

Life history and connectivity between essential ecological habitats of coastal fishes in temperate seas: case of sandeels species (Ammodytidae) and Sea bass (Dicentrarchus labrax, Linnaeus, 1758) in the Normand Breon Gulf, environmental tracers

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THESE

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Flora LAUGIER

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Histoires de vie et connectivité entre les habitats écologiques essentiels de poissons des mers côtières tempérées : le cas des lançons (Ammodytidae) et du bar européen (*Dicentrarchus labrax*, Linnaeus, 1758) dans le Golfe Normand Breton, une approche par les marqueurs environnementaux.

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INTRODUCTION GENERALE

1. Problématique et objectif principal

La migration peut être définie comme étant des mouvements dirigés alternatifs entre différents habitats et est réalisée par des organismes dans le but d'optimiser le succès reproducteur et la survie de l'espèce (i.e. la fitness) (Gross, 1996). Pour certaines espèces, la migration est obligatoire à certains stades de l'histoire de leur vie car leur tolérance physiologique ainsi que leurs besoins énergétiques ne correspondent pas à l'environnement présent (Bradford and Taylor, 1997, Pittman and McAlpine, 2003). Elle est optée, en règle générale, lorsque les bénéfices liés à la croissance et à la survie seront supérieurs aux coûts liés au déplacement (e.g. coût énergétique, mortalité) (Jonsson and Jonsson, 1993, Dahlgren and Eggleston, 2000). Il est possible de distinguer différents types de déplacements allers-retours, des mouvements journaliers aux migrations ontogéniques à l'échelle de la saison ou de l'année, coïncidant à différents stades de vie comme pendant la phase larvaire ou juvénile ou lors de la maturation des gonades. Ces migrations permettent d'optimiser certaines fonctions du cycle de vie (e.g. la croissance, la reproduction) (Pittman and McAlpine, 2003, Charles et al., 2004, Réveillac et al., 2008, Tabouret et al., 2011). Lorsqu'il n'y a pas de retour au point de départ, le terme de « dispersion » est préféré. La distance entre les habitats va également influencer les différents mouvements migratoires et aboutir à différentes stratégies d'utilisation des habitats (Pittman and McAlpine, 2000). Certaines espèces ont des cycles de vie caractérisés par de grandes boucles migratoires s'étalant sur plusieurs centaines voire milliers de kilomètres (e.g. anguille, saumon) et d'autres à des échelles spatiales plus réduites comme certains poissons récifaux mais ces espèces colonisent différents habitats pendant leur cycle de vie (Dahlgren and Eggleston, 2000, Hansen and Quinn, 1998, Aarestrup et al., 2009). D'autres espèces au contraire ne migrent pas et sont résidentes d'un même habitat (Pittman and McAlpine, 2000). Dans ce contexte, les divers habitats interconnectés par les voies

migratoires, ou par lesquels les individus passent lors de leur migration, assurent des fonctions indispensables au maintien des espèces et peuvent être qualifiés d'Habitats Ecologiques Essentiels (HEE), tels que les habitats de pontes (frayère), les habitats de repos (hivernage, estive), les habitats traversés par les individus en migration (corridor), ceux d'alimentation et de croissance, comme par exemple les zones de nourricerie pour les juvéniles (Figure 1) (Rijnsdorp et al., 2009; Petigas et al., 2013). Les habitats ayant des caractéristiques environnementales particulières ou rares et également situés à un endroit spatial stratégique pour le développement et le maintien d'une espèce lors de son cycle de vie peuvent être qualifiés d'HEE.

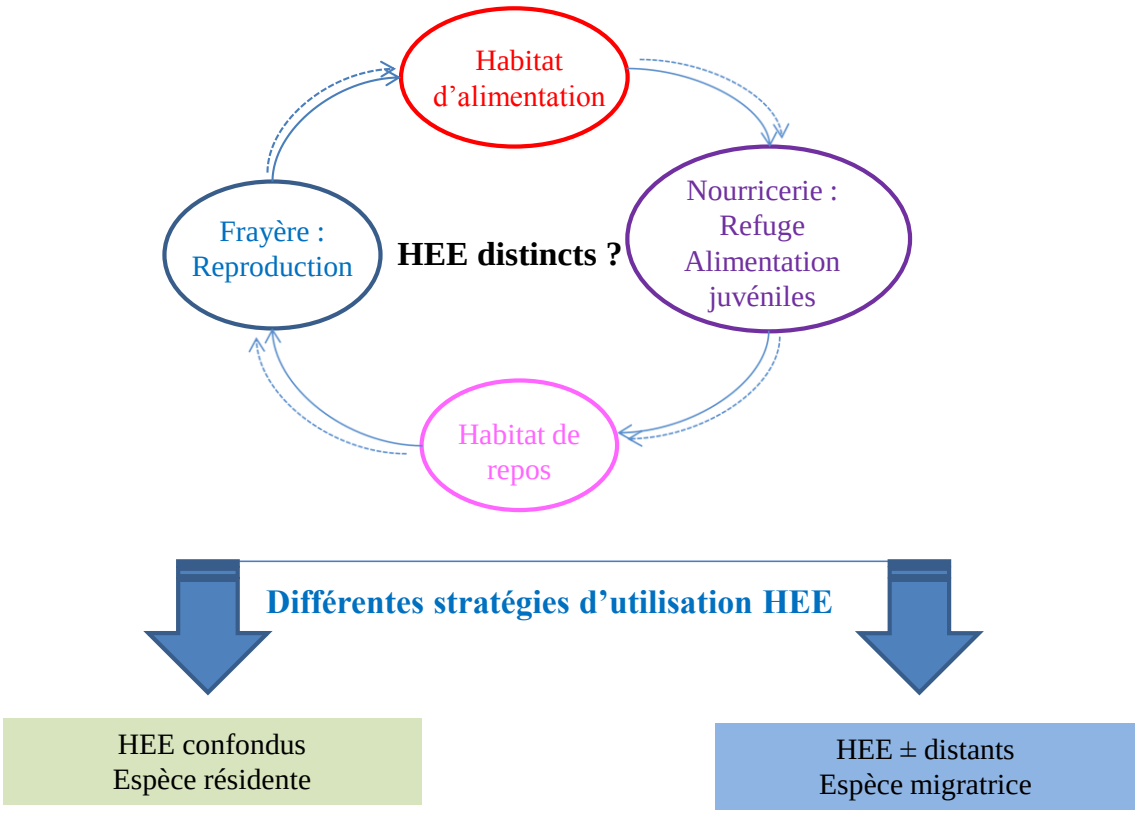


Figure 1 : Les Habitats Ecologiques Essentiels (HEE) peuvent être séparés spatialement et amener les espèces à effectuer des mouvements. Un espèce migratrice utilise différents HEE à l'échelle ontogénique, à l'inverse d'une espèce résidente.

Les différents choix de déplacements sont contrôlés par des facteurs intrinsèques (e.g. génétique) et modulés par l'environnement pour aboutir à différents phénotypes dans les populations, correspondant à une certaine plasticité (Via et Lande, 1985, Gross, 1996, Stearns, 2000). Cette plasticité dite phénotypique se mesure par différentes expressions de traits de vie (Via et Lande, 1985). Cependant selon les études, les traits de vie peuvent être considérés à différents niveaux d'organisation : individuel, populationnel ou communautaire ; et possèdent différentes significations selon les spécialités en biologie.

Dans ce travail de thèse, les traits de vie correspondent à des traits phénologiques, morphologiques ou physiologiques mesurablent à l'échelle individuelle, sans référence à l'environnement ou d'autres niveaux d'organisations (Violle et al., 2007). Les traits de vie seront utilisés et divisés en trois catégories: les traits d'histoire de vie, ceux liés à la morpho-anatomie et les traits biologiques (Figure 2).

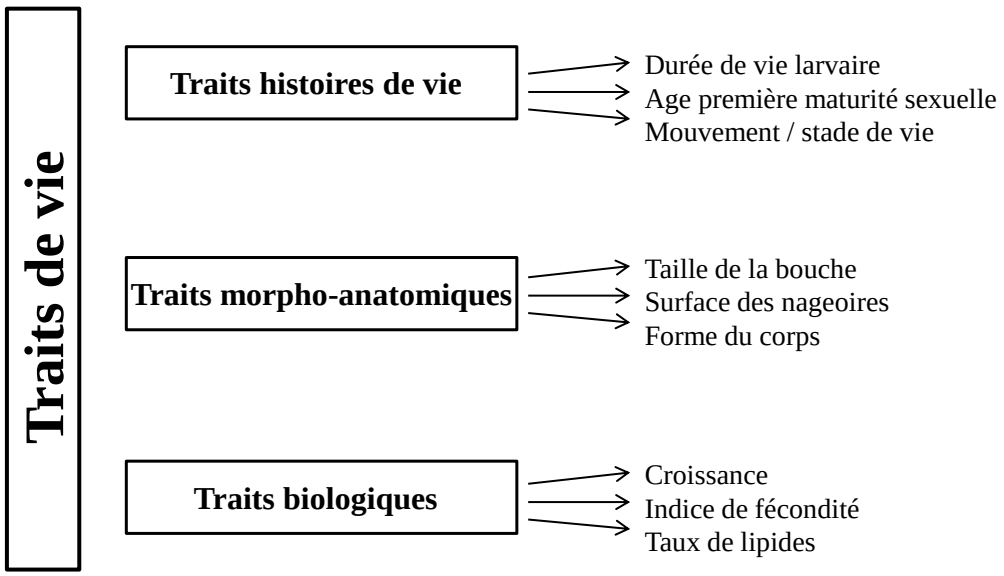


Figure 2 : Les traits de vie englobent l'ensemble des traits d'histoire de vie, morpho-anatomiques et biologiques. Trois exemples de traits de vie mesurés à l'échelle individuelle sont indiqués dans chacune des catégories.

Les traits d'histoire de vie sont à lier à la phénologie, tels que la durée de vie, l'âge à la première reproduction (Violle et al., 2007), c'est à dire un caractère intégrant une dimension temporelle. Dans ce cas, il est possible de considérer que les différents comportements de migration détectés à un stade de vie, par exemple via l'analyse des éléments chimiques dans l'otolithe ou encore la durée de la phase larvaire, sont des traits d'histoire de vie (Réveillac et al., 2008, Bond et al., 2015).

Les traits morpho-anatomiques sont par exemple la position et la forme des nageoires (Violle et al., 2007, McGill et al., 2006, Albouy et al., 2011). Certains traits morpho-anatomiques vont pouvoir renseigner sur des capacités de nage ou d'acquisition de nourriture (Chalcraft and Resetarits, 2003a, 2003b, Villéger et al., 2010).

Les traits biologiques vont être ceux renseignant sur le bon état de condition des individus comme la croissance, l'indice de fécondité, le régime alimentaire (Isnard et al., 2015).

La variabilité des traits de vie va permettre la mise en place de différentes stratégies d'utilisation des habitats entre les espèces. Cette variabilité des traits de vie est due à des tolérances différentes face aux conditions environnementales ou pressions de prédation et s'exprime au sein des communautés par diverses niches réalisées (McGill et al., 2006). En effet, la variabilité inter-espèce des traits morpho-anatomiques reliés à différentes fonctions comme la locomotion, l'acquisition de nourriture ou la reproduction, et également celle des traits d'histoire de vie, tels que le temps passé dans un habitat donné, permet ainsi la coexistence des espèces dans cet habitat et une stabilisation de la communauté (Bridcut and Giller, 1995, Labropouloua and Papadopoulou-Smith, 1999, Laffaille et al., 1999, Charles et al., 2004, Levine and HilleRisLambers, 2009). Par exemple, la coexistence de deux espèces de sole (*Solea solea* et *Solea senegalensis*) est possible dans un même estuaire car leurs préférences d'habitat (salinité, température, profondeur) et alimentaire (amphipodes vs

polychaetes et bivalves, respectivement) diffèrent, leur permettant de cohabiter dans la même zone. A ces traits, s'ajoute une période de reproduction décalée dans le temps permettant une colonisation des estuaires légèrement asynchrone (Cabral and Costa, 1999).

Cependant cette variabilité des traits de vie peut aussi s'exprimer à l'échelle d'une espèce (i.e. variabilité intraspécifique) et montrer l'existence de différentes stratégies d'utilisation des habitats, comme chez certaines espèces de salmonidés où certains individus ont un comportement résident et d'autres effectuent des migrations ontogéniques (Jonsson and Jonsson, 1993, McDowall, 2001, Bond et al., 2015). D'autres traits de vie tels que la croissance ou la forme et la taille de la bouche qui rendent compte du mode d'acquisition des proies, peuvent également varier entre les individus et induire une survie différentielle entre ces individus d'une même population (Jonsson and Jonsson, 1993, Chalcraft and Resetarits, 2003b Isnard et al., 2015).

La combinaison des traits de vie amène donc à rechercher l'existence de diverses stratégies de vie tant aux niveaux intra- qu'inter spécifiques (Rosecchi and Crivelli, 1995, McDowall, 2001, Salgado et al., 2004, Hunsicker et al., 2011, Bond et al., 2015).

Dans ce contexte, l'objectif de ce travail est de mieux comprendre les liens entre les traits de vie (i.e. traits morpho-anatomiques, d'histoires de vie et biologiques) et la connectivité entre les HEE à travers les différentes stratégies d'utilisations des habitats. Différents modèles biologiques ont été utilisés ayant *à priori* des traits de vie et des utilisations des HEE contrastés.

Les premiers modèles biologiques sont des espèces de la famille des Ammodytidae, ayant un cycle de vie court et plutôt considérées comme résidentes et très dépendantes de certains habitats. Certaines espèces de lançons sont susceptibles d'effectuer des mouvements de faible

amplitude entre les habitats pour se reproduire, passer l'hiver ou s'alimenter reflétant une faible connectivité entre les habitats (Engelhard et al., 2008, Jensen et al., 2011). Par ailleurs, plusieurs espèces de lançons coexistent en sympatrie et sont phylogénétiquement et morphologiquement proches. Pour mieux comprendre cette coexistence à l'échelle de la communauté, l'étude de la variabilité des stratégies de vie inter-espèces a été envisagée dans une première partie de la thèse.

Le deuxième modèle biologique utilisé est le bar européen, une espèce migratrice avec des traits de vie comme d'importantes capacités de déplacements, lui permettant de coloniser des HEE plus distants spatialement que ceux des lançons (Pawson et al., 2007, 2008, Quayle et al., 2009). Dans la deuxième partie, il s'agira de mieux comprendre les traits de vie au sein de cette espèce (i.e variation intraspécifique) en lien avec l'utilisation des HEE et plus particulièrement leurs habitats de nourricerie.

Pour répondre à ces questions complexes, nous avons choisi de combiner des méthodes d'analyses, en ayant recours à des marqueurs environnementaux en complémentarité des traits morpho-anatomiques mesurés à l'échelle individuelle.

L'otolithe va permettre de reconstituer l'environnement passé de l'individu, étant une pièce calcifiée, métaboliquement inerte et croissante pendant toute la vie du poisson. Il s'accroit de manière concentrique en alternant des couches de protéines et de carbonate de calcium. Il est composé à 90 % de minéraux et de 0,2 à 10 % de protéines (Hüssy et al., 2004). L'alternance de ces différentes couches se déposent selon différents rythmes journaliers, saisonniers et annuels et marqués sur l'otolithe par différentes macrostructures et microstructures (Panfili et al., 2002). Ces différentes structures sont les témoins d'événements passés du poisson et permettent de reconstituer son histoire de vie. Par exemple la phase larvaire est souvent repérée par un agencement des stries journalières très circulaires puis s'il y a un changement

d'habitat, il se peut qu'une structure secondaire apparaisse, caractérisée par un noyau accessoire et un changement d'axe de croissance (Campana, 1992, Wright, 1933, Panfili et al., 2002). L'otolithe va permettre d'enregistrer des données temporelles mais aussi environnementales à travers les éléments chimiques intégrés au sein de sa structure. Les éléments majeurs (supérieurs à 1000 ppm) sont le Na, Sr, les mineurs sont le K, Mg, Ba (1-100 ppm), et les éléments traces (1-1000 ppm) sont Li, Mn, Cu, Pb (Yoshinaga et al., 2000, Sturgeon et al., 2005). L'otolithe est entouré d'endolymphe mais les ions de l'eau traversent les membranes des branchies et de l'estomac, passent par le sang et arrivent dans l'endolymphe où ils sont incorporés dans l'otolithe, pendant la précipitation du calcium (Wright et al., 1992, Ibsch et al., 2004, Payan et al., 2004, Melancon et al., 2008). La source des éléments majeurs et traces est l'eau environnante, cependant à cause de ces différentes barrières biologiques, l'intégration des éléments n'est pas totale (Milton and Chenery, 2001, Lin et al., 2007, Marohn et al., 2009). De plus dans certains cas, l'alimentation (Sanchez-Jerez et al., 2002, Buckel et al., 2004) et d'autres facteurs externes (Bath et al., 2000, Martin and Thorrold, 2005, Brown and Severin, 2009, Miller, 2009, Woodcock et al., 2012) exercent une influence « mineure » sur l'incorporation de ces éléments dans l'otolithe. L'otolithe est considéré comme un « enregistreur » du milieu environnemental passé au cours de la vie du poisson. Différentes études ont étudié la signature microchimique multi-élémentaire de l'otolithe pour révéler les relations entre différents stocks ou populations, ou encore la connectivité entre les habitats pour différents stades de vie (Gillanders et al., 2003, Gillanders 2005). Les migrations sont étudiées à travers les variations des éléments en fonction de la macrostructure et la microstructure le long d'un axe de croissance de l'otolithe (du centre jusqu'au bord extérieur), renseignant sur les stades de vie et les évènements passés du poisson (e.g stress physico-chimique liés à un changement d'habitat, stress trophique, ou changement de saison, etc.) (Arai and Hirata, 2006, Feutry et al., 2011, Lord et al., 2011).

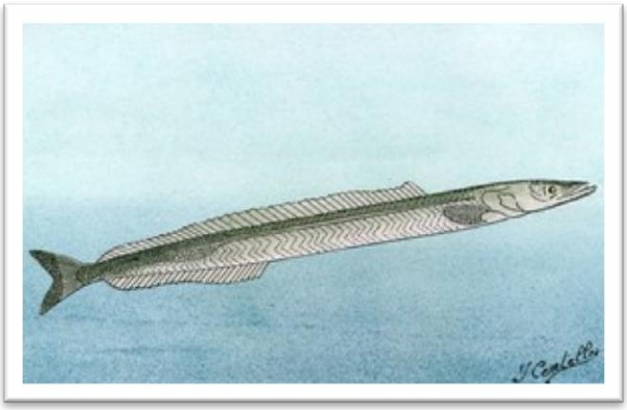
D'autres traceurs naturels tels que les isotopes stables sont utilisés pour étudier les relations trophiques et les sources d'alimentation des individus (Hobson and Welch, 1992). Les variations des rapports isotopiques entre les organismes sont dues à un phénomène dit de fractionnement ou de discrimination isotopique qui se produit lors d'une transformation entre les molécules de différentes masses, constituées d'isotopes différents. Les isotopes lourds (e.g. ^{13}C, ^{15}N) sont accumulés dans les tissus du consommateur pendant la croissance car les isotopes légers (e.g. ^{12}C, ^{14}N) sont eux préférentiellement mobilisés dans les réactions métaboliques (Peterson and Fry, 1987), ce qui en résulte un enrichissement du rapport d'azote ($^{15}N/^{14}N$, noté $\delta^{15}N$) et de carbone ($^{13}C/^{12}C$, noté $\delta^{13}C$) entre une proie et un consommateur (Kelly, 2000). Le fractionnement isotopique est variable selon l'élément. Pour l'azote, une augmentation du rapport $^{15}N/^{14}N$ d'environ 3 à 5 ‰ en moyenne est observée entre une proie et son consommateur (DeNiro and Epstein, 1981, Peterson and Fry, 1987). L'analyse du rapport isotopique $^{15}N/^{14}N$ se révèle donc très utile pour déterminer la position relative d'un organisme au sein d'un réseau trophique. D'autres travaux explicitent que ce rapport pourrait également servir comme un indicateur de la zone d'alimentation (Ménard et al., 2007, Chouvelon et al., 2012). Concernant le carbone, l'abondance naturelle de l'isotope lourd ^{13}C d'un organisme par rapport à sa nourriture augmente peu (0,5 à 1 ‰) (De Niro and Epstein, 1978). Le rapport $^{13}C/^{12}C$ d'un consommateur reflète donc assez bien celui des producteurs primaires du réseau trophique auquel il appartient et peut ainsi renseigner sur l'origine de la source de carbone, de la zone d'alimentation ou de l'habitat (marine *vs* eau douce, benthique *vs* pélagique) (Peterson and Fry 1987, Hobson and Welch, 1992, Hobson et al., 1994).

Grâce à cette approche combinée de plusieurs traceurs naturels nous pouvons aborder suivant plusieurs angles la diversité des traits de vie des espèces à l'échelle interspécifique puis intraspécifique.

2. Les modèles biologiques

2.1. Les Ammodytidae

2.1.1. Ecologie générale des lançons



Encore de nos jours les connaissances acquises sur les Ammodytidae des régions côtières tempérées restent très parcellaires, malgré leur importance pour le fonctionnement des réseaux trophiques marins côtiers (Anderwald et al., 2012, Engelhard et al., 2013) et l'exploitation halieutique dont ils sont l'objet dans certaines régions, tant par la pêcherie commerciale que par la pêche récréative (Augris, 2005, Engelhard et al., 2014). Ces connaissances manquent cruellement pour asseoir les principes de la gestion des habitats et des espèces notamment dans le contexte des activités prenant place dans les milieux côtiers (e.g. extraction de granulats, éolien marin).

Les espèces présentent en Europe

En Europe, il existe six espèces appartenant à trois genres (*Ammodytes, Hyperoplus* et *Gymnammodytes*). L'espèce la plus étudiée est *Ammodytes marinus* et est la plus nordique, présente du Groenland jusqu'à la Manche mais sa limite de répartition géographique sud reste encore mal définie. Sur les côtes normano-bretonnes, siège des travaux de cette thèse, quatre à

cinq espèces peuvent être présentes, *Ammodytes marinus*, *Ammodytes tobianus*, *Hyperoplus lanceolatus*, *Hyperoplus immaculatus*, *Gymnammodytes semisquamatus*.

Leur importance écologique

Ces espèces constituent une source trophique majeure pour 40 espèces d'oiseaux marins, 45 de poissons et 12 de mammifères marins (Robards et al., 2000, MacLeod et al., 2007, Eliasen et al., 2011; Engelhard et al., 2013, 2014, Learmonth et al., 2014). Les lançons se situent à un niveau intermédiaire dans le réseau trophique, étant consommés par les poissons piscivores et s'alimentant sur des organismes planctoniques (larves, copépodes) (Bauchot, 1987). Ainsi, ils représentent une source trophique très importante pour le soutien de certaines populations d'oiseaux marins au printemps et en été, leur abondance dans le milieu conditionnant le succès reproducteur et la survie de leurs prédateurs (Velando and Freire, 1999, Velando et al., 1999, Wanless et al., 2005, Robertson et al., 2014).

Des espèces dépendantes des milieux sableux

D'une manière générale, les différentes espèces de lançons fréquentent de façon presque exclusive les bancs de sable et ainsi représentent pour elles un habitat essentiel. Ils alternent un comportement d'enfouissement dans le sable (au repos la nuit et en hivernage) et une activité de nage et d'alimentation en pleine eau en journée et principalement pendant la période estivale (van Deurs et al., 2011b). Cette activité alternée est rythmée par des cycles courts (nycthéméraux ou/et tidaux), annuels (saisonnier) et à l'échelle ontogénique (Figure 3). Les bancs de sable grossiers (e.g. en zone intertidale ou les dunes hydrauliques) sont conséquemment considérés pour les lançons comme des habitats écologiques essentiels (i.e. HEE) c'est à dire des habitats indispensables au maintien des espèces, de par leur nature de

substrat, assurant les fonctions de reproduction, d'alimentation, de nourricerie et/ou de repos, (Rijnsdorp et al., 2009; Petigas et al., 2013).

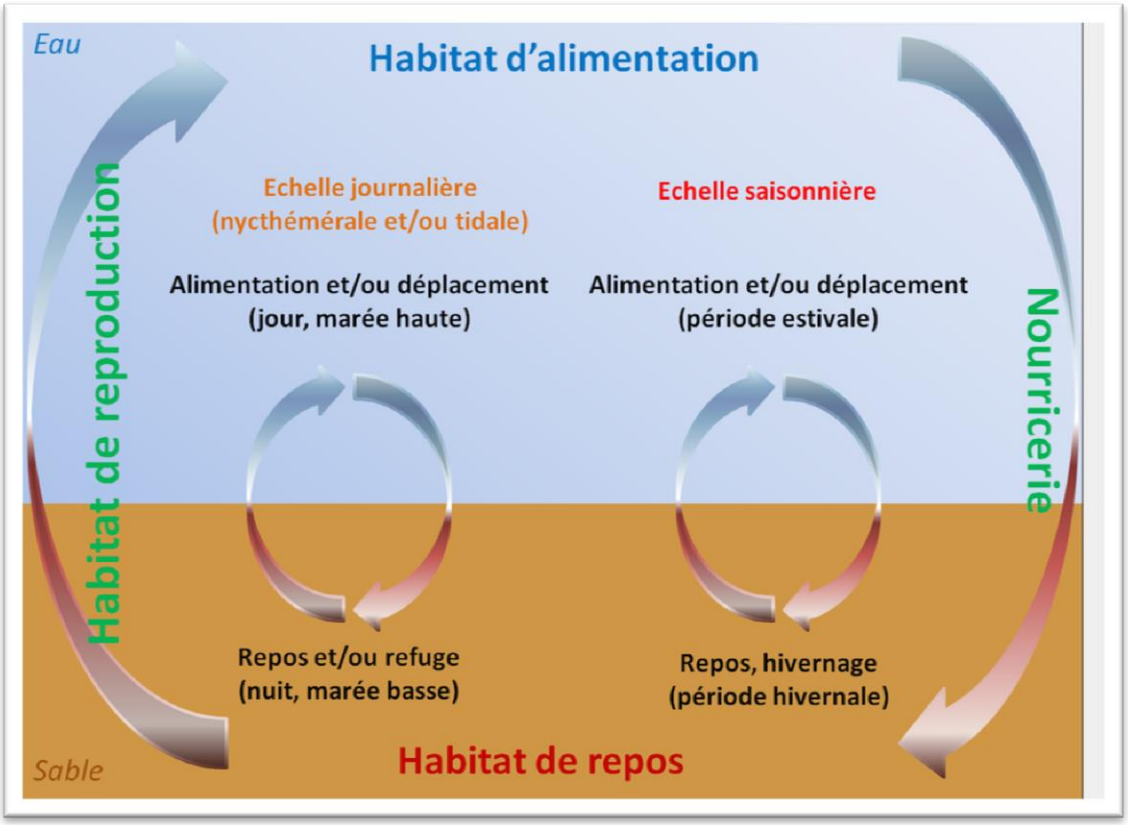


Figure 3 : Alternance de comportements enfoui et en pleine eau aux échelles journalière, saisonnière et ontogénique. L'effet de la marée est subit par les espèces présentes en zone intertidale. Les habitats de reproduction, d'alimentation, de nourricerie et de repos sont les habitats écologiques essentiels à l'échelle de la vie d'un individu.

Les cycles journaliers (Figure 3) présentent une alternance entre un comportement d'enfouissement dans le sable (repos) et une période d'alimentation en groupe en pleine eau comme le montre van Deurs et al. (2011a) en milieu contrôlé pour *Ammodytes tobianus*. L'enfouissement des lançons dans le sable est un moyen d'échapper à la prédation et permet la mise en place d'une stratégie d'économie de l'énergie (van Deurs et al., 2011b). L'activité de recherche alimentaire en pleine eau quant à elle peut être déclenchée par des facteurs internes (réserves énergétiques, vacuité de l'estomac) ou externes (quantités de proies,

lumière, température) (Winslade 1974, a, b, c) ou les deux selon leur degré d'importance (van Deurs et al., 2011a). L'alimentation évolue avec l'âge (Inoue et al., 1967). Les larves se nourrissent de phytoplancton, de diatomées, de dinoflagellés, tandis que les post-larves se focalisent sur les copépodes. Les adultes se nourrissent de proies plus grosses comme des macrocopépodes, larves de poisson, amphipodes, crustacés, annélides polychètes ou d'autres poissons et parmi eux potentiellement d'autres espèces de lançons (Eigaard et al., 2014).

Pendant la période hivernale (i.e. cycle saisonnier, Figure 1) les lançons sont rarement capturés dans la colonne d'eau et restent la plupart du temps enfouis dans le sédiment, c'est une période dite d'hivernage (Robards et al., 2000). Leur métabolisme énergétique est réduit grâce à une consommation d'oxygène ralentie et à leur capacité à tolérer de faibles concentrations en oxygène (Quinn and Schneider, 1991). Pendant le printemps et l'été, contrairement à l'hiver, la croissance est optimale, surtout pendant les deux premières années de vie des individus et va permettre d'accumuler des réserves suffisantes pour passer la période d'hivernage (Bellec, 1981, ICES, 1995, Bergstad et al., 2001, ICES, 2006, van Deurs et al., 2010, 2011b).

Pour le cycle de vie des lançons, les habitats sableux sont donc essentiels (reproduction, installation des post-larves, recrutement des juvéniles) (Figure 3). Les œufs sont démersaux et légèrement adhésifs au sable. Le temps d'incubation des œufs dépend de l'espèce, de la température et de l'oxygène. Les larves d'environ 6 mm (longueur totale) ont approximativement deux semaines après l'éclosion (Wright and Bailey, 1996). Les mâchoires et le tube digestif sont fonctionnels à l'éclosion pour *A. americanus* (Smigielski et al., 1984), contrairement à d'autres espèces comme *A. hexapterus* (Pinto, 1984). Toutes les espèces d'*Ammodytes* ont une vésicule vitelline qui est totalement résorbée au bout de deux semaines (Winslade, 1971, Wright, 1993). Ainsi, les larves de *A. americanus*, *A. personatus* et *A.*

marinus dépendent de leurs réserves vitellines pendant quelques semaines (Inoue et al., 1967) puis effectuent pour se nourrir des déplacements verticaux dans la colonne d'eau, proche de la surface le jour et du fond la nuit. Le recrutement des larves et post-larves annuel est largement variable, dépend des conditions environnementales comme la température, la courantologie et les ressources alimentaires… (Wright and Bailey, 1996). Les post-larves commencent à s'installer dans le sable à une taille de 35 à 55 mm (TL) correspondant à environ 35-40 jours de vie (pour *A. marinus*), amenant à un changement d'habitat de pélagique à semi-dermersal (Wright, 1993). Des études récentes sur *A. marinus* ont montré un comportement de migration limité et un haut degré de fidélité au site d'installation après la phase larvaire. Les zones de pêche correspondent aux zones d'installations des lançons et sont souvent réparties en forme de patch, or les échanges entre les zones de pêche sont faibles à une distance de 5 km tandis qu'à l'intérieur d'une zone, les échanges peuvent atteindre jusqu'à une vingtaine de kilomètres (Jensen et al., 2010). Une autre étude sur *A. marinus* montre que les déplacements de jour peuvent s'étendre à 15 km de leur site d'enfouissement nocturne (Engelhard et al., 2008). En effet, la vitesse de nage moyenne d'*A. tobianus* par exemple serait de 1 à 1.5 km/h (Kühlmannand Karst, 1967) et ne permet pas *à priori* de parcourir de longues distances journalières. Le mélange entre les populations est plutôt supposé être lié à la dérive des larves qui est majoritairement contrôlée par la courantologie et qui amène à des recrutements variables d'un site à l'autre (Reay, 1973, Christensen et al., 2008).

Caractéristique du sédiment de l'habitat de repos des lançons

Lorsqu'ils sont enfouis dans le sable, les lançons respirent dans l'eau interstitielle présente dans le sédiment qu'ils doivent réussir à faire circuler au niveau des branchies. De ce fait, le choix du sédiment est important et plusieurs facteurs interagissent (la concentration en oxygène, le renouvellement de l'eau interstitielle dû aux courants dans la zone, aux

mouvements d'eaux créés par les rides de sable, à la perméabilité du substrat ainsi qu'à la profondeur du banc de sable) (Wright et al., 2000). Ainsi les structures en rides de sable (« ripple marks ») généralement associées à des sables grossiers perméables et localisées dans des zones à forts courants sont des habitats favorables à ces espèces tandis qu'elles sont absentes des sédiments où la composition en argile dépasse les 10% (Wright et al., 2000). *Ammodytes marinus* par exemple occupe préférentiellement les sédiments composés de sable grossier avec des particules de gravier fin à moyen (2 à 16 mm) (Wright et al., 2000).

2.1.2.Bilan des connaissances sur les espèces présentes dans le Golfe Normand Breton

Le genre Ammodytes

Ammodytes tobianus est *a priori* la seule espèce de ce genre présente sur nos côtes. Peu d'informations sont disponibles mais d'avantages de publications existent sur les autres espèces d'*Ammodytes*, notamment sur *Ammodytes marinus*, l'espèce "nordique", très proche morphologiquement.

Les espèces du genre *Ammodytes* sont présentes dans des zones peu profondes, près des côtes, en général à moins de cinquante mètres de profondeur (Macer, 1966). *Ammodytes tobianus*, est l'espèce la plus abondante en domaine intertidal et est très rarement pêchée en zone subtidale (Reay, 1973, Jensen et al., 2004; van Deurs et al., 2011). Elle peut vivre jusqu'à 7 ans et mesure au maximum une vingtaine de centimètres (Reay, 1970). Les lectures d'âge basées sur l'otolithométrie peuvent être plus ou moins valides au vu de l'arrangement des zones opaques et translucides et la difficulté de lecture et de l'interprétation de ces zones (ICES, 1995, 2006).

La maturité sexuelle intervient aux alentours de deux ans (O'Connell and Fives, 1995) mais reste possible au bout d'un an. Pour *A. tobianus*, deux périodes de pontes possibles sont décrites, une au printemps et l'autre à l'automne (Reay, 1973, O'Connel and Fives, 1995, Kopp 1979). Les pontes automnales (e.g. *A. hexapterus*) requièrent environ 3 mois de maturation des gonades (Robards et al., 1999) tandis que celles printanières requièrent 5 à 7 mois de maturation (Reay, 1970). Toutes les espèces d'*Ammodytes* pondent dans le sable soit en intertidal soit plus au large mais toujours dans des eaux peu profondes (Robards et al., 2000). Pour toutes ces espèces également, la ponte semble avoir lieu dans l'habitat qu'elles occupent tout au long de l'année et aucune migration liée à la reproduction n'a été observée (Robards et al., 2000). Pour *A. marinus* trois habitats de ponte ont été identifiés (adultes avec des gonades matures et présence d'œufs) au sud des îles Shetland (au Nord de l'Ecosse), dans des eaux d'une profondeur inférieure à 50 m entre 1 et 2 kilomètres de la côte (Wright and Bailey, 1996). Cette espèce est cependant peu présente en zone intertidale au contraire d'*A. tobianus*.

Les stades larvaires et post-larvaires d'*A. tobianus* semblent se dérouler près de la côte puis, en concordance avec les deux périodes de ponte, deux recrutements ont lieu en zone intertidale, un printanier puis un autre à la fin de l'été ou en début d'automne (Langham, 1971, Reay, 1973). L'abondance du recrutement est variable entre les sites de la Manche (Reay, 1970). A la fin de la saison, les juvéniles du printemps mesurent entre 110 et 114 mm tandis que ceux recrutés en été mesurent de 78 à 85 mm. Pendant le début de l'été, les individus de deuxième année sont les plus abondants, puis ce sont les juvéniles qui dominent la population lorsqu'ils sont recrutés dans les populations côtières. Les individus les plus âgés semblent moins abondants au début de l'été ce qui permet de limiter la compétition trophique avec les

plus jeunes. Ensuite les individus plus âgés et matures sont de nouveau plus abondants à l'automne (Reay, 1973).

***Ammodytes tobianus*, est l'espèce d'Ammodytidae la plus abondante dans les habitats sableux intertidaux. Elle peut atteindre sa maturité sexuelle à un an et deux périodes de reproduction ont lieu (i.e. au printemps et à l'automne) pendant l'année. *A priori* cette espèce semble plutôt résidente et fidèle à son habitat de recrutement et est rarement capturée loin de la côte.**

Le genre Hyperoplus

Pour le genre *Hyperoplus*, les connaissances sont encore plus lacunaires, seules quelques études anciennes sur les distributions de fréquence de taille sont disponibles en Bretagne et aucune n'a été retrouvé en Europe.

Le lançon Jolivet ***Hyperoplus immaculatus*** est présent en Atlantique Nord, des Iles Britanniques au Sud Bretagne (*données non publiées*). Les individus inférieurs à 15 centimètres se nourrissent de zooplancton puis leur régime alimentaire peut évoluer vers l'ichthyophagie, favorisant des espèces de clupéidés ou d'ammodytidés. Leur taille maximale est d'environ 30 centimètres (Rankine and Morrison, 1989). Une seule ponte annuelle a lieu de décembre à février (Reay et al., 1986). Bellec en 1980-1981 étudie les communautés de lançons d'octobre à mars dans la Baie de Morlaix (Dune du Rater) dans les méga-rides qui se situent à environ 7-8 km de la côté la plus proche. Au vu des critères morphologiques énoncés, une des espèces décrites correspond à *Hyperoplus immaculatus*, ce qui est intéressant puisque c'est la seule étude décrivant les différentes cohortes et pour laquelle il met en évidence l'apparition de trois cohortes d'octobre à mars. Le groupe des individus âgés au maximum d'un an (groupe 0^{+}, tailles comprises entre 4 cm et 10 cm), ceux âgés entre deux

à trois ans (groupe 2^+, tailles entre 17.75 cm et 23.75 cm), les plus âgés (entre trois et cinq ans, tailles entre 24.5 et 29,5 cm). Les juvéniles de l'année (groupe 0^+) apparaissent en novembre puis en février. Leur apparition à ces deux périodes de l'année indique peut être que la période de reproduction de l'espèce n'est pas encore bien éclaircie. Le groupe 2^+ domine et reste constant pendant toute la période de l'étude. La stabilité de la taille moyenne de cette cohorte démontre une croissance quasiment nulle pendant la période hivernale (octobre à mars). Les individus les plus âgés apparaissent seulement aux mois de janvier et février. Les rapports gonado-somatiques atteignent les valeurs les plus fortes en janvier avec 16,5 % pour les femelles et 13,8 % pour les mâles et sont les plus élevés pour les individus du groupe 2^+. L'âge à la première maturité sexuelle semble donc être de deux ans. L'auteur situe également la ponte de janvier à mars.

Le cycle de vie d' *H. immaculatus* n'est pas encore très bien établi et la stratégie d'utilisation des HEE est encore mal identifiée entre les bancs de sable subtidaux et ceux intertidaux. La reproduction n'a pas été confirmée en zone subtidale, même si les d'individus âgés de deux ans voient leurs gonades devenir mâtures dans cette étude (Bellec, 1981). Par ailleurs la présence des juvéniles à deux périodes laisse encore un doute sur la période de reproduction. La faible présence d'individus plus âgés s'explique soit par un changement d'habitat, soit par une forte mortalité pendant l'hiver.

Hyperoplus lanceolatus occupe différents bancs en zone côtière ou plus au large, de la Norvège au Nord du Portugal. Cette espèce peut atteindre des tailles élevées, jusqu'à 40 centimètres et peut être piscivore (Kellnreitner et al., 2012). De manière générale, elle est moins abondante dans les communautés de lançon : en Baie de Morlaix (Le Rater) elle représente 22 % des captures (Bellec, 1981), sur la côte Est du Cotentin l'abondance est

inférieure à 10% des captures (Kopp, 1979) et en Baie de Saint Malo elle représente 2 % de la capture (*données personnelles*, 2012). Cependant, à la lecture des otolithes, sur les bancs de sable de la Baie de Morlaix, les individus appartenant au groupe 0^+ et 1^+ semblent absents et la classe d'âge la plus présente semble être celle des individus âgés de 2 ans (Bellec, 1981). La présence faible des individus les plus âgés souligne une mortalité estimée à 80% au cours de la troisième année de vie pour cette espèce (Macer, 1966). La lecture des otolithes indique une croissance forte pendant les deux premières années de vie, malgré une croissance très restreinte l'hiver. Les rapports gonado-somatiques ne cessent d'augmenter d'octobre à mars mais le suivi se finissant à ce mois, l'évolution n'a pu être suivie à terme (Bellec, 1981). La maturité sexuelle pour cette espèce est d'environ deux ans et la période de reproduction semble établie pendant l'été sur une période plus ou moins étalées (entre avril et août) en fonction d'un gradient latitudinal (Le Danois, 1913, Macer, 1966, Bellec, 1981, van Deurs et al., 2012).

H. lanceolatus **est une espèce moins abondante dont il est plus difficile de suivre l'évolution des cohortes dans le temps. Les juvéniles ne semblent pas présents dans les sites subtidaux (Bellec, 1981, *données personnelles*). Mais au regard des faibles présences de l'espèce dans les captures (Bellec, 1981), son histoire de vie n'a pas encore été bien démêlée.**

Le genre Gymnammodytes

Gymnammodytes semisquamatus, *a priori* est la seule espèce du genre présente sur nos côtes et est présente du sud de la Norvège à l'Espagne, généralement au large dans des bancs de sable coquillier (Corbin, 1950). Cette espèce semble s'alimenter sur le plancton et mesure en moyenne une quinzaine de centimètres. La ponte se déroulerait l'été (Reay et al., 1986).

Dans le rapport de Kopp (1979), cette espèce est la plus abondante (*e.g.* un tiers des captures annuelles) des espèces de lançon pêchées sur certains bancs de sable de la côte Est du Cotentin qui sont situés à environ 5 km de la côte. L'espèce semble donc inféodée aux bancs de sable subtidaux et n'est jamais capturée sur les bancs plus proches de la côte ou en zone intertidale. Jusqu'au mois d'août, la classe d'âge la plus abondante est représentée par les individus âgés de deux ans, puis ces individus disparaissent en septembre pour réapparaitre au début de la saison suivante. Les juvéniles de 1 an apparaissent à la fin de l'été (environ 100 mm). Les individus âgés de 3 et 4 ans sont présents d'avril à juin. Il semblerait qu'après la reproduction les individus quittent le banc, d'après l'observation de leur disparition après l'été ou que la mortalité soit élevée. Au vu des rapports gonado-somatiques, la maturité sexuelle intervient à deux ans et la reproduction semble avoir lieu pendant la période estivale (Kopp, 1979).

***G. semisquamatus* semble inféodée aux bancs de sables en zones subtidales pour tous les stades de vie. Cependant des mouvements semblent exister entre les bancs puisque certaines cohortes ne sont présentes qu'à une certaine période de l'année (Kopp, 1979). Comme pour les deux espèces d'*Hyperoplus*, son histoire de vie n'est pas encore clairement résolue.**

Traits de vie des quatre espèces de lançon étudiées



H. lanceolatus
- *TM: 40 cm*
- *R: été*
- *APR: 2 ans*
- *H: subtidaux mais apparition en zone intertidale*



H. immaculatus
- *TM: 30 cm*
- *R: hiver*
- *APR: 2 ans*
- *H: subtidaux mais apparition en zone intertidale*



G. semisquamatus
- *TM: 20 cm*
- *R: été*
- *APR: 2 ans*
- *H: subtidaux*



A. tobianus
- *TM: 20 cm*
- *R: été et automne*
- *APR: 1an*
- *H: intertidaux*

Figure 4 : Les principaux traits de vie des 4 espèces de lançon. TM : taille maximale, R: période de reproduction, APR: âge à la première reproduction, H: habitats sableux subtidaux ou intertidaux. Photographies réalisées pour les mesures morpho-anatomiques.

2.1.3. Les pressions sur les communautés de lançon

Peu d'études ont mesuré l'effet de l'extraction de granulat sur les communautés de lançon. De Groot (1979) montre qu'après l'extraction, la topographie (bathymétrie et granulométrie) du site est modifiée et que les communautés benthiques voient leur biomasse, leur densité et leur diversité spécifique diminuer. Cependant, les communautés benthiques peuvent revenir s'installer au bout de quelques années (trois années) dans le cas d'une exploitation ponctuelle (De Groot, 1979). Mais si l'exploitation est régulière les communautés benthiques ne reviendront jamais à leur stade d'origine. Les œufs de lançon sont déposés sur le sable et y adhèrent. Groot (1979) précise que lorsque les œufs sont recouverts totalement de fines particules le développement embryonnaire peut être stoppé. De ce fait le dragage peut

provoquer une diminution du succès de l'éclosion. Le mécanisme de sélection de la zone de ponte est peu connu pour les espèces d'Ammodytidae. L'enfouissement de ces espèces dans le sable, les rend vulnérables à cette exploitation et l'auteur préconise l'interdiction de l'exploitation totale d'une même zone (De Groot, 1979). Récemment, des modèles écosystémiques (Ecopath et EcoTroph) montrent que les extractions de granulats entraineraient une réaction en chaine tout le long du réseau trophique, allant jusqu'à modifier les biomasses des groupes n'étant pas directement impactés (Briffault, 2011).

Dans certains pays de l'Europe du Nord (e.g. Norvège, Danemark), les lançons sont économiquement importants et leur stocks sont fluctuants et ont décliné depuis quelques années, du fait de la surpêche mais également du changement climatique qui modifie les réseaux marins (Wanless et al., 2004; Frederiksen et al., 2007, 2011).

Par ailleurs, des études sur l'implantation de champs d'éoliennes off-shores ont également été menées pour mesurer l'impact de ces constructions sur les communautés de lançon et montrent qu'après sept années de suivis aucun changement significatif n'a été observé (van Deurs et al., 2012, Stenberg et al., 2015).

2.2. Le bar européen (*Dicentrarchus labrax*, Linnaeus, 1758)



Le bar européen, est présent dans la famille des Moronidae et deux espèces sont présentes dans le genre *Dicentrarchus*. *Dicentrarchus labrax* est présent dans l'Atlantique Nord-Est, du Maroc au Sud de la Norvège et est une espèce commune de nos côtes très recherchée par les pêcheurs professionnels ou amateurs. Il est décrit comme une espèce migratrice pouvant parcourir une centaine de kilomètres selon des études récentes de marquages acoustiques et tags archives (Pawson et al., 2007, 2008, Quayle et al., 2009). Probablement due à leur grande capacité de déplacement, seulement une légère différenciation génétique a été démontrée entre les individus capturés dans différents bassins maritimes (Irlande vs Golfe de Gascogne, Manche et Ecosse) (Fritsch et al., 2007). Une autre différenciation génétique sépare les populations de bar entre la Méditerranée et l'Atlantique via Gibraltar et deux populations sont également distinguées en Méditerranée (Occidentale vs Orientale) (Tine et al., 2014).

Cette espèce euryhaline est également très dépendante des estuaires (Elliot et al., 2007) avec certains individus plus tolérants à la dessalure puisque retrouvés dans les estuaires ou les lagunes (Guinand et al., 2014, 2015).

Les mâles seraient matures à environ 4 ans (35 cm approximativement) et les femelles vers 6 ans (42 cm environ) (Pickett and Pawson, 1994). Les adultes en cours de maturation se regroupent en milieu hauturier en hiver pour se reproduire (Figure 5) (Fritsch, et al., 2007, Pawson et al., 2007). La reproduction a lieu de janvier à avril et suivant la latitude, ils fraient plusieurs fois provoquant ainsi un décalage spatial et temporel dans les pontes (Fritsch, 2005, Vinagre et al., 2009). Le déclenchement de la saison de ponte plus avancé aux latitudes plus basses, est contrôlé par la température et la photopériode (Vinagre et al., 2009). Ces phénomènes ont été observés à des échelles locales comme au Portugal (Vinagre et al., 2009), en Irlande (Kennedy and Fitzmaurice, 1972), en France (Bretagne) (Chevalier, 1980) et en Espagne (Arias, 1980).

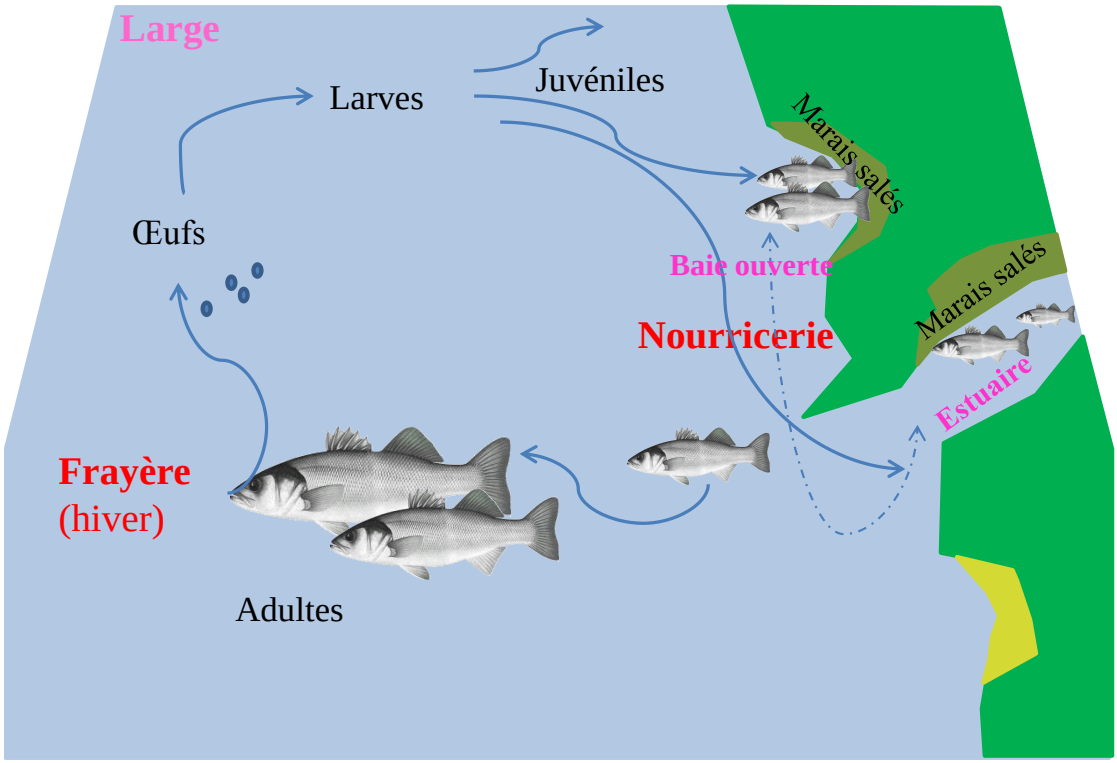


Figure 5 : Schéma théorique du cycle de vie du bar européen, *Dicentrarchus labrax*. Migrations ontogéniques entre les Habitats Ecologiques Essentiels.

La phase embryonnaire dure environ 6 jours (Ré et al., 1986) et les larves sont dispersées passivement par les courants vers la côte en moyenne pendant 20 jours correspondant à la période entre l'éclosion et la résorption de la vésicule vitelline (*i.e.* coïncidant aux premières prises alimentaires suite à l'ouverture de la bouche) (Jennings and Pawson, 1991, Parlier, 2006). La distance variable entre les zones de naissance et de nourricerie peut impliquer une croissance et une survie différentielle des larves. Les post-larves d'environ 15 à 20 mm (longueur totale) sont recrutées environ 90 jours après la ponte (vers le printemps ou le début de la saison estivale selon la latitude) dans différents habitats propices à leur croissance: les zones de nourricerie (Figure 5) (Jennings et al., 1992, Reynolds et al., 2003). L'accroissement journalier dans les otolithes de bar permet de reconstruire de manière précise leur histoire de vie (Regner and dulcic, 1994, Aguilera et al., 2009).

Les habitats de nourricerie sont des zones reconnues pour leur forte abondance en nourriture favorisant la croissance des juvéniles, ayant un rôle de protection contre les prédateurs car souvent de faible profondeur et permettent ainsi une bonne contribution au recrutement adulte, rapportée à la surface de l'habitat (Paterson and Whitfield, 2000, Beck et al., 2001). Cependant, si l'on se borne à ces conditions pour définir le statut de nourricerie d'un habitat donné, les grandes zones contribuant significativement aux stocks adultes ne sont pas considérées comme telles (Dahlgren et al., 2006). Dahlgren et al. (2006) suggèrent alors le terme d'Habitat Effectif pour les Juvéniles (HEJ) intégrant ainsi les habitats pour lesquels la contribution des juvéniles au stock adulte est supérieure à la contribution moyenne totale, c'est à dire calculée sur tous les habitats utilisés par les juvéniles. La contribution des juvéniles au stock dépend de la survie larvaire pendant la phase de migration et également de la survie et de la croissance des juvéniles dans les nourriceries (Beck et al., 2001).

