



HAL
open science

Ecologie trophique de l'espèce ingénier *Crepidula fornicata* et implications pour le fonctionnement de son habitat

Thibault Androuin

► **To cite this version:**

Thibault Androuin. Ecologie trophique de l'espèce ingénier *Crepidula fornicata* et implications pour le fonctionnement de son habitat. Ecosystèmes. Université de Bretagne occidentale - Brest, 2018. Français. NNT : 2018BRES0106 . tel-02826777

HAL Id: tel-02826777

<https://theses.hal.science/tel-02826777v1>

Submitted on 7 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

THESE DE DOCTORAT DE

L'UNIVERSITE
DE BRETAGNE OCCIDENTALE
COMUE UNIVERSITE BRETAGNE LOIRE

ECOLE DOCTORALE N° 598
Sciences de la Mer et du littoral
Spécialité : « *Ecologie Marine* »

Par

Thibault ANDROUIN

Ecologie trophique de l'espèce ingénieur *Crepidula fornicata*, et implications pour le fonctionnement de son habitat

Thèse présentée et soutenue à Plouzané, le 13 décembre 2018

Unité de recherche : Laboratoire d'écologie benthique côtière (IFREMER-DYNECO)

Rapporteurs avant soutenance :

Réjean TREMBLAY
Xavier de MONTAUDOUIN

Professeur, Université du Québec à Rimouski
Professeur, Université de Bordeaux

Composition du Jury :

Président du jury : Fred JEAN

Professeur, Université de Bretagne Occidentale

Rapporteur : Réjean TREMBLAY
Xavier de MONTAUDOUIN

Professeur, Université du Québec à Rimouski
Professeur, Université de Bordeaux

Examineurs : Dominique DAVOULT
Laurent BARILLÉ
Francis ORVAIN

Professeur, Université Pierre et Marie Curie
Professeur, Université de Nantes
Maitre de conférences, Université de Caen - Basse Normandie

Dir. de thèse : Stanislas DUBOIS
Enc. de thèse : Antoine CARLIER
Co-enc. de thèse : Priscilla DECOTTIGNIES

Cadre de recherche, centre Ifremer de Brest
Cadre de recherche, centre Ifremer de Brest
Maitre de conférences, Université de Nantes

Le travail de préparation de cette thèse de doctorat a été financé par l’Ifremer et la Région Bretagne et soutenu par le projet français LabexMer (ANR-10-LABX-19-01). Ce travail a également bénéficié d’un financement de la fondation TOTAL pour la Biodiversité et la Mer.

À mes parents,

La science fondamentale n'est pas un luxe...
Léo Blanchard

REMERCIEMENTS

On est aujourd'hui le 22 août 2019, ça fait 8 mois que la soutenance est passée, le manuscrit de thèse est corrigé, tout est prêt pour le dépôt final, à l'exception des remerciements que je n'arrive pas à terminer... Je crois que j'ai peur de ne pas remercier suffisamment les gens qui m'ont aidé, et qu'une fois que je l'aurai fait, l'aventure de ma thèse au LEBCO sera terminée, ce qui me rend très, très nostalgique.

Alors en premier lieu, je souhaite remercier mes trois chef.fe.s, Stanislas Dubois, Antoine Carlier et Priscilla Decottignies. Merci à tous les trois pour m'avoir guidé, pour m'avoir laissé prendre à bras le corps cette thèse, pour avoir laissé libre cours à mon imagination tout en sachant dire STOP lorsqu'une 10^{ème} futur-ex manip se profilait... Antoine et Stan, merci de m'avoir supporté au labo pendant 3 ans (+ 6 mois de stage pour Antoine :O). Merci de toujours avoir été réactif, que ce soit durant la thèse avec mes « petites » bourdes, durant l'écriture des articles pour vos corrections, ou encore à la toute fin de la thèse pour les dernières corrections à « l'arrache ». Merci pour votre intérêt et pour votre engagement dans mon travail, je suis vraiment heureux d'avoir fait cette thèse avec vous. Antoine, merci pour le temps énorme que tu m'as accordé, que ce soit lors de l'écriture mais surtout au jour le jour pour répondre à toutes mes questions, merci pour toutes ces discussions constructives, pour ta patience et pour ta capacité à me cadrer tout en restant calme ! Stan, merci pour avoir toujours été présent lorsqu'il le fallait (surtout ce soir d'hiver 2018 quand je t'ai annoncé par sms que j'avais confondu étuve et -80°C et que je t'ai demandé de me faire confiance pour replanifier 2 chapitres de thèse la veille d'un stage de 6 mois --'...). Merci également pour ta rigueur et ton analyse critique, et pour être capable de prendre du recul sur notre travail. Priscilla, bien que nous ayons moins échangé durant la thèse avec la distance, je te remercie pour ton implication dans ce travail, et notamment pour nous avoir ouvert les portes de la ferme aquacole du Croisic, et grandement aidé lors de la phase d'expérimentation. Je remercie également Alex Bec et Cédric Hubas qui m'ont aussi accompagné durant la thèse en tant que membre externe du comité de thèse. Merci à vous pour votre regard extérieur et pour tous vos conseils.

En parlant de chef, merci à Philippe Cugier et Cédric Bacher pour leur tolérance à mon égard (et celui de mes collègues de bureau !) pour nous avoir laissé faire du bureau une succursale de notre chambre-cellier d'appartement... ainsi que pour le bruit qui pouvait émaner de nos murs ! J'en profite d'ailleurs pour accuser ouvertement Philippe d'avoir subtilisé notre petit ballon de basket !

Je remercie l'ensemble du jury qui bien voulu examiner cette thèse. Merci aux deux rapporteurs, Réjean Tremblay et Xavier de Montaudouin pour leurs corrections et leur avis éclairés sur ce manuscrit. Merci également à Fred Jean, Francis Orvain, Laurent Barillé et

Dominique Davoult pour leurs commentaires et leurs questions. Ce fut un plaisir de discuter avec vous ! Un mot particulier pour Dominique qui est un peu responsable de ma passion pour ce compartiment « benthique ». Merci de m'avoir fait confiance avant la thèse, cela m'a permis de développer mes idées et nul doute que cette étape a été cruciale pour mon développement scientifique.

Un énorme merci à Martin Marzloff, jeune chercheur aux multiples talents, et qui a su garder tout sa fougue et sa curiosité de thésard. Véritable couteau suisse de la science, il est aussi à l'aise sur un bateau les mains dans la vase que derrière un ordinateur pour faire du « modelling » ! Merci pour ton intérêt dans mon travail, pour ces discussions sur la manière d'écrire un article, sur les statistiques dans leur immensité et sur la science en générale.

Un grand merci à Gauthier Schaal et Fabienne Legrand pour avoir fait partie intégrante de ce projet. Fabienne, merci pour ton implication technique, analytique et d'encadrement durant le stage de Gwendoline ! Monsieur Schaal, merci pour les discussions scientifiques que nous avons eues à de multiples reprises. J'espère avoir gagné ma moustache d'isotopiste ! Merci également pour m'avoir permis d'effectuer quelques heures d'enseignements où j'ai pris beaucoup de plaisir !

A big thank to Lubos Polerecky. I won't forget your "Look at the row data".. ☺. Thank you for your kindness, your accuracy during the writing process of my first article, and your time during our multiple skype sessions! I really enjoyed our two weeks together in Brest!

Merci à Cédric Hubas pour m'avoir accueilli à Concarneau et m'avoir intronisé au monde des pigments. En plus d'être un scientifique rigoureux qui sans cesse renouvelle ses techniques d'analyses, tu es aussi quelqu'un avec qui il est bon de travailler.

Comme il est important de travailler avant tout avec des gens qu'on apprécie, je remercie Christine Dupuy pour bien plus que de la science. D'abord étudiant puis trésorier de ton association, je fini ton collaborateur ! Un grand merci pour ta science des microbes ainsi que pour ton sens de l'humain.

Merci à Bruno Jésus, je garde en tête le « keep it simple » pour les prochaines manips ! Merci pour m'avoir accueilli à Nantes pour me former à l'imagerie hyperspectrale, ainsi que pour mes questions en urgence lors de la manip au Croisic.

Merci à Ewan Pelleter et Gilles Chazot pour leur intérêt à propos des calcifications de crépidules. Je suis très heureux d'avoir pu refaire un peu de « géologie » à vos côtés !

Je remercie chaleureusement Vincent Vallée et Gwendoline Lefebvre pour leur précieux travail réalisé pendant leur stage de master 2. Laisser ses données de thèse à quelqu'un, ça peut être tout l'un ou tout l'autre, mais heureusement pour moi, ce fût tout l'un les deux fois ! Je fus un réel plaisir que de participer à votre encadrement et de contribuer à votre formation.

Gwendoline, grâce à toi c'est rien de moins que 2 chapitres que j'ai pu faire ! Vincent, malgré que tes travaux n'apparaissent que très peu dans ce manuscrit, sâches qu'ils seront valorisés :D Un grand merci également à Maïwenn Lescop pour m'avoir aidé au labo à finir toutes mes boulettes isotopiques dans les temps !

Merci à une bonne partie des techniciens de DYNECO pour m'avoir aidé à un moment ou un autre, que ce soit à propos du sédiment (David Le Berre et Matthias Jacquet), du matos de labo et/ou d'embrayage.. (Julien Quéré et Erwan Legall) ou encore des lipides (Xavier Phillipon). Un grand merci aussi aux petits comiques qui se reconnaîtront et qui rendent la science plus légère ;)

Côté benthos, un grand merci à Céline Cordier pour avoir sauvé mon échantillonnage au Croisic. 7h de route, 10h d'échantillonnage et 6h de sommeil, le tout en mangeant 2 sandwiches... J'ai vraiment été un tyran sur ce coup-là et je m'en excuse ! Je n'aurais jamais fini sans toi ! Merci à Jean-Do(x) pour le travail de labo, notamment le long tri de sédiment, et pour les diverses réparations ! Je m'excuse encore pour cette histoire d'armoire et de perceuse, une erreur de jeunesse, ainsi que pour la brosse à dent, une seconde erreur de jeunesse.. Xavier, merci pour tout le travail de terrain effectué durant la thèse, notamment au Croisic et dans l'eau fraîche de l'Élorn. Merci également pour les anecdotes, les coins de pêche, ainsi que pour avoir hurlé dans le couloir que je prenais encore des vacances (je sais c'est ta voix normale.. :). Place maintenant à la maman de cette équipe benthos : Touria Bajjouk. Merci pour ton empathie et ta gentillesse à toute épreuve. Je te remercie également pour ton soutien dans la fin de la rédaction : l'humain dans la science compte et tu en es l'incarnation ! Et merci pour cette pastilla d'anthologie une fois la thèse terminée ! Dans la même veine, je remercie la douce dame Jacqueline, qui a toujours su m'épauler dans ma croisade contre Notilus, Sigma et autre HR access.. Tu nous manque et je te souhaite une douce retraite ! Kindless rime également avec Amélia Curd qui, malgré une thèse et une famille, a su trouver du temps pour plonger dans l'eau froide hivernale pour récupérer des crépidules. Dans la même lancée, merci Aline Blanchet-Aurigny pour tes conseils, techniques et analytiques, ainsi que pour ton regard toujours pointilleux. En parlant de sens critique, merci à Alain Menesguen (le bonnet rouge de la science), jeune retraité dont les paroles résonnent encore dans ma tête. Merci pour ton savoir sur la crépidule et sur la rade de Brest en générale. Plus généralement, je remercie du fond du cœur toute l'équipe du LEBCO (Mickaël, Yoann, Fernando, Claire, Carmen, Flavia, Jacques, Pierre-Olivier et tous les autres) pour sa bonne humeur, l'ambiance chaleureuse et la bienveillance qui y règne. Merci d'avoir toléré mes excès de zèle en tant que responsable du CCC et accepté de manger de la nourriture douteuse. Merci également à tous les stagiaires qui par leur passage apportent joie et rebondissement dans la vie du labo, surtout au creux de l'hiver.

Un grand merci à Elisabeth Bondu et son acolyte Elodie Benon de l'EDSM pour leur bienveillance à mon égard, et ce malgré mes légers déboires administratifs... L'arnaqueur vous remercie !

Je remercie également le crew du navire Albert Lucas, avec qui j'ai passé de très bon moment en mer ! Merci à Daniel, François, Franck et Alban pour leur bonne humeur (sauf quand la niskin ne percute pas ou lorsque j'oublie mon casque ou encore lorsque je porte une montre en or). Je remercie également les plongeurs de l'IUEM, Thierry, Isabelle, Emilie et Erwan, ainsi que Aude Leynaert pour avoir participé à l'échantillonnage en rade de Brest.

Je remercie aussi Oanez Lebeau, Clément Tanvet et Rudolph Corvaisier de la plateforme LIPIDOCEAN pour les analyses isotopiques. Je remercie en particulier Oanez pour d'avoir été si arrangeante avec moi et pour avoir passé dans les temps tous les échantillons !

Je clôture cette partie par celui qui contrôle les entrées et sorties de l'Ifremer. Bien que notre histoire ait mal, voir très mal débutée lors de mon stage de master, on s'est très bien rattrapé pendant les 3 ans de thèse. Alors merci à Albert Moreau pour nos discussions de début de soirée lorsque mon badge, perdu ou inactif, ne passait pas. Merci pour son assouplissement à mon égard et pour avoir été conciliant bien des fois 😊

La vie n'est rien sans les copains.

Quand on passe la majeure partie de ses journées au bureau, il est vital de s'y sentir comme à la maison. J'ai eu la chance de partager mon bureau avec des gens aussi variés qu'intéressants. Nikolaos Alexandridis, Auriane Jones et Bastien Taormina. Le premier est grec, la seconde est franco-américaine, et le dernier franc-comtois. Nikos, 3 ans déjà que tu es parti, mais tes mimiques résonnent encore dans ma mémoire (ton lavage de lunette bijournalier, ton art psychorigide de verser tes grains de Nescafé soluble, ou encore ta préparation de sandwichs artisanaux pour ne pas avoir à faire à manger le soir). Déjà présent lors de mon stage de M2, ton regard mécanistique sur les choses (parfois rigide) m'a souvent éclairé ! En ce qui concerne Auriane Jones, elle allait très bien dans le décor du bureau, arrivée de Dinard, un peu farfelue et toujours le smile (sauf le matin de bonne heure), on a pu ensemble partager certaines galères de la vie de jeunes benthologue en écologie trophique ! Merci à toi d'avoir animé le bureau avec tes histoires de pépin en tout genre, mais également d'avoir essuyé les plâtres de la science avant moi ! Bastos, Bast, Damien, Baptiste, Ismaël, Baster, Fabien ou encore Mlle Jaormina. Tant de nom pour décrire un seul homme, Bastien Taormina alias la bastos du siècle. Merci d'avoir partagé mon bureau et même ma vie durant cette dernière année (tant de gens doivent encore penser que l'on forme un mignon petit couple). Merci d'avoir parlé science, partagé des cafés, partagé ta voiture, placardé des articles gorafi dans les toilettes, plongé en eaux troubles, nettoyé l'aquarium, critiqué nos chefs respectifs (c'est une joke hein ;), me laisser enfermer tutu, ... Merci également pour

m'avoir aidé sur le terrain sans pour autant apparaître dans les co-auteurs, merci d'avoir fait la fermeture de la frime bien des fois, de m'avoir transporté dans ton coffre (oups), bref une belle aventure. Quand je t'ai vu, je me suis dit « encore un mec qui va faire de la faune fixée.. » mais tu es bien plus ! Bon courage pour ta dernière ligne droite bromingo, je vais louper ta soutenance mais je suis et serai de tout cœur avec toi !

