, il y a une faible corrélation entre la distance à la mer et le %TOC, avec des valeurs de TOC plus élevées vers l'amont.

En outre, il y a de fortes corrélations entre la granulométrie (représentée par le pourcentage de grains < 63 μm) et les paramètres organiques qui sont positives avec les pourcentages de TOC et TN et négative avec le C/N. En effet, plus la station est caractérisée par un pourcentage de silt et d'argile élevé, plus la quantité de TOC et d'azote est importante, et le rapport C/N faible. Dans les nuages de points illustrant ces relations (Figure V.7), on observe systématiquement deux groupes : 1) une grande majorité de sites avec un sédiment vaseux et 2) une minorité de sites avec un sédiment sablo-vaseux à sableux. Ces deux groupes montrent différentes relations entre granulométrie et paramètres organiques.

Tableau V.2: Matrice de corrélation basée sur un test de Spearman entre les paramètres environnementaux (la distance à la mer normalisée, la durée d'émergence normalisée, RD/CS normalisé, le pourcentage de silts et d'argiles, le pourcentage de TOC et TN, le rapport C/N et le rapport $\delta^{13}\text{C}$) Les tests de corrélation sont basés sur l'ensemble des stations à l'exception des paramètres annotés d'un «¹» pour lesquels quelques stations ne présentent pas de données (voir Tableau supplémentaire V.S4). Les chiffres indiquent le coefficient R, et les couleurs le niveau de significativité.

	Distance normalised	Emergence normalised	RD/CS normalised	Silt and clay	¹ TOC	¹ TN	¹ C/N	¹ $\delta^{13}\text{C}$
Distance normalised	-0.10	0.52	-0.04	-0.24	-0.19	-0.17	0.61	
Emergence normalised	-0.10	-0.13	-0.23	-0.02	-0.06	0.09	-0.18	
RD/CS normalised	0.52	-0.13	-0.06	0.02	0.03	-0.06	0.77	
Silt and clay	-0.04	-0.23	-0.06	0.31	0.39	-0.43	0.05	
¹ TOC	-0.24	-0.02	0.02	0.31	0.89	0.35	-0.11	
¹ TN	-0.19	-0.06	0.03	0.39	0.89	-0.23	-0.06	
¹ C/N	-0.17	0.09	-0.06	-0.43	0.35	-0.23	-0.21	
¹ $\delta^{13}\text{C}$	0.61	-0.18	0.77	0.05	-0.11	-0.06	-0.21	

R Spearman p-value

	ns		<0.1		<0.05		<0.01
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La Figure V.7 explore les différentes corrélations significatives décrites ci-dessous. La corrélation entre le pourcentage de TOC et le pourcentage de grains inférieurs à 63µm (représentant les sédiments silteux et argileux) est surtout due aux 13 stations à sédiment sableux, présentant une plus faible teneur en TOC. En effet, quand on enlève ces 13 stations, la corrélation n'est plus significative. Quelques stations se situent en dehors du quantile de régression (95%) et ne semblent pas suivre la tendance principale. Il s'agit des stations Auray₂₀₂₀ 8B, Belon 6B, 5B, 5A qui présentent des valeurs plus élevées de TOC. Ces stations sont toutes situées dans la partie amont de l'estuaire à des endroits où l'hydrodynamisme a été transformé par des constructions anthropiques (un pont à Auray, un barrage dans le Belon). Auparavant, Parent et al., (2021) ont interprété de telles valeurs, particulièrement élevées, comme dues à un enrichissement anthropique en matière organique.

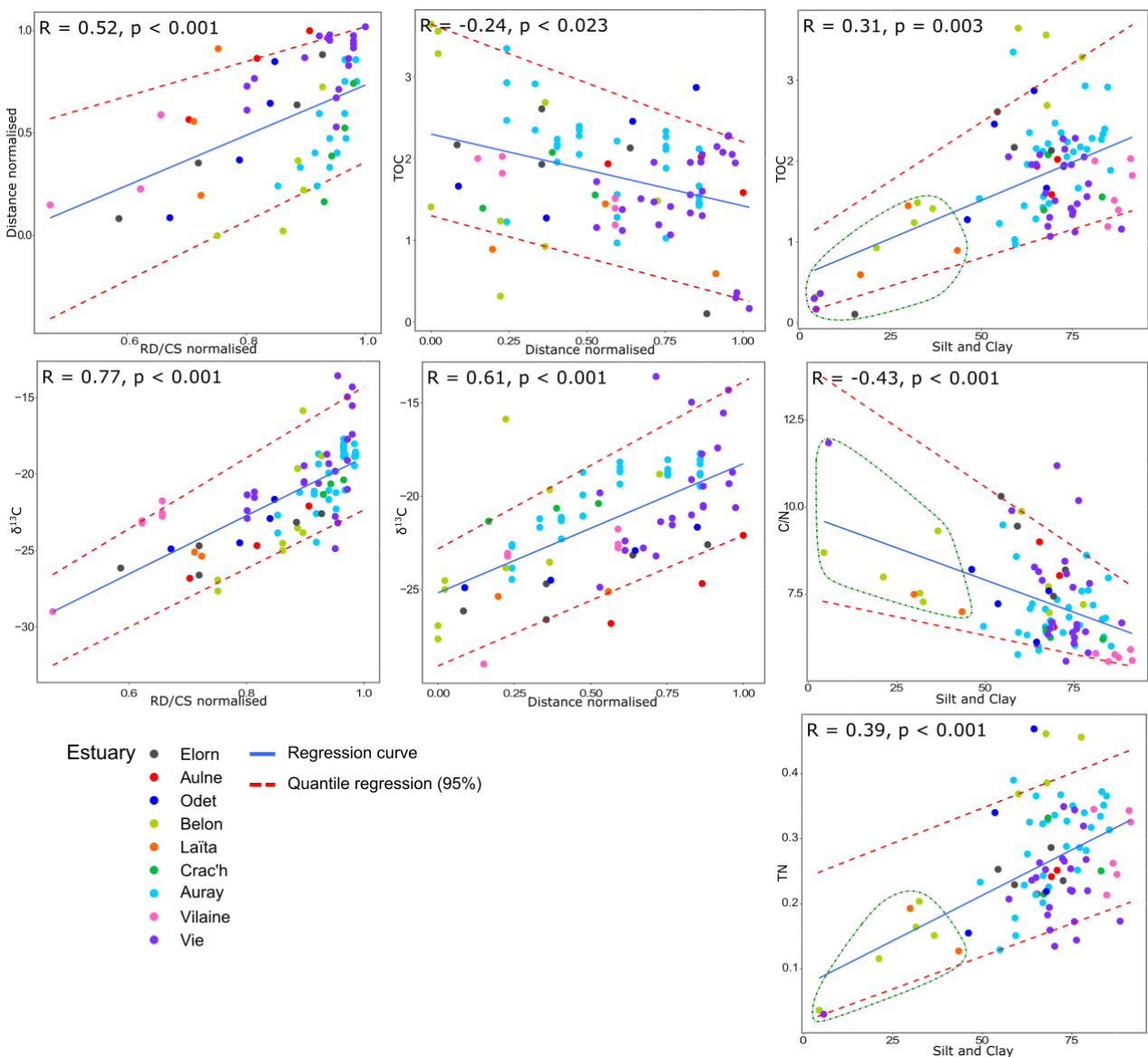


Figure V.7 : Relations entre les différents paramètres environnementaux. Seules les relations discutées dans le texte sont présentées ici. Les stations présentant un sédiment plus sableux sont entourées en vert. Les résultats des tests de corrélations sont basés sur des tests de Spearman.

3.2. Les communautés de foraminifères

Les valeurs d'abondance par station sont variables et sont comprises entre 3 (Belon 5A) et 3000 ind/50cc (Vilaine 1A) avec une médiane de 500 ind/50cc (Figure V.7A, Tableau supplémentaire V.5). Les plus fortes valeurs ont été trouvées dans les estuaires de la Vilaine et d'Auray. La richesse spécifique par station est comprise entre 2 (Belon 5A) et 13 (Vie₂₀₁₈ 3) avec une médiane de 9 (Figure V.7B). Les plus fortes valeurs ont été observées dans l'estuaire d'Auray et les valeurs les plus faibles dans la Vilaine, le Belon et l'Aulne. Cependant, il faut noter que la différence d'effort d'échantillonnage influe probablement les maxima de diversité observés (p. ex. 3 échantillons étudiés chacun pour l'Aulne et la Laïta contre 90 pour Auray). Les valeurs d'indices de diversité de Shannon sont relativement faibles, elles sont comprises entre 0.63 (Belon 5A) et 1.84 (Auray₂₀₁₉ 1C) avec une médiane de 1.17 (Figure V.7C).

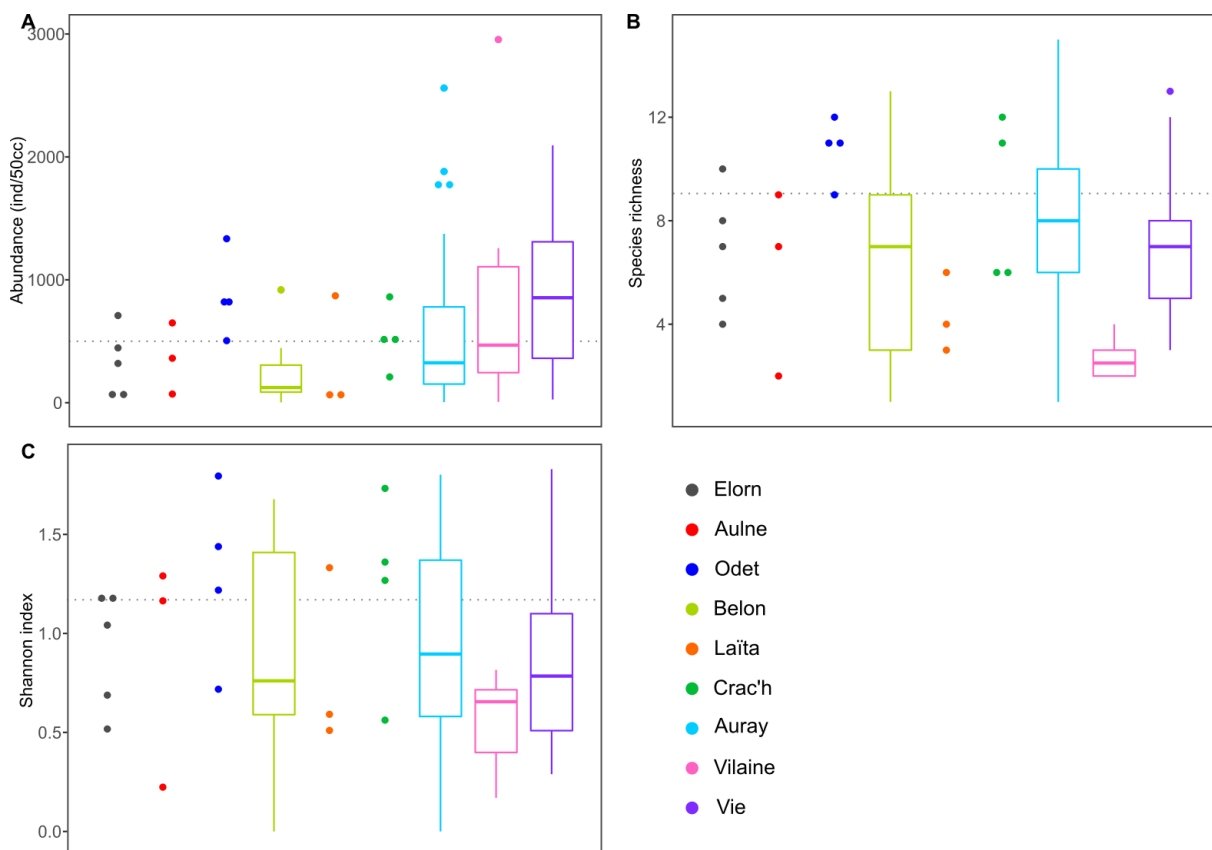


Figure V.8 : Distribution des valeurs d'abondance (A), de richesse spécifique (B) et d'indices de Shannon (C). Des boxplots sont utilisés pour les estuaires comportant plus de cinq stations. La ligne grise représente la valeur médiane pour l'ensemble des stations.

Le jeu de données contient au total 43 taxons (dans la fraction >125µm) dont 23 représentent plus de 2.5% de l'assemblage dans au moins une station. Les assemblages de foraminifères des échantillons étudiés sont décrits ci-dessous, estuaire par estuaire, du nord vers le sud. Dans ces descriptions, les différentes espèces seront caractérisées soit comme des « espèces marines », soit comme des « espèces estuariennes ». Selon les données de la littérature (p.ex., Murray, 1979; Debenay & Guillou, 2002; Martins et al., 2016), les

espèces estuariennes sont restreintes aux environnements estuariens, tandis que les « espèces marines » ont été décrites à la fois en milieu marin ouvert et dans les estuaires. Ces caractérisations seront plus amplement détaillées dans la discussion.

Dans l'estuaire de l'Elorn (Figure V.9), la densité variait entre 60 (station 4) et 700 individus (station 3A) ind/50cc. La richesse spécifique fluctuait de 4 (station 4) à 10 (station 2) espèces. L'indice de Shannon était minimal à la station 1 (H' : 0.5), autour de 1 aux stations 2, 3A et 3B puis diminuait à nouveau à la station 4 (H' : 0.7). La station 1, située à l'embouchure montrait une communauté atypique, fortement dominée par l'espèce porcelanée *Quinqueloculina oblonga*, qui représentait ~90% de l'assemblage avec une densité de 285 ind/50cc. Cette espèce était accompagnée par des espèces marines tel qu'*Ammonia beccarii*, *Haynesina depressula*, et des *Elphidium* (*E. williamsoni*, *E. gerthi*, *E. margaritaceum*). Les stations 2, 3A, 3B, 4 présentaient une communauté estuarienne plus classique, et étaient dominées par *Haynesina germanica* (60 à 80%), suivi d'*Ammonia* spp. (10 à 20%, équitablement partagés entre *A. aberdoveyensis* et *A. veneta*), *Elphidium oceanense* (2-6%) et *Q. oblonga* (5-15%). Les stations situées plus en amont (3A, 3B, 4) avaient une composition très comparable, avec en plus quelques individus d'espèces agglutinées (3-8%, *Ammotium salsum* et *Trochammina inflata*). L'estuaire de l'Elorn est le seul où *Ammonia confertitesta* n'a pas été observée morphologiquement, et où *A. aberdoveyensis* était l'espèce d'*Ammonia* qui comptait le plus d'individus.

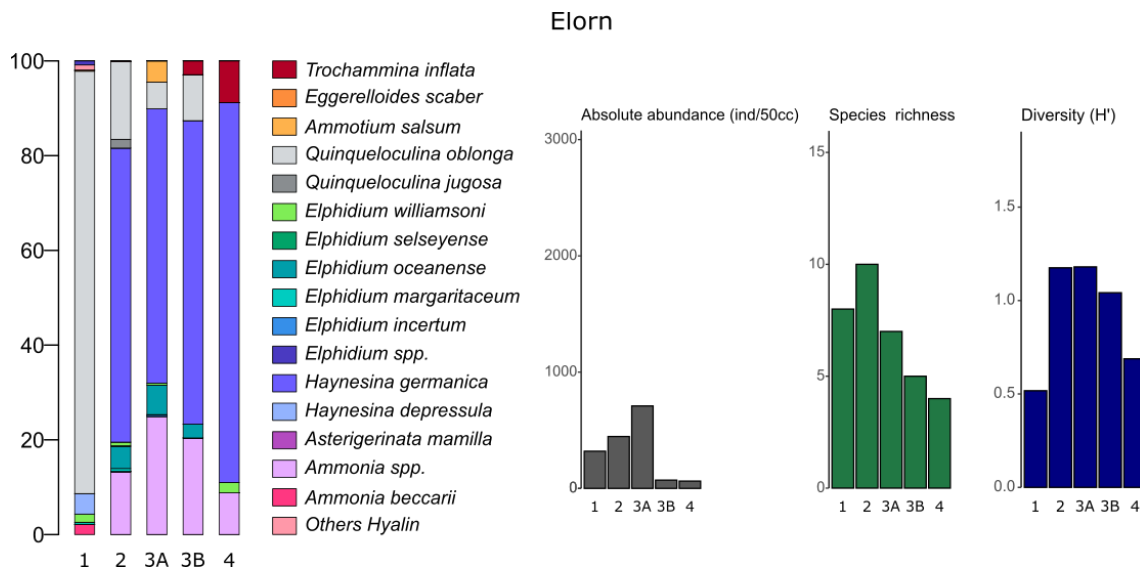


Figure V.9 : Estuaire de l'Elorn. A gauche, l'historgramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. A droite, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

Dans l'estuaire de l'Aulne (Figure V.10), la densité des foraminifères fluctuait entre 70 (station 3) et 650 ind/50cc (station 1) avec une densité décroissante d'aval vers l'amont. La richesse spécifique décroissait également d'aval vers amont et variait de deux (station 3) à neuf (station 1) espèces. L'indice de Shannon

suivait la même tendance, variant de 1.3 dans la station la plus en aval jusqu'à 0.2 dans la station la plus en amont. L'estuaire de l'Aulne présentait une communauté typiquement estuarienne. La station 1, la plus en aval, contenait la richesse spécifique la plus élevée, avec 9 espèces, dominées par *Haynesina germanica* (50%) et *Ammonia* spp. (33%), puis par plusieurs espèces d'*Elphidium* (*E. oceanense*, *E. williamsoni*, *E. margaritaceum*) ainsi que deux espèces porcelanées (*Quinqueloculina oblonga* et *Miliolinella subrotunda*). La station 2 présentait un peu moins d'espèces mais était toujours représentée par *H. germanica* (60%), *Ammonia* spp. (20%), *E. oceanense* (10%) et *Q. oblonga* (7%). Enfin la station 3 présentait la richesse spécifique la plus faible, composée uniquement de spécimens de *H. germanica* (94%) et *Ammonia* spp (6%). Parmi les spécimens d'*Ammonia* spp., la plupart des individus observés (~80-90%) étaient des *A. veneta*, mais quelques individus des deux autres espèces étaient aussi présents.

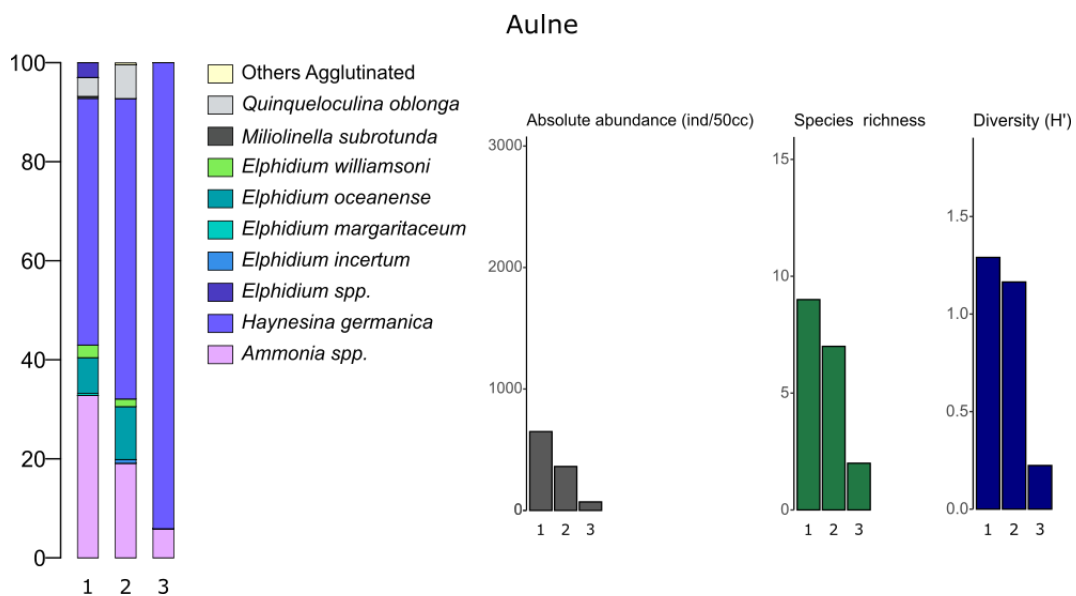


Figure V.10 : Estuaire de l'Aulne. A gauche, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. A droite, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

Dans l'estuaire de l'Odet (Figure V.11) la densité variait entre 500 (station 1) et 1300 (station 3) ind/50cc. La diversité variait peu, avec entre 9 et 12 espèces dans toutes les stations et des valeurs de H' variant de 0.7 à 1.8. Dans l'estuaire de l'Odet, les communautés des quatre sites étaient dominées par *H. germanica* (20 à 80%, diminuant vers l'amont), *Elphidium oceanense* (3 à 25%, augmentant vers l'amont), *Ammonia* spp. (10 à 30%), *E. williamsoni* (1 à 23%, augmentant vers l'amont) ainsi que quelques individus de *Quinqueloculina jugosa* (<5%) et *Quinqueloculina oblonga* (<2%). La station située la plus en amont contenait en plus des espèces agglutinées (*Miliammmina fusca*, *Ammotium salsum*, *Ammobaculites agglutinans*) qui atteignaient ~18% de l'assemblage. Les *Ammonia* spp. présentes dans l'estuaire de l'Odet étaient principalement des *A. veneta* (~80-90%), mais quelques individus de *A. aberdoveyensis* et *A. confertitesta* ont aussi été observés.

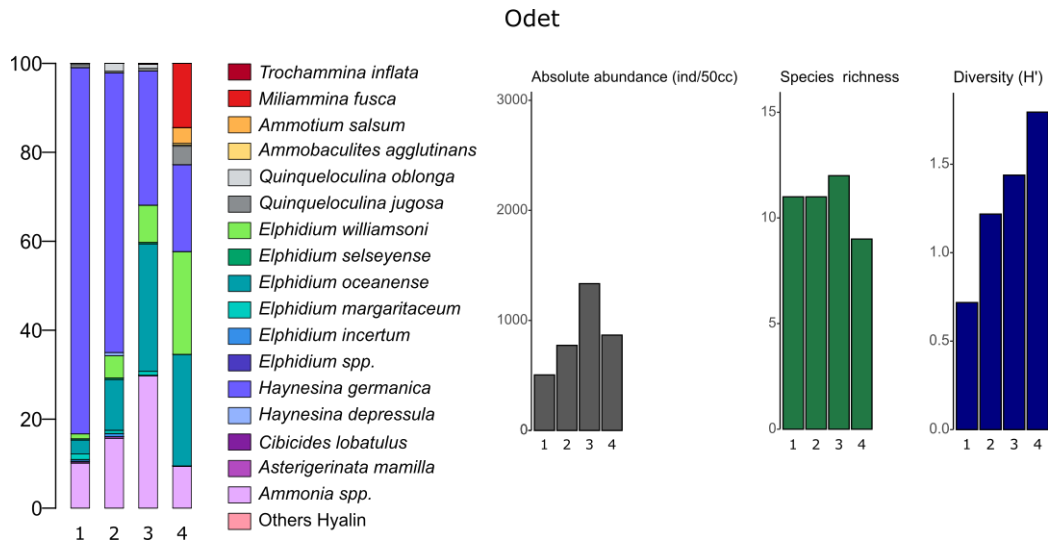


Figure V.11 : Estuaire de l'Odet. A gauche, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. A droite, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

Dans l'estuaire du Belon (Figure V.12), les densités observées étaient relativement faibles. Hormis la station 1 qui contenait 900 ind/50cc, toutes les stations contenaient moins de 500 ind/50cc. La station 5A présentait une abondance minimale avec seulement 3 individus de *H. germanica*. La richesse spécifique variait de 1 (station 5A) à 13 (station 1A) espèces et l'indice de Shannon variait de 0 à 1.7. Les communautés de foraminifères de l'estuaire du Belon étaient assez variables d'une station à l'autre. Dans les stations 3A et 4A, *H. germanica* était fortement dominante (82 – 86%) et était accompagnée d'*Ammonia* spp., *E. williamsoni*, *E. margaritaceum*, *E. oceanense*, *Quinqueloculina jugosa* et *Q. oblonga*. La communauté de la station 5B ressemble aux deux précédentes et ne contenait que trois espèces, une très large majorité d'individus de *H. germanica* (95%) accompagnés de quelques spécimens d'*E. williamsoni* et d'*Ammonia* spp. Ensuite, les stations 3B et 4B se démarquaient par une communauté composée d'un mélange d'espèces estuariennes (c.-à-d., *H. germanica* (18 – 50%), *Ammonia* spp. (7 - 11%), *E. williamsoni*, *Q. oblonga*, *Miliolinella subrotunda*), et d'espèces marines (c.-à-d., *Cibicides lobatulus* (22 - 54%), *Asterigerinata mamilla*, *E. crispum* (uniquement trouvé à la station 4B), et *Haynesina depressula* (dans la station 3B)). Les communautés présentes à ces stations ressemblaient quelque peu à celle de la station 1, située dans la partie aval. Cette station présentait la diversité la plus riche (S : 13 et H' : 1.7) avec les espèces marines *Haynesina depressula* (21%), *Asterigerinata mamilla*, *Elphidium margaritaceum* ainsi que les espèces estuariennes *Haynesina germanica* (39%), *Ammonia* spp., *E. oceanense*, *E. williamsoni*, *Miliolinella subrotunda*, *Quinqueloculina oblonga* (21%). Les stations 6A et 6B, situées en amont d'un barrage, contenaient principalement des espèces agglutinées tel que *Miliamina fusca* (72-77%) et *Ammobaculites agglutinans* (5-22%). A la station 6A, ces espèces étaient accompagnées de *H. germanica* (12%), *Elphidium incertum* (8%), et quelques individus de *E. williamsoni* et *Ammonia* spp. Les différentes espèces d'*Ammonia* spp. n'ont pas été étudiées pour cet estuaire.

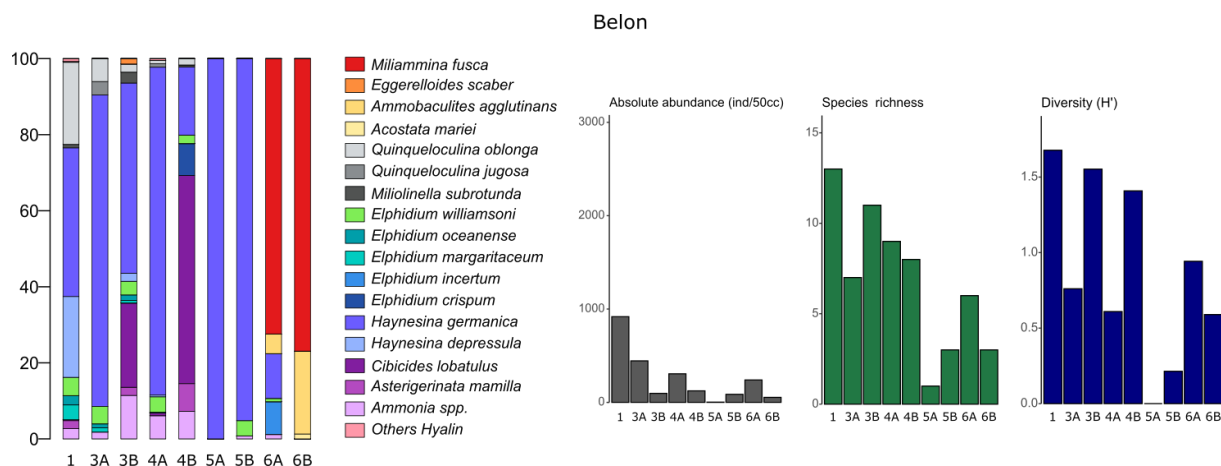


Figure V.12 : Estuaire du Belon. A gauche, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. A droite, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

Dans l'estuaire de la Laïta (Figure V.13), les densités faisaient partie des plus faibles du jeu de données, et variaient entre 60 (stations 1 et 3B) et 870 (station 2) ind/50cc. La richesse spécifique variait de trois (station 3B) à six (station 1) espèces. La valeur de l'indice de Shannon la plus élevée était observée à la station 1 (H' : 1.3). Les stations 2 et 3 présentaient une valeur minimale (H' : 0.5). Les communautés de l'estuaire de la Laïta étaient dominées par *Haynesina germanica* (37 à 85%, jusqu'à ~750 ind/50cc à la station 2) et *Elphidium williamsoni* (4 à 39%). Aux stations 2 et 3, ces espèces étaient accompagnées par *Ammonia* spp. (10 et 6% respectivement) et à la station 1 par *Asterigerinata mamilla* (15%) ainsi que *Cibicides lobatulus*, *Elphidium margaritaceum* (4%) et *Quinqueloculina oblonga* (3%). Les différentes espèces d'*Ammonia* spp. n'ont pas été étudiées pour cet estuaire.

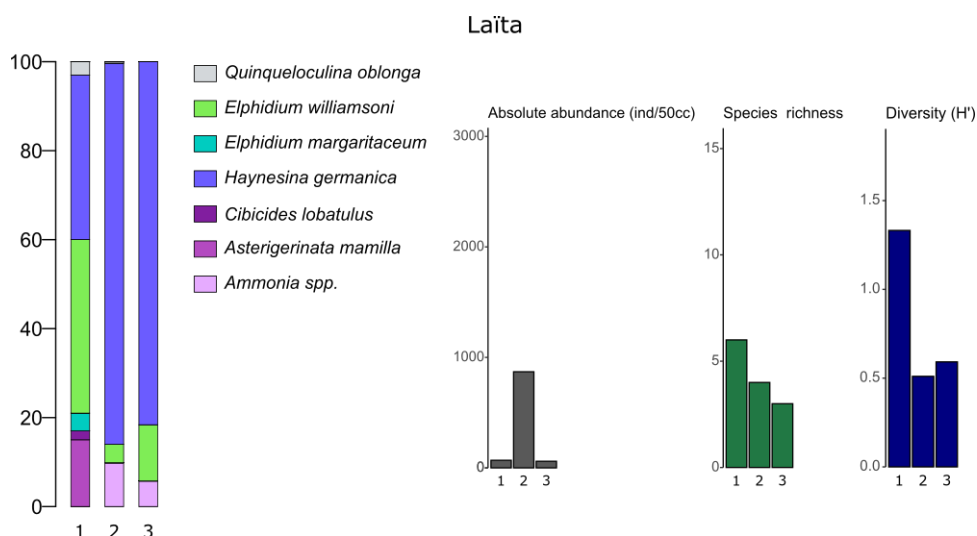


Figure V.13 : Estuaire de la Laïta. A gauche, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. A droite, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

Dans l'estuaire de Crac'h (Figure V.14), la densité variait entre 200 (station 2) et 900 (station 3) ind/50cc. La richesse spécifique variait de 6 (stations 2-3) à 11-12 (stations 1-4) espèces. Les valeurs de l'indice de Shannon, comprises entre 0.6 et 1.7, étaient maximales à la station 1, et minimales à la station 3. Les stations 2, 3 et 4 présentaient des communautés estuariennes typiques, contenant des *H. germanica* (45 à 84%), des *Ammonia* spp. (12 à 30%), des *Q. oblonga* (2 à 17%) et *Quinqueloculina jugosa* (1 à 9%). En plus de ces espèces, la station 4 montrait aussi de nombreux spécimens d'*Elphidium oceanense* (17%), ainsi que quelques individus d'espèces agglutinées (*Ammobaculites agglutinans*, *Ammotium salsum*, *Eggerelloides scaber* et *Haplophragmoides wilberti*). La station 1 située à l'embouchure présentait une communauté composée d'un mélange d'espèces marines, tels que *Haynesina depressula* (26%), *Ammonia beccarii* (1%), des espèces d'*Elphidium* (*E. incertum* (15%), *E. williamsoni* (3%), *E. margaritaceum* (2%)) et des espèces estuariennes comme *Haynesina germanica* (31%), *Ammonia* spp. (15%) et *Quinqueloculina oblonga* (6%). Les *Ammonia* spp. étaient principalement représentée par *A. veneta* (~50-90%), mais *A. aberdoveyensis* (~0-10%) et *A. confertitesta* (~0-45%) étaient présentes également, bien qu'en plus faibles quantités.

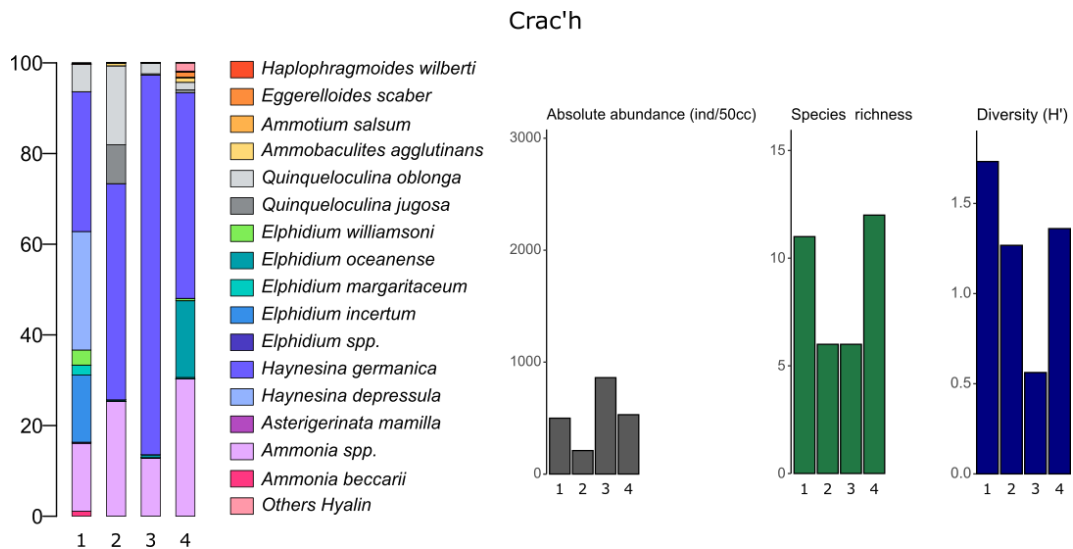


Figure V.14 : Estuaire de Crac'h. A gauche, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. A droite, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

Dans les échantillons récoltés dans l'estuaire d'Auray en juillet 2019 (Figure V.15), la densité variait entre 20-30 (station 2A et 2B) et ~1700 (station 1C) ind/50cc. Les trois réplicats avaient toujours des valeurs de densité comparables. La richesse spécifique variait de 1 (station 2A réplicats b et c) à 14 (station 1C réplicat a), et l'indice de Shannon était minimal dans les stations 2A et 5A ($H' \sim 0.1$) et maximal dans les stations 1B et 1C ($H' \sim 1.6$). A l'exception de la station 8B, les stations de l'estuaire d'Auray étaient toutes dominées par *Haynesina germanica* (40 à 100%). Cette espèce était toujours accompagnée par *Ammonia* spp. (0 à 25%, avec une dominance de *A. veneta*, quelques exemplaires de *A. aberdoveyensis* et de très rares individus de *A. confertitesta*). *Elphidium margaritaceum* (0 à 22%) et *Elphidium oceanense* (0 à 18%) étaient également présents, principalement au site 1. L'espèce *Haynesina depressula* n'était présente que dans les

stations situées en aval (1 à 4% aux stations 1B et 1C). Parmi les espèces porcelanées, *Quinqueloculina oblonga* était présente à presque toutes les stations, avec une tendance à augmenter vers l'aval. Elle ne représentait jamais plus de 6% de la communauté. A toutes les stations, des espèces agglutinées étaient présentes, incluant *Ammotium salsum*, *Ammobaculites agglutinans*, *Acostata mariei*, *Eggerelloides scaber*, et *Miliammina fusca*. Les agglutinées montraient une très forte augmentation vers la partie amont de l'estuaire, où la communauté trouvée à la station 8B était composée à 97% par des espèces agglutinées, principalement par *A. salsum*.

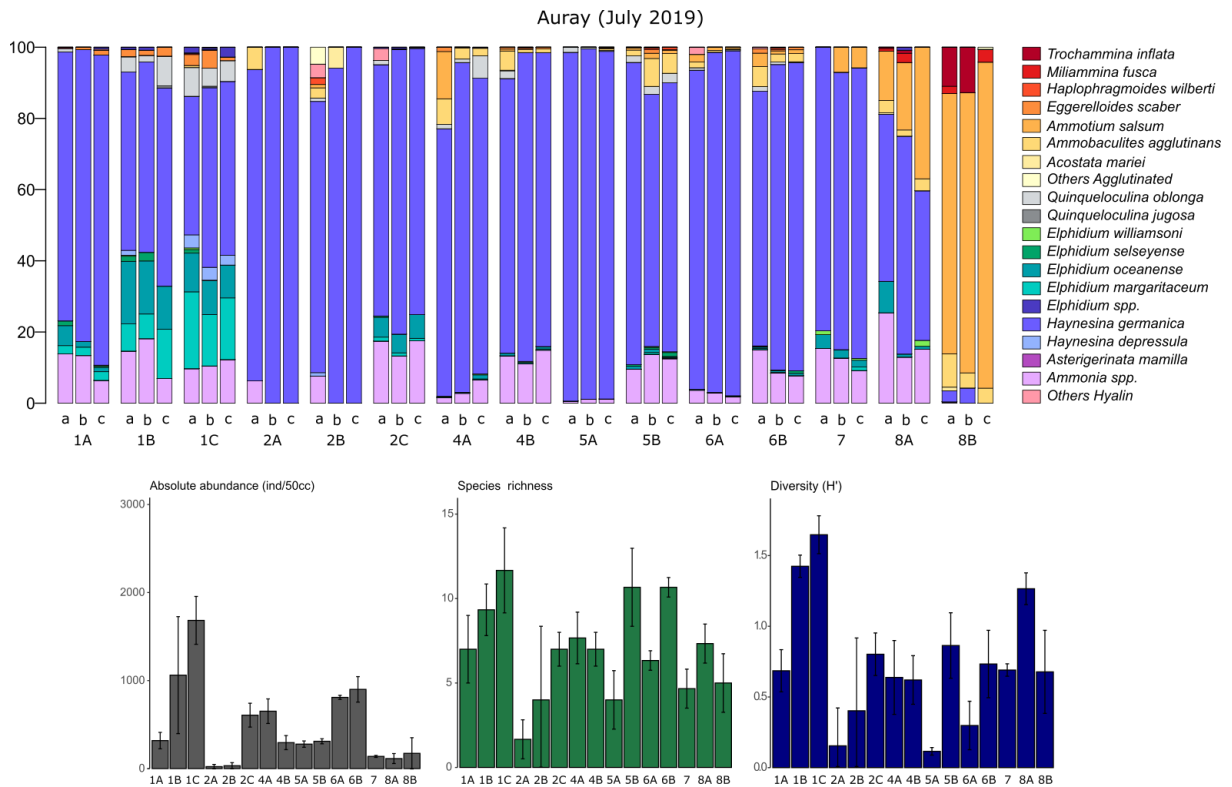


Figure V.15 : Estuaire d'Auray, campagne de juillet 2019. En haut, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. En bas, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu). Les trois replicats sont moyennés.

Concernant les échantillons récoltés dans l'estuaire d'Auray en septembre 2020 (Figure V.16), la densité variait entre 40 (station 4A) et 2560 ind/50cc (station 8B). La richesse spécifique était comprise entre 6 (station 2B) et 15 (station 1B, 1C et 4Ba) espèces. Les valeurs de l'indice de Shannon étaient assez stables et comprises entre 0.8 et 1.7. Les communautés étaient toutes composées principalement par *Haynesina germanica* (20 à 70%) et *Ammonia* spp. (0.3 à 30%). Les individus de l'espèce *Elphidium margaritaceum* étaient moins nombreux qu'en 2019 (0.3 à 2%). Des individus d'*Elphidium oceanense* (0 à 25%) étaient présents dans presque toutes les stations. Parmi les espèces porcelanées, *Quinqueloculina oblonga* (0-12%) était présente tout le long du gradient amont-aval, avec une tendance à augmenter vers l'aval. Elle était parfois accompagnée de *Quinqueloculina jugosa*, qui ne représentait pas plus de 4% de la communauté. Les espèces agglutinées, incluant *Ammotium salsum* (0 à 40%), *Ammobaculites agglutinans* (0 à 33%), *Acostata*

mariei (0 à 9%), *Eggerelloides scaber* (0 à 16%), et *Miliammina fusca* (0 à 20%), étaient bien plus nombreuses qu'en juillet 2019. Contrairement à juillet 2019, en septembre 2020 ces espèces n'étaient pas essentiellement confinées dans la partie amont mais étaient présentes tout le long du gradient amont-aval et notamment aux stations 2A et 2B. Concernant les espèces d'*Elphidium*, les *E. margaritaceum* étaient moins nombreux qu'en 2019. En revanche, les individus d'*E. oceanense* étaient plus nombreux en septembre 2020, et étaient répartis tout le long de l'estuaire, alors qu'ils étaient essentiellement localisés dans la partie aval en juillet 2019.

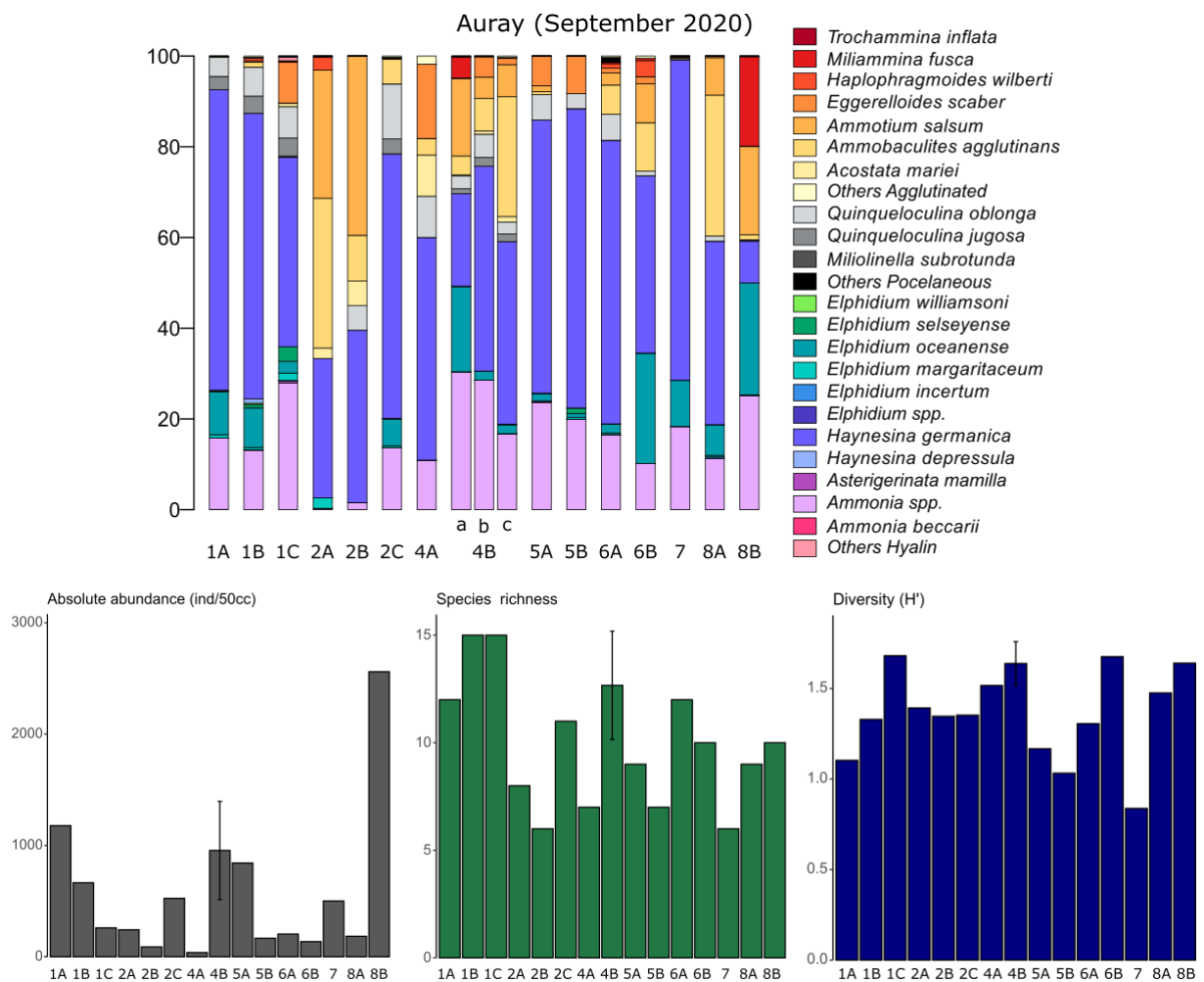


Figure V.16 : Estuaire d'Auray, campagne de septembre 2020. En haut, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. En bas, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

Dans les communautés récoltées dans l'estuaire de la Vilaine (Figure V.17), les densités variaient de 7 (station 3) à 3000 (station 1A) ind/50cc. Les valeurs de diversité spécifique étaient toujours très basses, entre 2-4 espèces par station et les valeurs de l'indice de Shannon en dessous de 1 (H' compris entre 0.2 et 0.8). Dans les stations de cet estuaire on observait une co-dominance d'*Ammonia* spp (33-97%) et *Haynesina germanica* (2-66%), excepté au site 2 où *H. germanica* était peu fréquente. Dans les stations 1A, 1C et 2A,

ces deux espèces principales étaient accompagnées de quelques individus d'*Elphidium oceanense* (0.6-0.8%) et *Elphidium selseyense* (0.3-3.4%). Dans cet estuaire, les *Ammonia* spp. appartenait presque exclusivement à l'espèce *A. confertitesta*.