Les marais salés, traversés par des criches (i.e. petits chenaux immergés entre les herbus), sont des zones propices au développement et à la croissance des juvéniles de bar (Mathieson et al., 2000, Laffaille et al., 2001, Lefeuvre et al., 2003, Cattrijsse and Hampel, 2006, Parlier, 2006). Ce sont des habitats à l'interface entre les domaines terrestres et marins et comptant parmi les systèmes les plus productifs de la planète (Lefeuvre et al., 2003). Mais ce sont également des zones présentant de fortes contraintes environnementales provoquées par une forte variabilité de la salinité et de la température. Par ailleurs ce sont des systèmes soumis au cycle des marées, n'étant par conséquent accessibles pour le necton qu'à marée haute, limitant leur exploitation (Cattrijsse and Hampel, 2006). Lorsque ces habitats ne sont pas accessibles ou disponibles, d'autres habitats doivent être utilisés comme les estuaires (Vasconcelos et al., 2007, 2010) ou tout autre habitat subtidal non soumis à l'influence des eaux douces. Bravo (2006) a montré au moyen d'analyses microchimiques réalisées sur des bars adultes capturés

sur des zones de frayère, que certains bars adultes ne passaient pas obligatoirement par les eaux saumâtres lors de leur stade juvénile; ainsi d'autres habitats de substitution non côtiers pourraient également jouer le rôle de nourricerie. Une autre étude basée sur les profils microchimiques d'otolithes (Ba/Ca) de juvéniles d'une espèce similaire, *Morone saxatilis*, montre un comportement soit résident, soit transitoire, dans des nourriceries en Caroline (USA) (Mohan et al., 2015). Dans le même esprit, deux études ont mis en évidence récemment une forte fidélité des juvéniles de bar à une partie bien définie d'un estuaire, indiquant une connectivité faible au sein de l'estuaire lui-même et entre l'estuaire et les marais salés adjacents (Green et al., 2012, Reis-Santos et al., 2015). Ces différentes études indiquent une forte variabilité dans l'utilisation des habitats côtiers et par conséquent une variabilité des histoires de vie au sein d'une même population de bar.

Par ailleurs, la qualité des marais salés en tant que zone de nourricerie, via les modifications de la composition et la structure de la végétation liées à l'invasion de certaines espèces végétales ou au pâturage, va modifier l'abondance et l'accessibilité des proies préférentielles (*e.g. Orchestia gamarella*) et impacter le régime alimentaire des juvéniles de bar (Laffaille et al., 2000, 2005). Cependant, les juvéniles de bar semblent pouvoir ajuster leur régime alimentaire et montrer un comportement allant d'opportuniste à spécialiste (Pickett and Pawson, 1994, Laffaille et al., 2000, Cardoso et al., 2015, Selleslagh and Amara, 2015). La recherche de nourriture est très importante pour les juvéniles et certains individus seraient plus tolérants aux périodes de jeûne et épuiseraient moins rapidement leurs réserves lipidiques (McKenzie et al., 2014). Cette différence de tolérance implique potentiellement différents comportements pour la recherche alimentaire et des vitesses de croissance différentielles.

Le bar européen (*Dicentrarchus labrax*) est une espèce migratrice euryhaline qui semble présenter une variabilité de comportements en termes d'utilisation des habitats

côtiers ou d'alimentation. Cependant les connaissances précises sur les différentes histoires et traits de vie au stade juvénile et leur niveau de dépendance à certains habitats ne sont pas encore clairs.

3. Objectifs scientifiques et organisation de la thèse

L'objectif principal de cette thèse est de mieux déterminer l'histoire de vie de différentes espèces de lançons *a priori* résidentes, très dépendantes des habitats sableux et à espérance de vie courte, puis d'une espèce migratrice et longévive, le bar européen.

Quatre espèces de lançons vivent en sympatrie sur nos côtes bretonnes. De fait, la littérature nous apprend que *A. tobianus* est plus résidente des bancs de sables intertidaux, que *G. semisquamatus* est considérée comme une espèce résidente du large, tandis que *H. lanceolatus* et *H. immaculatus* pourraient montrer des mouvements entre des bancs de sable de zones intertidales et subtidales. Si des études sur les mouvements ont été conduites sur les populations d'*A. marinus*, pour les autres espèces, les mélanges entre les populations et leurs histoires de vie restent très méconnus. L'utilisation conjointe des traceurs naturels tels que la composition élémentaire de l'otolithe et les isotopes stables sur ces espèces de petite taille peut s'avérer particulièrement pertinente.

La première partie de ce travail de thèse (**Partie 1- Article 1**) vise à mettre au point une méthode d'analyse permettant de reconstituer l'histoire de vie d'une espèce de petite taille à courte espérance de vie. Pour cela, nous avons recours à l'analyse de la microchimie de l'otolithe. La question à laquelle cette première partie devra répondre est : **la microchimie de l'otolithe permet-elle d'apporter des informations pertinentes sur l'histoire de vie individuelle d'*A. tobianus*?**

Si cette espèce montre des mouvements limités, nous supposons alors 1) que le bord externe de l'otolithe peut avoir une composition en éléments traces, distincte en fonction des sites de captures (où nous supposons que les individus ont séjourné suffisamment longtemps pour s'imprégner des éléments chimiques de leur environnement et pour peu que ces derniers soient discriminants d'autres habitats) et 2) que la signature microchimique ne varie pas du bord marginal au centre de l'otolithe autrement dit, tout au long de la vie des individus, synonyme supposé de sa sédentarité. Ces résultats seront confrontés à l'ontogénèse des individus retracée à partir des croissances déduites de l'analyse des distributions de tailles échantillonnées de façon mensuelle pendant une année et de la relation longueur totale de l'otolithe/longueur d'individus. Des vérifications méthodologiques seront apportées concernant de potentiels effets saisonniers et ontogéniques sur la stabilité de la microchimie de l'otolithe. Cette stabilité sera testée sur les signatures du site de capture, en comparant des individus capturés à différentes saisons et différents stades de vie (i.e. juvénile et adulte). Nous testerons à l'aide de différents modèles (Linear discriminant Analysis (LDA), Random Forest (RF), Artificial Neural Networks (ANN)) si nous pouvons distinguer des signatures de sites de captures sur des individus pêchés dans 3 sites intertidaux. Finalement ces démarches nous permettront de valider la pertinence de la microchimie de l'otolithe comme traceur potentiel de l'histoire de vie des espèces de lançon et de valider la fidélité d'*A. tobianus* en zone intertidale.

Le chapitre suivant de la thèse (**Partie 2- Article 2**), vise à analyser la partition de niche entre trois espèces sympatriques de lançon capturées en zone subtidale. Bien que ces espèces aient de fortes similarités morphologiques, elles présentent tout de même quelques différences de traits de vie (cf Figure 4, 2.1.2. Bilan des connaissances sur les espèces présentes dans le Golfe Normand Breton). Ces différences mineures pourraient révéler des régimes alimentaires

et/ou des capacités de déplacements ou de dispersions différentes et, par conséquent, différentes histoires de vie et utilisation des HEE. Les questions posées sont : **est-ce que les différentes espèces ont le même niveau de dépendance aux habitats subtidaux, autrement dit les individus sont-ils résidents permanents ou bien fréquentent-ils différents habitats écologiques essentiels au cours de leur cycle biologique ?**

Pour répondre à ces questions, nous avons capturé différentes espèces dans plusieurs sites du nord de la Bretagne et nous avons combiné différentes approches complémentaires : l'analyse des cohortes échantillonnées à différentes périodes de l'année nous permet d'évaluer la croissance des individus, l'otolithométrie permet d'identifier des phases de vie, l'âge et la taille aux différents stades, la composition élémentaire des otolithes dans les différentes zones macrostructurales décrites nous permet de détecter des changements d'habitats. L'analyse de la composition en isotopes stables du carbone et de l'azote nous permet de caractériser les relations trophiques entre les espèces, tandis que l'analyse des traits morpho-anatomiques nous informe sur les capacités de nage et alimentaire de différents individus.

Après avoir étudié la variabilité des histoires et l'utilisation des HEE entre les espèces de lançon à une échelle des communautés, les mêmes méthodologies seront appliquées pour approfondir les histoires de vie intra-espèce au sein du bar européen et plus particulièrement pour mieux comprendre l'utilisation des HEE de nourricerie des juvéniles.

La partie suivante de la thèse **(Partie 2)** permettra d'analyser la variabilité des traits de vie de jeunes bars européens capturés sur différentes nourriceries de la Manche Occidentale**.** En effet, les juvéniles de bar fréquentent les estuaires et les marais salés, malgré le coût physiologique nécessaire pour effectuer les déplacements pendant le flot et surtout faire face à la variabilité de la salinité. Le bénéfice est surtout assuré par l'abondance des proies benthiques et pélagiques concentrées dans les chenaux des marais salés (i.e. criches) et

l'absence de prédateurs. Or les caractéristiques environnementales varient d'un marais salé à l'autre en raison de leur accessibilité, leur surface respective, des habitats adjacents (vasières, zones sableuses, herbiers…) et des apports d'eau douce fluviale, ce qui provoque une variabilité de l'abondance, l'accessibilité, la diversité et la composition relative des communautés de proies, pouvant conditionner les performances de ces habitats en tant que nourricerie pour les bars. Les questions soulevées sont: **existe-il une variabilité des histoires de vie et traits de vie au sein des juvéniles de bar et ont-ils le même niveau de dépendance aux marais salés?**

Dans le premier chapitre (**Partie 2 - Article 3**), les signatures microchimiques des otolithes de juvéniles de bar capturés dans 5 marais salés du Golfe Normand Breton ont été analysées afin de retracer l'histoire migratoire de leurs phases larvaires marines puis littorales. Comme pour les lançons, sur chaque otolithe, les compositions élémentaires ont été analysées sur le bord externe, correspondant à la zone la plus récemment déposée et donc supposée caractéristique du site de capture. En outre, la fidélité aux différents habitats de nourricerie sera estimée à partir de l'analyse des signatures élémentaires des otolithes entre le recrutement sur la nourricerie et la capture. Les performances de croissance seront discutées au regard des histoires de vie et des caractéristiques des habitats.

Dans un deuxième chapitre (**Partie 2 - Article 4**), nous explorerons les réponses comportementales alimentaires des juvéniles dans ces habitats de nourriceries côtières avec les isotopes stables du C et du N et par les traits morpho-anatomiques liés à l'alimentation (telle que la forme de la bouche) et la motricité (capacités de nage révélées par les traits locomoteurs telles que la forme du corps et des nageoires), ainsi que par des performances révélées par les traits biologiques (croissance, indices de condition, etc.). La variabilité des comportements alimentaires sera aussi discutée à travers la qualité des habitats.

Une **discussion finale** permettra de faire le point sur les résultats de la thèse et notamment les avancées réalisées sur les traits de vie explorés pour ces 4 espèces de lançon présentes en communauté et l'utilisation des habitats à l'échelle ontogénique. Les avancées méthodologiques et leurs limites seront discutées pour évaluer les manques et identifier les perspectives d'étude pour mieux comprendre la connectivité entre les habitats et les populations de lançon à une échelle régionale. Nous discuterons également de la variabilité des traits de vie observée chez les juvéniles de bar et de l'importance de la qualité des habitats de nourricerie pour leur croissance. Nous évoquerons les limites de ces études et les perspectives futures pour faire le lien entre les nourricerie et le recrutement au stock adulte. Enfin, ces différents travaux nous permettrons de mettre en relation les capacités de déplacement et l'utilisation des HEE à travers la variabilité des traits de vie observée chez ces espèces à cycles biologiques contrastés et de mieux comprendre la connectivité entre les HEE et la structure de ces populations.

PARTIE 1:
LE CAS DE LA COMMUNAUTE DE LANÇON



Zone intertidale Lancieux

I. CONTEXTE DE L'ETUDE

Les études sur la connectivité entre les habitats et les migrations des espèces sont surtout centrées sur les espèces de grandes tailles effectuant de grandes migrations entre des habitats distants et contrastés (marins, eaux douces ou saumâtres) (Block et al., 2001, Milton and Chenery, 2003, Daverat et al., 2011, Bultel et al., 2014). Peu d'études se focalisent sur les espèces de petites tailles et à cycle de vie court à cause de la difficulté des méthodes de marquages et de suivies (Keith, 2003, Aldanondo et al., 2010).

Les Ammodytidae sont des espèces côtières de petites tailles et malgré leur importance écologique pour les réseaux marins, peu d'études se sont penchées sur la connectivité entre les habitats écologiques essentiels de ces espèces (Frederiksen et al., 2007, Engelhard et al., 2014).

Le premier chapitre de cette thèse vise à valider l'utilisation de la microchimie de l'otolithe comme outil pour comprendre l'histoire de vie des Ammodytidae, à travers l'exemple d'une des espèces présentes sur les côtes bretonnes. *Ammodytes tobianus* est *à priori* une espèce résidente et ayant une forte fidélité aux habitats sableux intertidaux (Reay, 1973, Jensen et al., 2004; van Deurs et al., 2011)

Puis le second chapitre tend à démêler les histoires de vie de 3 espèces de lançon très proches morphologiquement (Ida et al, 1994) et vivant en sympatrie sur une dune hydraulique. L'utilisation combinée de différents traceurs naturels tels que la microchimie des otolithes et les isotopes stables ainsi que les traits morpho-anatomiques sont utilisés pour mieux comprendre la cohabitation et l'utilisation des habitats écologiques essentiels au cours de leur cycle de vie.

II. ARTICLE 1 : LIFE HISTORY OF THE SMALL SANDEEL, *A. TOBIANUS*, INFERED FROM OTOLITH MICROCHEMISTRY. A METHODOLOGICAL APPROACH.

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Abstract

Knowledge of life history and connectivity between essential ecological habitats are relevant for conservation and management of species and some natural tracers could match to study the lifecycles of small or short-lived marine fishes. Although sandeels are central in marine food webs and key species, knowledge about population mixing and migration patterns is not completely unravelled. For the first time the use of the otolith microchemistry on sandeel species is evaluated through the case of the Small Sandeel. Variations in microchemical fingerprints of thirteen trace elements are performed with a Femtosecond LA-ICPM from the core to the margin of sagittal otolith and are compared within and between otoliths extracted from 34 fishes sampled in three different sites along the coast of the south-western English Channel in France. Firstly, preliminary investigations on the validity of the method revealed that Mg/Ca was the only ratio significantly dependant on fish ontogeny and sampling season. Secondly, the Mn/Ca, Zn/Ca, and Cu/Ca ratios enabled to significantly discriminate among sampling sites. Thirdly, microchemical fingerprints of each life stage varied significantly among sampling sites but not within them, suggesting high site fidelity over relatively short distances. Finally, the fingerprints of all life stages were significantly different from those of the larval and metamorphosis stages. The otolith microchemistry could detect change of signature relative to the shift from a pelagic behaviour to a resident bentho-pelagic behaviour during the middle of the juvenile stage in Small Sandeels. Hence, analysis of trace element fingerprints in otoliths appears to be a useful method to further studies on ontogenic habitat change, population mixing and variation of life history and be helpful for the management at local or regional scales of short-lived species such as those belonging to other Ammodytidae.

Keywords: Ammodytidae, short lifespan, multi-element signature, habitat use, intertidal beaches, biological tracer

1. Introduction

Identification of connectivity between essential habitats during the lifespan of species is key to the population ecology and management of fish. This has been studied in numerous marine fishes that move from estuaries or salt marsh systems during their juvenile stage to offshore habitats for growth and/or spawning (Hansen and Quinn, 1998; Fritsch, 2005; Aarestrup et al., 2009, Daverat et al., 2011; Mercier et al., 2012). Movements and migrations are also commonly studied in diadromous fish to study their land-ocean connectivity (Koutsikopoulos et al., 1995; Feutry et al., 2011; Bultel et al., 2014). However, such connectivity has most often been studied for large fish with long lifespans (Galuardi and Lutcavage, 2012) and with migration loops that lead fish across large distances (Secor and Zdanowicz, 1988; Block et al., 2001) and/or between contrasting habitats (e.g. marine to inland, estuarine to marine, etc.) (Milton and Chenery, 2003; Daverat et al., 2011; Isnard et al., 2015).

Focus on short, holobiotic migration loops, for example in coastal areas and for small fish with short lifespans is increasing (Goto and Arai, 2003; Keith, 2003; Aldanondo et al., 2010; Tabouret at al., 2011). This constitutes an opening not only from a fundamental perspective, but also from a management perspective. For example, many small coastal fishes, such as sandeels (Ammodytidae family), are keystone species of marine ecosystems. Their mid-trophic position in the foodweb make them a forage prey for top predators, including marine mammals, seabirds, and fish species (Wanless et al., 2005; Eliasen et al., 2011; Engelhard et al., 2013, 2014). Sandeels are also economically important, but their stocks appear to fluctuate and have declined in recent years through over-fishing or because of global change, which has modified the structure of marine foodwebs (Wanless et al., 2004; Frederiksen et al., 2007, 2011). In recent years, some studies on sandeel communities were conducted to examine the

impact caused by the development of offshore wind farms and marine sediment extraction for construction on these fishes (e.g. van Deurs et al., 2012). Indeed, well-oxygenated sand banks, preferably with a low fraction of silt and clay (Wright et al., 2000), are known to play important ecological roles for sandeels, which exhibit the unusual habit of alternating between pelagic swimming for feeding and lying buried in the sand substrate even at low tide in intertidal areas, at night and during winter (Winslade, 1974; Robards et al., 2000; Jensen et al., 2011; van Deurs et al., 2011). Sand banks could consequently be considered an essential ecological habitat (EEH) (e.g. spawning ground, nursery, feeding, or resting habitat) (Rijnsdorp et al., 2009; Petigas et al., 2013) for sandeel.

The Small Sandeel, *Ammodytes tobianus*, the most abundant sandeel species in intertidal sandy habitats, has a maximum age of 7 years old, can reach maturity at one year old and seems to spawn twice a year (spring and autumn) (Reay, 1973, Kopp, 1979; O'Connel and Fives, 1995), and it also remains unclear whether the Small Sandeel is an obligate intertidal spawner (Robards et al., 1999). The Small Sandeels are rarely caught offshore (Jensen et al., 2004; van Deurs et al., 2011). Despite the ecological and commercial importance of sandeels (Engelhard et al., 2014), studies have examined mainly the Lesser Sandeel, *Ammodytes marinus*, in the North Sea (Wright, 1993; Wright and Bailey, 1996; Wright et al., 2000; Frederiksen et al., 2011). Recent investigations on this specie have shown very limited migration behaviour with a high site fidelity to a 'home sand bank' after settlement and little mixing between grounds (Engelhard et al., 2008; Jensen et al., 2011). Furthermore, for the Lesser Sandeel, dispersion between sandy areas is suspected to occur via the drift of pelagic larvae controlled by ocean currents (Christensen et al., 2008). The study of the Lesser Sandeel movements between sandbanks has been tested in through field studies and models (Christensen et al., 2008; Engelhard et al., 2008; Jensen et al., 2011) but the mixing between populations and its life histories are not totally unravel.

As it is not currently possible to perform telemetry studies with small fish species, the purpose of the present paper is to validate, for the first time for an Ammodytidae specie, the Small Sandeel, the use of otolith microchemistry as a potential tracer of movements between habitats during the life history, which may exhibit little migration behaviour, except maybe during its larval stage. Otoliths are calcified inert structures located in the inner ear of fish that grow continuously throughout life (Campana and Neilson, 1985). Because they incorporate the chemical elements of the surrounding waters at the time of deposition, otoliths act as natural tags (Milton and Chenery, 2001; Lin et al., 2007; Marohn et al., 2009), and their structures (i.e. macrostructure and microstructure) as recorder of life stages (Campana, 1999). Furthermore, otolith microchemistry also provides powerful information on migration patterns and the habitat used during their life cycle (Gillanders, 2005; Arai and Hirata, 2006; Feutry et al., 2011; Lord et al., 2011; Mercier et al., 2012).

Our hypotheses are: 1) if the Small Sandeel exhibit comparable limited migration behaviour to the Lesser Sandeel, then their otoliths will have distinct microchemical fingerprints between individuals from different sandy beaches; and 2) if sandeels do not use different habitats during their lifecycle, reflecting a resident behaviour, the elemental composition will not vary within the otolith from the core to the margin.

In this study, first the variation in size class distribution of Small Sandeels over a one-year bimonthly survey is done to detect recruitment periods and growth rates. The use of the otolith macrostructure is validated by comparing the growth calculated from cohorts and from otoliths. Then, hypotheses are tested by comparing the elemental composition within an otolith's macrostructure and between otoliths of Small Sandeels captured in three nearby coastal areas of Northern Brittany and Normandy, France. Finally, to satisfy the validity of otolith microchemistry used for the first time on sandeel species, the stability of the microchemical signatures among seasons and ontogenic development are tested. The results

are used to discuss whether microchemical tracers of the otoliths are useful to analyse the life history traits of a coastal, non-migratory, short-lived fish species such as the Ammodytidae.

2. Materials and methods

2.1. Study area and fish sampling

The three studied intertidal sand banks are located in the south-west English Channel along the coast of the Norman-Breton Gulf. The main study site, Lancieux Bay, is located at the mouth of the Frémur estuary, and the other sites chosen for testing the site fidelity of Small Sandeels were Rotheneuf Bay and at the Chausey archipelago (respectively 20 and 40 km from Lancieux) (Figure 1).

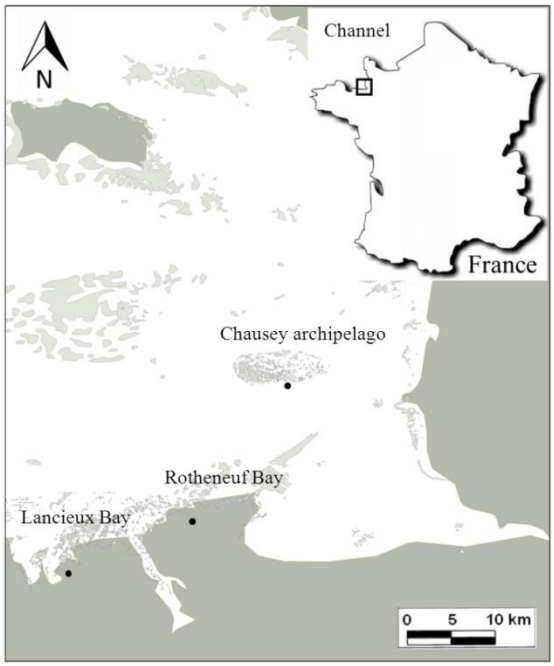


Figure 1: Location of the three intertidal sampling sites of Small Sandeels in the Norman-Breton Gulf (south-western English Channel).

Sandeels were sampled at Lancieux Bay with a shovel in the sand ripple marks of the beach at low tide twice a month for a year (February 2012 to January 2013). All of the fish

were stored at -20°C within one hour of capture for further identification and measurement (fork length (FL) in mm) at the laboratory.

In order to test ontogenic and seasonal variations in microchemical signature, 24 sagittal otoliths were extracted from juveniles and adults and in different seasons: 5 0-group juveniles caught in February and July, and 5 adults caught in July and 9 in November. Additionally, to identify spatial signatures among the three sites, sagittal otoliths were extracted in July, from 2 0-group juveniles and 3 adults caught at Chausey archipelago and 5 0-group juveniles at Rotheneuf Bay.

2.2. Otolith preparation and analysis

After extraction, the sagittal otoliths were washed three times in an ultra-pure water bath (milliQ 0.0055 µS). After the remaining tissues were removed under a binocular, otoliths were dried and stored in 1.5-mL plastic Eppendorf tubes. The left otolith extracted from each fish was embedded in araldite resin 2020 (Huntsman) with the sulcus acusticus downward. They were grounded in the sagittal plane up to the core with ultra-pure water and sandpaper with grains gradually decreasing from 2400 µm to 1200 µm, 9 µm, and 3 µm. Finally, the otoliths were rinsed with ultra-pure water and air-dried.

Otolith microchemical composition was assessed using 257 nm femtosecond laser ablation (Lambda 3, Nexeya, France) inductively coupled with plasma mass spectrometry (Elan DRCII, Perkin Elmer) (LA-ICPMS). This delivers 360 fs pulses at wavelengths of 1030 nm and can be operated at high repetition rates (up to 100 kHz). A 2D galvanometric scanner allows the fast movement of the laser beam (10 µm) at the surface of the sample to simulate virtual beam shaping when the laser is operated at a high repetition rate. Considering the otolith growth ring pattern, an elongated laser beam (10 × 50 μm^2) was simulated in order to preserve the high spatial resolution while keeping the highest signal sensitivity. The laser was

operated at 300 Hz with a pulse energy of 35 µJ while the scanner was doing a permanent 35-µm-wide, back-and-forth movement at a speed of 2 mm/s, resulting in a 20 × 50 µm laser beam. Combined with this back-and-forth movement, the sample was continuously moved along the posterior axis from the nucleus to the edge of the otolith at a speed of 5 µm/s, resulting in an uninterrupted ablation on the grounded surface. In order to prevent a blast effect on the nucleus, the ablation was started 200 µm before the nucleus. The ablation depth was evaluated at 10 µm.

At the beginning and end of each session, careful calibrations were carried out using NIST 610, 612, and 614 (National Institute of Standards and Technology). Quality control was systematically evaluated using pelletized CRM NIES 22 otolith powder (Certified Reference Material produced by the National Institute for Environmental Studies). ^{43}Ca was used as an internal standard for each ablation to correct for instrumental error in terms of ablation yield, sample transport and detection. Analysed isotopes were ^{86}Sr, ^{135}Ba, ^{138}Ba, ^{24}Mg, ^{26}Mg, ^{55}Mn, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{68}Zn, ^{57}Fe, ^{232}Th, and 238U, which are frequently used in microchemistry studies (Vasconcelos et al., 2011). Isotopes for which 75% of the measurements were above the limit of detection for at least one individual were retained. Furthermore, for elements with two isotopes (e.g. ^{63}Cu, ^{65}Cu), only the isotope with the highest natural abundance was kept after checking that no spectral interference was affecting the reliability of the result. After standardization by calcium (Campana, 1999), the remaining element ratios were Ba/Ca, Sr/Ca, Mn/Ca, Zn/Ca, Cu/Ca, Fe/Ca, and Mg/Ca.

To match the microchemical signatures with the different life stages of sandeels, macrostructural analyses of the otoliths (Figure 2) were performed. The larval stage (L) was clearly distinct on the otolith with a change in the growth axis (Figure 2A), which corresponds to settlement into sediment (Wright, 1993). Opaque zones (under transmitted light)

correspond to rapid growth in summer (S), whereas translucent zones were interpreted as low growth periods in winter (W) (ICES, 1995, 2006). Finally, we postulated that fish had spent enough time at the site of capture to assimilate a local fingerprint. The site of capture signature (C) was therefore considered to be the fingerprint measure within the external part of the otolith (Number 9, Figure 2B).

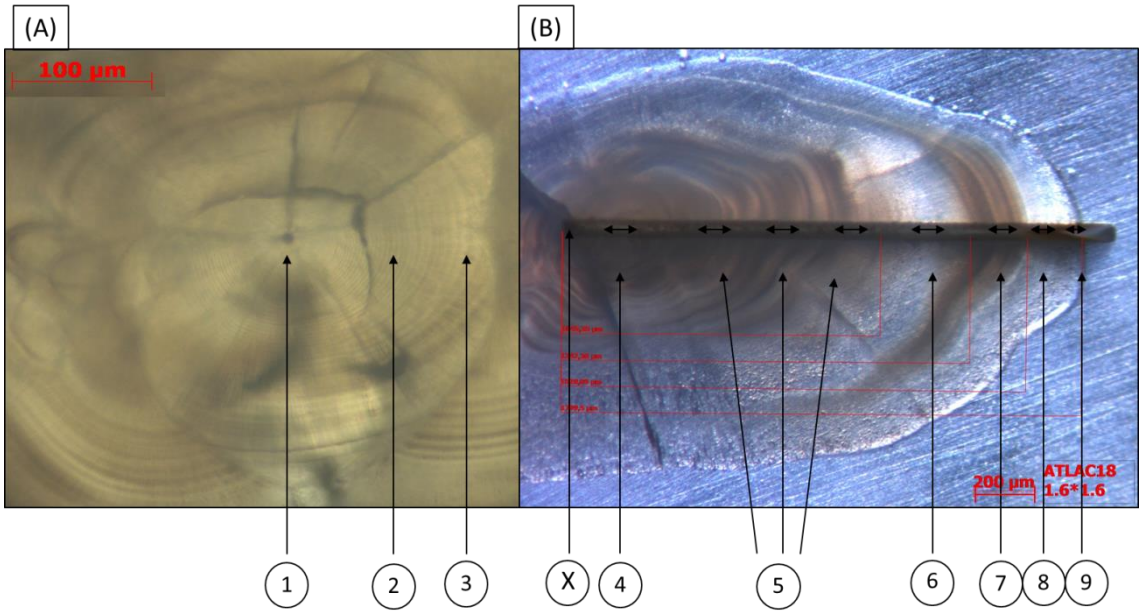


Figure 2: Microscopic photographs of an otolith from *Ammodytes tobianus* after sagittal section (transmitted light). (A): Focus on the circular larval zone: (1) nucleus, (2) daily increments, (3) end of the circular larval zone. (B): Otolith after the linear continuous ablation (X): (4) circular larval zone = L, (5) first summer growth season (S0) divided in three zones: beginning = S0b, middle = S0m, end = S0e, (6) first winter growth season = W0, (7) second summer growth season = S1, (8) second winter growth season = W1, (9) edge of otolith and signature of the capture site = C. Black horizontal arrows represent the distance considered to calculate the means of element ratios in different identified zones of the otolith.

After the continuous ablation, each otolith was photographed to measure larval, translucent, and opaque zones from the core to the end of otolith. The average of all of the element ratios was assessed at the centre of each zone except for the first summer growth zone (S0). This larger summer growth zone was divided into three sub-zones where means of element ratios were calculated (i.e. at the beginning (S0b, 20 µm after the larval stage),

middle (S0m), and end (S0e) of the S0 zone) (Figure 2B). The length of the ablation segment used to calculate the mean element ratios was adjusted to the width of each zone, which varied among fish and according to their location within the otolith. When the identified opaque or translucent zone measured more than 200 µm, the mean of element ratios was calculated over a 100 µm distance, and when it was inferior to 200 µm, the mean was calculated over a 50 µm distance. Finally, the signature of capture was calculated over 30 µm at 20 µm from the edge of the otolith.

2.3. Data analysis

At Lancieux Bay, sampling was operated twice a month and a total of 642 *Ammodytes tobianus* were measured. However, for simplification, the size-class distributions were plotted on a monthly basis. Following a cohort analysis, age classes were determined and enabled to estimate the season of birth of juveniles. Code, for example G0 A2011, was attributed to each individual and represents the cohort (i.e. age class: G0, G1, etc.), the season (S: spring; A: autumn) and the year of birth. Age estimations were validated using age readings of otoliths that were extracted for the microchemistry and macro-structural analyses (n = 24). The growth at one year of age was only calculated for two young cohorts (G1 and G0 A2011), for which it was possible to estimate the season of birth.

A linear model (LM) between the fork length (FL) of fish and the total length of their otolith (n = 44) with a Pearson correlation test (the normality and homoscedasticity of data were verified by Shapiro-Wilk and Bartlett tests) was performed to validate that otolith length was a proxy for fish length. This relationship permitted us to back-calculate Small Sandeel growth and to link different life stages of fish to LA-ICPMS results. The length of the larval stage and the first growth zone in otoliths (n = 24) were compared between the different cohorts using a linear model (LM).

Secondly, to validate the usefulness of otolith microchemistry in Ammodytidae, the potential ontogenic and seasonal effects were analysed with linear model (LM) on the microchemical fingerprint from the marginal zone (i.e site of capture (C), see Figure 2B) by comparing juveniles and adults sampled at different times (juveniles caught in February and July and adults in July and November).

For all LM the normality of residuals was verified with the QQ-plot and there was no violation of the assumption to apply the Gaussian distribution. When a significant difference was detected with the Anova (F-test) type III (for the un-balanced data ("car" R package)), a multiple pair-wise comparison (Tukey post-hoc test) was applied (multcomp" R package). For the highly unbalanced data, which occurred when analysing the otolith widths of the larval stage and the first growth zone, a bootstrap with 1000 iterations was applied after the Anova type III to verify the robustness of the results.

Finally, to distinguish the potential difference in habitat fingerprints on otoliths, the fingerprints of the marginal zone (C) of adults and juveniles captured at the three sites were compared (juveniles and adults from Lancieux and Chausey, only juveniles from Rotheneuf). Three different classification methods were used to estimate the contribution of elements measured in otoliths since some elements could provide more noise than real signal: linear discriminant analysis (LDA) and two learning methods, random forest (RF) and artificial neural networks (ANN), which are less demanding in terms of assumptions than LDA. For the LDA, capture fingerprint data met requirements for normality and homoscedasticity (i.e verified by Shapiro-Wilk and Bartlett tests), and all element ratios were standardized to give them the same weight due to differences in magnitude (Mercier et al., 2011). RF, is a tree classification method, which separates at each node the dataset in binary groups. This enables to randomly look for the group of element ratios that maximizes the homogeneity into the two

groups. Each group then splits again following the same procedure until no more homogeneity is found. ANN is a system of interconnected neurons, which computes values from input neurons to hidden and finally to output neurons and linked by a function (linear, logarithmic, etc.). To identify the best method to select an optimal element combination for the discrimination of site fingerprints, the maximal prediction accuracy (i.e. the percentage of correct assignment of the fish to their capture habitat) was tested according to the methods used (RF, LDA, and ANN) and for each possible combination of 1 to N chemical elements ($2 \times \exp^{(N - 1)}$ possible combinations). Furthermore, a cross-validation was performed for each element combination by testing 1000 replicates. For each cross-validation procedure, 75% of the fish (training data set) were randomly chosen to train the classifiers, the remaining 25% being used to measure the quality of prediction. Five hundred trees were built for the random forest method. For more details, see the R script named R_otolith_microchem_elements_and_method_selection.R (Mercier et al., 2011). To complete this analysis, a multivariate analysis of variance (MANOVA) type III (for un-balanced data, "car" R package) was used between the fingerprints of the three sites.

Lastly, to analyse site fidelity according to sites and life stages, microchemical fingerprints of each macrostructure (see Figure 2, L, S0b, S0m, S0e, W0, S1, W1, etc.) were compared with each other and to the capture fingerprint (C, site signature) with MANOVA type III.

All MANOVA were used with the Pillai's trace since it is relatively robust to deviations from multivariate normality (Johnson and Field, 1993). Indeed, half of the elements (i.e. Ba/Ca, Sr/Ca, and Fe/Ca) in the data based on different life stages of Lancieux fishes did not meet normality even after $\log_{10}$ transformation.

All the element ratios were transformed by log10. The threshold for rejection of the null hypothesis was defined at $p = 0.05$ and coded as follow: *: $0.05 > p > 0.01$; **: $0.01 > p >$

0.001; and highly significant ***: $p < 0.001$. All statistical analyses were performed using R software (R-2.15.1 R Development Core Team 2012).

3. Results

3.1. Growth estimation: validation from cohorts and otolith microstructures

Four species were caught at Lancieux Bay: *Hyperoplus lancelolatus, Hyperoplus. immaculatus, Gymnammodytes semisquamatus* and *Ammodytes tobianus*, the latter accounted for 73.04% (n = 642) of the catches.

At Lancieux Bay, fish sizes ranged from 38 to 175 mm (Figure 3). A maximum age of 5 years was observed, but most fishes were one or two years old. Whatever the sampling period, individuals from the G2 (131.62 ± 7.53 mm) and G3 (146.47 ± 8.34 mm) age classes were detected. At the beginning of the survey, G1 individuals born in spring 2011 (96.71 ± 7.83 mm) were still very abundant in February and April 2012, with an estimated age of around one year old (i.e. G1, Figure 3, black stars).

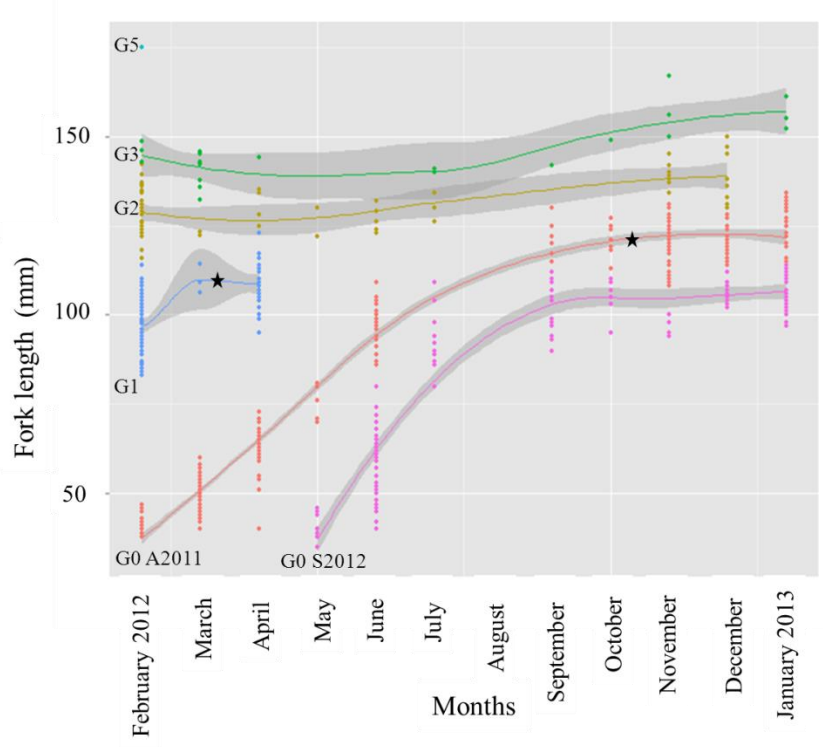


Figure 3: Mean (solid lines) and individual sizes (mm) (points) distribution of Small Sandeels sampled from February 2012 to January 2013 (with confidence interval of 95% around the mean in grey). Codes represent identified cohorts (i.e. individual of the same age class: G0, G1, etc.), back-calculated season of birth (S: spring; A: autumn), and year of birth. G0 A2011 = group 0 born in autumn 2011, G0 S2012 = group 0 born in spring 2012, G1 = group 1 born in spring 2011, G2 = group 2, G3 = group 3, G5 = group 5. Accurate estimation of size reached by fish at one year of age was only possible for two cohorts (G1 and G0 A2011), and represented by black stars.

During the survey, two newly recruited juvenile cohorts were detected; the first, born in autumn 2011 (G0 A2011, 41.37 ± 2.37 mm, Figure 3), appeared at the beginning of the survey in February 2012 and grew 7.93 mm between February and March, with a maximum growth rate in May (20.98 mm/month). For this cohort, the growth started to decrease at the end of the summer, was very low during the winter (0.46 mm between November and December) and reached one year of age during the autumn (Figure 3, black stars). Conversely, individuals born during the spring of that year (G0 S2012, 40.64 ± 3.56 mm, Figure 3), recruited in May, had higher growth at the beginning of their life (7.93 mm and 17.98 mm between the first two months, G0 A2011 and G0 S2012, respectively) and a higher maximal

growth rate in June (34.38 mm/month) than individuals from G0 A2011. Interestingly, at the end of their first year of growth, juveniles were almost the same size regardless of their recruitment period.

Finally, at one year old, individuals of G1 and G0A2011, from whom it was only possible to estimate the season of birth, measured 109.89 ± 4.08 and 120.75 ± 4.50 mm, respectively (Figure 3, black stars).

Fork length (FL) and the otolith total length (Toto) were highly correlated and can be expressed as FL = 0.0454 × Toto + 11.083 (R^2 = 0.94, Df = 41, $p < 2.2e^{-16}$ ***, Figure 4). The mean length of sandeels at one year of age was back-calculated from adult otolith diameter at the end of the first opaque zone of the otolith, and was estimated at 118.33 ± 7.15 mm.

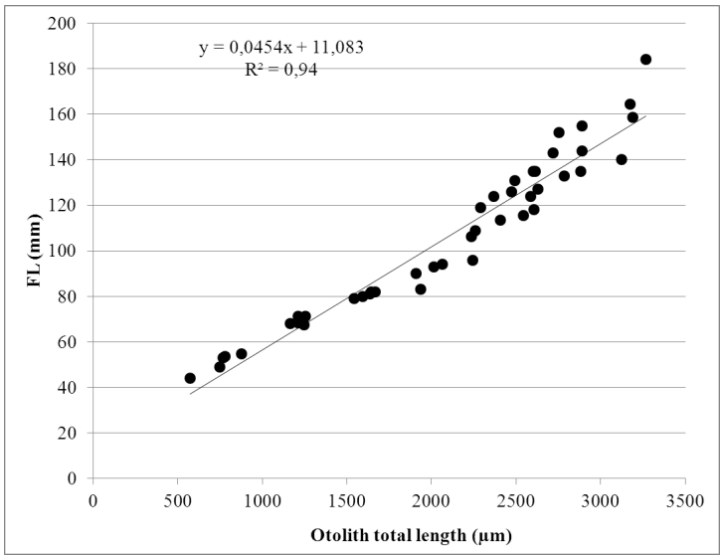


Figure 4: Relationship between fish fork length (FL in mm) and total length of sagittal otolith (mm) (antero-posterior axis, n = 44).

The larval zone had an average diameter of 265.62 ± 21.05 µm (n = 24) and did not change among cohorts (Df = 3, p = 0.30, Figure 5). However, the following first growth zone (i.e. S0) was significantly wider for individuals born in autumn (i.e. G0 A2011, 1927.52 ± 75.00 µm, n

= 3) than individuals born in spring (G1, 1323.63 ± 107.01 µm, n = 5; G0 S2012, 1398.17 ± 68.99 µm, n = 5), but also for older individuals (n = 11) (Df = 3, p = 2.02e^{-05} ***, Figure 5). G2 might also be born in spring considering their small S0 zone.

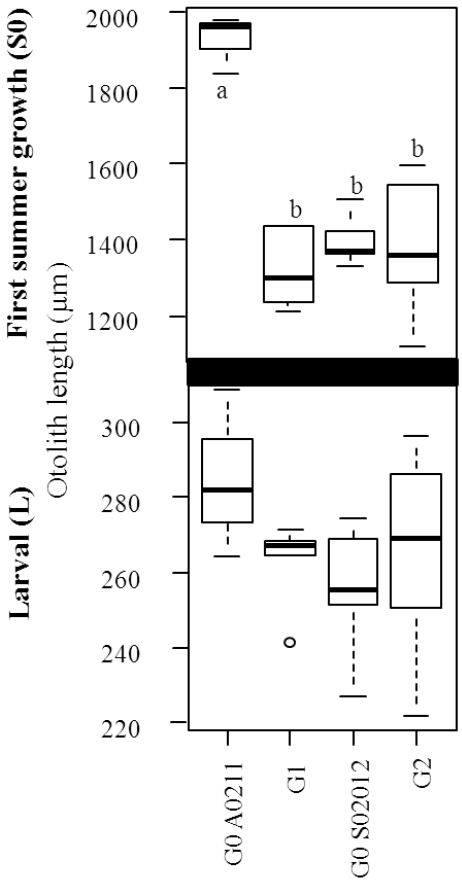


Figure 5: Comparison between otolith lengths at the larval stage (L) and at the first summer growth (S0) according to different cohorts captured at Lancieux (n = 24). Cohorts used are G1 = group 1 born in spring 2011, G0 A2011 = group 0 born in autumn 2011, G0 S2012 = group 0 born in spring 2012, G2 = group 2 years old. Letters in superscript indicate significant differences ($p < 0.05$) between the cohorts (S0) according to a linear model. No significance difference occurs at larval stage (NS).

3.2. Significance of microchemical fingerprints in otoliths

3.2.1. Ontogenic and temporal variation in otolith fingerprints

Mg/Ca varied significantly both according to season and fish age (Df = 3, Deviance explained per factor = 77%, p = 1.45e^{-06}***) and exhibited higher ratios in juvenile otoliths

and during the summer (Figure 6). The microchemical fingerprints of the other elements ratios were not different (mean ± sd of all individuals: Ba/Ca = $2.12e^{-06}$ ± $6.24e^{-07}$, Sr/Ca = $4.40e^{-03}$ ± $1.02e^{-07}$, Mn/Ca = $1.19e^{-05}$ ± $6.51e^{-06}$, Zn/Ca = $8.63e^{-06}$ ± $7.38e^{-06}$, Cu/Ca = $8.12e^{-06}$ ± $7.95e^{-06}$, and Fe/Ca = $2.02e^{-03}$ ± $2.40e^{-04}$). Meaning the variation in Mg/Ca ratio in otolith fingerprint is more due to seasonal effects and physiological changes during the fish's development than to ontogenic habitat changes (see Discussion). Therefore to avoid biaises, Mg/Ca was removed in further analysis.

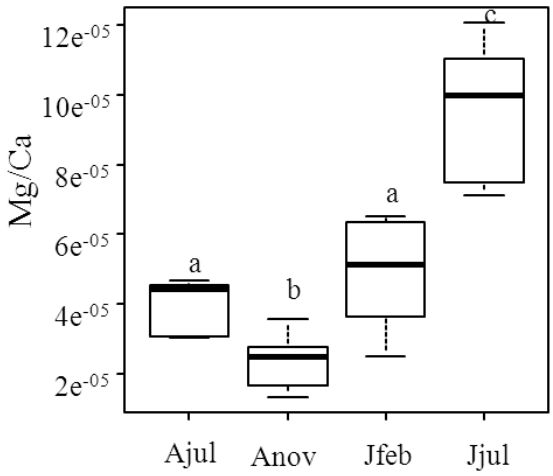


Figure 6: Comparison of Mg/Ca ratios from juveniles and adults otoliths according to months of capture at Lancieux (n = 24; adults in July (Ajul, n=5) and November (Anov, n=9), juveniles in February (Jfeb, n=5) and July (Jjul, n=5)). Letters in superscript indicate significant differences ($p < 0.05$) from a linear model.

3.2.2. Discrimination of sites and optimal element combination

The three classification methods performed (LDA, RF, and ANN) provided good maximal accuracy of prediction, falling between 78.44% and 83.79% (Table 1). LDA had the best maximal prediction accuracy (83.79%), and the best element combination was composed of Cu/Ca and Mn/Ca (Table 1). Capture signature in otoliths from Lancieux was significantly

different from those of Chausey (Manova, p = 0.0070**) and Rotheneuf (Manova, p = 0.032**) but Chausey and Rotheneuf were not globally different (Manova, p = 0.27). Mn/Ca ratios were significantly higher in otoliths from Rotheneuf and Chausey than those from Lancieux (Figure 7). Cu/Ca and Zn/Ca ratios were significantly higher in otoliths from Rotheneuf than the two other sites (Figure 7).

Table 1: Maximal classification success (± standard deviation, sd) and best combination of elements obtained by three classification methods: linear discriminant analysis (LDA), random forest (RF), and artificial neural networks (ANN). Microchemical fingerprints are inferred from the external zone (signature of capture) of the Small Sandeel otoliths from Lancieux (n = 24), Chausey (n = 5), and Rotheneuf (n = 5).

Method	Maximal accuracy (% ± sd)	Combination of elements
LDA	83.79 ± 12.35%	Cu/Ca, Mn/Ca,
RF	79.19 ± 12.67%	Cu/Ca, Mn/Ca, Sr/Ca, Zn/Ca
ANN	78.44 ± 13.79%	Cu/Ca, Fe/Ca, Mn/Ca, Zn/Ca

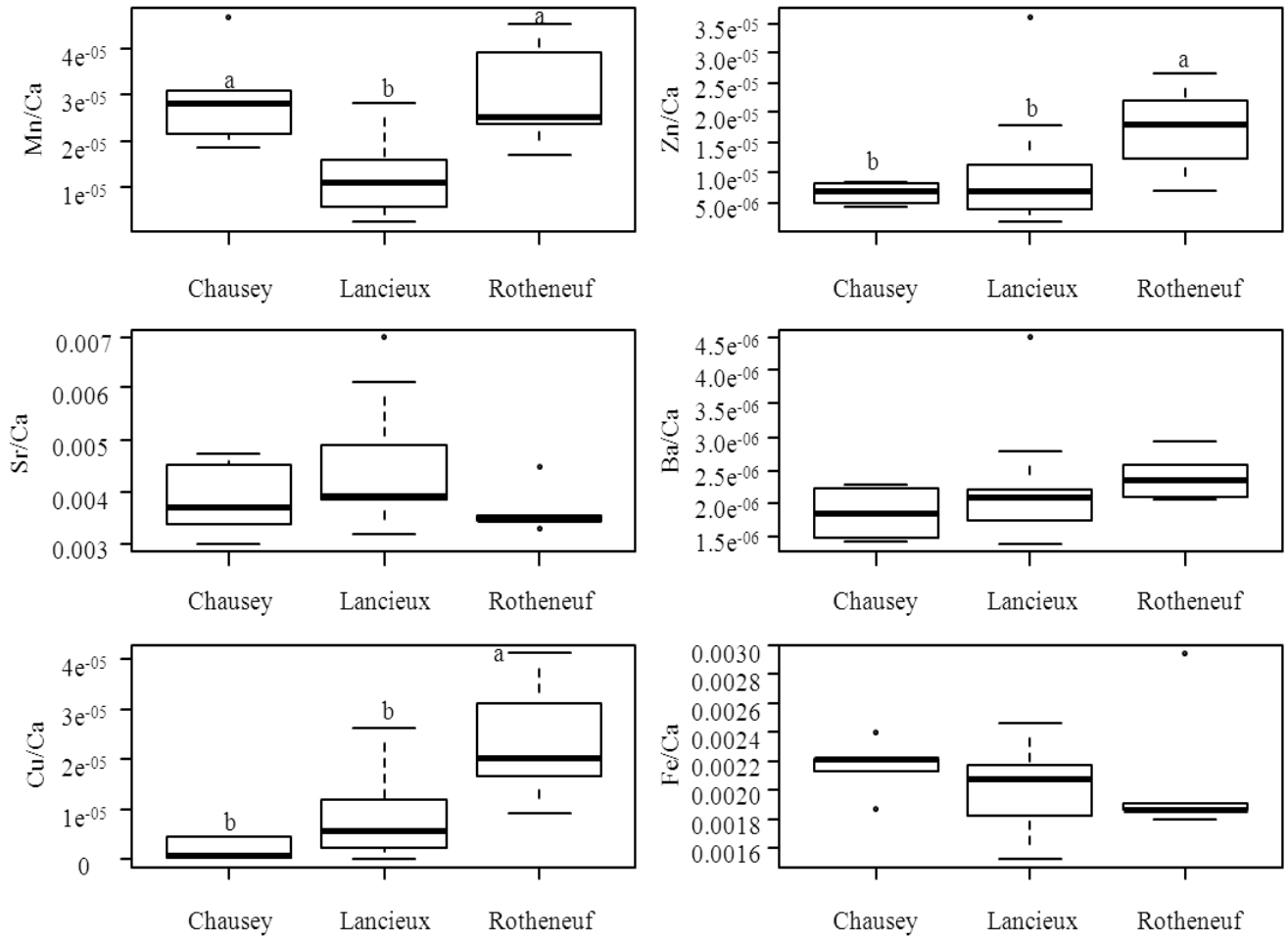


Figure 7: Boxplots comparing different element ratios from Small Sandeels otoliths from the three sites of capture. Element ratios are inferred from the external zone of the otoliths (capture fingerprint) of Lancieux (n = 24), Chausey (n = 5), and Rotheneuf (n = 5). Letters in superscript indicate significant differences (MANOVA, $p < 0.05$) between sites, when present.

3.3. Comparison of life stage signatures from Lancieux sandeels with signatures of sites of capture (Chausey, Lancieux, and Rotheneuf)

The large majority of the microchemical fingerprints found for the different life stages (i.e. macrostructural zones of otoliths) of Small Sandeels captured at Lancieux always appeared different from the Chausey and Rotheneuf capture signatures (Table 2). Among the elements, Cu/Ca, Mn/Ca, and Zn/Ca ratios were always significantly different from the Rotheneuf

capture signature, and Mn/Ca and Fe/Ca ratios differed significantly and the most frequently from Chausey capture fingerprint (Table 2).

Table 2: Multivariate analysis of variance (MANOVA type III) comparing the different microchemical signatures (corresponding to different life stages) of Small Sandeel otoliths from Lancieux (n = 24) with capture signatures of the otoliths from Chausey (n = 5) and Rotheneuf (n = 5). The macrostructure of the Lancieux otoliths is divided into several zones (see materials and methods): L = larvae; S0b = beginning, S0m = middle, S0e = end of first opaque zone corresponding to the first summertime growth period; W0 = first translucent zone corresponding to the first winter growth period; S1 = second opaque zone (second summertime growth period); W1 = second translucent zone (second winter growth period). Indicated p values are the mean of different p values calculated for each element ratio between pairwise analyses (Example: larval zone of Lancieux (L) versus capture signature of Chausey). Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant).