La science est grande, puisqu'on peut faire une thèse sur le cochon et ensuite un post doc en écologie benthique ! Natacha Go, qui passait pas mal de temps dans notre bureau, certes pour procrastiner à nos côtés mais également pour nous soutenir. Un grand merci à toi pour ton soutien pendant et en fin de thèse, pour les visites au beaj (promis j'étais content ! :)

Je remercie également l'upside down du bureau benthos, c'est-à-dire le bureau pelagos juste au-dessus de nos têtes. Pierre Ramond et Gabriel Métaignier. En plus des bières et cafés, merci pour m'avoir renseigné sur ce monde qu'est la protistologie. Je crois que mon plus grand rêve aurait été qu'on forme un seul et même bureau..

Je remercie mes 2 aventuriers, Douglas Couet et Louis Wilmotte, qui par leur passage à Brest et leur récit d'aventures, m'ont permis de garder en tête que la thèse est également une aventure !

Merci à mes 2 romanichels préférés, Valérian Le Roy et Thibaut Pollina, pour les aventures à pas cher, les bivouacs improvisés, la chasse aux crevettes bleues, la cuisson dont on rêve et tous les autres petits plaisirs de la vie.

Merci à mes anciens collègues de master OEM (Guilhem, Carolus, Flo, Marie & Marie, Martin, Perrine) et particulièrement Guilhem et Carolus pour la collocation de rêve durant la première année ! Le pled de Carolus, la trompette de Gui et les bières du soir, le roi de l'oignon vous remercie pour cette vie à trois !

Merci aux copains brestois, Louise, Margaux, Marc, Aurélien, Emilien, Ghassen, Justine, Sonia, Louis, Lise, Elyne, Robin pour les soirées, les multiples anniversaires surprises, surtout lors de la première année !

« Les amis, c'est des gens qu'on connaît, mais qu'on aime quand même ». Merci aux copains d'avant d'avoir été présent avant, pendant et j'espère après la thèse. Alex, JM, Léo, Franz, Hélène, Clélia, Ludo, Mario, notamment en faisant l'effort de venir me rendre visite, et ce malgré mes piètres talents de pêcheurs ! Je vais enfin, je l'espère, pouvoir passer plus de temps avec vous !

Je remercie également le Beaj café, qui m'a permis de finir cette thèse dans de bonnes conditions alimentaires. Mes 3 dernières semaines ont été bercé par l'odeur du cappuccino, tartines et autres soupes ! Ma bourse de jeune chômeurs y est également passée mais bon..

Serge, patron de la Convention, merci de nous avoir accueilli dans ton bar qui ressemble étrangement à notre bureau, longue vie à toi et ton bar !

Je fini ces remerciements par ma famille. A mes parents et sœurs pour leur soutien et pour leur compréhension durant ces 3 ans, pour mes absences téléphoniques et mes retards aux cadeaux de Noël. Merci à mes parents pour avoir tout fait pour me permettre d'en arriver là ! Cette thèse est pour vous.

Dernière arrivée en date dans la famille, la marquise Clémence Gourtay. Malgré 3 années de thèse, un océan et quelques mètres de neiges qui nous ont séparés pendant un long moment, on peut dire qu'on bien géré la crise ! Merci de ton soutien à toute épreuve, et également de ton aide pour les dernières relectures et la mise en page de la thèse, je n'aurai jamais fini sans toi... Longue vie à nous et à cette nouvelle aventure québécoise qui nous attend !

PUBLICATIONS PRÉSENTÉES DANS CE MANUSCRIPT

Androuin T., Polerecky L., Decottignies P., Dubois S. F., Dupuy C., Hubas C., Jesus B., Le Gall E., Marzloff M. P., Carlier A. Subtidal microphytobenthos: a secret garden stimulated by the engineer species *Crepidula fornicata*. (*Published* in *Frontiers in Marine Science*, *Marine Ecosystem Ecology*, DOI : 10.3389/fmars.2018.00475)

Androuin T., Dubois S. F., Decottignies P., Carlier A. The dark side of soft tissues: unexpected inorganic carbonate in the invasive slipper limpet *Crepidula fornicata* and its implications for stable isotope interpretations. (*Published* in *Rapid Communications in Mass Spectrometry*, DOI : 10.1002/rcm.8322)

Androuin T., Dubois S. F., Hubas C., Lefebvre G., Legrand F., Schaal G., Carlier A. Sharing is caring: ontogenic trophic niche differentiation in the invasive gregarious species *Crepidula fornicata* (*In preparation*)

Androuin T., Dubois S. F., Lefebvre G., Legrand F., Schaal G., Carlier A. Enemies together in close quarters: food partitioning between common suspension-feeders in an engineered benthic habitat. (*In preparation*)

Androuin T., Dubois S. F., Carlier A. *Crepidula* beds, a legacy for complex food web? (*In preparation*)

COMMUNICATIONS ET FORMATIONS

Présentations orales

Androuin T., Vallée V., Dubois S. F., Marzloff M. P., Carlier A. Disentangling physical and biological effects of the invasive slipper limpet by the use of biological trait analysis. Benthic Ecology Meeting, April 12-16 2017, Myrtle Beach, South Carolina, USA.

Androuin T., Polerecky L., Decottignies P., Dubois S. F., Dupuy C., Hubas C., Jesus B., Le Gall E., Marzloff M. P., Carlier A. Subtidal microphytobenthos: a « secret garden » stimulated by the engineer species *Crepidula fornicata*. 5^e colloque du groupe hyper-spectral – Société Française de Photogrammétrie et de Télédétection, 9-11 mai 2017, Plouzané, France.

Androuin T., Vallée V., Dubois S. F., Marzloff M. P., Carlier A. Disentangling physical and biological effects of the invasive slipper limpet by the use of biological trait analysis. Biological traits for benthic community ecology. 18-20 Juin 2017. Plouzané, France.

Androuin T., Polerecky L., Decottignies P., Dubois S. F., Dupuy C., Hubas C., Jesus B., Le Gall E., Marzloff M. P., Carlier A. Subtidal microphytobenthos: a « secret garden » stimulated by the engineer species *Crepidula fornicata*. Journée scientifique de Nantes, atelier hyperspectrale, 2 Juin 2017. Nantes, France.

Posters

Androuin T., Polerecky L., Decottignies P., Dubois S. F., Dupuy C., Hubas C., Jesus B., Le Gall E., Marzloff M. P., Carlier A. Subtidal microphytobenthos: a « secret garden » stimulated by the engineer species *Crepidula fornicata*. The British Phycological Society and Microphytobenthic Biofilm Symposium, January 8-11 2018, Southend-Essex, UK.

Androuin T., Dubois S. F., Hubas C., Lefebvre G., Legrand F., Schaal G., Carlier A. Trophic relationships between co-occurring benthic suspension-feeders, a matter of scale? International Conference on Application of stable Isotope Techniques to Ecological Studies, July-August 30-3 2018, Vina del Mare, Chili.

Androuin T., Dubois S. F., Decottignies P., Carlier A. The dark side of soft tissue: non-expected inorganic carbonates in the invasive slipper limpet *Crepidula fornicata* and implications for stable isotope interpretations. International Conference on Application of stable Isotope Techniques to Ecological Studies, July-August 30-3 2018, Vina del Mare, Chili.

Communications grand public

Interview pour Télé Bretagne Ouest (Tébéo) dans « instant été » le 29 septembre 2016 (présentation des habitats à crépidules).

Nuit européenne des chercheurs. Animation d'un stand sur les habitats de la rade de Brest en collaboration avec l'association des jeunes chercheurs de l'école doctorale des sciences de la mer (MERSIDOC). 30 Septembre 2016, Océanopolis, Brest, France.

Nuit européenne des chercheurs. Animation d'un stand sur l'espèce invasive *Crepidula fornicata* en collaboration avec le laboratoire LEBCO et la cellule communication de l'IFREMER. 29 Septembre 2017, Océanopolis, Brest, France.

Encadrements de stage

Vincent Vallée, Université de Bordeaux, stage de master 2 (2016). Effets ingénieurs auto- et allogénique de l'espèce invasive *Crepidula fornicata* (Linnaeus, 1758) sur la diversité structurelle et fonctionnelle des habitats benthiques colonisés de la rade de Brest. Encadrement : Antoine Carlier, Thibault Androuin.

Gwendoline Lefèbvre, Université de Bretagne Occidentale, stage de master 2 (2018). Diversité trophique horizontale associée à l'espèce ingénieur *Crepidula fornicata*. Encadrement : Gauthier Schaal, Thibault Androuin, Fabienne Le Grand, Antoine Carlier.

Enseignements

Aide au tri et à la détermination d'invertébrés benthiques lors de travaux pratiques de master 1 Sciences Biologiques Marines, au sein de l'unité d'enseignement Ecologie des Systèmes Marins (2015-2017) pour une durée de 48h. Responsable : Gauthier Schaal.

Formations suivies

Cytométrie : théorie et application (6h, IUEM, Plouzané)

Ethique dans la recherche (6h, cours informatique, Université de Bordeaux)

Plongée scientifique (60h, Station biologique de Roscoff)

Perfectionnement au langage informatique R (18h, Ifremer, Plouzané)

Cartographie à l'aide du logiciel QGIS (18h, Ifremer, Plouzané)

TABLE DES MATIÈRES

INTRODUCTION GÉNÉRALE.....	1
I – Les habitats benthiques : structuration et impacts des espèces invasives	1
Les espèces ingénieurs de l'écosystème.....	1
Les espèces invasives	3
Le double impact des espèces ingénieurs et invasives : le cas des mollusques.....	4
II – Le cas de l'espèce ingénieur et invasive <i>Crepidula fornicata</i>	7
Un concentré d'innovations biologiques.....	7
Des effets multiples sur les habitats	10
Niche trophique et compétition	12
La crépidule dans la rade de Brest.....	13
III – Objectifs de la thèse	16
CHAPITRE I : Influence de la crépidule sur la production primaire benthique	19
Méthode 1 : Imagerie hyper-spectrale.....	21
Article 1 : Subtidal microphytobenthos: the « secret garden » stimulated by the engineer species <i>Crepidula fornicata</i>	23
CHAPITRE II : Écologie trophique de <i>Crepidula fornicata</i>	45
Méthode 2 : Isotopes stables	47
Article 2 : The dark side of soft tissue: unexpected carbonates in the invasive slipper limpet <i>Crepidula fornicata</i> and its implications for stable isotope interpretations	49
Méthode 3 : Pigments photosynthétiques.....	67
Méthode 4 : Acides gras.....	69
Article 3 : Sharing is caring: ontogenic trophic niche differentiation in the invasive gregarious species <i>Crepidula fornicata</i>	71
CHAPITRE III : Interactions trophiques interspécifiques multi-échelles.....	99
Article 4 : Enemies together in close quarters: food partitioning between suspension feeders in an engineered benthic habitat.....	101
Méthode 5 : Analyse des traits biologiques.....	123
Article 5 : <i>Crepidula</i> beds, a legacy for complex food web ?	125

SYNTHÈSE & DISCUSSION GÉNÉRALE	149
I – Crépidule, carbonates et questionnements	151
Spécificités des tissus de crépidule.....	152
Implications pour le cheminement de la thèse.....	155
II – Écologie trophique associée aux bancs de crépidules.....	156
Analyse expérimentale de la stimulation du microphytobenthos subtidal.....	156
Quantification <i>in situ</i> du microphytobenthos subtidal.....	158
Relations trophiques intraspécifiques chez la crépidule.....	159
Relations trophiques interspécifiques au sein des suspensivores	162
Relations trophiques interspécifiques au sein de la communauté.....	164
III – Le MPB subtidal : un jardin encore secret.....	166
Des conditions du milieu particulières	166
Rôle trophique du MPB subtidal	171
IV – Impact à long terme sur l'écosystème de la rade de Brest	179
La crépidule en voie de disparition ?.....	179
L'envahisseur part, les coquilles restent.....	181
V – Espèces invasives et ingénieurs, pour ou contre ?.....	182
Une vision anthropocentrée de la nature	182
La facilitation comme aide à l'interprétation.....	183
Une décision difficile.....	184
 RÉFÉRENCES BIBLIOGRAPHIQUES	 188

LISTE DES FIGURES

Figure 1 : Concept d'ingénieur de l'écosystème illustré par les herbiers de phanérogames à <i>Zostera marina</i> et les sédiments bioturbés à <i>Arenicola marina</i>	2
Figure 2 : Étapes du processus d'introduction	4
Figure 3 : Résumé des effets autogéniques et allogéniques des mollusques invasifs.	6
Figure 4 : Chaîne de crépidules en activité de filtration.....	8
Figure 5 : Schématisation de l'orientation des flux de matière lors de la filtration et de la biodéposition chez <i>Crepidula fornicata</i>	9
Figure 6 : Valeurs isotopiques de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ de <i>Crepidula fornicata</i> et deux sources potentielles de matière organique (matière organique particulaire (MOP) et microphytobenthos (MPB)) issues de la littérature.	12
Figure 7 : Cartographie des principaux habitats de la rade de Brest de 1987 à 2018.....	14
Figure 8 : Prélèvement en plongée sur une zone à forte biomasse de crépidules.....	17
Figure 9 : Principe de fonctionnement de l'imagerie hyper-spectrale, et application sur les communautés photosynthétiques de l'huître <i>Magallena gigas</i>	21
Figure 10: Timeline of the experimental protocol and sampling design, top-view schematic of the hyper-spectral imaging system, and regions of interest within each mesocosm tank.	28
Figure 11: Top view of the sediment surface after 10 days in the experimental mesocosms with the control, dead and live treatments.....	32
Figure 12: Microphytobenthos (MPB) biomass (mean \pm SE of the three replicates) as a function of time measured at the surface in the inner bed sediment for the three treatments.....	33
Figure 13: Close-up images of microphytobenthos (MPB) biomass within <i>Crepidula fornicata</i> bed in dead and live treatments after 10 days.	35
Figure 14: Microphytobenthos (MPB) biomass as a function of time (mean \pm SE, n = 3) averaged over the three regions of interest in treatments with dead (A) and live (B) <i>Crepidula fornicata</i>	36
Figure 15: Concentrations (mean \pm SD, n = 4) of (A) proteins, (B) carbohydrates and (C) heterotrophic prokaryotes between treatments collected at days 0 and 10.....	37
Figure 16: Schematic diagram of the effects of <i>Crepidula fornicata</i> on microphytobenthos growth, discriminating between the effects of dead (physical engineering) and live (physical plus biological engineering) animals.	39