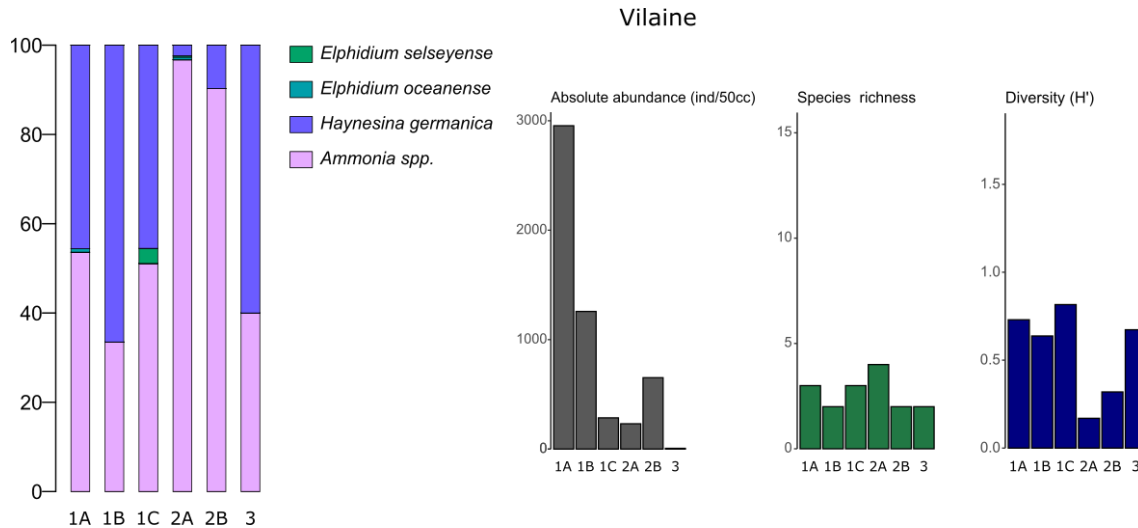


Figure V.17 : Estuaire de la Vilaine. A gauche, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. A droite, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

Dans les échantillons récoltés dans l'estuaire de la Vie en octobre 2018 (Figure V.18) la densité variait entre 50 (station 1) et 1800 (station 8A) ind/50cc. La richesse spécifique de cet estuaire fluctuait de 5 (station 10A) à 13 (station 3) espèces et l'indice de Shannon entre 0.3 (station 7A et 7B) et 1.8 (station 1). Les réplicats étaient assez similaires, tant en termes de densité que de composition spécifique. Les stations peuvent être divisées en trois groupes. Les stations portuaires 3, 4, 5, situées dans la partie aval, étaient principalement composées de *E. selseyense* (43 à 82%), accompagnée de *H. germanica* (9 à 48%) et *Ammonia* spp. (1 à 3.5%). Les communautés des stations 6, 7A, 7B, 7C, 8A, 8B, situées dans la partie intermédiaire de l'estuaire étaient essentiellement composées de *H. germanica* (63 à 94%) accompagnée de *Ammonia* spp. (4 à 27%) et *E. selseyense* (0 à 15%). Les assemblages des stations 9, 10A, 10B, situées le plus en amont, étaient dominées par *Ammonia* spp. (28 à 83%) accompagnée de *H. germanica* (34 à 70%). Bien que peu abondantes, les *Quinqueloculina* (*Q. oblonga* et *Q. jugosa*) étaient présentes à toutes les stations et représentaient entre 1 et 10% de l'assemblage. Plusieurs espèces d'*Elphidium* étaient présentes tout le long de l'estuaire (c.-à-d., *E. oceanense* (0 à 9%), *E. magellanicum* (0 à 6%), *E. williamsoni* (0 à 3%), *E. margaritaceum* (0 à 1%)). Deux stations, situées à l'embouchure, montraient des communautés très différentes des autres. La station 1 présentait une communauté assez riche (15 espèces) composée d'espèces représentatives d'un environnement marin comme les *Ammonia beccarii* (8 à 19%), *Cibicides lobatulus* (<1%) et plusieurs espèces d'*Elphidium* (p.ex., *E. crispum* (<6%), *E. magellanicum* (<6%), *E. macellum* (<1%), *E. incertum* (<2%)). Cependant des espèces estuariennes comme *Haynesina germanica* (20 à 30%),

Ammonia spp. (<3%), et *Elphidium selseyense* (35 à 50%) étaient aussi présentes à cette station. La station 2 présentait un assemblage atypique, avec une communauté fortement dominée par une seule espèce, *Elphidium incertum*, qui représentait 86-90% de l'assemblage. Les *Ammonia* spp. trouvées dans l'estuaires de la Vie étaient composées de *A. veneta* et *A. confertitesta* à part, égale ainsi que de rares exemplaires de *A. aberdoveyensis* (~10%). Les espèces agglutinées étaient quasiment absentes de cet estuaire.

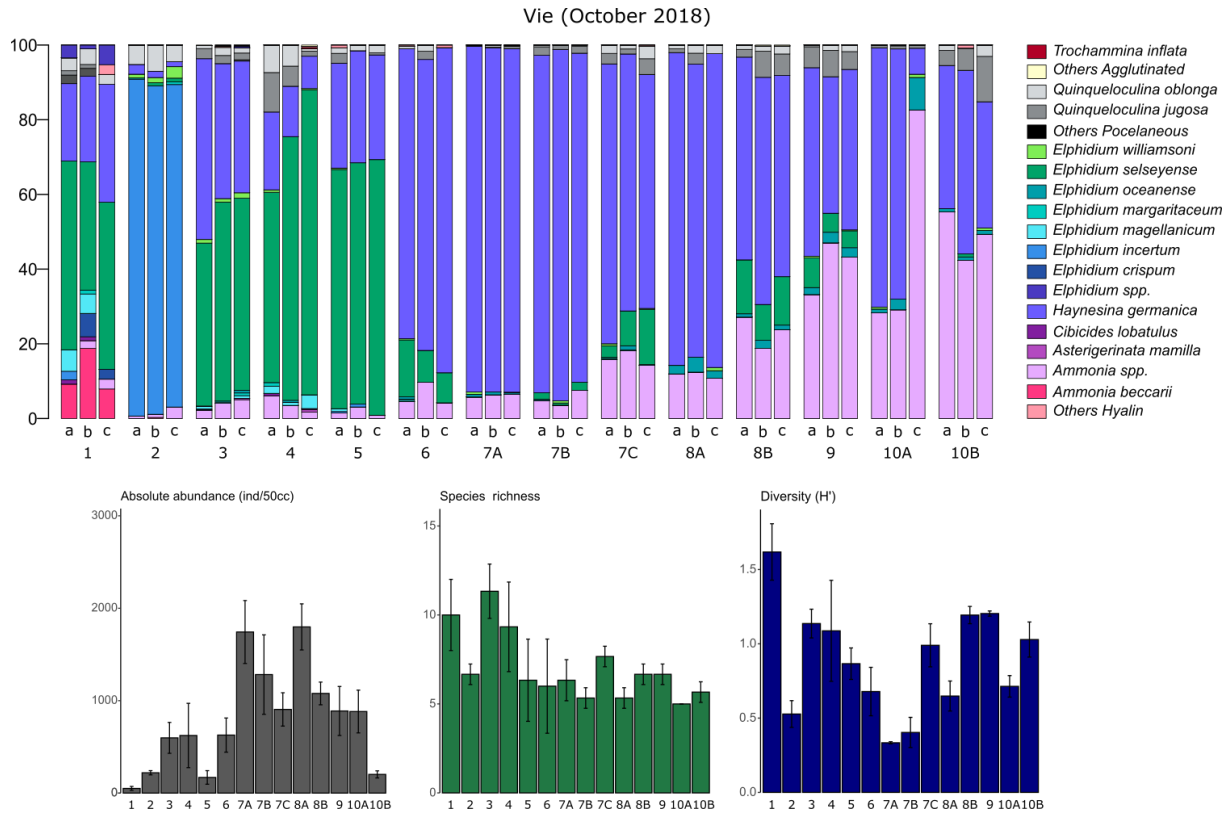


Figure V.18 : Estuaire de la Vie, campagne d'octobre 2018. En haut, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. En bas, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu). Les trois réplicats sont moyennés.

Dans les échantillons récoltés dans la Vie en juin 2020 (Figure V.19) la densité variait entre 300 (station 10A) et 2000 (stations 4, 8B, 9) ind/50cc. La richesse spécifique variait de 3 (station 10A) à 10 (station 9) espèces. Concernant l'indice de Shannon, un groupe de stations présentaient des valeurs autour de 0.5 (stations 2, 4, 7A, 8A, 8B, 9, 10A, 10B) tandis que les autres stations (3, 5, 7B, 7C) présentaient un H' autour de 1. Les communautés des stations portuaires 3, 4, 5, étaient très majoritairement représentées par *E. selseyense* (52 à 87%), accompagnée de *H. germanica* (9 à 35%) et *Ammonia* spp. (2 à 14%). Les assemblages des stations des parties intermédiaires et amont de l'estuaire (7A à 10B) étaient toutes dominées par *H. germanica* (63 à 92%). *E. selseyense* était encore présente dans les stations 7B et 7C (16 et 20%) mais disparaissait progressivement vers les stations situées le plus en amont. Les *Ammonia* spp. étaient présentes tout le long de l'estuaire et représentaient entre 2 et 15% de la communauté. Les espèces porcelanées (*Q. oblonga* et *Q. jugosa*) étaient très peu présentes dans l'ensemble de l'estuaire et représentaient moins de 2%

de l'assemblage. La station 2 présentait à nouveau un assemblage atypique avec une communauté dominée par une seule espèce, *Elphidium incertum*, qui représentait 87% de l'assemblage.

Lors de la campagne d'octobre 2018, les stations situées en amont (stations 9, 10A et 10B) contenaient un grand nombre d'individus d'*Ammonia* spp., ce qui n'était pas le cas en juin 2020 (en moyenne 280 ind/50cc, soit ~45% de la communauté en 2018 contre 90 ind/50cc, soit ~10% de la communauté en 2020). Comme pour la campagne d'octobre 2018, quelques spécimens agglutinés, appartenant aux espèces *Trochammina inflata* et *Entzia macrescens*, ont été observés dans les échantillons de juin 2020, mais ces espèces ne dépassaient pas 1% de la composition de l'assemblage.

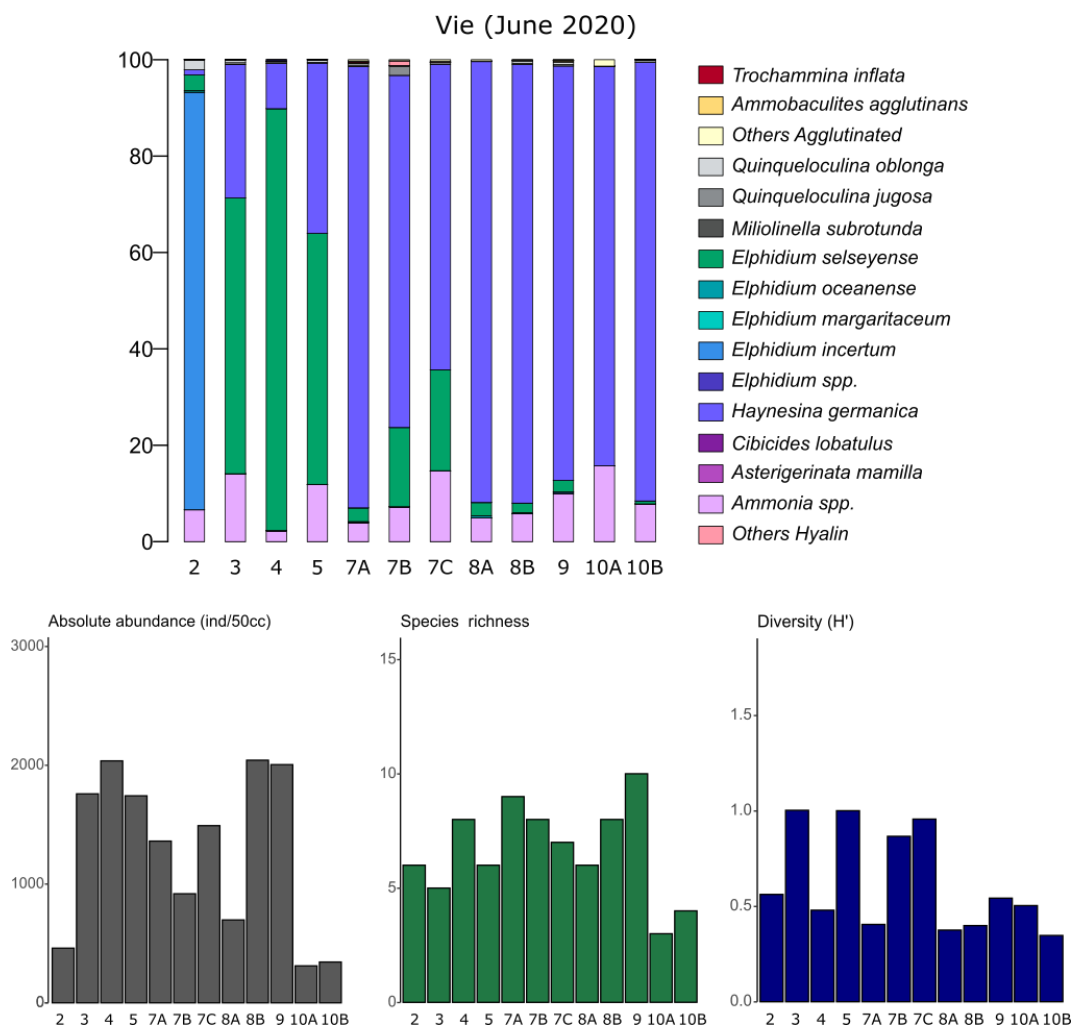


Figure V.19 : Estuaire de la Vie, campagne de juin 2020. En haut, l'histogramme représente la composition des assemblages de foraminifères benthiques exprimés en abondances relatives dans chacun des échantillons. En bas, les histogrammes représentent les densités absolues (gris), la richesse spécifique (vert) et l'indice de diversité de Shannon (bleu).

3.3. Relation entre les foraminifères et les paramètres environnementaux

3.3.1. Relation entre l'abondance et la diversité avec les paramètres environnementaux.

Les Figures V.20 et le Tableau V.3 présentent les corrélations entre les assemblages de foraminifères et les paramètres environnementaux. Les 13 stations contenant moins de 40% de silts (voir Figure V.2) ont été écartées de ces analyses. Ces stations montrent des relations particulières entre granulométrie et matière organique et sont souvent caractérisées par des communautés de foraminifères très différentes des autres, avec plus d'espèces marines.

L'abondance présente une corrélation positive avec la distance à la mer normalisée et le MII, avec des densités plus importantes vers l'aval. L'abondance affiche une corrélation négative plus faible ($R = -0.34$) avec le %TOC, ce qui peut être partiellement expliqué par le fait que la distance à la mer normalisée est négativement corrélée au %TOC.

La richesse spécifique montre des corrélations positives avec les trois paramètres constituant le MII (distance à la mer, émergence et décharge fluviale normalisées) et le $\delta^{13}C$ ainsi qu'une corrélation négative avec le % de grains $<63\mu m$. Pour l'indice H' les résultats sont très semblables, à l'exception d'une plus faible corrélation avec la distance à la mer. Ces résultats indiquent que la diversité a une forte tendance à augmenter de l'amont vers l'aval (avec une influence marine plus importante), et à diminuer quand les sédiments sont plus argileux. Il est à noter que la diversité ne montre pas de corrélation significative avec le %TOC.

Tableau V.3: Matrice de corrélation basée sur un test de Spearman entre les paramètres environnementaux (la distance à la mer normalisée, la durée d'émergence normalisée, RD/CS normalisé, le MII, le pourcentage de silts et d'argiles, le TOC, le rapport C/N et le rapport $\delta^{13}C$) et les paramètres biologiques (abondance, indice de Shannon, richesse spécifique). Les chiffres indiquent le coefficient R la couleur le niveau de significativité. 13 stations caractérisées comme trop sableuses (<40% de silts) ont été écartées de ces analyses.

	Distance normalised	Emergence normalised	RD/CS normalised	MIII	Silt and Clay	TOC	C/N	$\delta^{13}C$
Abundance	0.42	0.11	0.05	0.39	-0.09	-0.34	-0.06	0.23
S	0.34	0.26	0.51	0.53	-0.34	-0.03	0.15	0.50
H'	0.09	0.33	0.37	0.32	-0.43	-0.11	0.10	0.15

R Spearman p-value

	ns		<0.1		<0.05		<0.01
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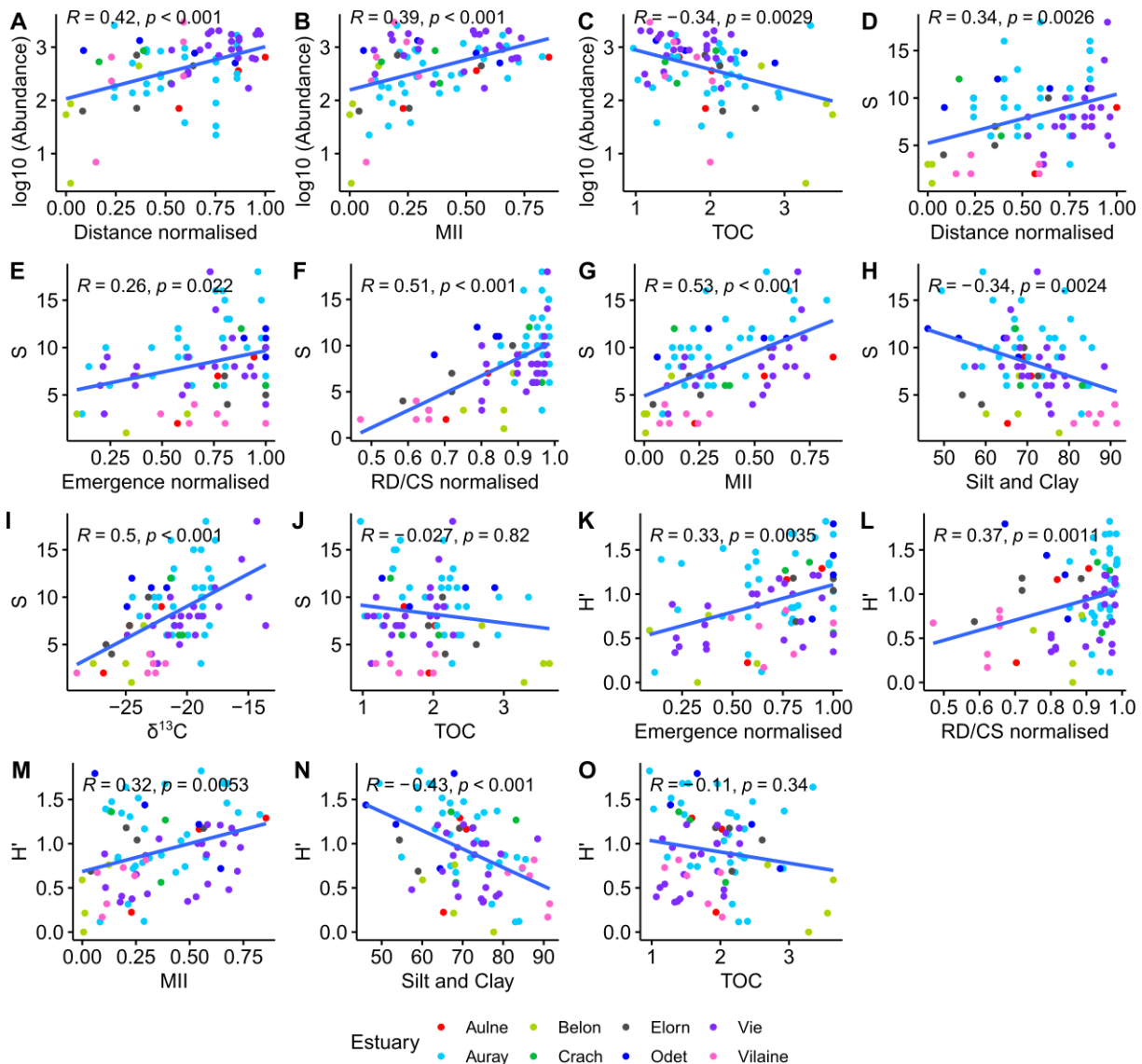


Figure V.20 : Relations entre l'abondance transformée en logarithme, la richesse spécifique (S), l'indice de diversité de Shannon (H') avec les paramètres environnementaux. Seules les relations discutées dans le texte sont présentées ici. Treize stations caractérisées par un sédiment plus sableux (<40% de silts) ont été écartées de ces analyses. Tous les coefficients de corrélations et valeurs de probabilité sont basés sur des corrélations de Spearman.

3.3.2. Relation entre les principales espèces et les paramètres environnementaux

Le Tableau V.4 synthétise les résultats des tests de corrélations de Spearman entre les abondances relatives des principales espèces et les paramètres environnementaux. Ici encore, les 13 stations contenant moins de 40% de silts ont été écartées de ces analyses. Les corrélations de Spearman cherchent à mettre en évidence des relations monotones, qui ne sont pas nécessairement linéaires. Néanmoins, des relations « en cloche », où une espèce a un optimum à une certaine valeur de la variable explicative, ne sont pas mises en évidence par cette méthode.

Les figures V.21 à V.23 montrent la nature de ces relations pour les corrélations significatives. Ces graphes montrent une courbe de régression linéaire ou exponentielle en fonction de la tendance observée. Dans la

majorité des plots, on observe que la corrélation est basée sur assez peu de points, et qu'il y a beaucoup de points à valeur zéro. Les points zéro peuvent avoir deux causes : 1) soit ils représentent des stations dans un estuaire où l'espèce est présente, mais où les conditions de la station ne sont pas favorables à son développement, ou 2) des estuaires où l'espèce n'est pas présente. Cette dernière possibilité traduit la forte spécificité des assemblages de foraminifères des estuaires étudiés, avec certaines espèces présentes uniquement dans quelques estuaires.

Les *Ammonia* spp. montrent uniquement une corrélation positive avec le temps d'émergence normalisé, ce qui indique que la densité relative de ce taxon a tendance à diminuer vers le haut de la vase (Figure V.21A). *Haynesina germanica* montre uniquement une corrélation significative positive avec le pourcentage de grains <63µm, traduisant une préférence pour des sédiments plus fins (Figure V.21B).

Les espèces *Elphidium selseyense*, *E. margaritaceum*, *Quinqueloculina oblonga* et *Q. jugosa* présentent toutes des corrélations positives avec la distance à la mer normalisée, RD/CS normalisée (ainsi qu'avec le MII) et le $\delta^{13}\text{C}$ (Figure V.22). En effet, la corrélation des trois premiers paramètres est surtout due à une forte augmentation des espèces concernées à des valeurs très élevées du paramètre explicatif. Ces espèces semblent donc uniquement proliférer dans des conditions d'influence marine maximale. *Quinqueloculina oblonga* et *E. margaritaceum* affichent également une corrélation négative avec le pourcentage de grains <63µm, suggérant une tolérance amoindrie pour des sédiments très vaseux. Il est important de noter que les relations concernant *E. margaritaceum* et *E. selseyense* sont principalement dues à quelques stations situées dans un seul estuaire (estuaire de la Vie pour *E. selseyense* et Auray pour *E. margaritaceum*).

Les espèces *E. oceanense* et *E. williamsoni* ne suivent pas ces tendances. *Elphidium oceanense* montre, tout comme les *Ammonia* spp., une corrélation positive avec l'émergence normalisée, suggérant une préférence pour le bas des vasières (Figure V.21C).

Les espèces agglutinées *Ammotium salsum* et *Miliammina fusca* montrent une corrélation négative avec la distance à la mer normalisée (et avec la MII pour *A. salsum*), indicatrice de leur habitat dans la partie amont dans les estuaires. *Miliammina fusca* montre également une corrélation négative avec le pourcentage de grains <63µm, reflétant une présence plus conséquente sur des sédiments plus sableux (Figure V.23).

Tableau V.4: Matrice de corrélation basée sur un test de Spearman entre les paramètres environnementaux (la distance à la mer normalisée, le temps d'émergence normalisé, RD/CS normalisé, le MII, le pourcentage de silts et d'argiles, le TOC, le rapport C/N et le rapport $\delta^{13}C$) et les espèces principales. Les chiffres indiquent le coefficient R, et la couleur le niveau de significativité. 13 stations caractérisées comme trop sableuses (<40% de silts) ont été écartées de ces analyses.

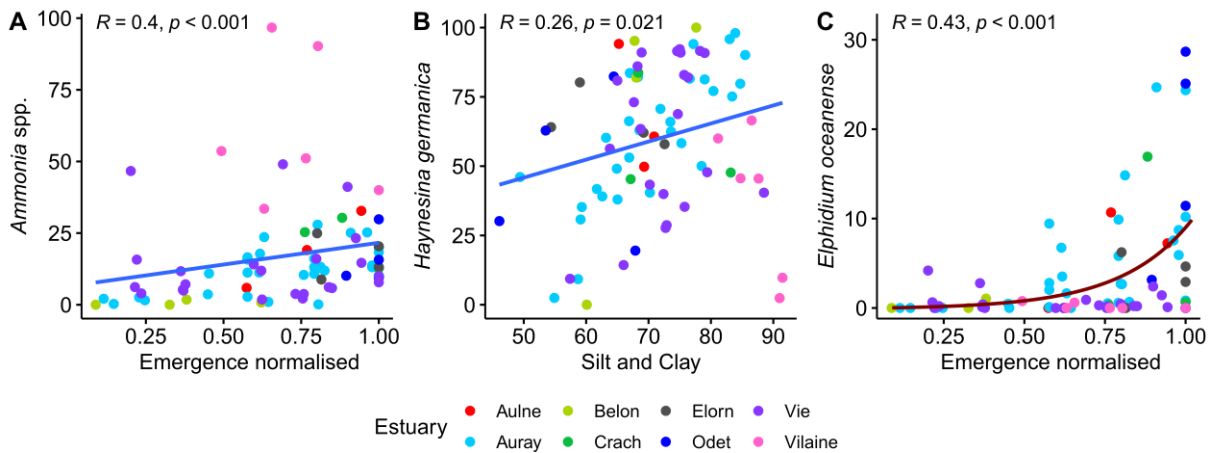
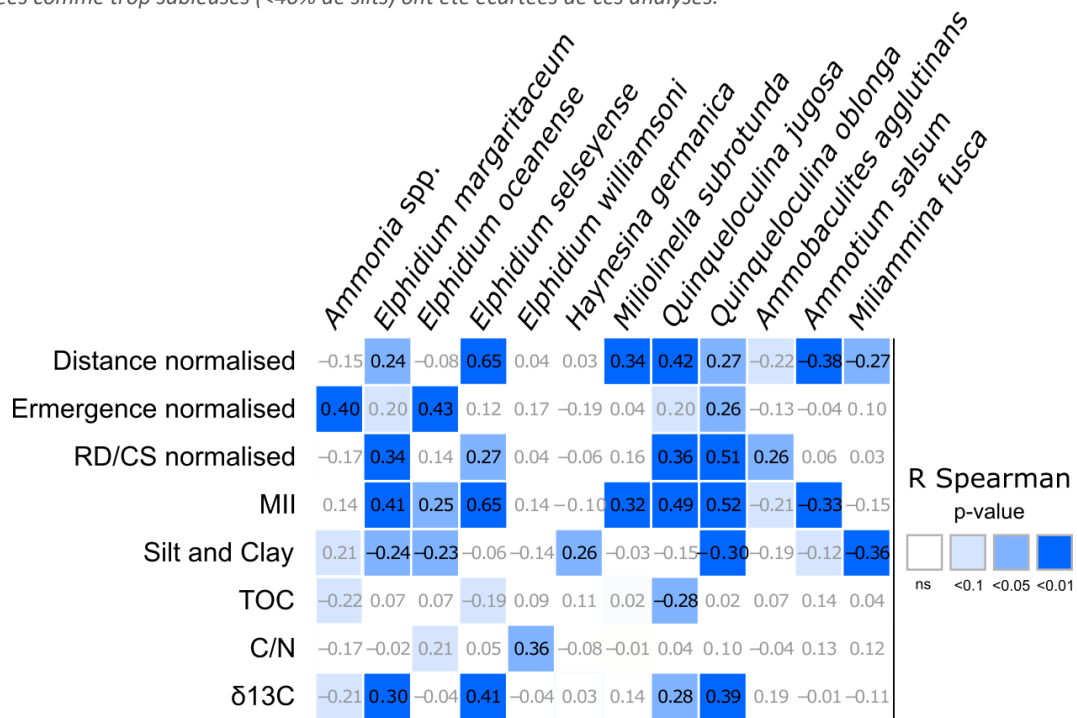


Figure V.21 : Relations entre l'abondance relative des espèces Ammonia spp., Haynesina germanica, Elphidium oceanense avec les paramètres environnementaux. Seules les relations discutées dans le texte sont présentées ici. Les 13 stations caractérisées par un sédiment plus sableux (<40% de silts) ont été écartées de ces analyses. Les courbes de régression basées sur un modèle linéaire sont en bleues, celles sur un modèle exponentiel sont en brun foncé. Les résultats des tests de corrélations sont basés sur des tests de Spearman.

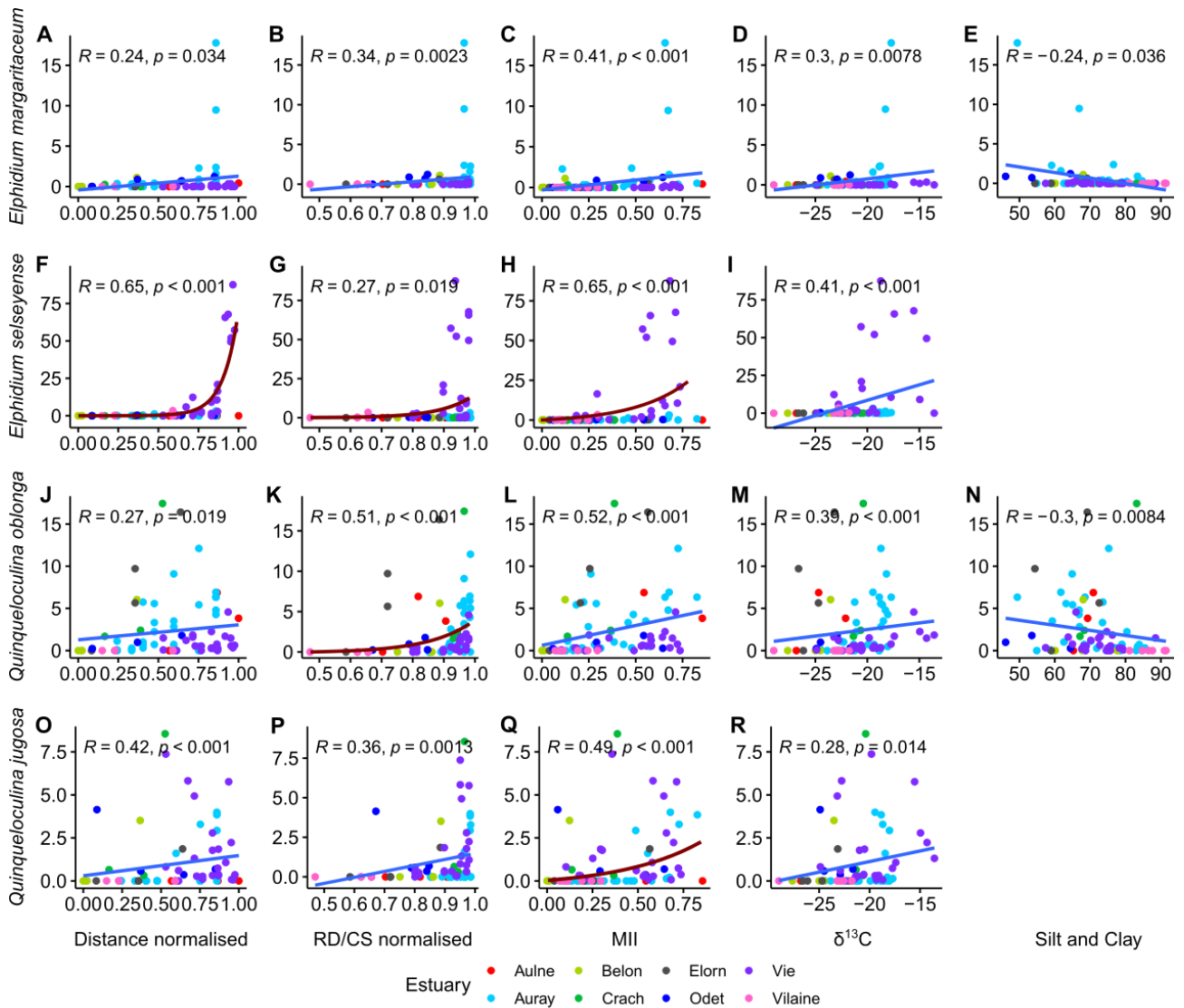


Figure V.22 : Relations entre l'abondance relative des espèces *Elphidium margaritaceum*, *E. selseyense*, *Quinqueloculina oblonga* et *Q. jugosa* avec les paramètres environnementaux. Seules les relations discutées dans le texte sont présentées ici. Les 13 stations caractérisées par un sédiment plus sableux (<40% de silts) ont été écartées de ces analyses. Les courbes de régression basées sur un modèle linéaire sont en bleues, celles sur un modèle exponentiel sont en brun foncé. Les résultats des tests de corrélations sont basés sur des tests de Spearman.

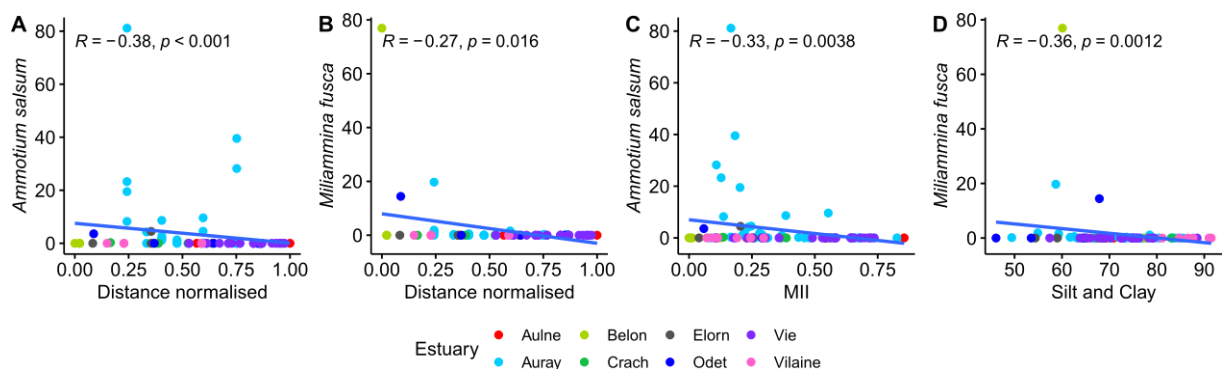


Figure V.23 : Relations entre l'abondance relative des espèces *Ammotium salsum* et *Miliammina fusca* avec les paramètres environnementaux. Seules les relations discutées dans le texte sont présentées ici. Les 13 stations caractérisées par un sédiment plus sableux (<40% de silts) ont été écartées de ces analyses. Les résultats des tests de corrélations sont basés sur des tests de Spearman.

3.3.3. Analyse en Composantes Principales

Afin de visualiser la répartition des espèces de foraminifères dans les différents estuaires en fonction des paramètres environnementaux, une ACP non standardisée, basée sur les abondances relatives (non normalisées) a été réalisée. L'application d'un modèle « broken stick » aux eigenvalues (Tableau V.6) suggère que les trois premiers axes sont significatifs. Ensemble, ces axes expliquent 78.5% de la variance totale (Figure V.24). Les loadings des espèces sur les trois premiers axes sont donnés dans le Tableau V.7.

Sur l'axe 1, qui explique 47.0% de la variabilité, le côté positif est fortement dominé par *Haynesina germanica*, tandis qu'*Ammonia spp.* et *Elphidium selseyense* se positionnent sur le côté négatif de cet axe. Sur l'axe 2, qui explique 18.5%, les *Ammonia spp.* sont du côté positif, opposées à *E. selseyense* qui est du côté négatif. Finalement, l'axe 3, qui explique encore 12.9%, est occupé par les espèces agglutinées *Miliammina fusca*, *Ammotium salsum* et *Ammobaculites agglutinans* du côté positif. Du côté négatif, *Elphidium selseyense*, *Ammonia spp.* et *Haynesina germanica* contribuent le plus.

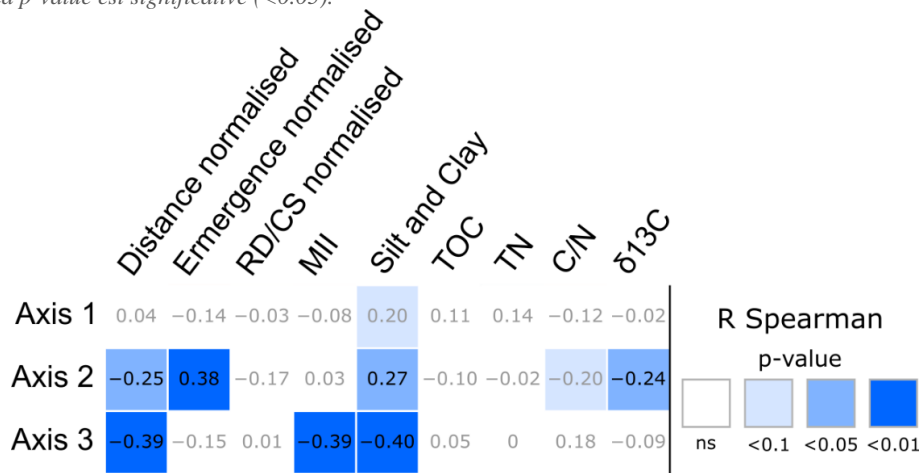
L'axe 1 est totalement dominé par les trois taxa les plus abondants sur les vasières estuariennes. Dans le Tableau V.5 on peut observer que cet axe n'est corrélé avec aucun paramètre environnemental. Par contre, l'axe 2 montre une corrélation positive avec l'émergence normalisée et le pourcentage de silts et d'argiles et une corrélation négative avec la distance à la mer. En effet, il a déjà été noté que les *Ammonia spp.* sont moins abondantes en haut des vasière (Figure V.24), tandis que *E. selseyense* apparaît surtout dans la partie aval des estuaires.

L'axe 3 est chargé positivement par les trois espèces agglutinées indicatrices de la partie amont des estuaires. Cet axe est corrélé négativement avec la distance à la mer normalisée. L'axe 3 montre aussi une corrélation négative avec le pourcentage de silts et d'argiles.

Dans le biplot des axes 1 et 2 (Figure V.24A) un premier groupe de stations (groupe I) est situé sur la partie positive, où la communauté est largement dominée par *Haynesina germanica*. En opposition, les stations du groupe II, situées dans le quadrant en haut à gauche, montrent un plus fort pourcentage d'*Ammonia spp.* Le cas extrême est trouvé aux stations Vilaine 2A et 2B (96 et 90% d'*Ammonia spp.*). Le groupe III, en bas à gauche, réunit des stations avec une forte dominance d'*Elphidium selseyense*. Ces stations se trouvent toutes dans l'estuaire de la Vie. Le groupe IV, situé à gauche du centre, contient des stations qui ne sont pas dominées par une des trois espèces mentionnées. Finalement, le groupe V, avec des valeurs négatives sur l'axe 1, regroupe certaines stations avec de faibles pourcentages de *Haynesina germanica*. Ce groupe, qui paraît hétérogène, regroupe les stations riches en espèces agglutinées (Belon 6A, 6B, Auray_{2019/2020} 8B) ainsi que la station Vie_{2018/2020} 2 avec une communauté fortement dominée par *Elphidium incertum*.

Sur le biplot des axes 2 et 3 (Figure V.24B), la distinction entre les groupes II, III et V est encore plus explicite. On voit maintenant que le groupe II, qui réunit des stations riches en *Ammonia* spp. est surtout lié à l'estuaire de la Vilaine. Le groupe III, constitué des stations avec un pourcentage élevé d'*Elphidium selseyense*, contient des stations de l'estuaire de la Vie. Finalement, dans le groupe V, caractérisé par un pourcentage plus élevé d'espèces agglutinées, se trouvent des stations des estuaires d'Auray et du Belon. Ainsi cette analyse montre une très forte spécificité des communautés des différents estuaires et met en évidence l'importance de la biogéographie.

Tableau V.5: Matrice de corrélation basée sur un test de Spearman entre les paramètres environnementaux (le MII, la distance à l'embouchure normalisée, la durée d'immersion, le débit du fleuve rapporté à la surface, le pourcentage de silts et d'argiles, le TOC, le TN, le rapport C/N et le rapport $\delta^{13}C$) et les axes significatifs de l'ACP. Les chiffres indiquent le coefficient R pour les corrélations dont la p-value est significative (<0.05).



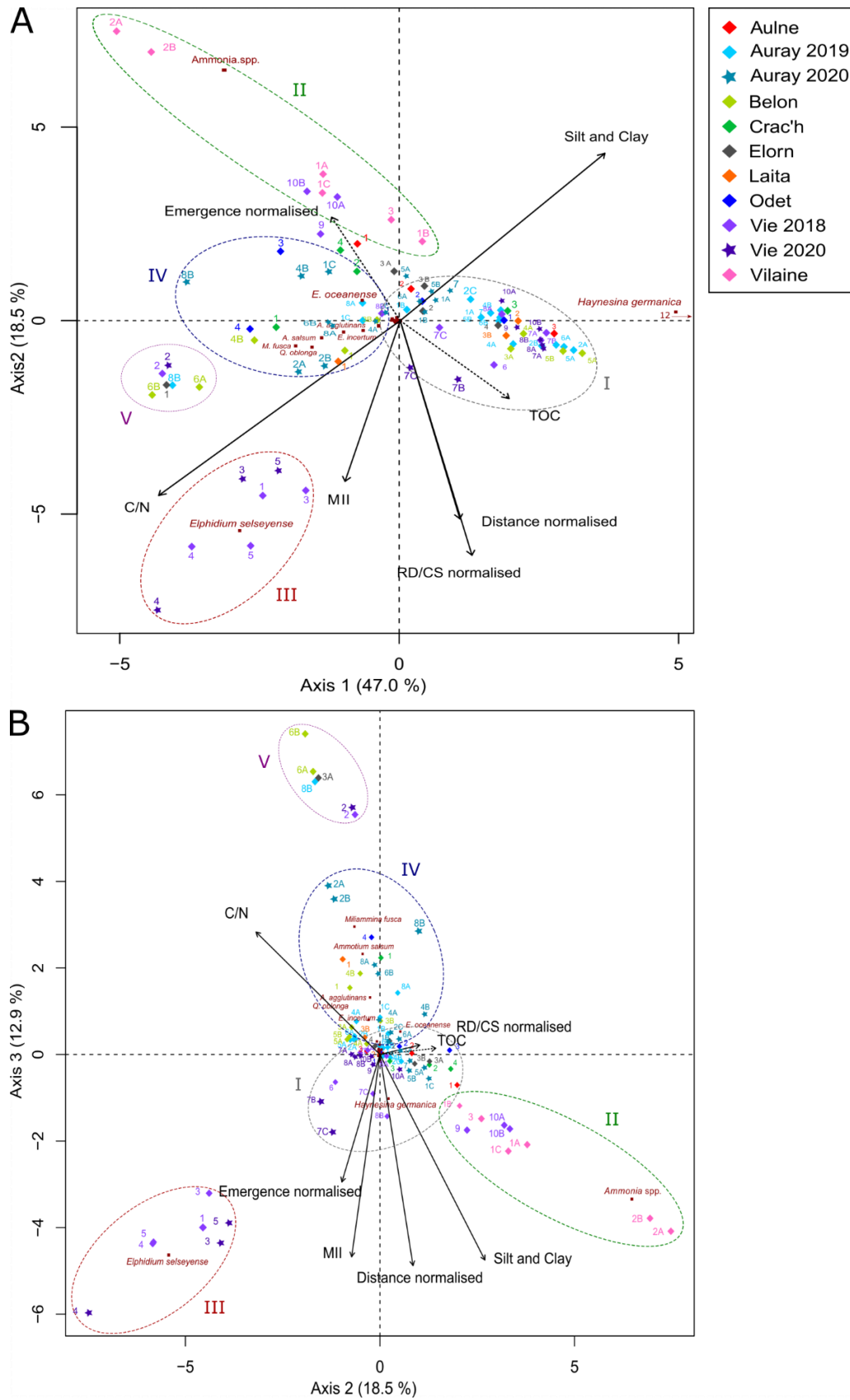


Figure V.24 : ACP basée sur les abondances relatives de foraminifères. A : axes 1 et 2, B : axes 2 et 3. L'analyse est non contrainte ; les paramètres environnementaux ne contribuent pas à la construction du plan axial ; leur corrélation avec les axes a été calculé après l'analyse. Les paramètres non significatifs sont indiqués en pointillé. Les ellipses correspondent aux groupes discutés dans le texte.

Tableau V.6: Eigen values et proportion expliquée des axes de la ACP basée sur les abondances relatives.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalues	857.21	337.59	236.17	122.86	95.26	57.34
Proportion expliquée	0.4704	0.1853	0.1296	0.0674	0.0522	0.0314

Tableau V.7: Scores des espèces sur l'ACP basée sur les abondances relatives.

	PC1	PC2	PC3
<i>Ammonia beccarii</i>	0,00	0,00	0,00
<i>Ammonia</i> spp.	-3,13	6,47	-3,34
<i>Asterigerinata mamilla</i>	-0,06	-0,02	0,04
<i>Aubignyna perlucida</i>	0,01	0,01	0,00
<i>Cibicides lobatulus</i>	-0,39	-0,08	0,30
<i>Discorbis vilerboana</i>	0,00	-0,01	0,00
<i>Elphidium crispum</i>	-0,06	-0,01	0,04
<i>Elphidium decipiens</i>	0,00	0,01	0,00
<i>Elphidium gerthi</i>	-0,01	0,01	-0,01
<i>Elphidium incertum</i>	-0,99	-0,30	0,80
<i>Elphidium magellanicum</i>	-0,02	-0,04	-0,03
<i>Elphidium margaritaceum</i>	-0,04	0,00	0,09
<i>Elphidium oceanense</i>	-0,66	0,52	0,53
<i>Elphidium selseyense</i>	-2,85	-5,43	-4,63
<i>Elphidium williamsoni</i>	-0,07	-0,01	0,22
<i>Haynesina depressula</i>	-0,05	-0,04	0,10
<i>Haynesina germanica</i>	12,60	0,22	-1,02
<i>Miliolinella subrotunda</i>	-0,01	0,00	0,01
<i>Quinqueloculina jugosa</i>	-0,12	0,01	-0,16
<i>Quinqueloculina oblonga</i>	-0,13	0,04	0,11
<i>Acostata mariei</i>	-0,05	-0,03	0,13
<i>Ammobaculites agglutinans</i>	-0,64	-0,26	1,32
<i>Ammotium salsum</i>	-1,39	-0,44	2,33
<i>Eggerelloides scaber</i>	-0,04	0,08	0,04
<i>Entzia macrescens</i>	0,01	0,00	0,00
<i>Haplophragmoides wilberti</i>	-0,02	-0,01	0,05
<i>Miliammina fusca</i>	-1,85	-0,65	2,96
<i>Morulaepecta bulbosa</i>	0,01	0,00	0,00
<i>Trochammina inflata</i>	-0,04	-0,03	0,13

4. Discussion

Indications de pollution dans les estuaires étudiés.

Afin de détecter des possibles pollutions aux stations étudiées, nous avons mesuré deux paramètres : les éléments-traces métalliques et la matière organique dans les sédiments.

Concernant les éléments-traces métalliques, nous avons pu observer un enrichissement majeur de Cd, Pb et Ag dans l'Aulne, tandis que quelques stations situées dans plusieurs estuaires présentant des enrichissements modérés ou mineurs pour un ou plusieurs de ces métaux. Plus généralement, nous avons observé des enrichissements modérés/mineurs en Cu et Zn dans la majorité des stations. Ces enrichissements semblent être des artefacts de la méthode de normalisation, avec un sédiment de référence ayant des concentrations naturellement plus basses de ces métaux que les échantillons analysés. Comme tous les estuaires sont concernés, l'explication probable est que les enrichissements en Cu et Zn sont dus aux faibles teneurs de ces métaux dans la carotte de référence. Cependant on ne peut pas exclure entièrement que dans certains cas, les facteurs d'enrichissements modérés pourraient correspondre à des apports d'origine anthropique.

Dans l'Aulne, les valeurs de plomb atteignent 232 mg/kg. Les teneurs naturelles du sédiment en plomb sont estimées à environ 30mg/kg (Bakke et al., 2010) et dans notre carotte de référence, la valeur était de 36 mg/kg de Pb. Ainsi les valeurs de Pb dans l'Aulne sont largement supérieures aux teneurs naturelles ; les facteurs d'enrichissement sont compris entre 5 et 8, ce qui correspond à un enrichissement majeur. Bien que la pollution au plomb soit la plus marquée, cet estuaire présente aussi des enrichissements en argent, en cadmium et en zinc. La source probable de contamination métallique dans l'estuaire de l'Aulne est une ancienne exploitation minière de plomb et d'argent située à Huelgoat, à 100km en amont de l'estuaire. Bien que la mine ne soit plus en activité depuis 1934, le site n'a pas été nettoyé et continue de contaminer la rivière. Au pied de la zone de rejet, les concentrations en plomb excèdent 2 000 mg/kg dans le sédiment (Nasri et al., 2021). Ces derniers auteurs ont aussi mesuré le sédiment dans l'estuaire et ont trouvé des valeurs similaires aux nôtres.