	Capture signature of Chausey		**Capture signature of Rotheneuf**	
	P value	**Significant element**	**P value**	**Significant elements**
L	**$6.17e^{-06}$ *** **	Mn/Ca ***, Sr/Ca ***, Cu/Ca **, Ba/Ca **, Fe/Ca **	**$1.85e^{-06}$ *** **	Mn/Ca ***, Sr/Ca ***, Cu/Ca **
S0b	**$8.38e^{-04}$ *** **	Mn/Ca ***, Fe/Ca **, Cu/Ca *	**$2.55e^{-04}$ *** **	Mn/Ca ***, Cu/Ca ***, Zn/Ca **
S0	**$3.89e^{-03}$ *** **	Fe/Ca **, Mn/Ca *	**$1.03e^{-05}$ *** **	Cu/Ca ***, Zn/Ca ***, Mn/Ca **
S0e	**$1.54e^{-02}$ * **	Mn/Ca **, Fe/Ca *	**$1.32e^{-04}$ *** **	Cu/Ca ***, Zn/Ca ***, Mn/Ca **
W0	**0.017 * **	Mn/Ca ***	**$1.89e^{-04}$ *** **	Mn/Ca ***, Cu/Ca ***, Zn/Ca **, Sr/Ca **, Ba/Ca *
S1	**0.020 * **	Mn/Ca **, Zn/Ca *	**$9.34e^{-04}$ ** **	Mn/Ca ***, Zn/Ca ***, Cu/Ca **

The microchemical signatures varied significantly from the centre (larval stage) to the margin of the otoliths. Two main differences occurred between the larval stage (L) and the beginning of the first growth stage (S0b) (Table 3). The larval stage was more enriched in Sr/Ca, Ba/Ca, Zn/Ca, and Cu/Ca than the S0b stage (Table 3). The second significant change was between this latter zone (S0b) and the first mid-growth stage (S0m) (Table 3). Then, the first mid-growth stage (S0m) did not differ significantly to the end of this zone (S0e). These three first zones (L, S0b, S0m) were significantly different from the Lancieux capture fingerprint, unlike for all of the following zones of the otolith (S0e, W0, S1, W1 and C), which did not vary significantly (Table 3).

Table 3: Multivariate analysis of variance (MANOVA) comparing, from the Lancieux Small Sandeels otoliths (n = 24), the microchemical fingerprints during the ontogeny. The otoliths were divided into several ontogenic stages inferred from macrostructural patterns (see materials and methods): L = larvae; S0b = beginning, S0m = middle, S0e = end of first opaque zone corresponding to the first growth period; W0 = first translucent zone corresponding to the first growth stop; S1 = second opaque zone (second growth period); W1 = second translucent zone (second growth stop period). The microchemical fingerprints of each of these ontogenic stages were compared to each other. Indicated p values are a mean of different p values calculated for each element ratio between pairwise analyses (Example: larval zone of Lancieux (L) versus capture signature of Lancieux). Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant).

	L	**S0b**	**S0m**	**S0e**	**W0**	**S1**	**W1**	**Capture Lancieux**
L		**1.13 e^{-09} *** Sr/Ca***, Ba/Ca***, Zn/Ca**, Cu/Ca*	**1.14e^{-06} *** Sr/Ca***, Cu/Ca***, Zn/Ca***, Ba***, Mn/Ca*	**3.12e^{-05} *** Sr/Ca***, Zn/Ca***, Ba/Ca***, Cu/Ca**, Mn/Ca*	**1.57e^{-05} *** Ba/Ca***, Sr/Ca**, Cu/Ca**, Zn/Ca**	**4.53e^{-04} *** Sr/Ca***, Ba/Ca***, Zn/Ca***, Cu/Ca*	**8.02e^{-03} ** Zn/Ca***, Cu/Ca*	**2.92e^{-03} ** Ba/Ca***, Sr/Ca *
S0b			**7.16e^{-03} ** Sr/Ca**, Cu/Ca*	**3.43e^{-03} ** Sr/Ca***	**4.85e^{-5} *** Sr/Ca***	**1.49e^{-02} * Sr/Ca**	**3.82e^{-02}* Sr/Ca**, Zn/Ca*	**1.07e^{-02}* Sr/Ca ***
S0m				NS	**3.02e^{-04}** Sr/Ca***	**3.66e^{-02}* Fe/Ca*	NS	**1.28e^{-02} ** Cu/Ca *, Zn/Ca *
S0e					NS	NS	NS	NS
W0						NS	NS	NS
S1							NS	NS

4. Discussion

4.1. Methodological validations

Results of growth estimation validate the correlation between otolith and fish length and permit to further link the different life stages of otolith to the continuous laser ablation. The definitive length of fish, almost reached at one year of age, was estimated from otoliths at 118.33 ± 7.15 mm and was corroborated by the size estimated from the two cohorts (respectively 109.89 ± 4.08 and 120.75 ± 4.50 mm, see Figure 3, black stars). This is in accordance with Reay (1973), who found fish sizes at one year of age ranging between 110 and 114 mm for two different years. Furthermore, the length of the first growth zone in otoliths can help to detect the season of birth; a small first opaque zone in the otolith corresponds to fish recruited in late spring or autumn and born in early spring, while a large zone corresponds to fish recruited in spring and born in the previous autumn, as Reay (1973) reported. This marked macrostructure of the otoliths was useful to reliably detect ontogenetic stages in the sandeel otoliths, and, given the relatively large size of the otoliths and large identifiable zones in the first year of life, it was possible to perform broad laser ablations providing enough material to detect fingerprint variations.

However, in order to definitely validate the use of microchemical fingerprints, we had to check their stability according to years, seasons, and age classes, as previously described in various fish species (Chittaro et al., 2006; Tanner et al., 2011; Mercier et al., 2012; Tournois et al., 2013). Among all the elements analysed in the Small Sandeel otolith, only Mg/Ca differed according to age class and season, which is consistent with several studies that found magnesium to be physiologically regulated (Martin and Thorrold, 2005; Tanner et al., 2011; Woodcock et al., 2012).

Three methods were used to verify whether microchemical variations in otoliths were reliable for detecting habitat changes. Although the random forest (RF) method has proven discrimination performances for recent microchemistry data sets (Mercier et al., 2012; Tournois et al., 2013), linear discriminant analysis (LDA) had the highest maximal accuracy in our study. This could be explained by the fact that our data set of capture sites exhibits multi-normality and homoscedasticity (Mercier et al., 2011). The best element combination was composed of Cu/Ca and Mn/Ca, and these elements are generally influenced by terrigenous inputs and tend to decline with distance from the shore (Kremling, 1985; Shiller, 1997; Laes et al., 2007) and have already been used to discriminate among coastal sites (Tanner et al., 2011). Our results suggest that the distinction between the three sites of capture was mainly due to a significant enrichment in manganese (Mn/Ca) in the outer perimeter ofotoliths from Chausey and Rotheneuf. In addition, enrichment in copper and zinc in the coastal sites at Lancieux and Rotheneuf enabled us to distinguish between the three sites. The observed differences could be explained by the geological composition of the sediments and of the water. Despite our weak number of individuals in Chausey and Rotheneuf sites, our results highlight the potential capacity of the otolith microchemistry of sandeels to imprint local trace elements and therefore to distinguish marine habitats, even over low geographical gradients, and confirm the interest of multi-elementary signatures as a spatial tracer. However, meaningful ecological interpretations of element concentrations remain difficult to make, especially because more information is required to understand the incorporation processes of chemical elements in fish and sandeel otoliths.

4.2. Site fidelity of the Small Sandeel according to age: insights from microchemical fingerprints of otoliths

Firstly, the microchemical signatures of the Chausey and Rotheneuf sites were never similar to those of Lancieux fishes (40 and 20 km away, respectively) suggesting that (i) environmental imprints may occur among nearby marine areas, and (ii) there was *a priori* no population connectivity between these intertidal sites. This is in accordance with Jensen et al. (2011), who found high fidelity of *A. marinus* to their nighttime burrowing sites, with a range that did not exceed 5km. Nevertheless, it appeared that diurnal movements could extend about 15 kilometres away from nighttime burrowing sites (Engelhard et al., 2008 for *A. marinus*). Finally, the swimming capacity of the Small Sandeel (1 to 1.5 km/h) (Kühlmannand Karst, 1967), suggested that fish caught in Lancieux were unable to reach either the Chausey archipelago or Rotheneuf Beach through daily movement, which does not exclude potential migrations over several days. Therefore, as telemetry is difficult for small fishes and traditional surveys and acoustic methods do not permit tracking individual behaviour, otolith microchemistry appears to be a potentially useful tool to determine the connectivity between sandeel stocks.

Secondly, the signatures of the larval stage (L) and the early juvenile growth stage (i.e. S0b, the beginning of the juvenile zone), including the metamorphosis stage (Wright, 1993), of fish caught in Lancieux, presented significantly distinct microchemical fingerprints from older stages. This first fingerprint change over the Small Sandeel lifespan could be explained by a change inhabitat, probably when the metamorphosis occurred. Indeed, the size estimated from otoliths, at the beginning of the juvenile zone (S0b), just after the larval stage, ranged between 24.96 ± 0.96 mm and 34.04 ± 0.96 mm. Even if no data were found for Small Sandeel, Wright described *A. marinus* larvae as undergoing metamorphosis over the length

range 35 to 55 mm TL, leading to a change from a pelagic to a semi-demersal habitat (Wright, 1993). This early life stage seems to occur for Small Sandeel in coastal waters according to Langham (1971), who never found larvae and post-larvae of the species in the Scottish offshore waters. Our sampling tended to confirm this result, since the smallest size of Small Sandeel settled (metamorphosed) detected at Lancieux was 38 mm. Contrary to the following stages, larvae are notably not in contact with sand during the night, which could explain the change in the microchemical fingerprint. Finally, the change in behaviour and habitat (pelagic to semi-demersal) and the influence of particular ecophysiological characteristics (growth and feeding) of these early stages (larval and during metamorphosis) on the microchemical composition of the otoliths cannot be excluded (Otake et al., 1997; Arai et al., 2000; Chittaro et al., 2006; Tanner et al., 2011).

Interestingly, the signature of the middle of the juvenile zone (S0m) differed from the capture signature of the Lancieux site (C), but not from the end of the juvenile zone (S0e) or from the second winter period (W1). At this stage (S0m), the estimated length of fish ranged from 45.10 ± 5.42 to 54.18 ± 5.42 mm. If differences in the first juvenile phase (S0b) and larval signatures are hypothetically due to a change in ecophase, as previously stated, this could be the beginning of settlement in the site in accordance with the mean sizes of recruitment in our samples of 41.37 ± 2.37 and 40.64 ± 3.56 mm according to the period (February 2012 (G0 A2011) and May 2012 (G0 S2012)). As the end of the first juvenile stage (S0e) did not differ from the next zones and the fingerprint of capture, it could correspond to a real settlement of juveniles. Observed differences in microchemical signatures and the recruitment size could result from i) a delay of signature incorporation in the otoliths (Yokouchi et al., 2011), or ii) a behavioural difference in habitat use compared to older individuals. Then, the growth and survival of *Ammodytes marinus* larvae are controlled and supported by the zooplankton peak concentration and the increase of temperature enhancing

the growth at the optimal time of match-mismatch (Gurkan et al., 2013), so variation in the time of hatching can lead to potentially different larval growth (Wright and Bailey, 1996) and juvenile size at recruitment. Accordingly, our individuals used for microchemistry and the otolith-length relationship were born in different years and probably seasons (G2, G1 (born in spring 2011) and G0 S2012), which might have effects on size at arrival at Lancieux compared to those of recruits of the year (G0 A2011 and G0 S2012).

Microchemical results finally support that the Small Sandeel exhibit high fidelity to the Lancieux site, where they have been captured during all stages of their lifecycle after the beginning of their settlement. Based on microchemistry and the sizes of recruitment and juvenile growth, intertidal beaches seem to act as nursery, growth, and resting habitats for the species, especially when they are overwintering (Wright et al., 2000; van Deurs and Steffensen, 2011) and we cannot exclude their potential role as spawning habitat for this species.

Conclusions and perspectives

To conclude, despite the small size of the sample used for this study, it confirms that exchanges could be limited between sand banks and sandeel body morphology restricts their movements in the short-term (i.e. foraging behaviour) and longer periods in their lifecycle (Engelhard et al., 2008; Jensen et al., 2011). This method is also able to detect ontogenic habitat shifts in the Small Sandeel, notably the settlement stage in intertidal beaches. Otolith microchemistry appears to be a relevant tool for investigating the life history of short-lived fishes such as Ammodytidae. It also provides a complementary approach to molecular methodologies to unravel population mixing on a geographical scale relevant to conservation and management.

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III. ARTICLE 2 : CONTRASTED LIFE HISTORIES OF THREE SYMPATRIC SANDEELS CROSS VALIDATED BY OTOLITH MICROCHEMISTRY, STABLE ISOTOPES AND FUNCTIONAL TRAITS

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Abstract

Fishes species can be morphologically and genetically closely related but exhibit different niche partitioning to enable the co-existence in communities. Sandeels are important preys for many predators in coastal areas but they are closely related both from ecological and morphological perspectives. We investigated the niche partitioning through several natural tracers to unravel the life history and behaviours of three sandeel species inhabiting the same subtidal sand bank in a northern site of Brittany, France. We sampled the community in May and September 2012 to follow the length frequency distributions, and the otolith microchemistry, isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$) and morphological traits were analyzed on individuals in May. Firstly, life history traits were inferred from the elemental compositions in otolith of these three species. Sr/Ca and Mg/Ca patterns suggested ontogenetic effect of the three species while site fidelity was assessed for *Hyperoplus lanceolatus*. Our results show that *Hyperoplus* species (i.e *H. immaculatus* and *H. lanceolatus*) were not present on the site during their entire life cycle and had better swimming capacities. On the contrary *Gymnammodytes semisquamatus* was resident on subtidal sand banks and exhibited lower locomotion capacities. Additionally, $\delta^{15}N$ values and oral gape indicated different feeding behaviours and possible predation of the larger *H. lanceolatus* species on *H. immaculatus*. Even if they are closely related, the three species have different life histories and feeding behaviours allowing them to live in sympatry in sandy habitats by reducing competition. But the population connectivity and habitat functionality still needs to be better understood through regional scale studies including *H. immaculatus* and *G. semisquamatus*.

Keywords: *Hyperoplus lanceolatus*, *Hyperoplus immaculatus*, *Gymnammodytes semisquamatus*, elemental fingerprint, swimming capacity, food acquisition, $\delta^{15}N$ and $\delta^{13}C$

1. Introduction

Niche theory explains that stable co-existence is sustained by species with different or overlapping niches stabilizing the dynamic of communities (Chase and Leibold, 2003, McGill et al., 2006, Levine and HilleRisLamberts, 2009). However, common practice in ecology pool species together in guild or functional group or trophic level, assuming they are functionally comparable according to similarities in morphology, diet, behaviour but this process cause a loss of information as each species has a different function in communities (Chalcraft and Resetarits, 2003a, Resetarits and Chalcraft, 2007). Even species as fish predator with some similarities in morphology or phylogeny (same genus) have a specific function in the community (Resetarits and Chalcraft, 2007). A broad number of closely related fish species of a same genus or family live in sympatry in coastal marine areas and the co-existence is only possible if they have some dissimilar niches through: 1) different diets (Labropoulou and Papadopoulou-Smith, 1999, Laffaille et al., 1999); 2) different life histories, *i.e.* sharing common habitats during one or several stages of their life cycle, while having separate habitats at other stages (Cabral and Costa, 1999, Charles et al., 2004).

A number of small fish, as sandeels (Ammodytidae), play a key role in food webs and thereby in ecosystem functioning. They are predated by marine mammals, seabirds and fishes (MacLeod et al., 2007, Engelhard et al., 2013, Robertson et al., 2014). Despite their economic and ecologic importance, little is understood about their biological cycle, their essential habitats and connectivity and population structure (Engelhard et al., 2014, Laugier et al., 2015). Sandeels have an uncommon behaviour, sharing their time between pelagic habitats for feeding and sand banks where they shelter at night and during winter (Winslade, 1974, Jensen et al., 2011). Moreover, during their life cycle, these species possibly move between Essential Ecological Habitats (EEH) (e.g. spawning ground, nursery, feeding, or resting habitat)

(Rijnsdorp et al., 2009; Petigas et al., 2013). Nevertheless, recent investigations on *Ammodytes marinus* (i.e a Nordic species) and *A. tobianus* suggested limited migration behaviour with a high site fidelity to a 'home sand bank' after settlement and little mixing between grounds (Engelhard et al., 2008; Jensen et al., 2011, Laugier et al., 2015). This low displacement behaviour, could be compensated by dispersion between sandy areas via the drift of pelagic larvae controlled by ocean currents (Christensen et al., 2008).

Four sandeel species are present in the West coasts of France (Brittany) living together and seem to exhibit different life cycle: *Ammodytes tobianus* is mostly linked to intertidal sandy habitats (van Deurs et al., 2011, Laugier et al., 2015), *Gymnammodytes semisquamatus* is considered as an offshore or subtidal species (Kopp, 1979) whereas *Hyperoplus lanceolatus* and *Hyperoplus immaculatus* are present in intertidal and/or offshore sand banks. Moreover, the three latter species live in same sandbanks and have contrasted reproduction periods. Indeed, *H. lanceolatus* and *G. semisquamatus* breed during summer (Le Danois, 1913, Bellec, 1981, Reay, 1986, Kopp, 1979) whereas *H. immaculatus* breeds during winter (Reay, 1986). These three species are closely related and morphologically very similar, making their distinction very difficult at small sizes (van Deurs et al., 2012). At small sizes, the distinction between them is mainly based on the mouth shape and the dorsal fin, which are related to feeding and locomotion functions. These dissimilarities between functional traits could reveal contrasted diets and/or movements/dispersion capacities as shown for other fish species (Schaefer et al., 2002, Chalcraft and Resetarits, 2003b, Albouy et al., 2011).

Consequently, if contrasted life histories and diets exist among sandeel species, one expects contrasted imprints of markers such as stable isotope ratios of carbon and nitrogen, trace element composition of otoliths and morphological features.

Otolith microchemistry represents a reliable method to retrace life history of fish ontogenetic movements (Campana, 1999, Bond et al., 2015). Indeed, the otolith margin contains the most recently synthesized material and includes trace elements that were present in the recently used habitats. Variation of the trace element composition from the centre to the margin of the otolith provides information about environmental shifts during ontogeny of the fish from the most recent periods to the larval stage (e.g. Bouchard et al., 2015, Clarke et al., 2015). Recently, some life history features of the Small Sandeel, *A. tobianus*, have been inferred from otolith microchemistry (Laugier et al., 2015). The stable isotope analysis informs about the diet, the trophic food web and act also as a spatial tracer to detect movement. Theory is based on an enrichment between a consumer and his prey and the isotopic signatures of basal resources are reflected into tissues of organisms through the food web (Hobson, 1999, Peterson and Fry, 1987). The ecomorphological traits are the reflect of contrasted feeding behaviours and life histories (Schaefer et al., 2002, Resetarits and Chalcraft, 2007) .

The aim of this study is to investigate niche partitioning according to the life history traits, the habitat use and the diet of three quite similar sympatric sandeel species of subtidal sand banks of the Western Chanel. To this end, several ecological tracers were used: the otolith elemental composition along transects from the centre to the margin, $\delta^{13}C$ and $\delta^{15}N$ stable isotopes in muscles and ecomorphological characters.

2. Material and methods

2.1. Study area and fish sampling

Three Sandeels species *H. lanceolatus*, *H. immaculatus* and *G. semisquamatus* were collected during May and September 2012 with a four panel fishing trawl (width: 15.90

meters, length: 22.80 meters, 60 mm mesh size in the wings and 3 mm at the bottom) in Lannion Bay, south-west Channel along the coast of the Norman-Breton Gulf (Figure 1). We did not catch any larvae and small juveniles (< 6 cm TL) due to the selectivity of the nets. The total length (TL) and weight (W) of 1037 fishes were measured to nearest g and mm, respectively. Subsamples of 55 fishes were stored at -20°C within one hour of capture for further identification and measurement at the laboratory. To test the spatial variations of microchemical signature in *H. lanceolatus*, specimens were provided from another subtidal site at Hébihens Isles (n = 7) and one coastal site at Lancieux Bay (n = 15), both located in Saint Malo Bay.

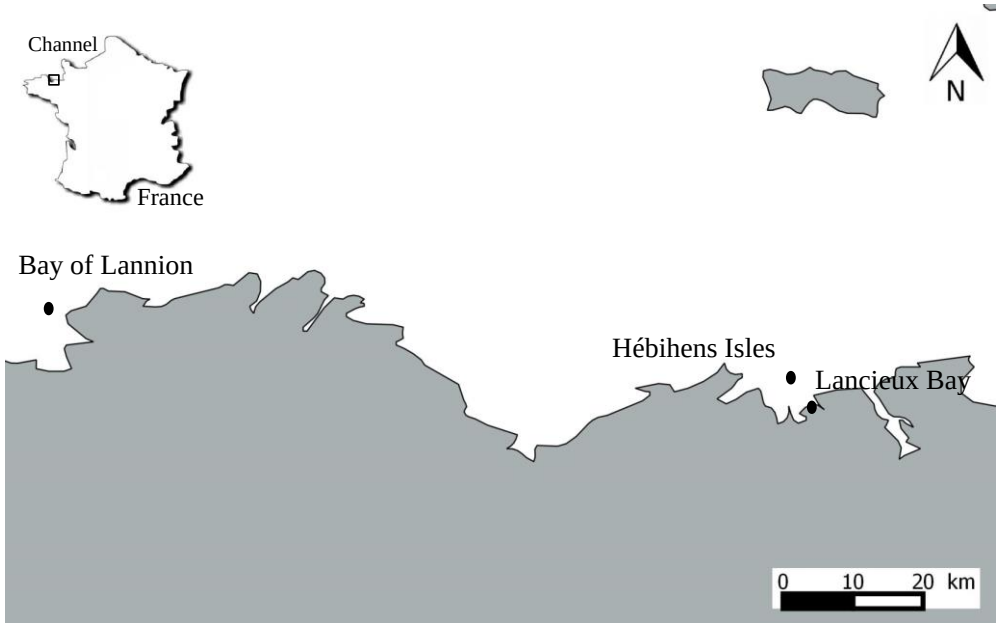


Figure 1: Location of the three sampling sites of sandeels in the Norman-Breton Gulf (south-western English Channel). The three species were captured in subtidal sandbanks in the Bay of Lannion. For the spatial analysis, *H. lanceolatus* was also captured in Hébihens Isles (subtidal sandbanks) and in intertidal sandbanks at Lancieux Bay.

2.2. Functional traits measurement, stable isotope analysis and otolith preparation

Ecomorphological traits were assessed to reflect food acquisition and locomotion capacities. Traits were measured on photographs of each fish using ImageJ software to calculate 16 functional indices (Mouchet, 2010) (Table 1).

For the three species collected in May (15 individuals per species), a piece of muscle was taken for isotopic analysis. Samples were immediately stored in an Eppendorf tube, frozen, freeze-dried, ground into a fine powder and 0.40 ± 0.05 mg were weighed in tin cups for stable isotope analyses. They were performed with EA3000 (EuroVector) elemental analyser coupled to IsoPrime (Elementar) mass spectrometer (Plateforme SF ICORE, Caen). The calibrations were carried out using isotopic international standards (IAEA, Vienne, Australie): Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The results are presented in the usual notation δ in parts per thousand (‰) relative to the deviation from standards. The analytical precision is based on replicate measurements of internal laboratory standards (n = 8) and the experimental precision is ± 0.17 ‰ and ± 0.07 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

After extraction, the otoliths (*H. hyperoplus,* n = 47; *H. immaculatus* n = 15; *G. semisquamatus,* n = 14) were washed three times in an ultra-pure water bath (milliQ 0.0055 µS) and the remaining tissues were removed under a binocular. Then the otoliths were dried and stored in plastic Eppendorf tubes. The left otolith extracted from each fish was embedded in araldite resin 2020 (Huntsman) with the sulcus acusticus downward. They were grounded in the sagittal plane up to the core with ultra-pure water and sandpaper with grains gradually decreasing from 2400 µm to 3 µm. Finally, the otoliths were rinsed with ultra-pure water and air-dried.

Table 1 : Description of the functional indices for the two functions calculated from the ecomorphological measurements (Mouchet, 2010).

Component	Functional indice	Code	Calculated indice
Physiological state	Fulton indice	K	weight / (fork length)^3
Type/size of food items captured	Oral gape surface	Osf	(mouth (maximum width* maximum height* corner width) / ((maximum width^2*maximum height)bod
Method to capture food items	Oral gape shape	Osh	maximum height mouth / maximum width mouth
Feeding position in the water column	Oral gape position	Ops	mouth height / head height
Prey detection/ visual acuity	Eye size	Edst	eye diameter / head height
Position of fish and /or prey in water column	Eye position	Eps[a]	eye position to the base of the head*eye position to the anterior end of the head /head height* body lengtl
Vertical position in the water coulmn and hydrodynamism	Body transversal shape	Bsh	body height / body width
Mass repartition along the body for hydrodynamism	Body transversal surface	Bsf	body width * body height / body weight
Pectoral fin use for maneuverability	Pectoral fin position	PFps	position of the pectoral fin / body heigth at the pectoral fin insertion
Pectoral fin use for propulsion	Aspect ratio of pectoral fin	Pfar	maximum pectoral fin length^2 / pectoral fin surface
Caudal fin use for propulsion	Caudal peduncle throttling	CPt	maximum caudal fin depth / peduncle minimal depth
Quality of propulsion and direction	Aspect ratio of caudal fin	Cfar	maximal caudal fin depth / caudal fin surface
Type of propulsion between caudal and pectoral fins	Fins surface ratio	Frt	2* pectoral fin surface / caudal fin surface
Acceleration and/or maneuverability efficiency	Fins surface to body size ratio	Fsfd[a]	((2* pectoral fin surface) + caudal fin surface + dorsal fin surface) / (∏ / 4* body width* body depth)
Dorsal fin use for maneuverability	Insertion dorsal fin	Dfi[b]	position of the dorsal fin to the head end / fork length
Dorsal fin use for propulsion	Aspect ratio of dorsal fin	Dfar[b]	base of the dorsal fin length / dorsal fin surface

[a]Adapted from Mouchet, 2010

[b]New indice created for the dorsal fin

The microchemistry composition of 76 otoliths was assessed using 257 nm femtosecond laser ablation (Lambda 3, Nexeya, France) inductively coupled with plasma mass spectrometry (Elan DRCII, Perkin Elmer) (LA-ICPMS), resulting to an uninterrupted ablation on the grounded surface (the ablation beam wasi about 50 µm large and 10 µm deep). Parameters of the laser and ICPMS followed exactly the same methodology as Laugier et al (2015). At the beginning and end of each session, careful calibrations were carried out using NIST 610, 612, and 614 (National Institute of Standards and Technology). Quality control was systematically evaluated using pelletized CRM NIES 22 otolith powder (Certified Reference Material produced by the National Institute for Environmental Studies). ^{43}Ca was used as an internal standard for each ablation to correct for instrumental error in terms of ablation yield, sample transport and detection. Analysed isotopes were ^{86}Sr, ^{135}Ba, ^{138}Ba, ^{24}Mg, ^{26}Mg, ^{55}Mn, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{68}Zn, ^{57}Fe, ^{232}Th, and ^{238}U, which are frequently used in microchemistry studies (Vasconcelos et al., 2011). Isotopes for which 75% of the measurements were above the limit of detection for at least one individual were retained. Furthermore, for elements with two isotopes (e.g. ^{63}Cu, ^{65}Cu), only the isotope with the highest natural abundance was kept after checking that no spectral interference was affecting the reliability of the result. After standardization by calcium (Campana, 1999), the remaining element ratios were Ba/Ca, Sr/Ca, Mn/Ca, Zn/Ca, Cu/Ca, Fe/Ca, and Mg/Ca.

To match the microchemical signatures with the different life stages of sandeels, we performed macrostructural analyses of the otoliths following the same methodology to Laugier et al, (2015). The larval stage (L) was clearly distinct on the otolith with a change in the growth axis, which corresponds to settlement into sediment (Wright, 1993). Opaque zones (under transmitted light) correspond to rapid growth in summer (S), whereas translucent zones were interpreted as low growth periods in winter (W) (ICES, 1995, 2006). Finally, we postulated that fish had spent enough time at the site of capture to assimilate a local

fingerprint. The site of capture signature (C) was therefore considered to be the fingerprint measure within the external part of the otolith (38 µm). After ablation, each otolith was photographed to measure larval, translucent, and opaque zones from the nucleus. The average of all of the element ratios was assessed at the centre of each zone. The length of the ablation segment used to calculate the mean element ratios was adjusted to the width of each zone, which varied among fish and according to their location within the otolith. When the identified opaque or translucent zone measured more than 200 µm, the mean of element ratios was calculated over a 100 µm distance, and when it was inferior to 200 µm, the mean was calculated over a 50 µm distance. This methodology has already prove it efficiency on the Small Sandeel species (Laugier et al., 2015).

2.3. Data analysis

In order to detect inter specific variations of elemental composition, a linear model was applied on the multi-elemental signature of the otolith external margin for the three species captured at the same campaign in May. Then, we compared, for each element, the signals found along the axis from the core to the margin of the otolith using a linear model (p value and the degree of correlation were indicated ($r^{2,}$ Spearman test)). The normality and homoscedasticity of the data set were tested by Shapiro and Bartlett tests, respectively.

In order to detect within species spatial variations, we compared the fingerprints of the marginal zone (C) of *H. lanceolatus* captured at three sites (Lannion Bay and two sites in the Saint Malo Bay: Hébihens Isles (subtidal site) and Lancieux Bay (intertidal site)). A multivariate analysis of variance (MANOVA) was used, and Pillai's trace was selected since it is relatively robust to deviations from multivariate normality (Johnson and Field, 1993). In addition, to test the seasonal stability of the signature, we compared the microchemical

fingerprints of marginal zones of *H. lanceolatus*'s otoliths captured in spring and autumn. The statistical significance of these fingerprint's variability was tested using a MANOVA.

Lastly, to analyze site fidelity according to life stages, microchemical fingerprints of each macrostructure (L, S0, W0, S1, W1, etc.) were compared to the capture fingerprints (C, site signature) with a MANOVA. Then these microchemical results were linked to the Bhattacharya model to corroborate the age of arrival on the site of capture and the habitat use strategy. This step was performed for the three species. Then, we also confronted the Bhattacharya model of the two campaigns (May and September) to detect potential cohorts newly recruited on the site of Lannion.

In order to estimate trophic variation among species, the stable isotope $\delta^{13}C$ and $\delta^{15}N$ ratios of individuals captured in May were analysed. The significance of the results was tested with a MANOVA. The size between the three species was compared using a linear model. Finally, to detect ecomorphological differences among species, we applied a linear model and a principal component analysis (PCA) on the set of 16 functional indices (see table 1).

For all the linear models (LM) the normality of residuals was verified visually with the QQ-plot and no violations of the assumption were detected to apply the Gaussian distribution. When a significant difference was detected with the Anova (F-test), a multiple comparison Tukey post-hoc test was applied ("multcomp" R package). The threshold for rejection of the null hypothesis was defined at $p = 0.05$ and coded as follow: *: $0.05 > p > 0.01$; **: $0.01 > p > 0.001$; and highly significant ***: $p < 0.001$. All statistical analyses were performed using R software (R-2.15.1 R Development Core Team 2012).

3. Results

3.1. Microchemical composition of otoliths according to sandeel species

The capture microchemical fingerprints (C) varied between sandeel species for all tested elements except for Cu and Fe (Table 2). *G. semisquamatus*' otoliths exhibited much higher Mn/Ca, Sr/Ca and Zn/Ca ratios than for *H. immaculatus* and *H. lanceolatus* (Table 2). Sr/Ca was the element ratio that differed the most between species (p value < $2.2*10^{-16}$ and 84 % of the deviance was explained, Table 2). Mg/Ca was not significantly different between *G. semisquamatus* and *H. lanceolatus* but higher for the third species. *H. lanceolatus* had lower ratios of Ba/Ca, Mn/Ca, Sr/Ca (Table 2).

Table 2 : Linear models (LM) testing the effect of species on the microchemical composition in the external margin of the otoliths (C: capture firngerprint) (n = 39; *G. semisquamatus* (GS), *H. immaculatus* (Hi) and *H. lanceolatus* (HL) captured in May at Lannion Bay). Effects are identified for each element (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant). For each element, the degree of freedom is 2 and the residual degrees of freedom are 38.

Element	Deviance residual	Deviance	F	P (F)	% Deviance explained /factor	Effect
Ba/Ca	$4.59*10^{-11}$	$5.86*10^{-11}$	15.65	**$9.52*10^{-6}$**	44	GS = Hi > HL ***
Mn/Ca	$5.34*10^{-11}$	$8.39*10^{-11}$	12.80	**$5.05*10^{-5}$**	39	GS > Hi * > HL ***
Sr/Ca	$1.54*10^{-4}$	$3.03*10^{-5}$	101.95	**< $2.2*10^{-16}$**	84	GS > Hi *** > HL ***
Mg/Ca	$1.80*10^{-9}$	$3.28*10^{-9}$	8.562	**$1.60*10^{-4}$**	35	GS = HL> Hi ***
Zn/Ca	$3.47*10^{-9}$	$3.07*10^{-9}$	22.61	**$2.69*10^{-7}$**	53	GS > HL *** > Hi ***
Cu/Ca	$3.84*10^{-11}$	$2.22*10^{-11}$	0.35	0.72	14	NS
Fe/Ca	$4.42*10^{-8}$	$2.53*10^{-6}$	0.31	0.71	2	NS

This led to analyse the three species separately because of the potential difference of element incorporation in the otolith. In addition, various relationships between element ratios and the distance along the posterior otolith axis occurred among the three species (Figures 2a, 2b, 2c). For the three species the link with Mg/Ca was highly correlated and significantly decreasing with the distance to the core of the otolith (Figure 2a, 2b, 2c). Sr/Ca increased significantly from the centre to the edge of the otoliths of *G. semiquamatus* and *H. immaculatus* but not in those of *H. lanceolatus*. For the latter species a strong negative correlation was detected for Mn/Ca and Ba/Ca (Figure 2c). As Sr/Ca and Mg/Ca were likely depending upon the ontogeny, they were excluded from further analysis.

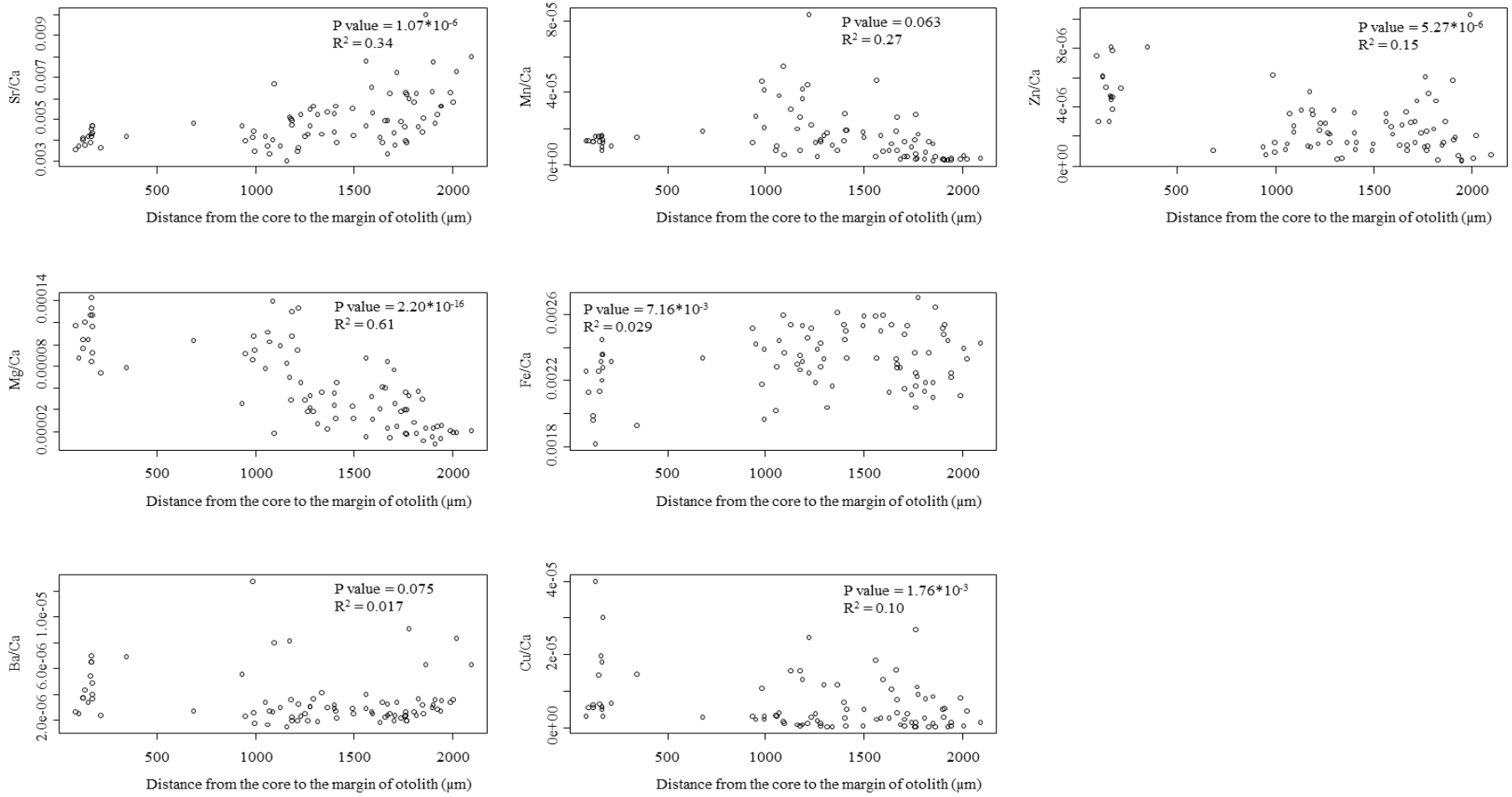


Figure 2a: Relationship between element ratio and the distance along the posterior otolith axis of *H. immaculatus*, P value (p) of the linear model and the Spearman correlation (R^2) are indicated.

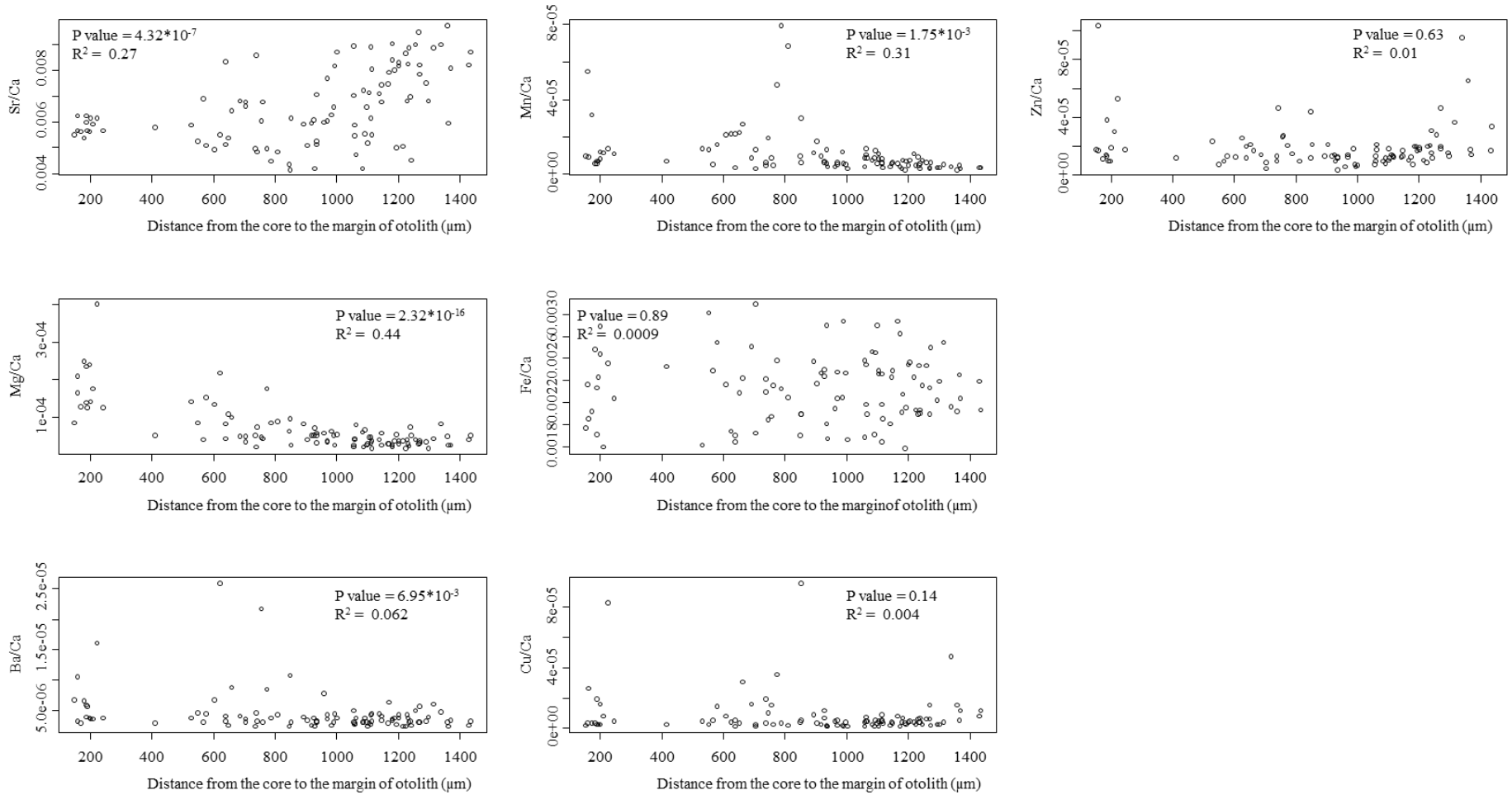


Figure 2b: relationship between element ratio and the distance along the posterior otolith axis of *G. semisquamatus* , P value (p) of the linear model and the Spearman correlation (R^2) are indicated.

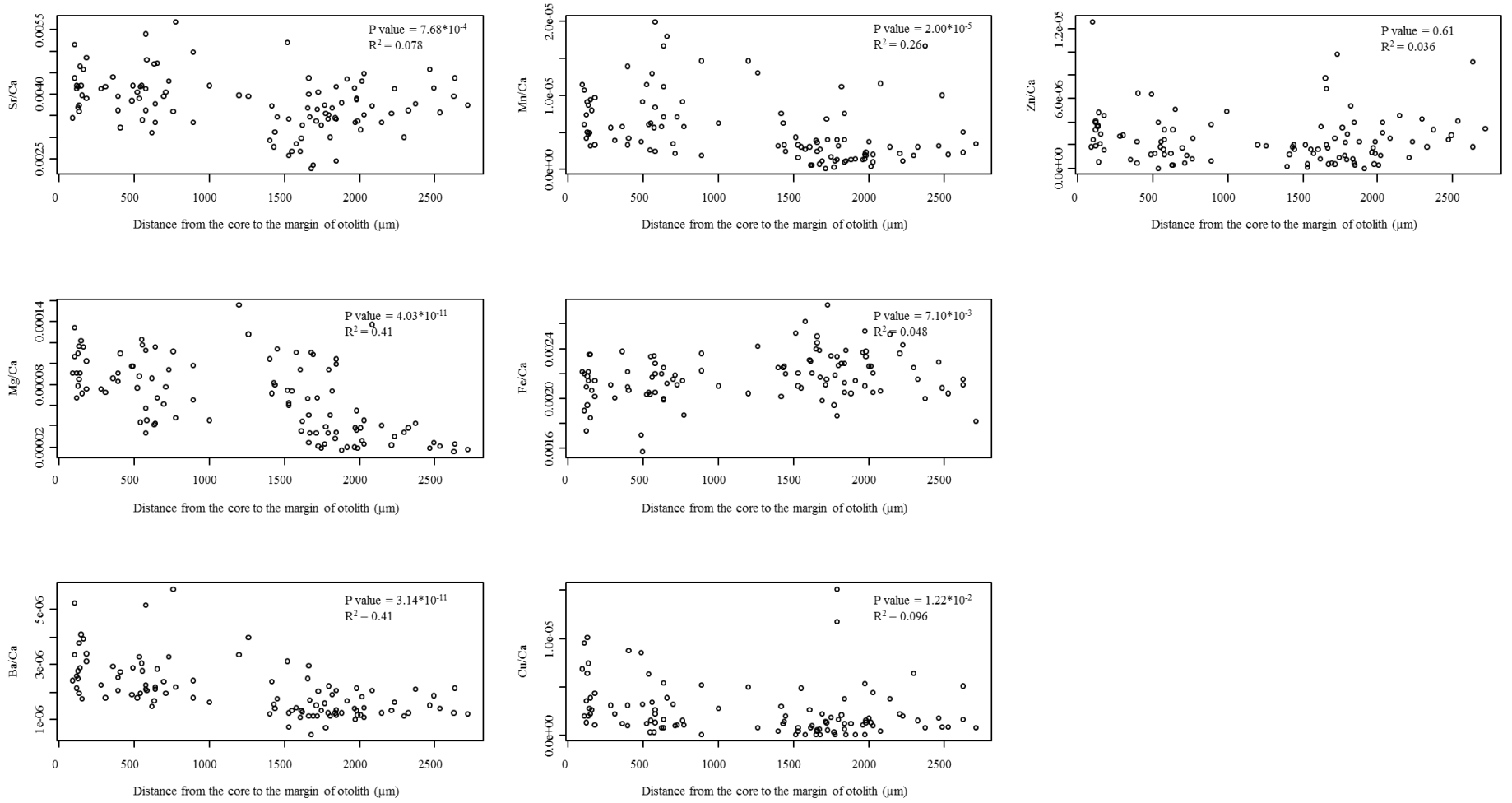


Figure 2c: relationship between element ratio and the distance along the posterior otolith axis of *H. lanceolatus*, P value (p) of the linear model and the Spearman correlation (R^2) are indicated.

3.2. Life history and habitat use of *H. lanceolatus*

3.2.1. Spatial and seasonal effects on the chemical signature

The elemental fingerprints of the marginal zone of *H. lanceolatus*'s otoliths captured in Lannion did not differ significantly between seasons (May vs September) (n = 25, MANOVA, p value = 0.50). Nevertheless, they were significantly different among the three sites (Table 3). Ba/Ca ratio was significantly higher in otoliths from Lancieux than those of Hébihens and Lannion bay, the two subtidal sand banks. Fe/Ca ratio was much higher in Lannion Bay than in the other sites. Mn/Ca was significantly lower Lannion Bay than in Hébihens.

Table 3 : Multivariate analysis of variance (MANOVA) comparing pairwise the microchemical fingerprints of the three sites of capture, inferred from the margin zone of the otoliths of Lancieux (n = 15), Hébihens (n = 7), and Lannion (n = 25). Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant).

	Lancieux		**Lannion**	
	p	**Elements**	**p**	**Elements**
Hébihens	**0,015 ***	Ba/Ca **, Fe/Ca **	0,072	Mn/Ca **
Lancieux			**$5{,}35*10^{-8}$ *****	Mn/Ca ***, Ba/Ca ***, Fe/Ca ***
Lannion				

3.2.2. Variation of microchemical signatures according to life stages

Life history of *H. lanceolatus* was inferred from the variations of trace element composition along the macrostructural zones of otoliths. The observed patterns varied among sites. In Lannion Bay, the element composition of larval zone (L), first summer (S0), first winter (W0) and second summer (S1) were different from that of the capture zone (marginal edge) (Table 4). For all these zones, Mn/Ca was discriminant, while Ba/Ca ratio was only discriminant until the first winter (W0) and Fe/Ca was only discriminant for the larval stage

(L). The older stages were not different from those of the capture zone suggesting a fidelity to the capture site starting from the second year of life.

Table 4: Multivariate analysis of variance (MANOVA) comparing the microchemical fingerprints of the different macrostructural zones of otoliths from Lannion sample (n = 15) with those of other sites of capture ((C): external zone of the otoliths) of Lancieux Bay (n = 15) and Hébihens (n = 7). 5 macrostructural zones were identified (see materials and methods): L = larvae; S0 = first opaque zone corresponding to the first summertime growth period; W0 = first translucent zone corresponding to the first winter growth period; S1 = second opaque zone (second summertime growth period); W1 = second translucent zone (second winter growth period). P values are the mean of different p values calculated for each element ratio between pairwise analyses (Example: signature of larvae zone (L) of Lannion fishes versus signature of site of capture (C) at Lannion, Lancieux and finally Hébihens). Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant).

	Capture signature of Lannion		**Capture signature of Lancieux**		**Capture signature of Hébihens**	
	p	Elements	p	Elements	p	Elements
L	**$9.09*10^{-8}$ *** **	Mn***, Ba**, Fe*	**$1.10*10^{-2}$ ***	Ba**, Fe**	**0.0019** **	Ba***
S0	**$5.44*10^{-5}$ *** **	Mn***, Ba***	NS		NS	
W0	**$3.15*10^{-5}$ *** **	Mn**, Ba***	**$1.50*10^{-3}$ ** **	Fe***, Zn**	**$4.10*10^{-3}$ ** **	Ba**, Zn*
S1	**$1.10*10^{-3}$ ** **	Mn**	**$5.49*10^{-5}$ *** **	Zn*, Ba*, Fe***	NS	
W1	NS		**$5.84*10^{-5}$ *** **	Ba***, Fe***	NS	
S2	NS		**$2.30*10^{-4}$ *** **	Ba***, Fe***, Zn**	NS	
W2	NS		**$3.80*10^{-2}$ ***	Fe***	NS	
S3	NS		NS		NS	

Secondly, the fingerprint of all the life stages of the individuals captured at Lannion differed from the signature of capture of Lancieux. Ba/Ca of capture zone was higher at Lancieux while Fe/Ca was higher in Lannion. Zn/Ca was lower in Lannion than in Lancieux. Lastly, only the composition larval stage (L) and the first winter period (W0) differed from the capture signature of Hébihens, (Table 4) mainly because of Ba/ca and secondarily to Zn/Ca for the first winter (W0).

3.2.3. Were H. lancelolatus resident?

The microchemical results (Table 4), suggested that sandeels may recruit on the site during the second year of their life according to otolith macrostructures and were validated by length frequency distribution of the population (Figure 3). In May, three length modal classes were detected and were attributed to three different age classes (i.e. cohorts) (Figure 3a). Otolithometry indicated that the first mode (22.5 cm) corresponded to 2 years old fishes, the second (28 cm) and third mode (31 cm) corresponding to 3 and 4 years old, respectively.

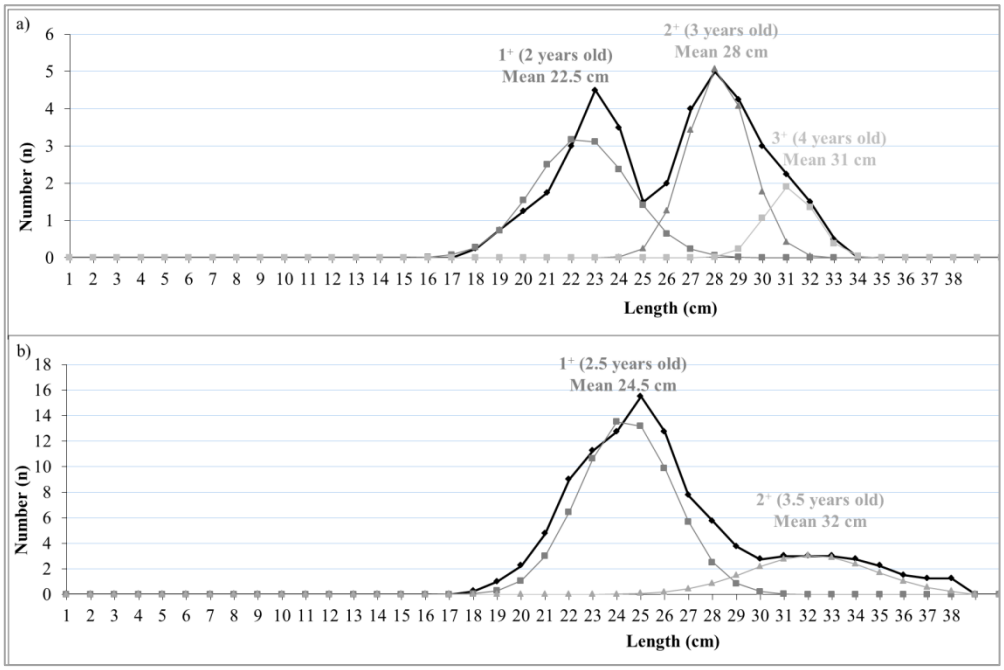


Figure 3: Length frequency distribution of *H. lanceolatus* caught by trawl in Lannion subtidal sand banks in a) May (n = 39) and b) September (n = 108). Black line corresponds to raw data; grey line to Bhattacharya model. Age range (inferred from otoliths) and the mean of length (cm) are indicated.