Supplementary Figure 17: <i>In situ</i> light spectra recorded at 0, 3 and 11.5 meter depth in the bay of Brest.....	43
Supplementary Figure 18: End-member spectra used to quantify microphytobenthos biomass from the log-transformed spectral reflectance data obtained from the experimental mesocosm using hyperspectral imaging.....	44
Figure 19 : Diagramme isotopique conceptuel illustrant l'enrichissement attendu entre un producteur primaire (Salsepareille), un consommateur primaire (Schtroumpft) et un consommateur secondaire (Gargamel).....	47
Figure 20: Relationships between nitrogen or carbon stable isotope ratios (‰) measured in the foot of <i>Crepidula fornicata</i> and individual size for both untreated (filled circles) and acid-treated (open circles) samples. (C) Correction model showing the differences of $\delta^{13}\text{C}$ value between acid-treated and untreated samples, according to individual size.	55
Figure 21: Organic carbon content (%) in foot of <i>Crepidula fornicata</i> in untreated (white) and acid-treated (grey) samples.....	56
Figure 22: Example of an <i>in toto</i> histological cut of the slipper limpet <i>Crepidula fornicata</i> foot (A) before acidification (t0) and (B) 1'20, (C) 1'40, (D) and 2'40 after 1 M HCl acidification.	56
Figure 23: Scanning electron microscopy of the foot of adult of <i>Crepidula fornicata</i> at different magnification levels.....	57
Figure 24: Images of the slipper limpet <i>Crepidula fornicata</i> inhabiting a calcareous substrate and a non-calcareous substrate. Calcareous concretions in <i>Crepidula fornicata</i> soft tissue fixed on a non-calcareous substrate and close-up of a foot tissue sample from the same individual.	58
Figure 25: Relative contributions of benthic diatoms, macroalgae and C4 plants, and marine POM to the diet of <i>C. fornicata</i> of six size classes calculated with uncorrected (grey) and corrected (black) $\delta^{13}\text{C}$ values.....	59
Figure 26: Seasonal variations of $\delta^{13}\text{C}$ measured for <i>Crepidula fornicata</i> (open circles) and <i>Magallena gigas</i> (filled circles) (mean \pm SD; n = 5). Interspecific $\delta^{13}\text{C}$ comparisons are shown with (A) uncorrected and (B) corrected.	60
Figure 27: Multiple factorial analysis on the total pigment and fatty acid compositions between organic matter sources (Biofilm, PPOM, RPOM) and sampling period (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April).....	80
Figure 28: Total fatty acids, chlorophyll <i>a</i> and fucoxanthin concentrations (mean \pm SD, n = 3), and ratios between chlorophyll <i>b</i> , chlorophyll <i>c</i> , and fucoxanthin over chlorophyll <i>a</i> for the potential organic matter sources. S1 to S4 corresponds to sampling periods (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April).....	83

Figure 29: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic compositions (mean \pm SD, n = 3), and overall isotopic biplot for organic matter source (Biofilm, PPOM, RPOM) and sampling period (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April).....	84
Figure 30: Principal component analysis based on total fatty acids compositions of ontogenic classes of <i>Crepidula fornicata</i> (motile males, sessile males, sessile females) and sampling periods (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April).....	85
Figure 31: Relative abundance of fatty acids (FA) classes in the three ontogenic classes of <i>Crepidula fornicata</i> (motile males, sessile males, sessile females) (mean \pm SD, n = 5). S1 to S4 corresponds to sampling periods (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April).....	89
Figure 32: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic compositions (mean \pm SD, n = 5), and overall isotopic biplot for three ontogenic classes of <i>Crepidula fornicata</i> (motile males, sessile males, sessile females). S1 to S4 corresponds to sampling periods (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April).....	90
Supplementary Figure 33 : Photosynthetic available radiations (PAR) at the surface and at the bottom in the study site.....	97
Figure 34: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ compositions of suspension-feeding species (<i>Crepidula fornicata</i> , <i>Chlamys varia</i> , <i>Ostrea edulis</i> , <i>Calyptraea chinensis</i> , <i>Austrominius modestus</i>) and sampling dates (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April). Ellipses enclosed the standard ellipse area corrected for small sample size (SEAc).....	109
Figure 35: Principal component analysis based on the total fatty acid compositions of suspension-feeding species (<i>Crepidula fornicata</i> , <i>Chlamys varia</i> , <i>Ostrea edulis</i> , <i>Calyptraea chinensis</i> , <i>Austrominius modestus</i>) and sampling dates (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April).....	110
Figure 36 : Illustration de l'analyse par traits biologiques avec l'exemple de <i>Crepidula fornicata</i>	123
Figure 37: Sampling stations in the bay of Brest. S1 - S2: live <i>Crepidula</i> beds, S3 – S4: dead <i>Crepidula</i> beds, M: maerl beds.	129
Figure 38: Ordination of taxa and biological traits by fuzzy correspondence analysis. Distribution of taxa in clustered tropho-functional (TF) group and modalities of the four biological traits on the two first factorial plane of the analysis.	135
Figure 39: Biplot of mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of taxa sampled in dead and live <i>Crepidula</i> beds in summer and winter. Isotopic data of the maerl bed were taken from Grall et al., (2006).....	136
Figure 40: Comparison of six isotopic metrics calculated for the community in live (black) and dead (grey) <i>Crepidula</i> beds in winter and summer period.....	138
Figure 41: Comparisons of six isotopic metrics calculated for the three tropho-functional groups in live (black) and dead (grey) <i>Crepidula</i> beds, in winter and summer periods.	139

Figure 42: Comparisons of six isotopic metrics calculated for the community in dead <i>Crepidula</i> bed (black) and maerl bed (grey).	140
Figure 43: Summary of tropho-functional groups considered in this study, schematized by a few illustrative species.	142
Supplementary Figure 44: Size frequency distribution of <i>Crepidula fornicata</i> in the four sampled stations.	146
Figure 45 : Microscopie électronique à balayage élémentaire à l'échelle du tissu et à l'échelle d'une sphérule. Spectre élémentaire montrant la composition atomique des sphérules. Diffractogramme à rayon X pour analyser la cristallographie des sphérules.	153
Figure 46 : Valeurs isotopiques de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ de <i>Crepidula fornicata</i> et deux sources potentielles de matière organique (matière organique particulaire (MOP) et microphytobenthos (MPB)) issues de la littérature.	156
Figure 47 : Habitat à crépidules illustrant les espèces de suspensivores étudiés.	162
Figure 48 : Photographies au microscope électronique à balayage de diatomées épiphytes présentes à la surface d'une coquille de <i>Crepidula fornicata</i> échantillonnée à 5 m de profondeur dans la rade de Brest (Ní Longpuhirt et al., 2007) et à la surface d'une coquille de <i>Crepidula fornicata</i> provenant de notre expérimentation <i>ex situ</i>	170
Figure 49 : Valeurs de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ obtenues pour trois suspensivores à deux saisons (pré/post-efflorescence) et pour deux sites (Elorn/Lanvéoc).	172
Figure 50 : Evolution des fréquences de 4 groupes fonctionnels en fonction du poids total de crépidules (mortes + vivantes).	182

LISTE DES TABLEAUX

Table 1: Results of the RM-ANOVA applied to assess differences in microphytobenthos biomass between the three treatments in the inner bed sediment.....	34
Table 2: Results of the RM-ANOVA applied to assess differences in microphytobenthos biomass between two treatments and three regions of interest (ROI).....	36
Supplementary Table 3: Food source and trophic fractionation values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of organic matter sources used in the mixing model.....	65
Supplementary Table 4: $\delta^{13}\text{C}$ measured for <i>Crepidula fornicata</i> according to isotopic sample preparation in seven previous studies.....	66
Table 5 : Exemples de biomarqueurs utilisés pour le microphytobenthos.....	67
Table 6 : Exemples de biomarqueurs couramment utilisés en écologie trophique.	69
Table 7: Pigment composition of organic matter sources (Biofilm, PPOM, RPOM) and sampling period (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April) (% of total pigment concentration, mean \pm SD, n = 3).....	81
Table 8: Fatty acids (FA) composition of organic matter sources (Biofilm, PPOM, RPOM) and sampling period (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April) (% of total FA concentration, mean \pm SD, n = 5).	82
Table 9: Fatty acids (FA) composition of the ontogenic classes of <i>Crepidula fornicata</i> (motile males, sessile males, sessile females) at the four sampling date (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April) (% of total FA concentration, mean \pm SD, n = 5).	87
Table 10: Fatty acids (FA) used as dietary biomarkers in this study. BFA: branched FA, PUFA: polyunsaturated FA, SFA: saturated FA, LCSFA: long chain saturated FA (≥ 22 carbons).	108
Table 11: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ compositions (‰, mean \pm SD, n ≥ 3) of the five suspension-feeders (<i>Crepidula fornicata</i> , <i>Chlamys varia</i> , <i>Ostrea edulis</i> , <i>Calyptrea chinensis</i> , <i>Austrominius modestus</i>) at the four sampling date (S1, S2, S3, S4).	109
Table 12: Fatty acids (FA) composition of the five suspension-feeders (<i>Crepidula fornicata</i> , <i>Chlamys varia</i> , <i>Ostrea edulis</i> , <i>Calyptrea chinensis</i> , <i>Austrominius modestus</i>) on the four sampling dates (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April) (% of total FA concentration, mean \pm SD, n ≥ 3). BFA: branched FA; SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; NMI FA: non-methyl-interrupted FA; DMA: imethyl acetals FA.	111
Table 13: Results of Factorial twoway ANOVA showing the effects of species (<i>C. fornicata</i> , <i>C. varia</i> , <i>O. edulis</i> , <i>C. chinensis</i> , <i>A. modestus</i>) and sampling dates (S1 = 26 th February, S2 = 21 th March, S3 = 28 th March and S4 = 12 th April)..	113

Table 14: Biological traits associated with the five species encountered in this study, with a special reference to their trophic niche.....	118
Supplementary Table 15: Relative abundance of the main fatty acids (% of total FA concentration) of individuals green macroalgae sampled in May 2016 at the study site.....	121
Table 16: Biological traits, modalities and rationale behind the traits selection.	131
Supplementary Table 17: <i>p</i> -value from Kolmogorov-Smirnoff tests between pairs of the four sampled stations.....	146
Supplementary Table 18: Crepidula biomass and Crepidula shell weight in the four sampled stations (mean ± SD g per 0.1m ² , n = 3).....	147

LISTE DES ABBRÉVIATIONS

	<u>Français</u>		<u>English</u>
AG	acide gras	FA	fatty acid
AGB	AG branché	BFA	branched FA
	AG monoinsaturé	MUFA	monounsaturated FA
AGPI	AG polyinsaturé	PUFA	polyunsaturated FA
AGS	AG saturé	SFA	saturated FA
AGSLC	AG saturé à longue chaîne	LCSFA	long chain SFA
	aire standard de l'ellipse	SEA	standard ellipse area
	chromatographie liquide haute pression	HPLC	high pressure liquid chromatography
	diméthylacétals	DMA	dimethyl acetals
	distance du plus proche voisin	NND	nearest neighbour distance
	écart-type de la distance du plus proche voisin	SDNND	standard deviation NND
	espèce invasive	IS	invasive species
	esters méthyliques d'acides gras	FAME	fatty acids methyl esters
	inconnu	UK	unknown
	isotope stable	SI	stable isotope
	lipide neutre	NL	neutral lipid
MO	matière organique	OM	organic matter
MOP	matière organique particulaire	POM	particulate organic matter
	méthylés non interrompus	NMI	non-methyl-interrupted
MPB	microphytobenthos	MPB	microphytobenthos
	microscopie électronique à balayage	SEM	scanning electron microscopy
	MOP pélagique	PPOM	pelagic POM
	MOP re-suspendue	RPOM	re-suspended POM
	radiation disponible pour la photosynthèse	PAR	photosynthetically available radiation
	ratio isotopique	SIR	stable isotope ratio
	région d'intérêt	ROI	region of interest
	spectroscopie dispersive d'énergie	EDS	energy dispersive spectroscopy
	tropho-fonctionnel	TF	tropho-functional

INTRODUCTION GÉNÉRALE

I – Les habitats benthiques : structuration et impacts des espèces invasives

Les espèces ingénieurs de l'écosystème

En milieu marin, le compartiment benthique montre une grande diversité d'habitats. La classification européenne EUNIS (European Nature Information System) en dénombre plus de 1000 à travers l'Europe (Bajjouk et al., 2015). Ces habitats sont définis et classés de façon hiérarchique selon la profondeur, le type de substrat, les conditions hydrodynamiques et environnementales, ainsi que par les espèces ou groupes d'espèces caractéristiques. Bien que la nature du sédiment soit primordiale dans la structuration des communautés benthiques (Sanders, 1958 ; Dutertre et al., 2013), l'habitat biotique, et certaines espèces en particulier, peuvent jouer un rôle significatif sur cette structuration (Bouma et al., 2009 ; Reise et al., 2002). C'est le cas des espèces ingénieurs de l'écosystème. *Sensu stricto*, une espèce dite ingénieur de l'écosystème va créer, modifier ou entretenir un habitat en régulant directement ou indirectement la disponibilité d'une ressource pour les autres espèces (Jones, 1994). Cette « ingénierie » se fait par un changement d'état physique du matériel biotique ou abiotique (Jones, 1994). Bien que le concept soit complexe, on peut distinguer deux grands types d'ingénieurs de l'écosystème : autogénique et allogénique (Figure 1a). Le premier désigne une espèce, qui par sa seule structure physique (vivante ou morte), va engendrer des modifications de l'environnement. Le cas des herbiers de phanérogames marines en est un exemple (Figure 1b). Comparé à un substrat nu, sa présence va réduire l'hydrodynamisme au sein de l'herbier, ce qui va favoriser la sédimentation des particules fines, limiter la pénétration de la lumière, et ainsi modifier toute la biogéochimie du sédiment (Bouma et al., 2005 ; Widdows et al., 2008 ; Kombiadou et al., 2014). L'allogénie concerne les espèces pour lesquelles l'activité biologique est responsable de la régulation d'une ressource. Un exemple classique en milieu marin est l'arénicole *Arenicola marina*, un ver fouisseur endogé qui vit dans le sédiment (Figure 1c). Son activité de nutrition consiste à ingérer de grandes quantités de sédiment pour en extraire la matière organique, et à les expulser hors de son tunnel, créant le tortillon de sédiment qui indique sa présence. Par son activité de bioadvection, l'arénicole

oxygénise le sédiment, augmente les flux de nutriments vers la surface du sédiment et stimule ainsi le microphytobenthos (Chennu et al., 2015 ; Na et al., 2008 ; Volkenborn et al., 2007). L'influence d'une espèce ingénieur est souvent densité-dépendante (D'Andrea et DeWitt, 2009 ; Jones, 1994) et suit l'hypothèse de « *mass ratio* » qui prévoit que l'effet d'une espèce sur une propriété de l'écosystème est proportionnelle à son importance relative dans la communauté en terme d'abondance ou de biomasse (Grime, 1998). La notion d'ingénieur de l'écosystème est d'ailleurs à ne pas confondre avec celle d'espèce clé de voûte (« *keystone species* »), qui aura un effet démesuré sur un écosystème par rapport à sa biomasse ou son abondance, sans pour autant modifier de manière significative son habitat (Paine, 1969).

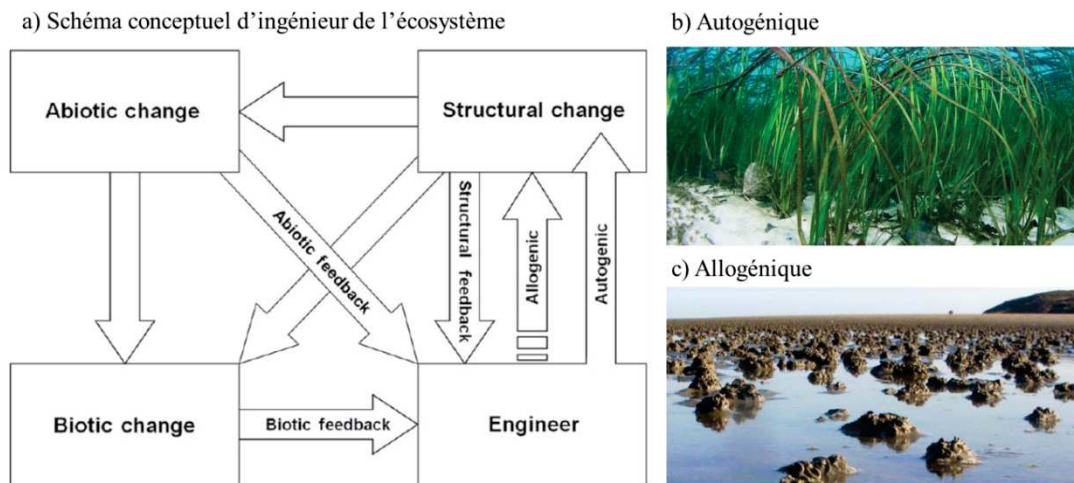


Figure 1 : (a) Concept d'ingénieur de l'écosystème d'après Jones (1997) illustré par (b) les herbiers de phanérogames à *Zostera marina* et (c) les sédiments bioturbés à *Arenicola marina*.