Concernant les enrichissements modérés à mineurs, les stations Elorn 3A, 3B, 4 et Vilaine 3 montrent des enrichissements modérés en cadmium et cobalt (seulement pour Vilaine 3), de l'ordre de deux fois la valeur de référence. La station 3 de la Vilaine est située à proximité du barrage d'Arzal, au milieu d'un petit port de plaisance. L'Elorn est situé à proximité de l'Aulne, ces deux fleuves se jettent dans la rade de Brest. La rade de Brest accumule des pollutions en métaux liées à l'agriculture et à l'industrie, et cette zone a aussi subi des pollutions suite aux conflits militaires de la deuxième guerre mondiale (Siano et al., 2021).

Concernant le Cu et Zn, les enrichissements modérés observés aux stations Auray₂₀₁₉ 1A, Vie₂₀₁₈ 6 (pour le Cu), et Vilaine 3 (pour le Zn), qui dépassent largement ceux des autres stations de ces estuaires, pourraient

refléter une introduction anthropique, peut-être dus à l'utilisation des peintures anti-fouling dans ces zones portuaires (Schiff et al., 2004; Singh & Turner, 2009; Dafforn et al., 2011).

En résumé, seul l'estuaire de l'Aulne montre une pollution métallique majeure manifeste. Dans une moindre mesure, certaines stations de l'Elorn, la Vilaine, Auray et la Vie suggèrent une pollution d'origine anthropique. Une attention particulière sera portée aux communautés de foraminifères de ces stations afin de voir si ces pollutions avérées ou supposées ont un impact sur les communautés de foraminifères. Hormis ces stations, les estuaires étudiés ne semblent pas être soumis à des pollutions métalliques majeures.

Concernant les mesures de matière organique, les valeurs de TOC mesurées dans cette étude varient de 0.1 à 3.6% et sont comparables à celles trouvées dans des estuaires des côtes de la Manche (Francescangeli et al., 2020). Afin de détecter un écart aux teneurs naturelles de TOC dans le sédiment, nous avons mis en relation le TOC avec la granulométrie (Figure V.7). En effet, de nombreuses études montrent un rapport entre ces deux paramètres (p.ex., Pelletier et al., 2010; Bianchi et al., 2018). Sur la base de la relation entre TOC et granulométrie, nous avons identifié quatre stations (Auray 2020 8B et Belon 6B, 5B, 5A) avec un pourcentage élevé de TOC par rapport à leur pourcentage de silt et d'argiles. Ces stations sont toutes situées dans la partie amont des estuaires, proches d'infrastructures humaines, qui ont probablement transformé l'hydrodynamisme (stations situées proche d'un pont dans l'estuaire d'Auray, et proches d'un barrage dans l'estuaire du Belon). La conséquence est que la teneur en matière organique de ces sites est typique pour la partie amont de l'estuaire (avec des valeurs plus élevées que dans la partie aval) mais la granulométrie est localement plus grossière.

Concernant les valeurs de rapports C/N et $\delta^{13}\text{C}$, la plupart des stations montre un gradient de valeurs d'amont vers l'aval, reflétant le mélange progressif de matières organiques continentale et marine (Figure V.6). Les stations Belon 4B et Vilaine 3, ainsi qu'une partie des échantillons de la Vie (2018), ne suivent pas la tendance générale.

Pour la station Belon 4B, le $\delta^{13}\text{C}$ particulièrement élevé suggère une matière organique plutôt marine. Ces valeurs sont en accord avec les observations de granulométrie (sédiment sableux indiquant un fort hydrodynamisme) et de la composition des communautés de foraminifères avec une forte contribution des espèces marines. Il apparaît donc que ce site est régulièrement atteint par des apports marins, malgré sa position plutôt à l'intérieur de l'estuaire. Inversement, la station Vilaine 3, qui se situe en amont du barrage d'Arzal, a été échantillonnée quand le barrage était fermé, et nos mesures reflètent donc un environnement d'eau douce. Parmi les stations de la Vie, les stations 7C et 8A sont caractérisées par un $\delta^{13}\text{C}$ anormalement élevé. Ces stations sont situées à l'intérieur de l'estuaire, immédiatement en aval et en amont d'un centre d'épuration d'eau, respectivement. Ensuite, les stations 3 et 4, qui sont situées dans des zones portuaires, montrent un $\delta^{13}\text{C}$ et un rapport C/N élevé par rapport à leur position dans l'estuaire (Figure V.6). Dans ce cas, les valeurs atypiques pourraient donc refléter une introduction anthropique de matière organique. En

résumé, il semble qu'à l'exception des stations Vie 3, 4, 7C et 8A il n'y a pas d'indications pertinentes d'introductions anthropiques de matière organique.

En combinant les données des éléments-traces métalliques et de la matière organique, nous arrivons à la conclusion qu'à l'exception de la forte pollution en Pb, Cd et Ag dans l'Aulne, les estuaires étudiés sont peu pollués, et la répartition des communautés devrait plutôt refléter les paramètres naturels. Néanmoins, quelques valeurs de $\delta^{13}\text{C}$ et de C/N anormales suggèrent des introductions anthropiques de matière organique, qui pourraient avoir un impact sur les communautés de foraminifères. Par la suite, les communautés présentes à ces stations seront considérées avec un intérêt particulier.

Répartition des communautés de foraminifères le long du gradient estuarien

De nombreux auteurs ont étudié la succession des espèces de foraminifères dans les milieux estuariens. Toutefois, ces études reposent souvent sur l'étude d'un ou deux estuaires (p.ex., Hayward, 2014; Camacho et al., 2015; Alves Martins et al., 2019; Rodrigues et al., 2020; Francescangeli et al., 2021; Ye et al., 2021). En effet, les synthèses résumant la distribution des différents assemblages dans les milieux estuariens basées sur l'étude de plusieurs estuaires sont plus rares. Debenay et al. (2000) ont proposé plusieurs schémas de répartition pour différents types de zones de transition (c.-à-d., estuaire tropical microtidal, estuaire tropical mésotidal, estuaire tempéré méso à macrotidal, lagune méditerranéenne, lagune tropicale). Dans le cadre de notre étude, nous nous sommes intéressés aux vasières intertidales des estuaires tempérés méso- à macrotidaux. Le modèle proposé par Debenay (2000) pour ces milieux décrit une succession de quatre assemblages dans le chenal principal le long du gradient amont-aval (Figure V.25). Dans ce modèle, l'assemblage situé à l'embouchure est le plus diversifié, le deuxième est co-dominé par *Ammonia tepida* (*Ammonia* spp. dans cette étude) et *Haynesina germanica*, le troisième est dominé par *Haynesina germanica*, et enfin la partie la plus en amont présente des assemblages dominés par *Miliammina fusca*.

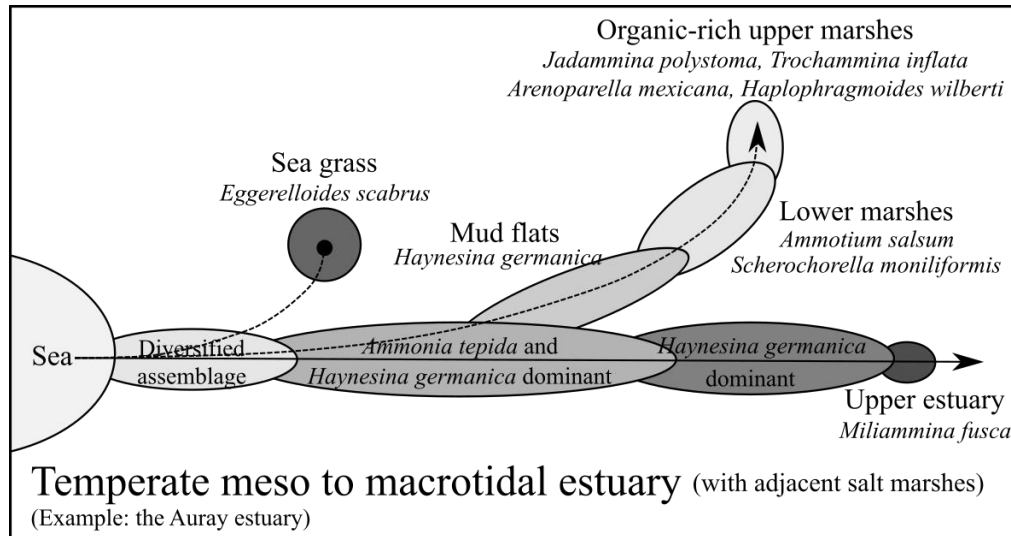


Figure V.25 : Schéma de la distribution des espèces de foraminifères le long des gradients estuariens. D'après Debenay et al., 2000

L'étude des assemblages récoltés pour notre étude présente des résultats similaires. Néanmoins, le plus grand nombre d'estuaires étudiés (trois pour Debenay, neuf dans notre étude) permet d'affiner ce modèle. Dans notre étude, les observations des communautés de foraminifères et la répartition des espèces sur l'ACP ont permis d'identifier quatre types de communautés de foraminifères dans les neuf estuaires étudiés (Figure V.26) :

1) une communauté typique des stations proches de l'embouchure, où la diversité est plus élevée, avec un mélange d'espèces estuariennes et marines (c.-à-d., *Ammonia beccarii*, *Elphidium crispum*, *Haynesina depressula*, *Asterigerinata mamilla*).

2) une communauté typique pour la partie aval de l'estuaire, dominée par *Elphidium margaritaceum*, *Elphidium selseyense*, et les espèces porcelanées *Quinqueloculina oblonga*, *Quinqueloculina jugosa* et *Miliolinella subrotunda*

3) une communauté du milieu de l'estuaire, qui est principalement composé d'*Haynesina germanica*, mais contient aussi *Ammonia* spp., *Elphidium oceanense* et *E. williamsoni*. Il est à noter qu'*Ammonia* spp. et *E. oceanense* sont plus nombreux dans les stations proches du chenal.

4) une communauté de la partie amont, qui est caractérisée par des abondances plus élevées d'espèces agglutinées (*Ammotium salsum*, *Miliammina fusca*, *Ammobaculites agglutinans* et *Trochammina inflata*).

Néanmoins, toutes les espèces typiques pour certaines zones estuariennes n'étaient pas présentes dans tous les estuaires. En effet, chaque estuaire présentait une succession semblable, mais pas toujours avec exactement les mêmes espèces. Par exemple, *Elphidium selseyense* était dominante dans la partie aval de l'estuaire de la Vie, mais presque absente dans les autres estuaires, où *E. margaritaceum* était parfois

fréquente dans cette partie de l'estuaire. Cela traduit une forte spécificité faunistique des neuf estuaires étudiés.

Quelques stations présentaient des assemblages atypiques par rapport à leur positionnement dans l'estuaire. A deux stations, les assemblages étaient fortement dominés par une seule espèce, qui était absente ou rare aux autres stations. Il s'agit de *Quinqueloculina oblonga* à la station Elorn 1 et d'*Elphidium incertum* à la station Vie 2 (en 2018 et 2020). Dans les deux cas, il s'agit de stations à sédiment sableux situées à l'embouchure des estuaires. Ensuite, certaines stations présentaient des assemblages apparemment en désaccord avec la position de ces stations dans l'estuaire. Par exemple, dans l'estuaire du Belon, les stations 3B et 4B, situées dans la partie intermédiaire de l'estuaire, présentaient des assemblages avec un pourcentage élevé d'espèces marines. La granulométrie plus grossière de ces stations (78-96% de sables) suggère un fort hydrodynamisme, responsable d'un transport d'espèces marines (vivantes) vers les parties intérieures de l'estuaire. Les stations Auray₂₀₂₀ 2A et 2B, situées dans la partie milieu/aval de l'estuaire présentaient un assemblage avec un pourcentage élevé (55 à 67%) d'espèces agglutinées typiques de la partie amont. Cette observation fait partie d'une tendance générale de densités (absolue et relative) élevées de ces espèces dans l'estuaire d'Auray en septembre 2020 (comparés à l'échantillonnage de juillet 2019 et aux autres estuaires). Les forts pourcentages de ces espèces ont notamment été observés aux stations plus élevées sur la vasière. Puisque ces espèces ont une tolérance élevée vis-à-vis des variations de salinité (elles se trouvent à la fois dans de l'eau saumâtre et dans les marais salants (Ellison & Murray, 1987; De Rijk, 1995)), leur forte densité pourrait refléter des conditions persistantes de valeurs extrêmes de salinité (c.-à-d., sursalure, sous-salure, forte variabilité à court terme) à cette période. En effet, en 2020 le débit fluviale avait été minimale dans les quatre semaines précédant l'échantillonnage et les températures avaient été assez élevées, conduisant à des salinités élevées sur le haut des vasières (salinité mesurée : 35.5). Ces deux exemples illustrent la difficulté à généraliser la répartition des foraminifères dans les estuaires, qui peuvent avoir des particularités morphologiques (en partie à l'origine de la forte spécificité des communautés de foraminifères), et qui sont soumis à une très forte variabilité temporelle. Ces deux phénomènes peuvent provoquer des déviations spatiales et temporelles importantes par rapport aux schémas généralisés de répartition.

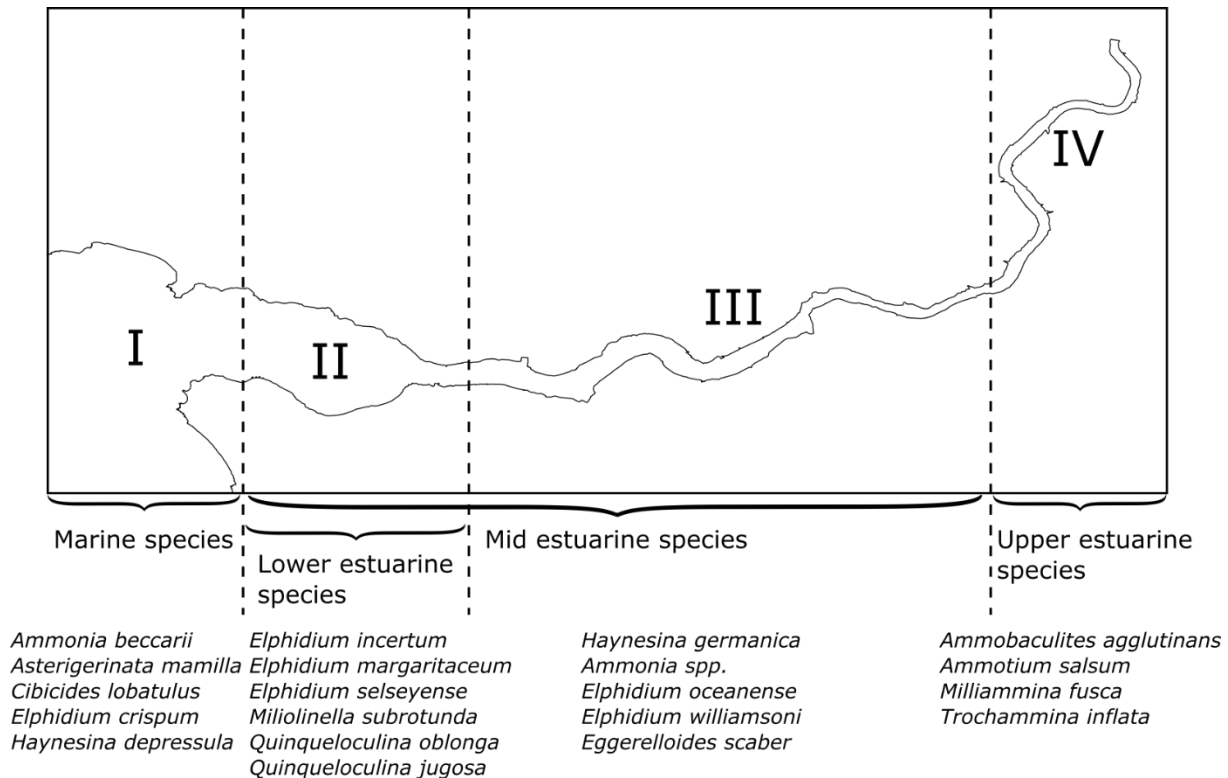


Figure V.26 : Schéma généralisé de la répartition des espèces dominantes de foraminifères le long du gradient amont-aval des estuaires de la façade atlantique française.

Sur la base de ces observations, et en nous inspirant de l'indice de confinement de Debenay (Debenay, 1995), nous proposons un indice de répartition des espèces de foraminifères estuariens (EFI, Estuarine Foram Index). Cet indice est calculé suivant la formule suivante :

$$EFI = 0.1 * \left(\%GroupeI + \frac{2}{3} * \%GroupeII + \frac{1}{3} * \%GroupeIII + 0 * \%Groupe4 \right)$$

Où le groupe I représente le pourcentage d'espèces marines (voir schéma plus haut, en Figure V.26), groupe II le pourcentage d'espèces de la partie aval, groupe III le pourcentage d'espèces de la partie intermédiaire de l'estuaire et groupe IV le pourcentage d'espèces de la partie amont. Ainsi, une valeur de 10 représente un assemblage uniquement composé d'espèces marines, et une valeur de 0 un assemblage uniquement composé d'espèces représentatives de la partie amont de l'estuaire.

Le but de cet indice EFI est de pouvoir caractériser la communauté des foraminifères d'un site estuarien avec un seul descripteur quantitatif. Ceci facilite la comparaison avec les paramètres environnementaux et avec le MII, sensé décrire les caractéristiques environnementales. Sur la Figure V.27, pour chacun des estuaires étudiés, nous présentons les valeurs du EFI le long du gradient amont-aval estuarien. Dans le cas de transects perpendiculaires sur la vase, seules les stations les plus proches du chenal sont représentées sur cette figure. La couleur des stations correspond aux scores du EFI, tels que :

EFI \geq 6 = Zone I, embouchure de l'estuaire sous forte influence marine ;

$4.5 \leq \text{EFI} < 6 = \text{Zone II}$, partie aval de l'estuaire ;

$3 \leq \text{EFI} < 4.5 = \text{Zone III}$, partie intermédiaire de l'estuaire ;

$\text{EFI} < 3 = \text{Zone IV}$, partie amont de l'estuaire.

Sur la Figure V.27, on peut voir que la grande majorité des stations correspondent à la zone III. Ceci est dû à la prépondérance de *Haynesina germanica* dans les assemblages. On peut aussi remarquer qu'une succession complète des quatre biozones n'est observée dans aucun des estuaires. Trois principaux cas de figure peuvent être dégagés :

- a) des estuaires avec la zone III uniquement (Aulne, Vilaine)
- b) des estuaires avec les zones II et III (Elorn, Laïta, Crac'h et Vie)
- c) des estuaires avec les zones III et IV (Odet, Auray)

Le Belon, avec une succession quelque peu anarchique, avec les zones I et II jusqu'au barrage de Guilly, et la zone IV dans la vasière derrière ce barrage, forme une exception.

La forte dominance de la zone III est sans doute en partie la conséquence du caractère opportuniste de l'espèce dominante *Haynesina germanica*. Cette espèce euryhaline a tendance à fortement dominer la communauté dans des conditions poly- à mésohalines. Malgré la dominance de *H. germanica* dans la zone III, un gradient de valeurs croissantes de l'amont vers l'aval peut être observé dans plusieurs estuaires (Elorn, Laïta, Crac'h, Tableau Supplémentaire V.S6). Le faible nombre de sites avec les zones II et IV, s'explique aussi par notre échantillonnage, focalisé sur les vasières intertidales en domaine estuarien, et qui a largement évité à la fois les zones proches des embouchures et la zone oligohaline.

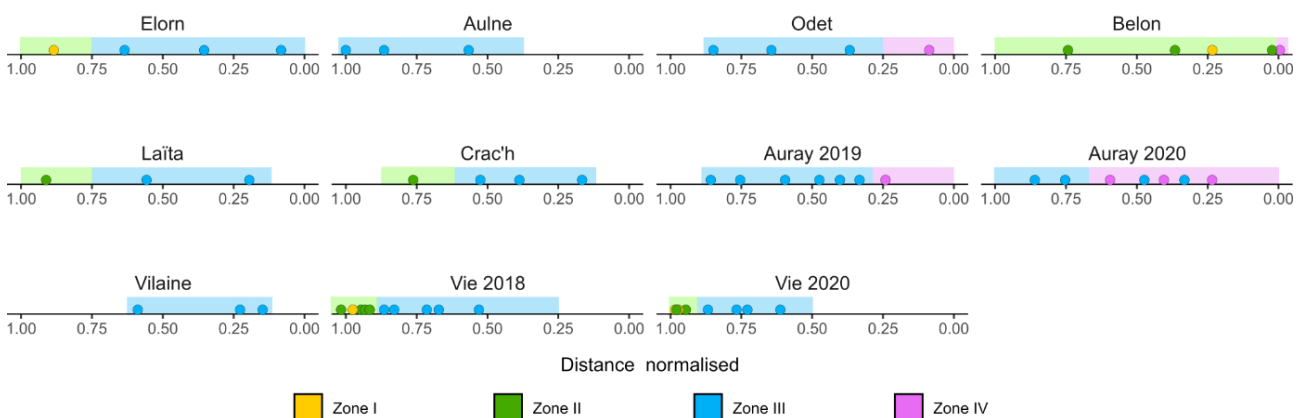


Figure V.27 : Succession des différents scores du EFI en fonction de la distance normalisée à la mer (où 1 correspond à l'embouchure de l'estuaire).

L'importance des différences biogéographiques

Dans le chapitre précédent, nous avons montré que tous les estuaires sauf le Belon montrent une succession typique de communautés. Cependant, les observations des communautés et les résultats de l'ACP indiquent que chaque estuaire possède ses propres particularités au niveau des communautés de foraminifères. Sur 43 espèces identifiées, seules *Haynesina germanica* et *Ammonia* spp. ont été observées dans tous les estuaires. En effet, plusieurs estuaires montrent des communautés particulières. Par exemple, l'estuaire d'Auray est caractérisé par de fortes abondances d'*Ammotium salsum* et d'*Ammobaculites agglutinans* tandis que l'estuaire de la Vie se démarque par une forte abondance d'*Elphidium selseyense* dans la partie aval. Ces différences biogéographiques importantes ont également été mises en avant par les analyses de metabarcoding dans les mêmes estuaires (Singer et al., soumis). Ainsi les facteurs environnementaux mesurés, qui ne montrent pas de différences systématiques entre ces estuaires, ne semblent pas suffire à expliquer la présence/absence et/ou densité absolue/relative de certaines espèces. Ces différences pourraient en partie être expliquées par un gradient climatique latitudinal. Néanmoins, l'analyse des données d'ADNe montre que la partie de la variabilité expliquée par ce paramètre est plutôt faible (8%) (Singer et al., soumis). Il semble donc que d'autres paramètres, tels que la géomorphologie et l'histoire de l'utilisation anthropique des estuaires, aient contribué à façonner les communautés de foraminifères dans ces environnements. Plus particulièrement, les invasions biologiques provoquées par l'homme ont pu jouer un rôle déterminant (voir chapitre 3). Finalement, les communautés de foraminifères trouvées dans les différents estuaires peuvent aussi s'expliquer en partie par les différents degrés de connectivité avec la mer ouverte et entre les estuaires. En effet, les estuaires à faible connectivité, qui ont connu peu d'échanges, ont pu développer des communautés avec une composition particulière au cours du temps (dérive écologique).

Relations entre les foraminifères et les paramètres environnementaux

Parmi les paramètres environnementaux étudiés, la granulométrie et les paramètres liés à l'influence marine (la distance à la mer, la durée d'émergence, la décharge fluviatile et le $\delta^{13}\text{C}$) ont montré des corrélations significatives avec la diversité et avec l'abondance relative de certains taxons (Tableaux V.3 et V.4). Certaines espèces (*Elphidium margaritaceum*, *Elphidium selseyense*, *Quinqueloculina oblonga*, *Quinqueloculina jugosa*) présentent des abondances plus élevées quand l'influence marine est élevée, d'autres (*Ammotium salsum* et *Miliammina fusca*) présentent des abondances plus élevées quand l'influence marine est faible. Il est intéressant de remarquer que le groupe « espèces estuariennes » (*Haynesina germanica*, *Ammonia* spp., *Elphidium oceanense* et *Elphidium williamsoni*) ne montre pas de préférence, ce qui souligne leur grande tolérance vis-à-vis de la variabilité de la salinité.

En revanche, le TOC ne semble pas influencer la répartition des foraminifères dans les estuaires étudiés. Le TOC ne montre pas de corrélation significative avec la diversité (Tableau V.3), ni avec les espèces

principales (Tableau V.4). Dans nos estuaires, le %TOC a tendance à diminuer de l'amont vers l'aval (Figure V.7), et ce gradient correspond à la succession de nos quatre principales zones estuariennes (Figure V.26). Dans nos estuaires peu touchés par la pollution anthropique, nous n'observons pas d'espèces précises qui seraient favorisées par de fortes valeurs en TOC, comme cela a été proposé par Bouchet et al. (2021).

Relations entre le MII et les foraminifères

Le MII est corrélé positivement avec le rapport $\delta^{13}\text{C}$, ce qui suggère qu'il reflète également l'équilibre entre nutriments d'origines marine et continentale. Concernant la communauté de foraminifères, le MII montre des corrélations positives avec la diversité (avec S : $R = 0.53$, $p < 0.001$; avec H' : $R = 0.32$, $p = 0.0053$) et l'abondance des foraminifères ($R = 0.39$, $p < 0.001$). De plus, cet indice a montré des corrélations avec l'abondance relative de certaines espèces individuelles. Plusieurs espèces d'*Elphidium* (*E. margaritaceum*, *E. selseyense* et *E. oceanense*) et les trois espèces porcelanées (*Miliolinella subrotunda*, *Q. jugosa*, *Q. oblonga*) montrent une corrélation positive tandis que l'espèce agglutinée *Ammotium salsum* a une corrélation négative. Ces résultats confirment les résultats précédents (chapitre 2) basés sur deux estuaires. Cependant, il est à noter que les différentes espèces d'*Elphidium* n'ont pas toutes montré la même relation avec le MII et ses composantes. Par exemple, les espèces *E. selseyense* et *E. margaritaceum* sont positivement corrélés avec le MII, tandis qu'*E. oceanense* et *E. williamsoni* ne sont pas corrélées avec le MII. Ainsi, contrairement à ce qui a été proposé auparavant, les espèces d'*Elphidium* ne semblent pas toutes avoir une préférence pour les zones plus marines.

Le MII montre une corrélation significative avec l'EFI ($R: 0.65$; Figure V.28). L'indice MII reflète donc plutôt bien la succession des communautés de foraminifères dans des conditions plutôt naturelles. Toutefois il y a une grande majorité de stations avec une valeur autour de 3.33, ce qui est dû à la forte dominance d'*Haynesina germanica* dans les communautés. Dans ce contexte, il est à noter que beaucoup de points aux deux extrêmes sur l'échelle du MII affichent un score d'EFI autour de 3.33. Le fait que *Haynesina germanica* domine fortement les communautés dès qu'on entre dans la zone polyhaline/mésohaline peut expliquer cette observation. En outre, notre identification des limites de l'estuaire pourrait ne pas correspondre à la réalité biologique. Pour déterminer la limite entre mer et estuaires ($\text{MII} = 1.0$), nous nous sommes uniquement basée sur la morphologie de l'embouchure. La détermination de la limite de l'intrusion saline, qui est déterminante pour la valeur du MII en amont, est également problématique dans les cas de constructions anthropiques qui limitent la propagation naturelle de l'eau de mer.

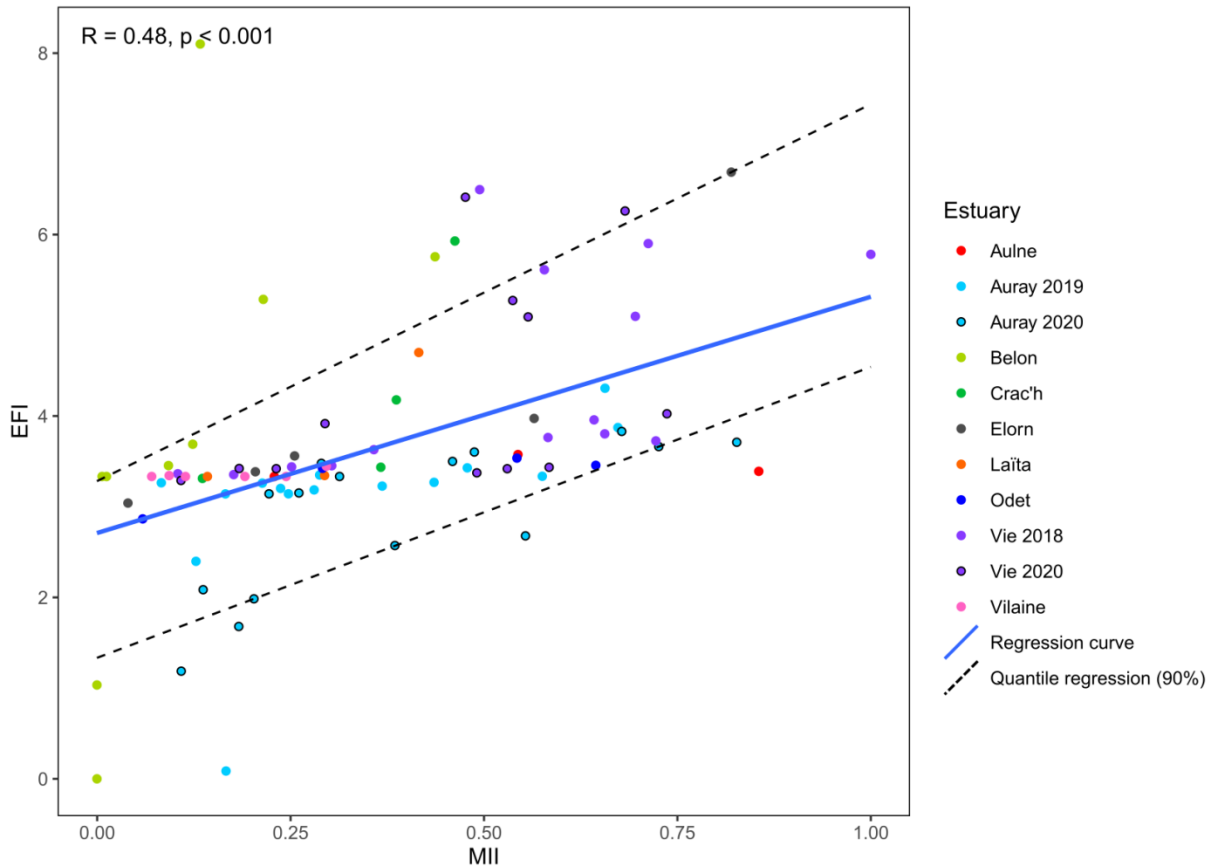


Figure V.28 : Corrélation entre le MII (intrusion marine) et l'EFI (succession des espèces). Les résultats des tests de corrélations sont basés sur des tests de Pearson.

Afin d'éviter le problème de la majorité des valeurs autour de 3.33 causé par la forte dominance de *H. germanica*, nous avons développé un deuxième indice EFDI (Estuarine Foraminiferal Diversity Index) basé sur la diversité alpha de chacun des quatre groupes d'espèces :

$$EFDI = \frac{Nbspp. Groupe I + \frac{2}{3} * Nbspp. Groupe II + \frac{1}{3} * Nbspp. Groupe III + 0 * Nbspp. Groupe IV}{Nbspp. dans les 4 groupes}$$

Cet indice aura une valeur entre 1 et 0.67 si la communauté contient uniquement des espèces des groupes I et II, entre 0.67 et 0.33 s'il y a des espèces des groupes II et III, et une valeur < 0.33 si la communauté comporte uniquement des espèces des groupes III et IV. La relation du EFDI avec le MII est montrée en Figure V.29.

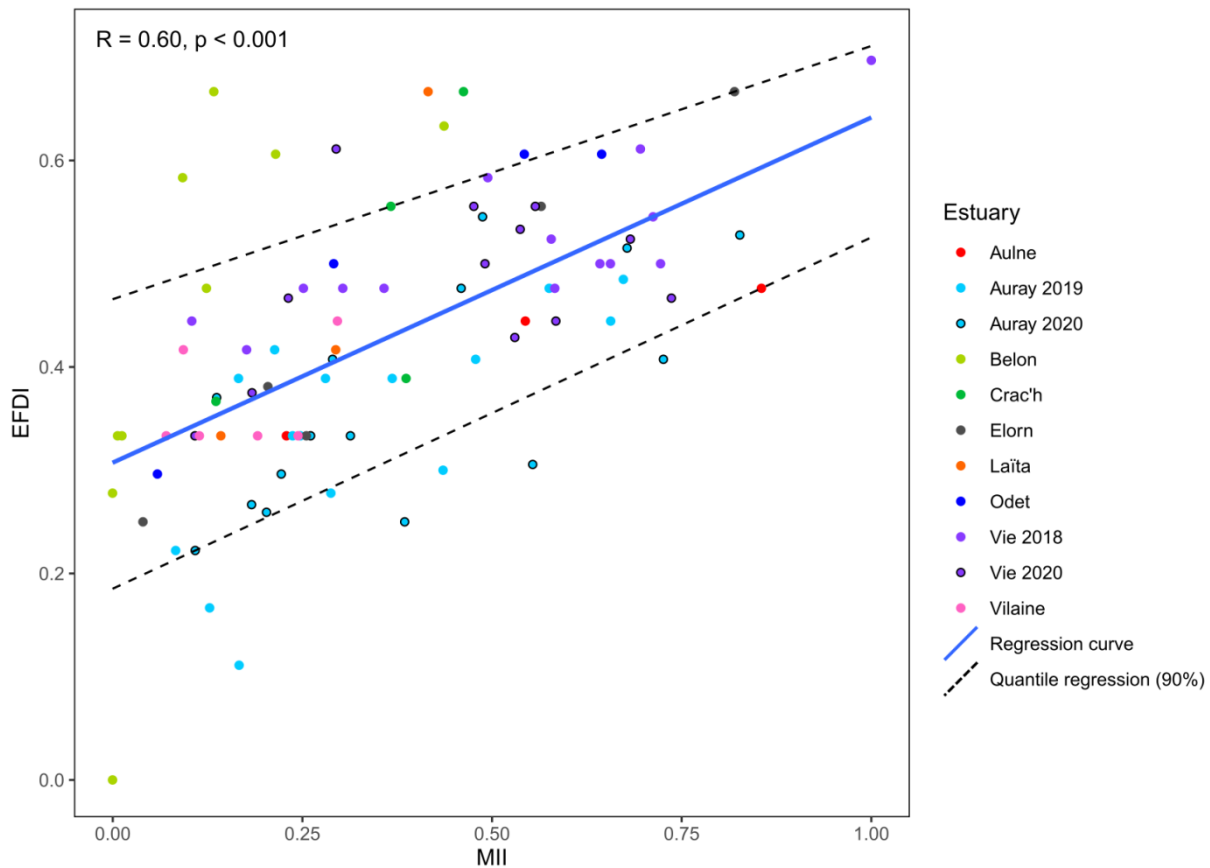


Figure V.29 : Corrélation entre les indices MII (intrusion marine) et EFDI (succession des espèces basée sur la diversité). Les résultats des tests de corrélations sont basés sur des tests de Pearson.

Une corrélation significative ($R = 0.60$, $p < 0.001$) peut être observée avec un coefficient de corrélation plus élevée (Figure V.29). Cela suggère que l'EFDI décrit mieux les zones de transition entre les différentes parties de l'estuaire. Dans la Figure V.29, quelques points sont situés sous le quantile de régression. La plupart des points appartiennent aux stations de l'estuaire d'Auray caractérisées par une forte diversité d'espèces agglutinées. Le point en bas à gauche de la figure appartient à la station Belon 6B, uniquement composée de l'espèce agglutinée *Miliammina fusca*.

Relations entre pollution et caractéristiques des assemblages de foraminifères

Dans cette étude, peu de stations ont montré des enrichissements notables en métaux, à l'exception de l'estuaire de l'Aulne, clairement contaminé au plomb. Cependant, les communautés de foraminifères observées dans l'Aulne ne sont pas très différentes de celles de certains autres estuaires typiques des conditions estuariennes. Ceci est d'abord visible sur l'ACP (Figure V.24), où les trois stations de l'Aulne se positionnent au cœur du principal nuage de points, en suivant un gradient amont-aval. Ce gradient s'explique par la fréquence relative d'*Haynesina germanica*, qui diminue de la partie amont vers la partie aval, de 95%

à Aulne 3 à 50% à Aulne 1. Cette diminution est accompagnée par une augmentation de la diversité et une augmentation de l'abondance relative d'*Elphidium oceanense* (0% à 10%) et de *Quinqueloculina oblonga* (0 à 7%). Cette évolution le long du gradient est assez typique, et similaire à ce qui a été observé dans l'Odette ou la Vilaine. L'absence d'anomalie de répartition des espèces suggère que les communautés de foraminifères ne sont pas sensibles au plomb. Cependant, des expériences en laboratoire ont montré les effets délétères de la pollution au plomb sur la structure interne de ces organismes (Frontalini et al., 2015) et sur la densité et diversité des communautés de foraminifères (Brouillette Price et al., 2019).

L'explication la plus plausible serait que, bien qu'abondant dans le sédiment, cet élément trace ne serait pas bio-disponible. En effet, même si la concentration de Pb dans le sédiment dépasse largement le seuil de forte pollution (cette étude ; Chiffolleau, 2017; Nasri et al., 2021), les mesures effectuées sur la chair d'huitres (~4 µg/g de poids sec ; Chiffolleau, 2017) et les intestins d'oursins (Guillou et al., 2000) n'indiquent pas de dépassement du seuil des recommandations OSPAR (fixée à 7.5 µg/g de poids sec). Ainsi, nos résultats suggèrent que dans l'Aulne, la biodisponibilité des métaux d'origine minière est limitée, et qu'il n'y a pas d'impact sur les communautés de foraminifères.

Comme indiqué précédemment, une approche alternative pour déterminer les éventuels effets de la pollution serait de considérer plus en détail les zones estuariennes avec une communauté particulière de foraminifères. Trois cas sont concernés par cette approche :

- 1) Les stations Elorn 1 et dans une moindre mesure Elorn 2 se distinguent par de très fortes abondances (absolue et relative) de *Quinqueloculina oblonga* (89 et 16% respectivement). Ces fortes valeurs n'ont été observées dans aucun autre estuaire. Par conséquent, dans l'ACP, la station Elorn 1 se positionne dans le groupe V, loin du nuage de points central. Il s'agit d'une station à sédiment très sableux, très pauvre en matière organique (0.1%) sans aucune indication de pollution métallique ou de caractéristiques particulières de matière organique. Il semble donc que la forte densité de *Q. oblonga* puisse plutôt s'expliquer par la nature grossière du sédiment.
- 2) Toutes les stations de la Vilaine sont caractérisées par de fortes abondances relatives d'*Ammonia* spp. (30 à 96%), largement supérieures à celles observées dans les autres estuaires. Dans ce cas également, il n'y a aucune indication d'une pollution métallique ou organique. Dans le chapitre 3, nous avons remarqué que l'estuaire de la Vilaine est le seul où *Ammonia confertitesta* est très fortement dominante (95%) parmi les espèces d'*Ammonia* spp. Ceci suggère que la tendance très opportuniste de cette espèce invasive, menant à des peuplements avec de très fortes densités, pourrait expliquer sa très forte dominance.
- 3) Les stations 3, 4 et 5, situées dans la partie aval de l'estuaire de la Vie sont caractérisées par des assemblages avec une forte contribution (55-85%) de l'espèce *Elphidium selseyense*, et cela pour les deux échantillonnages (octobre 2018 et juin 2020). Dans l'ACP, ces stations forment un nuage de points particulier, éloigné de toutes les autres stations (Figure V.24, groupe III). Ces stations, situées dans une zone

portuaire (station 3 : ponton de départ du bateau vers l'Île d'Yeu, stations 4 et 5 : sur des rampes dans des bassins semi-fermés pour les bateaux de pêche, station 5 à côté de la criée), affichent des enrichissements modérés en Cu, et surtout, des caractéristiques très particulières de la matière organique, avec un $\delta^{13}\text{C}$ (-20.6 à -14.3), un rapport C/N (5.6 à 9.9) et un %TOC élevé par rapport à la granulométrie, comparé aux autres stations de cet estuaire (Figure V.7). Auparavant, nous avons suggéré que ces valeurs pourraient indiquer des apports anthropiques de matière organique, originaires soit des activités de la pêche, soit des effluents de la station d'épuration située en amont de ces stations. Dans la littérature, *E. selseyense* a été considérée comme une espèce indicatrice de pollution (Sharifi et al., 1991; Debenay et al., 2001; Armynot du Châtelet & Debenay, 2010) sur la base d'abondances relatives plus élevées dans les stations les plus polluées qui étaient localisées dans les ports ouverts vers la mer. Néanmoins, cette espèce a également été décrite dans des baies estuariennes (Goubert, 1997; Gehrels & Newman, 2004; Armynot du Chatelet et al., 2009) ainsi qu'en milieu marin ouvert (Feyling-Hanssen, 1972; Miller et al., 1982; Darling et al., 2016; Choquel, 2021), apparemment dans des conditions plutôt naturelles, sans pollution anthropique importante. Nos observations dans l'estuaire de la Vie, en combinaison avec certaines observations rapportées dans la littérature, suggèrent que cette espèce, malgré son caractère ubiquiste, pourrait être avantagée par des introductions anthropiques de matière organique dans des milieux estuariens. Ce qui en ferait une des rares espèces pouvant être considérée comme indicatrice de stress anthropique.

En résumé, les sites échantillonnés dans cette étude ne présentent pas de pollution majeure ayant un impact incontestable sur les caractéristiques des communautés de foraminifères. Les quelques enrichissements modérés à mineurs observés ainsi que la présence d'activités humaines plus ou moins intense sur les berges ne permet pas de classer ces sites comme naturels. Néanmoins les perturbations existantes ne semblent pas impacter les communautés de foraminifères observées dans ces sites. Les répartitions observées dans cette étude reflètent donc probablement des distributions naturelles. Seuls l'abondance relative élevée d'*Elphidium selseyense* dans quelques sites de l'estuaire de la Vie pourrait être une réponse à une introduction anthropique de matière organique.

Les espèces bioindicatrices et les indices biotiques

Dans les eaux de transition, plusieurs études ont proposé d'utiliser certaines espèces de foraminifères comme bio-indicatrices de pollution (p.ex., Armynot du Châtelet et al., 2004; Ferraro et al., 2006; Bouchet et al., 2021).

Dans la littérature *Haynesina germanica* et *Ammonia* spp. sont considérées comme des espèces indicatrices de pollution (Alve, 1995; Ferraro et al., 2006; Armynot du Châtelet & Debenay, 2010; Caruso et al., 2011; Frontalini & Coccioni, 2011). Cependant, au vu de nos résultats, nous avons conclu que la très grande

majorité des sites étudiés ne subissent pas de pollution majeure. Or, ces deux espèces sont présentes dans tous les estuaires étudiés, presque à toutes les stations. *H. germanica* était présente dans 88 stations et *Ammonia* spp. dans 86 stations sur 90. Ces espèces semblent donc être représentatives du stress naturel qui caractérise les milieux estuariens, et il ne paraît pas envisageable de les utiliser comme indicatrices de pollution dans ces milieux.

Dans notre étude, seules les stations localisées dans la partie aval de l'estuaire de la Vie, présentent la combinaison d'une forte dominance d'une autre espèce que *Haynesina germanica* et *Ammonia* spp. (c.-à-d., *Elphidium selseyense*), accompagnée d'une signature particulière de la matière organique. Cette dominance récurrente à plusieurs stations pourrait être indicatrice d'une modification des communautés en réponse à une introduction de matière organique d'origine anthropique. *E. selseyense* a aussi été observée avec un pourcentage élevé (~40%) dans le port de Boulogne-sur-Mer (Francescangeli et al., 2020, sous le nom de *Criboelphidium excavatum*), qui est considéré comme pollué sur la base d'un indice basé sur les éléments traces métalliques. Cette espèce a aussi été observée avec un pourcentage élevé (~20%) dans la marina de Port Joinville (Ile d'Yeu ; Debenay et al., 2001).

Cependant, cette observation nécessite d'être corroboré par d'autres observations, avant que l'espèce *E. selseyense* puisse être considérée comme bio-indicatrice d'enrichissement organique. Ainsi, dans cette étude, l'absence de sites clairement pollués avec une réponse significative de la communauté de foraminifères, ne permet pas d'identifier des espèces indicatrices de pollution, à l'exception peut-être d'*E. selseyense*. De plus, la forte spécificité faunistiques des neuf estuaires étudiés, qui suggère un gradient biogéographique fort, complique encore l'identification d'espèces bioindicatrices. Ainsi, l'absence d'une espèce particulière dans un estuaire donné reflètera souvent les limites de sa répartition géographique plutôt que l'absence de pollution. Pour cette raison, de futures investigations devraient se focaliser sur des sites localisés clairement pollués (p. ex. des zones de rejets d'usine ou de stations d'épuration, des sites avec une activité portuaire très intense) pour voir si les communautés de ces stations dévient par rapport à des communautés plus naturelles dans les mêmes estuaires.

En milieu côtier, les indices de qualité écologique les plus fréquemment utilisés reposent sur la tolérance des espèces à l'enrichissement en matière organique (p. ex. Foram-AMBI (Alve et al., 2016; Jorissen et al., 2018), TSI-Med (Barras et al., 2014), FSI (Dimiza et al., 2016) et en partie le NQI_f (Alve et al., 2019)). Les vasières estuariennes sont des zones naturellement riches en matière organique, et pour les organismes vivant dans ces milieux la quantité de matière organique n'est probablement jamais un paramètre limitant. De plus, la grande variabilité de ce paramètre est en grande partie liée à la granulométrie. De ce fait, nous n'avons pas trouvé dans les estuaires étudiés, une relation forte entre les caractéristiques des communautés et le pourcentage de matière organique du sédiment. Il semble donc que les indices de qualité environnementale basés sur la tolérance/préférence des espèces pour certaines concentrations de matière

organique ne soient pas applicables dans les vasières estuariennes. Récemment, Bouchet et al., 2021 ont proposé une adaptation de l'indice Foram-AMBI pour les masses d'eau de transition et les ports. Bien que cet indice semble efficace pour les ports, nous déconseillons l'utilisation de cet indice pour les vasières estuariennes.

Une autre approche consiste à utiliser la diversité comme indication de l'état de santé de l'écosystème étudié (p. ex. $\exp(H'bc)$ (Bouchet et al., 2012, 2018a) et en partie le NQI_f (Alve et al., 2019)). Comme détaillé en introduction, une diversité élevée en milieu côtier traduit un écosystème en bon état de santé, tandis qu'une diminution de la diversité indique une perturbation. Cependant les écosystèmes estuariens sont naturellement caractérisés par une diversité faible, et un gradient de diversité croissant de l'amont vers l'aval. Les indices basés sur la diversité doivent donc être adaptés aux milieux estuariens. Une piste de réflexion pourrait être l'utilisation du MII. En effet l'indice d'intrusion marine a montré une corrélation positive avec la richesse spécifique, et également avec l'indice de Shannon (Figure V.20). Ainsi, une pondération des indices de diversité par le MII permettrait de différencier une baisse de la diversité due à la diminution de l'influence marine, de celle due à une perturbation anthropique. La comparaison du MII et des indices EFI et EFDI, qui présentent une caractérisation quantitative des communautés, constitue une autre piste prometteuse pour détecter des anomalies des communautés de foraminifères. Il faut maintenant tester ces méthodes sur un jeu de données comportant des sites incontestablement pollués par des introductions anthropiques.