Smaller length frequency distributions, equivalent to the young of the year were not present into the site. In September only two length frequency distributions were graphically identified but they contained fishes from the 2+ age group (24.5 cm) and 3+ to 5+ age group (32 cm) (Figure 3b). The fact that small fish were not present in the captures supported the results inferred from microchemistry that recruitment of *H. hyperoplus* occured on the site of Lannion during the second year of life.

3.3. Life history and habitat use for *H. immaculatus* and *G. semisquamatus* ?

In *H. immaculatus*'s otoliths the elemental compositions of the four first macrostructural zones were significantly distinct from those of the external edge ((C) capture signature) of otoliths. The discrimination was mainly due to Mn/Ca (Table 5), which decreased from the centre to the edge along the otolith posterior axis (Figure 2a). This suggested that fishes recruited on the Lannion site during their second winter at approximately 2 year olds. Only one mode was detected by Bhattacharya model, and contained 2+ age group fishes (20.5 cm) (Figure 4a). As, for *H. lanceolatus*, both methods converged and suggested a recruitment at 2 years old for *H. immaculatus*. In September, the 2+ cohort grew during the summer and older individuals of 3+ (22 cm) and 4+ (27 cm) years old were caught (Figure 4b).

Table 5: Multivariate analysis of variance (MANOVA) comparing the microchemical fingerprints of the macrostructure of *H. immaculatus* otoliths from the Lannion sample (n = 15) with that of their site of capture inferred from the margin zone of the otoliths.. The otolith was divided into several zones according to macrostructural characteristics (see materials and methods): L = larvae; S0 = first opaque zone corresponding to the first summertime growth period; W0 = first translucent zone corresponding to the first winter growth period; S1 = second opaque zone (second summertime growth period); W1 = second translucent zone (second winter growth period). Indicated P values are the mean of different P values calculated for each element ratio between pairwise analyses. Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant).

	Capture signature of Lannion	
	p	Significant elements
L	**$2.09*10^{-10}$ *** **	Mn ***, Zn **, Fe *
S0	**$5.90*10^{-5}$ *** **	Mn ***
W0	**$6.58*10^{-3}$ ** **	Mn ***
S1	**$8.22*10^{-5}$ *** **	Mn ***, Ba *
W1	NS	
S2	NS	

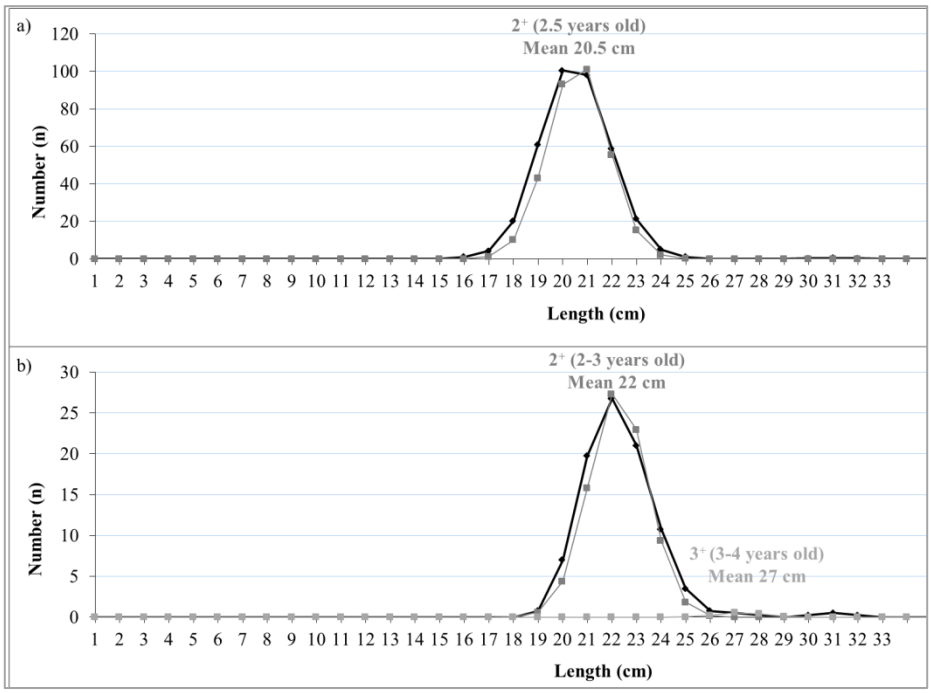


Figure 4: Length frequency distribution of *H. immaculatus* sampled in Lannion subtidal sand banks in a) May (n = 372) and b) September (n = 92). Black line corresponds to raw data and grey line, to modal decomposition using Bhattacharya model. For each mode, age range (years) inferred from otoliths and the mean of length (cm) are indicated.

For the third species, *G. semisquamatus*, elemental compositions of three macrostructures (L, S0, and S1) were significantly different from that of the external layer ((C) capture fingerprint of Lannion) of otoliths (Table 6). Interestingly, the elemental compositions of winters (W0 and W1) and that of the second summer (S2) were not significantly different from those of the marginal zone. The elements responsible for these differences were mainly Mn/Ca for all stages and Ba/Ca at the larval stage (Table 6). This suggested that fishes could recruit on the site during their first winter at approximately 1 year old and during the second winter at two years old.

Table 6: Multivariate analysis of variance (MANOVA) comparing the microchemical fingerprints of the macrostructure of *G. semisquammatus* 's otoliths from the Lannion sample (n = 14) with that of their site of capture inferred from the margin zone of the otoliths. The otolith was divided into several zones according to macrostructural characteristics (see materials and methods): L = larvae; S0 = first opaque zone corresponding to the first summertime growth period; W0 = first translucent zone corresponding to the first winter growth period; S1 = second opaque zone (second summertime growth period); W1 = second translucent zone (second winter growth period). Indicated p values are the mean of different p values calculated for each element ratio between pairwise analyses. Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant).

	Capture signature of Lannion	
	p	**Significant elements**
L	**0.025 ***	Mn *, Ba *
S0	**$8.07*10^{-3}$ ****	Mn ***
W0	NS	
S1	**$4.56*10^{-3}$**	Mn ***
W1	NS	
S2	NS	

Using the polymodal Bhattacharya model we detected three cohorts corresponding to fish of two, three and four years old (Figure 5). The three years old fish were dominant in May whereas in September the two years old individuals were much more abundant. Moreover, in September smaller individuals appeared and corresponded to the juveniles born during the year of sampling. Both methods converged to suggest that recruitment at Lannion site was possible at 1 (during the year of birth) and 2 years old.

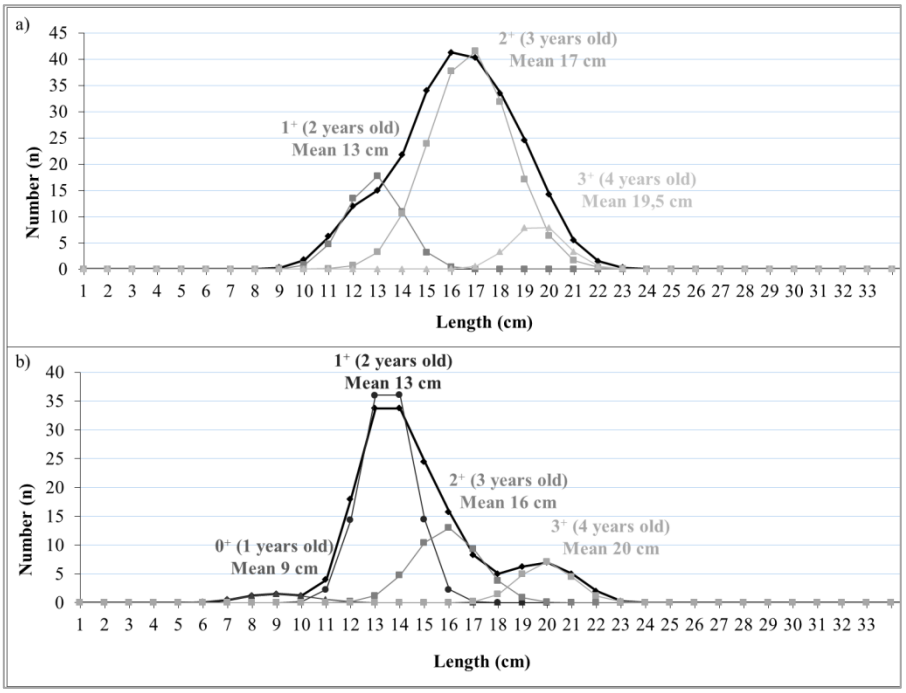


Figure 5: Length frequency distribution of *G. semisquamatus* sampled in Lannion subtidal sand banks in a) May (n = 252) and b) September (n = 168). Black line corresponds to raw data and grey line, to Bhattacharya model. Age range (inferred from otoliths) and the mean of length (cm) are indicated.

3.4. Trophic link

Firstly, significant differences were observed for the two stable isotopes between the three species (MANOVA, $p < 2.2*10^{-16}$ ***). Secondly, we observed a gap of approximately 3 ‰ $\delta^{15}N$ and 1 ‰ $\delta^{13}C$ between the two *Hyperoplus* species, *H. lanceolatus* being more enriched, meaning the latter could be potentially a predator of *H. immaculatus* (*H. lanceolatus*, $\delta^{15}N$ = 12.63 ± 0.93, $\delta^{13}C$ = -17.60 ± 0.33; *H. immaculatus*, $\delta^{15}N$ = 9.55 ± 0.85, $\delta^{13}C$ = -18.53 ± 0.46) (Figure 6). *G. semisquamatus* was positioned between the *Hyperoplus* species (*G. semisquamatus*, $\delta^{15}N$ = 11.24 ± 0.28, $\delta^{13}C$ = -18.67 ± 0.32) (Figure 6). This is surprising when comparing the specific sizes of the three species: (Linear model, $p = 3.24*10^{-14}$ ***) (*G. semisquamatus* 166.48 ± 10.32 mm, *H. immaculatus* 215.47 ± 15.52 mm, *H. lanceolatus*

263.68 ± 33.85 mm), one would expect *G semisquamatus* to have the lowest trophic level of the three species while it was intermediate between both *Hyperoplus* species.

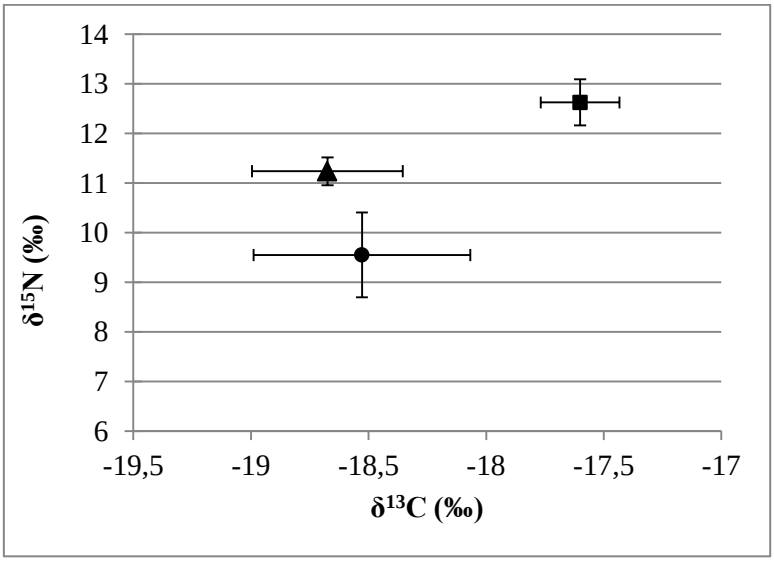


Figure 6: $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) muscle stable isotopic values for the three sandeels in May at Lannion (n = 15, for each species). Square: *H. lanceolatus*, triangle: *G. semisquamatus*, circle: *H. immaculatus*.

3.5. Functional traits can reflect swimming and feeding capacities

Strong functional divergences occurred between sandeel species of the community (Figure 7, Table 7). For food acquisition, the eye size (Edst) and the oral gape surface (Osf) were the most distinct traits and *H. lanceolatus* had the bigger mouth surface but smaller eye. The oral gape shape (Osh) was higher for *H. lanceolatus* meaning that this species had a taller than broad gape. For the locomotion function, three traits differed highly. The body transversal surface (Bsf) meant that *G. semisquamatus* was more hydrodynamic in relation to a lighter weight. The ratio of the caudal (CFar) and dorsal (DFar) fins were higher for *G. semisquamatus* corresponding to a smaller fin surface and suggesting a lower swimming capacity. The fin surface ratio (Frt) was also bigger for *Gymnammodytes* and smaller for *H. lanceolatus* corresponding to a more powerful caudal fin for the latter.

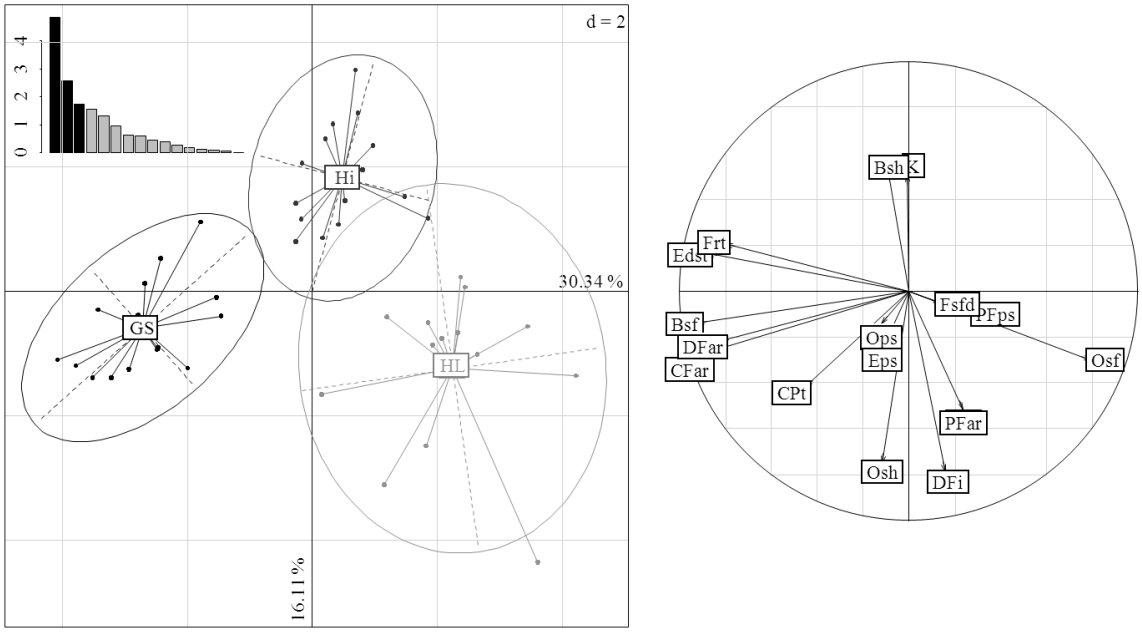


Figure 7: Functional differences linked to locomotion and food acquisition functions between the three sandeel species captured at the same date (May) at Lannion Bay, in a principal component analysis (PCA) (n = 44).

Table 7: Linear models (LM) testing the effect of species on the 16 function indices between the three sandeel species (n = 45; *G. semisquamatus* (GS), *H. immaculatus* (Hi) and *H. lanceolatus* (HL) captured at the same date (May) at Lannion Bay). Effects are identified for each indice (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant). For each element, the degree of freedom is 2 and the residuals degrees of freedom are 42.

Functional indice	Deviance residual	Deviance	F	P value (F)	% Deviance explained/factor	Effect
		Feeding				
K - Fulton indice	$2.23*10^{-6}$	$5.80*10^{-6}$	8.08	$1.07*10^{-3}$ **	28	GS = HL > Hi **
Osf - Oral gape surface	1.54	0.70	45.99	$2.63*10^{-11}$ ***	69	HL ***> Hi ** > GS
Osh - Oral gape shape	8.18	7.07	24.29	$9.77*10^{-8}$ ***	54	GS = HL > Hi ***
Ops - Oral gape position	$1.21*10^{-3}$	0.37	$6.82*10^{-2}$	0.93	0.20	NS
Edst - Eye size	0.25	0.08	65.01	$1.38*10^{-13}$ ***	73	GS > Hi *** > HL ***
		Locomotion				
Eps - Eye position	$4.31*10^{-5}$	$1.71*10^{-3}$	0.52	0.59	2	NS
Bsh - Body transversal shape	$7.48*10^{-2}$	0.90	1.74	0.19	8	NS
Bsf - Body transversal surface	1.95	0.26	155.36	$2.2*10^{-16}$ ***	88	GS > Hi *** > HL ***
PFps - Pectoral fin position	$1.56*10^{-2}$	$9.74*10^{-2}$	3.36	$4.42*10^{-2}$ *	14	HL ** > GS = Hi
PFar - Aspect ratio of pectoral fin	1.84	8.95	4.31	$1.99*10^{-2}$ *	17	HL > GS > Hi *
CPt - Caudal peduncle throttling	2.62	11.96	4.60	$1.57*10^{-2}$ *	18	GS = HL > Hi **
CFar - Aspect ratio of caudal fin	$2.93*10^{-2}$	$1.23*10^{-2}$	49.79	$8.28*10^{-12}$ ***	70	GS > Hi *** > HL ***
Frt - Fins surface ratio	1.46	1.31	23.51	$1.41*10^{-7}$ ***	53	GS > Hi ** > HL ***
Fsfd - Fins surface to body size ratio	1.97	80.03	0.52	0.6	2	NS
DFi - Insertion dorsal fin	$2.56*10^{-3}$	$3.73*10^{-3}$	14.43	$1.70*10^{-5}$ ***	41	Hi * > GS > HL**
DFar - Aspect ratio of dorsal fin	0.39	0.12	67.59	$7.44*10^{-14}$ ***	76	GS > Hi *** > HL ***

4. Discussion

Here we have deduced the different life histories of 3 sympatric sandeels caught at the same time in a subtidal sand bank of northern Brittany through different patterns of the movement, diets and capacities of locomotion and food acquisition. Otolith microchemistry has already proven it efficiency to improve understanding on ontogenetic fish movements and/or connectivity between habitats in the Small Sandeel (Laugier et al., 2015) and was applied in this study to determine the spatial niche partitioning.

The fundamental assumption is that the trace element composition in otolith represents environmental fingerprints (Campana, 1999). However, some other assumptions have to be verified due to possible interactions between exogenous and endogenous factors on the incorporation of element in otolith of marine fishes (Elsdon and Gillanders., 2004, Sturrock et al., 2012). Firstly, we noted strong interspecific differences for Sr/Ca, Mn/Ca, Zn/Ca and Ba/Ca ratios in the capture signature. These cases had already been noted for Sr, Mn and Mg (Hamer and Jenkins, 2007) and also for Ba, Cu, Zn, Pb, Li (Vasconcelos et al., 2007). Even species with close phylogenetic distances or individuals of different populations can have strong changes of otolith microchemical composition (Hamer and Jenkins., 2007, Clarke et al., 2011). In addition to this interspecific variation, we observed a possible ontogenetic effect (i.e. correlated to age) as Sr/Ca ratio increasing from the centre to the edge of otoliths for *G. semisquamatus* and *H. immaculatus*. Ontogenetic trend in otolith Sr/Ca ratio have already been observed for several marine species but the factors influencing Sr incorporation are not completely unraveled for fish species and family (Kalish, 1989, Brown and Severine, 2009, Walther et al., 2010, Sturrock et al., 2012). This phenomenon is more current for marine fishes (Brown and Severine, 2009) than for diadromous or freshwater fishes for which strontium is a good indicator of salinity changes and related habitat shifts (Gillanders, 2005).

Mg/Ca ratio is also related to fish ontogeny and decreased with age for the three species, as for the Small Sandeel (Laugier et al., 2015) and is probably related to physiological processes (Martin and Thorrold, 2005, Tanner et al., 2015, Woodcock et al., 2012). Regarding these assumptions Sr/Ca and Mg/Ca were removed when we compared different life stages and the species were studied separately regarding to the different compositions of capture fingerprint.

The discrimination among the three sites was significant for *H. lanceolatus* and was due to Mn/Ca, Fe/Ca and Ba/Ca ratios. Differences in Mn/Ca ratio between the two subtidal sites suggested that Mn/Ca inform about regional difference and it is a scavenged element, which tends to decrease with depth and distance from the shore (Laes et al., 2007). Ba/Ca, which was higher at the intertidal site and Fe/Ca at the two subtidal sites, could possibly act as local tracer with a gradient from the shore to the subtidal sand banks (Walther and Limburg, 2012). Sturrock et al (2012) indicate that elements with short turnover times (*e.g* Mn and Ba) may be better to study downscale changes than elements with longer turnovers (*e.g* Sr and Pb). Consequently, these elements with short turnovers should be more reactive to environmental changes, as freshwater inputs from river floods, and their variations in otoliths could indicate fast changes of water composition. In the future, other elements have to be analysed to increase the potential discrimination between habitats and other subtidal sites have to be added to map the different sandy habitats to ensure the understanding of habitat connectivity.

Habitat fingerprints (i.e three sites) were discriminated with success and the different otolith's signatures according to life stages of *H. lanceolatus* from Lannion were compared with it in order to infer the life history and explore site connectivity. Firstly, the signatures of the beginning of life were more concentrated in Mn/Ca and Ba/Ca, consequently the first life stages could be linked to near shore habitats while combination of otolith microchemical signatures and length frequency analysis converge to show that individuals recruit at two

years old on the Lannion site. The gonado-somatic ratio on 25 individuals was not enough to prove the spawning area status of the site, but the first sexual maturity is about two year olds and the reproduction takes place in summer (Le Danois, 1913, Bellec, 1981, Macer, 1966), so maybe maturation of the gonads takes place on subtidal sandy habitats. Secondly, between Lannion and Lancieux the connectivity was weak explained by the strong divergence of the microchemical fingerprints. To the contrary, some macrostructure elemental signatures comparing with Hébihens site exhibited some similarities, suggesting possible connectivity between these two subtidal sites. But due to the distance separating the two subtidal sites (superior to 100 km) and in accordance with Jensen et al. (2011), who found high fidelity of *A. marinus* to their nighttime burrowing sites, with a range that did not exceed 5 km, connectivity seemed unlikely despite the microchemical results. Nevertheless, it appeared that diurnal movements could extend about 15 kilometres away from nighttime burrowing sites (Engelhard et al., 2008 for *A. marinus*). More subtidal and intertidal areas and nearest to the site of Lannion should be included in the future to better describe the local and regional connectivity between populations and habitats.

For *H. immaculatus*, the length frequency distributions and the microchemical analysis converge to indicate that they could recruit on the site at approximately two years old and it was mainly the only year class detected on the site during the two seasons as Bellec (1981). The reproduction occurs in winter and the first age at sexual maturity is two year old (Bellec, 1981) but our samples did not permit to determine if they reproduced on the site. The life history of this species, which is genetically closely related to *H.lanceolatus*, indicated potential movements between proximate sand banks for the other size classes as these ones were not captured. The Mn/Ca and Ba/Ca ratios increased during the first summer growth, so juveniles could possibly inhabit sandy habitats close to the shore, and we had already seen juveniles of *H. immaculatus* on Lancieux site (*unpublished*).

For the smaller species *G. semisquamatus*, individuals of two, three and four year olds were captured on the site in May and some juveniles were observed in September. The chemical signature indicated a possible installation during their first year of live or at two year olds. This species has already been cited as the most offshore or subtidal sandeel species (Kopp, 1979) and our results confirm an earlier installation on the site compared to the two others and a more resident life history of this species.

The three species inhabited the subtidal sand bank at the same time but had various life histories. Only *G. semisquamatus* seemed resident during its life cycle and the two others could move between adjacent areas and the juveniles could potentially inhabit other sand banks more closely to the coasts (Reay, 1973). Even if sandeels are not enable to cover important distances (Engelhard et al., 2008, Jensen et al., 2011), differences of swimming capacities were observed between the three species and were in agreement with our previous results. The two *Hyperoplus* species had higher swimming capacities with higher caudal and dorsal fin surfaces and a more powerful caudal fin and consequently seemed more mobiles than *G. semisquamatus*. This could be the consequence of a different spatial repartition between these species inducing niche differentiation and allowing the co-existence in communities (Gross, 1987, Charles et al., 2004). In addition, the juveniles of the two *Hyperoplus* species were not present and reduced the food competition for juveniles's *G. semisquamatus* (Cabral and Costa, 1999). But when adults of the three species were present, mainly at two year olds for *Hyperoplus*, the food competition and predation cannot be excluded. We observed that the bigger species *H. lanceolatus* could probably predate *H. immaculatus* (maybe small individuals) due to his bigger surface and taller oral gape, and his higher enrichment in ^{15}N about 3 ‰. Additionally, the lower abundance of *H. lanceolatus* also converges to suggest a predatory behaviour. *Hyperoplus* species are also characterised by a single bicuspid tooth and could be related to a particular function for the food acquisition. The

gape shape is an important morphological adaptation and the final body size reflect the metabolic demand and are both related to the feeding behaviour (Chalcraft and Resetarits, 2004, Schafer et al., 2002). *G. semisquamatus* despite his smaller size compared to the two *Hyperoplus*, was more enriched in ^{15}N than *H. immaculatus*. This result was not expected but recently cannibalistic and piscivorous habits of the nordic species, *A. marinus*, have been observed (Eigaard et al., 2014). The morphological characteristics linked to the food acquisition and isotopic values observed for the three species revealed a potentially overlapping feeding behaviours for the species at two year olds in addition to the spatial segregation at different stages. Even if sandeel species are genetically, morphologically closely related and considered as forage fish species in food webs, all of them have a different function in communities (Chalcraft and Resetarits, 2003a).

Conclusion

The multi-tracers used in our study showed contrasted life histories and feeding behaviours, and consequently different niche partition allowing the sympatry in community for these sandeel species. But the nursery for *Hyperoplus* species was not identified and the spawning habitat with the eggs presence was not investigated. *Hyperoplus* species could be more mobile than the *Gymnammodytes* species but the spatial scale was not yet elucidated. The connectivity of populations between sandy habitats is not yet unravelled for the three sandeel species and further sites must be added to address this question for *H. immaculatus* and *G. semisquamatus*. Also genetic tools will be helpful and complementary to unravel the population and habitat connectivity (Miller et al., 2005), which is important facing the development of sand extraction.

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PARTIE 2 :

LE CAS DU BAR EUROPEEN, *DICENTRARCHUS LABRAX*



I. CONTEXTE DE L'ETUDE

La première partie de la thèse visait à comprendre les histoires de vie et l'utilisation des HEE (i.e. Habitats Ecologiques Essentiels) pour des espèces d'Ammodyidae plutôt résidentes de certains habitats au cours de leur cycle de vie.

La deuxième partie de la thèse est centrée sur une espèce migratrice, le bar européen, possédant de plus grandes capacités de déplacements que celles des lançons et pouvant donc utiliser des HEE plus distants au cours de leur cycle de vie (Jennings and Pawson, 1992, Pawson et al., 2007). La variabilité des traits de vie intra-espèce sera analysée pour mieux comprendre principalement l'utilisation des habitats de nourricerie côtière pour les juvéniles tels que les marais salés (Laffaille et al., 2000, 2005).

II. ARTICLE 3 : DIVERSITY OF LIFE HISTORIES OF JUVENILE EUROPEAN SEA BASS, *DICENTRARCHUS LABRAX*, REVEALED BY THE MICROCHEMISTRY OF THEIR OTOLITH.

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Abstract

Understanding the life cycle of marine migrant fish species is essential to better manage the populations and habitats but is still an intricate challenge regarding to the complex utilisation of habitats. Juveniles of the European sea bass forage in the salt marshes but their movements and life histories from the first life stages to their behaviour in coastal areas are not yet well defined. Juveniles were collected into 5 salt marshes and in one seagrass bed from the French coast of the English Channel. For the first time, the multi-elemental composition of grounded juvenile otoliths (n = 55) was continuously analysed from the centre to the edge by femtosecond laser ablation coupled to ICPMS in order to detect precisely habitat changes. Firstly, the microchemical profile through the entire life indicated a marine larval migration with a high Sr/Ba ratio and a progressive coastal arrival was detected around the post-larval stage in approximately 83.80 ± 17.94 days. The growth rate of the first life stages (i.e larval stage to post-larval stage) corresponding to the marine life period was not different between sites (3.06 ± 0.85 µm/day). After the coastal arrival, most of the juveniles spent time in brackish waters, while some had never or rarely spent time into it. Some fishes (Lessay site) mainly spent time in mesohalin (5-18 psu) waters but their juvenile otolith growth rate (7.26 ± 1.39 µm/day) and fish growth rate also were low. In contrast, the juveniles of the Mont Saint Michel Bay spent more time in polyhalin waters (30-18 psu) during their juvenile life time and were found to have better growth rates. The accessibility of salt marshes is complex due to tidal movement inducing probably variable habitat use strategies for juveniles and these strategies are discussed regarding to the habitats characteristics. Unravelling the life strategies of sea bass juveniles in coastal habitats is fundamental to the management of nursery habitats and the forthcoming contribution to adult stock.

Keywords: LA-ICPMS, multi-element signature, life history, nursery, salt marshes, biological tracer

1. Introduction

Understanding the life histories of marine fishes is especially important for the conservation and management of species but can be complex due to the migration between offshore spawning and growing areas such as coastal nurseries (Chapman et al., 2012). Migration occurs if the benefit is superior to the costs of the travelling between habitats (Gross, 1987). Nursery habitat status imply a high juvenile density, a refuge from predators, a better growth rate than in other habitats and a good contribution to adult recruitment regarding to the surface area (Beck et al., 2001) but the larger habitats that contribute significantly to the adult populations are not considered as nursery in this concept (Dahlgren et al., 2006). Dahlgren et al (2006) suggest the term Effective Juvenile Habitat (EJH) as a habitat that the contribution of juvenile to adult stock is greater than the overall average contribution calculated on all habitats used by juveniles. The contribution of juveniles to adult stock depends on the larval survival during the migration of the first life stages as well as survival and growing of juveniles in nurseries (Beck et al., 2001). Estuarine habitats (e.g. mudflats, salt marshes) are often considered as nurseries or EJH for multiple species but numerous pressures can affect the fitness of juveniles through modifications on their environment affecting the quality of their resources like for instance vegetation mowing, grazing, invasive species… (Laffaille et al., 2000, Lefeuvre et al., 2003, Cattrijsse and Hampel, 2006, Parlier et al., 2006, Vasconcelos et al., 2007, Kostecki et al., 2012). Tidal salt marshes are flooded during high tide and fishes come to forage with the rising tide and must leave with the ebb tide, also depending on a seasonal tidal cycle. Accessibility for fish is consequently reduced to only short immersion periods (few hours per day and only when the level of the tide is sufficient) depending on the geomorphology and the location of the marsh.

These complex fish movements induced by tidal nurseries coupled with larval migration from offshore spawning areas appeared crucial to unravel in order to manage and conserve both species and habitats but remain an intricate challenge.

Major life stages and migrations due to ontogenetic habitat changes can be studied through structures and elemental compositions of otolith (Panfili et al., 2002, Sturrock et al., 2012). Otolith microchemistry has already proved its efficiency to unravel life histories of fish, to discriminate nursery fingerprints and finally to link nursery habitats to adult stocks (Vasconcelos et al., 2007, 2011, Mercier et al., 2012). Otolith is an inert structure growing continually, that incorporates the trace elements of the surrounding water and consequently reflects the environment characteristics into the entire life of fish (Gillanders, 2005). Sr and Ba are notably highly correlated to salinity and consequently are used to study the fish movements between different habitats and more often with contrasted salinities. Although some physiological parameters, food consumption or temperature can influence the elemental otolith composition, the water chemistry is one of the main source contributing (Izzo et al., 2015). Most studies exploring fish habitat relationship with microchemistry tracers used only a part of the otoliths (e.g. their external part to determine the capture fingerprint) which only reflect the last few days of life but the real challenge is to apprehend the movement between essential habitats occurring across the entire lifespan of individuals by considering the whole otolith (Lord et al., 2011, Feutry et al., 2012, Pécheyran et al., 2014, Laugier et al., 2015, Reis-Santos et al., 2015).

The European sea bass (*Dicentrarchus labrax*) is a commercial key species with a migratory behaviour but precise knowledge about the movements and life histories from the first life stages to the behaviour of juveniles in coastal areas are not yet well defined. The multi-elemental fingerprint of capture in sea bass juveniles has shown their fidelity to estuary and possibly low connectivity between areas within estuary (Reis-Santos et al., 2015) but it is

still reflect only the last few days of life. Recently, microchemistry profile (Ba/Ca) on otoliths from *Morone saxatilis* juveniles, a similar species, revealed contrasted behaviour, transient and resident strategies, in nurseries in Carolina (USA) (Mohan et al., 2015).

European sea bass has a offshore winter spawning period and the first post-larvae recruitment occurs in estuaries approximately 3-4 months after, depending on the distance between areas and environmental conditions (Reynolds et al., 2003). Time of recruitment of post-larvae differs according to authors and latitude. It occurs at about 15-20 mm in March in Mont Saint Michel Bay and June in South of England (Jennings et al., 1992, Laffaille et al., 2001). These different arrival times observed could also reflect different life histories of juveniles. Daily increment in sea bass otolith allows to accurately determine the life history of juveniles through their growth rate correlated to that of fish (Regner and Dulcic, 1994, Aguilera et al., 2009). For sea bass, it increases rapidly at the end of the metamorphosis corresponding to the late post-larvae stage and the beginning of the juvenile stage at 14 mm (standard length) and 53-54 days (Regner and Dulcic, 1994). Furthermore, as observed for some other juvenile fishes (eel, flounder, gobies, *Kuhlia spp*, gilthead sea bream) a clear change of otolith growth axis indicates a change of habitat and migration phase from offshore to coastal habitats (Daverat et al., 2011, Lord et al., 2011, Feutry et al., 2012, Mercier et al., 2012, Isnard et al., 2015).

The aim of this study is to better understand the change of habitat and the life history of sea bass juveniles in coastal nurseries from 5 salt marshes (the candidate nursery habitat for the species according to Laffaille et al., 2000, Parlier, 2006, Cattrijsse and Hampel, 2006) and one seagrass bed (considered here as an alternative nursery habitat) from the French coast of the English Channel. The water element composition was firstly assessed to verify the relationship with the salinity under several environments. Life histories of juveniles was explored through the otolith elemental composition according to the otolith structure and

notably i) the marine larval stage and ii) the juvenile growth stage in the supposed nursery habitats until the capture. Additionally, the signature of capture was analysed to determine the potential salt marshes discrimination. Finally, both life history and growth performance characterizing juveniles from the different nurseries sampled were discussed according to the environmental parameters.

2. Materials and methods

2.1. Study area and fish sampling

Juveniles of European sea bass were sampled by fyke nets (4 mm mesh, 20 m long and 1.80 m height) during ebb tide in creeks of 5 salt marshes in the Normand Breton Gulf (French coast of the English Channel) from October to November 2011 and in a seagrass bed from a distant site, Brest (i.e Dinan Anse precisely), in June 2012 (n = 5) (Figure 1).

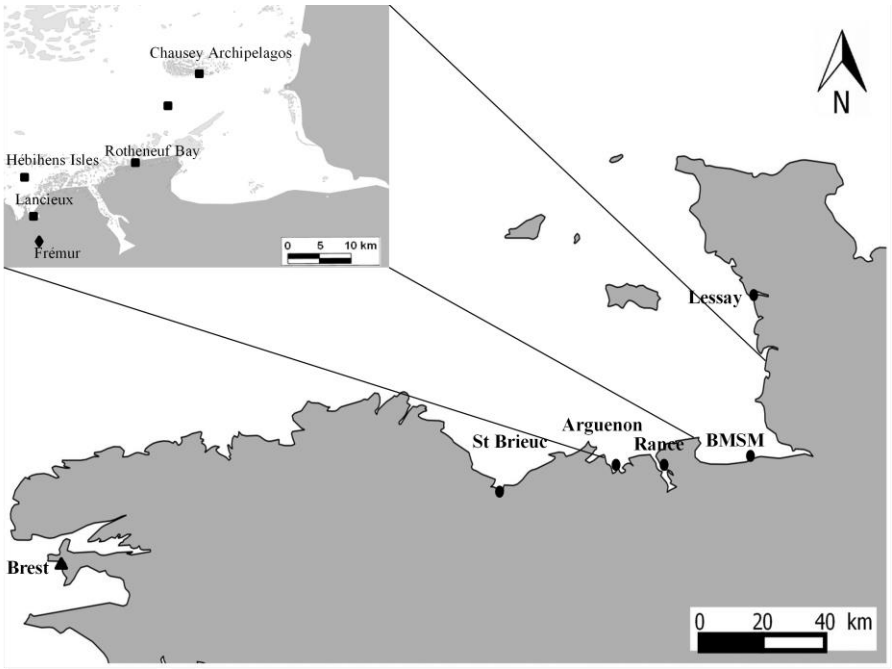


Figure 1: Location of the sampling sites in the Normand Breton Golf (south-western English Channel). Fish samples are localised by circles and triangle (Brest), and water samples for salt marshes (circle), fresh water (diamond), sea surface waters (square).

The Lessay is the northern site and is characterized by a heavily sheep grazed salt marsh and dominated by a monospecific *Elytrigia maritima* lawn (Table 1). The Mont Saint Michel Bay (MSMB) receives the output water of 3 rivers covering a drainage of 2700 km^2. Due to high tidal ranges of up to 15 m, and huge sediment accretion the tidal flats cover 240 km^2 including 200 km^2 of mudflats and 40 km^2 of salt marshes which are among the biggest in Europe (Lefeuvre et al., 2000). About 2/3 of the salt marsh is sheep grazed and therefore covered by *Puccinellia maritima* monospecific assemblages and 1/3 is ungrazed and used to be covered by a succession of communities ranging from *P. maritima* in the low marsh, *Atriplex portulacoides* in the middle marsh and *Elytrigia sp*. in the high marsh. However, to eutrophication *Elytrigia sp*. has invaded up to about 73 % of the salt marsh (Valéry et al., 2004). The Rance ria is strongly modified by a tidal plant built in 1963. The estuary covers 5.29 km^2 including 10% of salt marshes dominated by *Elytrigia sp*. and *A. portulacoides* according to the distance to the sea and the elevation. A recent proliferation of *Ulva spp.* occurred over recent years due to eutrophication. The catchment area of the Rance covers a drainage of 1400 km^2. The Arguenon is a lowly modified estuary covering about 1.2 km^2 including c.a. 0.55 km^2 of *A. portulacoides* and *Atriplex sp.* salt marshes. The catchment of the Arguenon river covers c.a. 601 km^2. The Saint Brieuc Bay has a tidal flats cover 27 km^2 with 25 km^2 of mudflats and 1.12 km^2 of salt marshes. The Bay is drained by three rivers Gouët, Urne and Gouessant. The Yffiniac Anse is a 11.4 km^2 natural reserve since 1998. The catchment area of Urne is subject to low cattle grazing and covers a drainage of 108 km^2 in the Yffiniac Anse. This bay has a high tidal range of 13 m and the salt marshes are flooded when the level is above 10.90 m. The vegetation where the fishes are captured is dominated by *A. portulacoides* (Table 1).

In each site, 10 juveniles were sampled, except in Brest where only five were sampled. The fish were measured to nearest mm (fork length (FL)) and then stored at -20°C within one hour

after capture. sea bass were smaller in Lessay (Table 1) and the largest ones were sampled in Brest (125.35 ± 12.60) due to sampling 8 months later.

Table 1: Environmental parameters of the salt marshes. Plant cover: E: *E. maritima*, A: *A. portulacoides*, P: *P. maritime*, S: *S. maritime*. Fork length (FL) (mm, mean ± sd) of the juveniles used for microchemical analysis. N (number of individual).

Site	Tidal flat (km^2)	Salt marshes (km^2)	Catchment area (km^2)	Accessiblity (%)	Plant cover	Sheep grazing	FL (mm)	N
MSMB	240	40	2700	44.38	E, A, P	High	81.64 ± 5.63	10
Rance	5.29	1.39	1400	84.11	E, A, P, S	No	87.57 ± 11.78	10
Lessay	5.8	0.56	154	54.25	E, S	High	67.07 ± 11.96	10
Arguenon	1.2	0.55	601	94.25	E, A, P	No	81.71 ± 6.71	10
St Brieuc	27	1.12	108	93.97	E, A, P	Low	87.82 ± 13.86	10

2.2. Water sampling

Water samples were collected at subsurface in 5 salt marshes to measure Sr/Ca and Ba/Ca as these ratios have proven to be particularly powerful in studies of the environmental migratory history of fish (Walther and Limburg, 2012). Additional sites corresponding to different salinities were added (Figure 1). The salinity was measured during the sampling. Samples were treated and analysed according to Tabouret et al. (2010). Briefly, for each sample, 50 mL of water were filtered through 0.45 µm syringe-driven filter (Millipore) into new (acid leached/washed) PP tubes, acidified with 500 µL of ultrapure nitric acid (JT Baker, Ultrex II) and stored refrigerated until analysis. Water samples were analyzed using solution-based ultra-sensitive inductively coupled plasma mass spectrometer (ICP-MS, Bruker Aurora Elite; www.bruker.com) following a 20-fold dilution step and standard addition for Ba and a 100-fold step dilution for Ca and Sr. Internal standard of Indium (2 µg L^{-1}) was used to correct for instrument drift. The general performance of the procedure was checked using the certified reference nearshore CASS-4 (NRCC, Canada), the latter being diluted and spiked

with Ba as in saline samples. The error of analytical reproducibility was less than 5% for both Ca, Sr and Ba. As for otoliths, Sr and Ba were standardised to calcium (i.e. Sr/Ca, Ba/Ca) and converted to weight ratio (mg Sr g^{-1} Ca for Sr/Ca and µg Ba g^{-1} Ca for Ba/Ca), thus giving concentration ratio values.

2.3. Microchemistry analysis and otolithometry

The sagittal otoliths were extracted and washed three times in an ultra-pure water bath (milliQ 0.0055 µS). After the remaining tissues were removed under a binocular, otoliths were dried and stored in 1.5-mL plastic Eppendorf tubes. The left otolith extracted from each fish was embedded in araldite resin 2020 (Huntsman) with the *sulcus acusticus* downward. They were grounded in the sagittal plane up to the core with ultra-pure water and sandpaper with grains gradually decreasing from 2400 µm to 1200 µm, 9 µm, and 3 µm. Finally, the otoliths were rinsed with ultra-pure water and air-dried.

Otolith microchemical composition was assessed using a high repetition rate 257 nm femtosecond laser ablation (Lambda 3, Nexeya, France) inductively coupled with plasma mass spectrometry (Element XR Thermo Scientific) (LA-ICPMS). In order to improve spatial resolution and sensitivity, an elongated beam shape (10 x 30 µm) parallel to the growing rings was simulated by using the special feature of this laser allowing the combination of high repetition rate (up to 100 kHz) with a fast and precise laser beam movement driven by 2D galvanometric scanner. The laser was operated here at 1000 Hz with a pulse energy of 18mJ and a beam diameter of 20 µm. The galvanometric scanner was set to move the laser beam according to a permanent 20-µm-wide, back-and-forth movement (at a speed of 1 mm/s), resulting in a virtual 10 × 30 µm laser beam perpendicular to the posterior axis. Combined with this back-and-forth movement, the sample was continuously moved along the posterior axis from the nucleus to the edge of the otolith at a speed of 5 µm/s, resulting in an

uninterrupted ablation on the grounded surface (Figure 2). Taking into account the ICPMS acquisition rate set to 1.6 s, each data point along the transect corresponded to roughly 8 µm. In order to prevent a blast effect on the nucleus, the ablation was started 200 µm before the nucleus. The ablation depth was evaluated between 5 and 10 µm. In order to prevent any contamination, a fast and soft laser pre-cleaning of the otolith surface was carried out prior each ablation along the selected transect. The laser parameters used for this pre-cleaning (4 µJ, stage speed 50 µm/s) were adjusted so that the ablation depth was kept within the range of 1-2 µm.

At the beginning and end of each session, careful calibrations were carried out using NIST 610 and 612 (National Institute of Standards and Technology). Quality control was systematically evaluated using pelletized CRM NIES 22 otolith powder (Certified Reference Material produced by the National Institute for Environmental Studies). ^{43}Ca was used as an internal standard for each ablation to correct for instrumental error in terms of ablation yield, sample transport and detection. Analysed isotopes were ^{7}Li, ^{27}Al, ^{59}Co, ^{60}Ni, ^{111}Cd, ^{208}Pb, ^{88}Sr, ^{138}Ba, ^{24}Mg, ^{55}Mn, ^{63}Cu, ^{66}Zn and ^{56}Fe, which are frequently used in microchemistry studies (Chang and Geffen, 2013). After standardization by calcium (Campana, 1999), the remaining element ratios were Ba/Ca, Sr/Ca, Mn/Ca, Zn/Ca, Cu/Ca, Li/Ca, and Mg/Ca, for which 75% of the measurements were above the limit of detection.

Furthermore, ages (in days) of 5 sea bass in the 5 salt marshes were estimated from daily growth increments and total diameter (µm) in otoliths. Two macrostructures were observed in the otoliths grounded to the core on the sagittal plane. The first zone included the core and the larval stage characterised by regular circular increments (Regner and Dulcic, 1994). The second zone was characterised by a change of growth axis and is thought to correspond to the juvenile stage that starts once the fish has recruited, after the metamorphose, into the costal

habitat (i.e nursery) (Figure 2) (Parlier, 2006). For each zone, otolith growth rate (µm/day) was estimated.

The signature of the larval stage was calculated on 36 µm and represents approximately 10 days. This mean was calculated 20-30 µm after the centre (i.e the nucleus) to avoid the peak of barium due to the maternal influence (Figure 2). The signature of the sites of capture was calculated on 17 µm length corresponding to the last 2-3 days before the fish capture.

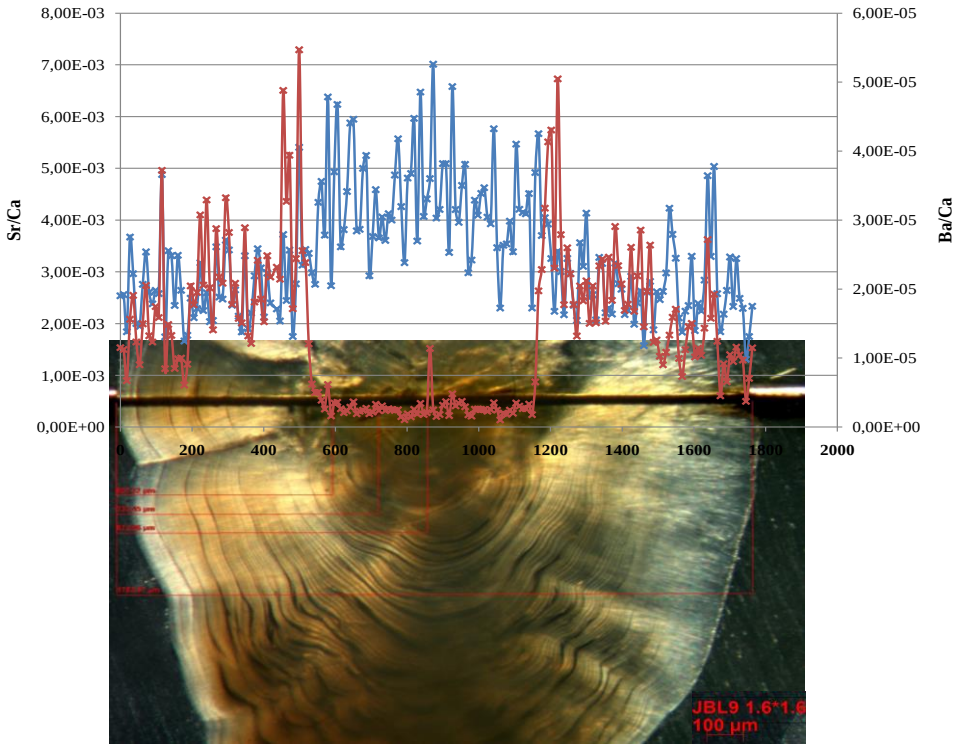


Figure 2: Microscopic photograph of an otolith from *Dicentrarchus labrax* after sagittal section (transmitted light). The laser ablation transect appears as a black line. First otolith macrostructural zone includes the hatching and the coastal arrival of first stages (i.e larvae and post-larvae) from 600 µm to 1200 µm on the laser transect. The second macrostructural zone extends from the growth axis change, corresponds to juvenile stage in costal habitats and extends from 1200 µm to the otolith edge. The nucleus is visible at 876 µm and corresponds to the observed Ba/Ca peak.

2.4. Data analysis

The signature of the sites of capture was analysed by a multivariate analysis of variance (MANOVA) type III (for un-balanced data). MANOVA were used with the Pillai's trace since it is relatively robust to deviations from multivariate normality (Johnson and Field, 1993).

Water elemental ratios (Sr/Ba) were compared with water salinity from each site. Then the salinity was compared with the Sr/Ba ratio of otoliths. The signature of capture represented different coastal salinities and the signatures of the larval stage were used to represent the marine salinity (superior to 34 psu), because the spawning occurs in offshore areas. The relation allowed to determine 4 Sr/Ba groups corresponding to 4 salinity thresholds: halin (superior to 34 psu), euhalin (30-34 psu), polyhalin (18-30 psu) and mesohalin (5-18 psu). Four groups were created to better reflect the change of environment between offshore and coastal areas such as the estuaries.

From all fishes per site, Sr/Ba average profile was calculated from the centre to the edge of otoliths and life histories were firstly graphically determined. Secondly, to specify individual life history of fish regarding to the water salinity encountered during his life, each measure of Sr/Ba in otolith profile was assigned to one of the 4 Sr/Ba groups corresponding to a different salinity. Percentages of salinity groups that the fish has encountered during his life were calculated according to the two macrostructural zones of otoliths corresponding 1) to the larval phase and 2) to the juvenile growth phase in costal habitats.

Then to determine if different life histories existed for the fishes captured in different sites, the percentages of salinity groups were compared with linear model (LM) between sites.

Finally to precise the inter-individual variability of the juvenile's costal life history, the percentages of salinity groups found for each individual, corresponding to the juvenile life stage into otolith, was represented.

Growth rates of fish during the larval and juvenile phases (see 2.3. Microchemistry analysis and otolithometry) were compared with linear models (LM) between sites.

For all LM the normality of residuals was verified with the QQ-plot and there was no violation of the assumption to apply the Gaussian distribution. When a significant difference

was detected with Anovas (F-test) type II or type III (for the un-balanced data ("car" R package)), a multiple pair-wise comparison (Tukey post-hoc test) was applied (multcomp" R package). The threshold for rejection of the null hypothesis was defined at $p = 0.05$ and coded as follow: *: $0.05 > p > 0.01$; **: $0.01 > p > 0.001$; and ***: $p < 0.001$. All statistical analyses were performed using R software (R-3.1.3 R Development Core Team 2014).

3. Results

3.1. Multi-elemental signature of sites of capture

The capture fingerprint representing the 2-3 last days of life permitted to compare different sites imprints (Table 2). Signature of Brest was significantly distinct from the others. MSMB (Bay of Mont St Michel) was also significantly different of all sites, except from that of Arguenon, which appeared not significantly different neither from Rance nor St Brieuc. Lessay was distinct to Rance, MSMB and Brest. Saint Brieuc and Arguenon sites were both less dissimilar. Note that the dicriminant elements are very variable according to the pairs of sites comparison.

Table 2: Multivariate analysis of variance (MANOVA type III) comparing the different microchemical signatures of capture (corresponding to the 5 salt marshes and Brest) of sea bass otoliths. MSMB corresponded to the Bay of Mont St Michel. Indicated p values are the mean of different p values calculated for each element ratio between pairwise analyses (Example: MSMB versus Rance). Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant).