La structure et le fonctionnement des communautés a longtemps été formalisée selon des interactions intra et interspécifiques de compétition, prédation, parasitisme et mutualisme (Connell, 1961 ; Menge 1976). L'idée qui en découle est que la ressource, au sens large (habitat, nourriture, etc.), devient d'autant plus limitante que la diversité et la densité des individus augmentent. Le concept d'espèce ingénieur, à travers les transformations qu'il implique, a ouvert la voie au concept de la facilitation (Bruno et al., 2003 ; Halpern et al., 2007). La facilitation peut être vue comme une propriété émergente d'un système dans le sens où elle propose que la présence d'une espèce améliore l'existence d'une autre, sans bénéfice réciproque. Très prolifique, ce concept a montré qu'un grand nombre de relations interspécifiques dans la nature relevait de la facilitation. C'est par exemple

le cas en milieu intertidal ou les rudes conditions du milieu sont atténuées par l'action d'ingénieurs de l'écosystème (Bouma et al., 2005 ; 2009 ; Bruno et al., 2003 ; Jones et al., 1997 ; Jones, 2017 ; Wright et al., 2017). Cette facilitation s'exerce à différentes échelles de temps et d'espace (Hastings et al., 2007). Alors qu'une structure physique persistante (ex : récif biogénique) aura un effet même après la mort de l'organisme qui l'a créé (Gutiérrez et al., 2003 ; Sheehan et al., 2015), la production d'une source de matière organique facilitée localement (ex : microphytobenthos) pourra être exportée et participer au fonctionnement d'habitat adjacents moins productifs (Chennu et al., 2015 ; Rigolet et al., 2014). Lorsque qu'une espèce tire un bénéfice trophique de la facilitation, on parle de « *gardening* ». Cette hypothèse a été démontrée pour la première fois chez l'arénicole, capable d'assimiler des microorganismes dont la présence a été stimulée par son activité de bioadvection du sédiment (Chennu et al., 2015 ; Hylleberg, 1975). Cette hypothèse a été vérifiée chez d'autres espèces ingénieurs intertidales tels que les récifs d'huitres (Decottignies et al., 2007 ; Echappé et al., 2018), de moules (Asmus et Asmus, 1991 ; Engel et al., 2017) ou d'hermelles (*Sabellaria alveolata*, Jones, 2017). Le *gardening* peut également profiter aux autres espèces associées à la communauté (Jones, 2017 ; Rigolet et al., 2014).

Les espèces invasives

Une espèce est considérée comme introduite si elle remplit 4 critères : 1) elle est non-indigène dans l'écosystème considéré, 2) sa présence est liée directement ou indirectement aux activités anthropiques, 3) sa dispersion a créé une discontinuité géographique avec son aire d'origine, et 4) elle se reproduit par elle-même dans l'aire d'introduction, sans action de l'homme. Si la population introduite constitue une population suffisante pour devenir autonome en termes de reproduction, l'espèce est dite naturalisée (Stiger-Pouvreau et Thouzeau, 2015 ; Figure 2). En Europe, plus de 11 000 espèces sont considérées comme introduites tout milieux confondus, mais 15% seulement sont qualifiées d'invasives. Le milieu marin compte 87 espèces qui sont décrites comme invasives (Katsanevakis et al., 2014). Le caractère invasif fait référence à la capacité de prolifération de l'espèce, capable de générer des impacts significatifs pour l'écosystème receveur et les espèces qu'il abrite (IUCN, <http://www.iucn.org>).

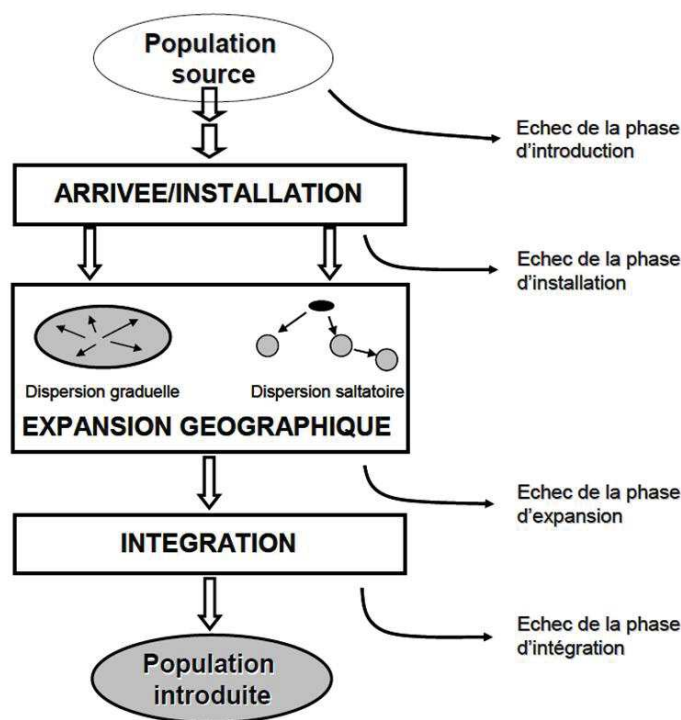


Figure 2 : Étapes du processus d'introduction (Richard 2005, modifié d'après Williamson, 1996).

Liée aux activités anthropiques, l'augmentation constante de ces espèces introduites est aussi intimement liée au changement global (Bax, 2003 ; Vitousek, 1997). Qui plus est, le milieu marin est particulièrement sensible aux invasions biologiques à cause de son caractère diffusif (faible barrière géographique et écologique, potentiel de dispersion), et dont les principaux vecteurs d'introduction sont les eaux de ballast, l'aquaculture et le biofouling (Padilla et al., 2011 ; Stiger-Pouvreau et Thouzeau, 2015).

Le double impact des espèces ingénieurs et invasives : le cas des mollusques

L'arrivée d'une espèce qui combine les caractères invasif et ingénieur est susceptible d'affecter de manière plus importante l'écosystème colonisé (Crooks, 2002 ; Vitousek, 1990). Ces effets sont d'autant plus importants lorsque cette espèce est à la fois un ingénieur auto et allogénique. C'est le cas de certaines espèces de mollusques et en particulier des bivalves. Ce phylum est présent sur tous les types d'habitat (substrats durs et meubles) et peut représenter de très fortes biomasses. En Europe, 36 % des espèces invasives sont des mollusques (Gouletquer, 2002 ; Stiger-Pouvreau et Thouzeau, 2015).

Premièrement, les mollusques sont des ingénieurs autogéniques de par leur production coquillière (Gutiérrez et al., 2003). En fortes densités, ces structures biogéniques diminuent l'hydrodynamisme au niveau de la couche limite de fond et stabilisent le sédiment à l'interface (Friedrichs et Graf, 2009) (Figure 3b). Une autre conséquence de la présence de ces coquilles, plus ou moins importante selon leur structure et leur degré de fragmentation, est l'apport de complexité et d'hétérogénéité à la matrice sédimentaire (Gutiérrez et al., 2003). En effet, les coquilles vont fournir une diversité de micro-habitats pour les invertébrés benthiques mobiles et fixés (Lejart et Hily, 2011 ; Ricciardi et al., 1997 ; Sellheim et al., 2010 ; Zaiko et al., 2009), servant de refuge à certains (Stewart et al., 1999 ; Beekey et al., 2004), et de substrat à d'autres (Botts et al., 1996) (Figure 3b). Enfin, par leur persistance au-delà de la durée de vie des individus, ces amas de coquilles constituent un héritage pour l'écosystème, avec des conséquences à plus ou moins long terme pour les communautés associées (Gutiérrez et al., 2003).

Deuxièmement, les mollusques sont des ingénieurs allogéniques par leur activité biologique (Figure 3c). Une majorité d'entre eux étant filtreurs, ils intensifient le couplage benthopélagique et produisent de grandes quantités de biodépôts sur le sédiment, qui vont l'enrichir en matière organique (Stewart et al., 1998). L'apport de matière organique a un effet sur la diversité de la macrofaune associée, entraînant dans la plupart des cas une augmentation de production secondaire, par l'intermédiaire d'un cortège peu diversifié d'espèces opportunistes (Ozersky et al., 2012 ; Stewart et al., 1998). Cet apport va également modifier la structure des communautés microbiennes et donc les flux biogéochimiques du sédiment (Newell et al., 2002). Ces flux peuvent à leur tour modifier la biomasse et la composition des communautés de producteurs primaires benthiques et planctoniques (Asmus et Asmus, 1991 ; Cloern, 1982 ; Echappé et al., 2018 ; Engel et al., 2017 ; Porter et al., 2004 ; 2013). Le métabolisme (respiration, excrétion, calcification) des mollusques contribue aussi directement à la modification des cycles biogéochimiques du carbone, de l'azote ou du phosphore (Arnott et Vanni, 1996 ; Gardner et al., 1995 ; Lejart et al., 2012). Une autre conséquence de la filtration est la diminution de la turbidité et l'augmentation de la pénétration de la lumière. Si davantage de lumière atteint le compartiment benthique, cela peut contribuer à stimuler la production primaire benthique (Porter et al., 2004 ; Newell et al., 2002).

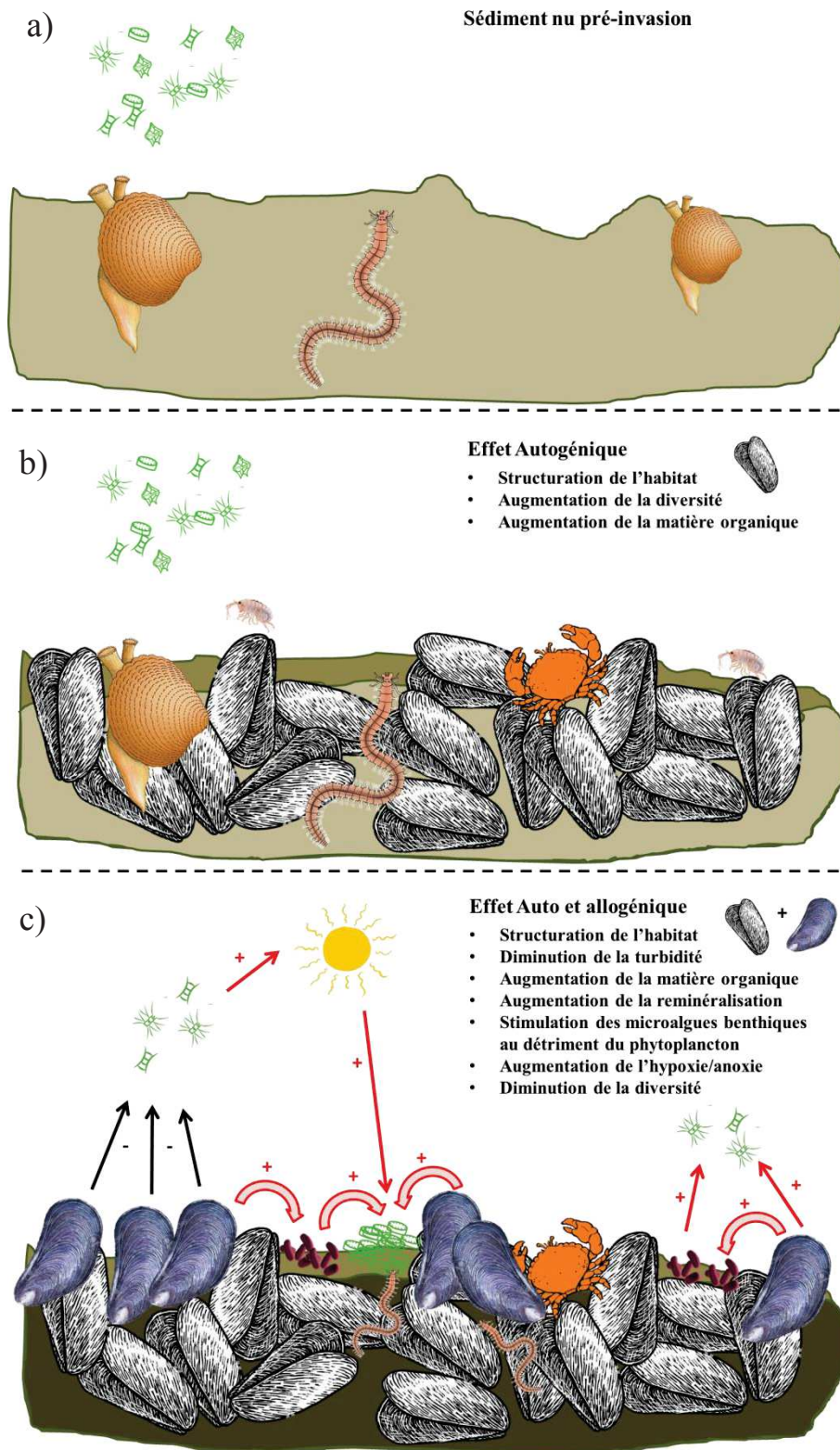


Figure 3 : Résumé des effets autogéniques et allogéniques des mollusques invasifs. (a) sans espèce ingénieur, (b) effet autogénique, (c) effet auto et allogénique.

À travers ces effets sur les producteurs et les consommateurs, c'est donc tout le réseau trophique de l'écosystème qui peut potentiellement être modifié. En effet, outre la compétition directe entre une espèce invasive et les espèces natives (Zwerschke et al., 2016), les effets indirects dus à la transformation de l'habitat sont susceptibles d'influer sur le fonctionnement trophique de l'écosystème (Beekey et al., 2004 ; Donadi et al., 2013 ; van der Zee et al., 2015). Par exemple, la colonisation par la moule zébrée *Dreissena polymorpha* a engendré un changement complet du réseau trophique d'un lac par l'intermédiaire de la stimulation du microphytobenthos par ses biodépôts (Ozersky et al., 2012).

Les exemples cités ci-dessus illustrent les effets de mollusques bivalves, les gastéropodes étant bien moins représentés en termes d'espèce ingénieur de l'écosystème. Une espèce fait néanmoins exception à la règle : c'est la crépidule, ou *Crepidula fornicata* (Linnaeus, 1758).

II – Le cas de l'espèce ingénieur et invasive *Crepidula fornicata*

Un concentré d'innovations biologiques

La crépidule est un gastéropode de la famille des *Calyptraeidae*. En plus d'être un modèle biologique pour la biologie du développement des métazoaires (Henry et al., 2016), sa dimension invasive a suscité un grand intérêt de la part des chercheurs et des politiques locales pour comprendre son écologie et mieux gérer sa prolifération. L'espèce est originaire de la côte Est des États-Unis et s'est implantée en Europe suite à trois vagues d'introduction : une première en Angleterre dans les années 1870 suite à l'introduction de l'huître américaine *Crassostrea virginica*, une seconde lors de la deuxième guerre mondiale via les navires alliés ayant séjournés en Angleterre, et une dernière dans les années 1970 suite aux importations de l'huître creuse *Magallana gigas* (anciennement *Crassostrea gigas*) en provenance du Japon et des États-Unis. De nombreuses études ont été consacrées à la reproduction (Dupont, 2004 ; Le Cam, 2009 ; Le Gall, 1980 ; Richard, 2005), la physiologie (Noisette, 2013), la dynamique (Bohn, 2012 ; Coum, 1979 ; Richard, 2005 ; Rigal, 2010) ou encore la génétique des populations de crépidules (Dupont, 2004 ; Riquet, 2012). Un aspect moins souvent mis en valeur est l'écologie de cette

espèce, et notamment trophique, en lien avec son mode de vie très particulier d'association en chaîne (Figure 4).