5. Conclusion

Cette étude menée sur neuf estuaires localisés sur la côte atlantique française contribue à l'amélioration des connaissances sur la répartition des foraminifères en milieu estuarien. Grâce à une étude des enrichissements en éléments traces métalliques et à l'analyse de la matière organique nous avons conclu que la très grande majorité des stations étudiées dans neufs estuaires de la façade atlantique ne présentaient pas de pollution majeure ayant un impact visible sur les communautés de foraminifères. Ainsi, cette étude a surtout permis de mieux connaître la répartition naturelle de ces organismes dans les vasières estuariennes et de comprendre le rôle structurant des paramètres naturels. Il apparaît que les communautés de foraminifères peuvent être divisés en quatre groupes : les assemblages typiques d'embouchure (présence d'espèce à préférence marine tel que *Ammonia beccarii*, *Haynesina depressula*), les assemblages caractéristiques de la partie aval (p. ex. *Elphidium margaritaceum*, *E. selseyense*, *Quinqueloculina oblonga* et *Q. jugosa*), les assemblages du milieu de l'estuaire (caractérisés par de fortes abondances d'*Haynesina germanica*, puis d'*Ammonia* spp. accompagnées d'*Elphidium oceanense*, *E. williamsoni*), et les assemblages de la partie amont (constitués d'espèces agglutinées telles qu'*Ammotium salsum*, *Ammobaculites agglutinans* ou

Miliammina fusca). Le MII et ses composants (distance à la mer normalisée, temps d'émergence normalisé, et la décharge fluviale normalisée) ont montré de bonnes corrélations avec la distribution des foraminifères en termes de diversité mais aussi d'abondances relatives de certains taxa. En revanche, dans notre jeu de données, obtenu surtout aux sites estuariens non soumis à de forts enrichissements en matière organique, le TOC ne paraît pas être un paramètre contrôlant la distribution des communautés. Finalement, nous proposons deux nouveaux indices pour décrire quantitativement les caractéristiques des communautés de foraminifères des estuaires : l'EFI, basée sur les abondances relatives des quatre groupes d'espèces dans les estuaires, et l'EFDI, basée sur la diversité de chacun de ces groupes dans les assemblages. La comparaison du MII avec ces deux indices semble particulièrement prometteuse pour pouvoir distinguer des assemblages avec une composition qui n'est pas en accord avec la position dans l'estuaire du site étudié. De telles anomalies peuvent être la conséquence d'une perturbation anthropique.

Matériel supplémentaire

Tableau supplémentaire V.S1: Concentrations en éléments traces métalliques (mg/kg) mesurées dans le sédiment.

	Station	Cr	Ni	Cu	Zn	Cd	Pb	V	Co	As	Mo	Ag	Sn	Sb	Ba	U	Th
Aulne	Aulne 1	58.9	22.4	14.0	317	1.94	143.0	87	7.67	12.1	1.64	0.66	1.29	1.53	237	2.45	7.66
	Aulne 2	69.2	28.3	21.3	419	2.50	193.0	101	10.00	18.0	0.97	0.92	6.73	2.02	248	2.26	7.95
	Aulne 3	71.5	30.2	22.7	508	3.99	232.0	97	10.60	16.8	1.05	1.26	4.51	2.45	257	2.47	8.12
Auray 2019	Auray ₂₀₁₉ 1A	76.4	28.0	61.5	168	0.55	41.8	98	8.95	16.2	2.04	0.30	6.29	1.04	341	3.00	7.65
	Auray ₂₀₁₉ 1B	63.9	23.4	16.2	123	0.47	35.9	83	7.79	11.4	2.03	0.23	5.15	0.87	412	2.65	5.79
	Auray ₂₀₁₉ 1C	59.0	21.8	13.4	107	0.39	32.4	77	7.41	11.1	3.03	0.22	4.75	0.89	369	2.76	5.46
	Auray ₂₀₁₉ 2A	81.8	30.3	23.1	154	0.56	43.4	104	9.11	17.1	2.75	0.32	6.50	0.99	295	3.28	9.39
	Auray ₂₀₁₉ 2B	77.0	28.5	20.2	139	0.50	41.5	99	8.64	15.8	2.89	0.29	6.12	0.95	287	3.46	8.78
	Auray ₂₀₁₉ 2C	71.8	26.2	18.6	125	0.48	37.4	90	7.98	16.4	2.25	0.29	5.72	0.91	289	2.95	7.89
	Auray ₂₀₁₉ 4A	66.0	24.5	14.6	108	0.39	35.0	86	7.90	13.3	4.39	0.24	5.23	0.94	358	3.31	6.73
	Auray ₂₀₁₉ 4B	75.5	28.1	22.6	146	0.53	41.6	98	8.88	14.6	2.69	0.30	6.14	0.97	334	3.39	8.15
	Auray ₂₀₁₉ 5A	79.2	29.2	25.7	173	0.58	44.4	98	9.14	18.0	1.55	0.37	7.07	1.02	297	3.01	8.70
	Auray ₂₀₁₉ 5B	82.5	30.7	24.7	170	0.60	46.0	103	9.49	18.0	1.16	0.37	7.19	1.00	308	2.93	9.20
	Auray ₂₀₁₉ 6A	71.4	26.2	21.8	160	0.58	42.6	88	8.39	17.8	1.39	0.36	6.19	0.95	313	2.88	7.54
	Auray ₂₀₁₉ 6B	72.6	27.5	23.3	162	0.60	42.8	90	8.58	18.2	1.31	0.41	6.17	0.89	277	2.84	8.10
	Auray ₂₀₁₉ 7	87.0	33.4	30.4	196	0.66	50.7	106	10.10	21.1	1.20	0.46	7.46	1.07	294	3.25	9.94
	Auray ₂₀₁₉ 8A	71.1	27.8	25.5	173	0.63	42.5	86	8.45	19.2	1.59	0.43	6.34	0.88	272	2.98	7.92
	Auray ₂₀₁₉ 8B	40.3	15.9	12.7	126	0.44	35.2	49	5.51	12.2	0.57	0.25	4.61	0.68	470	1.92	4.21
	Auray 2020	Auray ₂₀₂₀ 1A	77.3	26.8	27.6	159	0.34	45.7	91	8.59	14.6	2.52	0.20	6.54	1.03	300	2.99
Auray ₂₀₂₀ 1B		68.8	24.8	21.6	141	0.32	34.8	88	8.20	14.0	1.80	0.18	4.02	0.93	283	2.51	5.77
Auray ₂₀₂₀ 1C		68.4	25.5	17.3	132	0.29	34.9	91	8.56	12.7	1.69	0.15	4.52	1.00	312	2.57	6.10
Auray ₂₀₂₀ 2A		55.6	20.2	22.3	123	0.31	34.7	72	6.30	11.4	2.49	0.20	4.37	0.83	311	2.58	6.02
Auray ₂₀₂₀ 2B		77.2	27.0	21.5	143	0.34	34.3	92	8.20	14.1	2.82	0.22	14.70	0.80	256	2.87	6.87
Auray ₂₀₂₀ 2C		62.7	22.7	15.2	120	0.24	31.8	83	7.18	14.0	1.39	0.17	6.96	0.74	241	2.52	6.63
Auray ₂₀₂₀ 4A		61.6	22.3	15.1	131	0.33	38.3	81	7.54	12.3	2.11	0.19	10.70	0.83	339	2.70	6.00
Auray ₂₀₂₀ 4B		57.1	20.9	12.7	106	0.22	30.6	76	7.08	10.2	2.10	0.12	6.48	0.77	323	2.56	5.14
Auray ₂₀₂₀ 5A		75.7	28.4	22.7	175	0.36	40.9	96	8.92	19.5	3.87	0.25	7.30	0.89	285	2.77	8.29
Auray ₂₀₂₀ 5B		86.1	32.2	36.5	201	0.39	43.3	106	10.00	23.2	1.17	0.26	9.69	1.02	266	2.78	8.63
Auray ₂₀₂₀ 6A		77.9	29.7	25.4	196	0.52	46.5	98	9.28	18.9	1.97	0.39	5.65	1.07	304	3.27	8.39
Auray ₂₀₂₀ 6B		80.5	30.8	25.6	198	0.49	44.9	101	9.92	16.2	1.34	0.32	8.71	0.97	286	3.13	8.67
Auray ₂₀₂₀ 7		78.3	29.0	28.1	194	0.43	42.7	94	9.17	20.6	0.99	0.33	7.05	0.88	249	2.58	8.17
Auray ₂₀₂₀ 8A		76.8	29.1	28.0	200	0.44	44.0	93	9.19	22.5	0.89	0.43	6.14	0.86	271	2.77	8.23
Auray ₂₀₂₀ 8B		75.0	30.0	28.6	247	0.66	44.4	93	10.20	22.9	1.13	0.36	5.84	0.98	279	3.45	7.75
Belon		Belon 1	36.8	16.9	10.1	68	0.20	18.1	46	5.85	11.7	0.72	0.18	3.39	0.34	214	1.01
	Belon 3A	63.0	26.1	24.4	150	0.60	32.7	80	8.06	17.9	1.46	0.34	6.22	0.76	266	1.84	7.21
	Belon 3B	28.2	14.2	7.9	63	0.22	14.6	34	4.81	7.3	0.53	0.17	2.88	0.19	199	1.04	2.79
	Belon 4A	41.4	18.3	12.7	95	0.36	20.9	51	6.64	9.6	0.58	0.19	4.38	0.37	288	1.17	4.88
	Belon 4B	20.2	8.6	4.3	38	0.15	11.7	22	3.53	3.0	0.35	0.12	1.71	0.20	199	0.77	0.75
	Belon 5A	68.9	27.7	28.1	172	0.90	37.7	83	8.68	18.5	1.15	0.41	7.38	0.72	296	2.55	8.69
	Belon 5B	62.6	24.6	24.8	163	0.85	33.9	74	8.02	16.1	1.10	0.39	7.07	0.64	320	2.20	7.47
	Belon 6A	25.5	10.5	6.4	64	0.39	39.2	34	5.03	5.7	0.28	0.23	3.86	0.29	411	1.43	3.10
Belon 6B	35.2	13.6	11.3	97	0.75	24.2	45	6.24	10.9	0.83	0.30	5.41	0.37	322	2.88	4.61	
Crac'h	Crac'h 1	9.5	3.5	1.6	37	0.03	19.7	13	1.40	1.7	0.16	0.05	1.82	0.72	339	0.64	1.40
	Crac'h 2	63.0	22.4	39.1	155	0.22	34.1	80	7.23	13.2	2.10	0.19	8.91	0.90	235	2.40	6.71
	Crac'h 3	76.8	28.9	43.9	182	0.23	40.8	102	9.35	18.3	1.21	0.21	12.50	1.11	265	2.82	8.16
	Crac'h 4	61.4	22.1	31.1	211	0.21	38.0	80	7.67	13.4	0.92	0.23	7.06	0.99	343	2.62	6.62
Elorn	Elorn 1	51.9	18.7	18.0	100	0.13	27.0	73	5.25	8.6	1.15	0.10	6.73	0.63	339	1.79	4.66
	Elorn 2	59.9	22.2	33.9	202	0.59	51.6	78	7.03	17.6	0.87	0.33	6.63	0.98	270	2.05	6.90
	Elorn 3A	59.4	25.4	36.7	324	1.55	56.7	72	10.10	17.3	0.85	0.41	4.40	0.94	282	2.01	6.52
	Elorn 3B	61.5	24.0	34.4	241	1.03	50.2	77	8.25	13.8	0.89	0.36	7.07	0.94	294	2.35	6.98
Elorn 4	61.8	27.6	37.4	361	1.45	53.3	75	10.60	16.5	0.85	0.39	5.04	0.93	302	2.34	6.94	
Laita	Laita 1	22.2	8.3	4.0	55	0.12	13.1	28	3.63	7.4	0.31	0.05	1.24	0.28	207	0.61	1.99
	Laita 2	36.3	15.2	9.1	146	0.61	21.0	46	7.02	7.9	0.53	0.12	4.53	0.40	280	1.27	3.82
	Laita 3	29.5	13.7	7.6	156	0.75	18.9	36	6.73	6.5	0.44	0.14	4.55	0.37	268	0.93	2.92
Odet	Odet 1	58.8	23.4	20.4	170	0.46	37.6	82	7.84	13.4	1.94	0.29	8.28	1.07	253	2.38	6.11
	Odet 2	54.2	21.5	18.4	151	0.35	36.8	73	8.16	16.3	0.97	0.28	7.28	1.04	236	1.98	5.53
	Odet 3	30.2	11.4	12.6	104	0.31	31.9	41	5.40	10.2	0.76	0.23	11.70	0.92	233	1.81	4.22
	Odet 4	50.8	21.2	22.7	171	0.55	38.0	67	8.31	16.9	0.80	0.40	7.84	1.78	291	2.32	4.65
Vie 2018	Vie ₂₀₁₈ 1	18.1	12.0	9.4	40	0.10	10.8	33	4.00	8.4	0.30	0.20	1.60	1.20	293	1.10	2.60
	Vie ₂₀₁₈ 2	80.1	30.7	30.5	162	0.40	39.8	102	12.30	24.0	1.00	0.50	6.10	1.50	314	2.30	9.35
	Vie ₂₀₁₈ 3	62.1	22.8	20.9	121	0.30	32.9	77	9.50	13.2	0.90	0.40	4.50	1.20	332	2.30	7.25
	Vie ₂₀₁₈ 4	11.6	7.9	5.0	27	0.10	12.7	22	3.00	7.4	0.90	0.10	1.00	0.80	241	0.80	1.92
	Vie ₂₀₁₈ 5	51.7	22.3	17.8	104	0.30	28.7	71	8.30	15.5	0.80	0.40	3.80	1.00	213	1.70	5.94
	Vie ₂₀₁₈ 6	52.5	22.6	26.5	128	0.30	30.0	73	8.90	16.7	2.40	0.40	4.00	1.00	218	1.70	6.16
	Vie ₂₀₁₈ 7A	60.3	24.9	31.4	138	0.30	33.9	82	9.80	19.3	0.90	0.40	4.70	1.20	245	1.90	6.91
	Vie ₂₀₁₈ 7B	62.8	28.5	51.3	195	0.40	39.2	84	10.60	16.4	1.30	0.40	5.80	1.30	287	2.20	7.46
	Vie ₂₀₁₈ 7C	63.9	25.1	19.3	127	0.30	35.9	82	10.								

	Vie ₂₀₂₀ 3	67.7	26.4	21.7	131	0.22	35.0	92	9.94	19.3	0.84	0.24	5.27	1.13	307	2.10	8.97
	Vie ₂₀₂₀ 4	18.7	9.0	5.0	31	0.07	12.2	27	4.75	7.6	0.43	0.11	1.54	0.84	271	0.82	2.37
	Vie ₂₀₂₀ 5	63.0	25.5	20.0	113	0.17	34.3	90	9.62	22.1	1.09	0.23	5.07	1.07	230	1.95	7.97
	Vie ₂₀₂₀ 7A	43.6	18.7	32.0	87	0.17	26.1	61	7.15	13.6	0.83	0.19	3.57	0.80	229	1.67	5.72
	Vie ₂₀₂₀ 7B	62.4	24.7	21.6	113	0.20	34.3	87	9.62	19.5	0.94	0.33	4.89	1.01	236	1.94	7.97
	Vie ₂₀₂₀ 7C	63.8	25.4	17.5	118	0.24	36.9	90	9.83	17.9	0.91	0.26	5.14	1.09	258	2.19	8.40
	Vie ₂₀₂₀ 8A	60.8	24.0	18.2	127	0.23	34.7	86	9.51	17.8	0.93	0.25	4.88	1.06	250	2.09	7.93
	Vie ₂₀₂₀ 8B	51.9	23.3	15.8	102	0.20	30.7	74	8.52	14.2	0.91	0.23	4.24	0.95	245	1.93	6.85
	Vie ₂₀₂₀ 9	60.7	23.5	19.3	138	0.29	34.1	86	9.53	16.9	1.03	0.26	4.76	1.14	280	2.30	7.83
	Vie ₂₀₂₀ 10A	51.2	20.1	17.5	110	0.24	30.1	71	8.16	15.8	0.78	0.26	4.28	0.91	273	2.22	6.96
	Vie ₂₀₂₀ 10B	53.4	19.5	16.7	98	0.23	30.2	65	7.89	13.5	0.68	0.24	3.67	0.88	314	1.89	6.35
Vilaine	Vilaine 1A	75.0	29.5	18.2	158	0.35	42.2	93	12.20	21.4	1.05	0.41	5.45	1.12	335	2.07	8.27
	Vilaine 1B	87.0	35.7	21.9	182	0.31	49.8	113	14.30	25.0	1.29	0.41	6.66	1.36	325	2.16	9.68
	Vilaine 1C	84.2	35.2	21.7	180	0.32	48.7	109	13.80	24.1	1.05	0.38	6.19	1.26	302	2.07	9.36
	Vilaine 2A	90.5	39.5	25.1	215	0.41	53.2	118	16.30	25.4	1.14	0.49	6.60	1.38	316	2.16	9.86
	Vilaine 2B	92.0	40.7	25.0	222	0.40	53.1	120	16.50	24.4	1.05	0.43	6.64	1.41	332	2.22	10.11
	Vilaine 3	85.5	48.8	35.9	395	1.75	45.1	116	27.10	23.8	0.75	0.54	5.82	1.58	498	2.62	9.10
	Référence carotte VIVAN 8	81.9	43.9	18.8	135	0.90	36.7	119	14.2	23.4	30.2	0.50	4.50	1.50	310	13.4	10.30

Tableau supplémentaire V.S2 : Facteur d'enrichissement calculés sur la base des concentrations en éléments traces métalliques mesurées dans le sédiment.

	Station	EF (Cr)	EF (Co)	EF (Ni)	EF (Cu)	EF (Zn)	EF (Cd)	EF (Pb)	EF (V)	EF (As)	EF (Mo)	EF (Ag)	EF (Sn)	EF (Sb)	EF (Ba)	EF (U)
Aulne	Aulne 1	0.97	0.73	0.69	1.00	3.15	2.90	5.23	0.98	0.70	0.07	1.76	0.39	1.37	1.03	0.25
	Aulne 2	1.10	0.91	0.84	1.47	4.01	3.60	6.83	1.10	1.00	0.04	2.39	1.94	1.74	1.04	0.22
	Aulne 3	1.11	0.94	0.87	1.53	4.76	5.62	8.01	1.03	0.91	0.04	3.19	1.27	2.07	1.05	0.23
Auray 2019	Auray ₂₀₁₉ 1A	1.26	0.85	0.86	4.41	1.67	0.83	1.53	1.10	0.93	0.09	0.81	1.88	0.93	1.48	0.30
	Auray ₂₀₁₉ 1B	1.39	0.98	0.95	1.53	1.62	0.92	1.74	1.23	0.87	0.12	0.83	2.03	1.03	2.36	0.35
	Auray ₂₀₁₉ 1C	1.36	0.98	0.94	1.34	1.48	0.82	1.66	1.22	0.90	0.19	0.84	1.99	1.11	2.24	0.39
	Auray ₂₀₁₉ 2A	1.10	0.70	0.76	1.35	1.25	0.69	1.30	0.96	0.80	0.10	0.69	1.58	0.72	1.04	0.27
	Auray ₂₀₁₉ 2B	1.10	0.71	0.76	1.26	1.20	0.64	1.33	0.97	0.79	0.11	0.69	1.60	0.74	1.09	0.30
	Auray ₂₀₁₉ 2C	1.14	0.73	0.78	1.29	1.20	0.70	1.33	0.98	0.91	0.10	0.75	1.66	0.79	1.22	0.29
	Auray ₂₀₁₉ 4A	1.23	0.85	0.86	1.19	1.23	0.67	1.46	1.11	0.87	0.22	0.73	1.78	0.96	1.77	0.38
	Auray ₂₀₁₉ 4B	1.17	0.79	0.81	1.52	1.36	0.75	1.43	1.04	0.79	0.11	0.75	1.73	0.82	1.36	0.32
	Auray ₂₀₁₉ 5A	1.14	0.76	0.79	1.62	1.51	0.76	1.43	0.97	0.91	0.06	0.87	1.86	0.81	1.13	0.27
	Auray ₂₀₁₉ 5B	1.13	0.75	0.78	1.47	1.41	0.75	1.40	0.97	0.86	0.04	0.82	1.79	0.75	1.11	0.25
	Auray ₂₀₁₉ 6A	1.19	0.81	0.82	1.59	1.62	0.88	1.59	1.00	1.04	0.06	0.98	1.88	0.86	1.38	0.29
	Auray ₂₀₁₉ 6B	1.13	0.77	0.80	1.58	1.52	0.84	1.48	0.95	0.99	0.06	1.03	1.74	0.76	1.14	0.27
	Auray ₂₀₁₉ 7	1.10	0.74	0.79	1.68	1.50	0.76	1.43	0.92	0.94	0.04	0.95	1.72	0.74	0.98	0.25
	Auray ₂₀₁₉ 8A	1.13	0.77	0.82	1.76	1.66	0.90	1.50	0.93	1.07	0.07	1.12	1.83	0.76	1.14	0.29
Auray ₂₀₁₉ 8B	1.20	0.95	0.88	1.66	2.28	1.19	2.34	1.00	1.27	0.05	1.23	2.50	1.11	3.71	0.35	
Auray 2020	Auray ₂₀₂₀ 1A	1.40	0.90	0.91	2.18	1.75	0.56	1.85	1.13	0.93	0.12	0.60	2.16	1.02	1.44	0.33
	Auray ₂₀₂₀ 1B	1.50	1.03	1.01	2.05	1.86	0.64	1.69	1.32	1.07	0.11	0.63	1.60	1.11	1.63	0.33
	Auray ₂₀₂₀ 1C	1.41	1.02	0.98	1.55	1.64	0.54	1.61	1.29	0.92	0.09	0.52	1.69	1.12	1.70	0.32
	Auray ₂₀₂₀ 2A	1.16	0.76	0.79	2.03	1.55	0.58	1.62	1.03	0.83	0.14	0.68	1.66	0.95	1.72	0.33
	Auray ₂₀₂₀ 2B	1.41	0.87	0.92	1.71	1.59	0.56	1.40	1.15	0.90	0.14	0.66	4.89	0.80	1.24	0.32
	Auray ₂₀₂₀ 2C	1.19	0.79	0.80	1.25	1.38	0.41	1.34	1.08	0.93	0.07	0.54	2.40	0.77	1.21	0.29
	Auray ₂₀₂₀ 4A	1.29	0.91	0.87	1.38	1.66	0.63	1.79	1.17	0.91	0.12	0.66	4.09	0.94	1.88	0.35
	Auray ₂₀₂₀ 4B	1.40	1.00	0.95	1.36	1.57	0.48	1.67	1.27	0.87	0.14	0.48	2.88	1.03	2.09	0.38
	Auray ₂₀₂₀ 5A	1.15	0.78	0.80	1.50	1.60	0.50	1.39	1.00	1.04	0.16	0.62	2.02	0.74	1.14	0.26
	Auray ₂₀₂₀ 5B	1.26	0.84	0.88	2.32	1.77	0.52	1.41	1.06	1.18	0.05	0.62	2.57	0.81	1.03	0.25
	Auray ₂₀₂₀ 6A	1.17	0.80	0.83	1.66	1.78	0.71	1.56	1.00	0.99	0.08	0.96	1.54	0.88	1.20	0.30
	Auray ₂₀₂₀ 6B	1.17	0.83	0.83	1.62	1.74	0.65	1.45	1.00	0.82	0.05	0.75	2.30	0.77	1.10	0.28
	Auray ₂₀₂₀ 7	1.21	0.81	0.83	1.88	1.81	0.60	1.47	0.99	1.11	0.04	0.83	1.98	0.74	1.01	0.24
	Auray ₂₀₂₀ 8A	1.17	0.81	0.83	1.87	1.84	0.62	1.50	0.97	1.21	0.04	1.06	1.71	0.71	1.10	0.26
Auray ₂₀₂₀ 8B	1.22	0.95	0.91	2.02	2.42	0.97	1.61	1.03	1.30	0.05	0.95	1.72	0.87	1.20	0.34	
Belon	Belon 1	2.21	2.03	1.90	2.66	2.48	1.10	2.42	1.89	2.46	0.12	1.72	3.71	1.11	3.40	0.37
	Belon 3A	1.10	0.81	0.85	1.85	1.58	0.95	1.27	0.95	1.10	0.07	0.98	1.97	0.72	1.22	0.20
	Belon 3B	1.27	1.25	1.19	1.55	1.71	0.92	1.47	1.06	1.16	0.06	1.29	2.36	0.47	2.37	0.29
	Belon 4A	1.07	0.99	0.88	1.42	1.48	0.84	1.20	0.90	0.87	0.04	0.82	2.06	0.53	1.96	0.18
	Belon 4B	3.41	3.43	2.72	3.19	3.89	2.24	4.40	2.57	1.76	0.16	3.41	5.26	1.85	8.86	0.80
	Belon 5A	1.00	0.72	0.75	1.77	1.50	1.18	1.22	0.82	0.94	0.05	0.97	1.94	0.57	1.13	0.23
	Belon 5B	1.05	0.78	0.77	1.82	1.66	1.31	1.27	0.85	0.95	0.05	1.08	2.17	0.59	1.42	0.23
	Belon 6A	1.04	1.18	0.80	1.13	1.58	1.43	3.55	0.94	0.81	0.03	1.50	2.85	0.64	4.41	0.35
Belon 6B	0.96	0.98	0.69	1.34	1.59	1.85	1.47	0.84	1.05	0.06	1.34	2.69	0.55	2.32	0.48	
Crac'h	Crac'h 1	0.85	0.72	0.59	0.61	1.98	0.20	3.94	0.80	0.54	0.04	0.75	2.96	3.51	8.03	0.35
	Crac'h 2	1.18	0.78	0.78	3.19	1.76	0.37	1.43	1.03	0.87	0.11	0.57	3.04	0.92	1.16	0.28
	Crac'h 3	1.18	0.83	0.83	2.94	1.70	0.33	1.40	1.08	0.99	0.05	0.54	3.50	0.93	1.08	0.27
	Crac'h 4	1.17	0.84	0.78	2.58	2.43	0.37	1.61	1.04	0.89	0.05	0.72	2.44	1.03	1.72	0.30
Elorn	Elorn 1	1.40	0.82	0.94	2.11	1.64	0.33	1.63	1.35	0.82	0.08	0.43	3.30	0.92	2.42	0.30
	Elorn 2	1.09	0.74	0.75	2.69	2.22	0.97	2.10	0.97	1.12	0.04	0.97	2.20	0.98	1.30	0.23

	Elorn 3A	1.14	1.13	0.91	3.09	3.77	2.72	2.44	0.95	1.17	0.04	1.29	1.54	0.99	1.44	0.24
	Elorn 3B	1.11	0.86	0.81	2.70	2.62	1.69	2.02	0.95	0.87	0.04	1.06	2.32	0.92	1.40	0.26
	Elorn 4	1.12	1.10	0.93	2.95	3.96	2.40	2.16	0.93	1.05	0.04	1.15	1.66	0.92	1.45	0.26
Laïta	Laïta 1	1.41	1.32	0.98	1.11	2.10	0.70	1.85	1.20	1.63	0.05	0.54	1.43	0.96	3.45	0.24
	Laïta 2	1.19	1.33	0.93	1.30	2.91	1.83	1.54	1.03	0.91	0.05	0.64	2.71	0.72	2.44	0.25
	Laïta 3	1.27	1.67	1.10	1.43	4.07	2.93	1.82	1.07	0.99	0.05	0.98	3.57	0.87	3.06	0.24
Odet	Odet 1	1.21	0.93	0.90	1.83	2.12	0.86	1.73	1.16	0.96	0.11	0.99	3.10	1.21	1.38	0.30
	Odet 2	1.23	1.07	0.91	1.82	2.07	0.72	1.86	1.13	1.30	0.06	1.03	3.01	1.28	1.42	0.27
	Odet 3	0.90	0.93	0.64	1.64	1.88	0.84	2.12	0.84	1.07	0.06	1.13	6.35	1.49	1.84	0.33
	Odet 4	1.37	1.30	1.07	2.67	2.80	1.36	2.29	1.24	1.60	0.06	1.76	3.86	2.63	2.08	0.38
Vie 2018	Vie ₂₀₁₈ 1	0.88	1.11	1.08	1.99	1.17	0.52	1.17	1.11	1.43	0.05	1.76	1.41	3.14	3.75	0.31
	Vie ₂₀₁₈ 2	1.08	0.96	0.77	1.79	1.32	0.48	1.19	0.99	1.69	0.16	1.51	1.14	2.77	4.17	0.31
	Vie ₂₀₁₈ 3	1.08	0.95	0.74	1.58	1.27	0.50	1.27	1.02	1.15	0.05	1.26	1.46	1.13	1.19	0.22
	Vie ₂₀₁₈ 4	0.76	1.14	0.96	1.44	1.08	0.55	1.85	1.03	1.19	0.13	1.24	1.49	1.11	1.18	0.21
	Vie ₂₀₁₈ 5	1.09	1.01	0.88	1.64	1.33	0.57	1.36	1.03	1.23	0.05	1.30	1.55	1.20	1.18	0.21
	Vie ₂₀₁₈ 6	1.07	1.04	0.86	2.36	1.58	0.54	1.37	0.98	0.97	0.06	1.24	1.79	1.22	1.28	0.23
	Vie ₂₀₁₈ 7A	1.10	1.03	0.85	2.49	1.52	0.56	1.38	0.97	0.88	0.12	1.29	1.52	1.15	1.23	0.24
	Vie ₂₀₁₈ 7B	1.06	1.03	0.90	3.77	1.99	0.59	1.48	1.09	0.97	0.05	1.30	1.49	1.19	1.18	0.23
	Vie ₂₀₁₈ 7C	1.11	1.02	0.81	1.46	1.33	0.54	1.39	0.97	1.08	0.05	1.28	1.51	1.16	1.24	0.22
	Vie ₂₀₁₈ 8A	1.15	1.22	0.85	1.62	1.32	0.55	1.35	0.93	1.07	0.08	1.29	1.43	1.20	1.45	0.23
	Vie ₂₀₁₈ 8B	1.06	1.02	0.82	1.60	1.30	0.58	1.37	0.99	1.09	0.06	1.30	1.77	1.27	1.43	0.24
	Vie ₂₀₁₈ 9	1.04	1.05	0.78	1.72	1.35	0.56	1.36	0.91	1.07	0.04	3.19	1.42	1.20	1.59	0.22
	Vie ₂₀₁₈ 10A	1.13	1.04	0.81	1.75	1.37	0.60	1.35	0.94	1.13	0.04	1.18	1.49	1.07	1.12	0.19
Vie ₂₀₁₈ 10B	1.04	1.08	0.91	1.76	1.35	0.54	1.38	0.91	0.80	0.04	1.24	1.42	1.18	1.52	0.24	
Vie 2020	Vie ₂₀₂₀ 2	1.00	0.84	0.63	1.18	1.07	0.29	1.13	1.00	1.40	0.06	0.95	1.49	2.43	3.81	0.27
	Vie ₂₀₂₀ 3	0.95	0.80	0.69	1.33	1.11	0.28	1.09	0.98	1.22	0.05	0.59	1.46	0.93	0.96	0.19
	Vie ₂₀₂₀ 4	0.99	1.46	0.89	1.14	1.01	0.35	1.44	0.92	1.04	0.05	0.68	1.43	0.96	1.33	0.22
	Vie ₂₀₂₀ 5	0.99	0.88	0.75	1.37	1.08	0.25	1.21	0.94	1.08	0.04	0.85	1.40	0.87	0.98	0.19
	Vie ₂₀₂₀ 7A	0.96	0.91	0.77	3.07	1.15	0.35	1.28	0.92	0.94	0.04	0.64	1.40	0.89	1.02	0.20
	Vie ₂₀₂₀ 7B	0.98	0.88	0.73	1.48	1.08	0.28	1.21	0.94	0.99	0.04	0.65	1.41	0.92	1.05	0.20
	Vie ₂₀₂₀ 7C	0.96	0.85	0.71	1.14	1.07	0.32	1.23	0.93	0.91	0.05	0.69	1.42	0.95	1.19	0.22
	Vie ₂₀₂₀ 8A	0.96	0.87	0.71	1.26	1.21	0.33	1.23	0.95	0.95	0.04	0.67	1.39	1.00	1.19	0.23
	Vie ₂₀₂₀ 8B	0.95	0.90	0.80	1.26	1.14	0.34	1.26	0.88	1.00	0.04	0.77	1.41	0.90	1.30	0.25
	Vie ₂₀₂₀ 9	0.97	0.88	0.70	1.35	1.34	0.42	1.22	0.89	0.93	0.04	0.77	1.32	0.95	1.65	0.23
	Vie ₂₀₂₀ 10A	0.93	0.85	0.68	1.38	1.21	0.40	1.22	0.88	0.76	0.03	0.64	1.27	0.90	1.54	0.23
	Vie ₂₀₂₀ 10B	1.06	0.90	0.72	1.44	1.17	0.41	1.33	0.88	0.95	0.03	0.55	1.34	0.86	1.14	0.18
	Vilaine	Vilaine 1A	1.14	1.07	0.84	1.21	1.45	0.49	1.43	0.96	1.14	0.04	1.02	1.51	0.93	1.35
Vilaine 1B		1.13	1.07	0.86	1.24	1.43	0.37	1.44	1.01	1.14	0.05	0.88	1.58	0.97	1.12	0.17
Vilaine 1C		1.13	1.07	0.88	1.27	1.46	0.39	1.46	1.00	1.13	0.04	0.83	1.52	0.93	1.07	0.17
Vilaine 2A		1.15	1.20	0.94	1.39	1.66	0.48	1.52	1.03	1.13	0.04	1.01	1.53	0.96	1.07	0.17
Vilaine 2B		1.14	1.18	0.94	1.35	1.67	0.45	1.47	1.02	1.06	0.04	0.87	1.50	0.95	1.09	0.17
Vilaine 3		1.18	2.16	1.26	2.16	3.30	2.19	1.39	1.09	1.15	0.03	1.21	1.46	1.19	1.82	0.22

Tableau supplémentaire V.S3 : Eigen values et proportion expliquée des axes de la ACP basée sur les éléments trace métallique (avec une normalisation).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15
Eigenvalues	7.57	2.67	1.26	1.06	0.75	0.48	0.39	0.28	0.22	0.13	0.10	0.04	0.03	0.01	0.007
Proportion expliquée	0.504	0.179	0.084	0.071	0.050	0.032	0.026	0.019	0.015	0.008	0.007	0.003	0.002	0.0008	0.0005

Tableau supplémentaire V.S4 : Valeurs de TOC (percentage of total organic matter), TN (percentage of total nitrogen), et les rapports C/N et $\delta^{13}C$.

Estuary	Station	TOC%	TN%	C/N	$\delta^{13}C$	% grains < 63 μ m	Estuary	Station	TOC%	TN%	C/N	$\delta^{13}C$	% grains < 63 μ m
Elorn	Elorn 1	0.10	NA	NA	-22.60	15.1	Auray 2020	Auray ₂₀₂₀ 1A	2.12	0.32	6.57	-18.04	66.9
	Elorn 2	2.13	0.29	7.44	-23.16	69.2		Auray ₂₀₂₀ 1B	1.48	0.23	6.53	-18.85	68.5
	Elorn 3A	1.93	0.24	8.20	-24.70	72.6		Auray ₂₀₂₀ 1C	1.45	0.23	6.33	-19.47	61.7
	Elorn 3B	2.61	0.25	10.32	-26.62	54.4		Auray ₂₀₂₀ 2A	1.03	0.18	5.76	-18.85	59.1
	Elorn 4	2.17	0.23	9.46	-26.15	59.0		Auray ₂₀₂₀ 2B	2.15	0.37	5.89	-18.56	65.0
Aulne	Aulne 1	1.58	0.24	6.55	-22.10	69.3		Auray ₂₀₂₀ 2C	2.11	0.35	6.03	-18.70	75.3
	Aulne 2	2.02	0.25	8.03	-24.68	70.9		Auray ₂₀₂₀ 4A	1.28	0.21	5.99	-18.17	64.9
	Aulne 3	1.94	0.22	9.01	-26.82	65.2		Auray ₂₀₂₀ 4B	0.97	0.15	6.40	-18.46	59.3

Odet	Odet 1	2.87	0.47	6.12	-21.66	64.4	Vie 2018	Auray ₂₀₂₀ 5A	2.35	0.33	7.22	-18.72	63.2
	Odet 2	2.46	0.34	7.22	-22.92	53.5		Auray ₂₀₂₀ 5B	2.05	0.29	7.12	-21.06	73.5
	Odet 3	1.27	0.16	8.21	-24.50	46.1		Auray ₂₀₂₀ 6A	2.24	0.33	6.84	-21.22	73.6
	Odet 4	1.66	0.22	7.59	-24.90	67.9		Auray ₂₀₂₀ 6B	1.96	0.28	7.08	-22.29	62.6
Belon	Belon 1	1.48	0.20	7.28	-18.82	32.4	Auray ₂₀₂₀ 7	2.35	0.37	6.40	-21.67	71.9	
	Belon 3A	2.69	0.39	6.97	-23.54	68.0	Auray ₂₀₂₀ 8A	2.47	0.34	7.34	-22.59	70.2	
	Belon 3B	0.93	0.12	7.99	-19.65	21.1	Auray ₂₀₂₀ 8B	3.35	0.39	8.58	-24.46	58.7	
	Belon 4A	1.24	0.16	7.53	-23.84	31.4	Vie ₂₀₁₈ 1	0.17	NA	NA	NA	4.5	
	Belon 4B	0.32	0.04	8.70	-15.87	4.3	Vie ₂₀₁₈ 2	0.30	NA	NA	NA	4.0	
	Belon 5A	3.29	0.46	7.20	-24.53	77.6	Vie ₂₀₁₈ 3	2.27	0.27	8.45	-14.31	72.4	
	Belon 5B	3.56	0.46	7.72	-25.00	67.8	Vie ₂₀₁₈ 4	2.08	0.26	7.90	-15.54	66.0	
	Belon 6A	1.41	0.15	9.32	-26.94	36.5	Vie ₂₀₁₈ 5	2.15	0.27	8.09	-17.41	72.9	
Belon 6B	3.65	0.37	9.88	-27.65	60.1	Vie ₂₀₁₈ 6	1.96	0.24	8.15	-17.73	65.0		
Laita	Laita 1	0.59	NA	NA	NA	16.7	Vie ₂₀₁₈ 7A	1.34	0.22	6.09	-19.11	75.0	
	Laita 2	1.45	0.19	7.50	-25.11	29.8	Vie ₂₀₁₈ 7B	1.56	0.27	5.81	-21.00	79.0	
	Laita 3B	0.89	0.13	7.00	-25.37	43.4	Vie ₂₀₁₈ 7C	1.95	0.25	7.70	-14.96	74.7	
Crac'h	Crac'h 1	NA	NA	NA	NA	3.9	Vie ₂₀₁₈ 8A	1.47	0.14	10.19	-13.58	76.3	
	Crac'h 2	1.55	0.25	6.20	-20.39	83.2	Vie ₂₀₁₈ 8B	1.96	0.24	8.28	-23.20	63.8	
	Crac'h 3	2.08	0.33	6.26	-20.65	68.3	Vie ₂₀₁₈ 9	1.51	0.13	11.20	-22.77	70.2	
	Crac'h 4	1.39	0.21	6.49	-21.34	67.1	Vie ₂₀₁₈ 10A	1.72	0.22	7.81	-24.88	79.4	
Auray 2019	Auray ₂₀₁₉ 1A	1.66	0.25	6.61	-18.77	76.6	Vie ₂₀₁₈ 10B	1.16	0.17	6.67	-19.82	88.5	
	Auray ₂₀₁₉ 1B	1.43	0.20	7.07	-18.27	66.9	Vie ₂₀₂₀ 2	0.36	0.03	11.85	NA	5.6	
	Auray ₂₀₁₉ 1C	1.53	0.23	6.58	-17.71	49.4	Vie ₂₀₂₀ 3	1.95	0.35	5.57	-20.61	72.7	
	Auray ₂₀₁₉ 2A	2.27	0.33	6.78	-18.87	83.0	Vie ₂₀₂₀ 4	2.05	0.21	9.90	-18.71	57.4	
	Auray ₂₀₁₉ 2B	1.88	0.31	6.01	-18.84	85.5	Vie ₂₀₂₀ 5	2.28	0.34	6.63	-19.33	75.8	
	Auray ₂₀₁₉ 2C	2.33	0.37	6.27	-18.04	83.4	Vie ₂₀₂₀ 7A	2.05	0.32	6.42	-19.46	78.3	
	Auray ₂₀₁₉ 4A	1.56	0.24	6.41	-18.69	67.0	Vie ₂₀₂₀ 7B	1.60	0.25	6.34	-20.50	67.7	
	Auray ₂₀₁₉ 4B	2.22	0.33	6.74	-19.35	68.3	Vie ₂₀₂₀ 7C	1.30	0.19	6.69	-20.58	68.7	
	Auray ₂₀₁₉ 5A	2.40	0.35	6.82	-19.93	83.9	Vie ₂₀₂₀ 8A	1.41	0.22	6.39	-21.54	74.6	
	Auray ₂₀₁₉ 5B	2.28	0.32	7.19	-21.08	80.4	Vie ₂₀₂₀ 8B	1.07	0.16	6.68	-21.18	68.9	
	Auray ₂₀₁₉ 6A	2.17	0.29	7.59	-21.13	77.2	Vie ₂₀₂₀ 9	1.19	0.18	6.52	-21.37	68.2	
	Auray ₂₀₁₉ 6B	2.15	0.28	7.61	-21.32	79.0	Vie ₂₀₂₀ 10A	1.12	0.17	6.50	-22.40	75.7	
	Auray ₂₀₁₉ 7	2.91	0.37	7.97	-21.21	84.7	Vie ₂₀₂₀ 10B	1.38	0.22	6.25	-22.90	75.1	
	Auray ₂₀₁₉ 8A	2.93	0.34	8.62	-22.69	78.5	Vilaine 1A	1.19	0.21	5.56	-21.76	84.7	
Auray ₂₀₁₉ 8B	1.23	0.13	9.49	-23.86	54.9	Vilaine 1B	1.51	0.26	5.77	-22.57	86.5		
						Vilaine 1C	1.39	0.25	5.68	-22.77	87.6		
						Vilaine 2A	2.03	0.34	5.91	-23.06	91.0		
						Vilaine 2B	1.82	0.33	5.59	-23.22	91.5		
						Vilaine 3	2.00	0.35	5.80	-29.00	81.1		

Tableau supplémentaire V.S5A : Données d'abondances (en ind/50cc) dans l'estuaire de l'Aulne.

Estuary	Station	<i>Ammonia</i> spp.	<i>Elphidium decipiens</i>	<i>Elphidium gerthi</i>	<i>Elphidium incertum</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium williamsoni</i>	<i>Haynesina germanica</i>	<i>Miliolinella subrotunda</i>	<i>Quinqueloculina oblonga</i>
Aulne	1	212,7	5,5	13,8		2,8	47,0	16,6	323,2	2,8	24,9
	2	69,1			2,8		38,7	5,5	219,6		24,9
	3	4,1							66,3		

Chapitre 5: Répartition des foraminifères dans les vasières de neuf estuaires de la façade atlantique française

Tableau supplémentaire V.S5B : Données d'abondances (en ind/50cc) dans l'estuaire d'Auray.

Estuary	Station	Foraminifera Species																													
		<i>Ammonia beccarii</i>	<i>Ammonia spp.</i>	<i>Asterigerinata mamilla</i>	<i>Aubignyna perlucida</i>	<i>Elphidium decipiens</i>	<i>Elphidium gerthi</i>	<i>Elphidium incertum</i>	<i>Elphidium macellum</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium selseyense</i>	<i>Elphidium williamsoni</i>	<i>Haynesina depressula</i>	<i>Haynesina germanica</i>	<i>Stainforthia fusiformis</i>	<i>Miliolinella subrotunda</i>	<i>Miliolid sp.</i>	<i>Quinqueloculina jugosa</i>	<i>Quinqueloculina oblonga</i>	<i>Acostata mariei</i>	<i>Ammobaculites agglutinans</i>	<i>Ammonium salsum</i>	<i>Eggerelloides scaber</i>	<i>Haplophragmoides wilberti</i>	<i>Miliammina fusca</i>	<i>Moridaplecta barbosa</i>	<i>Trochammina inflata</i>			
Auray 2019	1 A	36,2			0,5				7,5	10,3	2,1	0,3		257,1					1,4				1,9					0,5			
	1 B	115,4			2,9	0,4			116,3	150,5	8,5	0,9	4,6	569,2				3,7	65,3	0,2			23,7								
	1 C	182,0			17,6	11,7			305,2	167,2	6,8	2,8	55,9	767,6				1,8	108,8		3,7		48,5		2,6	0,7					
	2 A	0,2												21,9							0,2										
	2 B	1,8		0,9									0,2	26,9					0,2	0,7	0,2		0,5			1,2					
	2 C	99,4		8,3	1,4			0,2	5,5	35,5	0,9			451,9						3,5			0,2	0,2							
	4 A	25,0			0,6				1,2	3,4		1,0		539,2								27,0	31,4	2,8							
	4 B	39,1							0,3	1,8		0,2		239,9							0,2	8,5	0,7	1,4		0,2					
	5 A	2,8												272,8								0,5	0,2	0,2							
	5 B	36,6	0,2						0,7	2,1	2,1	0,5		240,8								15,0	3,0	1,4	1,2			0,2			
	6 A	22,6	0,2	5,5						0,8	0,6			760,8								6,5	9,5								
	6 B	97,4		1,8					1,5	4,6	2,5	0,6	0,6	723,7							0,2	32,4	18,6	6,9	1,4			0,2			
	7	17,0							0,5	3,7		0,7		110,5									6,2								
	8 A	22,8								5,8		0,5		55,9							0,2	3,5	23,5			1,2		0,5			
	8 B	0,5												4,3							1,2	13,7	134,3			3,9		15,0			
	Auray 2020	1 A	0,7	185,8			0,7			8,3	111,2	0,7	2,1	1,4	780,4				34,5	50,4		2,1									
1 B			87,0	0,7					3,5	58,0	4,8	2,1	6,2	418,5				25,6	42,1		7,6		2,1	3,5		2,1					
1 C			72,5	1,4		0,7			4,1	6,9	8,3			108,4	1,4	0,7		10,4	18,0	2,1			23,5		0,7						
2 A			0,7						5,5					74,6						5,5	80,1	68,4		6,9	0,7						
2 B			1,4											33,8						4,8	9,0	35,2									
2 C			71,8			0,7			2,1	31,1		0,7		305,9				17,3	63,5		29,0		1,4					1,4			
4 A			4,1											18,6						3,5	1,4		6,2								
4 B			241,3		0,2	0,5				94,8	0,2	0,9			302,3				14,0	29,9	6,2	119,9	109,1	12,4	0,2	21,6		0,2			
5 A			198,9	2,8						13,8	0,7				507,6							5,5	11,0	55,2							
5 B			33,1							0,7	1,4	2,1			109,8									13,8							
6 A			33,8							0,7	4,1				127,8			2,1		11,7		13,1	5,5	2,1	2,1	0,7	0,7	0,7			
6 B			13,8								33,1				53,2						1,4		14,5	11,7	2,1	4,8	0,7				
7			91,9								51,1				354,3									1,4			1,4				
8 A			20,7							0,7	12,4				74,6						2,1		57,3	15,2	0,7						
8 B			643,0							4,1	631,9				236,9						2,1	4,1	29,7	499,3			504,8		4,1		

Tableau supplémentaire V.S5C : Données d'abondances (en ind/50cc) dans l'estuaire de du Belon.