	MSMB	Rance	St Brieuc	Arguenon	Lessay	Brest
MSMB		**$1.96e^{-03}$**** Mg***, Mn**, Ba*	**$1.33e^{-04}$***** Mn**, Sr**	NS	**$1.40e^{-02}$*** Mg**, Mn**, Cu*, Sr*, Ba*	**$1.23e^{-02}$*** Li**, Mn**, Mg*
Rance			NS	NS	**$5.14e^{-03}$**** Zn, Sr*, Ba***	**$2.68e^{-02}$*** Ba**
St Brieuc				NS	NS	**$1.40e^{-02}$*** Zn*, Sr*, Ba*
Arguenon					NS	**$3.96e^{-02}$*** Zn**
Lessay						**$1.69e^{-02}$*** Zn**, Sr*
Brest						

3.2. Life histories of sea bass juveniles

A strong correlation appeared between the water Sr/Ba ratio and the water salinity from the study sites and completed with additional water samplings (Figure 3a). The Sr/Ba ratio followed an exponential curve according to the salinity ($Sr/Ba = 7.23e^{0.141Salinity}$, $r^2 = 0.87$, n = 11, Figure 3a). Additionally, a correlation was assessed between the water salinity and the Sr/Ba ratio of otoliths. The Sr/Ba ratio followed an exponential curve according to the salinity ($Sr/Ba = 63.84e^{0.087Salinity}$, $r^2 = 0.61$, n = 85, Figure 3b). The salinities in salt marshes of Rance (24 psu) and Arguenon (9 psu) were not included in the relation because they were related to a rainy event (lower salinities than expected) and did not reflect the last days before the fish capture and consequently they did not match with the signatures of capture.

Otolith Sr/Ba ratios were compared with salinity and classified in 4 groups, with the exponential relationship, corresponding to 4 salinity thresholds. Halin group (superior to 34

psu) corresponded to Sr/Ba values superior to 1217, euhalin group (34-30 psu) included 1217-860 Sr/Ba values, polyhalin group (30-18 psu) was between 860-304 Sr/Ba values and mesohalin group (18-5 psu) contained values between 304 and 0 Sr/Ba.

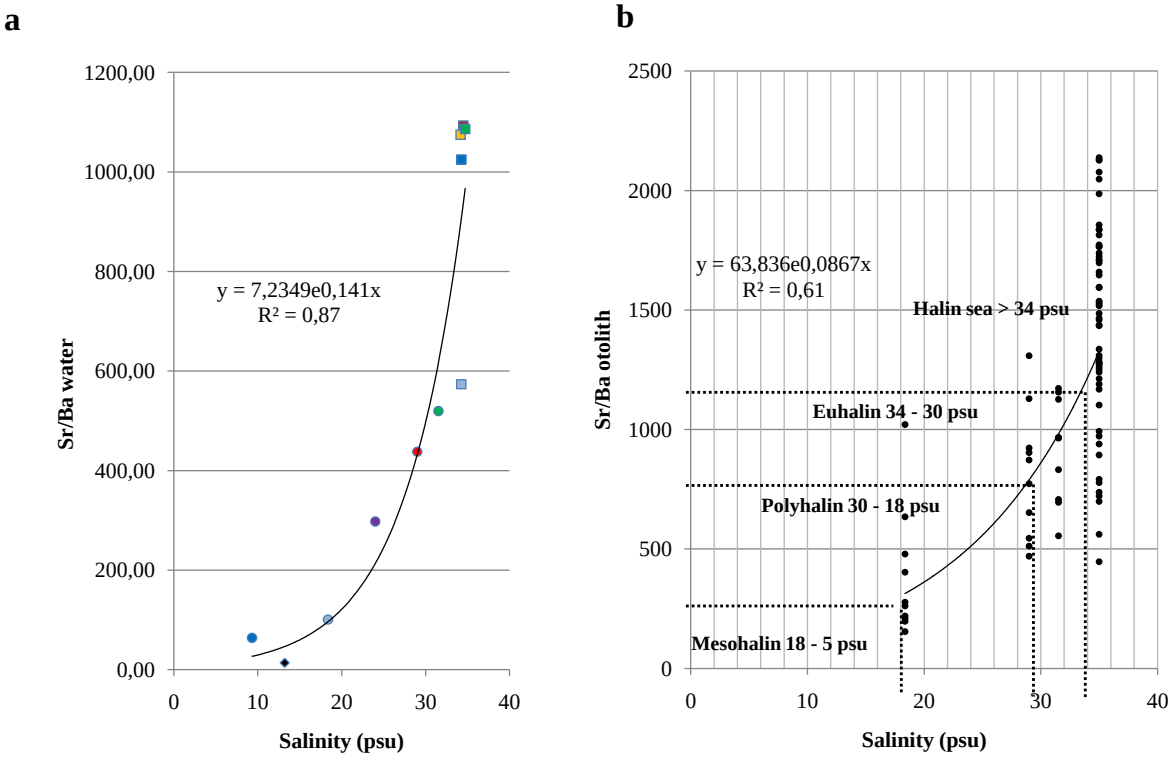


Figure 3: Salinity of salt marshes and sea surface waters with a) Sr/Ba water (n = 11), b) Sr/Ba otolith (n = 85). Water samples of the 5 salt marshes (circle): Arguenon (dark blue), Lessay (light blue), Rance (purple), MSMB (red), St Brieuc (green). Additional water samples were collected: fresh water in the Frémur river (black diamond); sea surface waters (square): Lancieux (brown), Hébihens Isles (light blue), Rotheneuf Bay (orange), Chausey Archipelago (green), between Chausey Archipelago and the continent (bleu). 4 salinity thresholds were defined: halin sea (superior to 34 psu), euhalin (30-34 psu), polyhalin (18-30 psu) and mesohalin (5-18 psu).

During the larval stage (i.e from 0 to 400 µm on the otoliths, depending on the sites) the Sr/Ba ratios were high (Figure 4) corresponding to 54.20 ± 19.00 % halin and 22.28 ± 11.21% euhalin salinities measured in otolith. Then a decrease of Sr/Ba ratios occurred, when the growth axis was changing (i.e. 400-500 µm on the otoliths approximately, depending on the sites), due to the progressive arrival of the fishes on coastal waters (Figure 4) and progressively lower salinities were detected in otoliths with 21.99 ± 9.78 of polyhalin and 1.10 ± 2.19 of mesohalin salinities. This phenomenon indicated a sea water migration phase

between the hatching and the arrival on coastal nurseries. We also observed a difference of salinities groups detected between the individuals of the different sites (Figure 5). Larvae of Lessay spend clearly more time in halin waters (84.54 ± 12.54 %) than Rance or Brest. Maybe these patterns reflected different migrations before the coastal arrival.

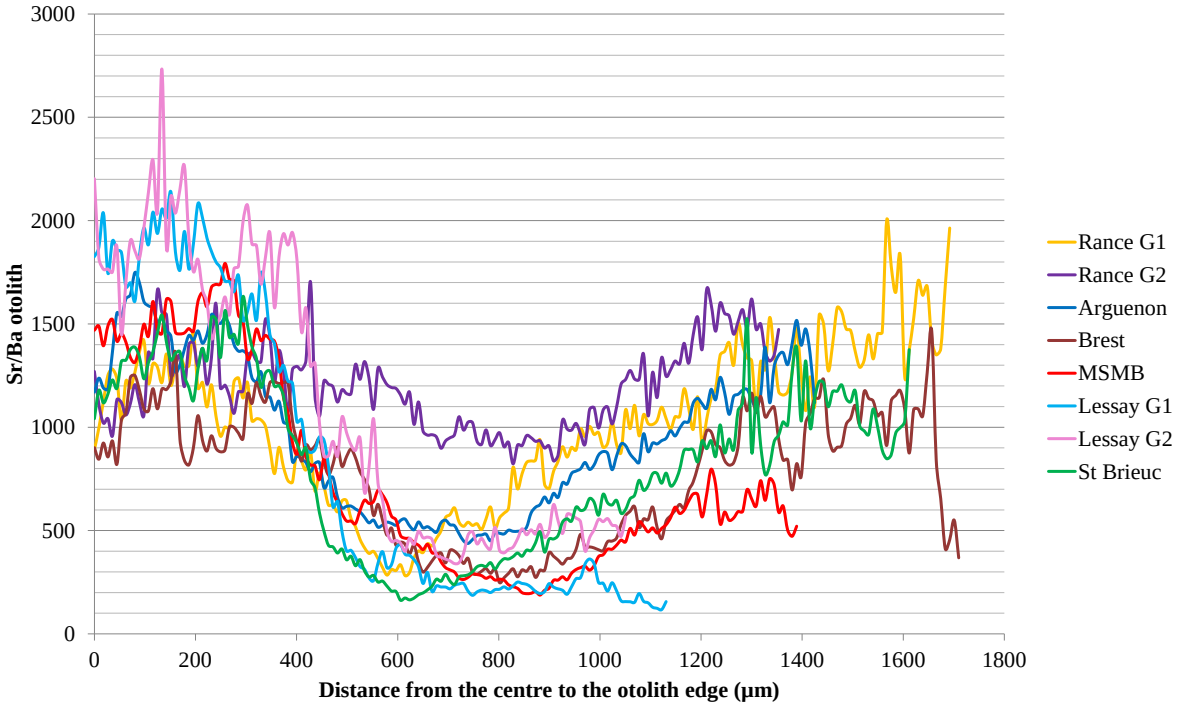


Figure 4: Sr/Ba ratio average profiles per site (n = 10) along the sagittal otolith (from 200 µm before the core to the edge) for the 5 salt marshes and Brest. The change of growth axis is observed approximately between 400 and 500 µm, depending on the sites. Rance and Lessay had two different profiles (Group 1= G1, Group 2 = G2), G2 showed a minor decrease in Sr/Ba ratio at the change of growth axis compared to other fishes in the same site.

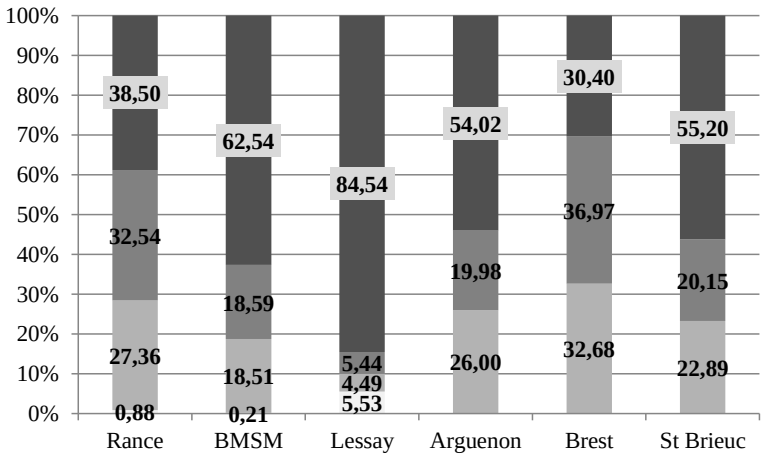


Figure 5: Groups of salinity detected during the larval stage for juveniles captured in 5 sites. 4 salinity groups: halin water (superior to 34 psu) in dark grey, euhalin (30 - 34 psu) in medium grey, polyhalin (18 - 30 psu) in light grey and mesohalin (5 - 18 psu) in white.

After the change of growth axis into otoliths, the Sr/Ba profiles declined hardly related to habitats with lower salinities, except for the Group 2 from Rance (Figure 4). Afterward these declines, the profiles were fluctuating according to sites but generally Sr/Ba ratios increased again, indicating movements into different salinities. The group 1 of Lessay showed a stable and low Sr/Ba ratio until the capture (light blue profil, Figure 4).

Rance had the highest percentage of halin water compared to the other sites (Figure 6a). The percentage of euhalin water in otoliths was different for the juveniles of Arguenon and Rance compared to Lessay and MSMB which had the lowest percentage (Figure 6b). Lessay and Rance had low percentage of polyhalin water compared to the other sites (Figure 6c). In the salinity range between 5 and 18 psu, the Lessay was significantly higher than all the other sites (Mesohalin, Figure 6d).

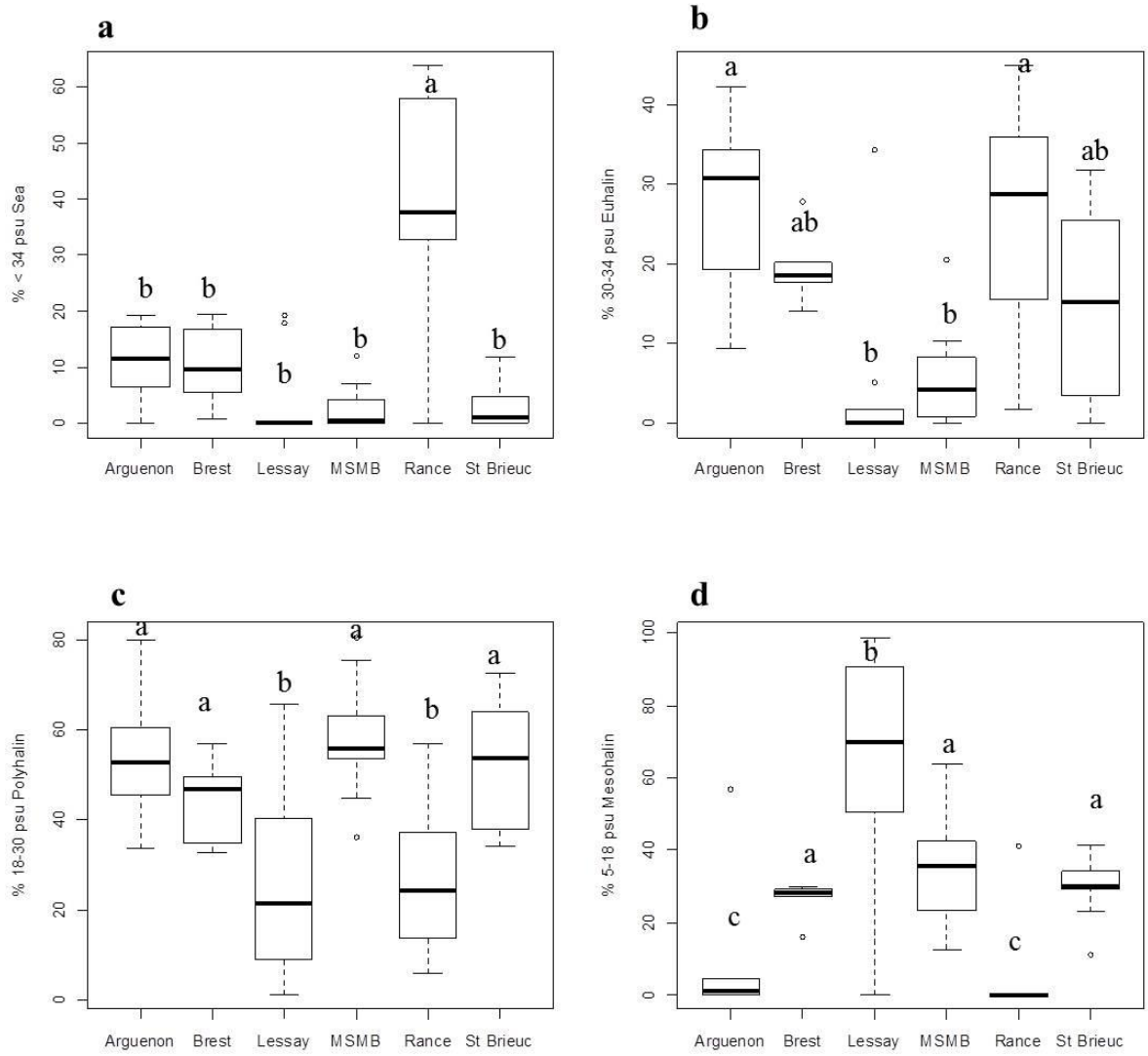


Figure 6: Comparison of salinity groups (%) detected in the juvenile otolith part between sites. a) Halin sea, $p = 2.29e^{-11}$ ***, b) Euhalin, $p = 6.48e^{-06}$ ***, c) Polyhalin, $p = 8.76e^{-06}$ ***, d) Mesohalin, $p = 1.69e^{-09}$ ***. Letters in superscript indicate significant differences ($p < 0.05$) from a linear model.

As water salinities detected in otolith were different between sites (Figure 6), the individual life histories were explored (Figure 7). More precisely, during the juvenile stage the percentage of time spent in the 4 salinity groups also exhibited individual differences within and between sites reflecting different life histories (Figure 7). At Lessay, two different strategies were observed: one individual spent most of his time in higher salinities (individual 1: 34.38 % of juvenile time spent in halin waters, 65.63 % in euhalin waters) than other

individuals spent the majority of their time in mesohalin waters (46.43 to 98.8 %) (Figure 7). On the contrary, fishes from Rance spent the majority of their juvenile time in halin waters (ranging from 32.09 to 63.85 %) and euhalin waters (10.77 to 44.95 %). Note that one individual (number 10, Figure 7) exhibited only 0 % of juvenile time spent in halin waters, and 1.75 % in euhalin but 57.02 % in polyhalin waters (18-30 psu) and 41.23 % in mesohalin waters. At the Mont Saint Michel Bay (MSMB) the percentage of time spent in halin waters is not high and 5 fishes spent only between 0.01 to 12.05 % of their juvenile time in this salinity. Juveniles spent the majority of their time (36.28 to 80.68 %) in polyhalin water and 12.50 to 63.72 % in mesohalin water (Figure 6). For Arguenon, only one individual exhibited more polyhalin and mesohalin salinities. Individuals from MSMS, Saint Brieuc and Brest showed quite similar patterns but Brest exhibited less variability of the salinity detected in their otoliths (Figure 7).

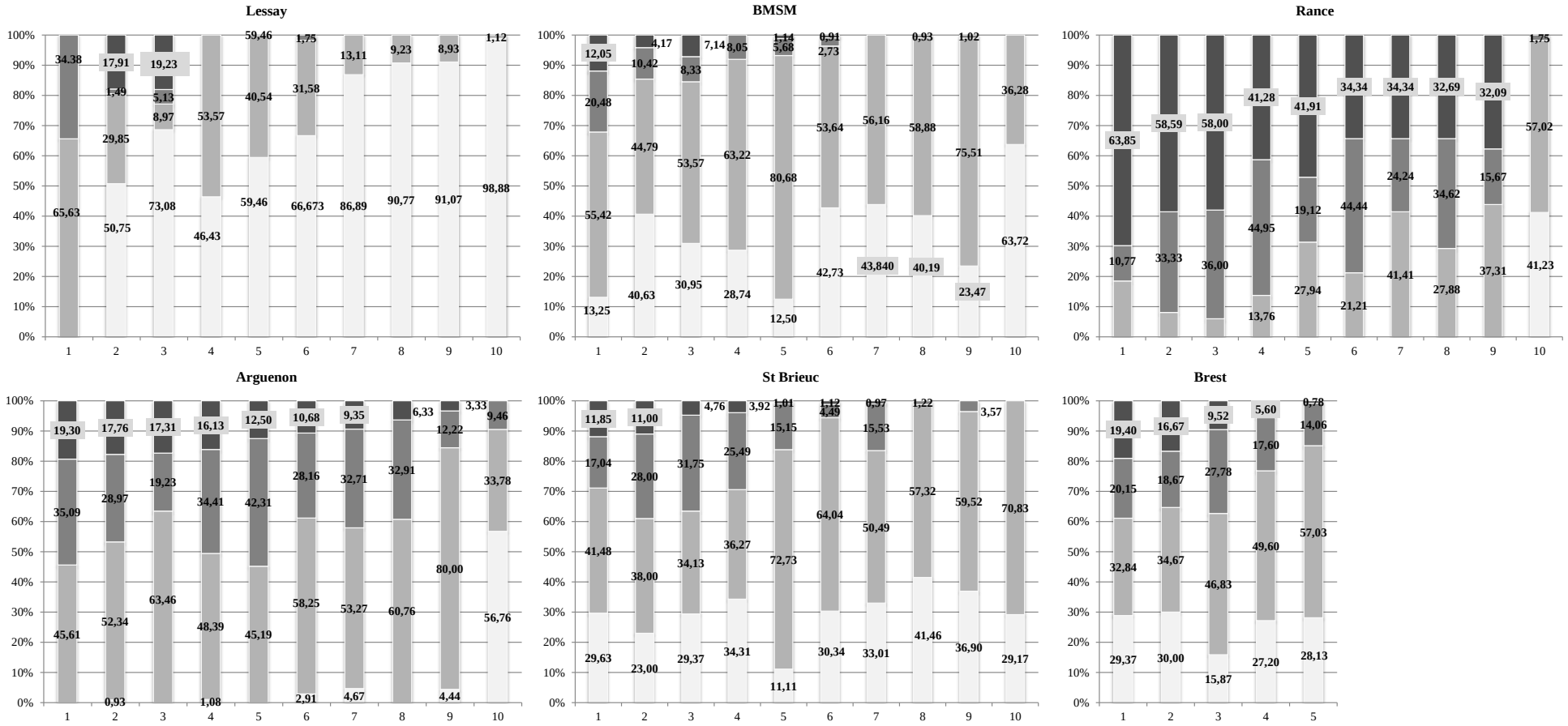


Figure 7: Individual life history: the composition of salinity groups (%) detected in the juvenile otolith part for each individual (i.e. one histogram bar) in the 6 sites. 4 salinity groups: halin water (superior to 34 psu) in dark grey, euhalin (30 - 34 psu) in medium grey, polyhalin (18 - 30 psu) in light grey and mesohalin (5 - 18 psu) in white.

3.3. Growth rates of fish and otolith

The fish fork length was highly correlated to the otolith length (FL = 0.056*Otolith length - 28.21, n = 55, r^2 = 0.84, p = < $2.2e^{-16}$***). No significant differences were observed between sites for growth rate of the first life stages (i.e larval stage to post-larval stage) corresponding to the marine life period (3.06 ± 0.85 µm/day, p = 0.11) and for the duration of the migration to the coast (83.80 ± 17.94 days, p = 0.9491). The variability in Rance is important due to two individuals, with a minimum of 58 to 132 days of sea migration. The Mont Saint Michel Bay (MSMB) had the highest fish growth rate (0.54 ± 0.06 mm/day, p = $1.60e^{-02}$ *) and all other sites have similar growth rate values (Figure 8a). The juvenile otolith growth rate was also higher for MSMB (11.02 ± 1.53 µm/day) than Lessay (7.26 ± 1.39 µm/day), Arguenon and Saint Brieuc but identical to Rance (Figure 8b, p = $1.65e^{-03}$ **).

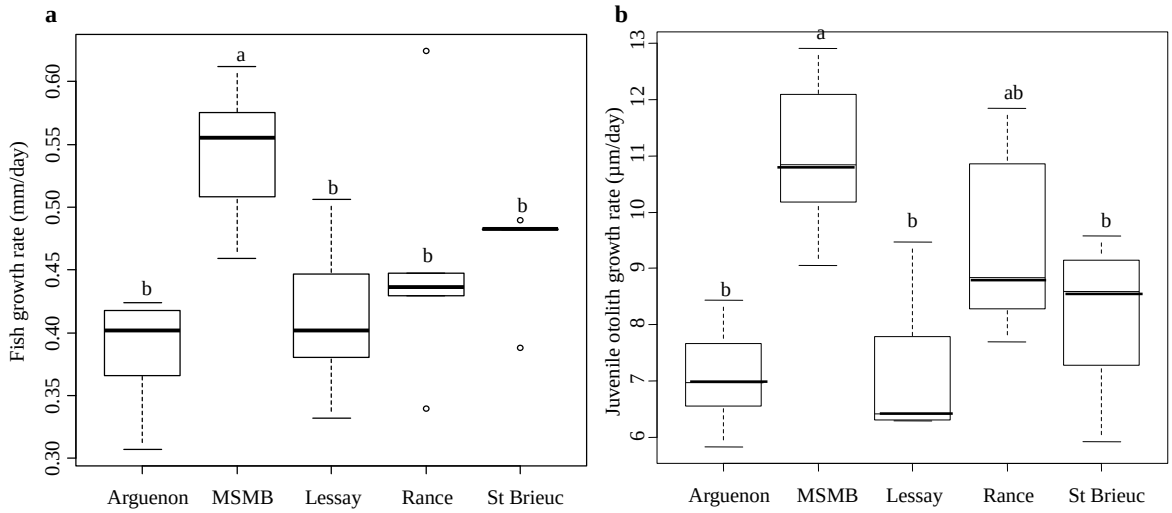


Figure 8: Comparison between sites of a) fish growth rate (mm/day) and b) otolith growth rate in the juvenile part (µm/day). Letters in superscript indicate significant differences (p < 0.05) from a linear model.

4. Discussion

For the first time on *Dicentrarchus labrax*, the otolith microchemistry along the entire life of juveniles had been reconstructed and showed the marine larval migration and the arrival into more brackish waters at the end of the post-larvae stage for the majority of the fishes captured in the salt marshes on the Normand Breton Golf and seagrass habitat. Nevertheless some patterns were distinct and few fishes had never or rarely spent time into mesohalin waters suggesting contrasted strategies of coastal habitats use. The growth rate of juveniles was related to movements or/and to quality of salt marshes and led to management implications of some coastal nurseries. 4 salt marshes had distinct capture fingerprints highlighting the potential of otolith trace element composition as reliable markers of adults' life history during their juvenile life and further to link the contribution of coastal nursery to adult stocks.

Fishes spending time in different habitats will have distinct otolith compositions but spatial scale and intrinsic characteristic of site can imply variations of this composition. Studies on the capture fingerprint of nurseries and isotopic composition in muscle for sea bass have shown distinct signature and revealed site fidelity (Green et al., 2012, Reis-Santos et al., 2015). In our study, 3 salt marshes (Rance, Lessay, MSMB) in Normand Breton Gulf were spatially distinct to other sites and also from Brest due to Mn/Ca, Ba/Ca, Sr/Ca, Zn/Ca, Mg/Ca and Li/Ca ratios. Brest was the only site where juveniles were captured on seagrass and was also the most distant site and served as an out-group showing a clear microchemical fingerprint. 4 of these element ratios (Ba/Ca, Sr/Ca, Mg/Ca, Li/Ca) have already shown differences within and between estuaries along the Portuguese coast (Vasconcelos et al., 2008, Reis-Santos et al., 2015). Salt marshes of St Brieuc, Arguenon and Rance were not significantly different from each other. Maybe the water characteristics and/or geological

bedrock cannot permit to have a distinct otolith multi-elemental fingerprint in this part of the Normand Breton Gulf, even if Sr/Ba in water of Arguenon was clearly different but due to a low salinity. However considering that the signature of capture represents the 2-3 last days of life and the distance between these sites (20 - 40 km), it remains unlike that the juveniles could migrate over such a distance during this small period.

The water composition (Sr/Ba) was a good proxy of salinity to reconstruct the past environment of sea bass. We observed a different relationship between the Sr/Ba water and Sr/Ba otolith with salinities and it was due to discrimination coefficients during the integration of element in otolith (Walther and Limburg., 2012). Nevertheless, a relation was found between salinity and otolith but some sites were not included on the relationship due to rainy event representing by lower salinities than expected (Walther and Limburg., 2012) and consequently did not match with otolith signature of capture. The relationship between the waters and otoliths is not obvious as their time scales are different, the otolith integrates chemical element on average per day or several days (equilibrate time estimate when a fish move to a novel environment) (Elsdon and Gillander, 2005, Lowe et al., 2009, Macdonald and Crook, 2010, Miller, 2011) and the water chemistry can changes more quickly and frequently, even at a daily scale. To perform a better relationship a salinity monitoring during the season of capture with microchemical analysis of water will be done in forthcoming works.

Firstly, a marine migration was observed in all juvenile otoliths and for the first time the microchemistry confirmed a progressive coastal arrival corresponding to the change of otolith growth axis for this species. During these first 2-3 months, fishes moved from offshore to coastal areas and the duration was not significant from sites, although a variability of this oceanic migration duration occurred (varied from 58 to 132 days in Rance). This result was in accordance with results from Reynolds et al, (2003), which found a large variability of

oceanic migration duration for sea bass in the South of England (2 to 4 months) back calculated according to the distance between hatching and nursery areas. Even if the duration of the migration was similar, the life history regarding to the salinity was different and larvae from Lessay had spent more time in sea water, whereas some others could arrive on coastal areas earlier. This stage is mainly dependant on the hydrodynamic of the spawning area and can lead to larval retention (James et al., 2002). The marine growth rate was not different whatever the nursery considered and quite similar to *Sparus aurata* (2.8 ± 0.4 µm/day) (Isnard et al., 2015). To precise the duration of marine migration and otolith growth rates, additional otoliths will be added in further studies as the daily rings were only counted precisely on 5 otoliths per site (i.e corresponding to the number of otoliths where the rings were clearly visible).

At their arrival on the shoreline, 14 fishes (25.45 % of juveniles) were not joining mesohalin waters at the post-larvae stage but stayed into polyhalin waters (30-18 psu) and on the contrary 18 individuals (32.72 % of juveniles) did not spend time in halin water at the juvenile stage meaning that juveniles stayed in estuaries or coastal waters exclusively. A non-negligible part of individuals exhibited a different life history regarding the nursery habitats. The variation of the intraspecific life history was detected and may be link to the different individual tolerance of salinity face to the environmental characteristics (leading to "euryhalin or freshwater phenotypes") and to their genetic background (Guinand et al., 2014, 2015). Furthermore, this declined of Sr/Ba was due to the increase of salinity and not to a physiological event, as some microchemistry profiles did not exhibit the decrease of Sr/Ba corresponding to the decrease of salinity (e.g Rance individuals). Conversely, for other species, a change of otolith composition were observed at the post-larvae stage and mainly linked to physiological process during the first life stages (Daverat et al., 2011, Woodcok et al., 2012, Mohan et al., 2015, Laugier et al., 2015).

Furthermore we observed different life histories during the juvenile life stage corresponding to different ranges of salinity. The juveniles captured in Lessay spent more time in fresh waters compared to other sites and these fishes captured in the Lessay salt marshes had a low growth rate comparing to MSMB. Juveniles captured in MSMB had a better growth rates (i.e body and juvenile otolith part) but lower than those of Tagus Estuary (0.65 mm/day) (Cabral and Costa, 2001) and spent more time in polyhalin waters than Lessay 's fishes, corresponding to range of salinities of the salt marshes and the estuaries. The Lessay was accessible on 54.25 % of the annual tides, which was quite similar to MSMB and the two sites were affected by sheep grazing but the height of plant cover at Lessay was lower reflecting a higher pressure on the plant structure with a sparse *A. portulacoides* (see Table 1). As, juveniles captured in Lessay had a low juvenile growth rate, it could be related to the high sheep grazing observed in these salt marshes reducing *A. portulacoides* abundance. This change of vegetation induces a modification of the composition of preys available for the young juvenile sea basses, for example, restricting the *O. gammarella* abundance which was their main food item (Laffaille et al., 2000, 2001, 2005). But the growth differences could also be related to the availability of prey as the salt marshes area of MSMB is 70 times wider than Lessay (see Table 1). However the microchemistry revealed high freshwater in otolith for Lessay's fishes, so maybe the prey abundances inside the salt marshes was higher to sandy areas (i.e outside the salt marshes) due to the type of sediment (Vasconcelos et al., 2010) and fishes consider that the favourable trade off was inside the salt marshes. Juveniles captured in Arguenon, Rance and St Brieuc had similar growth rates but had different life histories regarding to the salinity encountered. On the contrary Brest, St Brieuc and MSMB exhibited quite similar life histories and are three open systems comparing to the Lessay, Rance, Arguenon.

Seagrass is considered as a shelter from predators and provide high food resources through the diversity of preys (Ouisse et al., 2012, Duarte, 2002). Juveniles captured in seagrass outside and far from the 3 rivers of the Rade of Brest showed strong variability in the salinity encountered through their life (see Figure 4) but a low inter-individual variability. These juveniles captured later (8 months later) were a bit bigger and could explain potentially bigger swimming capacities to move between adjacent areas (i.e brackish waters in the Rade of Brest and marine environments) than those of Lessay which were the smallest and could be more resident to salt marshes.

Cabral and Costa (2001) as already noted that the sea bass juveniles were in high densities in a salinity ranged from 13-17 psu and 22-28 psu in estuaries which correspond to the mesohalin and polyhalin waters founded in our otoliths during the juvenile life time. The different salinities encountered into otolith of juvenile sea basses reflected various life histories as some juveniles never spent time in brackish waters during their coastal arrival and conversely some spent mainly their time in brackish waters at the beginning and during their juvenile life. Juvenile sea basses exhibited several life history reflecting a strong inter-individual variability which consist to optimize their fitness. These life histories reflect a trade-off between the salinity tolerance, the costs and the growth benefit due to the change of habitat (Gross, 1987, Guinand et al., 2015). The growth was influenced by the choice of life strategy but also by the habitat quality encountered during their larval and juvenile stages (impacted habitat, high availability of prey, accessibility, system size, temperature..) (Vasconcelos et al., 2010). Growth is a measurable life history trait and can be easily influenced by diet which provide useful information about the habitat quality and can be analyze through the stable isotopes (Isnard et al., 2015, Mohan et al., 2015). Feeding and life strategies can also induce morphological adaptations (i.e size and form of mouth, body size,

visual acuity, swimming capacities) (Shaefer et al., 2002, Cardoso et al., 2015, Selleslagh and Amara, 2015).

Even if some growth rates and salinities detected in otolith were different between sites and seemed to reveal some patterns, a direct link between these life history traits was not clearly established (tested by GLM models) and maybe explained by the low samples considering for the otolith growth rate. But a new model will be require to integrate these different information which reflect different time scales, as the growth is one *t* instant measure and the life strategy is a measure on the entire lifecycle.

Conclusion

Although intraspecific diversity of life strategies is quite common for fishes, (Jonnson and Jonnson, 1993, Secor et al., 2001, Charles et al., 2004, Kerr et al., 2009, Mohan et al., 2015), diverse life strategies have been highlighted for the first time in the entire life of the European sea bass juveniles captured in coastal habitats at a accurate time scale through the femtosecond laser on the otolith surface (Pécheyran et al., 2014, Tanner et al., 2015). The function of these salt marshes as nursery or EJH have to be precise measuring the juvenile contribution to adult stocks regarding to the multi-elemental fingerprint of juvenile in the otolith of adult fishes (Beck et al., 2001, Dahlgren et al., 2006, Vasconcelos et al., 2011).

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III. ARTICLE 4 : VARIABILITY OF FEEDING AND LIFE TRAITS TO YOUNG EUROPEAN SEA BASS IN CONTRASTED NURSERY HABITATS OF THE WESTERN CHANNEL

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Article en préparation

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Abstract

Costal habitats are known to be nurseries for fish species and salt marshes play an important role for the growth of sea bass juveniles. Because of the tidal range, fishes can only exploit salt marshes during high flood tide. This particularity is thought to induce different feeding behaviours and dependency to these habitats. The different feeding behaviours can be associated to ecomorphological adaptations such as the food acquisition or the swimming capacities. Sea bass juveniles were sampled in 5 salt marshes of the south-western English Channel and other habitats, which represented potential alternative nurseries when the salt marshes were not accessible. The $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios of muscles were analysed to reflect recent diet of the fishes. Firstly, the isotopic signatures of juveniles exhibited differences between sites at the regional scale and the juveniles of Lessay were carbon depleted whereas those of the Mont Saint Michel were more carbon enriched. Secondly, strong intra-site variability was also detected corresponding to an enrichment of the signatures of preys from the salt marshes compared to those of the bays. These strong differences of the signature preys can also explain the regional variability through various feedings. The signature of juveniles of the Mont Saint Michel represented a mixture of prey from both salt marshes and bay, suggesting that sea basses exploit these two adjacent habitats. Furthermore, the different feeding behaviours were linked to functional traits depicting bigger swimming performance of Mont Saint Michel Bay's juveniles and an adaptation to exploit this bigger salt marsh and bay. Additionally, the habitat quality could affect the feeding behaviours and consequently the fitness of juveniles. This study improves our understanding of the dependency of sea bass juveniles to different types of costal nurseries.

Key words: *Dicentrarchus labrax*, stable isotopes, diet, ecomorphological traits, salt marshes

1. Introduction

Salt marshes are important habitats for resident and transient fish species (European sea bass, mullet, eel, flounder and gobie) and play several functions as feeding and shelter areas (Paterson and Whitfield, 2000, Cattrijsse and Hampel, 2006). These habitats are ecotones between terrestrial and marine systems and are among the most productive systems with a high primary production (i.e. dry organic material) (Lefeuvre et al., 2003). Fishes move into creeks of salt marshes during the flood tide to feed on organisms and return back to neighbouring areas (e.g. mudflats) at the ebb tide (Cattrijsse and Hampel, 2006). Common crustacean species as crab *Carcinus maenas*, shrimp *Crangon crangon*, *Palaemonetes spp*., mysid *Neomysis integer*, amphipods *Corophium volutator*, *Orchestia gamarella*, and polychaetes as *Hediste diversicolor* occupying the salt marshes (Cattrijsse and Hampel, 2006) can be preyed by fishes but their accessibilities are limited by short immersion periods (few hours per day and only when the level of the tide is sufficient) depending on the tidal range, the quality of the water, the geomorphology and the location of the marsh. The feeding behaviour of coastal fishes is not completely unravel, which limits our understanding of the relative importance of salt marshes compared to other coastal habitats and their functions as essential ecological habitats. This remains nevertheless crucial for management of fish stocks and key habitats such as nurseries.

European sea basses *Dicentrarchus labrax* of the western channel, a commercial key species, spawn in late winter in offshore areas and the post-larvae recruitment occur in coastal areas approximately 3-4 months after (Reynolds et al., 2003). Juveniles are known to explore estuaries where they exploit abundant preys. These latter are present both in the pelagic and benthic habitats but also in temporary flooded areas which are exploited by juveniles during flood tides. Among them, salt marshes appear to serve as particularly interesting nursery

habitats (Laffaille et al., 2001, Hampel and Clattrijsse, 2004, Parlier, 2006). Some different behaviours are reported in litterature for sea bass juveniles. They seem able to adjust their diet from opportunistic (Pickett and Pawson, 1994, Laffaille et al., 2000, Cardoso et al., 2015) to specialist feeders (Selleslagh and Amara, 2015) and also vary according to prey availability and metabolic requests (Schaefer et al., 2002). Some changes in plant communities and sheep grazing can also modify the availability and accessibility of salt marsh preys to fishes and modify their diet (Laffaille et al., 2000, 2005). In addition the habitat quality (i.e. anthropogenic pressure) could impact the feeding strategy from generalist to specialist (Selleslagh et al., 2012). Moreover the feeding behaviour depends on the habitat use strategy (i.e. resident or visitor) (Cardoso et al., 2015, Mohan et al., 2015) and could be related, for some fish species, to anatomical changes in predation mechanisms according to the increase of swimming capacities, the position in the water column, the mouth form and/or the visual acuity (Schaefer et al., 2002, Villeger et al., 2010, Spitz et al., 2014, Cardoso et al., 2015, Selleslagh and Amara, 2015). Some studies using stable isotopes report for sea bass juveniles a low connectivity within and between salt marshes and estuaries and consequently a strong site fidelity to a particular part of an estuary (Green et al., 2012, Reis-Santos et al., 2015). They also suggested that their isotopic composition differences where mainly due to some distinct habitat signatures and not necessarily to change of diet (Green et al., 2012, Reis-Santos et al., 2015). All of these different behaviours and fidelities did not provide a clear habitat use strategies for sea bass juveniles in specific areas: the salt marshes.

Stable isotope composition has been used to study trophic link in food webs, diets and patterns of migration (Hansson et al, 1997, Ménard et al., 2007). The assumption is that primary producers at the base line with distinct isotope compositions influence isotope ratios of predators through propagation in the food web. For example, halophyte C3 salt marsh plants have a depleted carbon ratio (-30 to -26 ‰) as *Atriplex portulacoides, Elytrigia*

maritima, Sueda maritima, Puccineli maritima (Créach et al., 1997, Gray and Mogg, 2001, Cloern et al., 2002, Eley et al., 2016) compared to marine particulate organic matter (-25.0 to -23.1 ‰) and macroalgaes (-22 to -11 ‰) (Cloern et al., 2002, Riera et al., 2009, Green et al., 2012). Consequently if fishes are residents, they will exhibit the signature of the sources of the habitat where they were sampled but if they move between habitats their signatures will be intermediate between the different habitats or sources explored. The fish juveniles with fast growth rates have a rapid metabolism and their isotopic composition reflects recent diet across weeks (Herzka et al., 2005, Buchheister and Latour, 2010). Recently the development of mixing models based on $\delta^{13}C$ and $\delta^{15}N$ provides information on the contribution of the prey items to the diet of organism and are a complementary approach to better understand the origin of isotopic signature (Parnell et al., 2010, Kostecki et al., 2012, Spitz et al., 2013).

We investigated the hypothesis that the dependency to the salt marshes and the feeding behaviour of juvenile sea basses varied and potentially according to nursery habitat characteristics. First we examined the isotopic composition of juvenile sea basses sampled in 10 sites distributed along the French coast of the Channel. Secondly to better understand the inter-site variability we analysed the isotopic signature at a local scale (i.e. intra-site variability) in relation to isotopic composition of sources. Finally the dependency to the salt marshes and the fish feeding behaviour was discussed regarding to morpho-anatomical traits, habitat quality and habitat use strategies.

2. Material and methods

2.1. Study area and fish sampling

Juveniles of sea bass were collected by fyke nets (4 mm mesh, 20 m long and 1.80 m height) during ebb tide in creeks of 5 salt marshes in the Normand Breton Gulf from the 13 October to the 3 November 2011 (Figure 1). The 5 salt marshes were chosen for their

different characteristics regarding to their location, size (tidal flat and salt marsh surfaces), accessibility and pressures (plant invasion and sheep grazing) (Table 1). The tidal flats of MSMB cover 240 km^2 including 200 km^2 of mudflats and 40 km^2 of salt marshes which are among the largest in Europe (Lefeuvre et al., 2000). The accessibility for the nekton to the salt marshes depends on the tidal range and varies among the 5 salt marshes (Table 1). For example the Lessay salt marshes are accessible during 54.25 % of the year, whereas the small salt marsh of Arguenon is accessible 94.25 % of the year and is a lowly modified estuary.

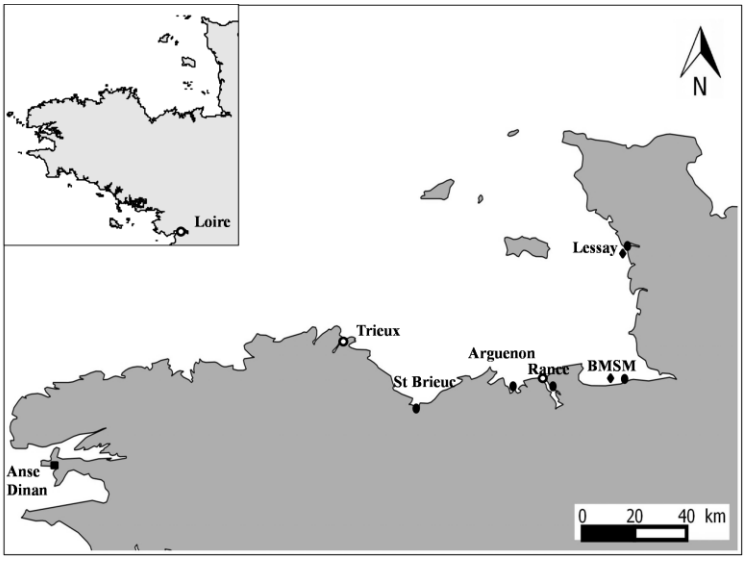


Figure 1: Location of the sampling sites in the Normand Breton Gulf (south-western English Channel) and Loire Estuary. Different sampling habitats were represented by filled circles for salt marshes: Lessay, BMSM, Rance, Arguenon, St Brieuc; open circles for estuaries: Trieux, Rance, Loire; diamonds for Bays in front of salt marshes: Lessay, BMSM and a square for the seagrass bed: Anse Dinan (i.e Brest).

Table 1: Environmental parameters of the 5 salt marshes sampled. Plant cover: E: *E. maritima*, A: *A. portulacoides*, P: *Puccinellia maritima*, S: *Sueda maritima*.

Site	Tidal flat area (km^2)	Salt marshes area (km^2)	Catchment area (km^2)	Accessibility (%)	Plant cover	Sheep grazing
MSMB	240	40	2700	44.38	E, A, P	High
Rance	5.29	1.39	1400	84.11	E, A, P, S	No
Lessay	5.8	0.56	154	54.25	E, S	High
Arguenon	1.2	0.55	601	94.25	E, A, P	No
St Brieuc	27	1.12	108	93.97	E, A, P	Low

The plant cover represents the main structure of the vegetable on each salt marsh and can be explain by plant invasion or sheep grazing (Table 1). In the MSMB, about 2/3 of the salt marsh is sheep grazed and therefore covered by *Puccinellia maritima* monospecific assemblages and 1/3 is ungrazed and used to be covered by a succession of communities ranging from *P. maritima* in the low marsh, *A. portulacoides* in the middle marsh and *Elytrigia sp*. in the high marsh. However, to eutrophication *Elytrigia sp*. has invaded up to about 73 % of the salt marsh (Valéry et al., 2004). The Lessay is also characterized by a heavily sheep grazed salt marsh and dominates by a monospecific *Elytrigia maritima* lawn. The Saint Brieuc salt marshes are quite preserved with a 11.4 km^2 natural reserve since 1998, so the pressures such as the sheep grazing is low (Table 1).

Additional fishes were sampled in 2012 in different habitats: in subtidal areas from three estuaries (Rance, Trieux and Loire, see Figure 1) (with a small trawl), in a seagrass bed with small seine (Anse Dinan outside the bay of Brest) and using fyke nets into mussel cultures corresponding to an adjacent habitat of salt marshes of MSMB, hereafter called “bay” (Figure 1).

Consequently, to test year variability on stable isotope compositions, other juveniles were fished in the salt marshes of Rance and Lessay in 2012.

In the 5 salt marshes in 2011, 15 juveniles were measured to nearest mm (fork length (FL)) and then stored at -20°C within one hour after capture. In 2012, 5 individuals per site were analysed (Trieux estuary, Lessay salt marshes, bay MSMB, Dinan Anse) except in the Loire Estuary (n = 7), in Rance Estuary (n = 3) and in Rance salt marshes (n = 3). A total of 108 juveniles were analysed.

To understand the isotopic signature at the local scale (i.e. intra-site variability), the potential sources and prey items of juveniles (salt marsh plants, seaweeds, mysids,

amphipods, crustaceans, polychaetes, bivalves, gobies, etc.) were collected in Lessay and MSMB salt marshes and in their two associated bays (Figure 1). They represented different functional groups (sources, primary and secondary consumers).

2.2. Stable isotope analysis

Stable isotope analyses were achieved on muscle for sea bass juveniles and all organisms sampled in the two sites (i.e Lessay and MSMB). Samples were immediately stored in an Eppendorf tube, frozen, freeze-dried, ground into a fine powder. To avoid erroneous $\delta^{13}C$ values due to inorganic carbon content in shells or cuticles, crustaceans and polychaetes were decarbonated by HCl 0,1N evaporation, according to the method recommended by LIENSs lab, La Rochelle, France. For small species, several individuals were pooled together. In case of decarbonation, two replicates were performed, one to obtain $\delta^{13}C$ value (i.e. decarbonated) and another for $\delta^{15}N$ value (i.e. without decarbonatation). No lipid extraction was done on the muscle samples as the lipids content in white muscle is very low (Sweeting et al, 2007) and because all C/N ratios were below the threshold of 4 as recommended by McConnaughey and McRoy, (1979) and Sweeting et al, (2007). All samples were weighed (0.40 ± 0.05 mg) in tin cups for stable isotope analyses. They were performed with Flash EA 1112 elemental analyser coupled to IRMS Thermo Scientific Delta V Advantage mass spectrometer (La Rochelle lab, France). The calibrations were carried out using isotopic international standards (IAEA, Vienne, Australie): Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The results are presented in the usual notation δ in parts per thousand (‰) relative to the deviation from standards. The analytical precision is based on replicate measurements of internal laboratory standards (n = 10) and the experimental precision is ± 0.03 ‰ and ± 0.08 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. In addition, the C/N ratio was used as a complementary indice of condition as it reflects the ratio lipid to protein, consequently more the ratio is high and more

the lipids are present into the muscle which represents energy reserves (McConnaughey and McRoy, 1979, Sweeting et al, 2007).

As the fish size can affect the isotopic composition we tried to collect the same fish size whatever sites and years (mean 82.65 ± 16.40 mm). Nevertheless, the fishes from Dinan Anse were significantly longer than those of other sites (excepted those from Rance estuary) and Lessay fishes were smaller than others except from those of Trieux estuary, Rance 2012 and in the mussel cultures in the MSMB.

2.3. Locomotion and food acquisition capacities

Ecomorphological traits were assessed to reflect food acquisition and locomotion capacities on fishes collected in the 5 salt marshes in 2011 and at Dinan Anse. The assumption is that fish may need to adapt to local conditions and food availability which is likely rely on swimming (i.e. movements between feeding habitats and between feeding and resting habitats) and feeding capacities (i.e. mouth size). Traits were measured from photographs of each fish using ImageJ software to calculate 16 functional indices (Mouchet, 2010) (Table 2).

2.4. Data analysis

The isotopic compositions between years, habitats, sites and within sites were tested with linear models. Additionaly to understand the intra-site structure for two sites, Lessay and MSMB, all the values were plotted graphically according to trophic groups of the preys (sources, primer (C1) and secondary consumers (C2)) captured into salt marshes and the adjacent bays.

Table 2 : Description of the functional indices for the two functions calculated from the ecomorphological measurements (adapted from Mouchet, 2010).

Component	Functional indice	Code	Calculated indice
Physiological state	Fulton indice	K	weight / (fork length)^3
Type/size of food items captured	Oral gape surface	Osf	(mouth (maximum width*maximum height*corner width)/((maximum width^2*maximum height)body)
Method to capture food items	Oral gape shape	Osh	maximum height mouth / maximum width mouth
Feeding position in the water column	Oral gape position	Ops	mouth height / head height
Prey detection / visual acuity	Eye size	Edst	eye diameter / head height
Position of fish and /or prey in water column	Eye position	Eps[a]	eye position to the base of the head*eye position to the anterior end of the head /head height*body length
Vertical position in the water column and hydrodynamism	Body transversal shape	Bsh	body height / body width
Mass repartition along the body for hydrodynamism	Body transversal surface	Bsf	body width * body height / body weight
Pectoral fin use for maneuverability	Pectoral fin position	PFps	position of the pectoral fin / body heigth at the pectoral fin insertion
Pectoral fin use for propulsion	Aspect ratio of pectoral fin	Pfar	maximum pectoral fin length^2 / pectoral fin surface
Caudal fin use for propulsion	Caudal peduncle throttling	CPt	maximum caudal fin depth / peduncle minimal depth
Quality of propulsion and direction	Aspect ratio of caudal fin	Cfar	maximal caudal fin depth / caudal fin surface
Type of propulsion between caudal and pectoral fins	Fins surface ratio	Frt	2* pectoral fin surface / caudal fin surface
Acceleration and /or maneuverability efficiency	Fins surface to body size ratio	Fsfd[a]	((2*pectoral fin surface) + caudal fin surface + dorsal fin surface) / (∏ / 4*body width*body depth)
Dorsal fin use for maneuverability	Insertion dorsal fin	Dfi[b]	position of the dorsal fin to the head end / fork length
Dorsal fin use for propulsion	Aspect ratio of dorsal fin	Dfar[b]	base of the dorsal fin length / dorsal fin surface

[a]Adapted from Mouchet, 2010

[b]New indice created for the dorsal fin

Then, we used SIAR R package to model the variations of juvenile diets according to sources. Models provide insights into inter-site and intra-site variations of the feeding behaviour for Lessay and MSMB. $\delta^{13}C$ and $\delta^{15}N$ values of the potential prey items (i.e. sources) caught in salt marshes and in bays were used to measure the relative contribution of these food sources to the diet. The Trophic Enrichment Factor (TEF) was set to 1 ‰ for carbon and 2.4 ‰ to 3.4 ‰ for nitrogen for the primary and secondary consumers, respectively (Zanden and Rasmussen, 2001, Sweeting et al., 2007, Kostecki et al., 2010, Spitz et al., 2013). A tolerance of 0.1 ‰ was chosen for all model runs according to Phillips and Gregg (2003) recommendations. In MSMB exclusively, we modelled the diets of juveniles of salt marshes and those captured in the bay (i.e intra-site variability).

A matrix of dissimilarity based on the Gower coefficient was calculated on the ecomorphological traits value related to swimming capacities for each pair of individuals (Gower, 1971). A dendrogram was achieved from an Ascending Hierarchical Classification (AHC) (average method) on this matrix to separate juveniles in groups according to their dissimilarities. Then each fish was individually assigned to a group in order to analyze the distribution of groups within and between sites. Finally, each group was characterized by its ecomorphological traits using a linear model. This methodology was not applied to the ecomorphological traits related to the food acquisition due to the low dissimilarity between juveniles. In this case, ecomorphological differences were directly compared between sites with linear model.