Figure 4 : Chaîne de crépidules en activité de filtration.

Un élément fort du succès écologique de la crépidule est sa stratégie de reproduction très efficace qui s'effectue entre individus d'une même chaîne. L'hypothèse du « *size-advantage* » prévoit un succès reproductif plus important pour les hermaphrodites séquentiels. Si les femelles ont un succès reproductif avec la taille ou l'âge plus grand que les mâles, le changement de sexe protandre sera favorisé (Munoz et Warner, 2003). Cette hypothèse s'applique à la crépidule et explique en partie ce succès écologique (Broquet et al., 2015). En effet, la crépidule est un hermaphrodite protandre, c'est-à-dire que chaque individu passe successivement du sexe mâle au sexe femelle. Ce changement de sexe est en lien avec la diminution progressive de la mobilité, les immatures et les jeunes mâles étant mobiles alors que les mâles plus âgés et les femelles sont sessiles. Ce changement de mobilité se fait probablement sous un contrôle hormonal (Dupont, 2004 ; Hoagland, 1975). Les deux sexes se retrouvent donc au sein d'une même chaîne de crépidule. La présence de mâles et de femelles à proximité immédiate facilite la reproduction, d'autant plus que la crépidule est capable de stocker le sperme qui sera ensuite sélectionné avant fécondation (Beninger et al., 2016). De plus, les femelles incubent les larves, diminuant leur mortalité lors de la phase pélagique de dispersion (Collin, 2006). Un élément important lié à la vie en chaîne est de pouvoir attirer les individus les plus jeunes. Il existe chez la crépidule un

INTRODUCTION GÉNÉRALE

chimiotactisme en faveur des recrues qui s'expliquerait par des phéromones émises par les adultes (Cahill et al., 2015), le biofilm jouant également un rôle pour la survie des jeunes crépidules (Pechenik et al., 2015). Enfin, la forme lisse et convexe de la coquille peut être vue comme un substrat idéal pour la fixation des jeunes (Woods, 1989).

Barillé et al., (2006) ont introduit le concept de « *Feeding community ecology* » à propos de cette espèce. Le concept prévoit que la filtration mesurée à l'échelle de la colonie (chaîne de crépidule) serait supérieure à la somme de celles mesurées à l'échelle de chaque individu, démontrant la synergie des parties constituant le tout. Bien que très intéressant, il ne semble pas à notre connaissance avoir été testé expérimentalement. Ce concept pourrait être élargi à d'autres traits biologiques. Chez certaines ascidies coloniales par exemple, les individus ont leur propre siphon inhalant mais partagent un même siphon exhalant, augmentant ainsi l'entrée d'eau et la sortie des biodépôts. De manière analogue, l'orientation des crépidules sur une chaîne n'est sans doute pas le fruit du hasard. En effet, les coquilles sont légèrement décalées les unes par rapport aux autres pour donner cette forme de « croissant » caractéristique, qui pourrait ainsi favoriser le flux d'eau entrant et concentrer l'éjection des biodépôts (Figure 5).

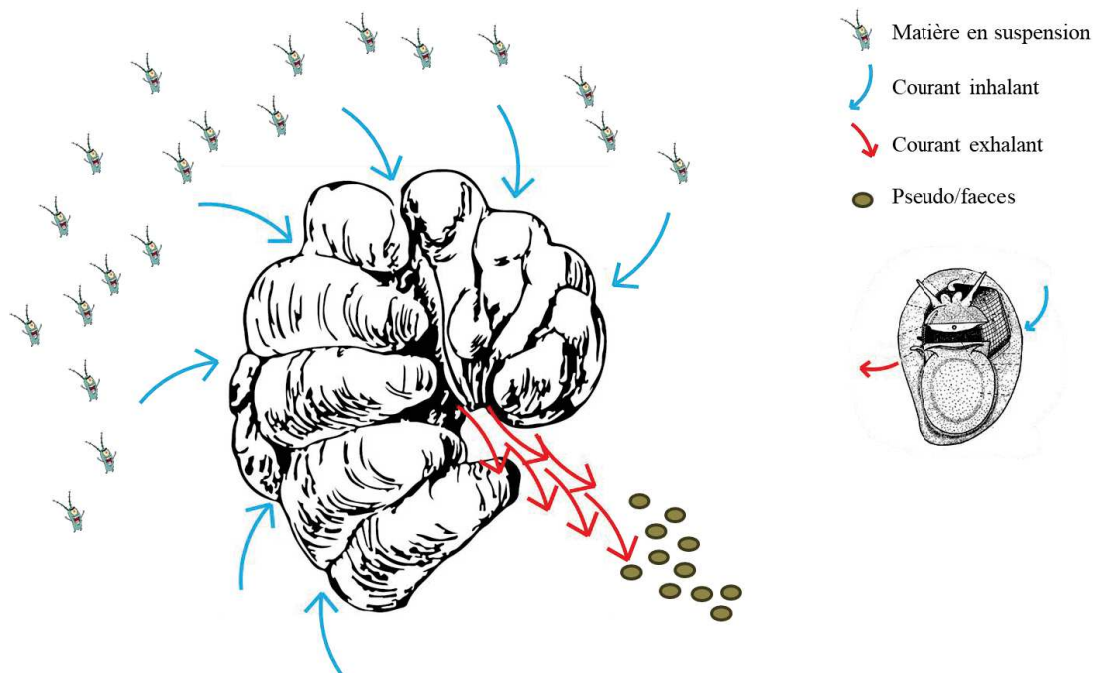


Figure 5 : Schématisation de l'orientation des flux de matière lors de la filtration et de la biodéposition chez *Crepidula fornicata*.

Cette idée s'appuie aussi sur le fait que la crépidule est sessile (adultes) et qu'elle vit à l'interface eau-sédiment dans des milieux assez calmes. Le mode de vie en chaîne d'individus éviterait donc une accumulation des biodépôts sur les chaînes elles-mêmes. En fonctionnant en synergie et maximisant l'effort de reproduction et l'effort d'alimentation, la chaîne de crépidules pourrait donc être assimilée à une entité fonctionnelle à part entière. A l'image des espèces coloniales, la structure en chaîne de la crépidule peut être vue comme un « super-individu ». Parmi la cinquantaine d'espèces que compte le genre *Crepidula*, seul *C. fornicata* montre des accumulations de plus de 4 individus par chaîne (synthèse d'après Le Cam, 2009), et démontre encore une fois l'exception écologique que représente cette espèce, même au sein de son genre.

Des effets multiples sur les habitats

Par son comportement grégaire, cette espèce est également un ingénieur de l'écosystème, à la fois physique et biologique, qui va modifier grandement l'habitat qu'elle colonise (Beudin, 2014 ; Guérin, 2004 ; Martin, 2005 ; Richard, 2005). Ces différents impacts, qui ont fait l'objet de nombreux travaux scientifiques, sont résumés ici à la lumière du concept d'ingénieur de l'écosystème.

Concernant son effet physique, les coquilles de crépidule structurent la matrice sédimentaire, fournissant des niches spatiales à tout type de macrofaune, que ce soit à l'intérieur ou à la surface des coquilles (Miller et DuBar, 1988 ; de Montaudouin et al., 1999 ; Sauriau et al., 2002 ; Woods, 1989). En ce qui concerne la mégafaune, le résultat est plus contrasté. Alors que la crépidule tend à augmenter la richesse spécifique des habitats colonisés (de Montaudouin et al., 2017), certaines espèces sont au contraire exclues. C'est le cas de la coquille Saint-Jacques *Pecten maximus* (Thouzeau et al., 2000) et de la sole *Solea solea* (Le Pape et al., 2004), espèces à fort intérêt commercial. Cette exclusion spatiale est due au fait que la crépidule atteint souvent de très fortes densités et un recouvrement proche de 100 % lorsqu'elle est présente, empêchant ainsi le recrutement des *post* larves de coquille Saint-Jacques (Thouzeau et al., 2000). D'un point de vue hydro-sédimentaire, l'effet physique de la crépidule modifie la couche limite de fond. A micro-échelle, la densité de crépidule tend à diminuer l'érosion du sédiment et donc à créer un effet de protection (Moulin et al., 2007). On retrouve cet effet à macro-échelle où

les simulations hydrodynamiques montrent que l'effet physique des crépidule influence le bilan sédimentaire de la rade de Brest (Beudin, 2014).

Du point de vue de son activité biologique, les effets majeurs de la crépidule sont liés à son activité de filtration et de biodéposition. En intensifiant le couplage benthopélagique, la crépidule est capable de réguler la production primaire phytoplanctonique (Chauvaud et al., 2000 ; Cugier et al., 2010). Une des conséquences de cette régulation est la biodéposition de matière organique sur le sédiment. Contrairement aux bivalves qui montrent souvent des capacités de sélection des particules grâce à leur branchies et leur palpes labiaux (Cognie et al., 2001 ; Beninger et Le Pennec, 2016), la crépidule ne possède pas de tels mécanismes (Beninger et al., 2007). Elle sécrète de grandes quantités de mucus au niveau de ses branchies pour capter la matière en suspension (Shumway et al., 2014). Bien que sa capacité de rétention soit très élevée (Barillé et al., 2006), la crépidule montre une faible efficacité d'ingestion, une partie de la matière captée (10 %) enrichie en matière organique va se déposer directement sur le sédiment, et s'ajouter à la fraction de pseudofèces (pré-ingestion) et de fèces (post-ingestion). Tout cela contribue à l'enrichissement en matière organique des habitats colonisés par la crépidule (Ehrhold et al., 1998 ; Manac'h, 1995 ; Martin et al., 2007). Cet apport de matière organique stimule la respiration des microorganismes et favorise l'anoxie du sédiment (Martin et al., 2006 ; Thouzeau et al., 2011), excluant les espèces sensibles et favorisant les espèces opportunistes (de Montaudouin et al., 1999). Considérant les fortes densités, le métabolisme intrinsèque de la crépidule joue également un rôle dans le fonctionnement de l'écosystème. Les flux liés à la respiration, la calcification et l'excrétion de l'animal dominent les flux de l'habitat colonisé (Martin et al., 2006). A l'échelle de l'écosystème, les crépidules sont également susceptibles de moduler la durée, l'intensité et la composition des blooms phytoplanctoniques (Foullaron et al., 2007 ; Claquin et al., 2010). Enfin, comme de nombreuses espèces invasives, la crépidule n'a pas ou peu de prédateurs et peut constituer une perte de productivité pour l'écosystème (Arbach Leloup et al., 2008 ; Cugier et al., 2010).

Niche trophique et compétition

Dès le début de sa phase de prolifération, la crépidule a été vue comme un potentiel compétiteur pour les espèces natives telles que l’huitre plate (Barnes et al., 1973), la coquille Saint-Jacques (Thouzeau et al., 2000) ou d’autres espèces volontairement introduites à des fins aquacoles comme l’huitre creuse (Blanchard et al., 1997). Plusieurs études utilisant le traçage isotopique naturel (voir encadré méthode n°2) ont montré que la crépidule n’était probablement pas un compétiteur trophique pour bon nombre d’espèces suspensivores qui partagent son habitat (Decottignies et al., 2007ab ; Dubois et al., 2014 ; Guérin, 2004 ; Grall et al., 2006 ; Lefebvre et al., 2009 ; Riera et al., 2002 ; 2007 ; Richard, 2005 ; Sauriau et al., 2002). Plusieurs hypothèses ont été proposées pour expliquer cette différence de niche trophique. La première était une contribution plus importante de microalgues benthique dans son régime alimentaire (Figure 6).

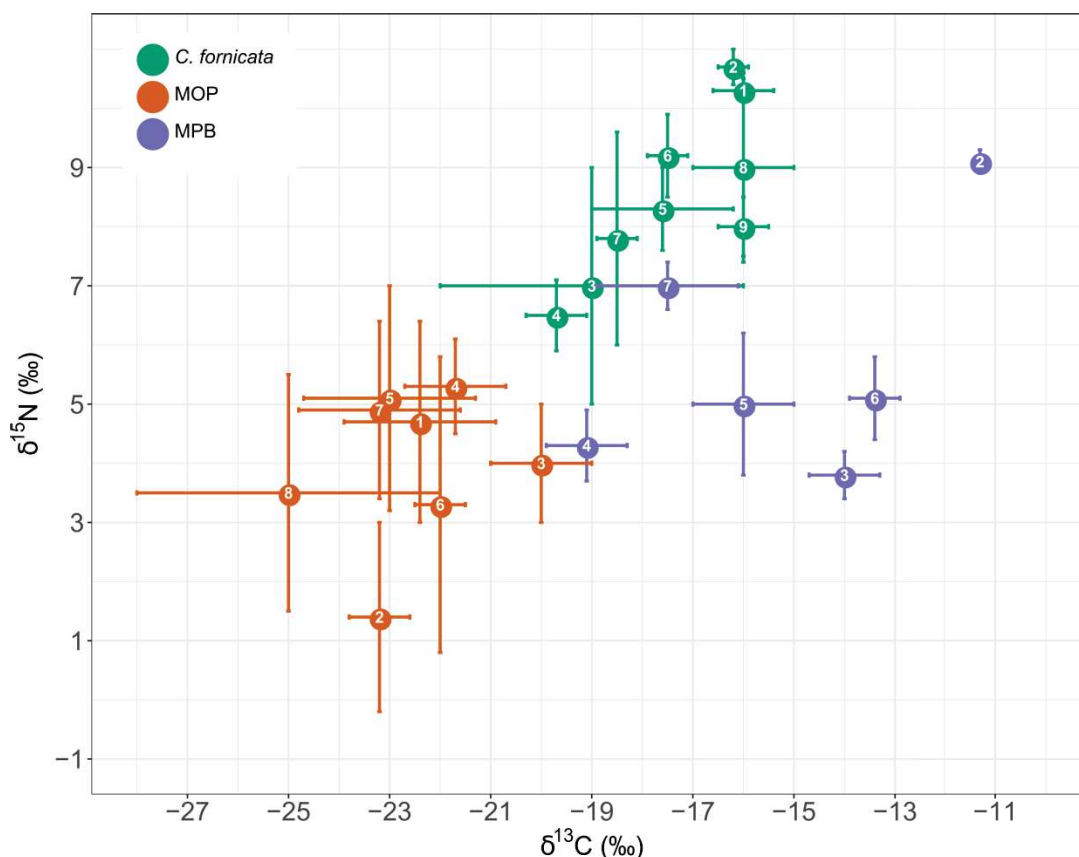


Figure 6 : Valeurs isotopiques de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ de *Crepidula fornicata* et deux sources potentielles de matière organique (matière organique particulaire (MOP) et microphytobenthos (MPB)) issues de la littérature. 1 : Grall et al., 2006 ; 2 : Riera et al., 2002 ; 3 : Riera, 2007 ; 4 : Dubois et al., 2014 ; 5 : Sauriau et al., 2002 ; 6 : Decottignies et al., 2007a ; 7 : Lefebvre et al., 2009 ; 8 : Richard, 2005 ; 9 : Guérin, 2004.