Estuary	Station	Foraminifera																	
		<i>Ammonia spp.</i>	<i>Asterigerinata mamilla</i>	<i>Cibicides lobatulus</i>	<i>Discorbis vilerboana</i>	<i>Elphidium crispum</i>	<i>Elphidium incertum</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium williamsoni</i>	<i>Haynesina depressula</i>	<i>Haynesina germanica</i>	<i>Mitiloinella subrotunda</i>	<i>Quinqueloculina jugosa</i>	<i>Quinqueloculina oblonga</i>	<i>Acostata mariei</i>	<i>Ammobaculites agglutinans</i>	<i>Eggerelloides scaber</i>	<i>Miliammina fusca</i>
Belon	1	24,5	19,6	2,1	6,3			35,8	22,6	43,9	195,2	359,0	8,2		198,4	1,9			
	3A	7,9						4,9	4,7	20,3		364,5		15,6	26,9				
	3B	11,0	2,1	21,4				0,7	1,4	3,5	2,1	48,3	2,8		2,1			1,4	
	4A	18,6		2,1	1,4				0,7	12,4	1,4	263,8		2,8	2,8				
	4B	9,0	9,0	67,7		10,4					2,8		22,1	0,7	2,1				
	5A												2,8						
	5B	0,7								3,5		82,2							
	6A	2,8					20,7			2,1		28,3					12,4		174,0
6B															0,7	11,7		41,4	

Tableau supplémentaire V.S5D : Données d'abondances (en ind/50cc) dans l'estuaire de de Crac'h.

Estuary	Station	Foraminifera																
		<i>Ammonia beccarii</i>	<i>Ammonia spp.</i>	<i>Asterigerinata mamilla</i>	<i>Aubignyna perlucida</i>	<i>Discorbis vilerboana</i>	<i>Elphidium incertum</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium williamsoni</i>	<i>Haynesina depressula</i>	<i>Haynesina germanica</i>	<i>Quinqueloculina jugosa</i>	<i>Quinqueloculina oblonga</i>	<i>Ammobaculites agglutinans</i>	<i>Annotium salsum</i>	<i>Eggerelloides scaber</i>	<i>Haplomagnum wilberti</i>
Crac'h	1	5,5	74,6	1,4		0,7	73,9	11,0		16,6	130,5	154,0		30,4				
	2		53,2						0,7			100,1	18,0	36,6	1,4			
	3		109,8	1,4					5,5			721,0	2,8	20,7				
	4		160,9		9,7			1,4	89,8	2,8		240,3	3,5	9,0	4,8	1,4	5,5	1,4

Tableau supplémentaire V.S5E : Données d'abondances (en ind/50cc) dans l'estuaire de l'Elorn.

Estuary	Station	Foraminifera																
		<i>Ammonia beccarii</i>	<i>Ammonia spp.</i>	<i>Asterigerinata mamilla</i>	<i>Elphidium gerthi</i>	<i>Elphidium incertum</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium selceyense</i>	<i>Elphidium williamsoni</i>	<i>Haynesina depressula</i>	<i>Haynesina germanica</i>	<i>Stainforthia fusiformis</i>	<i>Quinqueloculina jugosa</i>	<i>Quinqueloculina oblonga</i>	<i>Annotium salsum</i>	<i>Eggerelloides scaber</i>	<i>Trochammina inflata</i>
Elorn	1	6,9			2,8		1,4			5,5	13,8		3,5		285,2		0,7	
	2		58,7	0,7			2,8	20,7	0,7	3,5		276,9		8,3	73,2			
	3A		176,8			2,8		44,2		2,8		410,9			40,1	31,8		
	3B		14,5					2,1				45,6			6,9			2,1
	4		5,5							1,4		50,4						5,5

Tableau supplémentaire V.S5F : Données d'abondances (en ind/50cc) dans l'estuaire de la Laïta.

Estuary	Station	<i>Ammonia spp.</i>	<i>Asterigerinata mamilla</i>	<i>Cibicides lobatulus</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium williamsoni</i>	<i>Haynesina germanica</i>	<i>Quinqueloculina oblonga</i>
Laïta	1		10,4	1,4	2,8	26,9	25,6	2,1
	2	85,6				35,9	745,9	2,8
	3	3,5				7,6	49,0	

Tableau supplémentaire V.S5G : Données d'abondances (en ind/50cc) dans l'estuaire de l'Odet.

Estuary	Station	Species																
		<i>Ammonia spp.</i>	<i>Asterigerinata mamilla</i>	<i>Cibicides lobatulus</i>	<i>Discorbis vilerboana</i>	<i>Elphidium incertum</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium selseyense</i>	<i>Elphidium williamsoni</i>	<i>Haynesina depressula</i>	<i>Haynesina germanica</i>	<i>Quinqueloculina jugosa</i>	<i>Quinqueloculina oblonga</i>	<i>Ammobaculites agglutinans</i>	<i>Ammotium salsum</i>	<i>Miliammina fusca</i>	<i>Trochammina inflata</i>
Odet	1	51,1	1,4	0,7	0,0	2,1	6,2	15,9	1,4	5,5	0,0	415,7	3,5	1,4	0,0	0,0	0,0	
	2	121,5	2,8	0,0	0,0	5,5	5,5	88,4	2,8	38,7	5,5	486,2	2,8	13,8	0,0	0,0	0,0	
	3	397,8	0,0	0,7	0,7	0,0	11,7	382,6	4,1	111,9	0,0	402,6	7,6	13,1	0,0	0,0	0,0	0,7
	4	82,2	0,0	0,0	0,0	0,0	0,0	217,5	0,0	200,3	0,0	169,2	35,9	2,1	3,5	31,1	125,0	

Tableau supplémentaire V.S5H : Données d'abondances (en ind/50cc) dans l'estuaire de la Vie.

Estuary	Station	Species																						
		<i>Ammonia beccarii</i>	<i>Ammonia spp.</i>	<i>Asterigerinata mamilla</i>	<i>Aubignyna perlucida</i>	<i>Cibicides lobatulus</i>	<i>Discorbis vilerboana</i>	<i>Elphidium crispum</i>	<i>Elphidium gerthi</i>	<i>Elphidium incertum</i>	<i>Elphidium macellum</i>	<i>Elphidium magellanicum</i>	<i>Elphidium margaritaceum</i>	<i>Elphidium oceanense</i>	<i>Elphidium poeyanum</i>	<i>Elphidium selseyense</i>	<i>Elphidium williamsoni</i>	<i>Haynesina germanica</i>	<i>Miliolinella subrotunda</i>	<i>Quinqueloculina jugosa</i>	<i>Quinqueloculina oblonga</i>	<i>Ammobaculites agglutinans</i>	<i>Trochammina inflata</i>	
Vie 2018	1	6,7	0,7			0,5		1,6		0,5	0,9	2,3	0,2		0,5	21,6		12,0	0,9	0,5	1,8			
	2	0,2	3,2							194,1			0,7			1,4	3,9	4,1			12,4			
	3		24,1	0,9				0,2		0,2		3,4	2,5	2,1	299,7	6,9	231,4	0,9	12,8	9,1				
	4		23,0	0,9		1,4						10,6	1,4	1,8	427,3	1,4	87,9		35,5	30,8			0,5	
	5		2,5				0,5			0,5		0,5			112,1	0,2	48,1		2,1	3,0				
	6		39,0				1,8					0,5	0,5	0,9	61,8	0,5	512,9		4,9	4,6				
	7A		107,5											11,5	0,9	3,7	1607,0		5,3	5,8			1,2	
	7B		73,9											1,2	21,4	1,8	1157,0		23,5	3,7				
	7C		147,3		0,6							0,9	1,8	4,3	76,7	2,5	628,6		23,6	19,0				
	8A		209,5											48,1	0,5	5,5	1480,7		21,4	32,5				
	8B		247,7											15,7	131,7		608,7		55,2	17,5				
	9		376,6											22,1	48,8	1,8	375,7		51,6	12,0				
	10A		367,2											30,8			473,1		7,1	0,7				
	10B		100,8											1,8	0,5	0,5	80,3		14,7	3,2				
Vie 2020	2		30,4						399,2			1,4			15,2		4,8			9,7				
	3		247,9												1006,9		487,6		6,2	11,0				
	4		44,2			2,8			5,5						1783,1		190,6		1,4	6,9			2,8	
	5		207,2												907,5		616,0	1,4	1,4	9,7				
	7A		53,9										2,8	38,7		1247,2		4,1	4,1	2,8	4,1			
	7B		65,6	0,9											151,0		671,3		16,9	1,8				
	7C		218,2				2,8							1,4	312,2		946,1		5,5					
	8A		34,5											2,8	19,3		638,8		0,7					
	8B		118,8											4,1	40,1		1859,1		5,5	11,0			2,8	
	9		200,3						5,5			0,7			47,7		1725,1		5,5	11,0	5,5	2,8		
	10A		49,0														258,3							
10B		26,9													2,1		312,8		2,1					

Tableau supplémentaire V.S5I : Données d'abondances (en ind/50cc) dans l'estuaire de la Vilaine.

Estuary	Station	<i>Ammonia spp.</i>	<i>Elphidium oceanense</i>	<i>Elphidium selseyense</i>	<i>Haynesina germanica</i>
Vilaine	1A	1584,3	22,7		1347,6
	1B	421,3			836,3
	1C	145,7		9,7	129,8
	2A	223,8	1,4	0,7	5,5
	2B	588,8			63,6
	3	2,8			4,1

Tableau supplémentaire V.S6 : Score des indices MII (Marine influence index), EFI (Estuarine foraminifera index) et EFDI (Estuarine foraminiferal diversity index)

Estuary	Station	MI	EF	EFD	Estuary	Station	MI	EF	EFD	Estuary	Station	MI	EF	EFD
Elorn	Elorn 1	0,82	6,69	0,67	Auray 2019	Auray ₂₀₁₉ 1A	0,48	3,43	0,41	Vie 2018	Vie ₂₀₁₈ 1	1,00	5,78	0,70
	Elorn 2	0,56	3,97	0,56		Auray ₂₀₁₉ 1B	0,67	3,87	0,48		Vie ₂₀₁₈ 2	0,49	6,50	0,58
	Elorn 3A	0,20	3,39	0,38		Auray ₂₀₁₉ 1C	0,66	4,31	0,44		Vie ₂₀₁₈ 3	0,70	5,10	0,61
	Elorn 3B	0,26	3,56	0,33		Auray ₂₀₁₉ 2A	0,08	3,26	0,22		Vie ₂₀₁₈ 4	0,71	5,90	0,55
	Elorn 4	0,04	3,04	0,25		Auray ₂₀₁₉ 2B	0,17	3,14	0,39		Vie ₂₀₁₈ 5	0,58	5,61	0,52
Aulne	Aulne 1	0,86	3,39	0,48		Auray ₂₀₁₉ 2C	0,58	3,34	0,48		Vie ₂₀₁₈ 6	0,72	3,73	0,50
	Aulne 2	0,54	3,58	0,44		Auray ₂₀₁₉ 4A	0,25	3,14	0,33		Vie ₂₀₁₈ 7A	0,18	3,35	0,42
	Aulne 3	0,23	3,33	0,33		Auray ₂₀₁₉ 4B	0,44	3,27	0,30		Vie ₂₀₁₈ 7B	0,30	3,45	0,48
Odet	Odet 1	0,64	3,46	0,61		Auray ₂₀₁₉ 5A	0,29	3,35	0,28		Vie ₂₀₁₈ 7C	0,66	3,80	0,50
	Odet 2	0,54	3,54	0,61		Auray ₂₀₁₉ 5B	0,37	3,23	0,39		Vie ₂₀₁₈ 8A	0,25	3,44	0,48
	Odet 3	0,29	3,42	0,50		Auray ₂₀₁₉ 6A	0,21	3,26	0,42	Vie ₂₀₁₈ 8B	0,64	3,96	0,50	
	Odet 4	0,06	2,87	0,30		Auray ₂₀₁₉ 6B	0,28	3,19	0,39	Vie ₂₀₁₈ 9	0,58	3,76	0,48	
Belon	Belon 1	0,44	5,76	0,63		Auray ₂₀₁₉ 7	0,24	3,20	0,33	Vie ₂₀₁₈ 10A	0,10	3,36	0,44	
	Belon 3A	0,12	3,69	0,48		Auray ₂₀₁₉ 8A	0,13	2,40	0,17	Vie ₂₀₁₈ 10B	0,36	3,63	0,48	
	Belon 3B	0,21	5,29	0,61		Auray ₂₀₁₉ 8B	0,17	0,09	0,11	Vie ₂₀₂₀ 2	0,48	6,41	0,56	
	Belon 4A	0,09	3,45	0,58		Auray ₂₀₂₀ 1A	0,49	3,60	0,55	Vie ₂₀₂₀ 3	0,54	5,27	0,53	
	Belon 4B	0,13	8,10	0,67		Auray ₂₀₂₀ 1B	0,83	3,71	0,53	Vie ₂₀₂₀ 4	0,68	6,26	0,52	
	Belon 5A	0,01	3,33	0,33		Auray ₂₀₂₀ 1C	0,68	3,83	0,52	Vie ₂₀₂₀ 5	0,56	5,09	0,56	
	Belon 5B	0,01	3,33	0,33		Auray ₂₀₂₀ 2A	0,11	1,19	0,22	Vie ₂₀₂₀ 7A	0,18	3,42	0,38	
	Belon 6A	0,00	1,03	0,28	Auray ₂₀₂₀ 2B	0,18	1,68	0,27	Vie ₂₀₂₀ 7B	0,29	3,92	0,61		
Belon 6B	0,00	0,00	0,00	Auray ₂₀₂₀ 2C	0,73	3,66	0,41	Vie ₂₀₂₀ 7C	0,74	4,02	0,47			
Laita	Laita 1	0,42	4,70	0,67	Auray ₂₀₂₀ 4A	0,26	3,15	0,33	Vie ₂₀₂₀ 8A	0,23	3,42	0,47		
	Laita 2	0,29	3,34	0,42	Auray ₂₀₂₀ 4B	0,55	2,68	0,31	Vie ₂₀₂₀ 8B	0,53	3,42	0,43		
	Laita 3B	0,14	3,33	0,33	Auray ₂₀₂₀ 5A	0,29	3,48	0,41	Vie ₂₀₂₀ 9	0,58	3,43	0,44		
Crac'h	Crac'h 1	0,46	5,93	0,67	Auray ₂₀₂₀ 5B	0,46	3,50	0,48	Vie ₂₀₂₀ 10A	0,11	3,29	0,33		
	Crac'h 2	0,39	4,18	0,39	Auray ₂₀₂₀ 6A	0,22	3,14	0,30	Vie ₂₀₂₀ 10B	0,49	3,37	0,50		
	Crac'h 3	0,37	3,43	0,56	Auray ₂₀₂₀ 6B	0,38	2,57	0,25	Vilaine 1A	0,19	3,33	0,33		
	Crac'h 4	0,14	3,31	0,37	Auray ₂₀₂₀ 7	0,31	3,33	0,33	Vilaine 1B	0,24	3,33	0,33		
					Auray ₂₀₂₀ 8A	0,14	2,08	0,37	Vilaine 1C	0,30	3,45	0,44		
					Auray ₂₀₂₀ 8B	0,20	1,98	0,26	Vilaine 2A	0,09	3,34	0,42		
									Vilaine 2B	0,11	3,33	0,33		
									Vilaine 3	0,07	3,33	0,33		

Chapitre 6

Synthèse

Les objectifs de ce travail de thèse étaient de 1) mieux comprendre la répartition naturelle des foraminifères dans un milieu estuarien, afin de 2) pouvoir donner des pistes concernant le développement d'une méthode de bio-indication adaptée au suivi des vasières estuariennes.

Le **chapitre 2** s'inscrit directement dans cette thématique en décrivant la répartition des espèces de foraminifères dans les estuaires d'Auray et de la Vie, et en investiguant leurs relations avec les paramètres environnementaux. Les **chapitres 3** et **4** se concentrent plus particulièrement sur la morpho-espèce *Ammonia tepida*, qui peut être dominante dans les écosystèmes estuariens. Des analyses récentes ont démontré que cette morpho-espèce est composée de plusieurs espèces pseudo-cryptiques dont l'une est suspectée d'être exotique, voire invasive. Le **chapitre 3** étudie la répartition actuelle de ces trois espèces d'*Ammonia*, tandis que le **chapitre 4** se focalise sur l'histoire de l'espèce supposément exotique/invasive dans l'estuaire de la Loire. Le **chapitre 5** élargit l'approche suivie dans le deuxième chapitre à neuf estuaires de la façade atlantique. Dans ce dernier chapitre nous proposons des indices qui décrivent quantitativement la position des communautés le long du gradient estuarien.

1. Répartition des foraminifères en milieu estuarien

1.1. Description des communautés estuariennes

Pour répondre au premier objectif de cette thèse, nous avons étudié la répartition des foraminifères dans neuf estuaires de la façade atlantique française. Grâce à ces observations, (décrites aux **chapitres 2 et 5**), nous proposons un modèle de succession des espèces le long du gradient estuarien. Ce modèle (Figure VI.2) est inspiré par celui de Debenay (Figure VI.1) (Debenay et al., 2000). Le modèle de Debenay, est basé sur trois estuaires et propose : 1) un gradient longitudinal amont-aval avec trois espèces morphologiques dominantes : *Haynesina germanica*, *Ammonia tepida*, et *Miliammina fusca*, et 2) un gradient transversal, du schorre au chenal, avec des espèces calcaires (p.ex., *Haynesina germanica*) dans la partie basse de la vasière et des espèces agglutinées (*Ammotium salsum*, *Trochammina inflata*, *Haplophragmoides wilberti*) sur le schorre. Sur la base de nos observations dans les neuf estuaires étudiés, nous avons affiné ce modèle, en identifiant plusieurs espèces typiques pour chacune des différentes zones de l'estuaire. Chaque estuaire présente une succession d'espèces appartenant à quatre groupes : partie marine, partie aval, partie intermédiaire et partie amont des estuaires. Cependant, les espèces typiques de chaque groupe peuvent être différentes d'un estuaire à un autre. En effet, chaque estuaire présente une communauté avec un assemblage spécifique d'espèces de foraminifères. Plusieurs facteurs peuvent expliquer les différences observées entre les estuaires étudiés : 1) la géomorphologie et le microclimat, 2) l'histoire de l'occupation et l'utilisation anthropiques, et 3) l'histoire de la biocénose (p.ex., dérive écologique ; invasions biologiques), en partie en réponse aux deux premiers facteurs. Cependant, malgré des dissimilarités dans leurs assemblages, nos neuf estuaires sont caractérisés

par une proximité géographique, et présentent deux taxons en commun, qui occupent toujours une place dominante dans les assemblages : *Haynesina germanica* et *Ammonia* spp. Contrairement à nos observations sur la façade atlantique l'espèce morphologique *Ammonia tepida* ne dépasse pas 3% de l'assemblage total dans cinq estuaires des côtes françaises de la Manche (Francescangeli et al., 2020). Cette divergence liée probablement à la différence biogéographique doit être prise en compte. Pour cela l'étude d'autres estuaires permettrait d'affiner ce modèle.

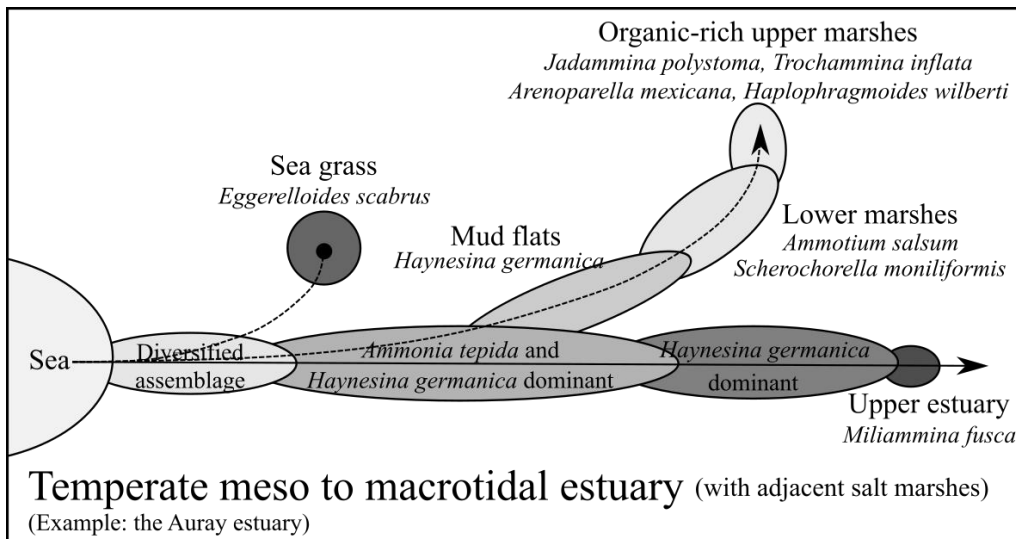


Figure VI.1 : Succession des espèces selon le gradient longitudinal (amont-aval) et transversal. D'après Debenay et al., 2000.

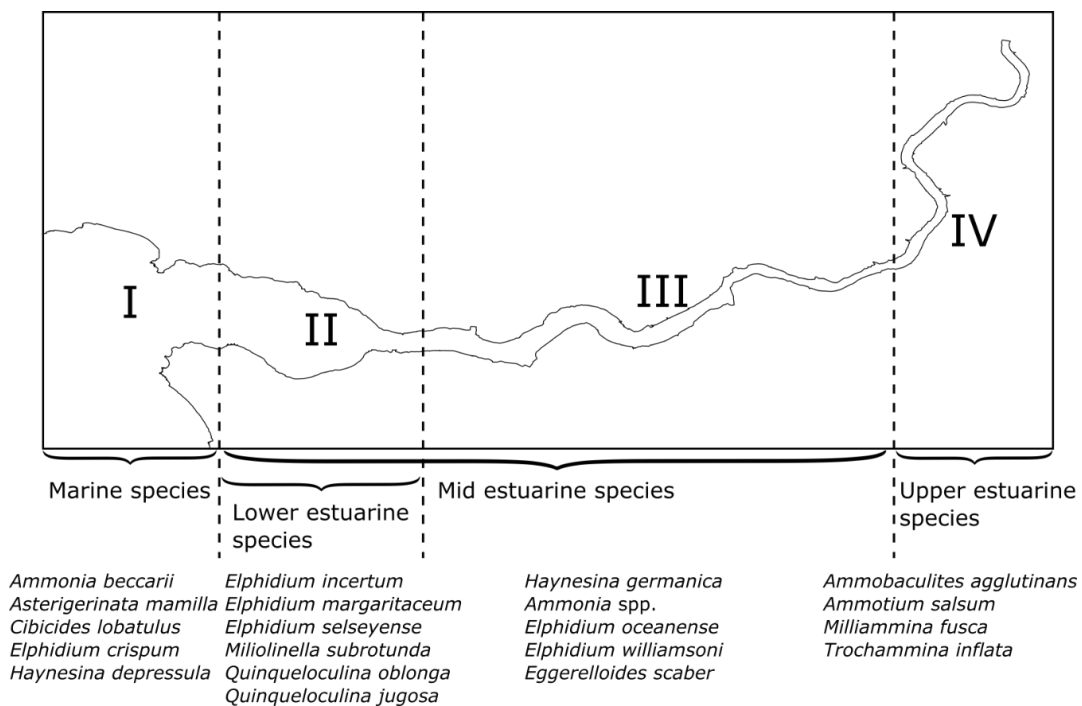


Figure VI.2 : Succession des espèces le long du gradient estuarien. Figure issue du chapitre 2.

Notre modèle décrit donc une succession de quatre groupes d'espèces. En fonction des caractéristiques intrinsèques de chaque estuaire, tous ces groupes ne sont pas forcément présent dans chaque estuaire. Cette représentation partielle peut d'abord être expliquée par notre effort d'échantillonnage focalisé sur les vasières estuariennes. Cela a mis de côté les stations marines et celles où la salinité était très basse. Ensuite, cette succession incomplète peut aussi provenir des constructions humaines, comme les barrages, qui modifient fortement les gradients estuariens naturels. Enfin, la morphologie naturelle des estuaires pourrait être à l'origine de transitions abruptes entre différentes zones estuariennes, qui expliqueraient l'absence de communautés typique d'une certaine zone.

Afin de représenter la succession des quatre groupes d'espèces de façon quantitative, nous proposons deux nouveaux indices. L'indice EFI (Estuarine Foraminiferal Index) est basé sur les abondances relatives cumulatives des espèces de chaque groupe, tandis que l'indice EDFI (Estuarine Diversity Foraminiferal Index) est basé sur la diversité spécifique au sein de chacun de ces quatre groupes. Ces indices permettent de résumer la communauté de foraminifère à une station donnée avec une seule valeur, qui décrit sa position par rapport à la succession des quatre biozones estuariennes. Ces deux indices, EFI et EDFI, sont complémentaires et doivent être utilisés simultanément. L'indice EFI prend en compte la succession d'espèces dominantes le long du gradient estuarien, tandis que l'indice EDFI est plutôt basé sur la biodiversité de chaque zone, et tente de résoudre le problème de la très forte dominance de *Haynesina germanica* dans une grande partie de l'estuaire. L'indice EDFI présente l'avantage additionnel qu'il peut également être utilisé pour des données semi-quantitatives issues du metabarcoding.

1.2. Réponses des foraminifères aux paramètres naturels

Dans la littérature scientifique, il y a un consensus selon lequel la salinité est le paramètre principal structurant la répartition des organismes dans les milieux estuariens (Scott et al., 1980; Cearreta, 1988; De Rijk, 1995; Attrill, 2002; Ysebaert et al., 2003; Crain et al., 2004; Horton & Murray, 2007; Telesh & Khlebovich, 2010). Cependant, il est difficile de mesurer ce paramètre dans les estuaires de façon précise, car il fluctue en permanence en fonction des cycles de marée, des phénomènes de crue du fleuve, et pour les vasières, en fonction des événements météorologiques (précipitations et évaporations). Ainsi, une mesure ponctuelle ne reflète que très partiellement la gamme de salinité à laquelle sont soumis les organismes vivant sur la vasière. Dans ce contexte, il est important de disposer d'un outil qui puisse estimer le degré d'influence marine auquel est soumise la communauté échantillonnée. A cette fin, nous avons développé un indice d'influence marine (MII) (Jorissen et al., 2022) qui traduit de façon quantitative l'influence des eaux marines à un point d'échantillonnage et à une date donnés. Cet indice inclut trois métriques qui contrôlent le degré d'influence marine : la distance du point d'échantillonnage de la mer, la durée d'émergence à marée basse et la décharge fluviale (dans les quatre semaines avant l'échantillonnage). Cet indice reflète non seulement la salinité, mais aussi un plusieurs autres variables, liées à l'intrusion d'eau marine dans l'estuaire,

tel que l'apport en nutriments d'origine marine et l'hydrodynamisme. D'autres paramètres non inclus dans le MII, tels que la température, la granulométrie du sédiment ou l'oxygénation de l'eau de fond peuvent également influencer les communautés de foraminifères. Néanmoins, nos résultats suggèrent que les paramètres inclus dans le MII et ceux corrélés à cet indice soient les plus importants pour décrire la répartition des communautés. En effet, nos données montrent que l'indice est corrélé avec plusieurs caractéristiques des communautés, ce qui suggère qu'il peut être utilisé pour résumer les relations entre les paramètres naturels du milieu et la composition des communautés de foraminifères. Une possible utilisation de cet indice pour décrire les conditions de référence est traitée en section II.1.a.

Dans cette thèse, nous avons observé très peu d'indications indiscutables de pollution anthropique, et par conséquent, nous n'avons pas observé de réponses évidentes des communautés de foraminifères à de telles pollutions.

Afin de mieux connaître la réponse des communautés de foraminifères à de telles perturbations, pour pouvoir développer une méthode de bio-indication efficace, de futures études devraient donc se focaliser sur des zones de forte pollution avérée. De telles études permettraient de vérifier que les caractéristiques des communautés montrent une dérive par rapport à celles répondant essentiellement aux paramètres naturels. La nature de ces éventuelles dérives, en termes de diversité ou de composition de la communauté, devrait permettre d'identifier des bio-indicateurs de pollution et de construire un indice robuste de qualité environnementale.

1.3. Focus sur le genre *Ammonia* spp.

Les espèces du genre *Ammonia* sont très communes dans les milieux côtiers et estuariens (Hayward et al., 2004; Bird et al., 2020), et font partie des premières espèces de foraminifères décrites (Brünnich, 1771). Malgré cela, les espèces composant ce genre ont longtemps été déterminées de façon limitée. En effet, au cours des cinquante dernières années, les espèces de ce genre ont souvent été attribuées (sur la base de la morphologie) à une à trois espèces, avec une distribution cosmopolite (Hayward et al., 2021). Les progrès en barcoding des cellules individuelles des foraminifères ont permis de clarifier la taxonomie des espèces de ce genre. Au sein de l'ancienne espèce (= morphogroupe) *Ammonia tepida*, on distingue aujourd'hui les espèces *A. veneta*, *A. aberdoveyensis* et *A. confertitesta* (correspondant aux phylotypes T1, T2 et T6 respectivement) (Richirt et al., 2019; Hayward et al., 2021). Cependant, étant donné que cette révision du genre *Ammonia* est récente, la plupart des études antérieures à 2020 portant sur les écosystèmes estuariens utilisent l'ancienne taxonomie. Au vu de la prédominance de ces taxa dans les milieux estuariens (p.ex., Cearreta, 1988; Alve & Murray, 1994; Debenay et al., 2000; Camacho et al., 2015; Martins et al., 2016; Mojtahid et al., 2016; Saad & Wade, 2017; Francescangeli et al., 2021), il est nécessaire de comprendre et décrire précisément la répartition des trois espèces présentes dans nos estuaires.

Il est probable que la distribution de ces trois espèces reflète différentes exigences écologiques. Dans un contexte de bio-indication, il est crucial de mieux comprendre leur répartition et niches écologiques, ainsi que leur tolérance aux perturbations environnementales. Cette question est abordée dans le **chapitre 3**. Grâce à des observations sous microscope électronique à balayage (MEB), nous avons analysé la morphologie de 578 individus d'*Ammonia* répartis dans 64 stations appartenant à sept estuaires. Pour aucune des trois espèces, nous n'avons observé un gradient amont-aval clair au sein de la partie centrale de l'estuaire (Figure VI.2 : la biozone III). Nous n'avons pas non plus observé de différences des relations vis-à-vis des paramètres environnementaux (tels que la granulométrie et le TOC) entre les trois espèces. En revanche, les espèces *A. veneta* et *A. aberdoveyensis* présentent une légère préférence pour les stations les plus proches du chenal, suggérant une tolérance limitée vis à vis de l'émersion. Nous avons aussi démontré que les trois espèces ne sont pas présentes dans les mêmes proportions dans les neuf estuaires étudiés. En effet, l'espèce *A. confertitesta* est rare, voire absente, dans les estuaires les plus au nord (Elorn, Aulne, Odet, Crac'h et Auray), tandis que plus au sud, cette espèce est commune (estuaire de la Vie), voire fortement dominante (estuaire de la Vilaine). Nous suggérons que ces différences géographiques peuvent être expliquées par le caractère invasif de cette espèce (Pawlowski & Holzmann, 2007; Schweizer et al., 2011; Richirt et al., 2021). En effet, les patrons de distribution d'*A. confertitesta* pourraient correspondre à différents stades de remplacement des espèces autochtones (*A. veneta*, *A. aberdoveyensis*). Des conditions favorables pour ce remplacement pourraient se produire après de fortes crues du fleuve, qui créent de nouveaux habitats en amenant du sédiment et/ou en emportant une grande partie des organismes. Ces niches nouvellement libérées favoriseraient *A. confertitesta*, qui recoloniserait plus rapidement grâce à son opportunisme plus élevé.

Ces résultats amènent à la question si l'aire de répartition d'*A. confertitesta* est toujours en expansion. Cette question est importante pour le suivi des communautés de foraminifères dans les estuaires. Si l'extension est toujours en cours, il faut s'attendre à de futurs changements des communautés en lien avec la densité croissante d'*A. confertitesta*. Dans un tel cas, les méthodes de bio-indication devraient être ajustées progressivement pour prendre en compte l'expansion de cette espèce. Dans cette optique, nos résultats ont soulevé plusieurs questions complémentaires. L'analyse morphologique des différentes espèces d'*Ammonia* dans l'estuaire de la Vie montre une co-dominance d'*A. veneta* et d'*A. confertitesta* à toutes les stations (Figure VI.3). Nous avons émis l'hypothèse que cette co-dominance est un état transitoire, c'est-à-dire que le remplacement progressif de l'espèce autochtone par l'espèce exotique est en cours. Une hypothèse alternative serait que cet assemblage mixte corresponde à un état d'équilibre, où les deux espèces cohabitent de manière pérenne. S'il s'agit d'un état transitoire, de futures études devraient montrer un remplacement complet de *A. veneta* par *A. confertitesta* dans cet estuaire. Cette observation illustre une question plus générale, concernant l'ensemble des estuaires des côtes européennes, notamment celle de la vitesse d'installation d'*A. confertitesta*, lors de la colonisation d'un nouvel estuaire. L'étude de plusieurs carottes sédimentaires dans des milieux actuellement colonisés par *A. confertitesta* permettraient d'obtenir plus d'informations sur les modalités de la colonisation de cette espèce.

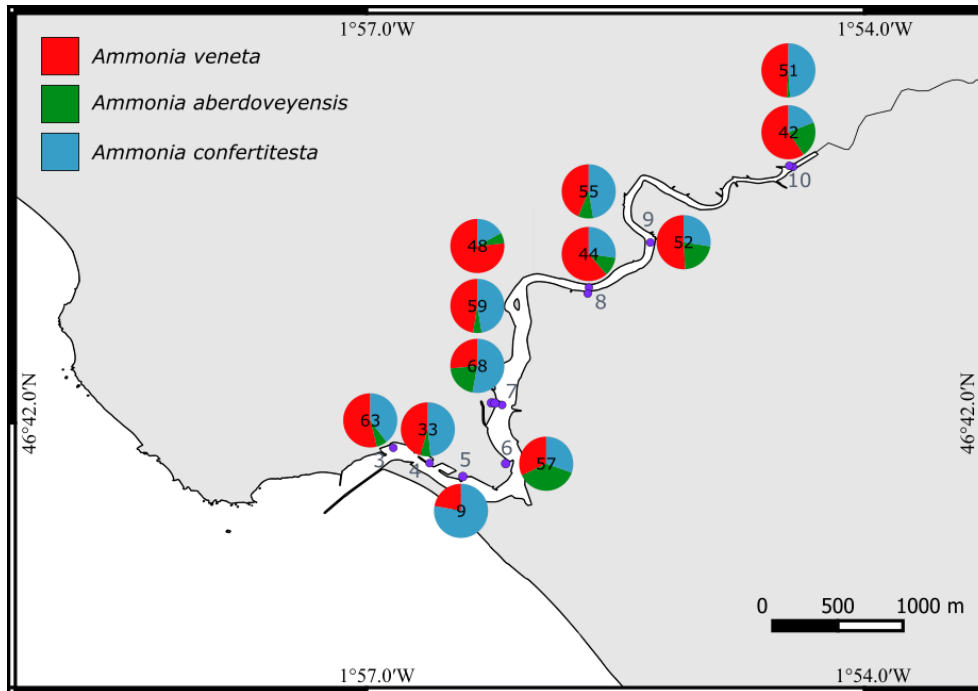


Figure VI.3 : Observations morphologiques des individus d'*Ammonia veneta* (rouge), *A. aberdoveyensis* (vert) et *A. confertitesta* (bleu) dans les échantillons de l'estuaire de la Vie (récoltés en octobre 2018). Les nombres d'individus identifiés par station sont indiqués au centre de chaque diagramme. Ces résultats sont issus du chapitre 3.

Pour répondre au moins partiellement à cette question et après avoir étudié l'expansion spatiale de cette espèce supposée invasive, il semblait utile d'étudier l'aspect temporel. Pour cela, dans le **chapitre 4**, nous avons étudié la succession des assemblages du genre *Ammonia* dans deux carottes sédimentaires, prélevées dans l'estuaire de la Loire (Durand, 2017). Plus précisément, les objectifs de ce chapitre étaient 1) de confirmer le caractère invasif d'*A. confertitesta* ; 2) de dater l'époque d'introduction de cette espèce ; et 3) d'étudier la vitesse de remplacement de l'espèce autochtone par l'espèce supposée invasive. Dans les deux carottes sédimentaires étudiées, la première apparition d'*A. confertitesta* a est observée dans des sédiments datés de plusieurs siècles, situés à plusieurs mètres de profondeur (2,50m pour la première carotte et 1,50m pour la deuxième). Les sédiments les plus profonds contiennent uniquement *A. aberdoveyensis*. Plus haut dans les carottes, les deux espèces sont observées ensemble sur un large intervalle, représentant environ deux siècles. Et enfin, seuls les individus d'*A. confertitesta* sont présents dans les sédiments près de la surface. Ceci démontre que *A. confertitesta* est bien une espèce invasive et que son introduction est bien plus ancienne que ce qui a été suggéré précédemment (c.-à-d. une introduction remontant au milieu voir au début du 20^e siècle) (Pawlowski & Holzmann, 2007; Schweizer et al., 2011; Bird et al., 2020; Richirt et al., 2021, 2022). Nos résultats suggèrent une longue période de cohabitation des deux espèces d'*Ammonia*, avant que la population de l'espèce autochtone ne décline et disparaisse.

Néanmoins, ces résultats amènent d'autres questions. Nous avons observé un déclin de l'espèce *A. aberdoveyensis* suite à l'introduction de *A. confertitesta*, mais qu'en est-il du reste de la communauté de foraminifères ? Est-ce que l'introduction de cette espèce a aussi eu des conséquences pour d'autres espèces ?

L'étude plus complète des assemblages de foraminifères dans les carottes longues de la Loire permettrait de répondre à cette question à cet endroit. L'étude de carottes sédimentaires d'autres sites, comme par exemple dans l'estuaire de la Vilaine, où *A. confertitesta* est majoritaire aujourd'hui, est nécessaire afin de savoir si nos observations faites dans l'estuaire de la Loire peuvent être généralisées, ou si l'histoire de l'invasion est différente pour chaque estuaire. De telles études pourraient aussi indiquer si l'introduction de cette espèce invasive a transformé les communautés en place.

2. Quelques pistes pour développer des outils de biomonitoring pour le suivi des vasières estuariennes

2.1. Les conditions de référence

Les milieux estuariens ont la particularité d'être habités par des communautés peu diversifiées avec une forte proportion d'espèces opportunistes (Dauvin, 2007; Elliott & Quintino, 2007). Pour être capable de détecter une anomalie de distribution des organismes liée aux activités anthropiques, il est nécessaire de parfaitement connaître les conditions de référence, c'est-à-dire la distribution naturelle de ces organismes (Borja et al., 2012). En mesurant l'écart entre la communauté naturelle (les conditions de référence) et la communauté observée (de Paz et al., 2008), on peut estimer le degré de perturbation de la communauté étudiée. En pratique, trois approches peuvent être utilisées pour déterminer les conditions de référence :

1) Il est possible d'utiliser des sites actuels avec des caractéristiques environnementales comparables aux conditions naturelles (p.ex., des aires marines protégées), et de décrire les communautés trouvées dans ce(s) site(s). Cependant, cette méthode est difficilement applicable dans les milieux estuariens où chaque estuaire présente un contexte géologique, morphologique et biologique particulier.

2) Ensuite, il est possible de modéliser un état de référence à partir des données actuelles (Muxika et al., 2007; van Loon et al., 2018). Ces états de référence modélisés sont généralement basés sur les valeurs de diversité observée aux sites les moins affectés par des activités anthropiques. A titre d'exemple, pour l'étude de la macrofaune, certains auteurs ont utilisé les meilleures valeurs de chacune des métriques trouvées dans les sites échantillonnés (p.ex., en utilisant les percentiles (Puente et al., 2010; Van Hoey et al., 2013; van Loon et al., 2015b)). Pour l'étude des foraminifères, des auteurs ont utilisé la granulométrie pour établir des conditions de références, sur la base de l'observation que les apports de matière organique sont étroitement corrélés avec la granulométrie du sédiment (Barras et al., 2014). Cependant, cette méthode ne peut pas être appliquée dans les estuaires, où la salinité est le principal paramètre contrôlant les communautés.

3) Finalement, avec des organismes fossilisables, il est possible d'utiliser les archives sédimentaires pour faire une reconstitution historique de la succession des communautés au cours du temps, et ainsi avoir des informations concernant les caractéristiques des communautés avant l'impact des activités anthropiques (p.ex., Barmawidjaja et al., 1995; Alve et al., 2009; Polovodova Asteman et al., 2015; Hess et al., 2020).

Puisque dans la majorité des cas, l'impact anthropique actuel ne peut pas être complètement supprimé, les conditions de référence obtenues avec ces méthodes ne correspondent pas forcément aux objectifs de restauration. L'objectif sera plutôt d'obtenir une bonne qualité environnementale dans un contexte d'utilisation anthropique.

Dans cette thèse, nous avons apporté des éléments à l'application de ces différentes méthodes dans les vasières intertidales estuariennes. Dans les chapitres 2 et 5, nous avons proposé des pistes pour modéliser des conditions de référence. Dans le chapitre 4, nous avons étudié des archives sédimentaires pour obtenir plus d'information sur l'invasion de l'espèce *Ammonia confertitesta*.

Dans la suite de ce chapitre, nous discuterons plus en détail deux des méthodes permettant de reconstituer ces conditions de référence. D'abord la méthode développée dans cette thèse, qui consiste à comparer l'indice MII (qui résume les paramètres environnementaux naturels) et les indices EFI/EFDI (qui résument la composition de la faune), et ensuite l'apport potentiel des études de carottes sédimentaires.

a) La comparaison des indices MII et EFI/EFDI

Dans les **chapitres 2** et **5**, nous proposons deux indices qui permettent la comparaison entre les conditions environnementales (MII) et la composition des faunes (EFI, EFDI). Ces indices pourraient permettre de mieux déterminer si la composition de la communauté est en équilibre avec les conditions environnementales, et, si ce n'est pas le cas, de relever des anomalies de distribution dues à des perturbations.

Le MII est un indice reflétant l'influence marine ressentie par les organismes à la station échantillonnée. Comme détaillé dans le **chapitre 2**, cet indice résume dans une seule métrique les principaux paramètres qui contrôlent les communautés de foraminifères. Dans notre zone d'étude, cet indice montre de bonnes corrélations avec d'autres paramètres environnementaux influencés par les apports marins, comme le pourcentage de silts et d'argiles, et le rapport $\delta^{13}\text{C}$, qui dépend du mélange de matière organique marine et continentale. Cet indice a d'abord été testé sur un jeu de données restreint (27 stations réparties dans deux estuaires, **chapitre 2**), et ensuite sur un jeu de données étendu (90 stations réparties dans neuf estuaires, **chapitre 5**). Dans les deux cas, nous avons observé une corrélation positive entre le MII et la richesse spécifique. Ainsi, la richesse spécifique est naturellement plus basse aux stations où l'influence marine est moins importante. De plus, les résultats du **chapitre 5** montrent des corrélations significatives entre le MII et

les abondances relatives des espèces de différents taxons (c.-à-d., *Quinqueloculina jugosa* et *Q. oblonga*, *Elphidium margaritaceum* et *E. selseyense*, *Ammotium salsum*). Ces résultats sont donc prometteurs pour l'utilisation du MII comme descripteur des paramètres environnementaux influençant la répartition naturelle des communautés.

Cet indice pourrait être utilisé pour déterminer si la communauté observée est en équilibre avec les conditions naturelles. En effet, le MII montre de très bonnes corrélations à la fois avec l'indice EFI et l'indice EFDI. Ceci suggère que la comparaison entre le MII et le EFI ou EFDI peut servir d'outil pour déterminer si la composition de la communauté reflète les conditions naturelles ou si cette communauté est affectée par d'autres facteurs, possiblement d'origine anthropique. Dans le second cas, on s'attend à observer une déviation par rapport à la courbe de régression qui décrit la relation entre les paramètres naturels du milieu (le MII) et la composition des communautés de foraminifères (EFI/EFDI) (Figure VI.4). Un écart par rapport à la courbe de référence, notamment avec un score d'EFI/EFDI plus bas par rapport à sa valeur de MII associée, serait révélateur d'une anomalie de la composition des communautés par rapport aux conditions environnementales du site, et pourrait être le signe d'une perturbation anthropique.

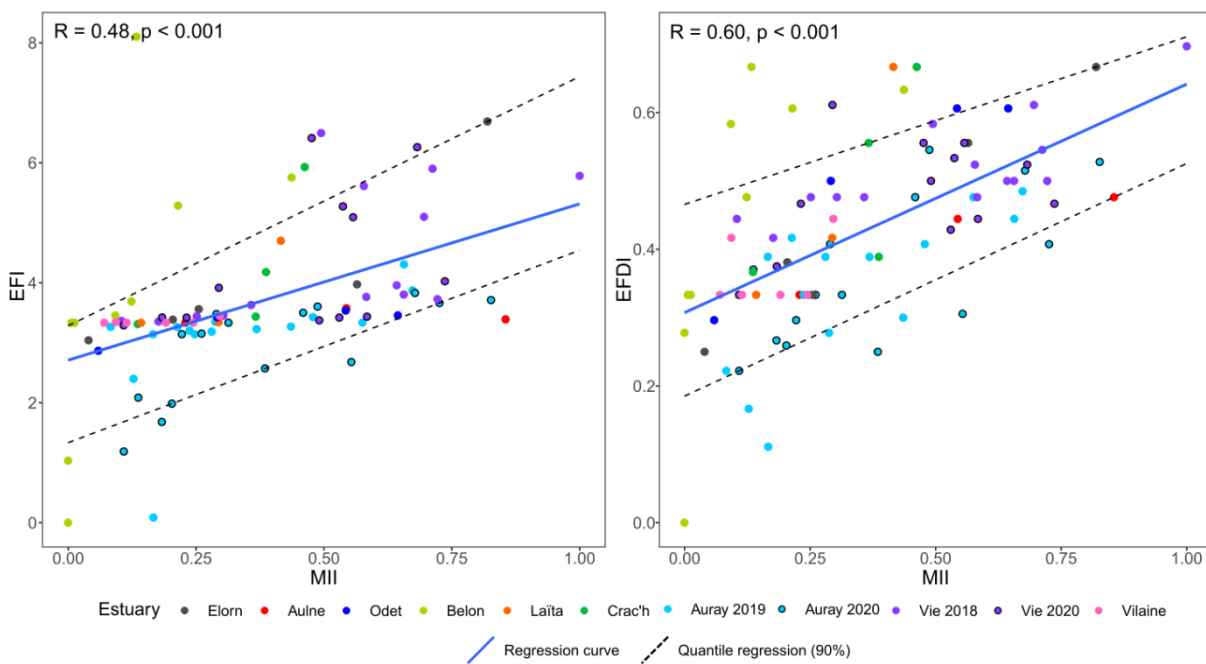


Figure VI.4 : Relations entre les indices EFI, EFDI et le MII. Cette figure est issue du chapitre 5.

Il semble nécessaire d'utiliser deux métriques décrivant les communautés à cause de la nature de la succession d'espèces dans les estuaires. Comme expliqué dans la première partie, même s'il y a une succession récurrente d'espèces dans les estuaires étudiés, la plupart des assemblages observés dans la zone III, qui correspond à la partie centrale de l'estuaire, sont très fortement dominés par l'espèce *Haynesina germanica*. Cette dominance diminue quelque peu la résolution de l'indice EFI, car toutes les stations dominées par cette espèce afficheront un score similaire, masquant ainsi d'éventuelles successions des

espèces avec des abondances plus faibles. Pour cette raison, l'utilisation simultanée de l'indice EFDI, qui est basé sur la diversité de chacun des quatre groupes estuariens, est conseillée. Cet indice est plus diagnostique, mais est moins robuste, car l'observation d'un seul exemplaire d'une espèce peut faire varier la valeur de l'indice.