For all LM the normality of residuals was verified with the QQ-plot and there was no violation of the assumption to apply the Gaussian distribution. When a significant difference was detected with the Anova (F-test), a multiple pair-wise comparison (Tukey post-hoc test) was applied ("multcomp" R package). The threshold for rejection of the null hypothesis was

defined at p = 0.05 and coded as follow: NS = non-significant;*: 0.05 > p > 0.01 ; **: 0.01 > p > 0.001 ; and highly significant ***: p < 0.001. All statistical analyses were performed using R software (R-3.1.3 R Development Core Team 2014).

3. Results

3.1. Ontogenic, temporal, habitat and inter-site variabilities

Firsltly the ontogenic effect was tested on these two isotopes. $\delta^{15}N$ was not influenced by the fish size (linear model, pvalue > 0.05). Conversely, $\delta^{13}C$ was significantly correlated to fish size of the juveniles (n = 104, pvalue = $1.15*e^{-4}$, r^2 = 0.14). This correlation was mainly affected by the small size of the Lessay juveniles and their carbon depletion. These results indicated that stable isotope enrichments in muscle were not only controlled by an ontogenic effect.

Year effect was only significant for juveniles in Lessay salt marshes, which had ^{13}C more enriched muscle (1.44 ‰) in 2012 compared to 2011 but no differences in $\delta^{15}N$ values were observed (Figure 2, Table 3). The large carbon variability in 2012 was due to one individual with very depleted muscle (-28.05 ‰) and another one very enriched (-16.08 ‰). The sea bass isotopic ratios in Rance were identical whatever the year (Table 3), even if the large $\delta^{13}C$ variability in 2012 was due to one depleted sample (-24.88 ‰) (Figure 2). These results suggested the stability of the signature for individuals caught in the same area even at different times.

The isotopic composition of sea bass muscles showed strong $\delta^{13}C$ and $\delta^{15}N$ differences according to sampling location, respectively ranging from -23.02 ± 4.45 to -15.84 ± 0.60 ‰ and from 13.90 ± 0.24 to 17.71 ± 1.30 ‰ (Figure 2).

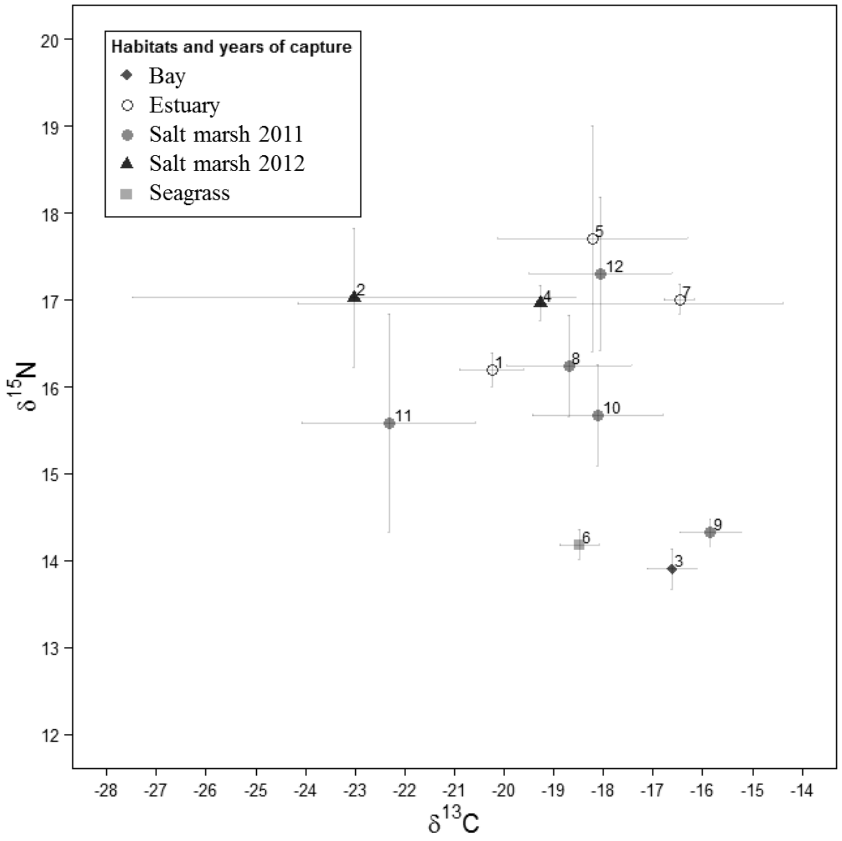


Figure 2: $\delta^{13}C$ and $\delta^{15}N$ (mean ± sd) signatures of *Dicentrarchus labrax* muscles distinguished according to locations, habitat types and years: 1 - Loire Estuary; 2 - Lessay 2012; 3 - Bay MSMB; 4 - Rance 2012; 5 - Rance estuary; 6 - Dinan Anse; 7 - Trieux estuary; 8 - Arguenon; 9 - MSMB, 10 - St Brieuc; 11 - Lessay 2011; 12 - Rance 2011.

Table 3: Linear models (LM) applied on the isotopic signatures testing the annual variability at Lessay and Rance (2011 and 2012), the habitat effect (salt marsh, estuary, bay, seagrass) and finally the inter-site differences for the 5 salt marshes. Df is the degree of freedom.

	Df	**F**	**p**
Lessay - year			
$\delta^{13}C$	1	0.27	0.61 NS
$\delta^{15}N$	1	5.71	0.028 *
Rance - year			
$\delta^{13}C$	1	0.76	0.40 NS
$\delta^{15}N$	1	0.44	0.51 NS
Habitats			
$\delta^{13}C$	4	1.18	0.32 NS
$\delta^{15}N$	4	11.75	$< 1.27e^{-6}$ ***
Inter site - salt marshes			
$\delta^{13}C$	4	45.11	$< 2.2e^{-16}$ ***
$\delta^{15}N$	4	27.84	$< 8.95e^{-14}$ ***

A habitat effect was significant for the nitrogen isotope and only the seagrass was not different to the Bay of MSMB. No significant difference was detected for $\delta^{13}C$ values between habitats (Table 3).

Then, strong and significant inter-site differences in carbon and nitrogen isotope compositions were detected between the 5 salt marshes in 2011 (Figure 2 and Table 3). Fishes from Lessay salt marshes were significantly depleted in carbon (-22.32 ± 1.74 ‰) compared to all other sites (Figure 2). Fish from Rance salt marshes (-18.06 ± 1.43 ‰) were not significantly different in carbon isotope composition from Arguenon and St Brieuc. Moreover, fishes from MSMB (-15.84 ± 0.61‰) were enriched in carbon compared to other sites. Additionally, the $\delta^{15}N$ values of the sea bass juveniles captured in the 5 salt marshes exhibited significant differences (Figure 2, Table 3). MSMB had the lowest value (14.32 ± 0.16 ‰). The middle group included Lessay, St Brieuc, and Arguenon (respectively, 15.59 ± 1.26, 15.67 ± 0.58 and 16.25 ± 0.58 ‰) and Rance salt marshes had the highest ^{15}N enrichment.

Finally, inter-site differences were high and the habitat effect was hard to discuss due to the lack of habitats in a same location. In order to explain these inter-site differences at the regional scale, the food webs of Lessay and MSMB, which represented the extreme values, were analysed.

3.2. Intra-site differences in Lessay and MSMB

3.2.1. Lessay site

Sea bass juveniles, sources and potential prey organisms captured at Lessay salt marshes, were $\delta^{13}C$ depleted (sea bass juveniles 2012: -23.02 ± 4. 45 ‰; 2011: -22.32 ± 1.74 ‰)

compared to the organisms of the bay, showing a clear gap between those two proximate habitats and probably two independent food webs (salt marshes vs bay) (Figure 3).

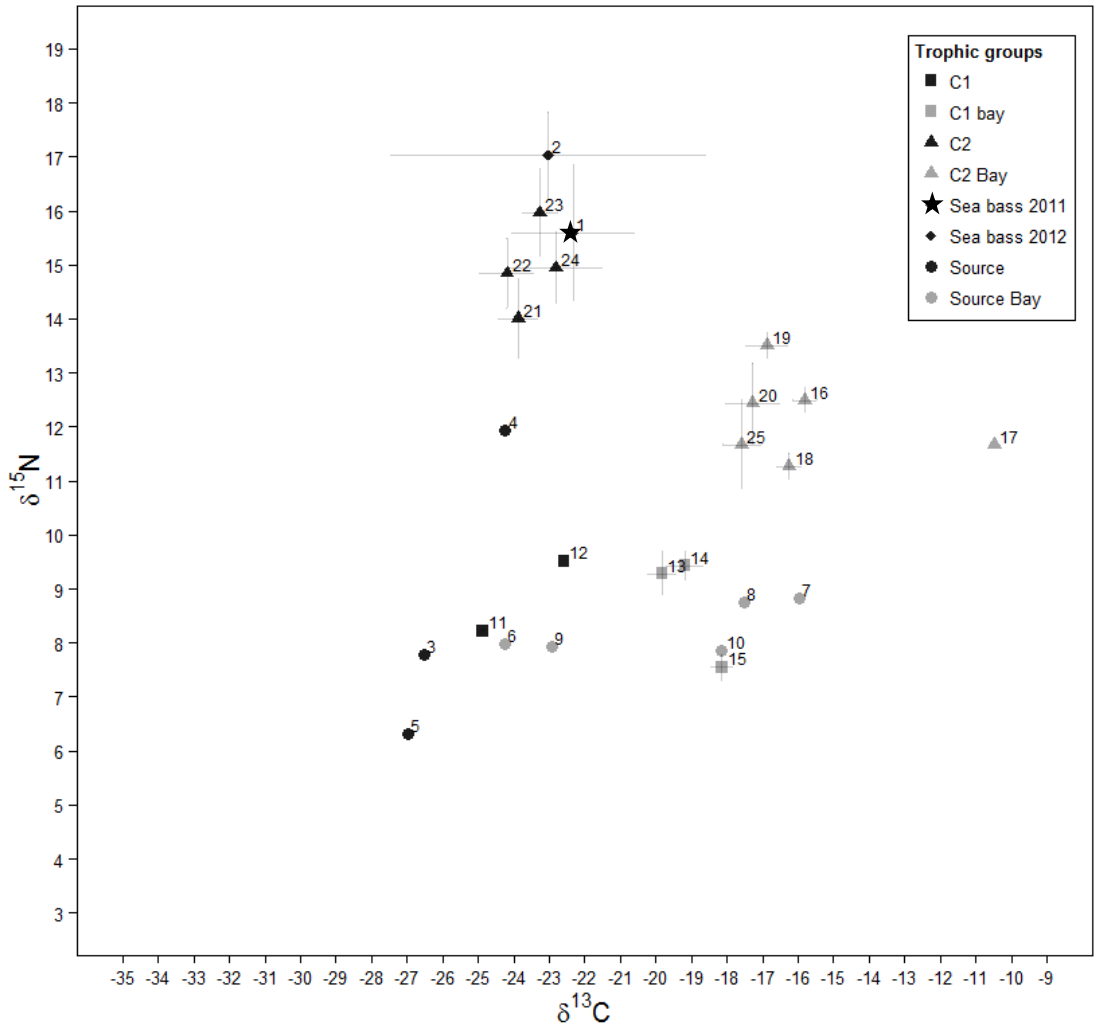


Figure 3: Isotope signatures of the food web of Lessay (mean and standard deviation). Black symbols corresponded to salt marshes organisms and grey symbols to organisms collected in the bay (i.e. outside of the salt marshes). The trophic groups represent sources, primer consumers (C1) and secondary consumers (C2). 1 - *D. labrax* 2011; 2 - *D. labrax* 2012; 3 - *S. maritima*, 4 - *A. portulacoides*, 5 - *E. maritima*; 6 - *Calliblepharis ciliata*; 7 - *Fucus serratus*; 8 - *Ascophyllum nodosum*; 9 - *Chondrus crispus*; 10 - *Halopitys incurva*; 11 - *Gammaridae*; 12 - *Sphaeromatidae*; 13 - *Crassostrea gigas*; 14 - *M. edulis*; 15 - *Cerastoderma edule*; 16 - *Nephtys sp*; 17 - *Leptosynapta inhaerans*; 18 - *Paraonidaes*; 19 - *Lumbrineridae*; 20 - *Nemerthe sp*; 21 - *C. maenas* (salt marsh); 22 - *Palaemons elegans*; 23 - *Pomatoschistus microps*; 24 - *C. crangon*; 25 - *C. maenas* (bay).

The salt marsh plants (*E. maritima* and *Sueda maritima*) were carbon depleted as the Gammaridae (-24.87 ± 0.07 ‰) (i.e amphipods) known to feed on plant detritus. In the bay, only red algae (*C. ciliata*) and (*C. crispus*) had low $\delta^{13}C$ values compared to the other species

of algae (for example: *F. serratus*, *A. nodosum*) but did not appear directly implicated neither in the salt marsh nor in the bay food webs. Note that some filter-feeders of the bay (*M. edulis* and *C. gigas*) were also more $\delta^{13}C$ depleted compared to the other organisms of the bay, probably due to consumption of a mix of plankton and particulate organic matter (POM, non-sampled in this site) from both food webs. Only *C. maenas* was sampled in salt marshes and bay and as other organisms found in the salt marsh, it exhibited the same $\delta^{13}C$ depleted pattern compared to the bay, confirming the hypothesis of a distinct food webs ($\delta^{13}C$ = -23.87 ± 0.55 ‰ and -17.58 ± 0.55 ‰ in salt marsh and bay, respectively).

3.2.2. MSMB site

In the MSMB, juveniles captured in the salt marshes had more ^{15}N and ^{13}C enriched muscles compared to those of juveniles from the bay ($\delta^{13}C$ and $\delta^{15}N$ respectively, $p = 3.10e^{-04***}$, p = 0.02*), but this difference appeared weak compared to the variability of the food web signatures (Figure 4).

Organization of the food webs in salt marshes and bay in MSMB appeared greatly different from those of the Lessay salt marshes (Figure 4). Sea bass juveniles captured in the salt marshes of the MSMB were more ^{13}C enriched (-15.84 ± 0.61 %) compare to juveniles of Lessay (-23.02 ± 4.45 %) and to the whole salt marsh food web (Figure 4). On the contrary the sources of salt marshes (i.e phanerogams) had depleted $\delta^{13}C$ and $\delta^{15}N$ ratios as the salt marsh of Lessay (Figure 4).

Interestingly, *Orchestia gammarella* (i.e amphipods) had similar signatures in MSMB and Lessay (-24.87 ± 0.07 ‰, 8.22 ± 0.03 ‰ ; -23.43 ± 0.48 ‰, 8.34 ± 0.86 ‰ in MSMB and Lessay, respectively). Red algae were carbon depleted (-34.39 and -33.28 ‰, *Plocamium sp.* and *Sphaerococcus coronopifolius*, respectively). The river POM (Particulate Organic Matter) was more ^{13}C depleted (-27.61 ± 2.29 ‰) than the sea POM (-23.63 ± 0.23 ‰) and the

phytoplankton (-19.90 ‰). The mysids collected in the salt marshes had almost the same isotopic composition than those collected into the bay (-15.89 and 11.07 ‰, -16.38 and 11.77 ‰, for the salt marshes and bay, respectively). *H. diversicolor* had an enriched value of carbon (-16.06 ± 0.16 ‰) compared to the other organism collected in the salt marshes.

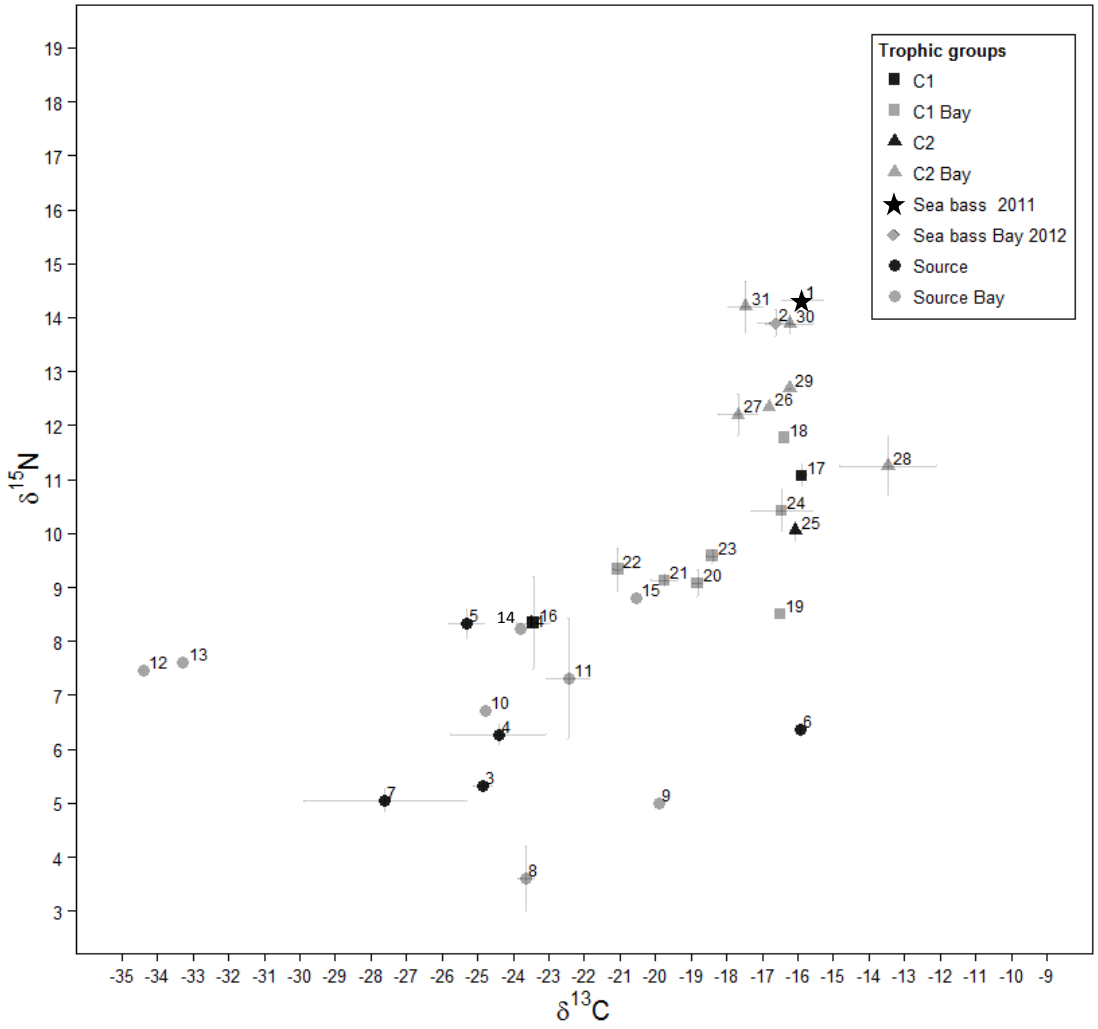


Figure 4: Isotope signatures of the food web of MSMB (mean and standard deviation). Black symbols corresponded to salt marsh organisms and grey symbols to organisms collected in the bay (i.e. outside of the salt marshes). The trophic groups represent sources, primer consumers (C1) and secondary consumers (C2). 1 - *D. labrax* 2011; 2 - *D. labrax* 2012 Bay; 3 - *E. maritima*; 4 - *P. maritima*; 5 - *A. portulacoides*; 6 - microphytobenthos; 7 - river POM; 8 - marine POM; 9 - phytoplankton Bay; 10 - *Taonia sp.*; 11 - *Ceramium sp.*; 12 - *Plocamium sp.*; 13 - *S. coronopifolius*; 14 - *Calliblepharis ciliata*; 15 - *Solieria chordalis*; 16 – Amphipoda (*Orchestia gammarellus*); 17 – Mysidacae (salt marsh); 18 - Mysidacae (bay); 19 - *Albra albra*; 20 - *Crepidula fornicata*; 21 - *M. edulis*; 22 - *Crassostrea gigas*; 23 - *Cerastoderma edule*; 24 - *Arenicola arenicola*; 25 - *H. diversicolor*; 26 - *Nephtys sp*; 27 - *Palaemon serratus*; 28 - *C. crangon*; 29 - *C. maenas*; 30 - *Pomatoschistus sp.*, 31 - *Loligo sp*.

The filter-feeders found in Lessay and MSMB bays (i.e *Mytilus edulis*, *Crassostera gigas* and *Cerastoderma edule*) had similar carbon values, ranging from -21 to -18 ‰ and were ^{15}N enriched compared to sources. The secondary consumers (e.g. *Nephtys sp*) had higher $\delta^{13}C$ and $\delta^{15}N$ ratios to the primary consumers and were similar to the Lessay Bay.

3.2.3. Mixing models for Lessay and MSMB

The stable isotopic mixing model showed differences of prey contributions: between Lessay salt marshes and MSMB salt marshes (Figures 5a and 5b) and between MSMB salt marshes and his adjacent bay (Figure 5b and 5c).

The common species for the three sites were crabs (*C. maenas*), shrimps (Palaemonidae: *P. elegans*, *P. serratus*), worms (*Nephtys sp*) (Figure 5a, b, c) and represented significant components of juvenile sea bass diets in these habitats.

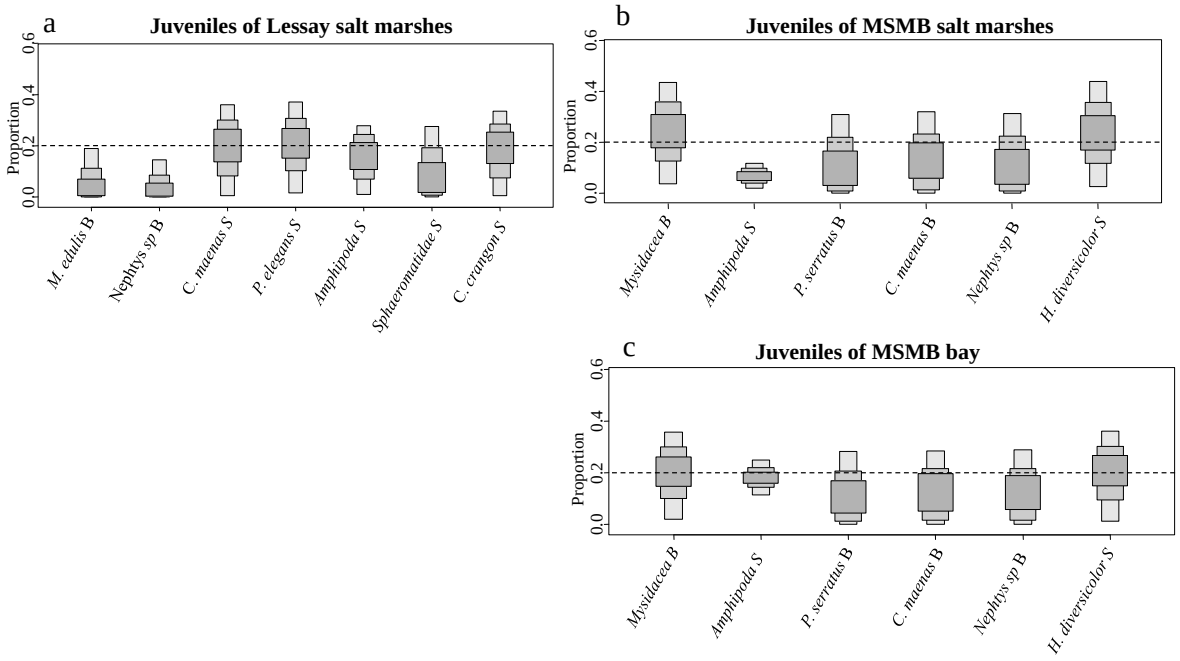


Figure 5: Proportion of modelled contributions of prey items (from the salt marshes (S) and from the bay (B)) to the diet of juvenile sea basses captured in a) Lessay salt marshes, b) MSMB salt marshes, c) MSMB bay. The dash line represented proportion equal to 20 %.

Between the two salt marshes of Lessay and MSMB, amphipods and sphaeromatidae were more consumed at Lessay site (respectively, 15.34 ± 7.22 and 12.65 ± 8.21 %) (Figure 5a and 5b). These species are affiliated to salt marsh plants. Sphaeromatidae were not observed in MSMB salt marshes during samplings.

The diet of juveniles caught in Lessay salt marshes were mainly composed by crabs *(C. maenas*), shrimps (*P. elegans, C. crangon)* and amphipods captured in salt marshes.

The diets of MSMB salt marshes were quite different than those of Lessay due to a lower contribution of the salt marsh preys (Figure 5b). As mysid isotopic signatures from MSMB salt marshes and bay were not significantly different, we used average signatures of all the mysids for the model. In the salt marshes of MSMB, juvenile sea basses mainly consumed mysids (23.80 ± 9.86 %) and *H. diversicolor* (24.67 ± 10.29 %) while amphipods was poorly consumed (6.82 ± 2.51 %) (Figure 5b). However, the contribution of amphipods increased to 18.25 ± 3.37 % for the juveniles of the bay (Figure 5c). The small change of preys items in the diet of juveniles captured in salt marshes and bay showed the connectivity between these two adjacent areas.

3.3. Morpho-anatomic and biological traits

3.3.1. Related to food acquisition

The ecomorphological traits related to the food acquisition showed small differences between individuals of the different sites. Juveniles captured in the MSMB had a bigger oral gape surface (Osf) than those of Rance, Arguenon and St Brieuc (Figure 6 a) but the position and shape of the oral gape was similar for all the juveniles (Figure 6 b, c). The ratio of the eye diameter on the head height (Edst) was lower for MSMB and Rance. The juvenile captured in

the MSMB had a lower K indice but the C/N ratio was also the lowest. Anse of Dinan and Lessay fishes had higher C/N ratio compared to MSMB.

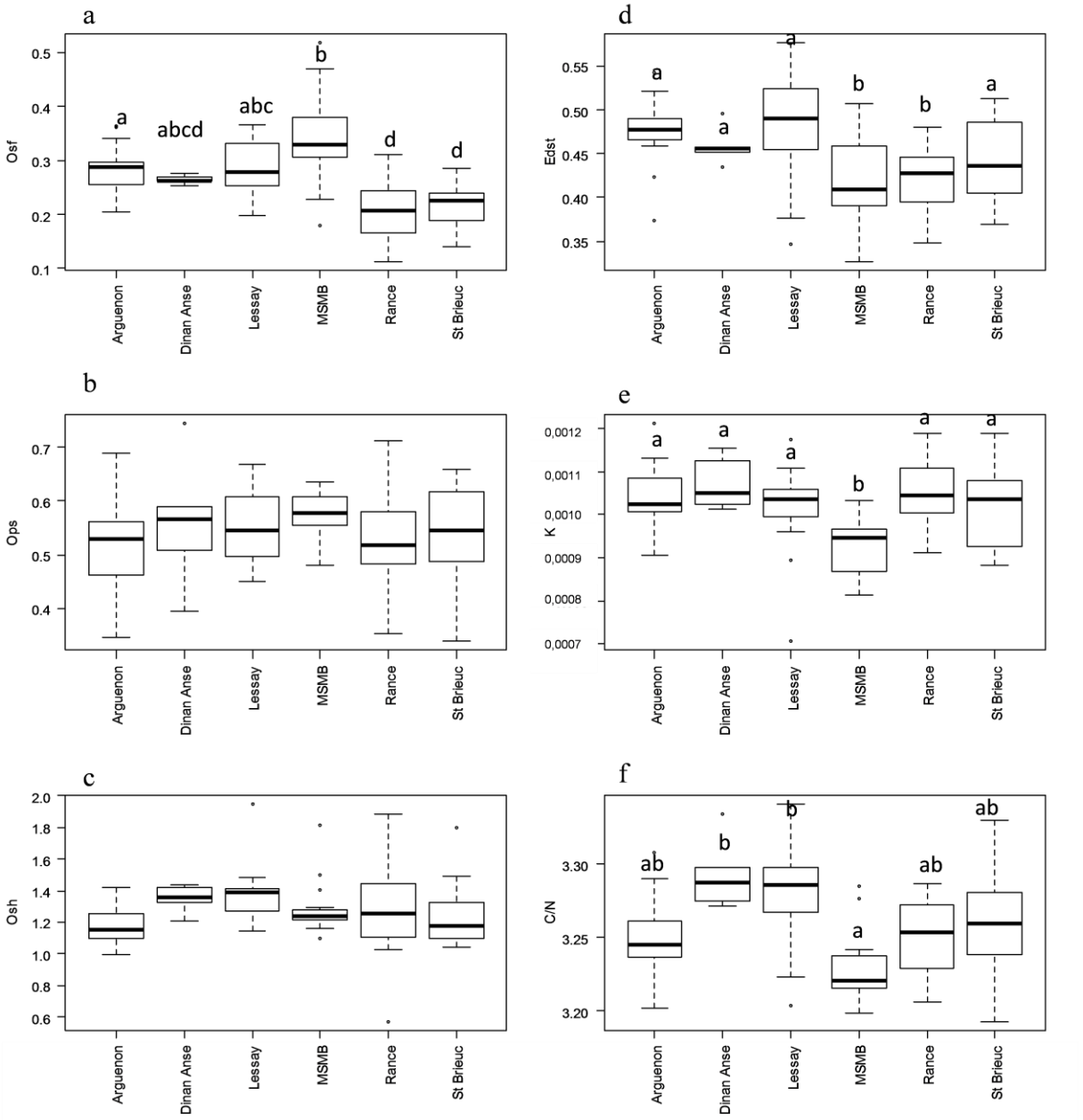


Figure 6: Ecomorphological traits related to food acquisition measured on sea bass juveniles captured on 5 salt marshes and on the seagrass bed (i.e Dinan Anse). 5 traits were originated from morphological measurements and 1 indice based on isotopic analysis a) Osf: Oral gape surface, b) Ops: Oral gape position, c) Osh: Oral gape shape, d) Edst: Eye size, e) K: condition indice, C/N: carbon/ nitrogen ratio. Different letters in superscript indicate significant differences ($p < 0.05$) from a linear model.

3.3.2. Related to locomotion capacities

The dendrogram allowed distinguishing 4 groups on the locomotion features at 58 % of dissimilarity (Figure 7). The group 4 gathered 60 % of all the juveniles; the group 3: 23.75 %, the group 2: 10 % and the group 1 included only 6.25 % of all the individuals. The first group was only composed by the individual of Dinan Anse and the second group clustered 46.67 % of Lessay juveniles (Table 4). The third group included the majority of Rance individuals (67 %) and few juveniles of Lessay and St Brieuc (Table 4). The totality of Arguenon, MSMB and the majority of St Brieuc fishes (66.7 %) were pooled in the group 4 (Table 4).

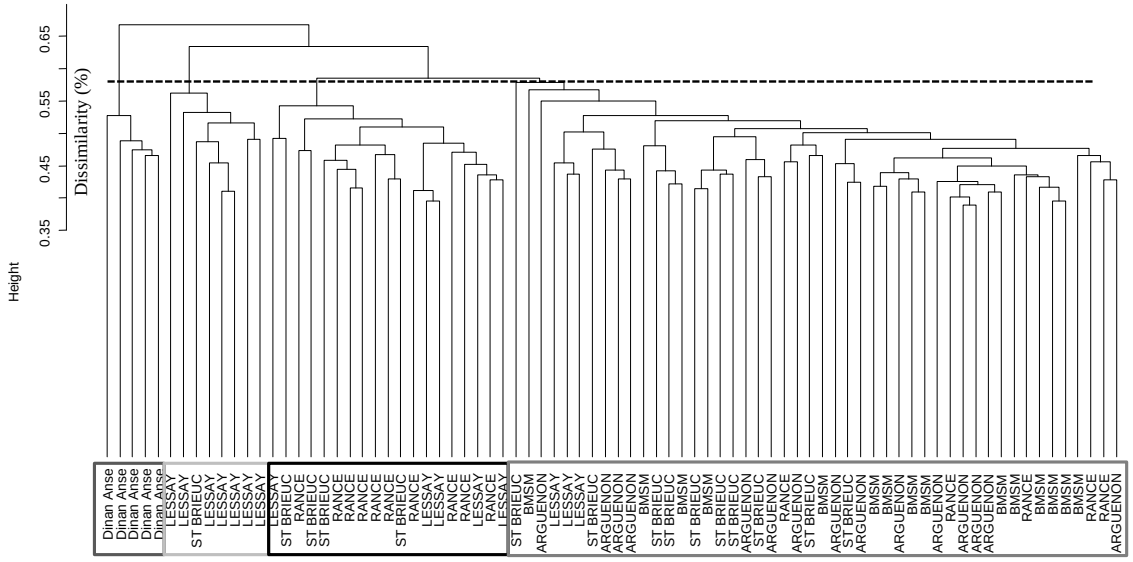


Figure 7: Dendrogram (average method) based on the dissimilarity of the locomotion traits between all the juveniles captured in the 5 salt marshes and Dinan Anse. Juveniles were classified in four functional groups (four different grey rectangles) corresponding to 58 % of dissimilarity.

Table 4: Percentage of individuals from sampling sites assigned to the 4 functional groups.

	Group 1	**Group 2**	**Group 3**	**Group 4**
Arguenon	0	0	0	100
St Brieuc	0	0	26.7	66.7
Lessay	0	46.67	33.33	20
MSMB	0	0	0	100
Rance	0	0	67	33
Dinan Anse	100	0	0	0

Several locomotion traits allowed separating the different functional groups of the ACH. The group 1 had significant low indices (PFar, DFar, CFar, Bsh) and a high Frt indice, and all these indices referred to bigger fin surfaces (Figure 8 a, b, c, d, f). On the contrary, the group 2 gathered the juveniles with low fin surfaces, with high PFar, DFar, CFar, DFi and low Frt and CPt indices (Figure 8 a, b, c, f, h, j). Differences between the group 3 and 4 was mainly due to PFar, CFar, DFar reflecting probably fishes with bigger pectoral fin surfaces but smaller dorsal and caudal fins for the group 4 (Figure 8 a, c, f). Moreover, the high Fsfd value of the group 4 corresponded to fishes having high fin surfaces compared to their body size (Figure 8 g), induced by differences in fin ratios and a higher body depth.

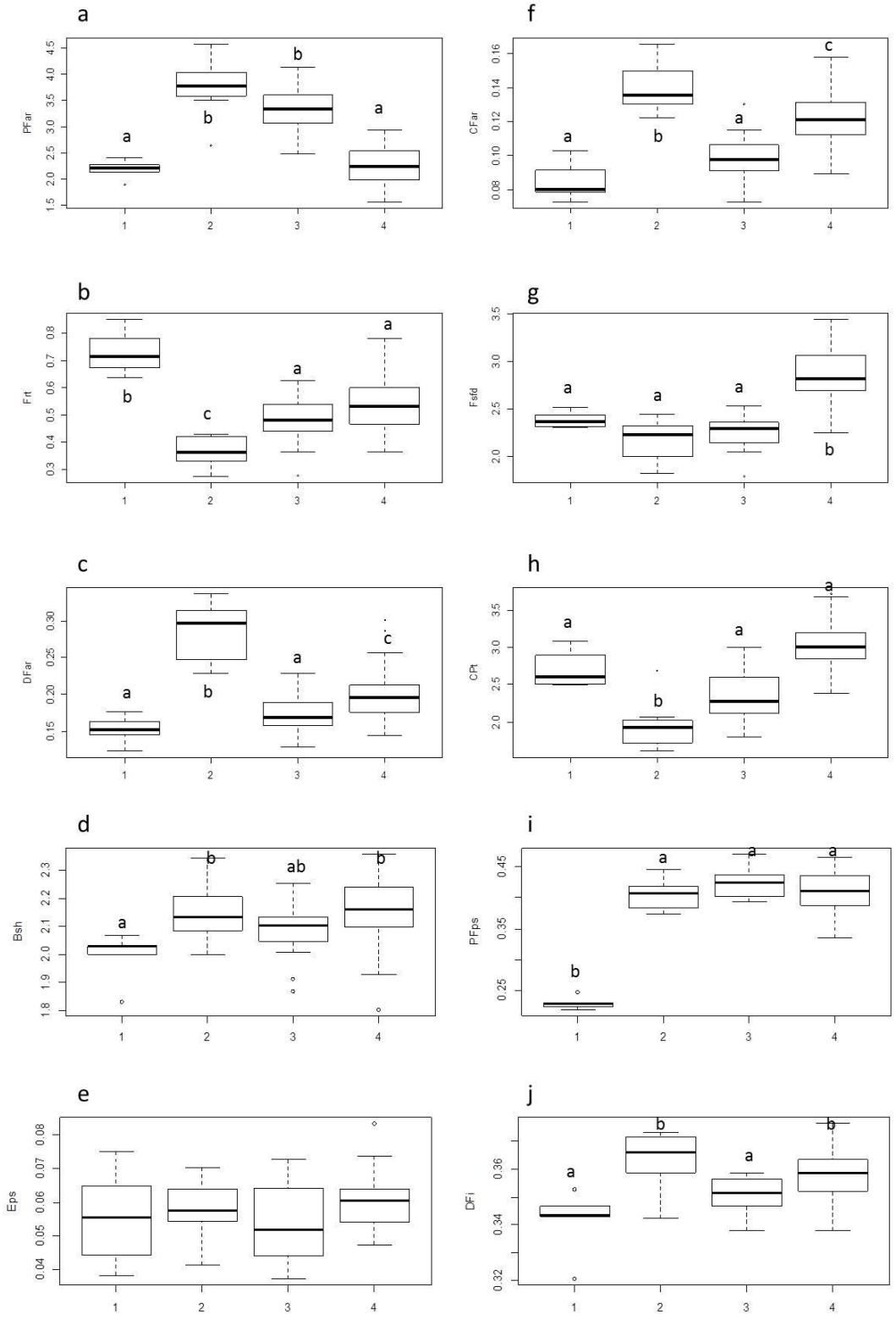


Figure 8: Ecomorphological traits related to locomotion capacities grouped in the four functional groups originating from the dissimilarity dendrogram. a) PFar: aspect ratio of pectoral fin, b) Frt: fins surface ratio, c) DFar: aspect ratio of dorsal fin, d) Bsh: body transversal shape, e) Eps: eye position, f) CFar: aspect ratio of caudal fin, g) Fsfs, fins surface to body size ratio, h) CPt: caudal peduncle throttling, i) PFps: pectoral fin position, j) DFi: insertion dorsal fin). Different letters in superscript indicate significant differences ($p < 0.05$) from a linear model.

4. Discussion

The sampling took place at the regional scale of the western Channel and the diets' diversity of juveniles appeared site depend and showed a different dependency to salt marshes for feeding, partly explained by the habitat quality and a geographical effect.

Indeed, the stable isotope composition of preys showed a clear intra-site difference providing a spatial signature of preys between the salt marshes and the bays. All the organisms captured in the two salt marshes were carbon depleted compared to their respective adjacent bay. Terrestrial influences are already known to induce carbon depletion in isotopic signature in food webs (Gillikin et al., 2006, Barnes et al., 2008, Cambiè et al., 2016). More precisely, the salt marsh plants (i.e C3 plants) are carbon depleted and have distinct signatures compare to marine organism (Créach et al., 1997, Cloern et al., 2002). These depleted carbon signatures of halophyte plants are incorporated in the food chain of detritivore organisms through the decomposition of the organic matter (Créach et al., 1997). This enabled to show the different prey contributions in the diet of sea bass juveniles captured in salt marshes and further their different feeding behaviours and dependency to salt marshes.

For juveniles captured in the salt marshes and bay (i.e. into the mussel cultures) of the MSMB, based on the mixing model, the prey contribution was mainly similar and composed by mysids and *H. diversicolor*, as reported by several authors (Boulineau - Coatanea, 1970, Laffaille et al., 2001, Martinho et al., 2008, Green et al., 2012, Cardoso et al., 2015). The mysids are marine inhabitants which can move into salt marshes with the flood explaining their marine signature even in salt marshes. *H. diversicolor* is a common infauna of salt marsh but submerged by sea water at each high tide, that could explain their carbon enriched isotope signature (i.e marine signature) even if they were captured in the salt marshes (see Figure 4) (Parlier, 2006, Green et al., 2012). *H. diversicolor* is a deposit feeder and filter feeder and could feed on benthic diatoms and plant detritus (Créach et al., 1997). *O. gammarelus* (i.e

amphipods) had a very similar signature to the salt marsh plants as their diet consisted of more than 50 % plant detritus (Créach et al., 1997). Interestingly, the latter was not the dominant prey for juveniles in salt marshes of MSMB, as might be expected. Laffaille et al. (2005) had already observed between 1997 and 2002 a major shift in the diet of sea bass juveniles switching from *O. gammarella* (i.e amphipods) to mysids in part of the MSMB salt marshes invaded by *E. athericus* (recently named *Elytrigia maritima*), modifying the habitat and abundances of *O. gammarella*. 10 years later, as the sampling of the sea bass of this study occurred, *E. maritima* cover the major part of MSMB salt marshes, probably increasing again the decrease of *O. gamarella.* Even if the salt marshes of MSMB are the largest system of the Western Channel, the juveniles did not seem to predate only on the salt marshes preys but rather on the available preys in the adjacent bay, showing that the juveniles were not exclusively dependent on the salt marshes for feeding and moved between habitats. Moreover, according to Laffaille et al. (2001) *M. edulis* was the most important food item of sea bass juveniles captured near to mussel cultures into the bay based on stomach content analysis. This result is not confirmed in our study, in which the contribution of *M. edulis* was not high enough to be considered by the mixing model. In this context, the isotopic composition could have revealed here an opportunistic feeding behaviour when the salt marshes are not accessible, as this habitat was accessible only 44.38 % of the time, or when the competition on preys is high in the salt marshes.

Conversely, at Lessay, the diet's juveniles was mainly based on preys of the salt marshes with depleted carbon signatures and was mostly composed by crabs (*C. maenas*) and shrimps (*P. elegans* and *C. crangon*), which are quite common preys for sea bass juveniles (Laffaille et al., 2001, Martinho et al., 2008, Green et al., 2012, Cardoso et al., 2015). Lessay salt marsh was dominated by *E. maritima* and *S. maritima* but *A. portulacoides* was less abundant due to invasion of *E. maritima* and probably to the sheep grazing as it was already demonstrated in

the MSMB (Laffaille et al., 2000). The isotope signature of amphipods was really distinct from *A. portulacoides* but closer to *S. maritima*, which was much more abundant than *A. portulacoides* in the habitat. The invasion of *E. maritima* and the sheep grazing which modify the vegetation structure, consequently impact the availability of preys in salt marshes (Laffaille et al., 2000, Lefeuvre et al., 2000, Laffaille et al., 2005). Thus, we observed that the contribution of amphipods to the diet of fishes in the salt marshes was not too high (i.e the fourth ingested prey). Moreover, the mysids did not contribute to the diet of juveniles and did not seem abundant in October in the system (*personal comment*) but its abundance is variable between estuaries and seasons (Martinho et al., 2008, Selleslagh and Amara, 2015).

Consequently, the strong difference of carbon signature between the Lessay and MSMB juveniles could be mainly explained by the potential different feeding behaviours, depending on the salt marsh preys for the Lessay and to marine preys for the MSMB. Elsewhere, the avalaibility and composition of preys in these sites and their adjacent bays (i.e different nature of subtract: sandy beaches around Lessay; mudflats for MSMB) were different and eventually induced these diet differences. The small carbon and nitrogen variabilities observed for sea bass juveniles of MSMB and Dinan Anse (i.e seagrass bed), contrarily to the others sites, might reflected the same individual feeding behaviour or a low prey diversity (Layman et al., 2007) or they could act as specialist feeders (Selleslagh and Amara, 2015). The feeding behaviour is linked to the availability of preys in the habitat but also to the energetic requirements and can explain the shift from opportunistic to specialist feeding behaviours (Selleslagh and Amara, 2015).

Regarding the variability and the intermediate carbon isotope composition of the juveniles in the other sites, we can suppose that they could have an opportunist feeding behaviour between the preys of the salt marshes and adjacent marine environments and did not exclusively feed on preys of one habitat.

Moreover, differences in $\delta^{15}N$ values between juveniles could be related to different prey $\delta^{15}N$ ratio and food web composition. For example the mysids have higher $\delta^{15}N$ value comparing to the zooplankton (Hansson et al., 1997, Selleslagh and Amara, 2015) and polychaetes are also more enriched (i.e secondary consumers). Lessay fishes seem to feed more on crustaceans and polychaetes than those of MSMB and could explain the $\delta^{15}N$ gap (2 ‰). Elsewehere, the secondary consumers in Lessay salt marshes had nitrogen enrichment as the crabs (*C. maenas*) and could impact the trophic level of sea bass juveniles in salt marshes.

Complementary indices are common used metrics of fish health and follow the assumption that a heavier and fatty fish has greater energy reserves and a better condition to support changes or stress conditions. K indice is a morphometric indice related to body length and weight and some studies shown a strong relationship with lipid contents for specific species (Mozsar et al., 2015). Lipids components are important energy reserves for body fish and are mainly composed by carbon. The assumption is based on an increasing relationship between the carbon-protein (C/N) ratio and the lipid concentration (Barnes et al., 2007 Fagan et al., 2011). Otherwise, the relationship between the C/N and lipids concentration shown interspecific differences and could be influenced by the habitat or the diet (Fagan et al., 2011). Juveniles of MSMB had low K indice and a low C/N ratio suggesting a low lipid contents and consequently lower energy reserves. Even if differences were observed, the range values were low and C/N ratios were very low comparing to sea bass juveniles caught in UK (Barnes et al., 2008). Fish health in our study was difficult to discuss regarding our results and might be considering among a larger population or several years.

Additionally, the low inter-annual variability of the carbon isotopic composition found for the Rance and Lessay's fishes, showed the fidelity behaviour to these coastal habitats and the

importance of the salt marshes for the growth and survival during this life time (Laffaille et al., 2001, Hampel and Clattrijsse, 2006, Martinho et al., 2008).

These assumptions have been also explored also through morphological adaptations as the oral gape and eye sizes known to be linked to feeding behaviour (Schaefer et al., 2007). The higher oral gape surface found for sea bass juveniles of MSMB could be related to their feeding on mysids, a pelagic prey that fish have to pursue with an open mouth (Schaefer et al., 2002). This manner to predate is already known for anchovies, sardines. The abilities to extend the upper jaw for some fishes have shown differences between fish species reflecting different feeding behaviour on polychaetes or preys in the water column (Schaefer et al., 2002) but this life trait was not measure on our juvenile but could be interesting to test afterward. The eye diameter for Rance and MSMB juveniles were smaller but maybe that the visual acuity was not necessary the more predominant factor to be a good predator.

Besides, morphological differentiations reflecting swimming capacities were more important than those related to the food acquisition. Half of the Lessay fishes were characterized by their small fin surfaces inducing probably lower dispersal capacities and were in agreement with the feeding behaviour found with the stable isotope results, indicating a higher salt marsh dependency. On the opposite, individuals of MSMB had bigger pectoral fin surfaces and high fin surfaces comparing to their body size, which could be related to better capacities than the Lessay juveniles and allow pursuing preys in the water column (for example mysids) or to move along long distance between feeding areas (Schaefer et al., 2002, Cardoso et al., 2015) as the tidal flat of the MSMB is 40 times bigger than the Lessay bay. St Brieuc and Arguenon juveniles were also pooled in the same group of MSBM but did not have the same isotopic ratios, so the same diet or feeding behaviour. Juveniles captured in Rance were mainly pooled in another group characterized by a bigger caudal fin surface,

which could be related to propulsion capacity. Additionally, the juveniles captured in Dinan Anse had the biggest fins surfaces and were a bit bigger; consequently they had the highest swimming capacities allowed them to exploit a diversity of habitats. The swimming capacities can exhibit capacities to pursue preys in the water column after ambush them of the substrate and relying to the distance to cover to find some food item (Schaefer et al., 2002). The Lessay is the one of the smallest system in our study and interestingly their swimming capacities are the lowest and they preferred to feed on the prey on salt marshes to enhance their growth, showing a strong dependency to this salt marsh, despite the high sheep grazing. On the contrary, the MSMB is the bigger system with 240 km^2 of tidal flat and 40 km^2 of salt marshes which could provide a large amount of food and juveniles captured in these habitats had better swimming capacities and were less depend to the salt marsh for feeding.

After the migration to coastal habitats, juveniles showed different diets and behaviours revealed by the isotopic composition and the habitat quality seemed to lead to more or less valuable fitness. But these different diets might be linked to the prey available, habitat characteristics and consequently may reveal an opportunistic behaviour between salt marsh and the adjacent bay.

Conclusion

The study provide evidence that different feeding behaviours between sea bass juveniles exist but the kind of habitat (i.e salt marshes, seagrass or estuary) do not seem to impact them, whereas the quality of some habitat (i.e salt marshes) can influence their diet and also their juvenile growth. The functional indices showed more or less distinct ecomorphological adaptations even if sometimes the distinction was weak. Nevertheless it was the first time that these ecomorphological traits were used to show intra-species differences as it used to be apply on fish communities (Villeger et al., 2010, Spitz et al., 2014). Utilisation of a

combination of several tracers (such as the microchemistry of otolith) will be complementary approach to unravel the life histories and the life cycle of sea bass and will permit a better understand of the function of these particular habitats.

Acknowledgements

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DISCUSSION GENERALE

Au cours de cette thèse nous avons, grâce à l'utilisation de méthodes complémentaires, apporté un éclairage nouveau sur les fonctions écologiques jouées par des habitats côtiers pour des espèces communes, les lançons et les bars, qui présentent des cycles biologiques très différents.

Nous avons caractérisé la phénologie de l'utilisation de différents types de milieux sableux du nord de la Bretagne par des populations de 4 espèces de lançon en répondant à la question: **est-ce que les différentes espèces ont le même niveau de dépendance aux habitats, autrement dit les espèces sont-elles résidentes permanentes ou bien fréquentent-elles différents habitats écologiques essentiels (HEE) au cours de leur cycle biologique ?**

En parallèle nous avons caractérisé la variabilité des traits de vie de bars juvéniles sur différents habitats de nourricerie, notamment les marais salés (ou schorres, herbus), des vasières et des zostères dans diverses baies et estuaires de la Bretagne et du Cotentin, en répondant à la question : **existe-il une variabilité des histoires de vie et traits de vie au sein des juvéniles de bar et ont-ils le même niveau de dépendance aux habitats de nourricerie?**

Ces études ont eu recours simultanément à plusieurs méthodes : l'analyse de traits alimentaires (régime alimentaire déduit des isotopes stables du carbone et de l'azote et des traits morpho-anatomiques liés à l'acquisition des proies) et de traits d'histoire de vie déduits de l'analyse morphologique et chimique des otolithes. Les approches étant combinées, il a été possible de montrer et de valider de manière croisée, l'existence de cycles migratoires tranchés entre des espèces apparentées et sympatriques (les lançons) et d'une variabilité des réponses biologiques des juvéniles de bar que ce soit à l'intérieur d'une même nourricerie littorale ou entre des habitats.

Nous présentons dans cette discussion les principales avancées obtenues pour les lançons, puis les bars et pour terminer nous proposons une synthèse générale de nos résultats afin de mieux comprendre **les liens entre les traits de vie et la connectivité entre les HEE à travers les différentes stratégies d'utilisations des habitats de ces espèces.**

Histoire de vie inter-espèce à l'échelle des communautés de lançon

Au début de notre travail, nous avons d'abord dû réviser attentivement les critères de distinction des espèces dont la détermination a probablement souvent été fausse tant les espèces sont cryptiques. Nous avons pu montrer que les communautés de Bretagne nord étaient composées de 4 espèces. Certaines études historiques ont probablement fait la confusion entre *Ammodytes marinus* et *A. tobianus*, la première espèce n'étant probablement pas présente, ou étant très marginale en Bretagne. De même une confusion d'identification a été détectée entre *A. tobianus* et *H. immaculatus*. De plus, *H. immaculatus* est décrite comme présente uniquement en Bretagne nord mais sa présence a finalement été révélée en Bretagne sud (*Données non publiées*).

Malgré l'importance des lançons pour le maintien de certaines espèces marines (Litzow et al., 2000, Wanless et al ., 2005, McLeod et al., 2007, Engelhard et al., 2014, Robertson et al., 2014), peu d'études se sont penchées sur l'histoire de vie des différentes espèces d'Ammodytidae et la connectivité entre leur HEE, les investigations de type marquage ou télémétrie étant limitées du fait de leurs petites tailles. Si *A. marinus* a été le plus étudié dans les pays du Nord de l'Europe, l'utilisation des traceurs naturels tels que les éléments chimiques de l'otolithe, les isotopes stables ou la morphométrie n'avaient pas été encore appliqués à ces espèces.