Le microphytobenthos, qui rassemble l'ensemble des microorganismes photosynthétiques se développant à la surface du substrat (MacIntyre et al., 1996), est en effet d'une grande qualité nutritive pour les consommateurs (Miller et al., 1996). En utilisant un autre type de marqueur trophique (voir encadré méthode 3 sur les acides gras), Dubois et al., (2014) ont suggéré que la crépidule pourrait également assimiler une part non négligeable de bactéries, ce qui lui conférerait une niche particulière, en plus de consommer du microphytobenthos.

Comme énoncé plus haut, le fait que la crépidule ne soit pas capable de sélectionner les particules parmi le *pool* de matière organique en suspension explique son régime opportuniste. C'est ce que l'on peut noter sur la Figure 6 où la variation du $\delta^{13}\text{C}$ moyen de la crépidule varie entre -20 et -16 ‰, en lien probablement avec des biomasses de microphytobenthos (MPB) disponibles plus ou moins importantes. Enfin, sa position très proche de l'interface eau-sédiment renforce l'idée que la crépidule assimile des sources telles que du MPB ou des bactéries se développant à la surface du sédiment.

La crépidule dans la rade de Brest

La rade de Brest est une baie semi-fermée de 180 km², dont la profondeur moyenne est de 5 m. Deux rivières fournissent l'essentiel des apports en eau douce : l'Aulne au sud (80 %, débit moyen : 24 m³ s⁻¹) et l'Elorn au nord (20 %, débit moyen : 6 m³ s⁻¹) (Troadek et al., 1997) (Figure 7). Les fleuves sont une source de nutriments importante qui supporte la production primaire en zones côtières, phénomène accentué dans la région Bretagne par l'agriculture intensive (Ménesguen et al., 2018b). Une forte eutrophisation dans une baie semi-fermée comme la rade de Brest pourrait être attendue. Cependant, le fort marnage (6 m) et l'étroit goulet à l'entrée de la rade (2 km de large) entraînent de forts courants, ce qui a pour effet de renouveler un tiers de son volume à chaque marée (Troadek et al., 1997). Cette caractéristique physique est un élément clé de son fonctionnement, permettant une forte production primaire tout en évitant l'eutrophisation (Le Pape et al., 1996).

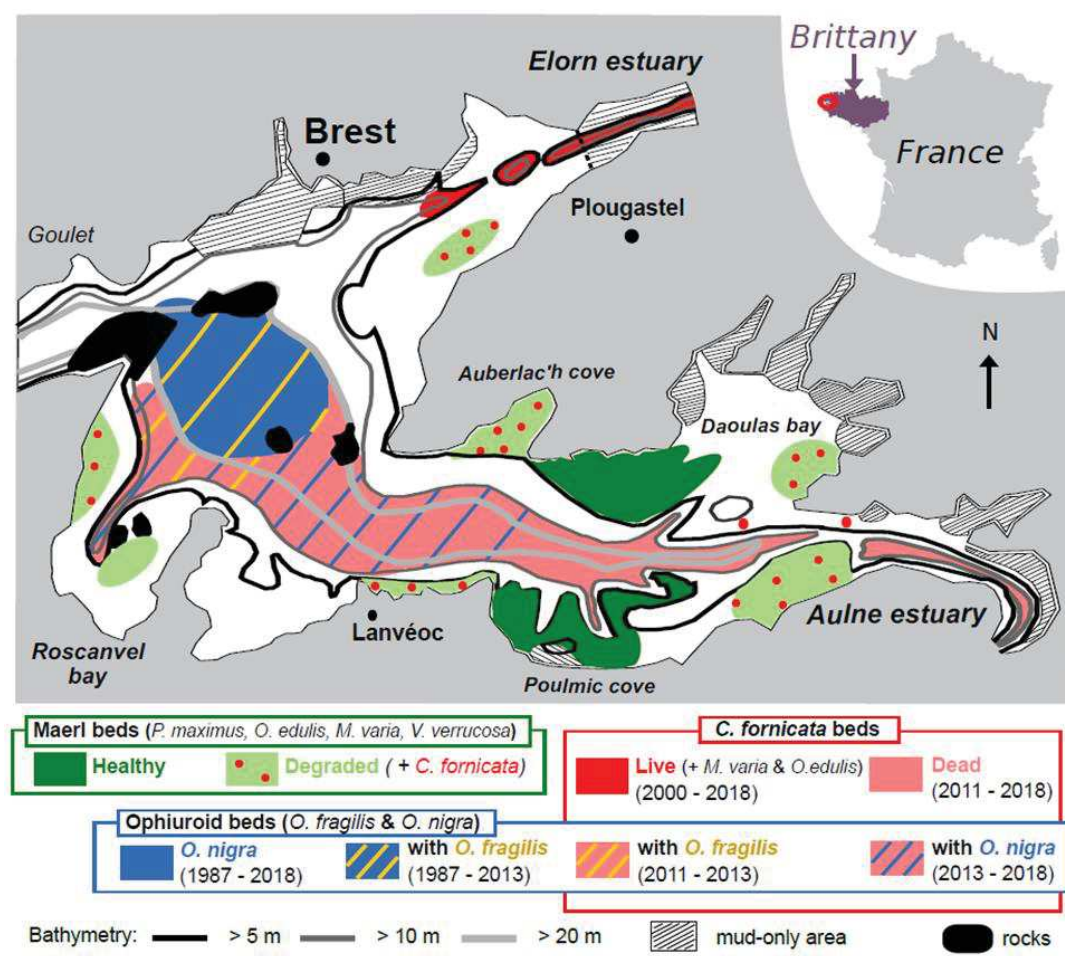


Figure 7 : Cartographie des principaux habitats de la rade de Brest de 1987 à 2018 (Go et al., données non publiées).

La mosaïque d'habitats benthiques de la rade de Brest s'explique grâce à la combinaison de deux facteurs : abiotique (hydrodynamisme) et biotique (espèce ingénier). Comme pour la masse d'eau, l'hydrodynamisme conditionne la sédimentologie. Les baies (Roscanvel, Daoulas) et estuaires (Aulne, Elorn) de la rade sont composés majoritairement de vase, alors que le centre rade est dominé par des sables plus ou moins hétérogène, et que la roche affleure aux zones de forts courants (Goulet, pointe de l'Auberlac'h). La présence de deux espèces biogéniques (le maërl et la crépidule) se superpose à cette cartographie sédimentaire et explique la diversité des habitats observés. Le maërl est une algue rouge corallinale qui forme des structures arbustives calcaires de l'ordre du centimètre. Situé sur les zones peu profondes (Figure 7), cette espèce ingénier (au sens autogénique) forme des bancs qui abritent une forte diversité benthique (Grall, 2002 ; Grall et al., 2006). La crépidule, pour les raisons évoquées précédemment, forme également des bancs subtidaux et participe à la structuration des fonds. L'espèce est décrite dans la rade

de Brest depuis les années 1950 (Cole, 1952). D'abord présente dans les chenaux de l'Aulne et de l'Elorn, sa distribution et son abondance n'ont cessé d'augmenter au cours des années 1990, atteignant localement des densités de plus de 2000 ind m⁻² (Chauvaud et al., 2000). Cette dynamique a ainsi produit une biomasse totale de 127 000 tonnes (poids frais avec coquille) en 2000, recouvrant près de 50 % de la rade de Brest (Guérin, 2004) (Figure 7). Récemment, un suivi a montré un déclin de la crépidule dans le bassin sud de la rade où les densités ont drastiquement diminuées (Blanchet-Aurigny et al., 2012a ; Carlier, données non publiées). Bien que des tentatives d'explications soient proposées, comme de fortes concentrations en métaux lourds (ex : argent, plomb) en provenance de l'Aulne auxquelles la crépidule serait particulièrement sensible (Ménesguen et al., 2018a ; Nelson et al., 1983), rien n'a encore été testé pour confirmer cette hypothèse.

La crépidule a été suggérée comme l'élément central de la régulation de la biomasse phytoplanctonique (Chauvaud et al., 2000). L'intensification du couplage benthopélagique (filtration et biodéposition) modifie les cycles biogéochimiques du compartiment benthique (Martin et al., 2006 ; Ragueneau et al., 2002 ; 2005), pouvant influencer la production primaire benthique et pélagique (Ní Longphuirt et al., 2007). Dans ce contexte, de récentes études ont montré que la production primaire benthique était sous-évaluée, et directement liée au MPB subtidal (Chatterjee, 2014 ; Ní Longphuirt et al., 2007). Très connu en milieu intertidal, l'importance du MPB a été largement démontré, que ce soit en termes de production primaire (Hernández Fariñas et al., 2017 ; MacIntyre et al., 1996), de source pour les réseaux trophiques côtiers (Miller et al., 1996) ou encore de stabilité du sédiment (Underwood et Paterson, 2003 ; Stal 2010). Son homologue subtidal reste moins étudié, et son rôle écologique est par conséquent souvent négligé (Cahoon, 1999). Néanmoins, la part de la production du MPB dans la rade de Brest est estimée à 30 % de la production totale, marquée par une contribution plus importante en fin d'hiver (Chatterjee et al., 2013 ; Ni Longphuirt et al., 2007). Le rôle du MPB dans le soutien du réseau trophique de la rade de Brest a été suggéré plusieurs fois, mais sans être véritablement testé (Chatterjee, 2013 ; Guérin, 2004 ; Grall et al., 2006 ; Richard, 2005).

III – Objectifs de la thèse

Au regard de la littérature existante, il semble que le MPB contribue de manière significative au régime trophique de la crépidule, que ce soit pour des individus intertidaux, où le rôle du MPB est déjà bien connu, ou pour des individus subtidaux, où le MPB reste peu étudié. Il convient donc de quantifier la contribution de cette source de matière organique dans les habitats subtidaux, et d'autant plus dans le cas d'une espèce invasive dominante en terme de biomasse. A la manière des espèces ingénieurs intertidales, la crépidule pourrait avoir un rôle dans la régulation de ce MPB. Le broutage mais aussi la stimulation du MPB par la crépidule pourrait conférer un avantage trophique à cette espèce, de même que pour celles partageant son habitat. Enfin, du fait de sa dynamique de population contrastée, la population de la rade de Brest constitue une zone d'exception permettant de tester les effets physiques (autogéniques) et biologiques (allogéniques) de cette espèce ingénieur. Cette thèse a donc pour but d'étudier le régime trophique de l'espèce ingénieur *Crepidula fornicata* et son implication pour le fonctionnement de son habitat. Elle se propose en particulier de tester l'hypothèse de *gardening* associée à cette espèce, c'est-à-dire la stimulation du MPB, son assimilation trophique par la crépidule et la communauté associée. Le manuscrit est structuré en trois chapitres abordant autant d'échelles d'observations.

Dans un premier chapitre, nous verrons comment la crépidule interagit avec le MPB subtidal, notamment en dissociant les influences physiques (hétérogénéité de l'habitat) et biologiques (excrétion particulière et dissoute) de cette espèce. Cette hypothèse de fertilisation sera testée en milieu contrôlé, et fera appel à une technique d'imagerie non invasive pour la quantification spatiale et temporelle du MPB : l'imagerie hyper-spectrale.

Dans un second chapitre nous nous intéresserons au régime trophique de la crépidule. Un article s'intéressera à l'histologie de cette espèce et montrera comment la technique de préparation des échantillons de tissus peut avoir une incidence sur l'analyse des marqueurs trophiques isotopiques. Il démontrera les conséquences de la particularité histologique de la crépidule sur l'interprétation de sa niche trophique. Le second article, à travers l'utilisation de deux marqueurs trophiques (acides gras et isotopes stables) tentera de déterminer si les changements

ontogéniques de certains traits biologiques de la crépidule (développement sexuel et capacité de mouvement) influencent sa niche trophique.

Dans le troisième et dernier chapitre, nous élargirons l'approche des marqueurs trophiques aux espèces qui partagent l'habitat de la crépidule. Un article se focalisera sur le groupe des suspensivores, et analysera comment la crépidule et les autres espèces se partagent la ressource trophique. Là encore une double approche acide gras et isotopes stables sera employée. Un autre article considérera l'ensemble des espèces d'invertébrés benthiques de l'habitat à crépidule et comparera la structure du réseau trophique de deux types de bancs de crépidules (mort vs. vivant). Une approche couplée traits biologiques et isotopes stables tentera d'expliquer les effets de la crépidule à l'échelle de la communauté en s'appuyant sur la dynamique contrastée de l'espèce dans la rade de Brest (Figure 8).



Figure 8 : Prélèvement en plongée sur une zone à forte biomasse de crépidules (embouchure de l'Elorn)

CHAPITRE I : Influence de la crépidule sur la production primaire benthique

Méthode 1 : Imagerie hyper-spectrale

L'œil humain est composé de 3 types de cellules sensibles à la qualité de la lumière appelées cônes. Ces cônes répondent à des radiations lumineuses appartenant à 3 domaines étendus de longueurs d'ondes (rouge : ~564 nm, vert : ~533 nm, bleu : ~437 nm), sur un spectre du visible qui s'étend de 380 à 780 nm. Toutes les couleurs que nous voyons sont donc un mélange de ces 3 couleurs recomposées par le cerveau. On dit que l'œil est trichromatique par association de ces trois larges bandes spectrales. Dans une plus grande mesure, la technologie hyper-spectrale permet des combinaisons de plusieurs centaines de bandes spectrales très précises de l'ordre du nanomètre (Figure 9). Ces images à haute résolution spectrale sont porteuses de nouvelles informations, notamment concernant les pigments photosynthétiques qui réagissent à la lumière (voir Méthode 3). Chaque pigment, qu'il soit de type chlorophyllien ou caroténoïdien, absorbe la lumière à une longueur d'onde spécifique (ex : chlorophylle *a* : 675 nm). Il est donc possible de détecter la présence de ces pigments et de les quantifier en analysant le spectre réfléchi. En fonction du caractère plus ou moins spécifique de ces pigments, on peut donc déduire des informations sur la présence de différents groupes taxonomiques de producteurs primaires. Le second avantage de cette technique optique est la résolution spatiale. En effet, il est désormais possible d'obtenir des valeurs quantitatives de pigment (ex : biomasse de chlorophylle *a*) pour chaque pixel de l'image, et ainsi réaliser des cartes de distribution de ces pigments sur une surface donnée. Étant non intrusives, ces mesures sont répétables dans le temps et permettent ainsi de suivre la dynamique d'un compartiment, tel que la croissance du MPB sur différentes zones d'intérêts.

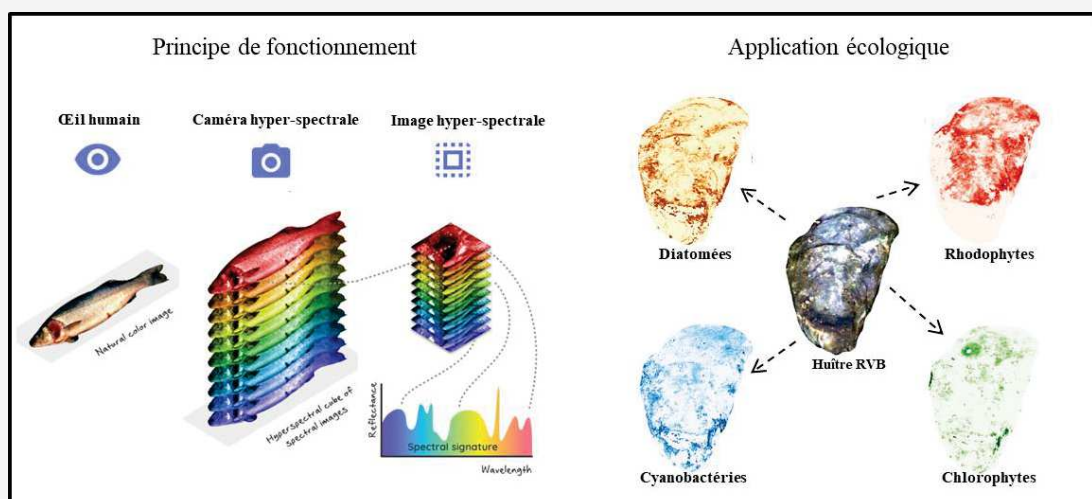


Figure 9 : Principe de fonctionnement de l'imagerie hyper-spectrale, et application sur les communautés photosynthétiques de l'huître *Magallena gigas* : 4 longueurs spécifiques montrent la distribution spatiale de 4 types de producteurs primaires (images tirées de Barillé et al., 2017).