Ces trois indices peuvent non seulement s'appliquer à une seule station, mais aussi servir à la comparaison de plusieurs stations à différents endroits dans un même estuaire, voire des stations issues de différents estuaires ou des échantillons récoltés lors de de saisons différentes. De futures études pourraient tester cette approche, de comparer le MII avec des indices décrivant la faune, sur d'autres modèles de communautés estuariennes sédentaires, tel que la macrofaune.

b) Les carottes sédimentaires

Après la mort des foraminifères, leurs coquilles minéralisées sont préservées dans la colonne sédimentaire. Les assemblages préservés permettent aux scientifiques de remonter le temps et d'étudier les communautés de foraminifères vieilles de plusieurs décennies à plusieurs millions d'années et ainsi d'obtenir des informations sur l'état de l'environnement dans le passé. Cette capacité des foraminifères de pouvoir fournir des informations sur la composition des communautés pré-impact anthropique a souvent été mise en avant dans le cadre du biomonitoring. Plusieurs auteurs ont ainsi étudié l'évolution historique des communautés de foraminifères vis-à-vis de l'augmentation des activités anthropiques (p.ex., Barmawidjaja et al., 1995; Cearreta et al., 2000, 2002; Scott et al., 2005; Dolven et al., 2013; Francescangeli et al., 2016; Daniel et al., 2019; de Jesus et al., 2020; Klootwijk et al., 2021). Cependant, l'utilisation des archives sédimentaires pour reconstituer la composition des communautés dans une époque sans impact anthropique et pour s'en servir comme conditions de référence, se heurte à plusieurs contraintes.

Pour les milieux estuariens, la première contrainte est de trouver des sites propices au prélèvement d'une carotte sédimentaire. En effet, les vasières intertidales sont des environnements très dynamiques et les dépôts sédimentaires y sont souvent fortement remaniés par le fort hydrodynamisme estuarien, et/ou par la forte activité de bioturbation des organismes (Andersen, 2017). Il devient alors difficile d'obtenir des archives continues, avec un taux de sédimentation stable au cours du temps. Un problème additionnel est que dans la majorité des cas, la non-linéarité de l'enregistrement ne devient évidente qu'après datation de la carotte.

La deuxième contrainte concerne les problèmes de transformations taphonomiques des assemblages. Pour certains groupes de foraminifères, les coquilles ne sont pas toutes conservées dans les taphocénoses (assemblages fossiles). Les tests organiques ne sont généralement pas préservés dans le sédiment (Pawłowska et al., 2016) et les tests agglutinés les plus fragiles subissent des pertes taphonomiques importantes (Schröder, 1988; Bender et al., 1995). De plus, l'étude des taphocénoses doit prendre en compte les déplacements post-mortem d'individus par les courants qui peuvent biaiser l'étude de l'assemblage

(Schönfeld et al., 2012), et affectant notamment les mesures de diversité. Enfin, des pertes taphonomiques importantes peuvent être dues aux activités des bactéries câbles (« cable bacteria »). Richirt et al. (2022) ont montré que ces bactéries peuvent générer une importante diminution du pH en subsurface conduisant à une dissolution des foraminifères à test calcaire. Ce phénomène découvert récemment (Nielsen et al., 2010) a été observé dans l'estuaire d'Auray (étudié dans cette thèse) et peut entraîner la perte des tests calcaires de foraminifères dans les archives sédimentaires (Fouet, Daviray et al., in prep).

La troisième contrainte concerne les invasions biologiques. L'étude menée dans le **chapitre 4** détaille l'introduction et l'implantation de l'espèce *Ammonia confertitesta* dans l'estuaire de la Loire. L'implantation de cette espèce s'est accompagnée de la disparition progressive de l'espèce autochtone *Ammonia aberdoveyensis*. De plus, les résultats présentés dans les **chapitres 2 et 3** montrent qu'aux certains sites *A. confertitesta* peut atteindre des densités très élevées, qui la rendent majoritaire dans les communautés. L'implantation d'*A. confertitesta* aurait donc pu causer une diminution de la biodiversité. Cependant, de telles expansions d'espèces invasives ne sont pas la conséquence directe d'une pollution, mais se font dans des conditions plutôt naturelles (quelques fois accrues par le dérèglement climatique), malgré l'origine éventuellement anthropique de l'introduction. Ainsi, il est possible qu'au cours du temps, il y ait eu des baisses de diversité notable, sans que le degré de pollution (ou la position sur le gradient estuarien) ait changé pour autant. L'utilisation d'archives historiques pour définir les conditions de référence peut donc poser un problème lors de la comparaison avec les communautés récentes, car l'implantation de ces nouvelles espèces a pu transformer les communautés de manière substantielle.

Finalement, l'utilisation des archives sédimentaires pour déterminer des conditions de référence ne permet pas de prendre en compte les transformations morphologiques (construction de barrages) et hydrologiques (changement climatique) qui ont eu lieu dans les estuaires. Ces changements sont souvent dus à des activités anthropiques qui modifient les caractéristiques de l'environnement naturel, sans pour autant augmenter le degré de pollution, ou diminuer la qualité écologique.

Malgré ces limitations, l'utilisation des archives historiques peut fournir des informations précieuses sur l'évolution des communautés en réponse aux modifications anthropiques de l'estuaire, et aux pollutions éventuelles (p.ex., impact des activités aquacoles, rejet de stations d'épuration, marée noire). Un choix judicieux des sites échantillonnés pour l'étude des archives historiques peut largement limiter l'impact des biais décrits ci-dessus. Dans ce contexte, il est essentiel de sélectionner des sites éloignés des constructions anthropiques (qui ont pu modifier l'hydrodynamisme naturel), où la vase est en accrétion. Il est aussi préférable de privilégier les sites où l'arrivée d'espèces invasives n'a pas eu lieu, ou n'a pas modifié la composition des communautés actuelles de façon substantielle.

2.2. Quelles méthodes d'analyse pour les communautés de foraminifères dans les vasières estuariennes ?

Depuis une décennie, des progrès significatifs ont été réalisés concernant les techniques de séquençage haut débit de l'ADN environnemental (Deiner et al., 2017). De plus en plus d'études proposent d'utiliser le metabarcoding à la place ou en parallèle de la technique de détermination sous loupe binoculaire (Pawlowski et al., 2014b; Cordier et al., 2017; Frontalini et al., 2020; Cavaliere et al., 2021). Pour la mise en place du biomonitoring des vasières intertidales, il est donc nécessaire de se poser la question du choix de la méthode utilisée en routine. Pour cela il faut comparer les avantages et les limitations de ces deux méthodes. En effet, les travaux entrepris dans le contexte du projet FORESTAT, dans lequel s'inscrit cette thèse, ont permis de mieux évaluer les atouts de chacune de ces deux approches.

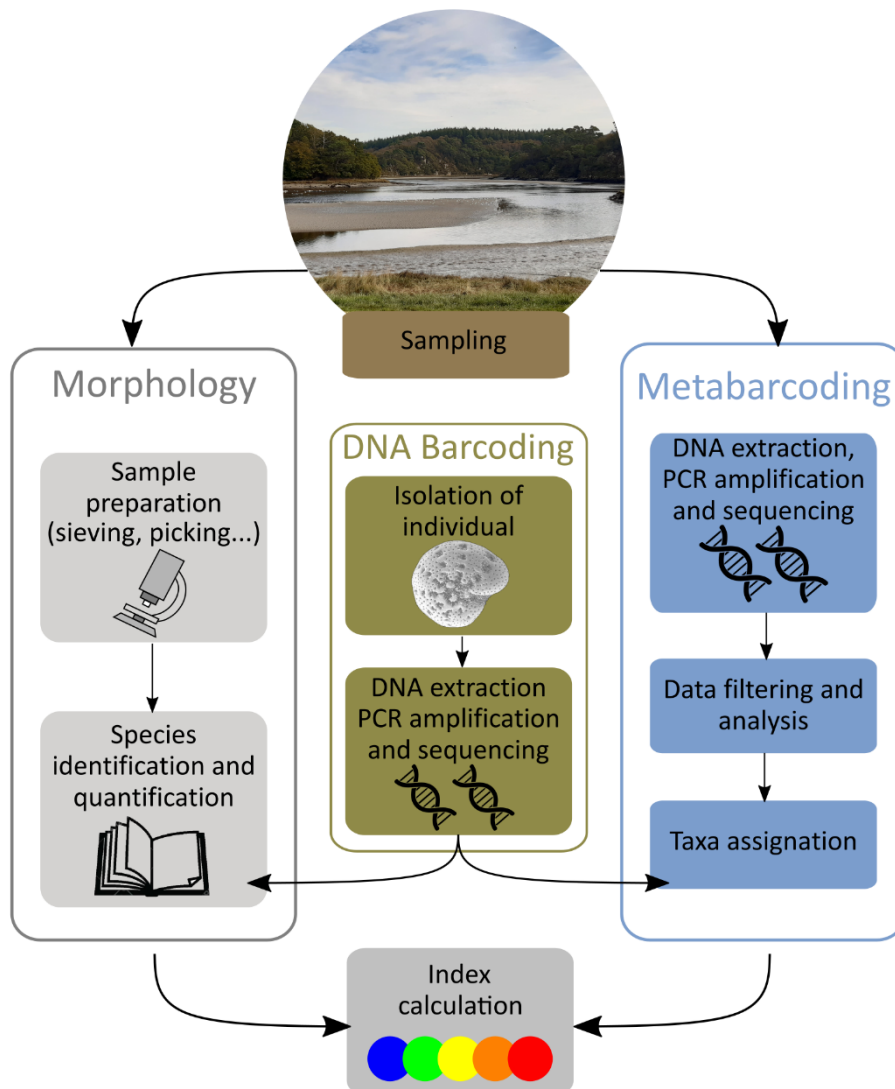


Figure VI.5 : Schéma des étapes clés des procédures de biomonitoring avec les approches morphologique et metabarcoding.

La Figure VI.5 récapitule succinctement les procédures des deux méthodes qui sont discutées dans ce chapitre. L'approche morphologique consiste en plusieurs étapes : 1) les échantillons de sédiment récoltés sont d'abord lavés et tamisés, 2) puis ils sont triés manuellement sous loupe binoculaire afin d'isoler les spécimens de foraminifères vivants (colorés au rose Bengal ou au CTG), 3) finalement, ces spécimens sont identifiés pour pouvoir réaliser un inventaire des différentes espèces observées et de leur abondance. L'approche metabarcoding suit les étapes suivantes : 1) l'ADNe est extrait du sédiment puis 2) les barcodes sont amplifiés avec des primers spécifiques ou universels et 3) les amplicons sont séquencés sur des plateformes de séquençage haut débit, 4) finalement, le jeu de données obtenu est filtré avec des outils bioinformatiques, et les séquences proches sont regroupées en OTUs (operational taxonomic unit) qui sont attribuées à une espèce grâce à une base de données de référence. Le barcoding de cellule individuelle permet de faire le lien entre ces deux méthodes, car il combine l'identification morphologique et le séquençage du barcode d'un même individu. Le barcoding permet donc d'affiner la taxonomie en clarifiant les relations entre les espèces synonymes et cryptiques. En outre, cette méthode permet d'identifier morphologiquement les individus auxquels appartiennent les séquences environnementales des différentes espèces dans la base de référence (utilisée pour l'identification des séquences provenant de l'ADNe). Dans l'approche morphologique et de metabarcoding, les inventaires obtenus sont ensuite interprétés grâce à des outils de biomonitoring qui attribuent une classe de qualité écologique à la communauté observée.

Les contraintes méthodologiques imposées par le biomonitoring

Le choix des méthodes sera influencé par deux contraintes inhérentes au biomonitoring. La rapidité ainsi que la simplicité de la méthode sont primordiales afin que les outils de biomonitoring puissent être utilisés en routine et soient utilisables par des non-experts (Bouchet et al., 2012).

Dans un premier temps, ces deux contraintes vont motiver le choix de la fraction de tamis utilisée pour l'observation morphologique. Le protocole FOBIMO (Schönfeld et al., 2012) recommande d'étudier la fraction de sédiment supérieure à 125 μ m. Cependant, les auteurs conseillent de regarder la fraction 63 μ m dans le cas où la diversité dans la fraction supérieure serait trop faible. En effet, par rapport à la fraction >125 μ m, la fraction >63 μ m contient plus de taxons, parce qu'elle inclut des juvéniles, mais aussi des adultes d'espèces de petite taille. Plusieurs études ont comparé les scores d'indices obtenus sur des résidus de tamisage différents (Bouchet et al., 2012; Lo Giudice Cappelli & Austin, 2019; Fossile, 2022; Klootwijk & Alve, 2022). Ces études montrent que même si la composition de l'assemblage peut être très différent entre les fractions, les tendances générales de la biodiversité restent les mêmes, quelle que soit la fraction étudiée. Il est certain que la fraction >63 μ m contient une diversité plus élevée que celle >125 μ m, grâce à la présence de petites espèces opportunistes (p.ex., les espèces des genres *Stainforthia*, *Epistominella*, *Bolivina*) (Mojtahid et al., 2006; Diz & Francés, 2008; Bergamin & Romano, 2016). Ces espèces vont donc augmenter

la diversité et également influencer les scores des indices basés sur les proportions relatives des espèces opportunistes. Néanmoins, la fraction $>125\mu\text{m}$ contient aussi des espèces opportunistes, permettant d'utiliser les indices de sensibilité écologique sur cette fraction sans problème. Les seuils des classes de qualité écologique des indices, qui sont largement basés sur le pourcentage d'espèces opportunistes, ne seront donc pas les mêmes pour des inventaires basés sur les fractions $>125\mu\text{m}$ et $>63\mu\text{m}$ (Bouchet et al., 2012).

Additionnellement, plus la fraction étudiée sera fine, plus les incertitudes de détermination, en particulier pour les individus juvéniles, seront importantes. En effet les individus juvéniles n'ont souvent pas encore développé tous les traits morphologiques, rendant dans certains cas l'identification taxonomique jusqu'à l'espèce particulièrement difficile, voire impossible (Hermelin, 1986; Schönfeld, 2012; Bouchet et al., 2012). Cette contrainte limite largement l'utilisation de cette fraction par des non-experts.

Enfin, l'analyse de la fraction $>125\mu\text{m}$ plutôt que celle $>63\mu\text{m}$ permet un gain de temps considérable (Schönfeld et al., 2012; Bouchet et al., 2012). Plus la fraction étudiée est fine, plus le temps de tri est long. Pour toutes ces raisons, dans un contexte de biomonitoring des vasières estuariennes, il est préférable d'étudier la fraction $>125\mu\text{m}$, car ce choix représente un compromis entre un temps d'analyse raisonnable et une quantité d'information suffisante pour utiliser des indices de qualité environnementale (Schönfeld et al., 2012).

Dans le contexte du biomonitoring, un des avantages avancé pour justifier le choix de la méthode de metabarcoding est qu'elle permet de traiter plus rapidement un plus grand nombre d'échantillons que la méthode morphologique (Pawlowski et al., 2014b, 2018). En effet, bien que des approches innovantes comme l'utilisation d'un « splitter » humide (Charrieau et al., 2018) ou la séparation par densité (Parent et al., 2018) aient accéléré la préparation des échantillons pour l'observation morphologique, l'étape de tri consistant à isoler les foraminifères du sédiment, reste une étape chronophage. Cependant, des méthodes de reconnaissance automatiques sont en cours de développement et pourraient à l'avenir substantiellement diminuer le temps d'analyse (Hsiang et al., 2019; Mitra et al., 2019; Marchant et al., 2020; Johansen et al., 2021; Ozer et al., 2022). En revanche, pour la méthode de metabarcoding, le traitement des données brutes et l'attribution des OTU à une espèce, en s'appuyant sur une base de données de référence, demande des connaissances spécifiques en bioinformatique (Lara et al., 2022). Lors de cette étape, plusieurs seuils devront être appliqués afin de nettoyer le jeu de données et d'enlever les contaminations ou artefacts présumés. Ce choix des seuils est déterminant pour le jeu de données final (Pawlowski et al., 2018). Par exemple, lors de l'élaboration du **chapitre 3** (portant sur la répartition des trois espèces d'*Ammonia* spp., basée à la fois sur la méthode morphologique et moléculaire) nous avons d'abord appliqué le seuil le plus strict concernant le nombre de séquences minimum pour considérer une OTU comme valide. Avec le seuil initialement retenu, l'espèce *Ammonia aberdoveyensis*, (couramment observée avec la méthode morphologique) était absente du

jeu de données basée sur l'analyse de metabarcoding. Il a donc fallu baisser ce seuil pour détecter cette espèce. Sans les connaissances apportées par l'approche morphologique, cette espèce, pourtant abondante dans les inventaires morphologiques, n'aurait pas été détectée avec le metabarcoding.

Un autre défi du metabarcoding dans le cadre du biomonitoring concerne la difficulté de relier les abondances des séquences aux abondances effectives des espèces dans les communautés (Elbrecht & Leese, 2015; Deiner et al., 2017). Pour le moment, le nombre de séquences obtenu n'est pas directement proportionnel aux nombres d'individus. En effet, la quantité de séquences obtenues par OTU va être influencée par de nombreux facteurs tel que le nombre de cycles d'amplification, la spécificité des primers, ou le nombre de copies par cellule (Pawlowski et al., 2014b, 2018). Pour ces raisons, certains auteurs proposent d'utiliser uniquement les données de présence/absence des espèces (Pawlowski et al., 2018). Or, les données de présence/absence montrent souvent des limites dans le cadre du biomonitoring. Pour pallier à ces limitations des nouvelles approches, telles que la correction par le biovolume (Singer et al., 2018), peuvent être envisagées pour limiter ces biais et pourraient finalement permettre l'utilisation d'une densité relative (Lara et al., 2022).

Un besoin d'améliorer les connaissances taxonomiques

La taxonomie des foraminifères, qui est traditionnellement basée sur des traits morphologiques du test, est encore sujette à discussion, à cause de l'absence d'informations concernant la génétique des individus, et l'existence de nombreux synonymes et/ou d'espèces pseudo-cryptiques (Darling et al., 2016; Jorissen et al., 2018; Bouchet et al., 2021). Cette confusion taxonomique est à la fois une contrainte pour la méthode morphologique (en compliquant la comparaison des jeux de données produits par différents opérateurs), mais aussi pour la méthode de metabarcoding, car elle est à l'origine d'erreurs dans la base de données de référence.

En effet, l'approche de metabarcoding permet de connaître les différentes OTUs présentes dans l'ADNe d'un échantillon (Pawlowski et al., 2018). Ces OTUs sont ensuite attribuées à des espèces dont le barcode est connu grâce à une base de données de référence. Cependant, le lien entre les OTUs et les espèces n'est pas toujours évident, plusieurs OTUs peuvent appartenir à une même espèce (diversité génétique intraspécifique) (Pawlowski et al., 2014a, 2018), ou inversement (Schweizer et al., 2011). De plus, malgré un effort de barcoding conséquent, beaucoup d'OTUs ne sont pas encore attribuées à une espèce connue (Pawlowski et al., 2014a, 2018; Cordier et al., 2017). Par exemple, Cavaliere et al. (2021) n'ont pu attribuer que 7% des OTUs à des espèces connues, et Pawlowski et al. (2014) 33.8%, dans des échantillons provenant du milieu marin ouvert. Dans les vasières estuariennes de nos sites d'étude (Singer et al., soumis (en annexe)), un quart des OTUs ont pu être attribuées. En revanche, toutes les espèces dominantes ont été détectées. Ceci peut

s'expliquer par la faible diversité spécifique des habitats estuariens en comparaison avec le milieu marin ouvert.

Afin de pallier à cette limitation, certains auteurs proposent d'utiliser une approche par machine learning pour développer des modèles prédictifs. Le but est d'attribuer des valeurs d'indices biotiques à partir des données de metabarcoding sans passer par l'attribution taxonomique (méthodes appelées « taxonomy free ») (Cordier et al., 2017, 2019b). L'objectif de ces méthodes est dans un premier temps d'entraîner le modèle à reconnaître la classe de qualité écologique grâce à un ensemble de données d'apprentissage constitué d'échantillons dont les valeurs d'indices biotiques sont connues grâce aux inventaires morphologiques. Dans un second temps, ces modèles pourraient déterminer les valeurs d'indices biotiques sans passer par les inventaires morphologiques.

Dans ce contexte, le DNA barcoding de foraminifères individuels permet de résoudre un grand nombre de problèmes taxonomiques. En effet, ces dernières années, des études combinant les approches morphologiques et moléculaires (par barcoding) ont permis à la fois de clarifier et redéfinir la taxonomie de certaines espèces. Concernant les espèces estuariennes, ce travail est bien avancé, et a été largement finalisé pour les espèces des genres *Ammonia* (Bird et al., 2020; Hayward et al., 2021), *Haynesina* et *Elphidium* (Schweizer et al., 2011; Pillet et al., 2013; Roberts et al., 2016; Darling et al., 2016). Cependant, ce travail n'a pas encore été effectué pour les espèces estuariennes agglutinées et porcelanées.

Les études basées sur la détermination morphologique ne prennent généralement pas en compte les espèces à test organique (monothalamides) (Holzmann et al., 2022). En effet, ces espèces, qui ne portent pas de coquille minéralisée, se désintègrent lors du séchage des échantillons (Gooday, 2002). De plus, le manque de caractéristiques morphologiques distinctives entrave l'identification taxonomique de ce groupe (Anikeeva et al., 2019; Goineau & Gooday, 2019). Additionnellement, les exigences écologiques des différents taxon sont mal connues, ce qui limite leur utilisation en biomonitoring (Schönfeld et al., 2012). Néanmoins, les études de metabarcoding prennent en compte les monothalamides et fournissent des informations concernant l'écologie de ces espèces (Pawlowski et al., 2014a; Holzmann et al., 2021, 2022). En effet, les espèces de monothalamides peuvent représenter la majeure partie des OTUs détectées dans les assemblages moléculaires (Pawlowski et al., 2014b; Cordier et al., 2019a; Singer et al., en révision). Il n'est pas encore clair si cette dominance reflète une réalité écologique ou si elle est expliquée par des biais méthodologiques (c.-à-d., taille du barcode plus court chez les monothalamides que dans les autres clades, le choix des amorces ou du kit d'extraction) (Singer et al., en révision).

En perspective, il sera utile d'homogénéiser la taxonomie utilisée par différents chercheurs avant de proposer un suivi des communautés de foraminifères. A cette fin, il semble pertinent d'organiser des ateliers de taxonomie sur les espèces de foraminifères estuariennes pour intercalibrer les différentes écoles taxonomiques comme cela a été proposé par plusieurs auteurs (Alve et al., 2016; Bouchet et al., 2021). De

plus, à l'instar des études concernant les genres *Elphidium* et *Ammonia*, il serait nécessaire de clarifier la taxonomie des espèces agglutinées et porcelanées estuariennes. De futures investigations combinant les approches morphologique et moléculaire (barcoding individuel) permettront d'identifier plus précisément ces taxons, tout en alimentant les bases de données de référence pour des analyses de metabarcoding.

Différentes approches, différents objectifs

Concernant les analyses des communautés actuelles, la méthode de metabarcoding détecte généralement une diversité plus importante qu'une analyse sous loupe binoculaire, notamment parce qu'elle inclut du matériel génétique issu d'individus dont la taille est inférieure à la taille de tamis utilisés avec la méthode morphologique (125µm, voir 63µm) (Pawlowski et al., 2014b). Ce matériel génétique englobe de manière indifférenciée des adultes, des juvéniles, des propagules, des stades de dormance biologiquement inactifs (Ross & Hallock, 2016), voire même des individus morts. Cette plus grande diversité peut être prise comme un avantage mais aussi comme un inconvénient. En effet, les propagules resteront en dormance et ne se développeront pas tant que les conditions de l'environnement ne leur seront pas favorables, et finiront par mourir (Alve & Goldstein, 2002)). Ainsi, la présence dans l'ADN environnemental d'espèces peu tolérantes aux perturbations anthropiques du milieu n'est pas nécessairement indicative d'une absence de telles perturbations, car les séquences peuvent provenir uniquement d'individus morts ou de propagules. En résumé, les différentes approches d'analyse ne fournissent pas les mêmes renseignements. La fraction >125µm représente les individus adultes et actifs dans l'écosystème, la fraction >63µm inclut d'autres espèces plus petites et des juvéniles, tandis que l'étude du sédiment complet (par metabarcoding) regroupe de façon non différenciée les adultes, les juvéniles, les propagules, des stades de dormance et les individus morts.

Dans de futures études, il serait intéressant de comparer l'ADNe extrait de sédiment brut avec l'ADNe extrait de différentes fractions de sédiment obtenues par tamisage. Les résultats permettraient d'avoir plus d'information sur l'origine des différents fragments génétiques (adultes, juvéniles, propagules, kystes), et de savoir si l'analyse de l'ADNe d'une fraction granulométrique précise donne les mêmes résultats que l'analyse sous microscope de cette même fraction.

En résumé, ces deux méthodes proposent deux visions complémentaires de la communauté de foraminifères. L'analyse de metabarcoding sur la totalité du sédiment met en évidence la présence de matériel génétique d'une espèce, elle renseigne sur les espèces qui pourraient potentiellement se développer si les conditions leur sont favorables. En revanche, l'étude morphologique, sur un sédiment tamisé, renseigne sur la présence d'individus matures et actifs qui ont pu se développer dans les conditions qui règnent sur le site.

Un bon exemple de l'intérêt de combiner ces deux approches a été démontré dans cette thèse. Dans le **chapitre 3**, les individus de l'espèce *Ammonia confertitesta* ont été détectés par metabarcoding, avec un nombre de séquences important dans tous les estuaires étudiés. En revanche, cette espèce n'était pas présente dans les inventaires morphologiques de tous les sites, et elle était abondante seulement dans quelques-uns. Ainsi, la complémentarité de ces deux approches nous a permis de comprendre que cette espèce s'est propagée dans tous les estuaires grâce au transport des propagules (Alve & Goldstein, 2003) mais que cette espèce a trouvé des conditions propices pour s'implanter et se développer massivement dans quelques sites seulement.

En perspective, pour donner un exemple plus concret de l'utilisation conjointe de ces deux méthodes, l'utilisation de metabarcoding et de détermination morphologique pourrait éventuellement valider l'utilisation d'*Elphidium selseyense* en tant qu'espèce indicatrice de perturbation anthropique. En effet, cette espèce montre des abondances élevées dans quelques stations de l'estuaire de la Vie mais elle est rare voire absente dans les autres sites étudiés. Nous avons émis l'hypothèse que sa présence pourrait être la conséquence d'apports anthropiques de matière organique. Afin de valider cette hypothèse, il faudrait savoir si sa présence est liée aux conditions particulières trouvées à ces sites, ou plutôt à sa répartition géographique naturelle. Si cette espèce est identifiée dans l'ADNe de tous les estuaires, il y a une forte indication que cette espèce s'est développée dans l'estuaire de la Vie à cause de conditions particulières. Dans le cas contraire, si des traces génétiques de cette espèce sont uniquement présentes dans les échantillons de l'estuaire de la Vie, il est plus probable que son développement dans ce site soit un phénomène biogéographique. Donc l'utilisation du metabarcoding devrait permettre de trancher entre ces deux possibilités, et de savoir si cette espèce peut être utilisée comme un indicateur d'apports anthropiques de matière organique.

En résumé, vu le caractère très complémentaire des informations fournies par l'analyse morphologique et l'analyse de metabarcoding, l'approche idéale pour évaluer et suivre l'état écologique d'un site estuarien serait de les utiliser simultanément. Une telle approche permettrait d'obtenir une vision d'ensemble, combinant la vision des communautés potentielles et avérées présentes sur le site étudié.

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Annexe 1

The Marine Influence Index (MII): A Tool to Assess Estuarine Intertidal Mudflat Environments for the Purpose of Foraminiferal Biomonitoring

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Concept Paper

The Marine Influence Index (MII): a tool to assess estuarine intertidal mudflat environments for the purpose of foraminiferal biomonitoring

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Abstract: In this paper, we propose a Marine Influence Index (MII), which is thought to give an integrated quantitative description of the complex of environmental parameters controlling foraminiferal faunas in estuarine intertidal mudflats. The MII contains three components: 1) the relative distance along the salinity gradient, 2) the emergence time relative to a reference tidal cycle, and 3) the relative importance of river outflow in the 30 days before sampling the foraminiferal faunas. Although these three parameters have all a strong relation with salinity, they implicitly also include other environmental parameters, like the introduction of marine and continental organic matter and biota, hydrodynamic energy, or temperature. To show the functioning of this new index, MII is calculated for 28 stations in the Auray and Vie estuaries, for two different periods. The next step will be to compare the MII with faunal data sets. Ideally, this comparison should allow us to find strong correlations between some characteristics of the foraminiferal assemblages and MII. If such strong correlations would indeed be found, any major deviation of this relationship could then be interpreted as due to strong anthropogenic disturbance.

Keywords: estuaries; bio-monitoring; foraminifera; anthropogenic impact; pollution

1. Environmental biomonitoring in coastal marine ecosystems

Coastal areas are essential for human societies, since they host about 10% of the global population [1]. Among coastal areas, estuaries provide significant commercial and recreational benefits. They are intensively used, for fisheries, aquaculture, navigation, tourism and many other human activities, and thereby supply huge ecosystem services. Since they constitute a physical entrance to low laying parts of the continent, they may be a vector for storm surges, often with dramatic consequences [2]. Estuaries also play a key role in the transfer of organic carbon, nutrients and pollutants from the continent to the ocean. Thereby, they strongly influence marine life and ultimately contribute to climate change. Like many other transitional water bodies, estuaries are of particular interest from a biological point of view. They are among the most productive natural ecosystems in the world, they may act like nurseries, provide shelter to migrating faunas, and are transited by species that alternate marine and freshwater habitats during their ontogeny.

The careful management of estuaries has been a priority of humankind at least since the Middle Ages. Today, management not only focuses on the physical protection against floods or storm surges, but is also strongly concerned with the quality of estuarine environments, as expressed by their biological, chemical and hydrodynamic characteristics. In Europe, the Water Framework Directive obliges all member states to monitor the quality of its coastal and transitional water masses, to evaluate their ecological status [3] and, if necessary, to take appropriate measures to guarantee a good quality of these ecosystems. To describe the ecosystem quality, a wide range of environmental indices has been developed, based on different characteristics, such as hydrodynamics, morphodynamics, chemical/physical properties, and especially, the biological inhabitants of the concerned ecosystems.

In this context, biotic indices, based on the characteristics of the faunas/floras (such as density, diversity and species composition) are considered particularly pertinent to evaluate the environmental quality. In fact, concentrations of chemical pollutants in the water column and/or in the interstitial waters of the sediment, and their comparison with reference conditions, may inform us about excess supplies resulting from anthropogenic activities. However, data on the enrichment of certain pollutants in the sediment or in the water column will neither inform us about their bio-availability, nor about their impact on biological

communities. Conversely, the density, diversity, species composition and functional traits of the living biota may provide valuable information about ecosystem functioning, and the extent to which it may be affected by pollutants. On the one hand, the presence, absence and/or relative abundance of certain stress-sensitive species (or functional traits) will inform us whether their tolerance levels have been surpassed or not. On the other hand, a dominance of stress-tolerant taxa, that often adopt an opportunistic life strategy, may indicate an elevated degree of environmental stress.

For marine ecosystems, the concept of Pearson and Rosenberg (1978) [4] has been pivotal. These authors showed the existence of a well-defined succession of species, with different ecological strategies, along organic matter enrichment gradients. Their conceptual model has been used to develop a large number of environmental quality indices. In the simplest form, these indices use the relative proportions of a number of faunal groups with different ecological requirements to obtain a quantitative measure of environmental quality. More elaborated indices are based on multivariate analyses of a wide range of biotic parameters [5–7].

A large number of biological groups has been proposed for these biotic indices, ranging from fishes, macro-invertebrates, macro-algae to micro-eukaryotes. Different faunal/floral groups will give information on different spatial and temporal scales, in function of their ecology and life strategies. Consequently, the environmental quality evaluation resulting from different indices is not necessarily identical.

Among microeukaryotes, foraminifera have increasingly been used over the last decennia to develop biotic indices. The international FOBIMO consortium has made significant efforts to standardise the sampling and sample treatment procedures [8]. The FOBIMO consortium has also started to adapt the AMBI-method [9], that was originally developed for macrofauna, to foraminifera. This has been achieved by attributing benthic foraminiferal taxa to different ecological groups and by testing these groups on regional datasets [10–12].

It is important to realise that in the original AMBI method [9] and in all similar methods, including most foraminiferal indices of environmental quality, species are listed in function of their response to organic matter gradients. This implicitly means that organic matter is considered as a major, integrative, stress factor. In view of the generally coherent and robust results yielded by most environmental indices based on the Pearson and Rosenberg paradigm, this hypothesis seems to be valid in most open marine ecosystems. The underlying rationale is that in coastal marine ecosystems, excess nutrient input leads to eutrophication (increase of biological production, organic matter deposition on the seafloor and hypoxia) [9]. In practise, nutrients and organic matter of anthropogenic origin will often be accompanied by a complex cocktail of other pollutants [13], some of which may be particularly harmful for marine life. In spite of this complexity, indices using the response of marine biota to organic matter as an overall marker of environmental quality, often give reliable results (e.g., [14,15]). The success of this approach may be explained by the fact that most stress-tolerant taxa living in open marine environments are not only resistant to an increased organic matter supply (and its consequences, like hypoxia), but also to a wide range of other stressors.

Another important aspect of most biotic indices is that they give a face value evaluation of the present-day quality of the monitored environments. These methods essentially oversee the fact that there may be considerable variety in environmental quality in natural ecosystems. In fact, under natural conditions, some ecosystems will receive much larger nutrient and organic matter supplies than other ones. This is especially the case for open marine ecosystems influenced by river outflow, or ecosystems subject to deposition of large amounts of fine-grained sediments, which tend to be naturally enriched in organic matter [16,17]. In such cases, biotic indices will often indicate moderate or even bad environmental quality. This contrasts with the idea that pristine ecosystems (without any influence of anthropogenic activities), should theoretically have an optimal environmental quality. Consequently, the values of biotic indices cannot always be taken at face value, but have to be compared with reference conditions. In practise, an Ecological Quality Ratio can be determined by dividing the index values of a study site by the one obtained for a reference site [18].

Today, the choice and description of reference conditions remain a major subsisting problem in environmental monitoring. A fundamental question is whether the reference conditions should represent pristine conditions, comparable to the situation in pre-industrial times. Today, most ecosystems on Earth are to a certain extent affected by human activities. Since there is no possibility to rapidly overturn this situation, it is generally considered more realistic not to use pristine ecosystems to define reference conditions, but rather ecosystems judged to have a good environmental quality. In practise, usually one or more reference

sites are selected, situated outside the direct influence of identified point sources of pollution. They are considered to represent the best environmental quality that can be found today in similar recent ecosystems and serve for comparison with the potentially polluted ecosystems under evaluation. These reference sites also represent the quality that has to be achieved by management decisions aiming to improve environmental quality.

In this context, foraminifera have a potential advantage. The preservation of their calcareous shells in the sediment record makes it possible, provided that a reliable age model can be obtained, to reconstruct the historical development of the foraminiferal community and to choose a certain time interval (for instance 1850, before the industrial revolution, or 1950) as « reference conditions » [19–21]. However, since important differences may exist between living foraminiferal faunas and sediment assemblages forming at the same site, the comparison of recent faunas with dead faunas preserved in the sediment record can almost never be straightforward. In fact, early diagenetic processes may lead to preferential loss of species with fragile tests, thereby introducing important taphonomic bias [22]. Also, important seasonal and interannual variability of living faunas may hamper the comparison with time-averaged sediment assemblages. Finally, more opportunistic species with a higher turnover ratio, often considered as pollution-tolerant, will produce more tests than stress-sensitive equilibrium species, thereby increasing their relative frequency in the sediment assemblages compared to the living faunas [23]. In spite of these limitations, foraminiferal sediment records may give valuable information about baseline conditions in pre-industrial times [19,24].

The foregoing short introduction on biomonitoring largely relates to open marine ecosystems. In view of the encouraging results of biomonitoring indices, it is not surprising that the same methods have been applied in estuaries and in other transitional water masses. However, it became rapidly clear that especially in estuarine ecosystems, the biotic indices very often indicated a poor or very poor environmental quality, even in cases where anthropogenic influence was apparently very limited. This negative evaluation is mainly caused by the generally low diversity and the dominance of a small number of opportunistic, stress-tolerant taxa. This observation has been termed the « estuarine quality paradox » [25,26]. The paradox is formed by the contradiction between apparently natural conditions, for which a good environmental quality is expected, and bad biotic index values. This contradiction results from the fact that even when they are in a natural state, most estuaries are stressed environments. This is due to two main reasons:

1) The presence of a strong salinity gradient, from fully marine conditions at the mouth of the estuary, to brackish and fresh water in the inner part of the estuary. Few species can cope with such a huge salinity range. Most marine and freshwater species are stenohaline, and can only bear minor salinity changes. It appears that estuaries are mainly inhabited by some more euryhaline marine species, whereas strictly estuarine species, living exclusively within estuaries, are rare or even not existing [27,28].

2) Many environmental parameters show a high spatial and temporal variability. This is the case for salinity, which may show a huge variability on a seasonal (due to changes in river discharge) as well as daily (weather forcing) and hourly (tidal cycles) scale. In intertidal areas, also other important ecological parameters (such as temperature, evaporation, resources, predation pressure, etc.) may vary dramatically spatially (e.g., due to different emersion times), seasonally or even during a single tidal cycle.

The present paper envisages strategies to overcome this problem. More precisely, we will investigate how, despite the “estuarine paradox”, foraminiferal assemblages can be used as indicators of environmental quality in intertidal estuarine mudflats. Our decision not to discuss all types of transitional water masses (TW's) but to concentrate on mid latitude estuarine intertidal mudflats is motivated by 1) the fact that TW's show a very large variability in environmental factors, which makes it difficult to consider them all together, and 2) estuarine intertidal areas are particularly stressed environments because of their emergence at low tide. We will concentrate here on French Atlantic estuaries, for which excellent environmental records are available on diurnal, seasonal and pluri-annual time scales. These estuaries are mostly tide-dominated (with a high-mesotidal to low-macrotidal regime), with the tidal currents being the main physical controlling factor. They are mostly characterized by a funnel-shaped mouth, and subtidal channels that are generally flanked by extensive intertidal mudflats.

It seems evident that in estuarine environments, an excess of anthropogenic stress can only be evaluated once the faunal response to the often severe natural stress is well known, and can be quantified. In this paper we will therefore investigate what are the main natural environmental parameters controlling the characteristics of foraminiferal communities in estuarine intertidal mudflats and how they can best be

described by an easy-to-handle index. We will then test this index on two contrasting French Atlantic estuaries for which environmental/foraminiferal datasets are available. In the companion paper of Fouet et al. (this volume) [29], we will describe these two foraminiferal datasets, and will investigate whether our description of the dominant controlling natural environmental parameters is adequate, and can explain part of the differences between the foraminiferal communities (density, diversity, species composition) within and between these two contrasting estuaries.

2. Factors controlling faunal distribution in estuaries

Our ultimate aim is to develop a reliable index of environmental quality in estuaries based on the available biota. Here, we will consider this question for foraminifera, that, similar to most other organisms, in estuaries show a low diversity and a strong dominance of few opportunistic taxa. To develop such an index, it is of paramount importance to understand first the natural distribution of the organisms used in the index, and how this distribution is determined/influenced by the complex interplay of environmental parameters. In this context, by "natural distribution", we do not mean the distribution found under pristine conditions, but rather the distribution found today at sites that are, as far as we can tell, not substantially affected by anthropogenic pollution. We suppose that even if today's ecosystems are all affected by human activities to some degree, the natural parameters are still the dominant controlling parameters of the faunal distribution. In principle, anthropogenic pollution should lead to deviations from the "natural distribution pattern". Evidently, such deviations can only be detected if the "natural distribution pattern" is perfectly known and the relations with the controlling parameters are well understood. In view of the stress-resistant character of most species living in estuaries, it seems probable that only major anthropogenic perturbations can be recognised as such.

In the literature, several attempts have been made to classify estuaries (and other transitional water masses) on the basis of morphology, hydrology, characteristics of the catchment area and climate (e.g., [28,30,31]). Although these studies present methods to classify, and to compare estuaries, they do not provide the means to compare individual sites, either within the same, or in different estuaries.

An extensive discussion has taken place about the main environmental parameters controlling faunal distribution in estuaries, for macrofauna as well as for foraminifera. It is generally accepted that estuaries are among the most productive natural ecosystems in the world [32], although high turbidity due to an important sediment load may hamper/reduce primary production [27]. Despite the chronic instability of all other physicochemical parameters, estuaries are not nutrient-limited. Therefore, it is generally agreed that not nutrients, but salinity is by far the most important controlling factor [33,34]. The relation between salinity and estuarine faunas is often illustrated with the conceptual Remane diagram [35], which shows how the relative proportions of marine, brackish and freshwater species change along the salinity gradient. In their excellent review, [36] argued that the Remane diagram was originally proposed for the Baltic Sea, a brackish sea, and can therefore not be applied straightforward to estuaries. They proposed a number of refinements to the model (Figure 1), and argued that the freshwater assemblage generally has a lower species diversity than the marine assemblage and does hardly penetrate into more saline waters. These authors further claimed that marine species also strongly dominate the polyhaline and mesohaline parts of the estuary, whereas exclusively estuarine/brackish species are scarce (Figure 1). In this context, on the basis of a principal component analysis of western North Atlantic estuaries, [37] demonstrated that marine species rapidly disappeared below a salinity of 24, whereas freshwater species were largely limited to salinities below 4. Many studies [38,39] show that these observations are also valid for foraminifera, with the possible exception of vegetated salt marshes, which host a number of species typical for these ecosystems [40].

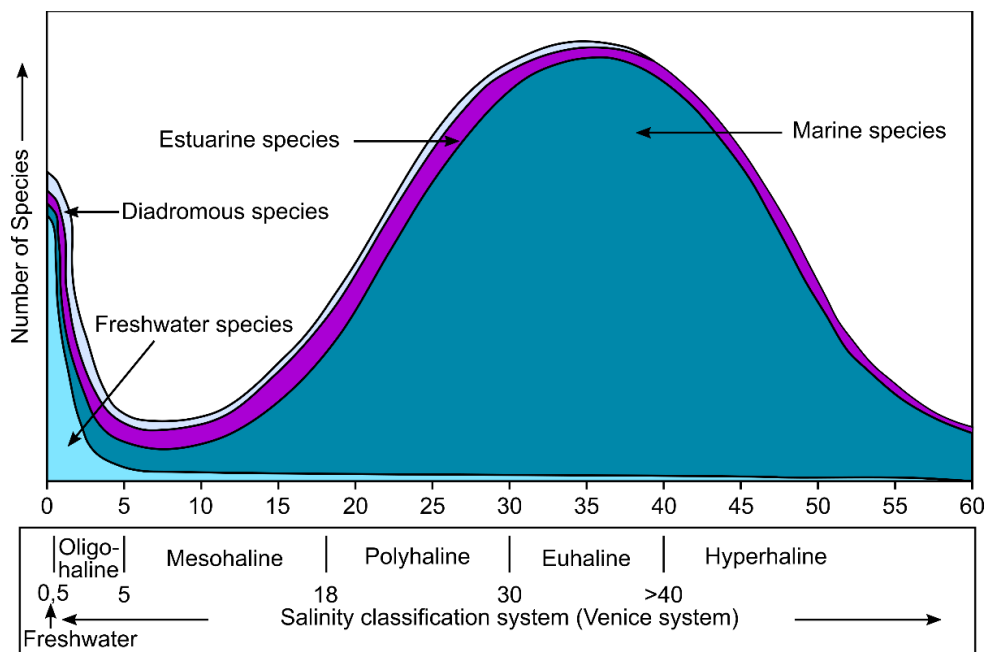


Figure 1. Conceptual model for estuarine biodiversity (number of species) changes covering the salinity continuum from freshwater to hyperhaline conditions (after Whitfield et al., 2012 [36]).

However, [41] argued that it is not the absolute salinity tolerance of the various species that decides on their distribution in the estuary, but rather their tolerance of salinity variation. It seems evident that compared to a rather stable brackish water basin like the Baltic Sea, the large (seasonal and diurnal) salinity fluctuations in tidally dominated estuaries must add an additional amount of stress.

Observations of similar faunal successions along transitional water transects with sometimes very different absolute salinity values led to the concepts of “marine vivification” [42] and “marinisation” [43]. These concepts imply that not salinity alone, but a complex of elements of marine origin (e.g., nutrients, marine flora/fauna, hydrodynamic energy) would be the main control on faunas living in transitional waters. Guelorget and Perthuisot (1992) [44] defined the term “confinement” as the time of renewal of these “vital elements of marine origin”.

Since salt is one of the elements of marine origin, there must evidently be a correlation between salinity and “confinement”. However, the absolute salinity values found in various parts of the estuary may be very different at different latitudes, with different water budgets (different ratio between evaporation versus precipitation and river discharge). Guelorget and Perthuisot [44] concluded that it is impossible to quantify their “confinement” factor.

Debenay and co-workers applied the “confinement” concept to the foraminiferal distribution in a number of tropical estuaries [45–47]. These studies proposed a method to calculate a “confinement index”, using the relative frequencies of three groups of foraminiferal species, corresponding to taxa occurring in the marine/outer estuary, middle estuary and inner estuary, respectively. This meant that instead of calculating a confinement index based on the physical properties of the estuary and then comparing the calculated index values with data on the faunal distribution, Debenay and co-workers did exactly the opposite. In our opinion, this approach is not entirely free from circular reasoning; high percentages of species considered typical of a limited marine influence (while living in the inner parts of the estuary), will unavoidably lead to high values of the confinement index there, indicative of limited marine influence.

Unfortunately, it is often far from easy to obtain reliable salinity measurements in an estuary. Especially in macrotidal estuaries, both the subtidal and intertidal areas experience huge temporal salinity variation on an hourly scale, as salinity rises with incoming tide and decreases with outgoing tide. A reliable picture of this pattern can only be obtained by continuous data logging, which is only possible for a limited number of points. Even when such a continuous record is available [48], it is not evident what precise salinity value(s) should be used to describe the ecosystem. For intertidal areas, which may every day emerge for many hours,

when they are affected by evaporation and/or precipitation, the problem is even larger. Therefore, [41] suggested to characterise the salinity regime by measuring the range between mean low-tide salinity and mean high-tide salinity. This may be a solution for the subtidal part of the estuary, but not for intertidal areas, where it is basically impossible to measure salinity at low tide.

Summarising, there is a consensus that salinity and/or marine factors related to salinity, are the main controls of faunal characteristics in estuaries. Together, these parameters can be regrouped under the term “marine influence”. This term not only refers to the introduction of salt, but also of marine organic matter, flora and fauna, and especially, tidal energy. Due to the tidal energy, this “marine influence” also partly controls factors as sediment grainsize, water column turbidity and oxygen concentration, and residence times of continental waters in the estuary. The latter factor may have important consequences regarding the use of river-born nutrients, primary production within the estuary, bottom and water column oxygen contents, as well as geochemical nutrient and organic matter recycling in the estuarine superficial sediments [49].

It appears therefore that a complex of “marine influence” factors, which largely exceeds salinity alone, constitutes the main control of estuarine biota. Many, if not all of these factors (salinity, strong tidal currents, hypoxia) may be causes of natural stress. In intertidal areas, additional stress results from emergence and faunal exposure during low tide, leading to major variations of temperature, salinity and predation pressure. In general, a decrease of marine influence leads to an increase of stress for marine species.