Les résultats du Chapitre 1 (Article 1), ont permis de confirmer la forte fidélité d'*A. tobianus* à son site de capture et de valider l'utilisation de la microchimie utilisée pour la première fois chez les Ammodytidae. Les résultats sur l'estimation de la croissance ont confirmé le lien fort existant entre la taille de l'otolithe et celle des poissons et a permis de relier les différents stades de vie à la microchimie. Pour cette espèce essentiellement capturée sur les bancs de sables intertidaux, deux cohortes annuelles de recrutement de juvéniles ont été observées conformément aux travaux de Reay (1973) qui décrit une période de reproduction automnale et une reproduction printanière. Cette différence se retrouve au sein même de la structure de l'otolithe avec des zones opaques (périodes de croissance forte estivale) et claires (croissance faible hivernale) logiquement agencées différemment selon que les individus sont issus de la première ou la deuxième période de recrutement annuel. Cette différence de macrostructure peut donc aider à l'identification de la période de ponte par la suite (Reay, 1973). Les changements de signature microchimique observés lors des premiers stades de vie indiqueraient une installation progressive des post-larves dans le sédiment vers des tailles comprises entre 24.96 ± 0.96 mm et 34.04 ± 0.96 mm correspondant certainement en même temps à la métamorphose des larves en juvéniles. Selon Wright (1993), la métamorphose correspond à un changement de comportement d'un mode de vie pélagique à semi-démersal. Nos travaux confirment ce changement d'habitat grâce à la localisation précise sur l'otolithe de cette phase (changement d'axe de croissance dans l'otolithe) pour laquelle nous mettons très clairement en évidence une modification de la signature microchimique. Après l'installation dans le sable les individus montrent peu de déplacements, corroborés par la présence d'individus adultes toute l'année ainsi que par la stabilité de la signature microchimique le long de l'otolithe. D'autre part, les résultats des analyses microchimiques des zones marginales des otolithes (i.e. signatures de capture) entre les 3 sites d'études (Lancieux, Havre de Rotheneuf et l'archipel de Chausey) ont permis de montrer : 1)

qu'il était possible de discriminer "microchimiquement" à partir d'otolithes, des sites même à faible échelle spatiale (entre 20 à 40 km) et 2) une faible connectivité à cette fine échelle, ce qui est plutôt en accord avec la vitesse de nage de ces poissons et les récentes études sur les faibles déplacements d'*A. marinus* (Jensen et al., 2011, Engelhard et al., 2008). Par conséquent ces habitats sableux intertidaux ou côtiers proches semblent être le lieu de croissance des juvéniles et probablement celui des adultes et également un lieu de repos des adultes en période hivernale. Cependant cette étude ne permet pas de statuer réellement sur la zone de reproduction puisque le suivi gonado-somatique tout au long de l'année n'a pas été réalisé mais nous présumons tout de même que ces bancs intertidaux constituent des habitats de reproduction (frayère) de cette espèce (*i.e.* maturation des gonades et pontes). L'histoire de vie d'*A. tobianus* approchée par la microchimie et le suivi de cohorte semble donc indiquer l'utilisation d'habitats intertidaux proches pour accomplir une grande partie de son cycle de vie, hormis une phase de dispersion larvaire pélagique. Par ailleurs, nous montrons que la microchimie de l'otolithe apparaît comme efficace pour démêler les histoires de vie et qu'elle apporte des éléments de réflexion sur la connectivité entre les habitats côtiers à petite échelle et sur les échanges potentiels entre les populations d'Ammoditydae.

Nos échantillonnages ont montré que plusieurs espèces d'Ammodytidae vivent en sympatrie sur les habitats sableux subtidaux (notamment les dunes hydrauliques) ce qui pose la question des mécanismes mis en place pour éviter la compétition entre ces espèces morphologiquement et phylogénétiquement très proches. Les histoires de vie des quatre espèces étudiées apparaissent différentes et permettent sans doute cette coexistence au sein de la communauté à travers une divergence de certains traits de vie (Article 2).

La plus grande des espèces, *H. lanceolatus*, apparaît plus mobile et les cohortes semblent occuper potentiellement divers habitats au regard des distributions des fréquences de taille détectées sur le site de Lannion (dunes hydrauliques subtidales à 4 miles des côtes que nous

qualifierons de site côtier proche) et de la microchimie de l'otolithe. Ainsi les individus s'y installeraient à environ deux ans, correspondant à l'âge de la maturité sexuelle (Le Danois, 1913, Bellec, 1981, Macer, 1966). Les juvéniles eux n'y sont pas capturés ce qui suggèrent qu'ils occupent d'autres habitats plus proches de la côte, hypothèse confirmée lors de nos échantillonnages en zone intertidale (*données non publiées*). La comparaison des résultats microchimiques entre nos trois sites d'études (Lannion, Iles Hébihens, Lancieux) distants d'une centaine de kilomètres, tendraient en faveur d'une connectivité inter-site très faible à l'échelle du cycle vital, confirmant également nos hypothèses sur *A. tobianus* (Article 1), stratégie d'utilisation des habitats qui semble être adoptée également par les autres espèces. Ces domaines vitaux peu étendus sont généralement confirmés par la littérature relative aux Ammodytidae (*A. marinus*; Engelhard et al., 2008, Jensen et al., 2011), la dispersion semblant n'être effectuée qu'au stade larvaire. L'investigation des capacités relatives des différentes espèces à se déplacer montre que *H. lanceolatus* possède les plus grandes capacités de locomotion avec des nageoires caudales et dorsales plus puissantes que les deux autres espèces et surtout que celles de *G. semisquamatus*. Ces résultats sont confirmés par une étude morphologique interne fine d'Ida et al, (1994) mettant en évidence des différences de structure de squelette de la nageoire caudale entre les genres. Ceci permet probablement à *H. lanceolatus* d'utiliser différents bancs de sable pour réaliser certaines étapes de son cycle de vie telle que la reproduction ou la croissance des juvéniles mais la connectivité entre sites serait restreinte à une échelle de l'ordre de la dizaine ou vingtaine de kilomètres (Figure 1). Pour *H. immaculatus*, comme pour *H. lanceolatus*, seuls les individus âgés (2 ans) sont présents sur le site d'étude de Lannion, résultats confirmés par les analyses microchimiques qui montrent l'acquisition de la signature du site à partir de deux ans. Par ailleurs aucun juvénile n'a pour le moment été capturé sur le site de Lannion. Finalement, l'espèce montrant le moins d'aptitudes morphologiques à la nage (*G. semisquamatus*) est aussi celle qui est la

plus résidente des bancs de sable subtidaux, les deux autres espèces étant probablement amenées à utiliser d'autres habitats du même type plus ou moins distants (Figure 1).

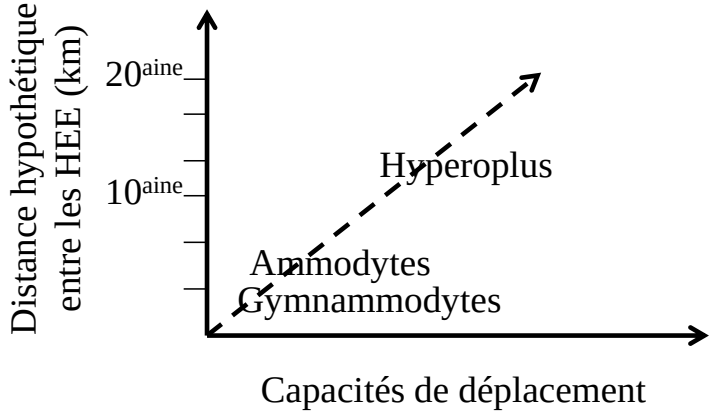


Figure 1: Relation entre la distance hypothétique entre les HEE et les capacités de déplacements des trois espèces. Les capacités de déplacements sont estimées via les patterns microchimiques et en tenant compte des classes d'âge présentes sur le site (Articles 1 et 2).

Ces différences d'utilisation des HEE des adultes et des juvéniles ainsi que les différentes périodes de reproduction permettent aux espèces de réduire la compétition interspécifique en utilisant différents habitats et également en décalant le recrutement des juvéniles dans les communautés (Cabral and Costa, 1999, Charles et al., 2004) (Figure 2). Cette compétition interspécifique peut également être d'ordre trophique, ce que nous avons exploré d'un point de vue morphologique à travers la mesure des capacités d'acquisition des proies entre les trois espèces, couplée aux ratios isotopiques stables. *H. lanceolatus* peut par exemple capturer des proies de relativement plus grande taille au vu d'une plus grande ouverture de la bouche, caractère confirmé par un niveau trophique sensiblement plus élevé, probablement ichtyophage, les autres espèces étant à dominante zooplanctonophage. Finalement ces traits reliés à l'acquisition de nourriture sont le corollaire de comportements différents et favorisent la cohabitation interspécifique (Labropoulou and Papadopoulou-Smith, 1999, Laffaille et al., 1999) (Figure 2). Même si ces espèces sont proches morphologiquement et

phylogénétiquement, leurs morphologies et leurs histoires de vie reflètent diverses tolérances ce qui leur permettent de coexister en communauté (Chase and Leibold, 2003, McGill et al., 2006, Levine and HilleRisLamberts, 2009).

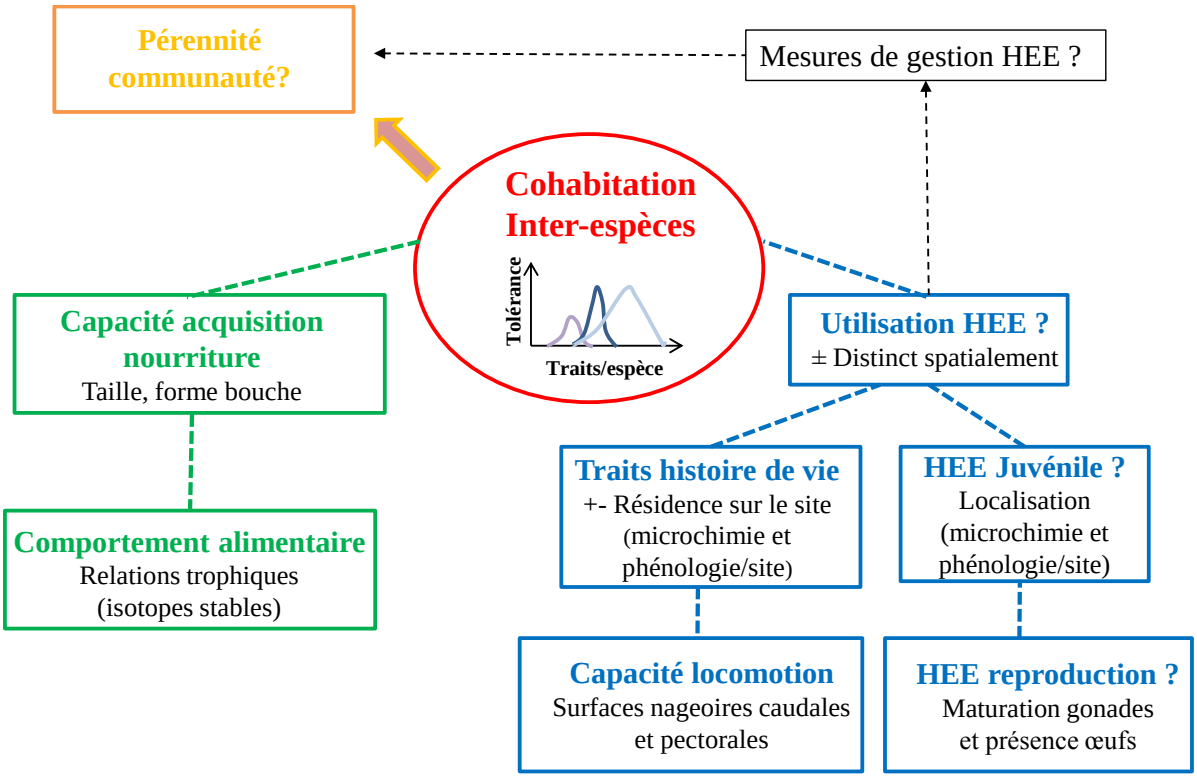


Figure 2: Schéma récapitulatif liant les différents traits de vie mesurés, l'utilisation des HEE et la cohabitation des espèces de lançon dans la communauté.

Limites et perspectives d'études :

Si ce travail de thèse a permis de mieux comprendre certains traits d'histoire de vie des espèces d'Ammodytidae, certaines limites ont été détectées et certains points manquent pour pouvoir statuer sur la fonction, l'identification et la localisation de tous les HEE utilisés pour toutes les espèces au cours de leur cycle vital. D'autre part, nous avons pu apporter des éléments sur la connectivité entre des habitats distants grâce à l'analyse microchimique des otolithes, mais ce travail mériterait d'être poursuivi pour mieux comprendre les niveaux

d'échange entre des sites proches. Ces connaissances sont indispensables pour définir des mesures de gestion pertinentes face au développement des zones d'extraction de granulat en mer et des champs d'éoliennes marines (Dauvin, 2008, Michel et al., 2013, Kermagoret et al., 2014).

Pour *H. lanceolatus* et *H. immaculatus* d'autres zones d'études (subtidales et intertidales) doivent être prospectées pour mieux comprendre le cycle de vie et identifier les HEE des juvéniles et de reproduction (*i.e.* habitats de nourricerie et de frayère). L'état de maturation des gonades devra être suivi sur un cycle annuel pour préciser la période de reproduction. Une nouvelle étude pourrait être réalisée pour analyser la connectivité des populations à une échelle régionale en croisant différents traceurs dont nous avons prouvé la pertinence (microchimie des otolithes, isotopes stables) et d'autres à explorer comme la génétique des populations. En effet les outils génétiques (gènes mitochondriaux ou les microsatellites non soumis à la sélection naturelle) permettront de renseigner les potentiels brassages entre les populations et leur structure sur des temps plus longs (Feutry et al., 2013, Martin et al., 2015).

Par ailleurs, le magnésium et le strontium semblent varier en fonction de l'âge des poissons et sont liés à l'ontogénie comme constaté pour d'autres espèces marines (Kalish, 1989, Martin and Thorrold, 2005, Brown and Severine, 2009, Walther et al., 2010, Sturrock et al., 2012, Woodcock et al., 2012). Les mécanismes d'intégration des éléments chimiques dans l'otolithe ne sont encore pas exactement compris et encore moins chez les Ammodytidae mais leur utilisation permet tout de même d'ouvrir de nouvelles perspectives d'études pour ces espèces de petites tailles avec un cycle de vie court.

Variabilité d'histoire de vie intra-espèce au sein du bar européen

Le bar européen a été choisi pour son cycle de vie différent et contrasté vis à vis de celui des lançons. En effet, il effectue une migration ontogénique entre des habitats de ponte hauturiers et des nourriceries côtières et fréquente par conséquent différents HEE distants au cours de son cycle de vie (Pickett et al., 2004, Fritsch et al., 2007, Pawson et al., 2007). De plus, différents traits de vie semblent varier au sein de l'espèce comme la tolérance à la salinité ou au jeûne ainsi qu'une certaine plasticité observée dans le comportement alimentaire des juvéniles (Guinand et al., 2014, 2015, McKenzie et al., 2014, Cardoso et al., 2015, Selleslagh and Amara, 2015). Compte tenu de ces caractéristiques, différentes stratégies tant dans l'utilisation des différents HEE qu'au sein des HEE eux-mêmes étaient suspectées.

Les articles 3 et 4 permettent de retracer l'histoire migratoire depuis la phase larvaire marine jusqu'au recrutement puis au développement dans différents habitats littoraux de nourricerie et de mesurer la dépendance des juvéniles à l'un des principaux habitats, selon les travaux de Laffaille et al., (2000, 2001, 2005) : les marais salés.

Les zones de frayère et de ponte se situent plus au large puis les larves effectuent une migration ontogénique vers la côte. Grâce à des techniques d'ablation laser très précises (ablation femtoseconde) couplées à des analyses par ICPMS, nous avons pu montrer les changements de la composition élémentaire des otolithes depuis le nucléus, la phase larvaire de dispersion, le recrutement en nourricerie, jusqu'à la capture sur les nourriceries. Grâce à l'interprétation de la microstructure de l'otolithe nous avons pu identifier les grandes phases ontogénétiques de la vie juvénile. La durée de vie larvaire (i.e jusqu'au changement d'axe de croissance) sur les bars juvéniles capturés lors de notre étude est de 83.80 ± 17.94 jours en moyenne, avec une forte variabilité (*e.g.* 58 à 132 jours en Rance), paradoxalement ne montre

pas de différences entre les juvéniles capturés dans les cinq marais salés du Golfe Normand Breton. La méthode d'analyse microchimique utilisée établit un lien très clair entre le rapport Sr/Ba des otolithes et la salinité du milieu dans lequel l'individu a séjourné, permettant ainsi de mettre en évidence différentes stratégies de dispersion des larves puisque certaines ont passé 85.54 % de leur temps en milieu marin (i.e. euhalin) (individus capturés au Lessay), d'autres 62.54 % (Baie du Mont St Michel, BMSM) contre 38.50 et 30.40 %, respectivement pour les individus capturés en Rance et à l'extérieur de la Rade de Brest. Différents patrons de migration semblent indiquer un rapprochement plus rapide vers la côte (eaux plus côtières puis saumâtres) pour les sites plus à l'ouest de la zone d'étude que ceux du Lessay et de la BMSM. Ces différentes histoires de vie larvaire sont à rapprocher à la localisation (peu connue) des frayères et des régimes de courant sévissant lors des trajets frayères-nourriceries : elles pourraient être mises en relation avec des modèles hydrodynamiques du Golfe pour mieux comprendre les processus de rétention et de dispersion des larves (Figure 3, Sandin et al., 2005, Réveillac et al., 2008, Feutry et al., 2013). Il serait également intéressant de connaître la distance entre les zones de frayères et les nourriceries pour essayer de connecter les différents HEE. Il est enfin intéressant de noter que ces histoire de vie larvaire contrastées ne semblent pas avoir d'effet sur les performances de croissance à ce stade qui restent identiques entre les sites.

En suivant les phases de vie identifiées sur l'otolithe, toutes les larves (n = 55), probablement au moment de leur métamorphose (i.e. identifiée par un changement de l'axe de croissance à une distance de 400-500 µm microns du nucléus) sont ou entrent dans des habitats relativement dessalés (polyhalin, entre 18 et 30 psu) sous influence plus ou moins forte des eaux continentales (estuaires…).

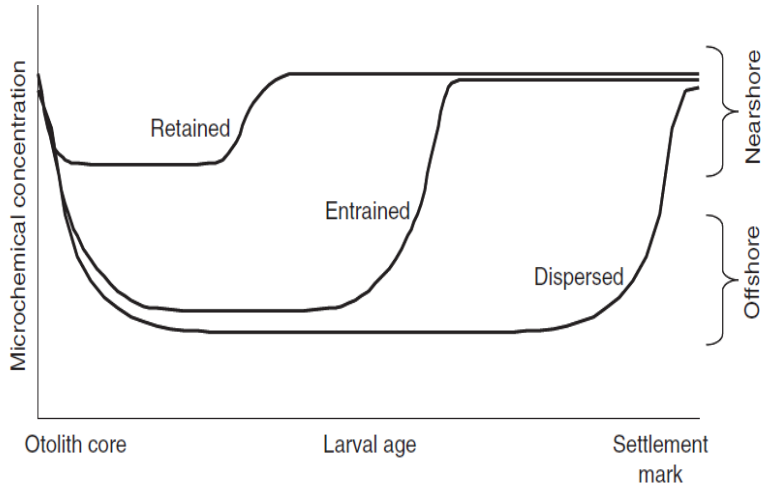


Figure 3: Différents patrons microchimiques correspondant à différents comportements de dispersion des larves en milieu marin vers la côte. Le patron "Retained" correspond à un processus de rétention des larves court et proche de la côte tandis que le patron "dispersed" indique une phase larvaire pélagique longue en milieu offshore avant la marque d'installation en milieu côtier (d'après Sandin et al., 2005).

Comme pour les larves, différents patrons d'occupation des marais salés entre et à l'intérieur des sites ont été détectés pour la phase de vie juvénile via les salinités inférées *via* les éléments chimiques de l'otolithe. Contrairement aux larves, ces histoires de vie témoignent par contre de performances de croissances contrastées entre les sites, effets que nous ne sommes pas en mesure d'interpréter mais pour lesquels nous proposons quelques hypothèses qui restent à explorer plus avant. D'un point de vue général, 25,45 % des juvéniles séjournent dans des gammes de salinités comprises entre 18 et 30 psu (jamais en dessous) et 32,72 % ne passent pas de temps dans des eaux avec une salinité supérieure à 34 psu. Ces deux patterns indiquent une dépendance aux milieux très côtiers, voire estuariens pour certains d'entre eux. Ces résultats sont confirmés sur d'autres sites : Cabral et Costa (2001) au Portugal ont déjà remarqué que les juvéniles de bar étaient présents dans les gammes de salinité comprises entre 13-17 et 22-28 psu dans les estuaires.

Cette importante variabilité des histoires de vie juvénile au sein de l'espèce pourrait être également liée à des capacités d'osmorégulation différentes entre les individus (McCormick,

2001, Guinand et al., 2014, 2015). Par exemple, les individus du Lessay ont passé beaucoup plus de temps en eau douce que les individus d'autres sites mais ont une croissance juvénile journalière faible. Sur ce site, les juvéniles présentent des traits locomoteurs suggérant de plus faibles capacités de nage que dans d'autres sites, leur permettant peut-être de parcourir des distances plus faibles. Les individus de la Baie du Mont Saint Michel fréquentent des habitats à plus forte salinité tandis qu'ils présentent des taux de croissance plus forts.

Ces différences de croissance peuvent être reliées à des histoires de vie différentes comme nous l'avons vu, mais aussi probablement avec la qualité du milieu d'accueil (Figure 4). En effet, nous avons constaté que les individus du Lessay avaient des signatures isotopiques très appauvries en $\delta^{13}C$ et plutôt expliquées par un réseau trophique basé sur les proies des marais salés alors que la qualité du marais semble impactée par le pâturage ovin (i.e. faible abondance d'obiones (*Atriplex portulacoides*) et des végétaux très ras). Il a en effet été démontré que le pâturage avait un impact fort sur l'abondance et l'accessibilité aux proies par les juvéniles de bars des marais salés en BMSM (Laffaille et al., 2000, Lefeuvre et al., 2000).

Par ailleurs, l'abondance des proies est probablement beaucoup plus importante en BMSM car ce système de Baie ouverte possède une étendue de marais salés environ 70 fois plus grande que celle du Lessay. Malgré cette grande étendue, nous avons constaté via l'analyse des isotopes stables du carbone, une alimentation basée sur des organismes marins comme les mysidacés. Ce constat peut cependant aussi s'expliquer par la faible accessibilité aux marais salés lors des cycles de marée (44.38 % du temps à l'année) (Laffaille et al., 2001, 2005) alors qu'elle sera proche des 80% sur les autres sites. Malgré ce régime tidal et les perturbations altérant la qualité des marais de la BMSM en tant que nourricerie pour le bar (auxquelles il faut rajouter l'invasion par le chiendent maritime diminuant aussi les abondances d'*Orchestia gammarella*, une des proies de prédilection des juvéniles de bar, (Laffaille et al., 2005)), la BMSM produit les juvéniles ayant les meilleures performances de croissance.

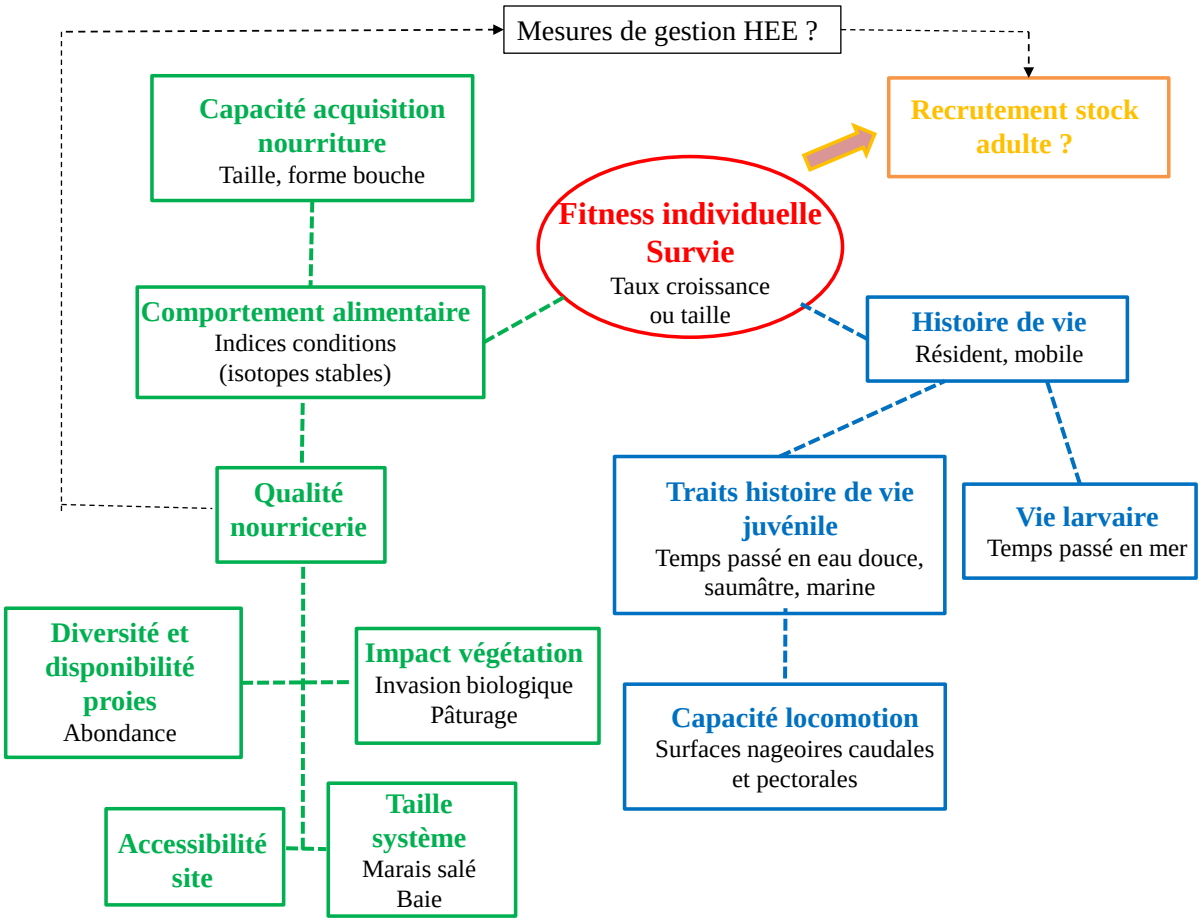


Figure 4: Schéma récapitulatif des liens explorés entre les différents traits de vie mesurés, l'utilisation des HEE et la fitness individuelle des juvéniles de bar européen.

Nous voyons que de nombreuses pistes peuvent expliquer ces résultats contrastés, complexes et probablement en interaction. Elles nécessiteront des investigations plus poussées afin d'en établir l'importance relative toujours en lien avec les stratégies individuelles mises en évidence dans ce travail et observées chez d'autres espèces de poissons côtiers (Nash and Geffen, 2000, Gilliers et al., 2006, Amara et al., 2007, Vasconcelos et al., 2009, Isnard et al., 2015).

Ces différences de croissances et de conditions des juvéniles pourraient contribuer également à des survies différentielles lors de la première année et peuvent conditionner, par la suite, différents recrutements aux stocks adultes (Sogard, 1997) (Figure 4). La variabilité

des traits de vie des juvéniles étudiée à travers une combinaison de traceurs confirme leur importance pour mieux comprendre au niveau individuel les performances de croissance, les histoires de vie larvaire et juvénile dans les habitats de nourriceries tels que les marais salés.

Limites et perspectives d'études :

La compréhension des processus de dispersion des larves en milieu marin est une étape essentielle du cycle de vie des poissons, ceux-ci conditionnant la connectivité entre les habitats essentiels à l'instar de ce qui prévaut chez de nombreuses espèces (Réveillac et al., 2008, Feutry et al ., 2012). De ce fait, la compréhension des mécanismes de dispersion est importante pour analyser et voire quantifier la connectivité entre les différents HEE de frayère et de nourricerie. L'otolithométrie est apparue comme un excellent auxiliaire de l'étude de l'histoire de vie des bars durant leurs stades précoces, en fournissant des estimations des durées de vie aux différents stades de développement, et sur la connectivité entre les habitats essentiels. Cependant, il s'agit d'une méthode lourde, nécessitant une technicité élevée et de nombreuses heures de travail. Un nombre plus important d'individus auraient probablement permis de diminuer la variabilité des mesures et mieux asseoir nos conclusions sur les histoires de vie individuelles en fonction des sites. Nous envisageons de faire un nouveau plan de ponçage sur lame mince sur 15 individus par site pour avoir une meilleure estimation de ces durées.

Par ailleurs, les signatures microchimiques correspondant au stade larvaire pourront être analysées pour mieux comprendre l'origine des larves (Zimmerman and Reeves, 2000) au sein du Golfe Normand Breton et faire le lien avec les frayères. Cette étape est cependant techniquement difficile et suppose des différences de concentrations des éléments chimiques entre les frayères or le milieu marin (offshore) est considéré comme plutôt stable. Cependant

nous avons déjà observé une forte variabilité du rapport Sr/Ba larvaire correspondant probablement à des différences de localisation plus ou moins proche de la côte.

La méthode utilisée pour délimiter les groupes de salinité repose sur la relation entre le rapport du Sr/Ba dans l'otolithe et la salinité. Ces relations sont difficiles à obtenir car les turnovers entre les changements de compositions dans l'eau et ceux de l'otolithe ne sont pas les mêmes. En effet des variations de salinité peuvent apparaître à des échelles courtes (heures et journalières) dans ces habitats soumis aux marnages et aux apports d'eaux douces tandis que l'otolithe intègre sur plusieurs jours les conditions environnementales passées (Walther and Limburg, 2012).

La proportion de bars juvéniles contribuant au stock adulte n'a pas été estimée dans ce travail de thèse mais sera envisagée par la suite à partir de l'analyse de la composition chimique de la partie juvénile dans les otolithes de bar adultes et de les comparer avec les signatures élémentaires caractéristiques de différentes nourriceries que nous avons étudiées. Selon le concept de Beck et al, (2001) et de Dalgren et al, (2006) ce type de travail peut permettre de statuer sur l'importance des différents habitats de nourricerie de la Manche pour les populations adultes de bar européen. Cela nécessiterait également de poursuivre l'effort de caractérisation des signatures microchimiques des otolithes de bars juvéniles capturés sur un plus grand nombre de nourriceries réparties en Manche et en Atlantique. Même si l'ICES (International Council for Exploitation of the Sea) définit des stocks différents entre la Mer du Nord et la Manche, aucune différenciation génétique n'a été observée (Fritsch et al., 2007), indiquant une forte connectivité entre les populations et les HEE, expliquée par des fortes capacité de dispersion et de migration lors de reproduction en Manche (Pickett et al., 2004, Pawson et al., 2007, Fritsch et al., 2007). Enfin, en lien avec le statut de cette espèce à fort enjeu économique, il est nécessaire de définir l'importance ou la qualité des habitats

écologiques essentiels comme les habitats de nourricerie pour mieux appliquer des mesures de gestions pour le maintien des populations (Figure 4).

Relations entre histoires de vie et connectivité des HEE

Ce travail de thèse s'est enrichi de différents modèles d'études pour comprendre les relations entre histoire de vie et connectivité des habitats écologiques essentiels (HEE). Les espèces d'Ammoditydae ont servi d'exemple d'espèce à faible dispersion et très dépendantes des habitats côtiers sableux mais présentant des différences d'utilisation des habitats entre les espèces (*i.e.* variabilité interspécifique). A l'opposé, le bar européen a montré un fort potentiel de dispersion larvaire puis différentes modalités d'utilisations des habitats de nourricerie par les juvéniles, reflétant également une variabilité intraspécifique des histoires de vie à ces différents stades.

Les capacités de dispersion mesurées chez les Ammodytidés à travers les profils microchimiques de l'otolithe ont montré différentes utilisations d'HEE. *A. tobianus* et *G. semisquamatus* utilisent probablement le même habitat ou ayant les mêmes caractéristiques lors de leur cycle de vie et ont de faibles capacités de dispersion. Ces traceurs semblent indiquer l'utilisation d'HEE non disjoints spatialement correspondant à une connectivité entre HEE réduite spatialement de l'ordre du kilomètre (Figure 5). Chez les deux espèces du genre *Hyperoplus* les capacités de dispersion sont plus importantes au vu des profils microchimiques, de la présence uniquement de certaines classes d'âge et de leurs capacités natatoires importantes révélées par les traits fonctionnels impliqués dans la locomotion. Ces caractéristiques leur permettent d'utiliser probablement des HEE distincts à une échelle un peu plus grande (Figure 5). Cependant les durées de vie larvaire n'ont pas été mesurées chez ces 4 espèces et pourraient peut-être s'avérer différentes et rendre compte d'un processus de dispersion et en conséquence des colonisations différentes.

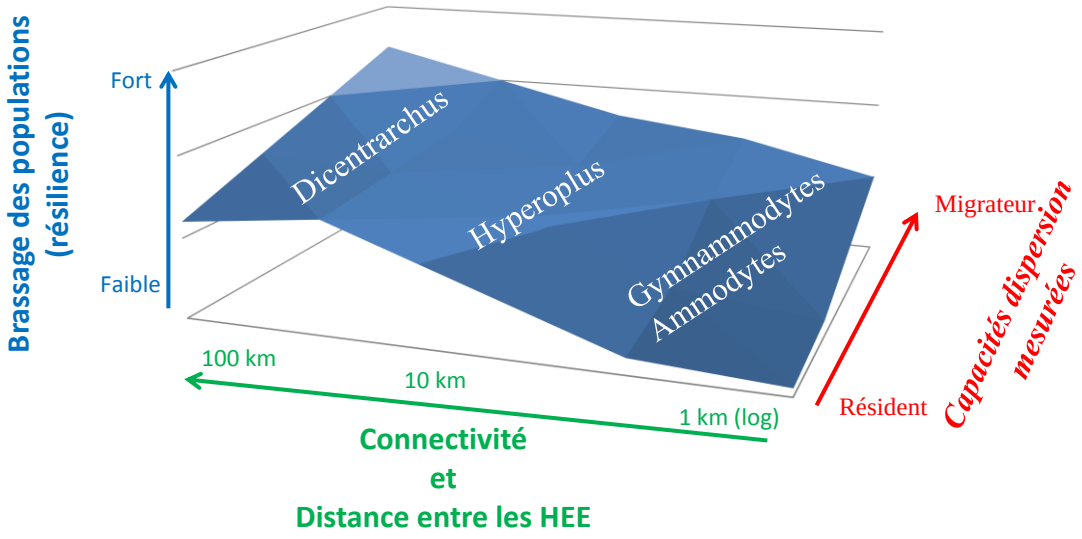


Figure 5 : Relations théoriques entre les capacités de dispersion mesurées chez les différents genres, la connectivité et la distance théorique entre les HEE et leur impact sur le brassage entre les populations. Les brassages entre les populations augmentent la résilience des populations face à des changements environnementaux ou pressions extérieures.

Pour le bar européen, nous avons mis en évidence que les capacités de dispersion sont plus importantes. Les larves effectuent depuis les zones de ponte une migration moyenne d'environ 3 mois, mais avec des durées pouvant être variables au sein des sites, avant d'être recrutées dans les habitats de nourricerie. Ces résultats concordent avec la bibliographie (Reynolds et al., 2003, Parlier, 2006). Les capacités de mouvement des juvéniles sont probablement plus importantes que celles des lançons au vu de la variabilité des profils microchimiques au niveau de la phase juvénile. Ces deux premiers stades de vie permettent déjà à l'espèce de parcourir de grandes distances et d'utiliser des habitats très distants spatialement (Figure 5). Mais ses capacités de dispersion sont encore plus importantes pour les adultes. En effet les déplacements vers les zones de reproduction en hiver peuvent s'étendre sur une centaine de kilomètres et les mouvements dans les zones côtières en période estivale ont été mesurés sur une vingtaine de kilomètres (Pawson et al., 2007, 2008). Les échanges entre les HEE à l'échelle régionale, voire à l'échelle de la Manche, induisent un brassage génétique des

populations important puisque aucune différenciation génétique n'a été réellement démontrée pour le moment à cette échelle géographique (Bonhomme et al., 2002, Fritsch et al., 2007).

A l'opposé, les faibles capacités de dispersion des Ammodytidés tendent en faveur d'une structure en métapopulations à des échelles spatiales plutôt locales (Figure 5). Une analyse via des marqueurs génétiques pour étudier la structure et le brassage génétique au sein des espèces de lançon sera engagée dans les trois prochaines années et est une perspective intéressante de poursuite des travaux présentés ici. En effet, le brassage génétique et la variabilité des histoires de vie permettent aux espèces d'augmenter leur capacité de résilience (Figure 5) (Secor, 2007, Kerr et al., 2010). Ces petites métapopulations seraient sans doute plus vulnérables que le bar européen, face aux changements environnementaux et aux pressions extérieures comme la détérioration de leurs habitats sableux.

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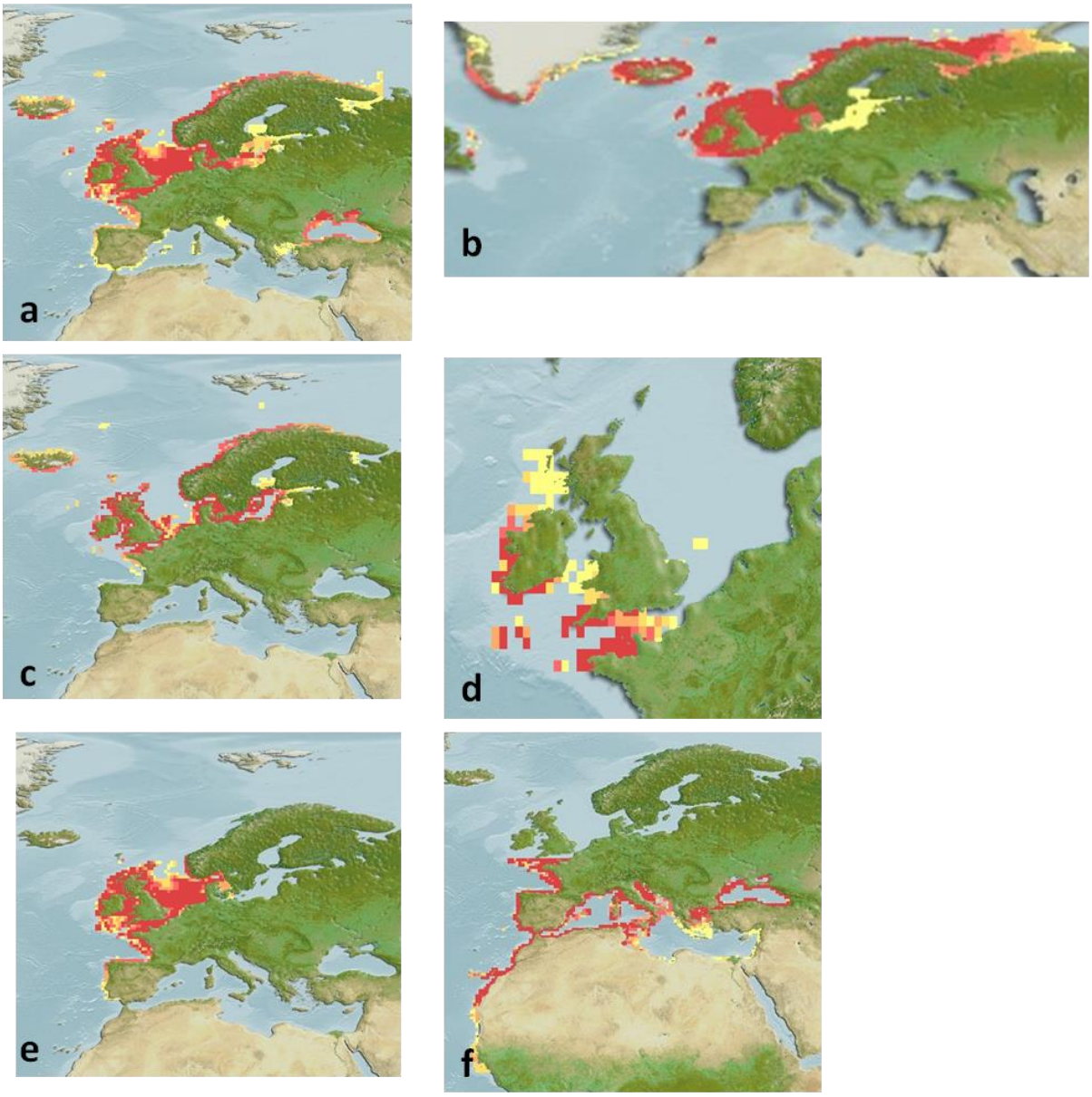
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ANNEXES

Annexe 1 : Distribution géographique des espèces dans l'hémisphère Nord

Figure 1 : Distribution géographique des six espèces de lançon présentes dans les mer et océans adjacents à la France. a) *Ammodytes tobianus*, b) *Ammodytes marinus*, c) *Hyperoplus lanceolatus*, d) *Hyperoplus immaculatus*, e) *Gymnammodytes semisquamatus*, f) *Gymannamodytes cicerelus*.



Annexe 2 : Identification morphologiques des espèces de lançon

Une synthèse sur les critères morphologiques permettant l'identification des espèces est réalisée à partir de trois ouvrages (Bauchot et Pras, 1980, Quéro et al., 2003, Louisy, 2002).

Pour simplifier le référencement des critères, une lettre est assignée aux trois ouvrages utilisés.

A = Bauchot, M. L. and A. Pras. (1980). Guide des poissons Marines d'europe (Marine Fish Guide from europe). *Delachaux & Niestle (Eds). Lausanne–Paris*, 427 p.

B = Quéro, J. C., P. Porché, and J. J. Vayne. (2003). Guide des poissons de L'Atlantique européen. *Delachaux & Niestlé (Eds)*. *Les Guides du Naturaliste, Lonay/Paris*. 465 p.

C = Louisy, P. (2002). Guide d'identification des poissons marins Europe et Méditerranée. *Ulmer (Eds), Paris*. 430 p.

Lorsqu'aucune référence bibliographique n'est citée, il s'agira de nos propres observations.

Les deux premiers critères facilement discernables sont la forme de la nageoire dorsale et anale ainsi que la forme de la pupille. Si les nageoires sont à bords sinueux (A, B, C) et que la pupille est en forme de poire (Annexe 3, Photographie b), il s'agit du genre *Gymnnammodytes* (cf **2**), dans le cas contraire les bords des nageoires dorsales sont droits (A, B, C) et la pupille est ronde, il s'agit du genre *Ammodytes* ou *Hyperoplus* (cf **3**).

(**2**) ***Gymnammodytes semisquamatus*** (i.e Gs) est l'espèce présente sur nos côtes car *Gymnammodytes cicerellus* est une espèce méditerranéenne (A, B, C).

G. semisquamatus a une ligne latérale avec des diverticules vers le haut et le bas (A, B) (ANNEXE 3, Photographie h) tandis que les autres genres ont une ligne latérale avec de temps en temps des diverticules vers le haut (Annexe 3, Photographie g, i). Cette espèce a une nageoire dorsale sinueuse avec 56-59 rayons (A). Elle mesure en moyenne 25 cm (A, B, C).

G. semisquamatus présente un repli de peau ventrolatéral uniquement au niveau des pectorales, contrairement à *G. cicerellus*, pour lequel ce repli atteint l'anus (B) (critère non pris en compte). Elle possède entre 53 et 59 rayons dorsaux (B). Elle mesure en moyenne 15 cm (A, C).

(**3**) Le genre *Ammodytes* se caractérise par une mâchoire supérieure protractile (A, B, C) et l'absence de véritables dents vomériennes (A, B) (Annexe 3, Photographie b) (cf **4**).

Le genre *Hyperoplus* présente une mâchoire supérieure moins protractile (A, B, C), mais des dents vomériennes sont bien visibles (A, B) (Annexe 3, Photographie f) (cf **5**).

Cependant le caractère protractile est délicat à utiliser. En effet, la lèvre supérieure est fixée par un isthme à la mâchoire chez *Hyperoplus*, celui-ci se détache souvent lorsque l'on veut vérifier le critère et s'apparente alors à une mâchoire protractile. Cependant en déployant la mâchoire au maximum, elle dépasse toujours la mâchoire inférieure chez *Ammodytes sp* et rarement chez *Hyperoplus sp*.

(**4**) Deux espèces se distinguent pour le genre *Ammodytes* et aucune ne présente de véritables dents vomériennes (A, B, Annexe 3, Photographie b).

Ammodytes tobianus a une base de la caudale écailleuse (A, B, C) et des écailles ventrales disposées en chevron (B). Un nouveau critère a été découvert par H. Jensen récemment (van Deurs et al., 2012) et décrit la présence d'une bande M à la base de la nageoire caudale et qui ne serait pas présent chez *A. marinus*. Selon les ouvrages le nombre de rayons dorsaux varie entre 49-58 (B) ou 50-56 (A). Le nombre de vertèbre est de 60 à 66 (A). Sur les tous les individus identifiés, 52 à 55 rayons dorsaux ont été observés. Nous observons que la ligne latérale est droite et que les écailles situées au-dessus forment des lignes obliques (Annexe 3, Photographie g). Elle mesure en moyenne 20 cm (A, B, C).

Tandis qu'***Ammodytes marinus*** ne présente pas d'écaille ni de bande M à la base de la nageoire caudale (A, B, C, van Deurs et al., 2012) et des écailles ventrales non disposées en chevron (B). Le nombre de rayon dorsaux varie entre 55 à 57 (A) ou 56 à 63 (B). Le nombre de vertèbre varie entre 66 à 72 (A). Elle mesure en moyenne 20 cm (A, B, C). La répartition géographique sud d'*Ammodytes marinus* est mal connue et elle semblerait absente de nos côtes bretonnes mais il faut cependant resté vigilant lors de l'identification.

(**5**) Il existe deux espèces pour le genre *Hyperoplus*.

Hyperoplus lanceolatus (i.e HL) a une tache noire sur le museau de la grosseur de l'oeil (A, B, C) (Annexe 3, Photographie c). Les dents vomériennes sont bien visibles dans la mâchoire supérieure (A, B) et la base du vomer est plus fine que la pointe des dents qui tend à diverger (Annexe 3, Photographie f). En général, le premier rayon de la nageoire dorsale est positionné en arrière de la pointe des nageoires pectorales (A, B). Le nombre de rayons dorsaux varie entre 52 à 61 (B) et 52 à 61 et de 65 à 69 vertèbres (A). Le nombre de rayons dorsaux observés varie entre 54 à 56. Elle mesure en moyenne 30 cm et 40 cm au maximum (A, B, C).

Hyperoplus immaculatus (i.e Hi) n'a pas de tache noire sur le museau mais ses mâchoires sont foncées (Annexe 3, Photographie a) (A). Les dents vomériennes sont bien visibles (A, B) et la base des dents est large tandis que les pointes des dents vomériennes sont plutôt convergentes. Le nombre de rayons dorsaux observé est compris entre 59-62 et la littérature en recense 59-62 (B) ou 60-67 (A). Le nombre de vertèbres varie entre 70 à 74 (A). Le premier rayon de la nageoire dorsale est situé en avant de la pointe des nageoires pectorales (A, B). La ligne latérale est droite et présente des petits diverticules (points) au-dessus (ANNEXE 3, Photographie i). Elle mesure en moyenne 25-30 cm et 35 cm au maximum (A, B, C).

Des erreurs d'identifications entre *A. tobianus* et *H.immaculatus* ont été constatées dans différentes campagnes de pêche et des rapports bibliographiques plus anciens. En effet, les

deux espèces peuvent avoir une pigmentation proche mais la seconde est beaucoup plus grande (taille couramment observée : *A. tobianus* 12 cm, *H. immaculatus* 22 cm), a une mâchoire inférieure plus sombre et une forme très pointue vers l'extrémité (Annexe 3, Photographie a), un nombre de rayons dorsaux plus grands et des dents vomériennes bien visibles avec une forme un peu différente d'*H. lanceolatus* (Annexe 3, Photographie f). Nous avons constaté qu'après la mort et souvent après la congélation, *A. tobianus* peut devenir plus verdâtre comme *Hyperoplus*, pouvant ainsi amener à des confusions dans l'identification en se basant uniquement sur la couleur du corps.

Annexe 3 : Distinction des critères morphologiques des espèces



Photographies : a) *A. tobianus* au-dessus et *H. immaculatus* au-dessous. *H. immaculatus* a des mâchoires supérieures foncées mais pas de tache noire circulaire comme *H. lanceolatus*. Sa mâchoire inférieure est plus pointue, b) *G. semisquamatus* pupille en forme de poire, c) *H.lanceolatus* mâchoire inférieure plus émoussée et tête plus fine chez le genre *Hyperoplus*, d) *A. tobianus* absence de dents vomériennes distinctes (forme plutôt collée), e) *H. lanceolatus* bouche protractile, la mâchoire supérieure se décolle un peu du museau. La tache noire sur le museau est bien visible, f) *H. lanceolatus* véritables dents vomériennes, g) *A. tobianus* ligne latérale droite et les écailles situées au-dessus sont alignées obliquement, h) *G. semisquamatus* ligne latéral présentant des diverticules courbés vers le bas et droits vers le haut, i) *H. immaculatus* ligne latérale droite avec quelques points juste au-dessus.

Annexe 4 : Life history of the Small Sandeel, *A. tobianus*, infered from otolith microchemistry. A methodological approach.

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Life history of the Small Sandeel, *Ammodytes tobianus*, inferred from otolith microchemistry. A methodological approach

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ABSTRACT

Knowledge of life history and connectivity between essential ecological habitats are relevant for conservation and management of species and some natural tracers could be used to study the lifecycles of small or short-lived marine fishes. Although sandeels are central in marine food webs and are key species, there is incomplete knowledge about population mixing and migration patterns. For the first time the use of the otolith microchemistry on sandeel species is evaluated in the case of the Small Sandeel. Variations in microchemical fingerprints of 13 trace elements are performed with a Femtosecond LA-ICPM from the core to the margin of sagittal otolith and are compared within and between otoliths extracted from 34 fishes sampled in three different sites along the coast of the south-western English Channel in France. Firstly, preliminary investigations on the validity of the method revealed that Mg/Ca was the only ratio significantly dependant on fish ontogeny and sampling season. Secondly, the Mn/Ca, Zn/Ca, and Cu/Ca ratios enabled us to significantly discriminate among sampling sites. Thirdly, microchemical fingerprints of each life stage varied significantly among sampling sites but not within them, suggesting high site fidelity over relatively short distances. Finally, the fingerprints of all life stages were significantly different from those of the larval and metamorphosis stages. The otolith microchemistry could detect change of signature relative to the shift from a pelagic behaviour to a resident bentho-pelagic behaviour during the middle of the juvenile stage in Small Sandeels. Hence, analysis of trace element fingerprints in otoliths appears to be a valuable method to further studies on ontogenic habitat change, population mixing and variation of life history and be helpful for the management at local or regional scales of short-lived species such as those belonging to other Ammodytidae.
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1. Introduction

Identification of connectivity between essential habitats during the lifespan of species is key to the population ecology and management of fish. This has been studied in numerous marine fishes that move from estuaries or salt marsh systems during their juvenile stage to offshore habitats for growth and/or spawning (Hansen and Quinn, 1998; Fritsch et al., 2005; Aarestrup et al., 2009; Daverat et al., 2011; Mercier et al., 2012). Movements and migrations are also commonly studied in diadromous fish to study their land-ocean connectivity (Koutsikopoulos et al., 1995; Feutry et al., 2011; Bultel et al., 2014). However, such connectivity has most often been studied for large fish with long lifespans (Galuardi and Lutcavage, 2012) and with migration loops by fish across large distances (Secor and Zdanowicz, 1998; Block et al., 2001) and/or between contrasting habitats (e.g. marine to inland, estuarine to marine, etc.) (Milton and Chenery, 2003; Daverat et al., 2011; Isnard et al., 2015).

The focus on short, holobiotic migration loops, for example in coastal areas and for small fish with short lifespans is increasing (Goto and Arai, 2003; Keith, 2003; Aldanondo et al., 2010; Tabouret et al., 2011). This is relevant not only from a fundamental perspective, but also from a management perspective. For example, many small coastal fishes, such as sandeels (Ammodytidae family), are keystone species of marine ecosystems. Their mid-trophic position in the foodweb make them a forage prey for top predators, including marine mammals, seabirds, and fish species (Wanless et al., 2005; Eliasen et al., 2011; Engelhard et al., 2013, 2014).