Article 1 : Subtidal microphytobenthos: the « secret garden » stimulated by the engineer species *Crepidula fornicata*

Thibault Androuin ¹, Lubos Polerecky ², Priscilla Decottignies ³, Stanislas F. Dubois ¹, Christine Dupuy ⁴, Cédric Hubas ⁵, Bruno Jesus ³, Erwan Le Gall ⁶, Martin P. Marzloff ¹, Antoine Carlier ¹.

¹ DYNECO-LEBCO, IFREMER, Plouzané, France

² Department of Earth Sciences – Geochemistry, Utrecht University, Utrecht, Netherlands

³ Lab. Mer Molécules Santé EA 2160, University of Nantes, Nantes, France

⁴ Littoral, Environnement et Sociétés (LIENSs) UMR 7266 CNRS, University of La Rochelle, La Rochelle, France

⁵ Muséum National d'Histoire Naturelle, UMR BOREA 7208 MNHN-Sorbonne Université-CNRS-UCN-UA-IRD, Concarneau, France

⁶ DYNECO-PELAGOS, IFREMER, Plouzané, France

Abstract

The slipper limpet *Crepidula fornicata* is an emblematic invasive species along the northeast Atlantic coast. This gregarious gastropod lives in stacks of several individuals and forms extended beds in shallow subtidal areas. The effects of this engineer species on the colonized habitat can be physical (e.g., presence of hard-shell substrates with uneven topography) or biological (e.g., nutrient enrichment by direct excretion or via biodeposition). We hypothesized that through biological activity, nutrient fluxes at the sediment-water interface are enhanced, leading to stimulated primary productivity by microphytobenthos (MPB) associated with *Crepidula* beds. To test this fertilization hypothesis, we conducted a 10-day mesocosm experiment using *C. fornicata* (live and dead) placed on top of sieved and homogenized sediment collected *in situ*. We used hyperspectral imaging to non-invasively map the development of MPB biomass, and to assess the potential influence of *C. fornicata* and its spatial extent. Our results showed that live *C. fornicata* significantly promote MPB growth through both physical and biological effects, with the biological effect dominating over the pure physical one. The highest stimulation was observed on the shells, suggesting that dissolved metabolic products excreted by *C. fornicata* were likely the main factor stimulating MPB growth in our short-term experiment. Our findings provide first direct evidence that stimulation of MPB growth by the biological activity of larger benthic epifauna occurs not only in intertidal but also in shallow subtidal habitats. More research is needed to assess the contribution of this fertilization effect to the trophic functioning of subtidal benthic systems.

Keywords: *Crepidula fornicata*, engineer species, subtidal microphytobenthos, fertilization, hyperspectral imaging.

1. Introduction

Microphytobenthos (MPB) refers to a complex assemblage of benthic unicellular eukaryotic algae (mainly diatoms) and cyanobacteria living at the sediment-water interface (MacIntyre et al., 1996). It plays a significant role in coastal ecosystems through its contribution to primary production (MacIntyre et al., 1996), food web functioning (Riera and Richard, 1996) and sediment stability (Underwood and Paterson, 2003). While intertidal MPB is fairly well-studied (Cartaxana et al., 2011; Juneau et al., 2015; Marques da Silva et al., 2017; Underwood, 2005), its subtidal counterpart has received relatively little attention. However, recent evidence suggests that MPB significantly contributes to shallow areas in terms of biomass (Chatterjee et al., 2013; Hernández Fariñas et al., 2017), primary productivity (Ní Longphuirt et al., 2007) and ultimately food webs (Rigolet et al., 2014).

MPB space-time dynamics is affected by a number of abiotic (e.g., light, temperature, nutrient availability, hydrodynamic conditions, sediment grain size) and biotic (e.g., grazing, competition) factors and their interactions (Azovsky et al., 2004; Jesus et al., 2005; Seuront and Spilmont, 2002). Among these factors, fertilization through the activity of benthic macrofauna is emerging as an important mechanism in benthic ecology (Chennu et al., 2015; Echappé et al., 2018; Engel et al., 2017). Fertilization, which refers to the ability of a species to directly or indirectly promote primary production, can be induced by several processes that affect nutrient availability at the sediment-water interface, e.g., by direct excretion of dissolved nutrients such as ammonia (Martin et al., 2006; Prins et al., 1997; van Broekhoven et al., 2015) or by enhancing organic matter mineralization through biodeposition (e.g., by suspension-feeders) or bioturbation (Kristensen, 2000; Mermillod-Blondin and Rosenberg, 2006). Quantifying the net effect of benthic macrofauna is, however, not straightforward, as it can control the MPB biomass both positively by fertilization and negatively by grazing (Asmus and Asmus, 1991; Porter et al., 2004; 2013). Recent studies demonstrated that MPB productivity in intertidal sediments can be stimulated by different types of engineering macrofauna such as lugworms, oysters and mussels (Chennu et al., 2015; Echappé et al., 2018; Engel et al., 2017). In this study, we aim to explore this phenomenon in subtidal

sediments, using the Atlantic slipper limpet *Crepidula fornicata* as a model of engineering organism in these habitats.

C. fornicata is a gregarious invasive species that spreads along most of the European coasts and is now well established in many shallow embayments (Blanchard, 2009). It is a suspension-feeder that forms very dense beds on the seafloor, with fresh biomass reaching up to 10 kg m⁻² (Blanchard, 2009). At high densities, this habitat-forming species builds complex 3D benthic habitats (Woods, 1989), and hence constitutes an interesting biological model to assess the effects of ecosystem engineering species on a colonized system (Jones et al., 2010). *C. fornicata* modifies its surrounding environment in two different ways, which we hereafter refer to as the physical and biological effects. First, their calcareous shells increase heterogeneity and topographic complexity of soft sediment bottoms (Gutiérrez et al., 2003), which enhances the local species diversity (Barnes et al., 1973; de Montaudouin et al., 2017; de Montaudouin and Sauriau, 1999) and modifies hydrodynamic conditions near the sediment-water interface (Moulin et al., 2007). Second, the filter-feeding activity of *C. fornicata* facilitates benthic-pelagic coupling (Chauvaud et al., 2000), and increases organic enrichment of the sediment through excretion of large amounts of particulate biodeposits (Ehrhold et al., 1998). Additionally, excretion of dissolved metabolic products, such as ammonia, can substantially enhance nutrient's availability in dense *Crepidula* beds (Martin et al., 2006). In most colonized areas, *C. fornicata* is a key species of the ecosystem functioning (Chauvaud et al., 2000; Martin et al., 2006).

To assess the potential of *Crepidula fornicata* for fertilization of subtidal MPB, we conducted a short-term (10 days) experiment where we monitored MPB biomass in sediment mesocosms amended with simplified *Crepidula* beds. We used hyper-spectral imaging to non-invasively monitor MPB biomass and thus infer its growth. To disentangle the role of the physical and biological effects of *C. fornicata*, we compared the MPB growth on bare sediments with that on beds with dead or live *C. fornicata*. Moreover, to gain insights into the spatial extent of the biological effect, we compared the growth within and outside of *Crepidula* beds and on shells, both for dead and live conditions. Additionally, to assess the effects of *C. fornicata* in a larger context, we also quantified other key components of MPB biofilms, namely the concentrations of proteins, carbohydrates and heterotrophic prokaryotes.

2. Material and Methods

2.1. Experimental setup

Sediment and stacks of *Crepidula fornicata* were collected from a dense *Crepidula* bed (biomass ~ 6 kg of fresh weight m^{-2}) in the bay of Brest ($48^{\circ} 23.36068' N$, $04^{\circ} 23.86901' W$) on March 1st 2017 (winter season) using a surface grab ($0.1 m^2$). One day after collection, *C. fornicata* stacks were cleaned of their epibionts and the empty shell at the base was removed as it often contains endobiont fauna. Half of the stacks were kept alive while the other half were unstacked, cleared of *C. fornicata* flesh and reassembled to stacks resembling the original ones using a non-invasive glue (coral glue, PREIS AQUARISTIK®). Subsequently, both the dead and live stacks were kept for one week in nets in a natural seawater pond to allow filter-feeding of live *C. fornicata* and partial recovery of MPB biofilms on the shells. No feeding was allowed during the experiment, so excretion of pseudofeces that occurred just after the installation of *C. fornicata* individuals onto the sediment resulted from their feeding activity in the pond.

The freshly collected sediment was first sieved through a $500 \mu m$ mesh to remove macrofauna that could potentially influence the experiment through their bioturbation and grazing, and then homogenized in a single large tank. After one night, the supernatant was discarded and 12 mesocosms (60 L glass aquaria, bottom area of $40 \times 40 cm^2$) were filled evenly with a 5 cm thick layer (after stabilization) of the slurry. After 24 h of decantation, each mesocosm was connected to a flow-through system and let stabilize for 7 days. During this time, the overlying seawater was replaced at a rate of $15 L min^{-1}$. The seawater was filtered ($5 \mu m$ mesh) and supplied from three dark $24 m^3$ tanks to prevent allochthonous primary production from entering the mesocosms. Illumination was supplied in a 12h:12h light:dark cycle using four light emitting diodes (LEDs) with different emission wavelengths (Supplementary Fig. 17). Light intensity (PAR) at the sediment surface was $4 \mu mol m^{-2} s^{-1}$, which simulated *in situ* light conditions in the bay of Brest at 11 m water depth during winter (Chatterjee et al., 2013). Temperature remained constant at $11.3 \pm 0.4 ^{\circ}C$ during the whole experiment.

After the sediment stabilization phase, mesocosms were divided into three treatments and incubated for 10 more days under the same conditions (illumination, temperature, water flow) as during the stabilization phase. The treatments, each performed in three replicate mesocosms, included a control treatment without *C. fornicata*, a treatment with reconstructed dead shell stacks, and a treatment with live stacks of *C. fornicata* (Fig. 10A). For the dead and live treatments, 9 stacks of equal initial fresh biomass (4–5 individuals per stack, total biomass 220 g, density 12 kg m⁻²) were placed on top of the sediment within a 15 cm diameter circle in the center of the mesocosms to mimic realistic biomass observed in the field in the bay of Brest (Guérin, 2004).

2.2. Hyper-spectral imaging of MPB biomass

Although extraction-based pigment analysis is a common way to directly quantify the biomass of microscopic primary producers (Wright et al., 1991), its invasive nature does not allow monitoring the biomass of a MPB biofilm in the same location through time. Remote sensing methods are a good alternative to quantify MPB pigments without interfering with the biofilm structure (Forster and Jesus, 2006; Paterson et al., 1998). Among them, hyper-spectral imaging, which is based on capturing back-scattered light with high spatial and spectral resolution, has been shown to provide accurate estimates of primary producers' biomass (Barillé et al., 2007; Carrère et al., 2004; Chennu et al., 2013; Combe et al., 2005; Jesus et al., 2008; 2014). Although often used air-borne or space-borne to study MPB distribution at large spatial scales (Echappé et al., 2018; Méléder et al., 2003), this approach can also be used at smaller scales, ranging from single cells to microbial communities such as mats and biofilms (Barranguet et al., 2004; Perkins et al., 2016; Polerecky et al., 2009a). Critically, the non-destructive nature of hyper-spectral imaging allows monitoring of temporal variations in pigment content in the same region of interest, thus allowing inference to the MPB growth (Chennu et al., 2013; Polerecky et al., 2009b).

Hyperspectral imaging of the mesocosms was done immediately after introducing the *Crepidula* stacks and after 4, 5, 6, 8, 9 and 10 days (Fig. 10A). Images were acquired in the VIS-NIR spectral range (400–1000 nm; spectral resolution 1.3 nm) using a Pika XC2 imager (RESONON Inc®). The imager was mounted on a translation stage (Fig. 10B) and controlled using the SPECTRONON

software (RESONON Inc®). The entire imaging system was mounted on rollers to allow movement from one mesocosm to another. Imaging was done by scanning the imager at 2.4 mm s^{-1} at 90 cm from the sediment water interface (SWI) at a nadir position, resulting in a spatial resolution of $250 \mu\text{m}$. Before each scan the water level was carefully lowered down to 10 cm above SWI to minimize light absorption and scattering in the water column. During scanning, which lasted about 12 min per scan, the imaged area was illuminated with 8 halogen lamps (downwelling light intensity at SWI of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$). To enable quantification of spectral reflectance corrected for light absorption in the overlying seawater, a white spectral reference plate was placed on the sediment surface and included in each scan (Chennu et al., 2013; Polerecky et al., 2009a). Because subtidal MPB vertically migrates in the sediment following a diurnal cycle (Ní Longphuirt et al., 2006), affecting spectral reflectance of sediments (Chennu et al., 2013), all mesocosms were scanned within 2 hours at the middle of the day.

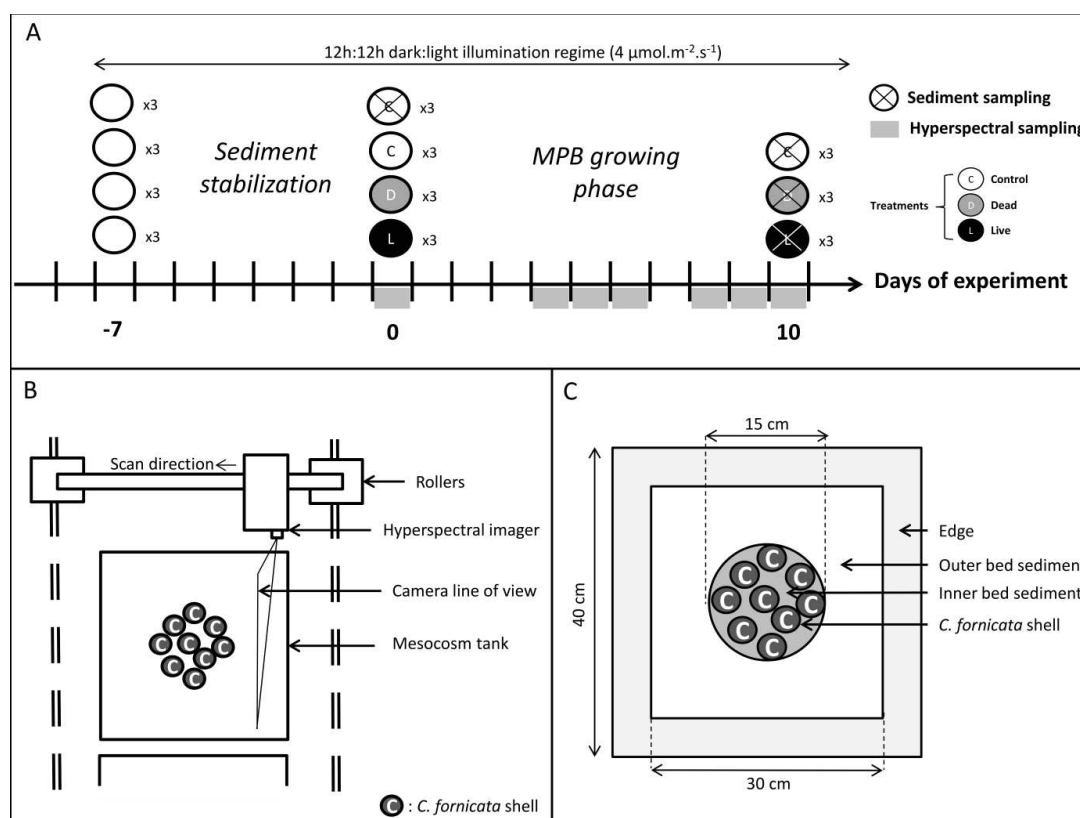


Figure 10: (A) Timeline of the experimental protocol and sampling design, (B) top-view schematic of the hyper-spectral imaging system, and (C) regions of interest within each mesocosm tank.