In this paper, we will propose a new Marine Influence Index (MII), with which we attempt to describe the cumulative effect of the above-mentioned stress factors affecting estuarine foraminifera. If the MII correctly describes the main factors controlling the foraminiferal assemblages, its values should show a strong correlation with the main faunal characteristics. If so, this index could serve to predict the characteristics of the fauna under natural conditions. Clear deviations from the natural distribution pattern as predicted from such a MII would then be due to factors non included in MII, such as anthropogenic pollution. Here, we will develop this Marine Influence Index and test it on two estuaries on the French Atlantic coast. In the companion paper of Fouet et al. [29], this MII will be tested on foraminiferal data of the same estuaries.

3. Ecology of estuarine foraminifera

Estuarine foraminiferal assemblages have been abundantly studied. Excellent reviews are given in [39,50–52]. It is important to note that many of the earlier studies in estuaries were based on total assemblages (i.e., counts of all foraminiferal tests, of living as well as dead individuals) and not only on living faunas, as we propose here. Until recently, the use of total assemblages was privileged because they were considered to give an average picture, not biased by the often important seasonal and spatial variability of the living assemblages [50]. Although total assemblages may be useful for comparison with fossil samples (for instance for the detailed reconstruction of ancient sea levels), their use for biomonitoring purposes has several pitfalls. In fact, the time slice recorded by a total assemblage may be very different between sites, due to the very different sedimentation rates found within single estuaries. In some cases, the time presented by a total assemblages may largely exceed the period characterised by anthropogenic impact. In other cases, bottom erosion, occurring frequently in estuaries may erode recent sediments, and lead to a mixture of living and much older dead foraminifera. Debenay et al. (2000) [50] argued that the potential bias (between living and total faunas) introduced by post-mortem transport and taphonomic losses may have been overestimated. We disagree with this opinion, and think that the higher diversity often recorded in estuarine total assemblages (compared to living faunas) is the direct consequence of a landward transportation of tests of marine species by tidal currents. The presence of such allochthonous taxa will mainly inform us about the hydrodynamics, and not about other local environmental conditions.

Unlike total faunas, living faunas reflect the environmental conditions prevailing during the weeks to months before sampling. Therefore, we judge that for biomonitoring studies, the use of living assemblages should be mandatory [8], in spite of the complexity created by important seasonal, interannual and spatial variability of the assemblages.

Like for macrofauna, the salinity gradient within the estuary, from fresh water in the inner part to marine salinity at the mouth, appears to be the dominant controlling parameter of the foraminiferal spatial distribution. This contrasts with open marine environments, where food availability and oxygen

concentration, and in shallower areas also temperature and the nature of the substrate, are the dominant controlling parameter [51,53]. Most foraminiferal taxa are stenohaline, limiting their habitat to the outer estuary, where diversity is maximal. Much less species seem to be adapted to the meso- and polyhaline conditions found in the middle estuary. Finally, foraminifera become scarce in the oligohaline upper estuaries, where thecamoebians flourish [39]. In this paper, we will not consider salt marshes, which host a highly specific fauna, usually strongly dominated by agglutinant taxa [54].

Many other parameters than salinity variation alone will influence the ecology of estuarine foraminifera. Temperature, food availability, oxygen concentration, sediment characteristics and hydrodynamics will all control to some extent the foraminiferal density, diversity and species composition. These factors may be especially important when their values are close to the upper or lower tolerance limits of individual species.

However, it appears that the natural distribution of foraminifera is foremost determined by salinity, or, more precisely, by salinity variation. In intertidal mudflats, which form the subject of this paper, biological stress caused by emergence at low tide, leading to abrupt changes in salinity and/or temperature, appears to be an important secondary factor. Because of the multiscale temporal and spatial variability of salinity in estuaries, this parameter is **not synchronized with foraminiferal life cycles**, and neither snapshot nor long-term salinity measurements are suitable to describe the influence of this factor on foraminiferal faunas. The optimal description of this major controlling environmental factor at each study site remains a major challenge that we will consider in this paper.

Salinity in estuarine waters depends on the mixing of marine and continental waters. Several factors play a role here:

- **Tidal influence** will diminish with increasing distance from the sea, and will vary along lunar-solar tidal cycles. Tides largely control the estuarine circulation and associated biological processes. The hydrodynamics of the rising and falling tide generates turbulence and causes vertical mixing between fresh and marine waters. Depending on the local tidal regime, river discharge and the morphology of the estuary, estuaries may be weakly to strongly stratified. Seasonal changes in river discharge and the dynamics of the salt-wedge intrusion also affect biological production [27]. For example, in a seasonally stratified estuary, the presence of the salt wedge in spring led to increased nutrient recycling and phytoplankton blooms [49]. The tidal range will, together with the elevation of the study site, also determine the emergence time of the various parts of the tidal flats.
- **River discharge**, that dilutes the sea water intruding by tidal processes into the estuary, may show major seasonal and interannual variability, depending on the climate regime. Consequently, sites located in the inner estuary may show major seasonal salinity changes, from almost marine to almost fresh water.
- **Local precipitation** is a second source of fresh water, which will especially affect the salinity of emerged mud flats during low tide.
- **Groundwater inflow** may be an important additional source of fresh water, which is often difficult to estimate.
- The **morphology of the estuary** may facilitate or hamper the propagation of the tidal wave and/or of marine waters in the estuary.

Together, these factors not only decide on salinity, but also determine the extent of “marine influence” in the different parts of the estuary. The term “marine influence” not only includes salinity, but also introduced marine organic matter and marine biota (as adults, larvae or propagules). This is important, since marine organic matter tends to be more labile than continental organic matter [55]. Marine biota may serve as food, or may fuel colonisation of the estuary by marine species, during (short) periods with suitable environmental conditions. Essentially, increased marine influence is the expression of a higher ratio between the volume of seawater entering the estuary and the freshwater supplies (through river runoff, groundwater input and precipitation). Increased marine influence may result in longer fresh water residence times in the estuary. This may lead to a more substantial utilisation of river-born nutrients within the estuary [49]. At low tide, the extent of “marine influence” will rapidly decrease, especially in case of intertidal estuarine mudflats subjected to strong precipitation and/or evaporation.

4. Quantifying the main parameters controlling foraminiferal distribution in estuarine intertidal mudflats

Here, we will try to quantify the main physical and hydrological parameters summarised under the term “marine influence”, since they appear to be responsible for most of the natural environmental stress in intertidal estuarine mudflats, to which the foraminiferal community is exposed.

Next, we will investigate the possibility to assemble these factors into an easily applicable Marine Influence Index (MII).

4.1. Position of the sampling point along the salinity gradient

Salinity appears to be the foremost factor controlling the ecology of estuaries, particularly in case of tide-dominated estuaries [56], as suggested by the Remane diagram (Figure 1). The factor “Salinity” includes absolute salinity values as well as the range of salinity variation. Because of strong temporal variability, it is already a challenge to measure these parameters in subtidal estuarine environments. This becomes almost impossible in intertidal mudflats, which are emerged at low tide. We suggest that the most integrative parameter related to salinity is the extent of the salt intrusion into the estuary. This is the point until which the salt water wedge enters the estuary at incoming tide. Beyond this point, the water is permanently fresh, even during the highest spring tides. However, the physical tidal influence may be felt further inland, in the fluvial estuary, where the incoming tidal wave may partly block the outflowing fluvial waters. Therefore, the upper limit of the estuary is often placed more inland than the salt intrusion [57]. In our study, that focuses on the influence of the inflowing marine waters on foraminiferal faunas, we will not consider the areas beyond the salt intrusion. Information concerning the salt intrusion is available for almost all French estuaries [58], but it is not clear when exactly these observations have been made. This is important, because the salt wedge intrudes much farther into the estuary in the dry season compared to the wet season. If data on the salt intrusion are not available, field measurements at high spring tide (during the dry season) can define its upper limit.

The relative position of the sampling point between the mouth of the estuary and the point of farthest salt intrusion can be used to characterise the position of the sampling point on the salinity gradient. This relative distance can be defined as:

$$X/S \quad (1)$$

where X is the distance (measured along the channel axis, in km) from the mouth of the estuary (defined on the basis of geomorphological criteria), and S is the length of the salt intrusion (in km). This ratio describes the relative position of the sampling point along the salinity gradient, irrespective of absolute salinity values, tidal range (micro- to macro-tidal) and the strength of the salinity gradient (which varies in function of the seasonal dilution by fresh waters). As such, this measure should be more robust than absolute salinity values, not only because these are challenging to obtain, but also since absolute salinity values may show large latitudinal variation, in function of different climate regimes.

It can be envisaged that the extent of the salt water intrusion does not diminish linearly with increasing distance from the mouth. In fact, it can be affected by the presence of mechanical obstacles, that may cause strongly localised reductions of salt water inflow. These obstacles may be of three different kinds:

1. **Turtuosity:** sea water will have more difficulty to enter a meandering estuarine channel than a rectilinear estuary, especially in case of a strongly curved shape of the channel. For this reason, we propose to measure the relative position of the sampling point with respect to the real distance from the inlet, considering all eventual curves of the main channel (i.e., definition of X in equation (1)).
2. **Abrupt narrowing of the estuary or channel sills** of geological origin will cause major constraints for salt water supplies to the upper parts of the estuary. Such “bottlenecks” will accentuate the non-linearity of the horizontal salinity gradient, and thereby shorten the length of the salt intrusion (i.e., S in Eq (1)). This factor, which is difficult to quantify, should be at least partly integrated in the decrease of marine influence with relative increasing distance from the mouth of the estuary.
3. **Manmade physical obstacles:** On the French Atlantic coast, many of the small and medium sized estuaries are closed upstream by a dam provided with a sluice. These dams/sluices are used to

prevent salt water to intrude the fluvial estuary beyond these dams. They are generally closed during rising tides in order to protect fields under cultivation from soil salinization. They open during some of the falling tides to regulate the outflow of riverine fresh waters into the estuary. This is done to keep fluvial waters available for irrigation purposes or to flush the lower parts of the estuary, to remove clay deposits in the navigation channel. When the dam/sluice is closed, salinity tends to be elevated until the dam is reached, with fresh water being present immediately landward of the dam. As a result, these dams artificially limit the salt intrusion, truncating abruptly the salinity gradient from marine to fresh waters. In such cases, we think that the (theoretical) natural salt intrusion should be used to define the relative position of the sampling point on the salinity gradient, and not the observed salt intrusion (until the mechanical obstacle). This natural salt intrusion (before the instalment of the manmade barrier) can be assessed by a careful study of the thalweg topography; normally salt water enters the estuarine valley until the point where the relief starts to increase, often abruptly.

4.2. *Altitude / Emergence time*

The altitude of the sampling site has a major impact on the ecology of the foraminiferal faunas living in intertidal areas, for three main reasons:

1. The elevation of a site may change the position of the foraminiferal assemblage with respect to the vertical salinity gradient, due to haline stratification. In highly stratified estuaries, the saltwater wedge enters the estuary below the outflowing superficial fresh water layer. If present, this vertical gradient will change on diurnal and seasonal timescales in response to incoming and outgoing tide, and to fluctuations in river discharge. Precipitation may be a secondary reason for the development of a vertical salinity gradient, even in case of estuaries with a homogenised water column. Whatever the exact reasons and salinity values, at higher elevation, the foraminiferal faunas will generally be confronted with lower salinity.
2. In combination with the tidal range, the elevation largely influences the time of emergence at low tide. The twice-daily emergence of the tidal mudflats exposes the intertidal habitats to major changes of moisture, temperature and salinity (due to evaporation and/or precipitation). In fact, in the intertidal realm, the time of emergence seems to be a much more important stress factor than slight salinity changes due to different positions of the sampling sites on the vertical salinity gradient. For macrofauna, emergence also leads to changes in predation pressure, as the contribution of fish decreases while predation by shore-birds and mud-snails becomes more important [59]. Little information is available for predation on foraminifera [60], although there are indications for selective predation by gastropods [61]. Together, these changes represent a substantial recurrent stress.
3. Hydrodynamic energy will progressively diminish from the estuarine channel to the most elevated parts of the intertidal mudflats. Tidal currents may displace foraminiferal faunas, leading to an underrepresentation of small species and introduction of allochthonous taxa in the intertidal realm. Hydrodynamics will also control sediment grain size and organic matter availability (as organic particles are preferentially bound to clays).

The question is how to describe these different effects of habitat elevation with a single quantitative proxy. The absolute altitude, i.e., the elevation above the lowest astronomical tide (chart datum), that is by definition a fixed value, cannot take into account the fluctuating ecological stress imposed by the emergence time variability. The emergence time depends mainly on the local tidal range. This is not only determined by the global tidal cycles (14-days and annual), but also by the spatial variability of the tidal range within the estuary. Due to the propagation of the tide wave in a complex estuarine geometrical system, a tidal asymmetry is produced (mainly by bottom friction and wave refraction), leading to amplitude and phase differences, from the river mouth to the inner estuary [62].

For these reasons, we suggest not to use absolute altitude, but rather the emergence time, that can be considered as an integrative factor for the environmental controls listed above.

The emergence time (ET, expressed as a fraction of total time) can be calculated for each sampling point on the basis of absolute altitude and the tidal tables, which are provided by SHOM for all French harbours [63]. We decided to use mean spring tide data (in France: tidal coefficient of 90) to calculate a reference

emergence time for all sites. The choice of conditions typical of a specific part of the 14-day tidal cycle is necessary to be able to compare sites at different altitude. Next, a spring tide situation was chosen, because during spring tide, every part of the intertidal mud flats is at least immersed part of the day. Conversely, during neap tide, the lower parts of the mudflats will be immersed all day, whereas the uppermost parts of the mudflats may never be reached by the rising tide.

It is important to realise that the relative emergence time (ET) is not determined by the global tidal cycles but by the local tidal range (micro- to macrotidal regime, up- to down-stream location), so that the tidal range parameter does not have to be further considered. Moreover, relative emergence time also partly expresses the hydrodynamics of a site. Short relative emergence times mostly concern intertidal areas located close to the channel axis where tidal dynamics is maximal. Inversely, sites with long relative emergence times will generally be far from the channel axis, and experience a much lower hydrodynamic energy.

A detailed description of the methods used to estimate absolute altitude and emergence time is added as supplementary material.

4.3. Importance of fluvial discharge

The extent of fluvial discharge is of paramount importance for the intrusion of the marine wedge. A large discharge volume will not only diminish the distance of the salt water intrusion, but will also lead to a smaller volume of salt water entering the estuary. In fact, some very large rivers, such as the Congo, have such large outflow volumes that intrusion of the salt wedge becomes almost impossible year round, even at high tide [57]. Fluvial discharge will, also in our mid latitude region, show large seasonal variability, with important maxima in winter/early spring and a minimum in summer. Generally, salinity (marine influence) largely decreases in the inner parts of the estuaries during winter runoff maxima. This may lead to a temporary strong diminution or even complete disappearance of foraminiferal faunas [64]. For all major French rivers, and for most major rivers worldwide, mean annual discharge volumes as well as detailed runoff data are available (<http://www.hydro.eaufrance.fr/>). However, such detailed data may be more difficult to obtain in other regions. It is important to evaluate which precise data (of what exact period) are relevant for the survey of benthic faunal constituents.

Foraminifera are supposed to have a rapid response to environmental change, as is shown by many examples of strong seasonal faunal variability, in response to changes of runoff volume and precipitation [54]. This rapid response is the direct result of their short life cycles (estimated from 6 months to 2 years [51]). Therefore, it is not relevant to compare the living foraminiferal community with year-averaged river discharge data. It seems more judicious to compare the faunal data with the conditions in the period immediately before sampling. Consequently, we decided to characterise the fluvial discharge regime by averaging the values of the 30 days before sampling.

Next, the volume of river discharge has to be related to the size of the estuary, to evaluate its impact on the introduction of marine waters into the estuary. In their comparison of tidal estuaries along the Atlantic coast of Europe, Middelburg and Herman [65] used (year-averaged) fresh water residence times to characterise the nine studied estuaries. They found that in general, river-dominated estuaries have short average fresh water residence times (up to 2 weeks), whereas strongly tidal-dominated estuaries have much longer average river water residence times (1 to 3 months). Residence times are important, because in case of longer fresh water residence times, estuarine environments are easier fuelled with marine nutrients, and riverine nutrients are recycled within the estuary, leading to increased biological productivity [64].

There is an extensive literature on the estimation of fresh water residence times in estuaries, that essentially shows that the calculation of such residence times is complex [66–68]. We suggest an easier way to compare the volume of river discharge with the size of the estuary (the total amount of water), notably to calculate for every sampling station the ratio between the average freshwater discharge (in the 30 days before sampling of the foraminiferal assemblages) and the cross section of the estuarine channel at the sampling point at mean high spring tide:

$$\text{RRO (Relative river outflow)} = \text{RD (river discharge)} / \text{CS (estuarine cross section)} \quad (2)$$

Since most estuaries become wider in seaward direction, this ratio will have the tendency to decrease from the inner to the outer parts of the estuary, although bottlenecks may lead to non-linear changes in RRO.

4.4. Distance from the main estuarine channel

There is a large difference between estuaries with limited width and narrow tidal flats, and estuaries with large tidal mudflats, sometimes with entire embayments emerging at low tide. In both cases, the main part of the in- and outflowing water volume is passing through an often narrow main channel, where the hydrodynamic energy is maximal. Hydrodynamic energy will decrease with increasing distance from the main channel. Consequently, tidal and fluvial currents are stronger in the upper parts of narrow mudflats, close to the main channel, than in the upper parts of very wide mudflats, which will only be attained at high tide. Hydrodynamic energy is probably also a major factor for the transport of tests and propagules of foraminifera and may therefore be an important vector for the (re)colonisation of estuarine habitats.

It appears that the decrease of hydrodynamic energy with increasing distance from the main channel is at least partly represented by the altitude/emergence time factor ET. In fact, the width of the estuary is also taken into account in the relative river outflow factor RRO, since it is the main parameter determining the surface area at the sampling sites. The RRO factor should therefore also be indicative at least of the hydrodynamic energy due to outflowing river water, which will be higher in estuaries with a high RD/CS ratio, in which large volume of river water flow out through a comparatively narrow estuary. We think therefore that it is not necessary to add a specific term for this factor to our index of marine influence.

5. Defining the Marine Influence Index (MII) for estuarine intertidal mudflats

The first three components discussed above together determine the level of marine influence in intertidal estuarine mudflats, that is consider as the main controlling factor of the foraminiferal faunas living there. They can be assembled into a Marine Influence Index (MII) in the following way:

$$MII = \alpha \left(1 - \frac{X}{S}\right) * \beta(1 - ET) * \gamma \left(1 - \sqrt{0.04 * \frac{RD}{CS}}\right) \quad (3)$$

where:

X = real distance from the sampling site to the mouth of the estuary, following the main estuarine channel. X is measured between the mouth of the estuary and a line starting from the sampling site, perpendicular to the channel axis.

S = distance of the natural salt intrusion into the estuary; the observed value when no obstacle is present, a theoretical value based on the thalweg topography when a manmade obstacle is present.

ET = emergence time, expressed as a fraction of a tidal cycle, calculated for mean spring tide conditions (in France: tidal coefficient 90).

RD = river discharge in the 30 days before sampling (in m³.sec⁻¹).

CS = surface area of the estuarine cross section at high tide (in m²).

Details about the calculation of S, ET and RD/CS are given in the supplementary material.

The equation is written in such a way that in case marine influence is total, all three individual parameters, as well as MII, are 1. The terms α , β and γ are constants, translating the respective weight of each of the three factors. The values of these constants have to be determined in future, on the basis of a comparison of faunal and environmental parameters in a wide range of estuarine mudflats.

In this equation, both $(1 - X/S)$ and $(1 - ET)$ are defined as normalised, dimensionless ratios, varying from 1.0, equivalent to maximum marine influence, to 0.0, no marine influence. This is not the case for the RD/CS ratio. We decided to normalise this factor by comparing its values to the one obtained in the Loire river estuary at Cordemais, 28 km from the mouth of the estuary. This site coincides with the administrative limit between the marine and fluvial domain, determined by the limit between mesohaline (5-18 PSU) and oligohaline (0.5-5 PSU) realms. The farthest point to which salty water can intrude is located at 60 km from the mouth of the estuary, slightly upstream of the town of Nantes.

The Loire river was chosen as a reference riverine system because it is the longest river in France (1013 km long), draining a catchment basin of 118,000 km², and evolves in the same climate regime as the studied estuaries on the Atlantic margin. Despite the fact that the tidal range at the entrance is 5.4 m (at spring tide)

and the tidehead (the farthest point upstream where a river is affected by tidal fluctuations) is located 97 km from the mouth, the estuary of the Loire river is the one with the strongest river influence along the French Atlantic Coast. The Loire estuary is affected by a mean annual freshwater discharge of $854 \text{ m}^3 \cdot \text{sec}^{-1}$, varying from 10-year floods of $5200 \text{ m}^3 \cdot \text{sec}^{-1}$ to summer low water flows of $140 \text{ m}^3 \cdot \text{sec}^{-1}$. Consequently, the freshwater residence time varies from 3 to 30 days [69]. At the Cordemais site, the fluvial estuary is about 750 m wide, with a cross section of about 3400 m^2 . This means that, when considering the mean annual discharge, RD/CS is $25 \text{ cm} \cdot \text{sec}^{-1}$.

For all other estuaries on the French Atlantic margin, that have a lower freshwater input than the Loire estuary, a normalised RRO (Relative river outflow) value, varying between 0.0 and 1.0, is calculated. First, the RD/CS value is divided by 25 (the reference value calculated for the Loire at the Cordemais site). In this way, all sites in all estuaries can be compared on the basis of a normalised RRO value. Finally, to give more weight to differences at the lower part of the 0.0 to 1.0 scale, we use the square root of the normalised RRO value for the MII calculation. In the remaining part of this paper, we will apply this Marine Influence Index on the Auray and Vie estuaries, for which we also dispose of foraminiferal records. We want to investigate how the MII differs 1) between estuaries with different characteristics, 2) between different parts of the estuaries, and 3) between different periods, with ample, or almost no river runoff.

6. Applying the Marine Influence Index (MII) to the Auray and Vie intertidal estuarine mudflats

In order to show how this Marine Influence Index describes spatial and temporal differences between and within different estuaries, we will apply the concept on two contrasting French estuaries on the Atlantic coast. In the companion paper of Fouet et al. [29], it is investigated whether the MII can be used to explain the faunal variability observed in these estuaries.

6.1. Auray estuary

The Auray estuary (Figure 2a) is a ria, a drowned river valley with a surface area of about 12 km^2 . It is part of the much larger Morbihan Gulf, a marine bay, and is a tide-dominated estuary. The entrance of the estuary is 950 m wide. Two small rivers flow out into the estuary, the Loc'h, with a mean annual discharge volume of $2.72 \text{ m}^3/\text{sec}$, and the Bono, with a mean annual discharge volume of $1.52 \text{ m}^3/\text{sec}$. Together, these two rivers drain a 60 km long catchment area of 324 km^2 . Total fresh water residence time is estimated at about 2 months, underlining the fact that the estuary experiences a strong marine influence. The estuary has a meso- to macrotidal regime, with a tidal range of 4 m at the mouth during mean spring tides. The salt wedge intrudes until 20 km from the mouth of the estuary. During the 2019 survey in the context of the European Framework Directive, the chemical properties of Auray estuary were evaluated as good, whereas the ecological state was evaluated as average [70]. The latter judgement was based on an indicator using fishes. Another indicator, based on macroalgae, indicated a good environmental quality. These results suggest that, in spite of an apparently strong anthropogenic pressure (oyster culture, recreational navigation), the living biota, including the foraminiferal community, are not subjected to severe stress of anthropogenic origin.

Annexe 1: The Marine Influence Index (MII): A Tool to Assess Estuarine Intertidal Mudflat Environments for the Purpose of Foraminiferal Biomonitoring

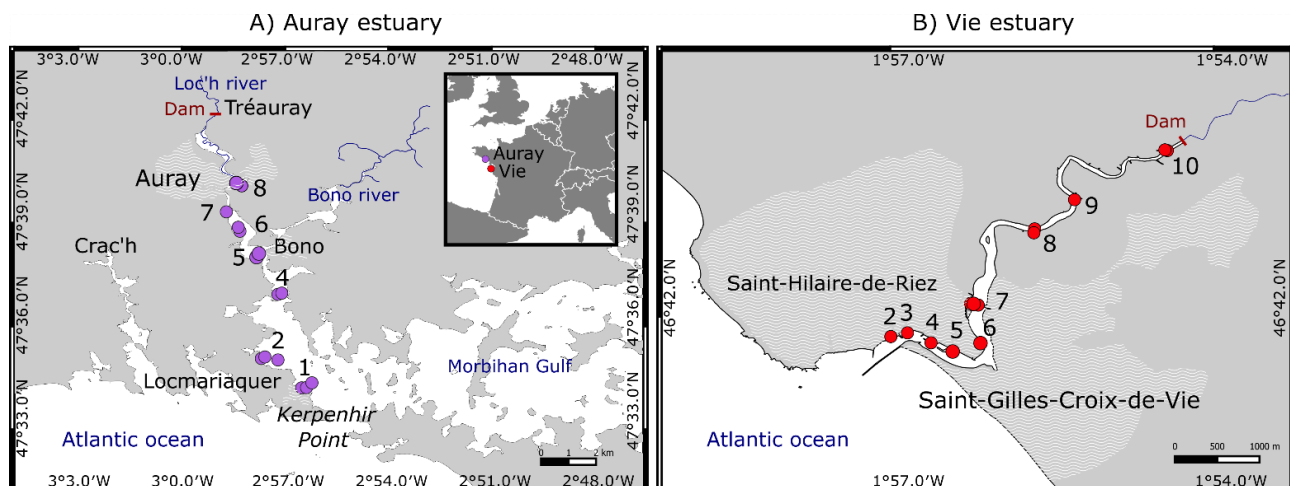


Figure 2. Schematic maps of the Auray and Vie estuaries on the French Atlantic coast with location of the sampling sites. Urbanised areas are indicated with a light grey shading.

In the Auray estuary, at seven sites, for a total of 15 stations, foraminiferal faunas were sampled from 10 to 12 July 2019 (Figure 2). The main characteristics of these sites are listed in Table 1. When several sampling stations were selected at different altitudes at a same site, the station at the highest elevation systematically has the suffix A, whereas stations B and C are positioned at progressively lower altitude.

Station	Distance to Sea (X, in km)	Normalized Distance to Sea (1-X/S)	Distance Perpendicular to Channel Axis (m)	Altitude (m)	Emergence Time (%)	Normalized Emergence Time (1-ET)	River Discharge/ Cross-Section (RD/CS, m/s)	RRO (Relative River Outflow)	MII (α , β and γ Set at 1.0)
1A	2.8	0.86	1150	2.52	42.3	0.58	0.03	0.97	0.48
1B	2.8	0.86	1055	1.29	19.7	0.80	0.03	0.97	0.67
1C	2.8	0.86	880	1.38	21.1	0.79	0.03	0.97	0.65
2A	4.9	0.75	1450	4.50	88.6	0.11	0.03	0.97	0.08
2B	4.9	0.75	1380	4.20	77.2	0.23	0.03	0.97	0.17
2C	4.9	0.75	880	1.38	21.1	0.79	0.03	0.97	0.57
4A	8	0.60	275	3.30	55.0	0.45	0.15	0.92	0.25
4B	8	0.60	210	1.37	21.1	0.79	0.15	0.92	0.43
5A	10.4	0.47	300	2.20	36.9	0.63	0.09	0.94	0.28
5B	10.4	0.47	60	1.21	17.8	0.82	0.09	0.94	0.37
6A	11.8	0.40	200	2.50	42.3	0.58	0.18	0.92	0.21
6B	11.8	0.40	50	1.49	24.3	0.76	0.18	0.92	0.28
7	13.2	0.33	100	1.31	20.4	0.80	0.30	0.89	0.24
8A	15	0.24	100	2.30	38.5	0.61	0.54	0.85	0.13
8B	15	0.24	65	1.29	19.7	0.80	0.54	0.85	0.17

Table 1. Environmental characteristics of the 15 sampling stations at 7 sites on intertidal mudflats in the Auray estuary. Altitudes refer to the French chart datum. The general methodology of the calculation of MII is explained in section 5; a more detailed explanation of the individual components is given as supplementary material.

The seven sampling stations are positioned between 2.8 (station 1) and 15.0 km (station 8) from the inlet of the Morbihan Gulf, at the Pointe de Kerpenhir. Since the salt wedge is observed until the Moulin de Tréauray, 19.8 km from the mouth of the estuary, the normalised distance from the mouth (1-X/S; column 2 in Table 1) varies from 0.86 (station 1, outer estuary) to 0.24 (station 8, inner estuary). Samples were taken between 1.29 and 4.5 m above French chart datum and consequently, the percentage of emergence (ET) varies from 17.8% (station 5B) to 88.6% (the highest station 2A), corresponding to normalised emergence times (1-ET) of 0.82 and 0.11, respectively.

The RRO values were calculated (see supplementary material for methodology) based on the river discharge volume recorded between 10 June and 9 July 2019, the 30 days before taking the foraminiferal samples (which are studied by Fouet et al., [29]). During this period, the discharge of the Loc'h river gradually decreased from 3.92 m³/sec on 12 June 2019 to 0.55 m³/sec on 9 July 2019, with an average value of 1.50 m³/sec. This value is about 55% of the yearly average of 2.72 m³/sec. For the Bono river (average yearly

discharge 1.52 m³/sec), no monthly data are available, but the yearly trend should be very similar, so that we decided to estimate the discharge during the 30 days before sampling at 0.84 m³/sec, using the same ratio as observed in the Loc'h river between the yearly average and the study period.

The obtained normalised RRO (Relative River Outflow) values vary from 0.85 (the most inland site 8) to 0.97 (site 1, closest to the mouth), showing that, compared to the Loire estuary, which serves as a standard (see paragraph 5), riverine influence is very weak in the Auray estuary.

In Table 1, the constants α , β and γ of the MII index (equation 3) are all set to 1, to give an equal weight to the three parameters (X/S, ET and RD/CS). The values of these constants have to be determined at a later stage by comparison with faunal patterns (considered as "natural") in a wide range of estuaries. In figure 3, the MII values, which vary from 0.67 (station 1B) to 0.13 (station 8A), have been plotted in a 3D diagram. The obtained isolines of MII connect points which should experience a similar degree of marine influence and should therefore host comparable faunas.

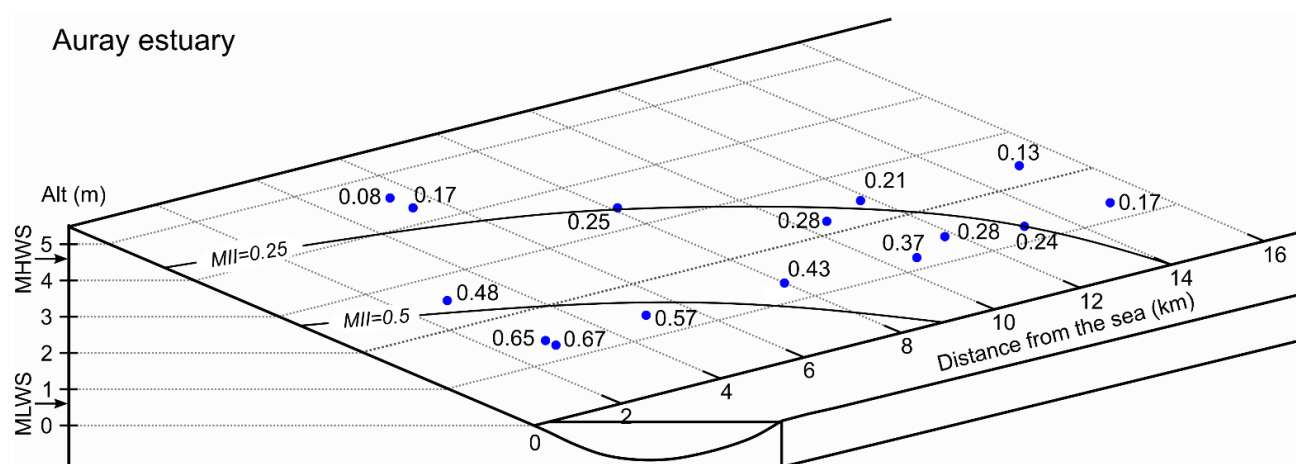


Figure 3. Overview of sampling sites in the Auray river estuary with values of the MII index, based on runoff data between 10 June and 9 July 2019. Altitudes refer to the French chart datum. See text for further explanation. Horizontal and vertical axes correspond to the distance to the sea and the elevation of the sampling stations, respectively. Tidal reference levels are indicated on the Y axis; MHWS: Mean high water springs; MLWS: Mean low water neaps.

6.2. Vie estuary

The Vie estuary (Figure 2B) is a typical lowland estuary. The Vie estuary receives fresh water from the Vie river, that is 63.5 km long, has a surface area of 0.95 km² and drains a catchment area of 84 km². The entrance of the estuary is about 200 m wide. Today, the salt wedge penetrates the estuary until a dam with a sluice at 8 km from the entrance. However, a detailed observation of topographical maps suggests that before the construction of this dam, salt water penetrated the estuary until about 16.8 km inland. In fact, the estuary shows a meandering pattern through the low coastal plain, with a nearly horizontal thalweg from the entrance of the estuary to the village of St. Maixent-sur-Vie, where it starts to rise abruptly. In our calculations of the normalised distance to the sea, we have used this putative value of 16.8 km.

The Vie river has a mean annual discharge volume of 1.18 m³/sec. Total fresh water residence time is estimated at about one week, showing that the Vie estuary is strongly river-dominated. The estuary has a meso- to macrotidal regime, with a tidal range of about 4 m at the mouth. During the 2019 survey in the context of the European Framework Directive, the biological status was evaluated as good, whereas the chemical status was not investigated [70].

Since our chemical analyses only show minor enrichment of Cu in some small harbors, and no enrichment of other toxic metals (Fouet et al., [29]), we conclude that also the Vie estuary is not affected by severe anthropogenic stress. This contrasts with the strong urbanisation and intensive recreational pressure in the lower part of the estuary (Figure 2B).

In the Vie estuary, 9 sites with a total of 13 sampling stations were selected for our study of the foraminiferal community (Figure 2B, Table 2), and were sampled from 23 and 24 October 2018. The main characteristics of the sampling stations are listed in table 2. The 13 sampling stations are positioned between

0.4 (station 2) and 7.7 km (station 10) from the mouth of the estuary. The normalised distance from the mouth (1-X/S) varies from 0.97 (station 2) to 0.53 (station 8). Samples were taken between 1.15 and 4.86 m above French chart datum and consequently, the percentage of emergence (ET) varies from 7.6 % (station 8B) to 77.9% (the highest station 10A), corresponding to normalised emergence times (1-ET) of 0.92 and 0.22, respectively.

Station	Distance to Sea (X, in km)	Normalized Distance to Sea (1-X/S)	Distance Perpendicular to Channel Axis (m)	Altitude (m)	Emergence Time (%)	Normalized Emergence Time (1-ET)	River Discharge/ Cross-Section (RD/CS, m/s)	RRO (Relative River Outflow)	MII (α , β and γ Set at 1.0)
2	0.4	0.98	185	3.35	48.32	0.52	0.01	0.98	0.49
3	0.5	0.97	65	2.00	27.21	0.73	0.01	0.98	0.69
4	0.7	0.96	90	1.82	24.49	0.76	0.01	0.98	0.71
5	0.9	0.95	170	2.67	37.84	0.62	0.01	0.98	0.58
6	1.9	0.89	50	1.44	16.44	0.84	0.02	0.97	0.72
7A	2.6	0.85	185	4.82	76.47	0.24	0.02	0.97	0.19
7B	2.6	0.85	140	4.18	62.25	0.38	0.02	0.97	0.31
7C	2.6	0.85	85	1.61	20.41	0.80	0.02	0.97	0.65
8A	4.6	0.73	35	4.21	62.91	0.37	0.05	0.96	0.26
8B	4.6	0.73	15	1.15	7.64	0.92	0.05	0.96	0.64
9	5.4	0.68	10	1.22	11.03	0.89	0.06	0.95	0.58
10A	7.7	0.54	40	4.86	77.92	0.22	0.06	0.95	0.11
10B	7.7	0.54	10	2.24	31.08	0.69	0.06	0.95	0.36

Table 2. Environmental characteristics of the 13 sampling stations at 9 sites on intertidal mudflats in the Vie estuary. Altitudes refer to the French chart datum. The general methodology of the calculation of MII is explained in section 5; a more detailed explanation of the individual components is given as supplementary material.

The RD/CS values were calculated on the basis of river discharge volume during the 30 days before taking of foraminiferal samples, from 23 September to 22 October 2018. During this period, discharge varied between 0.001 to 0.002 m³/sec for 23 September to 7 October 2018, and a maximum of 0.089 m³/sec measured on 21 October 2018. The average discharge during these 30 days was only 0.021 m³/sec, which is exceptionally low for this period (Figure 4). The obtained normalised RRO values vary from 0.98 (site 1) to 0.95 (the inner estuary site 10).

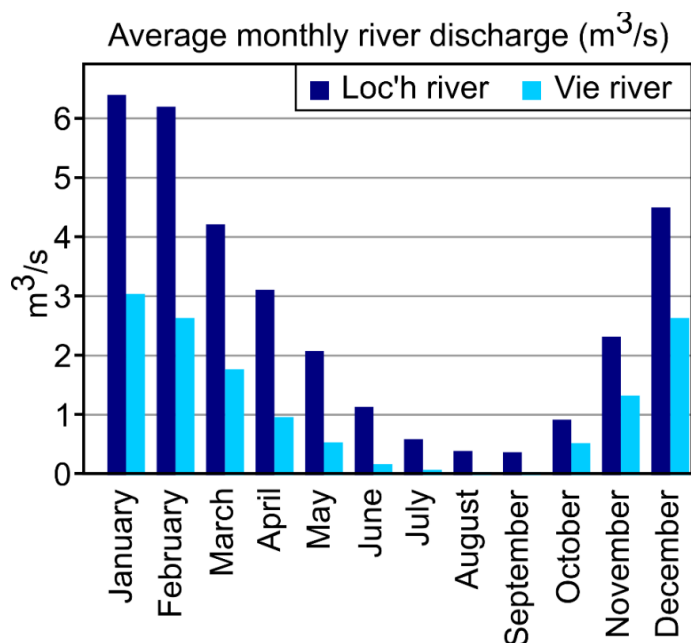


Figure 4. Average monthly discharge volumes for the Loc'h and Vie rivers (average of 49 years of observation). Source: <http://www.hydro.eaufrance.fr/>.

To calculate MII values, the scaling constants α , β and γ were all set to 1.0, so that the three parameters constituting the MII have been given equal weight. In figure 5, the MII values, which vary from 0.72 (station 6) to 0.11 (station 10A), have been plotted in a 3D diagram. The obtained isolines of MII connect points experiencing a similar degree of marine influence, which should potentially host comparable faunas.

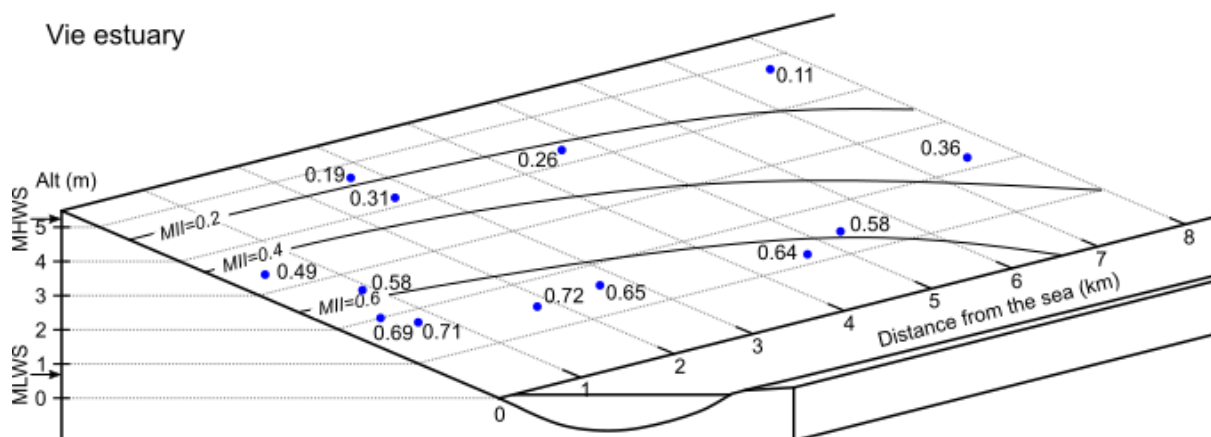


Figure 5. Overview of sampling sites in the Vie estuary with values of the MII index, based on runoff data between 10 June and 9 July 2019. See text for further explanation. Horizontal and vertical axes correspond to the distance to the sea and the elevation of the sampling stations, respectively. Tidal reference levels are indicated on the Y axis; MHWS: Mean high water springs; MLWS: Mean low water neaps.

6.3. Comparison of the two estuaries, impact of seasonal variation of river discharge

When we compare the MII values for the Auray and Vie estuaries, it can be noticed first that the range of index values is very comparable, from 0.67 to 0.13 for the Auray estuary, compared to 0.72 to 0.11 for the Vie estuary. Nevertheless, the values for the position of the sampling point on the salinity gradient are higher in the Auray estuary. This is due to the fact that we did not sample landward of the dam at 8 km from the mouth, that artificially blocks the salt water influence upstream. In both estuaries, riverine influence was minimal in the 30 days before sampling, as is expressed by the very low RD/CS values that vary from 0.03 to 0.54 in the Auray estuary and from 0.01 to 0.06 in the Vie estuary. These values are very small compared to the (year-averaged) value of 25.0 calculated for the Loire estuary at Cordemais. They show that in both estuaries, river discharge was very low in the 30 days before sampling, 2.34 and 0.02 m³/sec for the Auray and Vie estuaries, respectively.

To better show the influence of river discharge on the MII, we calculated for all sites of both estuaries the MII values on the basis of the year-averaged runoff volumes, of 4.29 and 1.19 m³/sec for the Auray and Vie estuaries, respectively (Tables 3 and 4).

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Auray Estuary	River Discharge/ Cross-Section (RD/CS, m/s)	RRO (Relative River Outflow)	MII (α , β and γ set at 1.0)	River Discharge/ Cross-Section (RD/CS, m/s)	RRO (Relative River Outflow)	MII (α , β and γ set at 1.0)
Station	June–July 2019			Average River Runoff		
1A	0.03	0.97	0.48	0.06	0.95	0.47
1B	0.03	0.97	0.67	0.06	0.95	0.66
1C	0.03	0.97	0.65	0.06	0.95	0.64
2A	0.03	0.97	0.08	0.05	0.96	0.08
2B	0.03	0.97	0.17	0.05	0.96	0.16
2C	0.03	0.97	0.57	0.05	0.96	0.57
4A	0.15	0.92	0.25	0.29	0.89	0.24
4B	0.15	0.92	0.43	0.29	0.89	0.42
5A	0.09	0.94	0.28	0.16	0.92	0.28
5B	0.09	0.94	0.37	0.16	0.92	0.36
6A	0.18	0.92	0.21	0.34	0.88	0.21
6B	0.18	0.92	0.28	0.34	0.88	0.27
7	0.30	0.89	0.24	0.54	0.85	0.23
8A	0.54	0.85	0.13	0.98	0.80	0.12
8B	0.54	0.85	0.17	0.98	0.80	0.16

Table 3. Auray estuary; MII calculated for observed river charge in June–July 2019 and for average river discharge.

Vie Estuary	River Discharge/ Cross-Ection (RD/CS, m/s)	RRO (Relative River Outflow)	MII (α , β and γ Set at 1.0)	River Discharge/ Cross-Section (RD/CS, m/s)	RRO (Relative River Outflow)	MII (α , β and γ Set at 1.0)
Station	October 2018			Average River Runoff		
2	0.01	0.98	0.49	0.29	0.89	0.45
3	0.01	0.98	0.69	0.50	0.86	0.61
4	0.01	0.98	0.71	0.34	0.88	0.64
5	0.01	0.98	0.58	0.32	0.89	0.52
6	0.02	0.97	0.72	1.40	0.76	0.57
7A	0.02	0.97	0.19	0.88	0.81	0.16
7B	0.02	0.97	0.31	0.88	0.81	0.26
7C	0.02	0.97	0.65	0.88	0.81	0.55
8A	0.05	0.96	0.26	2.98	0.65	0.18
8B	0.05	0.96	0.64	2.98	0.65	0.44
9	0.06	0.95	0.58	3.40	0.63	0.38
10A	0.06	0.95	0.11	3.40	0.63	0.08
10B	0.06	0.95	0.36	3.40	0.63	0.24

Table 4. Vie estuary; MII calculated for observed river charge in June–July 2019 and for average river discharge.

For the Auray estuary, the MII values change only marginally, but in the Vie estuary, the MII becomes much lower, with values below 0.5 in the whole inner part of the estuary. These values, calculated for year-averaged runoff volumes, show very well the large difference between the two estuaries. The Auray estuary is a ria, widely open into the marine Morbihan Gulf, and therefore more tide dominated than the Vie estuary, that has a narrow meandering course, and is a strongly river-influenced estuary. Of course, in the Vie estuary, river-influence is much less prominent in dry periods with minimal runoff volumes, which existed during the 30 days before our sampling in October 2018. In case we would have used maximal average river discharge values (3.12 m³/sec, average value for January, based on a 49-year record), MII values would be below 0.5 in the whole estuary, with the exception of sites 3 and 4, close to the mouth. This suggests that in winter, conditions in a large part of the Vie estuary are unfavourable for foraminifera.

7. Discussion and Conclusion

In this paper, we have investigated the dominant factors determining the natural distribution of foraminifera in intertidal estuarine mudflats. Next, we have attempted to assemble these factors in a single index of marine influence (MII), which ideally should give an overall characterisation of the natural conditions of intertidal estuarine sites. The aim of this MII is to make it possible to compare different sites in the same estuary, and/or to compare site in different estuaries.

Three environmental parameters have been selected to calculate the MII, because we hypothesize that they provide the best description of the environmental context in which the foraminiferal community develops:

1. The relative position of the sampling point on the gradient between fully marine water and fresh water,
2. The relative emergence time of the sampling point at mean spring tide conditions, expressed a percentage of the total length of a tidal cycle. This parameter is closely related to the absolute altitude (with respect to the level of the lowest astronomical tide) of the sampling point,
3. The relative importance of freshwater outflow, which is determined by dividing the average discharge volume in the 30 days before sampling by the cross-section area of the estuary at each sampling point.

All three parameters are clearly related to salinity, which is generally thought to be the dominant factor controlling estuarine faunas, but implicitly also include other controlling factors, such as the introduction of marine and continental organic matter and biota, temperature and hydrodynamic energy. In view of their short life cycles (estimated between 6 months to 2 years, [51]), and their high reactivity to environmental changes, a time lag of about 2 to 4 weeks between the environmental conditions and the foraminiferal response seems appropriate. For this reason, we calculate the average river discharge for the 30 days preceding the sampling of the foraminiferal faunas.

For the moment, we did not give different weights to each of the three theoretical factors constituting our MII. To be able to attribute such a relative weight, we added the constants α , β and γ in equation (3), but for the time being, these were set at one, giving equal weight to the three variables. The absolute values of α , β and γ will be determined in future, on the basis of a large-scale comparison of MII and the characteristics of the foraminiferal communities, in a large number of estuaries in different seasons. A multivariate analysis should make it possible to quantify the respective contribution of the three main components of MII to the multivariate model, and thereby, to determine the values of the weighing constants.