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Sandeels are also economically important, but their stocks appear to fluctuate and have declined in recent years through over-fishing or because of global change, which has modified the structure of marine foodwebs (Wanless et al., 2004; Frederiksen et al., 2007, 2011). In recent years, some studies on sandeel communities were conducted to examine the impact caused by the development of offshore wind farms and marine sediment extraction for construction on these fishes (e.g. van Deurs et al., 2012). Indeed, well-oxygenated sandbanks, preferably with a low fraction of silt and clay (Wright et al., 2000), play important ecological roles for sandeels, which exhibit the unusual habit of alternating between pelagic swimming for feeding and lying buried in the sand substrate even at low tide in intertidal areas, at night and during winter (Winslade, 1974; Robards et al., 2000; Jensen et al., 2011; van Deurs et al., 2011a,b). Sandbanks could consequently be considered an essential ecological habitat (EEH) (e.g. spawning ground, nursery, feeding, or resting habitat) (Rijnsdorp et al., 2009; Petitgas et al., 2013) for sandeel.

The Small Sandeel, *Ammodytes tobianus*, the most abundant sandeel species in intertidal sandy habitats, has a maximum age of 7 years old, can reach maturity at one year old and spawns twice a year (spring and autumn) (Reay, 1972, 1973; Kopp, 1978; O'Connel and Fives, 1995), and it also remains unclear whether the Small Sandeel is an obligate intertidal spawner (Robards et al., 2000). The Small Sandeels are rarely caught offshore (Jensen et al., 2004; van Deurs et al., 2011a,b). Despite the ecological and commercial importance of sandeels (Engelhard et al., 2014), studies have examined mainly the Lesser Sandeel, *Ammodytes marinus*, in the North Sea (Wright, 1993; Wright and Bailey, 1996; Wright et al., 2000; Frederiksen et al., 2011). Recent investigations on this species have shown very limited migration behaviour with a high site fidelity to a 'home sandbank' after settlement and little mixing between grounds (Engelhard et al., 2008; Jensen et al., 2011). Furthermore, for the Lesser Sandeel, dispersion between sandy areas is suspected to occur via the drift of pelagic larvae controlled by ocean currents (Christensen et al., 2008). The study of the Lesser Sandeel movements between sandbanks has been tested in through field studies and models (Christensen et al., 2008; Engelhard et al., 2008; Jensen et al., 2011) but the mixing between populations and its life histories are not totally unravel.

As it is not currently possible to perform telemetry studies with small fish species, the purpose of the present paper is to validate, for the first time for an Ammodytidae species, the Small Sandeel, the use of otolith microchemistry as a potential tracer of movements between habitats during the life history, which may exhibit little migration behaviour, except maybe during its larval stage. Otoliths are calcified inert structures located in the inner ear of fish that grow continuously throughout life (Campana and Neilson, 1985). Because they incorporate the chemical elements of the surrounding waters at the time of deposition, otoliths act as natural tags (Milton and Chenery, 2001; Lin et al., 2007; Marohn et al., 2009), and their structures (i.e. macrostructure and microstructure) as recorder of life stages (Campana, 1999). Furthermore, otolith microchemistry also provides powerful information on migration patterns and the habitat used during their life cycle (Gillanders, 2005; Arai and Hirata, 2006; Feutry et al., 2011; Lord et al., 2011; Mercier et al., 2012).

Our hypotheses are: a) if the Small Sandeel exhibit comparable limited migration behaviour to the Lesser Sandeel, then their otoliths will have distinct microchemical fingerprints between individuals from different sandy beaches; and b) if sandeels do not use different habitats during their lifecycle, reflecting a resident behaviour, the elemental composition will not vary within the otolith from the core to the margin.

This study firstly assesses the variation in size class distribution of Small Sandeels over a one-year bimonthly survey to detect recruitment periods and growth rates. The use of the otolith macrostructure is validated by comparing the growth calculated from cohorts and from otoliths. Then, hypotheses are tested by comparing the elemental composition within otolith macrostructure and between otoliths of Small Sandeels captured in three nearby coastal areas of Northern Brittany and Normandy, France. Finally, to validate the first use of otolith microchemistry on sandeel species, the stability of the microchemical signatures among seasons and ontogenic development are tested. The results are used to discuss whether microchemical tracers of the otoliths are useful to analyse the life history traits of a coastal, non-migratory, short-lived fish species such as the Ammodytidaes.

2. Materials and methods

2.1. Study area and fish sampling

The three studied intertidal sandbanks are located in the south-west English Channel along the coast of the Norman-Breton Gulf. The main study site, Lancieux Bay, is located at the mouth of the Frémur estuary, and the other sites chosen for testing the site fidelity of Small Sandeels were Rotheneuf Bay and at the Chausey archipelago (respectively 20 and 40 km from Lancieux) (Fig. 1). Sandeels were sampled at Lancieux Bay with a shovel in the sand ripple marks of the beach at low tide twice a month for a year (February 2012 to January 2013). All of the fish were stored at −20 °C within 1 h of capture for further identification and measurement (fork length (FL) in mm) at the laboratory.

In order to test ontogenic and seasonal variations in microchemical signature, 24 sagittal otoliths were extracted from juveniles and adults and in different seasons: 5 0-group juveniles caught in February and July, and 5 adults caught in July and 9 in November. Additionally, to identify spatial signatures among the three sites, sagittal otoliths were extracted in July, from 2 0-group juveniles and 3 adults caught at Chausey archipelago and 5 0-group juveniles at Rotheneuf Bay.

2.2. Otolith preparation and analysis

After extraction, the sagittal otoliths were washed three times in an ultra-pure water bath (milliQ 0.0055 μS). After the remaining tissues were removed under a binocular microscope, otoliths were dried and stored in 1.5-mL plastic Eppendorf tubes. The left otolith extracted from each fish was embedded in araldite resin 2020 (Huntsman) with the sulcus acusticus downward. They were grounded in the sagittal plane up to the core with ultra-pure water and sandpaper with grains gradually decreasing from 2400 μm to 1200 μm, 9 μm, and 3 μm. Finally, the otoliths were rinsed with ultra-pure water and air-dried.

Otolith microchemical composition was assessed using 257 nm femtosecond laser ablation (Lambda 3, Nexeya, France) inductively coupled with plasma mass spectrometry (Elan DRCII, Perkin Elmer) (LA-ICPMS). This delivers 360 fs pulses at wavelengths of 1030 nm and can be operated at high repetition rates (up to 100 kHz). A 2D galvanometric scanner allows the fast movement of the laser beam (10 μm) at the surface of the sample to simulate virtual beam shaping when the laser is operated at a high repetition rate. Considering the otolith growth ring pattern, an elongated laser beam (10×50 μm^2) was simulated in order to preserve the high spatial resolution while keeping the highest signal sensitivity. The laser was operated at 300 Hz with a pulse energy of 35 μJ while the scanner was doing a permanent 35-μm-wide, oscillating movement at a speed of 2 mm/s, resulting in a 20×50 μm laser beam.

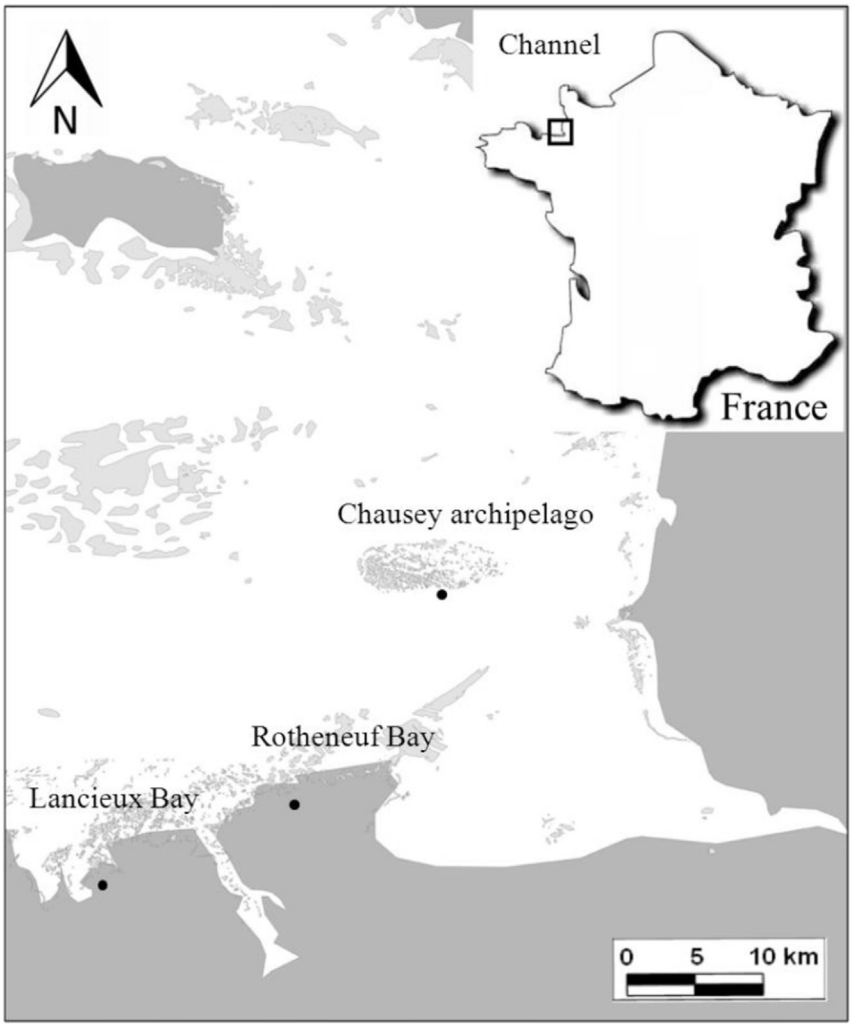


Fig. 1. Location of the three intertidal sampling sites of Small Sandeels in the Norman-Breton Gulf (south-western English Channel).

Combined with this oscillating movement, the sample was continuously moved along the posterior axis from the nucleus to the edge of the otolith at a speed of 5 μm/s, resulting in an uninterrupted ablation on the grounded surface. In order to prevent a blast effect on the nucleus, the ablation was started 200 μm before the nucleus. The ablation depth was evaluated at 10 μm.

At the beginning and end of each session, careful calibrations were carried out using NIST 610, 612, and 614 (National Institute of Standards and Technology). Quality control was systematically evaluated using pelletized CRM NIES 22 otolith powder (Certified Reference Material produced by the National Institute for Environmental Studies). ^{43}Ca was used as an internal standard for each ablation to correct for instrumental error in terms of ablation yield, sample transport and detection. Analysed isotopes were ^{86}Sr, ^{135}Ba, ^{138}Ba, ^{24}Mg, ^{26}Mg, ^{55}Mn, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{68}Zn, ^{57}Fe, ^{232}Th, and ^{238}U, which are frequently used in microchemistry studies (Vasconcelos et al., 2011). Isotopes for which 75% of the measurements were above the limit of detection for at least one individual were retained. Furthermore, for elements with two isotopes (e.g. ^{63}Cu, ^{65}Cu), only the isotope with the highest natural abundance was kept after checking that no spectral interference was affecting the reliability of the result. After standardization by calcium (Campana, 1999), the remaining element ratios were Ba/Ca, Sr/Ca, Mn/Ca, Zn/Ca, Cu/Ca, Fe/Ca, and Mg/Ca.

To match the microchemical signatures with the different life stages of sandeels, macrostructural analyses of the otoliths (Fig. 2) were performed. The larval stage (L) was clearly distinct on the otolith with a change in the growth axis, which corresponds to settlement into sediment (Wright, 1993). Opaque zones (under transmitted light) correspond to rapid growth in summer (S), whereas translucent zones were interpreted as low growth periods in winter (W) (ICES, 1995, 2006). Finally, we postulated that fish had spent sufficient time at the site of capture to assimilate a local fingerprint. The site of capture signature (C) was therefore considered to be the fingerprint measure within the external part of the otolith (Number 9, Fig. 2B). After the continuous ablation, each otolith was photographed to measure larval, translucent, and opaque zones from the core to the end of otolith. The average of all of the element ratios was assessed at the centre of each zone except for the first summer growth zone (S0). This larger summer growth zone was divided into three sub-zones where means of element ratios were calculated (i.e. at the beginning (S0b, 20 μm after the larval stage), middle (S0m), and end (S0e) of the S0 zone) (Fig. 2B). The length of the ablation segment used to calculate the mean element ratios was adjusted to the width of each zone, which varied among fish and according to their location within the otolith. When the identified opaque or translucent zone measured more than 200 μm, the mean of element ratios was calculated over a

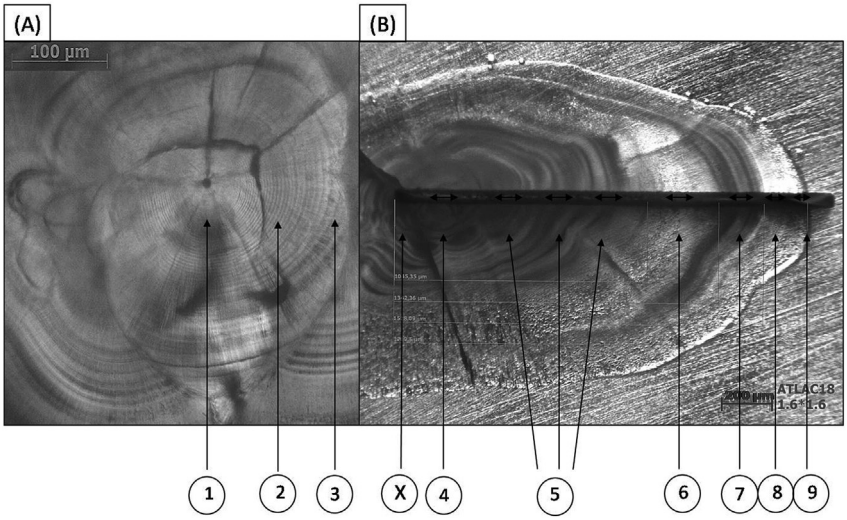


Fig. 2. Microscopic photographs of an otolith from *Ammodytes tobianus* after sagittal section (transmitted light). (A): Focus on the circular larval zone: (1) nucleus, (2) daily increments, (3) end of the circular larval zone. (B): Otolith after the linear continuous ablation (X): (4) circular larval zone = L, (5) first summer growth season (S0) divided in three zones: beginning = S0b, middle = S0m, end = S0e, (6) first winter growth season = W0, (7) second summer growth season = S1, (8) second winter growth season = W1, (9) edge of otolith and signature of the capture site = C. Black horizontal arrows represent the distance considered to calculate the means of element ratios in different identified zones of the otolith.

100 µm distance, and when it was inferior to 200 µm, the mean was calculated over a 50 µm distance. Finally, the signature of capture was calculated over 30 µm at 20 µm from the edge of the otolith.

2.3. Data analysis

At Lancieux Bay, sampling was twice a month and a total of 642 *Ammodytes tobianus* were measured. However, for simplification, the size-class distributions were plotted on a monthly basis. Following a cohort analysis, age classes were determined and enabled estimation of the season of birth of juveniles. A code, for example G0 A2011, was attributed to each individual and represents the cohort (i.e. age class: G0, G1, etc.), the season (S: spring; A: autumn) and the year of birth. Age estimations were validated using age readings of otoliths that were extracted for the microchemistry and macro-structural analyses (n = 24). The growth at one year of age was only calculated for two young cohorts (G1 and G0 A2011), for which it was possible to estimate the season of birth.

A linear model (LM) between the fork length (FL) of fish and the total length of their otolith (n = 44) with a Pearson correlation test (the normality and homoscedasticity of data were verified by Shapiro–Wilk and Bartlett tests) was performed to validate that otolith length was a proxy for fish length. This relationship permitted us to back-calculate Small Sandeel growth and to link different life stages of fish to LA-ICPMS results. The length of the larval stage and the first growth zone in otoliths (n = 24) were compared between the different cohorts using a linear model (LM).

Secondly, to validate the value of otolith microchemistry in Ammodytidae, the potential ontogenic and seasonal effects were analysed with linear model (LM) on the microchemical fingerprint from the marginal zone (i.e site of capture (C), see Fig. 2B) by comparing juveniles and adults sampled at different times (juveniles caught in February and July and adults in July and November).

For all LM the normality of residuals was verified with the QQ-plot and there was no violation of the assumption to apply the Gaussian distribution. When a significant difference was detected with the Anova (F-test) type III (for the un-balanced data ('car' R package)), a multiple pair-wise comparison (Tukey post-hoc test) was applied ('multcomp' R package). For the highly unbalanced data, which occurred when analysing the otolith widths of the larval stage and the first growth zone, a bootstrap with 1000 iterations was applied after the Anova type III to verify the robustness of the results.

Finally, to distinguish the potential difference in habitat fingerprints on otoliths, the fingerprints of the marginal zone (C) of adults and juveniles captured at the three sites were compared (juveniles and adults from Lancieux and Chausey, only juveniles from Rotheneuf). Three different classification methods were used to estimate the contribution of elements measured in otoliths since some elements could provide more noise than real signal: linear discriminant analysis (LDA) and two learning methods, random forest (RF) and artificial neural networks (ANN), which are less demanding in terms of assumptions than LDA. For the LDA, capture fingerprint data met requirements for normality and homoscedasticity (i.e verified by Shapiro–Wilk and Bartlett tests), and all element ratios were standardized to give them the same weight due to differences in magnitude (Mercier et al., 2011). RF, is a tree classification method, which separates at each node the dataset in binary groups. This enables to randomly look for the group of element ratios that maximizes the homogeneity into the two groups. Each group then splits again following the same procedure until no more homogeneity is found. ANN is a system of interconnected neurons, which computes values from input neurons to hidden and finally to output neurons and linked by a function (linear, logarithmic, etc.). To identify the best method to select an optimal element combination for the discrimination of site fingerprints, the maximal prediction accuracy (i.e. the percentage of correct assignment of the fish to their capture habitat) was tested according to the methods used (RF, LDA, and ANN) and for each possible combination of 1 to N chemical elements ($2 \times \exp^{(N-1)}$ possible combinations). Furthermore, a cross-validation was performed for each element combination by testing 1000 replicates. For each cross-validation procedure, 75% of the fish (training data set) were randomly chosen to train the classifiers, the remaining 25% being used to measure the quality of prediction. Five hundred trees were built for the random forest method. For more details, see the R script named R_otolith_microchem_elements_and_method_selection.R (Mercier et al., 2011). To complete this analysis, a multivariate analysis of variance

(MANOVA) type III (for un-balanced data, 'car' R package) was used between the fingerprints of the three sites.

Lastly, to analyse site fidelity according to sites and life stages, microchemical fingerprints of each macrostructure (see Fig. 2, L, S0b, S0m, S0e, W0, S1, W1, etc.) were compared with each other and to the capture fingerprint (C, site signature) with MANOVA type III. All MANOVA were used with the Pillai's trace since it is relatively robust to deviations from multivariate normality (Johnson and Field, 1993). Indeed, half of the elements (i.e. Ba/Ca, Sr/Ca, and Fe/Ca) in the data based on different life stages of Lancieux fishes did not meet normality even after $\log_{10}$ transformation.

All the element ratios were transformed by log10. The threshold for rejection of the null hypothesis was defined at $p = 0.05$ and coded as follow: *: $0.05 > p > 0.01$; **: $0.01 > p > 0.001$; and highly significant ***: $p < 0.001$. All statistical analyses were performed using R software (R-2.15.1 R Development Core Team 2012).

3. Results

3.1. Growth estimation: validation from cohorts and otolith microstructures

Four species were caught at Lancieux Bay: *Hyperoplus lanceolatus*, Hyperoplus *immaculatus*, *Gymnammodytes semisquamatus* and *Ammodytes tobianus*, the last accounted for 73.04% (n = 642) of the catches. At Lancieux Bay, fish sizes ranged from 38 to 175 mm (Fig. 3). A maximum age of 5 years was observed, but most fishes were one or two years old. Irrespective of the sampling period, individuals from the G2 (131.62 ± 7.53 mm) and G3 (146.47 ± 8.34 mm) age classes were detected. At the beginning of the survey, G1 individuals born in spring 2011 (96.71 ± 7.83 mm) were still very abundant in February and April 2012, with an estimated age of around one year old (i.e. G1, Fig. 3, black stars). During the survey, two newly recruited juvenile cohorts were detected; the first, born in autumn 2011 (G0 A2011, 41.37 ± 2.37 mm, Fig. 3), appeared at the beginning of the survey in February 2012 and grew 7.93 mm between February and March, with a maximum growth rate in May (20.98 mm/month). For this cohort, the growth started to decrease at the end of the summer, was very low during the winter (0.46 mm between November and December) and reached one year of age during the autumn (Fig. 3, black stars). Conversely, individuals born during the spring of that year (G0 S2012, 40.64 ± 3.56 mm, Fig. 3), recruited in May, had higher growth at the beginning of their life (7.93 mm and 17.98 mm between the first two months, G0 A2011 and G0 S2012, respectively) and a higher maximal growth rate in June (34.38 mm/month) than individuals from G0 A2011. Interestingly, at the end of their first year of growth, juveniles were almost the same size regardless of their recruitment period.

Finally, at one year old, individuals of G1 and G0A2011, from whom it was only possible to estimate the season of birth, measured 109.89 ± 4.08 and 120.75 ± 4.50 mm, respectively (Fig. 3, black stars).

Fork length (FL) and the otolith total length (Toto) were highly correlated and can be expressed as $FL = 0.0454 \times Toto + 11.083$ ($R^2 = 0.94$, Df = 41, $p < 2.2e^{-16***}$, Fig. 4). The mean length of sandeels at one year of age was back-calculated from adult otolith diameter at the end of the first opaque zone of the otolith, and was estimated at 118.33 ± 7.15 mm.

The larval zone had an average diameter of 265.62 ± 21.05 μm (n = 24) and did not change among cohorts (Df = 3, p = 0.30, Fig. 5). However, the following first growth zone (i.e. S0) was significantly wider for individuals born in autumn (i.e. G0 A2011, 1927.52 ± 75.00 μm, n = 3) than individuals born in spring (G1, 1323.63 ± 107.01 μm, n = 5; G0 S2012, 1398.17 ± 68.99 μm, n = 5), but also for older individuals (n = 11) (Df = 3, $p = 2.02e^{-05***}$, Fig. 5). G2 might also be born in spring considering their small S0 zone.

3.2. Significance of microchemical fingerprints in otoliths

3.2.1. Ontogenic and temporal variation in otolith fingerprints

Mg/Ca varied significantly both according to season and fish age (Df = 3, Deviance explained per factor = 77%, $p = 1.45e^{-06***}$) and exhibited higher ratios in juvenile otoliths and during the summer (Fig. 6). The microchemical fingerprints of the other elements ratios were not different (mean ± sd of all individuals: Ba/

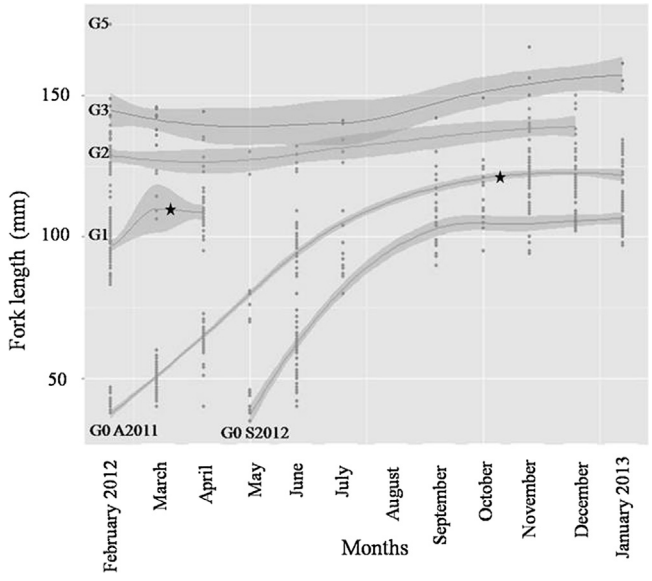


Fig. 3. Mean (solid lines) and individual sizes (mm) (points) distribution of Small Sandeels sampled from February 2012 to January 2013 (with confidence interval of 95% around the mean in grey). Codes represent identified cohorts (i.e. individual of the same age class: G0, G1, etc.), back-calculated season of birth (S: spring; A: autumn), and year of birth. G0 A2011 = group 0 born in autumn 2011, G0 S2012 = group 0 born in spring 2012, G1 = group 1 born in spring 2011, G2 = group 2, G3 = group 3, G5 = group 5. Accurate estimation of size reached by fish at one year of age was only possible for two cohorts (G1 and G0 A2011), and represented by black stars.

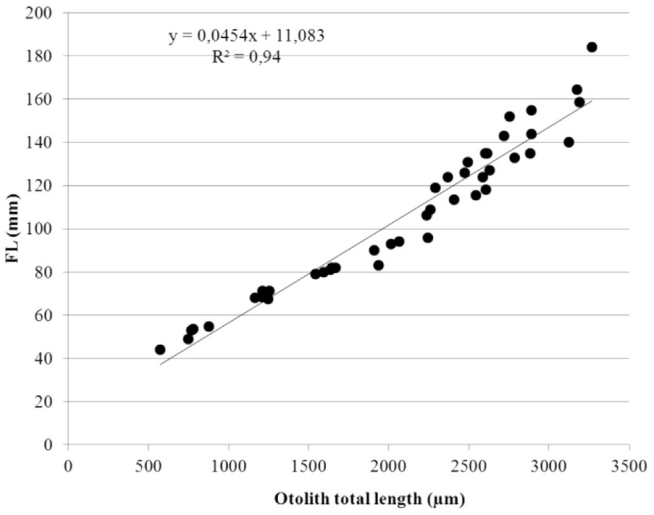


Fig. 4. Relationship between fish fork length (FL in mm) and total length of sagittal otolith (mm) (antero-posterior axis, n = 44).

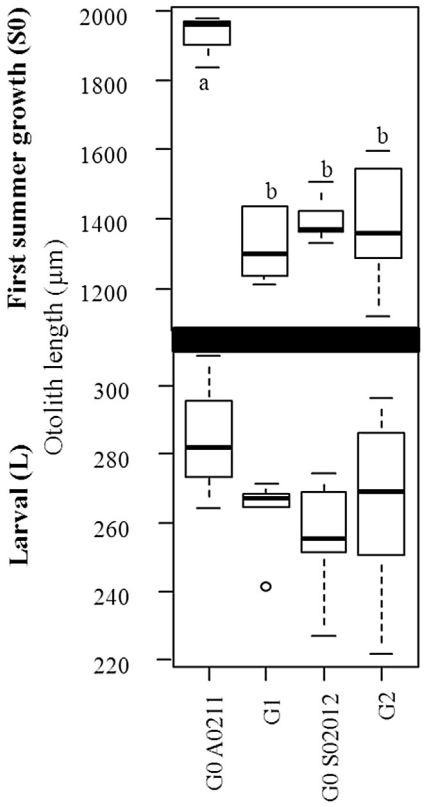


Fig. 5. Comparison between otolith lengths at the larval stage (L) and at the first summer growth (S0) according to different cohorts captured at Lancieux (n = 24). Cohorts used are G1 = group 1 born in spring 2011, G0 A2011 = group 0 born in autumn 2011, G0 S2012 = group 0 born in spring 2012, G2 = group 2 years old. Letters in superscript indicate significant differences ($p < 0.05$) between the cohorts (S0) according to a linear model. No significance difference occurs at larval stage (NS).

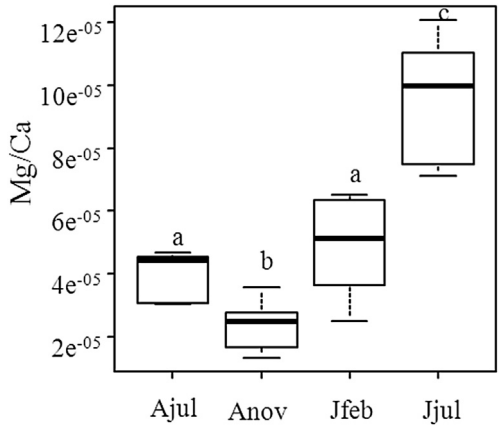


Fig. 6. Comparison of Mg/Ca ratios from juveniles and adults otoliths according to months of capture at Lancieux (n = 24; adults in July (Ajul, n = 5) and November (Anov, n = 9), juveniles in February (Jfeb, n = 5) and July (Jjul, n = 5)). Letters in superscript indicate significant differences ($p < 0.05$) from a linear model.

Ca = $2.12e^{-06}$ ± $6.24e^{-07}$, Sr/Ca = $4.40e^{-03}$ ± $1.02e^{-07}$, Mn/Ca = $1.19e^{-05}$ ± $6.51e^{-06}$, Zn/Ca = $8.63e^{-06}$ ± $7.38e^{-06}$, Cu/Ca = $8.12e^{-06}$ ± $7.95e^{-06}$, and Fe/Ca = $2.02e^{-03}$ ± $2.40e^{-04}$). Meaning the variation in Mg/Ca ratio in otolith fingerprint is more due to seasonal effects and physiological changes during the fish development than to ontogenic habitat changes (see Discussion). Therefore to avoid biaises, Mg/Ca was removed in further analysis.

Table 1
Maximal classification success (±standard deviation, sd) and best combination of elements obtained by three classification methods: linear discriminant analysis (LDA), random forest (RF), and artificial neural networks (ANN). Microchemical fingerprints are inferred from the external zone (signature of capture) of the Small Sandeel otoliths from Lancieux (n = 24), Chausey (n = 5), and Rotheneuf (n = 5).

Method	Maximal accuracy (% ± sd)	Combination of elements
LDA	83.79 ± 12.35%	Cu/Ca, Mn/Ca,
RF	79.19 ± 12.67%	Cu/Ca, Mn/Ca, Sr/Ca, Zn/Ca
ANN	78.44 ± 13.79%	Cu/Ca, Fe/Ca, Mn/Ca, Zn/Ca

3.2.2. Discrimination of sites and optimal element combination

The three classification methods performed (LDA, RF, and ANN) provided good maximal accuracy of prediction, falling between 78.44% and 83.79% (Table 1). LDA had the best maximal prediction accuracy (83.79%), and the best element combination was composed of Cu/Ca and Mn/Ca (Table 1). Capture signature in otoliths from Lancieux was significantly different from those of Chausey (Manova, $p = 0.0070^{**}$) and Rotheneuf (Manova, $p = 0.032^{**}$) but Chausey and Rotheneuf were not globally different (Manova, $p = 0.27$). Mn/Ca ratios were significantly higher in otoliths from Rotheneuf and Chausey than those from Lancieux (Fig. 7). Cu/Ca and Zn/Ca ratios were significantly higher in otoliths from Rotheneuf than the two other sites (Fig. 7).

3.3. Comparison of life stage signatures from Lancieux sandeels with signatures of sites of capture (Chausey, Lancieux, and Rotheneuf)

The large majority of the microchemical fingerprints found for the different life stages (i.e. macrostructural zones of otoliths) of Small Sandeels captured at Lancieux always appeared different from the Chausey and Rotheneuf capture signatures (Table 2). Among the elements, Cu/Ca, Mn/Ca, and Zn/Ca ratios were always significantly different from the Rotheneuf capture signature, and Mn/Ca and Fe/Ca ratios differed significantly and the most frequently from Chausey capture fingerprint (Table 2).

The microchemical signatures varied significantly from the centre (larval stage) to the margin of the otoliths. Two main differences occurred between the larval stage (L) and the beginning of the first growth stage (S0b) (Table 3). The larval stage was more enriched in Sr/Ca, Ba/Ca, Zn/Ca, and Cu/Ca than the S0b stage (Table 3). The second significant change was between this latter zone (S0b) and the first mid-growth stage (S0m) (Table 3). Then, the first mid-growth stage (S0m) did not differ significantly to the end of this zone (S0e). These three first zones (L, S0b, S0m) were significantly different from the Lancieux capture fingerprint, unlike for all of the following zones of the otolith (S0e, W0, S1, W1 and C), which did not vary significantly (Table 3).

4. Discussion

4.1. Methodological validations

Results of growth estimation validate the correlation between otolith and fish length and permit to further link the different life stages of otolith to the continuous laser ablation. The definitive length of fish, almost reached at one year of age, was estimated from otoliths at 118.33 ± 7.15 mm and was corroborated by the size estimated from the two cohorts (respectively 109.89 ± 4.08 and 120.75 ± 4.50 mm, see Fig. 3, black stars). This is in accordance with Reay (1973), who found fish sizes at one year of age ranging between 110 and 114 mm for two different years. Furthermore, the length of the first growth zone in otoliths can help to detect the

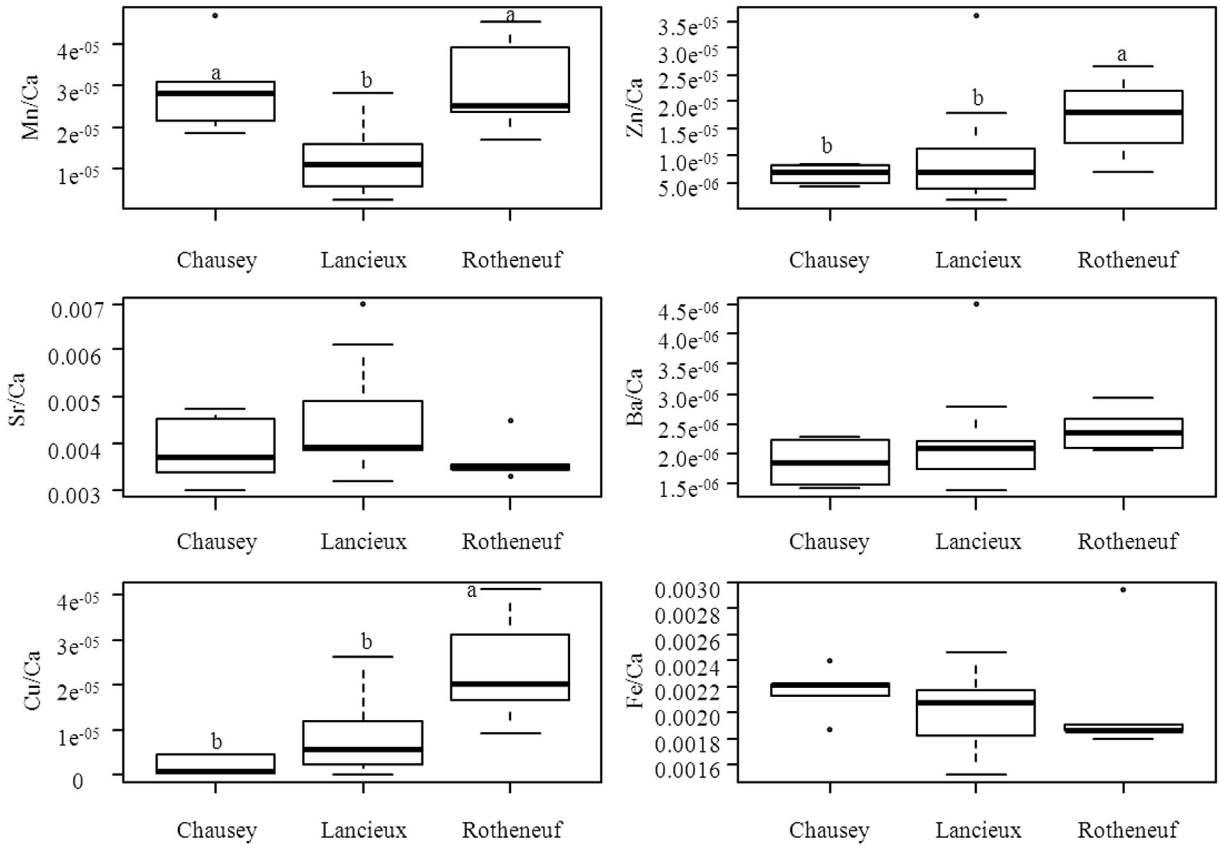


Fig. 7. Boxplots comparing different element ratios from Small Sandeels otoliths from the three sites of capture. Element ratios are inferred from the external zone of the otoliths (capture fingerprint) of Lancieux (n = 24), Chausey (n = 5), and Rotheneuf (n = 5). Letters in superscript indicate significant differences (MANOVA, $p < 0.05$) between sites, when present.

Table 2
Multivariate analysis of variance (MANOVA type III) comparing the different microchemical signatures (corresponding to different life stages) of Small Sandeel otoliths from Lancieux (n = 24) with capture signatures of the otoliths from Chausey (n = 5) and Rotheneuf (n = 5). The macrostructure of the Lancieux otoliths is divided into several zones (see materials and methods): L = larvae; S0b = beginning, S0m = middle, S0e = end of first opaque zone corresponding to the first summertime growth period; W0 = first translucent zone corresponding to the first winter growth period; S1 = second opaque zone (second summertime growth period); W1 = second translucent zone (second winter growth period). Indicated p values are the mean of different p values calculated for each element ratio between pairwise analyses (Example: larval zone of Lancieux (L) versus capture signature of Chausey). Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant). Bold characters indicate significant P Value.

	Capture signature of Chausey		Capture signature of Rotheneuf	
	P Value	Significant elements	P Value	Significant elements
L	**6.17e^{-06***}**	Mn/Ca***, Sr/Ca***, Cu/Ca**, Ba/Ca**, Fe/Ca**	**1.85e^{-06***}**	Mn/Ca***, Sr/Ca***, Cu/Ca**
S0b	**8.38e^{-04***}**	Mn/Ca***, Fe/Ca**, Cu/Ca*	**2.55e^{-04***}**	Mn/Ca***, Cu/Ca***, Zn/Ca**
S0	**3.89e^{-03***}**	Fe/Ca**, Mn/Ca*	**1.03e^{-05***}**	Cu/Ca***, Zn/Ca***, Mn/Ca**
S0e	**1.54e^{-02*}**	Mn/Ca**, Fe/Ca*	**1.32e^{-04***}**	Cu/Ca***, Zn/Ca***, Mn/Ca**
W0	**0.017***	Mn/Ca***	**1.89e^{-04***}**	Mn/Ca***, Cu/Ca***, Zn/Ca**, Sr/Ca**, Ba/Ca*
S1	**0.020***	Mn/Ca**, Zn/Ca*	**9.34e^{-04**}**	Mn/Ca***, Zn/Ca***, Cu/Ca**

season of birth; a small first opaque zone in the otolith corresponds to fish recruited in late spring or autumn and born in early spring, while a large zone corresponds to fish recruited in spring and born in the previous autumn, as Reay (1973) reported. This marked macrostructure of the otoliths was useful to reliably detect ontogenetic stages in the sandeel otoliths, and, given the relatively large size of the otoliths and large identifiable zones in the first year of life, it was possible to perform broad laser ablations providing enough material to detect fingerprint variations.

However, in order to definitely validate the use of microchemical fingerprints, we had to check their stability according to years, seasons, and age classes, as previously described in various fish species (Chittaro et al., 2006; Tanner et al., 2011; Mercier et al., 2012; Tournois et al., 2013). Among all the elements analysed in the Small Sandeel otolith, only Mg/Ca differed according to age class and season, which is consistent with several studies thatfound-magnesium to be physiologically regulated (Martin and Thorrold, 2005; Tanner et al., 2011; Woodcock et al., 2012).

Three methods were used to verify whether microchemical variations in otoliths were reliable for detecting habitat changes. Although the random forest (RF) method has improved discrimination performances for recent microchemistry data sets (Mercier

Table 3
Multivariate analysis of variance (MANOVA) comparing, from the Lancieux Small Sandeels otoliths (n = 24), the microchemical fingerprints during the ontogeny. The otoliths were divided into several ontogenic stages inferred from macrostructural patterns (see materials and methods): L = larvae; S0b = beginning, S0m = middle, S0e = end of first opaque zone corresponding to the first growth period; W0 = first translucent zone corresponding to the first growth stop; S1 = second opaque zone (second growth period); W1 = second translucent zone (second growth stop period). The microchemical fingerprints of each of these ontogenic stages were compared to each other. Indicated p values are a mean of different p values calculated for each element ratio between pairwise analyses (Example: larval zone of Lancieux (L) versus capture signature of Lancieux). Significant elements are identified for each pairwise analysis (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001; and NS = non-significant). Bold characters indicate significant P Value.

	S0b	S0m	S0e	W0	S1	W1	Capture signature Lancieux
L	**1,13 e^{-09}*** Sr/Ca***, Ba/Ca***, Zn/Ca**, Cu/Ca*	**1.14e^{-06}*** Sr/Ca***, Cu/Ca***, Zn/Ca***, Ba***, Mn/Ca*	**3.12e^{-05}*** Sr/Ca***, Zn/Ca***, Ba/Ca***, Cu/Ca**, Mn/Ca*	**1.57e^{-05}*** Ba/Ca***, Sr/Ca**, Cu/Ca**, Zn/Ca**	**4.53e^{-04}*** Sr/Ca***, Ba/Ca***, Zn/Ca***, Cu/Ca*	**8.02e^{-03}**** Zn/Ca***, Cu/Ca*	**2.92e^{-03}**** Ba/Ca***, Sr/Ca*
S0b		**7.16e^{-03}**** Sr/Ca**, Cu/Ca*	**3.43e^{-03}**** Sr/Ca***	**4.85e^{-5}*** Sr/Ca***	**1.49e^{-02}*** Sr/Ca**	**3.82e^{-02}*** Sr/Ca**, Zn/Ca*	**1.07e^{-02}*** Sr/Ca***
S0m			NS	**3.02e^{-04}**** Sr/Ca***	**3.66e^{-02}*** Fe/Ca*	NS	**1.28e^{-02}**** Cu/Ca*, Zn/Ca*
S0e				NS	NS	NS	NS
W0					NS	NS	NS
S1						NS	NS

et al., 2012; Tournois et al., 2013), linear discriminant analysis (LDA) had the highest maximal accuracy in our study. This could be explained by the fact that our dataset of capture sites exhibits multi-normality and homoscedasticity (Mercier et al., 2011). The best element combination was composed of Cu/Ca and Mn/Ca, and these elements are generally influenced by terrigenous inputs and tend to decline with distance from the shore (Kremling, 1985; Shiller, 1997; Laes et al., 2007) and have already been used to discriminate among coastal sites (Tanner et al., 2011). Our results suggest that the distinction between the three sites of capture was mainly due to a significant enrichment in manganese (Mn/Ca) in the outer perimeter of otoliths from Chausey and Rotheneuf. In addition, enrichment in copper and zinc in the coastal sites at Lancieux and Rotheneuf enabled us to distinguish between the three sites. The observed differences could be explained by the geological composition of the sediments and of the water. Despite the low number of individuals in Chausey and Rotheneuf sites, our results highlight the potential capacity of the otolith microchemistry of sandeels to imprint local trace elements and therefore to distinguish marine habitats, even over low geographical gradients, and confirm the interest of multi-elementary signatures as a spatial tracer. However, meaningful ecological interpretations of element concentrations remain difficult to make, especially because more information is required to understand the incorporation processes of chemical elements in fish and sandeel otoliths.

4.2. Site fidelity of the Small Sandeel according to age: insights from microchemical fingerprints of otoliths

Firstly, the microchemical signatures of the Chausey and Rotheneuf sites were never similar to those of Lancieux fishes (40 and 20 km away, respectively) suggesting that: (a) environmental imprints may occur among nearby marine areas, and (b) there was *a priori* no population connectivity between these intertidal sites. This is in accordance with Jensen et al. (2011), who found high fidelity of *Ammodytes marinus* to their night-time burrowing sites, with a range that did not exceed 5 km. Nevertheless, it appeared that diurnal movements could extend about 15 km away from night-time burrowing sites (Engelhard et al., 2008 for *A. marinus*). Finally, the swimming capacity of the Small Sandeel (1–1.5 km/h) (Kühlmann and Karst, 1967), suggested that fish caught in Lancieux were unable to reach either the Chausey archipelago or Rotheneuf Beach through daily movement, which does not exclude potential migrations over several days. Therefore, as telemetry is difficult for small fishes and traditional surveys and acoustic methods do not permit tracking individual behaviour, otolith microchemistry appears to be a potentially useful tool to determine the connectivity between sandeel stocks.

Secondly, the signatures of the larval stage (L) and the early juvenile growth stage (i.e. S0b, the beginning of the juvenile zone), including the metamorphosis stage (Wright, 1993), of fish caught in Lancieux, presented significantly distinct microchemical fingerprints from older stages. This first fingerprint change over the Small Sandeel lifespan could be explained by a change in habitat, probably when the metamorphosis occurred. Indeed, the size estimated from otoliths, at the beginning of the juvenile zone (S0b), just after the larval stage, ranged between 24.96 ± 0.96 mm and 34.04 ± 0.96 mm. Even if no data were found for Small Sandeel, Wright described *Ammodytes marinus* larvae as undergoing metamorphosis over the length range 35–55 mm TL, leading to a change from a pelagic to a semi-demersal habitat (Wright, 1993). This early life stage seems to occur for Small Sandeel in coastal waters according to Langham (1971), who never found larvae and post-larvae of the species in the Scottish offshore waters. Our sampling tended to confirm this result, since the smallest size of Small Sandeel settled (metamorphosed) detected at Lancieux was 38 mm. Contrary to the following stages, larvae are notably not in contact with sand during the night, which could explain the change in the microchemical fingerprint. Finally, the change in behaviour and habitat (pelagic to semi-demersal) and the influence of particular ecophysiological characteristics (growth and feeding) of these early stages (larval and during metamorphosis) on the microchemical composition of the otoliths cannot be excluded (Otake et al., 1997; Arai et al., 2000; Chittaro et al., 2006; Tanner et al., 2011).

Interestingly, the signature of the middle of the juvenile zone (S0m) differed from the capture signature of the Lancieux site (C), but not from the end of the juvenile zone (S0e) or from the second winter period (W1). At this stage (S0m), the estimated length of fish ranged from 45.10 ± 5.42 to 54.18 ± 5.42 mm. If differences in the first juvenile phase (S0b) and larval signatures are hypothetically due to a change in ecophase, as previously stated, this could be the beginning of settlement in the site in accordance with the mean sizes of recruitment in our samples of 41.37 ± 2.37 and 40.64 ± 3.56 mm according to the period (February 2012 (G0 A2011)

and May 2012 (G0 S2012)). As the end of the first juvenile stage (S0e) did not differ from the next zones and the fingerprint of capture, it could correspond to a real settlement of juveniles. Observed differences in microchemical signatures and the recruitment size could result from: a) a delay of signature incorporation in the otoliths (Yokouchi et al., 2011), or b) a behavioural difference in habitat use compared to older individuals. Then, the growth and survival of *Ammodytes marinus* larvae are controlled and supported by the zooplankton peak concentration and the increase of temperature enhancing the growth at the optimal time of match–mismatch (Gurkan et al., 2013), so variation in the time of hatching can lead to potentially different larval growth (Wright and Bailey, 1996) and juvenile size at recruitment. Accordingly, our individuals used for microchemistry and the otolith–length relationship were born in different years and probably seasons (G2, G1 (born in spring 2011) and G0 S2012), which might have effects on size at arrival at Lancieux compared to those of recruits of the year (G0 A2011 and G0 S2012).

Microchemical results finally support that the Small Sandeel exhibit high fidelity to the Lancieux site, where they have been captured during all stages of their lifecycle after the beginning of their settlement. Based on microchemistry and the sizes of recruitment and juvenile growth, intertidal beaches seem to act as nursery, growth, and resting habitats for the species, especially when they are overwintering (Wright et al., 2000; van Deurs et al., 2011b) and we cannot exclude their potential role as spawning habitat for this species.

4.3. Conclusions

Despite the small size of the sample used for this study, it confirms that exchanges could be limited between sandbanks and sandeel body morphology restricts their movements in the short-term (i.e. foraging behaviour) and longer periods in their lifecycle (Engelhard et al., 2008; Jensen et al., 2011). This method is also able to detect ontogenic habitat shifts in the Small sandeel, notably the settlement stage in intertidal beaches. Otolith microchemistry appears to be a relevant tool for investigating the life history of short-lived fishes such as Ammodytidae. It also provides a complementary approach to molecular methodologies to unravel population mixing on a geographical scale relevant to conservation and management.

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RESUME : Ces travaux de thèse ont pour objectif de mieux comprendre les stratégies d'utilisation d'habitats écologiques essentiels de poissons côtiers sur la façade Ouest de la Manche (Golfe Normand Breton et Baie de Lannion) en ayant recours à une multitude de traits de vie pour deux taxons avec des cycles biologiques contrastés. Le bar européen (*Dicentrarchus labrax*) sert de modèle d'espèce migratrice et longévive tandis que les quatre espèces sympatriques d'Ammodytidae représentent des espèces *a priori* résidentes et à cycle de vie court (*Ammodytes tobianus*, *Hyperoplus immaculatus*, *Hyperoplus lanceolatus* et *Gymnammodytes semisquamatus*). Une combinaison de différents traits de vie et marqueurs environnementaux est utilisée tels que les traits fonctionnels individuels (e.g traits morpho-anatomiques), les taux de croissance provenant de la microstructure des otolithes, les isotopes stables du carbone et de l'azote ainsi que l'analyse de la variation de la composition relative d'éléments métalliques traces dans l'otolithe *via* une ablation laser continue (e.g la microchimie de l'otolithe). Dans une première partie, l'utilisation conjointe de ces méthodes et de la phénologie des recrutements des espèces d'Ammodytidae sur leurs sites de capture ont révélé des différences interspécifiques d'utilisation des habitats sableux intertidaux et subtidaux permettant la cohabitation au sein de la communauté. Les deux espèces d'Hyperoplus pourraient exploiter différents habitats sableux au cours de leur cycle de vie comme des habitats subtidaux au stade adulte tandis que *G. semisquamatus* et *A. tobianus* semblent résider tout au long de leur vie, respectivement dans des habitats subtidaux et intertidaux. Dans une deuxième partie centrée sur le bar européen, l'étude de la microchimie et de la microstructure de l'otolithe et plus précisément la variation du Sr/Ba du centre jusqu'au bord extérieur de l'otolithe indique une migration larvaire toujours marine suivi d'une arrivée post-larvaire en eaux saumâtres, démontrant un recrutement dans des habitats côtiers. Puis lors de la croissance juvénile, différentes histoires de vie ont été identifiées au sein et entre les sites de capture : allant d'individus strictement marins alors que d'autres privilégient des eaux plus côtières de salinité variable. En parallèle, l'analyse des isotopes stables et des traits fonctionnels liés à la locomotion et à l'alimentation corroborent les différences d'utilisation des habitats de nourricerie détectées par la microchimie des otolithes. Par ailleurs, la qualité des sites de capture (ressources, invasions biologiques, pâturage et accessibilité du site) pourraient influencer les taux de croissances individuels observés indiquant des performances fonctionnelles variables entre les habitats de nourricerie du Golfe Normand Breton.

ABSTRACT: The objectives of our thesis were to precise the use strategies of essential ecological habitats by coastal fishes in the West French Channel (Norman-Breton Gulf and Lannion bay) using life traits for two taxa with contrasted biological cycles. European seabass (*Dicentrarchus labrax*) serves as model of migratory species with long lifespan and the four Ammodytidae sympatric species represent resident species with short lifespan (*Ammodytes tobianus*, *Hyperoplus immaculatus*, *Hyperoplus lanceolatus* et *Gymnammodytes semisquamatus*). For this purpose, a combination of complementary life traits and environmental tracers was used such as individual functional traits (e.g morpho-anatomical features), growth rates calculated *via* the otolith microstructure, carbon and nitrogen stable isotopes and otolith microchemistry analysis through a continuous laser ablation. In a first part, the combined use of these methods and the monitoring of sandeel species recruitment on each sites of capture highlighted strong interspecific differences of subtidal and intertidal habitat use allowing the cohabitation within the community. More precisely, the two Hyperoplus species could inhabit different sandy habitats during their lifecycle as subtidal habitats at the adult stage whereas *G. semisquamatus* and *A. tobianus* seem to stay all their life in subtidal and intertidal habitats respectively. In a second part, for the case of the European seabass, the microchemistry and microstructure study and more precisely Sr/Ba variation along the otolith axis from the center to the otolith edge indicated a marine larval migration followed by a post-larval arrival in brackish waters, proving a coastal recruitment. Then during the juvenile growth, different life histories were detected within and between sites of capture: some individuals spending time in marine waters and others in more coastal waters with variable salinities. Moreover, the stable isotopes analysis and the functional traits associated to the locomotion and food acquisition corroborate the differences of the nursery habitat use detected by the otolith microchemistry. Furthermore the habitat quality (resources, biological invasions, sheep grazing and site accessibility) could affect the individual growth rates observed and could indicate variable functional performances between the nursery habitats in the Norman-Breton Gulf.