To investigate whether this marine influence index (MII) correctly describes the distribution of foraminiferal faunas in intertidal estuarine mudflats, a comparison with faunal data is necessary. A first attempt is made in the companion paper of Fouet et al. (this volume) [29]. However, in this paper only a part of the large range of environmental variability is treated, especially with respect to river outflow, which was minimal at the time of sampling. In order to appreciate the efficiency of the MII as an overall descriptor of foraminiferal habitats in intertidal estuarine mudflats, a more exhaustive comparison with field studies is necessary. Such a more important study should ultimately also allow us to determine the values of the three constants.

The aim of the MII is to serve as a tool to predict the composition of foraminiferal assemblages in intertidal estuarine mudflats, in particular the relative frequencies (at different sites) of marine, estuarine and freshwater species, and of stress-tolerant taxa. It should be kept in mind that the MII presented here has been developed to quantify the complex environmental parameter "marine influence", that appears to be main responsible for the natural environmental stress characterising intertidal estuarine mudflats to which the foraminiferal community is exposed. It is evident that other factors than "marine influence" may affect foraminiferal communities as well. These may be physical/chemical (e.g., temperature, oxygen concentration, substrate grain size) or biological factors (predation, competition). However, most of these additional factors are in some way related to the distance to the sea and the emergence time as well, so that they are already partly included in the MII. The MII is therefore expected to explain a large part of, but not all of the characteristics of the foraminiferal community in natural conditions.

A comparison between the observed faunal characteristics and those expected on the basis of the MII should make it possible to detect sites at which the faunas are strongly impacted by anthropogenic pollution.

At such sites, the faunal composition should show major differences with the composition expected on the base of the MII, that can not be explained by other natural factors.

Summarising, the Marine Influence Index (MII) that is proposed here, is meant to be a first step in the development of a foraminiferal index of environmental quality, specific for estuarine intertidal mudflats. A phase of calibration of the index, by comparison with faunal data sets is now necessary, to investigate whether MII can indeed provide a reliable description of the complex of environmental parameters controlling the characteristics of the foraminiferal community in such environments.

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Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w14040676/s1>, Table S1: Tidal reference levels for St. Gilles Croix de Vie (Vie estuary) and four sites in the Auray estuary: Port Navalo (mouth of the estuary), Locmariaquer (2.8 km from the mouth), Fort-Espagnol (8.0 km from the mouth) and Auray (15.0 km from the mouth). Table S2: RD/CS and RRO (relative river outflow) calculated for four sites in the Loire estuary, using an average yearly discharge volume (RD) of 854 m³/s. Table S3: RD/CS and RRO (relative river outflow) calculated for four sites in the Loire estuary, using the discharge volume (RD) of 193 m³/s, the average value measured during low water conditions in October 2019. Table S4: Comparison of MII, Altitude, Distance to the sea, Normalised distance to the sea and Salinity, for the Auray and Vie estuaries. Figure S1: Schematic representation of the duration of emergence at a sampling point (arbitrary chosen at 2.6 m altitude) during a mean spring tide cycle, for the site Auray in the upper Auray estuary. In this example, the duration of emergence is 6 h 14'30", whereas the tidal cycle takes 12 h 19'. The corresponding reference emergence time will be 50%. Figure S2: Map of the Loire estuary with the position of the four localities used here. Figure S3: Relation between measured salinity and distance to the sea for the Auray estuary (in blue, July 2019) and Vie estuary (in orange, June 2020). Figure S4: Relation between measured salinity and normalised distance to the sea for the Auray estuary (in blue, July 2019) and Vie estuary (in orange, June 2020). Figure S5: Bottom water salinity (in the main estuarine channel) in Bono (black line) and Locmariaquer (grey line) between March 2006 and March 2008. After Diz et al. [48].

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Annexe 2

The estuarine quality paradox – eDNA metabarcoding provides a new vision of estuarine mudflat diversity

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The estuarine quality paradox – eDNA metabarcoding provides a new vision of estuarine mudflat diversity

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Keywords: Foraminifera, eDNA metabarcoding, mudflat, high-throughput sequencing

Abstract

Environmental biomonitoring is a prerequisite for efficient evaluation and remediation of ecosystem degradation due to anthropogenic pressure or climate change. Estuaries are key habitats subject to multiple anthropogenic and natural stressors at the crossroads of ocean and continent. Due to the multiple stressors, the detection of anthropogenic pressure is challenging. The fact that in estuaries, abundant natural stressors often lead to negative quality assessments has been coined the “estuarine quality paradox”. To solve this issue, the application of molecular approaches to successful bioindicators like Foraminifera is promising. However, sampling protocols, molecular procedures and data analyses remain to be validated before such tools can be routinely applied.

We conducted an environmental DNA survey of estuarine mudflats along the French Atlantic coast, using a metabarcoding approach targeting foraminiferal clades. The dominant taxa were different in the six estuaries. Our results further demonstrate that estuarine environments have only few active taxa dominating the community composition and a large stock of dormant or propagule stages. This underlines the importance of the so called “rare biosphere” in estuarine ecosystems. This diversity component constitutes an important species reservoir, with different species developing in response to various combinations of multiple stressors. Only 43% of the variance was explained by the physical and chemical parameters of the sediment and the climatic conditions. This suggests that other factors, such as ecological drift play a key role. Environmental DNA biomonitoring opens new perspectives to resolve the “estuarine quality paradox” by providing complementary information to the classical morphological approaches.

Introduction

Estuarine intertidal areas correspond to the ecotone where aquatic (oceans and rivers) and terrestrial ecosystems merge. They represent highly productive ecosystems (1) that provide numerous ecological (2) and economical (3, 4) services. Due to their unique properties, estuarine ecosystems are subject to both natural and anthropogenic environmental pressure from continental (nutrient input, pollution, floods, etc.) as well as oceanic sources (tides, salinity, etc.). For example, the tidal cycle will cause high variability of salinity and moisture content of different coastal habitats (5). River flow (low water levels, floods, etc.) will influence salinity as well, but may also cause erosion of banks and mudflats (5). To integrate these different parameters in a single index, we have recently developed and tested the “Marine Influence Index” (MII) which aims to provide an integrated assessment of the natural environmental conditions of estuarine sites (5, 6).

In view of the ecological and economical value of coastal areas, the European Commission adopted in October 2000 the Water Framework Directive (WFD, 2000/60/EC). This directive commits the Member States of the European Union to achieve good qualitative and quantitative status of all water bodies, including transitional and coastal waters. To achieve the objectives of the WFD, it is crucial to develop new approaches and methods to monitor environmental quality. The Foraminifera (Rhizaria, Eukaryotes) represent excellent model organisms to do so, as the species are known to respond differently to various environmental parameters. For example, different species are sensitive to organic enrichment (7, 8), heavy metal pollution (9), or ocean acidification (10–12). Compared to macrofauna or fishes, on which many indices are based, the advantage of foraminifera is their high potential species diversity and rapid response to environmental changes (13). The production of propagules and resistance stages is a well known adaptative mechanism to protect the organism against temporary adverse conditions. (14). Foraminifera have the ability to form inactive stages (i.e., dormant adults or juveniles and propagules), allowing them to survive periods of adverse conditions and rapidly respond to periods of favorable conditions (15). For example, propagules of sensitive species (e.g., porcelaneous taxa) appear to be able to withstand short periods of low pH conditions and to “bloom” once the conditions are close to their optimum. (16).

During the last decades, many studies have proposed foraminiferal biotic indices for open marine environments, especially for the shelf area. On the one hand, several indices based on morphological observations were developed, using different metrics such as alpha diversity (17) or an indicator species concept (18, 19). On the other hand, studies based on environmental DNA (eDNA) have provided indices based on assignments to a reference database (20) or on taxonomy free and machine learning (21). Nevertheless, these indices have their limits in estuarine environments. In fact, large spatial and temporal variations in natural environmental parameters (salinity, temperature, moisture, organic matter, and oxygen contents, etc.) as well as superimposed anthropogenic stressors impact the community composition. Consequently, the alpha diversity found in estuaries is low in comparison with fully marine environments (22, 23). Even in predominantly natural settings, species living in estuaries are highly adapted to extreme and highly variable environmental conditions requiring opportunistic life strategies. This strongly biases the results of environmental quality indices created in fully marine habitats, systematically leading to negative scores in estuaries (6). This apparent contradiction (low environmental quality values in apparently natural ecosystems) is known as the “estuarine quality paradox” (24, 25). Consequently, biomonitoring the environmental quality of estuarine ecosystems remains a major challenge (25, 26).

Here, we present a survey of foraminiferal diversity in estuarine mudflats based on high throughput sequencing (HTS). To assess the patterns and drivers of diversity, we used a metabarcoding approach using specific foraminiferal primers, targeting the V9 region of the 18S rRNA gene. Our dataset consists of 25 sites from various mudflats from six estuaries of the French Atlantic coast. At each site we have measured the major environmental parameters (sediment grain size, trace metals and organic matter) and have retrieved climatic data (temperature and precipitation). As eDNA metabarcoding allows the amplification of the whole diversity, including propagules and dormant stages (27), we hypothesize that (1) the diversity recovered by HTS will be high, but (2) only a fraction of this diversity will be represented by the active communities due to the potentially high density of inactive stages. In addition, as the estuaries are placed at the crossroads of continental and marine ecosystems, we expect (3) that the diversity will strongly react to both natural and anthropogenic environmental gradients.

Results

After removing non foraminiferal, low quality and chimeric reads, we obtained a dataset of 4,133,414 reads and a total of 162 ASVs (Datasets S2). Half of the reads (53.1%) and about a quarter of the ASVs (22.6%) were assigned with 100% confidence to the reference database (Fig. S1). The analyses of the alpha diversity of each replicate showed that a single replicate covers on average 63% of the total alpha diversity (Fig. S2). The number of ASVs in common between two replicates was moderate, so that a substantial part of the diversity was only present in one replicate (Fig. S2). Therefore, to reduce this sampling bias, further statistical analyses were carried out on combined replicates. The rarefaction curves (Fig. S3) demonstrated that the sequencing depth per site was sufficient, and the ASVs accumulation curve (Fig. S4) showed that the whole study adequately presented the total diversity of foraminifera. Each of the 162 ASVs was manually verified against our own reference database and on NCBI. All ASVs belonging to the same taxon have been combined in a final table count (Datasets S3). This final table count consists of 99 taxa corresponding to 18 taxa affiliated to monothalamids, 14 to Globothalamea and two to Tubothalamea. Finally, 65 environmental clades were affiliated to Foraminifera, but no better assignation could be provided (Fig. 2).

The proportion of the 65 environmental clades among the samples and estuaries was uneven with a higher number of unassigned taxa in Auray estuary. The proportion of Rotaliida (Globothalamea) was low, except for some samples where high numbers of reads of *Ammonia* and *Haynesina* could be observed (Auray ST02_A, Crac'h STO4_A, STO5_A and Odet STO4_A). Most of the monothalamids were affiliated to the Saccaminidae, which were dominant in most of the assemblages. A notable exception was the Laïta estuary where unclassified *Allogromida* and *Vellaria pellucida* were dominant.

About 67.5% of the dominant taxa (that represent more than 1% of the total number of reads) were present in five or six estuaries (Fig. S5). More than 80% of the taxa were present in at least four estuaries (Fig. S5). Finally, no dominant taxa were present in only one estuary. However, the relative abundance of the number of reads of the dominant taxa was highly variable among the studied estuaries (Fig. 3). A dominance of three to 12 specific taxa was typical for each of the six estuaries (Fig. 3). The communities often showed a dominance of one or two monothalamid taxa that were different in the various estuaries. For the Crac'h and Odet estuaries, the relative abundance of Globothalamea taxa was higher (Fig. 2) in comparison with the other estuaries. Even if every dominant species had a favorite estuary, they were also present in most of the other estuaries (Fig 3).

The NMDS analysis based on the dominant taxa (Fig. 4) showed that each estuary occupies a specific part of the ordination space. Both the Auray and Crac'h and the Elorn and Aulne estuaries plot in the same part of the ordination space. Conversely, the Odet and Laïta estuaries did not overlap with other estuaries. Although most of the taxa were in the center of the ordination space, some had a stronger influence on the ordination of the samples and estuaries. This was for instance the case for env_18, *Elphidium margaritaceum*, *Haynesina depressula* (Auray and Crac'h estuaries) and two monothalamids (GenBank accession numbers KP984731 and EU213249). The RDA analysis (Fig. 5a) showed that Foraminifera communities are significantly influenced by nine environmental variables (clay, Corg, distance to the sea, river discharge, Pb, Sn, U, PDM, and isothermality, $p < 0.05$). The first two axes explained 43.3% of the variance. The variance partitioning showed that the sediment and physical properties explained 15.9% of the variance, whereas the climatic data explained 8.2% (Fig. 5b). When both were combined, the percentage reached 17.2%. The residuals of this analysis correspond to 58.7%.

Discussion

Sampling and dataset consistency

Since this is the first published attempt to study foraminiferal eDNA living in estuarine mudflats with specific markers, our sampling strategy was not yet optimized for this purpose. At all sampling sites, two replicates of 5g sediment were collected. After analyzing the dataset, we noticed that on average, one replicate represents only 63% of the total sampling site diversity (Fig. S2). Nevertheless, the rarefaction and amplification curves clearly reach the “plateau” (Fig S3, Fig. S4) indicating that the sequencing depth is sufficient to cover the total diversity of a single replicate. Several explanations can be provided to explain this apparently contradictory observation: 1) The DNA extraction kit does not allow perfect DNA extraction of the cells (i.e., bead beating steps could be unable to break properly organisms provided with calcareous tests), 2) The quantity of sediment (i.e. 5g.) used as input may not cover the total diversity of the site and finally 3) the sampled area (for one replicate) is potentially too small to have a complete picture of the diversity of the site. However, to draw sound ecological conclusions, it is crucial to cover the total diversity. Therefore, more methodological work is needed to define the most efficient strategy (49, 50). To reduce sampling artifacts in our study we have decided to pool the replicates and consider them as single samples. The total diversity of the environment will be approached with this strategy.

To obtain a robust dataset that allows answering ecological hypotheses, different known biases (contamination, tag jumping, reads quality, assignation...) must be addressed (34). In this study, we choose to adopt the most stringent possible approach. To reduce the “tag jumping” effect we have used a unique double tag combination and have limited the number of samples per library to 35 (51). We have also improved the overall quality of our sequencing by including 1-4 nucleotides as spacers to increase the heterogeneity of the libraries (52). Next, we have checked the assignation of each ASV with the reference database in order to avoid potential bioinformatic misidentification. To remove diversity biases due to multiple variants that exist for some foraminiferal species (53), we have decided to pool the ASVs belonging to the same taxa. And finally, for our ecological model (Figs. 4 and 5), we have chosen to consider only taxa accounting for >1% of the total number of reads. This reduces the noise of the rare biosphere in our ecological model (41). All these steps tend to increase the robustness of our dataset and strengthen our interpretation and conclusions.

Estuarine foraminiferal eDNA diversity

We were able to assign most of our ASVs to species level (Datasets S2), demonstrating the advantage to use specific markers for specific clade investigation. As expected, due to the extreme environmental conditions in estuaries, the alpha diversity of foraminifera is low compared to open ocean habitats (54). The number of foraminiferal species observed at the investigated sites based on the morphological observation of the 125 μ m fraction of the sediment (only species with a mineralized or agglutinated shell were observed) ranges from two to 19 ((6) and unpublished data). In our study we observed 99 taxa resulting in a ratio of about five between taxa observed in eDNA and in morphological studies. The high proportion of monothalamids found in the eDNA dataset can be explained by a combination of factors. For example, the shorter barcode size in some monothalamids (i.e. saccaminids), compared to Globothalamea or Tubothalamea would promote their amplification and sequencing. Another explanation could be that monothalamids are easier to extract with the DNA kit as they have an organic test (55). A primer bias in favor of this group could also be possible. Finally, a naturally higher abundance of monothalamids compared to mineral shelled foraminifera (Globothalamea and Tubothalamea) could also be hypothesized (54, 56, 57). The much lower abundance or total absence of monothalamids in morphological studies can be explained by the fact that organic shelled foraminifera (dominant in of monothalamids) are usually ignored in this methodology.

Even if the number of environmental clades found in our study is high (representing more than 50% of the taxa), they represent only a fraction of the total number of assigned reads (Fig S1). This demonstrates that the effort of single cell barcoding in mudflats is sufficient to reliably assign the majority of ASVs. Consequently, the reference database for these ecosystems can be considered as accurate, even if an additional effort should be made to clarify the monothalamids taxonomy. We expect that when new sequences of monothalamid species will become available, some environmental clades will be identified morphologically. Therefore, their intra-individual variance will be better known and consequently the alpha diversity will decrease.

Our results show that natural environmental gradients have a strong impact on species composition. In function of the habitat properties, a specific selection of often opportunistic species will be present in each estuary. Our analyses suggest that the community composition in Auray and Crac'h estuaries is similar (Fig 4 and Fig 5), even if the dominant taxa are not the same in both estuaries (Fig S5, Fig 3). It appears that the small geographic distance between the two estuaries has led to more frequent or intense exchanges between the two communities (Fig 1). A similar pattern can be observed for the Aulne and Elorn estuaries.

Generally, only a few taxa dominate each sample, what is in line with the conclusion of morphological based studies (Fouet et al., 2022). Environmental DNA based studies have the advantage to detect propagules and dormant stages. Additionally, they present a semi-quantitative evaluation of the community composition. In our dataset, the distribution of dominant species that represent more than one percent of the total of the reads is very different between the samples, much more so than in the morphological inventories (Fouet et al., 2022; non published data). Together with the low alpha diversity of the morphology based (i.e., 2-19 (6)) analyses of the same sites, this suggests that only a limited number of taxa is able to occupy the ecological niches with adult specimens. The other taxa present in eDNA may stay in a dormant or propagule stage and wait for a “black swan event” to partly replace the dominant species.

Environmental parameters

Sediment characteristics, environmental properties and climatic data explain 43% of the variance in the RDA model (Fig. 5a); nine variables have a significant impact on the community composition (Fig 5a). The sediment grain size (in our model the clay percentage), that is known to significantly impact the foraminiferal communities (Armynot du Châtelet *et al.*, 2009), affects part of the community distribution on mudflats (Fig. 5). The percentage of organic carbon is often used in fully marine environments as an indicative parameter in morphology based quality indices (19, 59). Although this parameter also seems to influence the community distribution in our molecular study (Fig. 5), this may be partly due to the strong (negative) correlation with grain size. Two physical parameters (distance to the sea and river discharge) particularly affect the first axis of the RDA (Fig 5). These two variables are both related to the salinity of the estuary (5). The salinity is considered as the most important factor driving estuarine diversity for fishes (60), phytoplankton (61) and also foraminifera (6). Next, two variables related to the climate (Isothermality, Precipitation of the Driest Month (PDM)) were also significant in the model. Isothermality quantifies the extent of day-to-night temperature differences compared to summer-to-winter (annual) oscillations (62). Estuarine mudflat environments have large temperature gradients both at a daily (tidal effect) and monthly (spring and neap tides) scale, leading to low isothermality. PDM corresponds to extreme conditions in which a minimal amount of fresh water enters the system. PDM and river discharge both reflect the influence of freshwater input into the estuary, demonstrating the importance of this key element in the model. Finally, the Pb enrichment factor could also affect the estuarine foraminiferal communities (Fig 5, Datasets S1). It has been shown that a high Pb concentration can have an important impact on the abundance of dominant foraminiferal species (63).

Even if these variables undoubtedly influence the community composition, their contribution should be carefully evaluated. In fact, together these factors explain 43% of the total variance of the dataset, so that 57% remains unexplained in the RDA model (Fig. 5). This means that other factors are important drivers of diversity as well. This is underlined by the important differences observed between estuaries (Figs. 3 and 4), that are not corresponding to equally large differences in the adult community (as shown by morphological studies), and appear unrelated to the environmental characteristics discussed before. Several additional factors can be involved. For instance, more qualitative environmental factors such as the morphology, history and anthropogenic use of the estuaries could affect the community characteristics. However, such factors should also affect the composition of the adult assemblages, which is not evident. Therefore, we hypothesize that ecological drift could explain a significant part of the variance (64). In fact, stochastic processes are expected to become more significant with low population size and diversity (65), therefore this process can be especially pertinent in estuarine ecosystems.

Putative impact of anthropogenic stressors

Due to the cumulative effects of natural and anthropogenic stressors as well as potential qualitative environmental factors and ecological drift, the development of bioindicators in estuarine environments is still a challenge. Even if some metallic trace enrichment factors (i.e. Pb) and organic carbon can have an anthropogenic source, it was impossible to evaluate the impact of anthropogenic sources individually in our model (Fig. 5). Nevertheless, eDNA metabarcoding allows a fast evaluation of the current state of foraminiferal diversity. We have demonstrated that the community composition is highly specific for certain estuaries (Fig 3 and 4). Communities significantly influenced by

anthropogenic stressors should appear as outliers in the ordination model. As we have not observed such patterns, we can conclude that the foraminifera living in the estuaries along the French Atlantic Coast are not substantially influenced by anthropogenic stressors.

Estuarine quality paradox and rare biosphere

The low alpha diversity observed in the estuaries makes it even more difficult to solve the estuarine quality paradox. This paradox was initially based on morphological observations. One advantage of HTS in comparison to classical morphological studies is that it not only considers adult stages, but also includes monothalamids, propagules and dormant stages, thereby potentially strongly increasing alpha diversity. As HTS data are semi-quantitative, we can assume that due to the intrinsic mudflat properties, the dominant taxa (corresponding to >1% of the total of reads) in our study correspond to the adult and the most active stages of foraminifera. Nevertheless, the distribution of the reads of these dominant taxa is very different between the studied estuaries. It appears that the dominant species are present in all estuaries, but they do not develop everywhere; they have a high density of reads only at part of the sites. Environmental DNA approaches are therefore a powerful tool, which not only assesses the living and dominant species but also species that are potentially present, and that await more favorable conditions to develop. However, a limitation of the eDNA approach is the impossibility to distinguish the different categories (dormant, propagules, juveniles and adults). A dual approach, combining morphological as well as eDNA studies, to assess the different fractions of living and dormant stages seems therefore the ideal strategy to obtain a more complete overview of the foraminiferal communities.

Our study demonstrates that foraminiferal eDNA is particularly well adapted to assess the diversity in estuarine mudflats. The sampling strategy and the choice of eDNA extraction kits still need optimization. We suggest increasing the number of replicates and sites or/and the quantity of raw material and optimizing the bead biting step to extract more DNA from calcareous foraminifera. To obtain a reliable dataset several crucial factors (tag jumping, assignation, reference database...) must be carefully assessed. The environmental parameters, sediment characteristics as well as the climatic data partly explain the community composition. However, other factors like ecological drift, or the anthropogenic occupation history of the estuary appear to have a substantial effect as well. Finally, as the dormant and propagule stages of foraminifera are an important part of the diversity, it is crucial to characterize this component. The combination of morphological information, mainly concerning the adult living community, and HTS eDNA allows to obtain a more complete picture of the diversity. By monitoring the evolution of the diversity, we can detect shifts in the community due to “black swann” events and anthropogenic impact. This should ultimately allow us to overcome the estuarine quality paradox.

Materials and Methods

Studied estuaries and sampling sites

Samples were collected in six estuaries located along the north French Atlantic coast: Elorn, Aulne, Odet, Laïta, Crac’h, Auray (Fig 1, Datasets S1). These estuaries are subjected to a meso- to low macrotidal regime with a tidal range of about 4 to 5.5 m at the inlet. In all, 25 sites were sampled in September and October 2020. At each site, one to three stations (n=35) were sampled across the mudflat if it was large enough (A to C from upper to lower mudflat). Two

replicate samples of 5g of the first centimeter of the sediment were collected and stored rapidly at -20°C prior to DNA extraction. At each station, other replicates were collected for sediment grain size analysis, organic matter and heavy metal measurements. All samples were collected at low tide on mudflats.

The physical properties of each site were further characterized using the Marine Influence Index (5). The index includes the normalized measurements of the distance to the sea, the emergence time at low tide and the river discharge (Datasets S1). The detailed protocol and the rationale behind this index is explained in (Jorissen *et al.*, 2022). Climatic data were obtained by using the coordinates of each site to extract the value of the 19 variables extrapolated from monthly measurements of temperature and precipitation (Datasets S1). To this purpose, we used the finest 30 arc second resolution grids of the WorldClim database (28).

Sediment grain size, trace metal concentrations and organic matter contents were assessed to obtain sediment properties. Protocols for these methods are detailed in Fouet *et al.*, (2022). In brief, the sediment grain size was analyzed (non-decarbonated) with a *Mastersizer 3000 laser diffraction particle size analyser* (Malvern Pananalytical Ltd, Malvern, UK). In this study three different fractions are considered: clay (<4µm), silt (4-63µm) and sand (>63µm) (Datasets S1). Three samples (LAI_1A, ELO_1A and CRA_1A) are considered as outliers because of their high sand percentage (> 80%). These sites can not be considered as mudflat environments and will not be included in the ecological models. Trace metal concentrations were analyzed with an Inductively Coupled Plasma Mass Spectrometer (Thermo Scientific® X-Series 2 ICP-MS) on freeze-dried sediment, after total acid digestion (HCl, HNO₃ and HF) (29). Seventeen chemical elements (V, Cr, Co, Ni, Cu, Zn, As, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Pb, Th and U) were measured. Enrichment Factors (EF) were calculated by comparison with an unpolluted reference sample (6, 30) (Datasets S1). Most samples showed low enrichment factors, suggesting predominantly natural conditions, except in the Aulne estuary where a strong enrichment of Pb (>5) was observed (Datasets S1). Elemental carbon (%Corg) and nitrogen (%N) contents were measured on decarbonated freeze-dried sediment with a CHONS Elemental Analyser (EA Vario PYRO cube; Elementar®, Langenselbold, Germany) (Datasets S1). The results are in the range of values usually found in mudflat environments (31).

DNA extraction, amplification, sequencing

Total DNA was extracted from the sediment with the FastDNA Spin Kit for Soil (MP Biomedicals) according to the manufacturers' instructions. In our case, as the estimated diversity should be low (6), we expected to have deleterious effects of highly homogeneous nucleotide composition. This limits the number of high-quality reads generated per Illumina run. In order to reduce these effects and increase the overall quality of our sequencing, we included 0 to 4 nucleotides between the tags and the primers to increase the heterogeneity (32). To reduce the tag jumping effect, we used a dual-indexing approach (33). Primers, tag sequences and library information can be found in Datasets S4, and Datasets S1.

PCR was carried out with AccuPrime™ Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific). The 37F hypervariable region (34) (amplicon size: 135-190) was amplified using the specific foraminiferal primers s14F1 (35) and s15r (36). For each DNA extraction, three PCR replicates were done and pooled before the quantification. The PCR conditions consist of an initial denaturation of 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 50 °C for 45 s and extension at 68 °C for 90 s plus a final extension at 68 °C for 10 min. PCR

product pooled replicates were quantified using the QuBit HS dsDNA (Invitrogen). Each sample was then pooled with the same amount of DNA and purified using the NucleoSpin™ Gel and PCR Clean-up XS kit (Macherey-Nagel). Library preparation and Illumina MiSeq (paired-end, 2x250bp) sequencing were performed at ID-Genecodiagnosics (Geneva, Switzerland). Sequences are available on European Nucleotide Archive via project number PRJEB55114.

Bioinformatics and taxonomic assignment

Tags and primers were removed from the reads using cutadapt v. 3.4 (37). Clustering of the reads was done using the R package DADA2 (v. 1.16; (Callahan et al., 2016)). Raw reads were quality controlled by truncating (forward and reverse length of 120 bp) and filtering them to a maximum number of ‘expected errors’ of two. Amplicon sequence variants (ASV) were dereplicated if identical, clustered and pair-end reads merged using a minimum overlap of 12 bp and maximum mismatch of 0 bp. Chimeras were removed using the ‘pooled’ method (38). The ASVs were roughly assigned taxonomically using VSEARCH v. 2.18.0 (Rognes et al., 2016) using our foraminifera reference database based on NCBI. Then, all ASVs were verified manually by comparison with the GenBank database using BLAST, and quick neighbor joining tree analyses were performed to place phylogenetically ambiguous ASVs. The reads of the ASVs were finally merged for each species or environmental clade.

Data analysis

Statistical analyses were performed using R (v4.0.3) and the package vegan (v2.5-7) (39), if not specified otherwise. We performed distribution plots showing the number of reads and ASVs versus the similarity value with the reference sequences (single cell barcoded species) (40). Then, to determine whether the sequencing depth is sufficient to obtain an accurate estimate of taxon richness, we constructed rarefaction and accumulation curves with the *rarecurve* and *specaccum* functions, respectively. Representation of the alpha diversity of the species that represent more than one percent of the dataset (41) was computed using the *ggheatmap* function of the heatmaply (v1.3.0) package (42) and the *upset* function of the UpSetR (v4.0.5) package (43). We assessed the β -diversity (similarity patterns) among foraminiferal communities by non-metric multidimensional scaling (NMDS). NMDS was based on Bray-Curtis dissimilarities retrieved from the sequence relative abundance.

To assess relationships between the taxa and the environmental variables, we first assessed the collinearity between the explanatory variables. We performed a stepwise selection based on the variance inflation factors (VIF) with the recommended threshold of ten (44). Stepwise selection was performed using the custom script (<https://beckmw.wordpress.com/2013/02/05/collinearity-and-stepwise-vif-selection/>), applied in Singer *et al.*, (2019). The following variables were selected for further analyses: Corg, clay (%), distance to the sea, altitude, river discharge, trace elements (V, Co, Cu, As, Mo, Sn, Ba, Pb, U) and bioclimatic variables (Isothermality, Mean Temperature of Wettest Quarter (MTWQ) and Precipitation of Driest Month (PDM)).

Then we performed a partial redundancy analysis (RDA) on Hellinger transformed data (46). The significance of the variables and ordination axes (first and second) was assessed using a one-way analysis of variance (ANOVA; 1000 permutations, p value threshold = 0.05) (47). Finally, in order to evaluate the percentage of variance in foraminiferal communities explained by sediment characteristics and climatic variable, we performed variation partitioning (*varpart* (48)).

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Availability of Data and Materials

Raw sequences (fastq files) are available on European Nucleotide Archive (ENA) via the project number PRJEB55114. The data generated or analyzed during this study are included in this published article: the ASV table counts, ASV taxonomy and sample metadata are provided as additional files.

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Figures and Tables

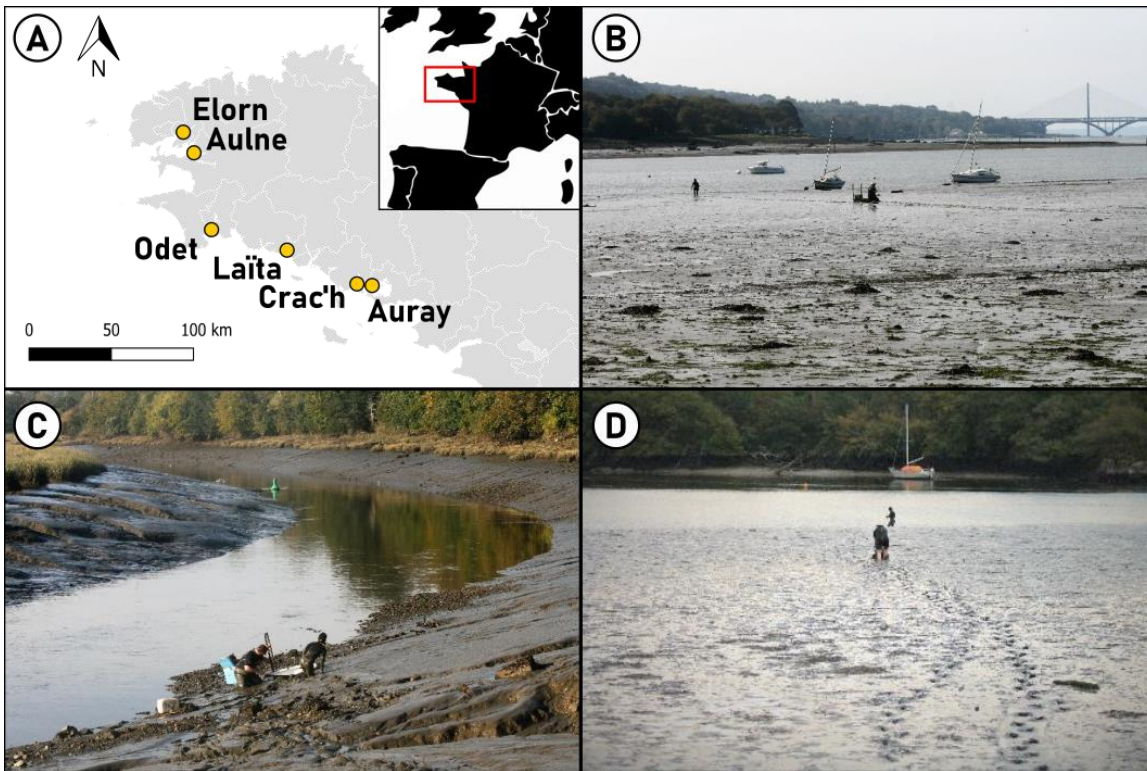


Figure 1. A) Map of the six estuaries (Elorn, Aulne, Odet, Laita, Crac'h and Auray), B) Picture of Elorn ST03_A and ST03_B C) Picture of Elorn ST04_A D) Picture of Laita ST03_A.

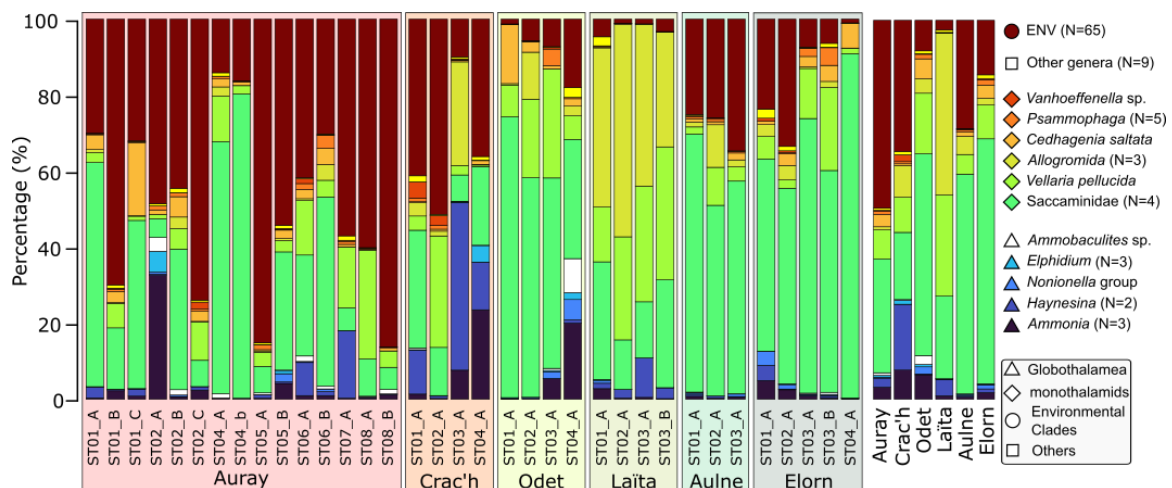


Figure 2. Distribution of the foraminifera taxa living in 6 French coast Estuaries. Taxa were obtained using an eDNA metabarcoding approach targeting specifically foraminifera. Sequencing was performed using a MiSeq platform. The taxa in blue are related to Rotaliida, in dark red the environmental clades that were not assigned to a known reference. Other colors represent the taxa affiliated to the Monothalamids.

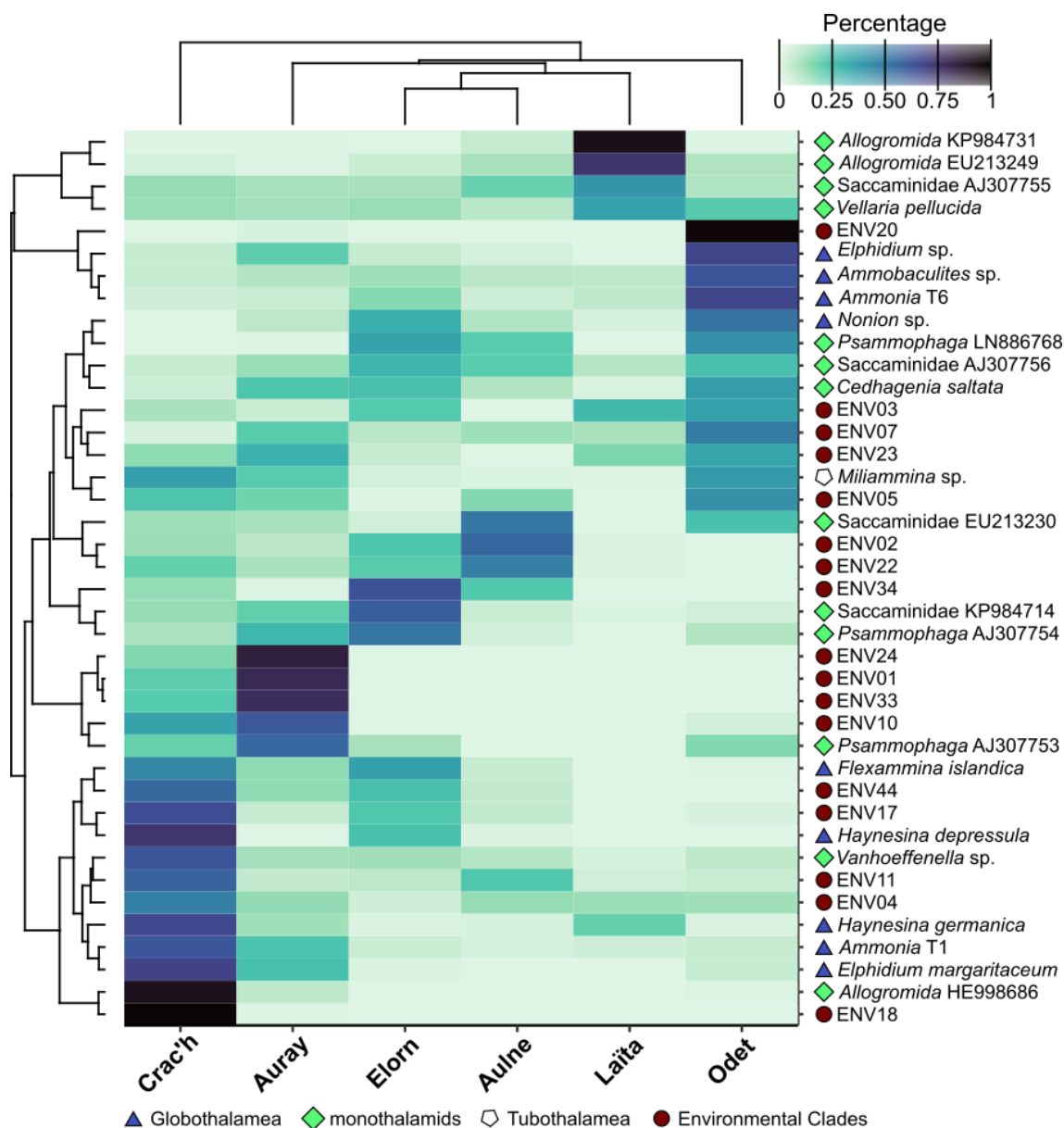


Figure 3. Heatmap depicting the dominant foraminiferal diversity and relative abundance in French coast estuaries. The taxa were obtained with an eDNA metabarcoding approach using specific primers for Foraminifera. Sequencing was done with a MiSeq platform. The distribution of the relative abundance of the reads across the estuaries is uneven and specific taxa are strongly related to a certain estuary.

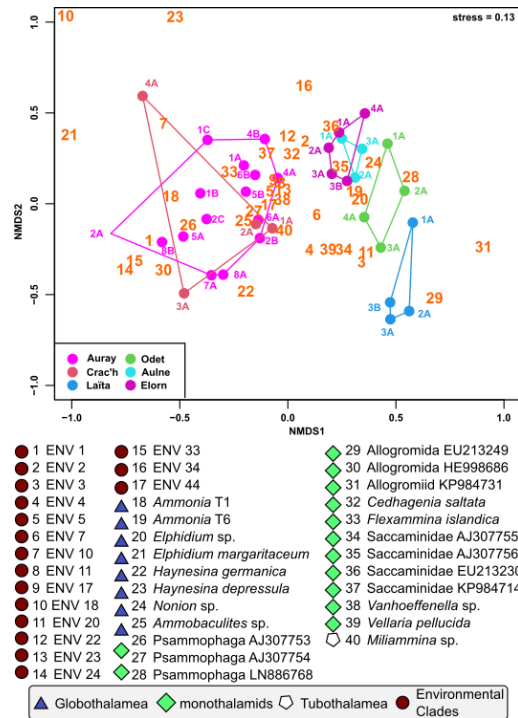


Figure 4. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities of 35 samples from Auray, Odet, Crac'h, Aulne, Laita and Elorn estuary. Stress value = 0,13. The NMDS is constructed with the dominant taxa that represent more than 1% of the total number of reads.

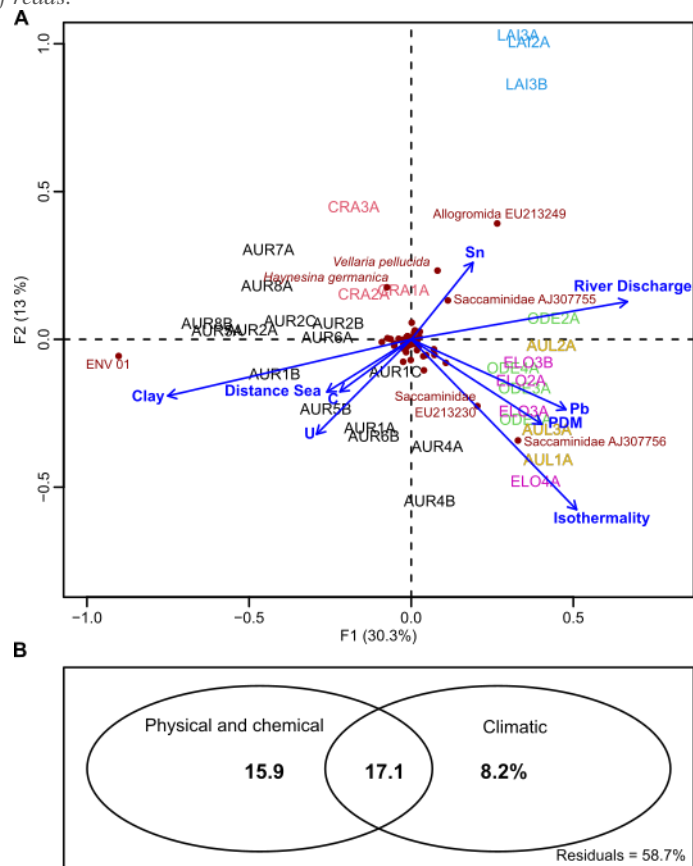


Figure 5. A) Redundancy analysis (RDA) of dominant foraminifera taxa extracted from sediment sample collected from six estuaries from the French Atlantic coast. Significant environmental variables ($P < 0.05$) are represented by arrows. B) The variance partitioning results for community composition among the components of the physical and chemical parameters and the climatic data. Residual values are also displayed. The analysis was performed using the varpart function of the Vegan package in R software.

Titre : Répartition des communautés de foraminifères dans les estuaires de la façade atlantique.

Mots clés : vasières estuariennes, foraminifères, biomonitoring, impact anthropique, paramètres environnementaux, *Ammonia*.

Résumé : Les estuaires sont des habitats complexes, présentant des conditions fluctuant selon les cycles de marée et les crues du fleuve. Les organismes peuplant ces milieux sont exposés à des variations extrêmes des paramètres environnementaux. De ce fait, les communautés estuariennes sont caractérisées par une faible diversité et une majorité d'espèces tolérantes. Les estuaires sont aussi soumis à de fortes pressions anthropiques, qui peuvent encore amplifier le stress exercé sur les organismes. Pour cette raison, les indicateurs d'état écologique développés dans les milieux côtiers montrent systématiquement une mauvaise qualité écologique. Pour développer une méthode de bio-indication fiable, il est d'abord primordial de parfaitement comprendre la répartition naturelle des organismes. Pour répondre à cette problématique, nous avons étudié la répartition des communautés de foraminifères dans les vasières intertidales de neuf estuaires. Afin de synthétiser les relations entre les paramètres environnementaux et les caractéristiques des communautés de foraminifères, nous proposons trois nouveaux indices. L'indice MII reflète l'équilibre entre influence marine et fluviale pour un point donné dans un estuaire, au moment de l'échantillonnage. Nous avons observé une succession de quatre biozones, présentant des communautés typiques de chacune des parties de l'estuaire. Nous proposons deux indices biotiques (EFI et EFDI) qui décrivent quantitativement la position de la communauté observée dans cette succession. La comparaison du MII avec l'EFI et l'EFDI devrait permettre de reconnaître des sites présentant un déséquilibre pouvant être le signe d'une perturbation anthropique. La deuxième partie de cette thèse se concentre sur trois espèces pseudocryptiques du genre *Ammonia*, un taxon majeur dans nos estuaires. D'abord, nous montrons de grandes différences de répartition biogéographique entre ces espèces, qui corroborent le caractère invasif d'*Ammonia confertitesta*. Ensuite, sur la base d'une investigation de deux carottes sédimentaires, nous montrons que l'introduction de cette espèce est beaucoup plus ancienne que généralement supposée, et remonte à plusieurs siècles. La répartition actuelle des trois espèces d'*Ammonia* montre que l'expansion spatiale de cette espèce invasive est toujours en progression. Les connaissances obtenues dans cette thèse permettent d'améliorer la compréhension de l'écologie des foraminifères dans les milieux estuariens et apportent ainsi une base pour le développement d'une méthode de bio-indication.

Title: Distribution of foraminiferal communities in estuaries of the French Atlantic coast.

Keywords : estuarine mudflats, foraminifera, biomonitoring, anthropogenic impact, environmental parameters, *Ammonia*.

Abstract: Estuaries are complex habitats, with fluctuating conditions according to tidal cycles and river floods. The organisms inhabiting these systems are exposed to extreme variations of environmental parameters. As a result, estuarine communities are characterised by low diversity and a majority of tolerant species. Estuaries are also subjected to strong anthropogenic pressure that can further increase the stress on organisms. For this reason, ecological status indicators developed in coastal environments systematically show poor ecological quality. In order to develop a reliable bio-indication method, it is first essential to fully understand the natural distribution of organisms. To achieve this, we studied the distribution of foraminiferal communities on intertidal mudflats of nine estuaries. To synthesise the relationships between environmental parameters and the characteristics of foraminiferal communities, we propose three new indices. The MII reflects the balance between marine and fluvial influence for a given point in an estuary at the time of sampling. We observed a succession of four biozones, with specific communities typical of each part of the estuary. We propose two biotic indices (EFI and EFDI) that quantitatively describe the position of the observed community on this succession. The comparison of the MII with the EFI and EFDI should allow to recognise sites with a deviation that may be indicative of a major anthropogenic disturbance. The second part of this thesis focuses on three pseudocryptic species of the genus *Ammonia*, a major taxon in our estuaries. First, we show large differences in biogeographic distribution between these species, which corroborate the invasive character of *Ammonia confertitesta*. Secondly, based on an investigation of two sediment cores, we show that the introduction of this species is much older than generally assumed, and dates back several centuries. The current distribution of the three *Ammonia* species shows that the spatial expansion of this invasive species is still in progress. The knowledge obtained in this thesis improves the understanding of the ecology of foraminifera in estuarine environments and thus provides a basis for the development of a bio-indication method.