2.3. Hyper-spectral image processing

Hyper-spectral image processing employed linear spectral unmixing to extract a proxy for MPB biomass in the studied biofilms from the measured reflectance spectra. Similar approach was successfully used to study the dynamics of MPB populations growing on artificial substrates (Polerecky et al., 2009b). First, the spectral reflectance, R , was calculated by normalizing the spectrum of back-scattered light measured in a given pixel with the mean spectrum co-registered in the same scan from the spectral reference plate. The reflectance was then log-transformed to account for the exponentially decreasing light in MPB biofilms due to strong absorption and scattering (Chennu et al., 2013). Finally, the end-member spectra were defined as (1) the spectrum of the MPB-free substrate (both MPB-free sediment and shells had similar reflectance spectra), (2) a wavelength-independent offset describing the general “darkness” of the MPB-covered substrate, and (3) a spectrum containing features typical for MPB pigments including the absorption maxima due to chlorophyll a (Supplementary Fig. 18). The 3rd end-member spectrum was determined by subtracting the $\log(R)$ spectra acquired from an MPB-covered and MPB-free substrate, and subsequently removing a constant off-set so that the result became zero at the wavelength of 730 nm. A detailed analysis revealed that all $\log(R)$ spectra could be satisfactorily decomposed into a linear combination of these three end-member spectra, suggesting that there was no significant change in the phototrophic community composition during the experiment. Therefore, the magnitude of the 3rd end-member spectrum was assumed to represent the MPB biomass in the studied biofilms. Since no calibration was performed, the biomass is presented in relative units.

The linear spectral unmixing was performed pixel-by-pixel using the Look@MOSI program (Polerecky et al. 2009a; <http://www.microsen-wiki.net>), and the resulting images were subsequently processed in Matlab. Specifically, signals representing the MPB biomass were averaged over three regions of interest (ROIs): (1) inner bed sediment, corresponding to the sediment in-between stacks inside the *Crepidula* beds, (2) outer bed sediment, corresponding to the sediment outside but close to the *Crepidula* beds, and (3) shells, corresponding to the *C. fornicata* shells (Fig. 10C). A 15 cm diameter circle in the centre of the control mesocosms was used as the reference ROI for the inner bed sediment. ROIs were drawn manually

based on the true-color images of the scene constructed from the bands acquired at wavelengths 672 nm (red), 550 nm (green) and 490 nm (blue).

2.4. Sediment sampling and analysis

Analysis of the concentrations of total carbohydrates, proteins, and abundances of heterotrophic prokaryotes in the sediment was done on samples collected at the beginning and at the end of the incubation experiment (Fig. 10A). This choice of time-points was restricted by the fact that sampling required for these analyses is destructive. For this purpose, the overlying water was slowly removed to avoid sediment resuspension, and 4 cm² cores were gently pressed into the sediment at random locations, avoiding areas close to the edge of the mesocosms (Fig. 10C). The top cm of these sediment cores was then sampled using a syringe, homogenized, and divided into three Eppendorf tubes for later analyses. Four cores were sampled in three control mesocosms at the beginning and three control mesocosms at the end of the experiment, whereas eight cores were sampled at the end of the experiment in the dead and live treatments to allow comparison between the outer and inner bed sediment (Fig. 10C).

Total carbohydrates concentrations were measured with the colorimetric method (Taylor and Paterson, 1998). Specifically, 200 µL of the sediment sample was added to 200 µL of 5 % phenol and 1 mL of sulphuric acid. After incubation at 30 °C for 35 min, the absorbance of the supernatant was measured at 488 nm. Calibration curves were prepared using glucose.

Total protein concentrations were determined using the modified Lowry method (Frolund et al., 1996). Specifically, 250 µL of the sediment sample was added to 250 µL of 2 % SDS (Sodium Dodecyl Sulphate) and 700 µL of a mix of chemicals as described by Frolund et al. (1996), and incubated for 15 min at 30 °C. Subsequently, 100 µL of Folin reagent (diluted 5:6 with distilled water) was added to this mixture and incubated for 45 min at 30 °C. Absorbance of the supernatant was measured at 780 nm. Calibration curves were prepared using bovine serum albumine (BSA).

Abundance of heterotrophic prokaryotes was measured by flow cytometry. Specifically, 1.5 ml of sediment was fixed in 4 ml of filtered (0.2 µm) formaldehyde solution (2 % final concentration) and stored at -80°C. Abundances

were determined according to the protocol described by (Lavergne et al., 2014) and expressed in cell/cm³ of wet sediment.

2.5. Statistical analysis

Since MPB biomass was monitored through time, we performed a repeated-measures ANOVA (RM-ANOVA) with the intra-subject factor “Time” and inter-subject factors “Treatment” and “ROI”. Because this test compares mean MPB biomass that increased over the period of 10 days, a significant difference in mean values between given treatments corresponds to a significant difference in growth rate between those treatments. Two models were used to test for different effects of *C. fornicata* on the MPB development. The first model compared MPB biomass in the inner bed sediment through time as a function of the three treatments (i.e., control, dead and live), with the aim to quantify the importance of the physical and biological effects at a local scale. The second model compared the three ROIs (i.e., outer, inner and shells) between the dead and live treatments, with the aim to assess the spatial extent of the biological effect of *C. fornicata*. For each RM-ANOVA, variance-covariance matrix sphericity was verified using Mauchly test. When significant, p-values were re-calculated using the Greenhouse-Geisser correction. When F-tests were significant, *post hoc* comparisons were calculated using contrasts for factor interactions and using Bonferroni-corrected p-values. Concentrations of carbohydrates, proteins and heterotrophic prokaryotes were compared between time, treatments and ROIs. Differences were assessed using the Kruskal-Wallis test based on ranks, followed by a *post hoc* analysis using Bonferroni-corrected Mann-Whitney tests. Statistical analyses were performed in R (version 3.3.0; (R Core Team, 2012) using packages ‘car’, ‘plyr’, ‘phia’ and ‘ggplot’.

3. Results

After 10 days of incubation, the effect of *Crepidula fornicata* on the MPB distribution in the mesocosms was clearly visible (Fig. 11). Patches with highest MPB biomass occurred within the inner bed sediment of live treatments, whereas lowest MPB biomass was found on the shells from the dead treatment. For all treatments, MPB biomass decreased with the distance from the seawater inlets (located at the top of the images in Fig. 11), indicating that more turbulent

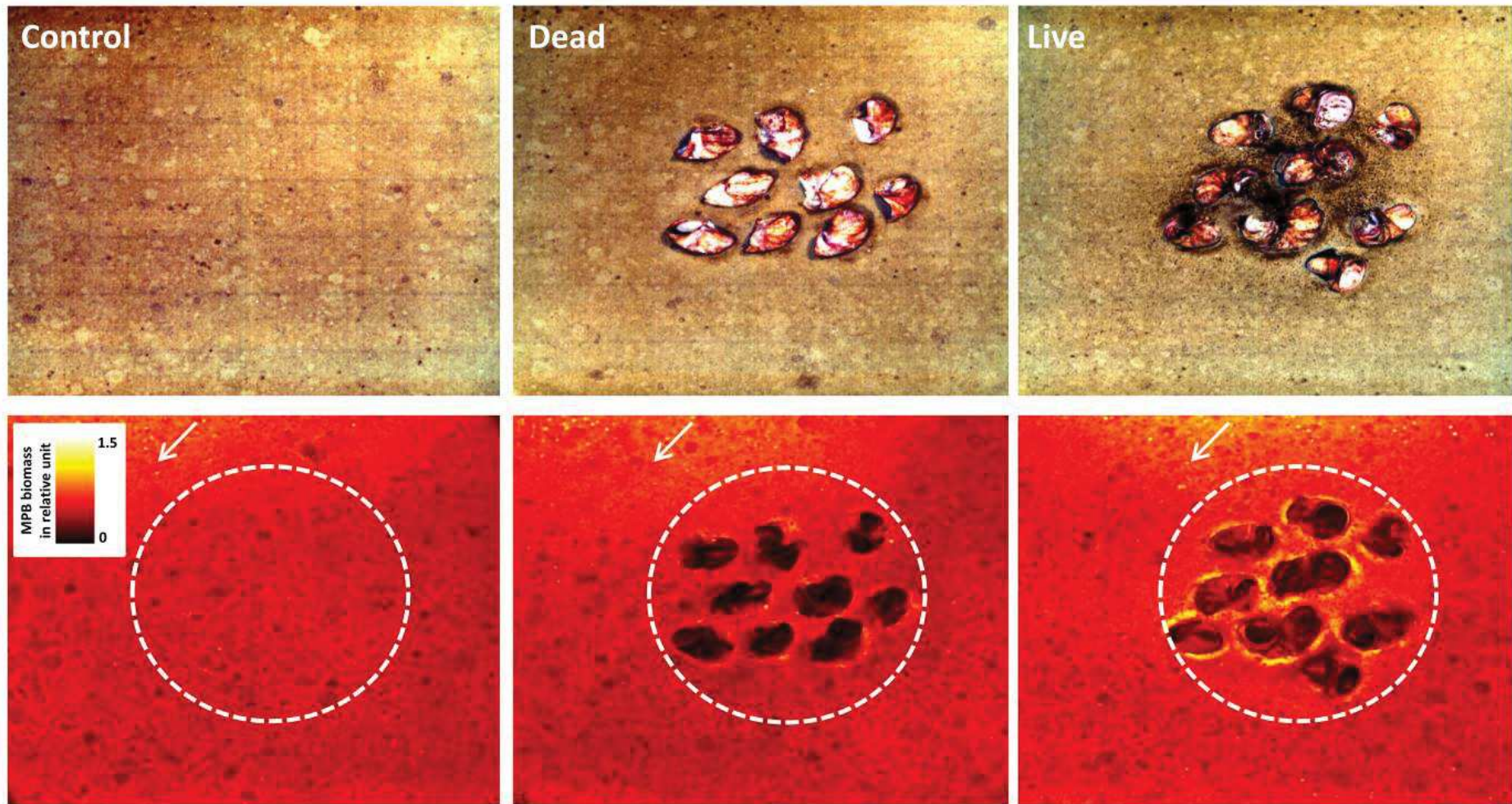


Figure 11: Top view of the sediment surface after 10 days in the experimental mesocosms with the control, dead and live treatments. Examples of true-color images (top row) and the corresponding microphytobenthos (MPB) biomass maps (in relative units; bottom row). White arrows indicate overlying seawater flow direction. Dashed circles represent the inner bed sediment area.

hydrodynamic conditions in the water-column also had a positive effect on MPB growth.

3.1. Physical vs. biological effect in the inner bed sediment

For all treatments, MPB biomass in the inner bed sediment increased approximately linearly over the course of the experiment (Fig. 12). The mean MPB biomass was significantly different between treatments ($p < 0.0001$, Table 1). *Post hoc* multiple comparisons revealed that the MPB biomass was significantly higher (by ~7 %) in the dead treatment than in the control ($p = 0.03$), was significantly higher (by ~12 %) in the live than in the dead treatment ($p < 0.01$), and was significantly higher (by ~19 %) in the live than in the control treatment ($p < 0.0001$). These results imply that both physical and biological effects of *C. fornicata* on MPB growth occurred in our experiment and that the biological effect was stronger than the physical effect. Moreover, these effects appear to be additive, i.e., there is no synergic effect (interaction between physical and biological effects) in the live treatment.

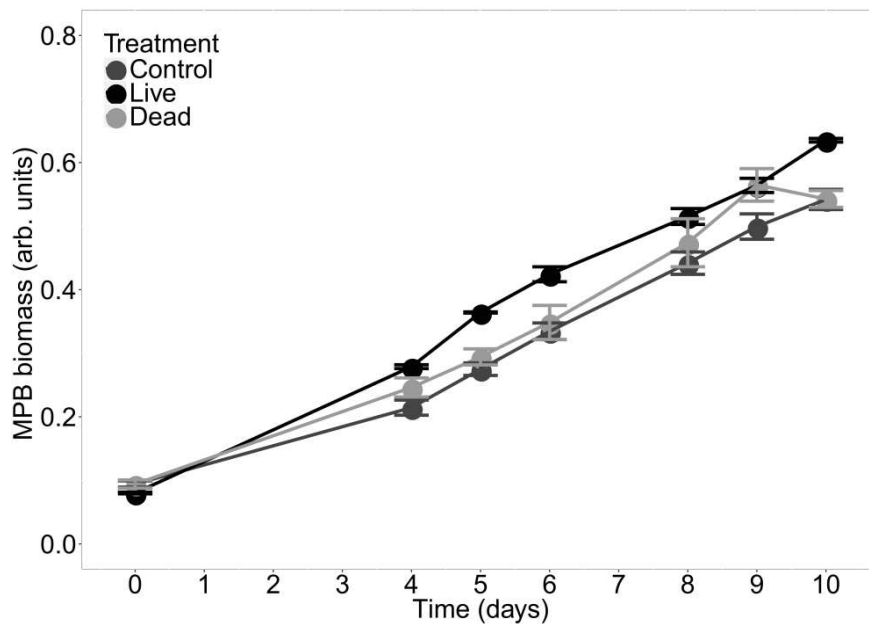


Figure 12: Microphytobenthos (MPB) biomass (mean \pm SE of the three replicates) as a function of time measured at the surface in the inner bed sediment for the three treatments.

Table 1: Results of the RM-ANOVA applied to assess differences in microphytobenthos biomass between the three treatments in the inner bed sediment.

RM-ANOVA					
		num Df	den Df	approx F	p-value
(Intercept)		1	20	104.01	3×10^{-9}
Treatment		2	19	19.81	3×10^{-5}
<i>Post hoc</i> test					
	Value	num Df	den Df	approx F	p-value
Control - Dead	-0.023	1	20	5.64	0.028
Control - Live	-0.066	1	20	40.5	1×10^{-5}
Dead - Live	-0.042	1	20	11.1	7×10^{-3}

3.2. Spatial extent of the biological effect

The high spectral resolution of the hyper-spectral imaging-based pigment analysis was instrumental in discriminating between pseudofeces and MPB biomass. While both categories appeared dark-brown in the true-color images (Fig. 13B), the 2nd and 3rd end-member spectra identified pseudofeces and MPB, respectively. Images of the magnitude of the 2nd end-member showed that pseudofeces were distributed on one side of live stacks (e.g., white arrow ① in Fig. 13D), predominantly in the direction of ex-current flow associated with filtration of *C. fornicata* individuals (which all are oriented in the same way), and that they were clearly absent around the dead *Crepidula* stacks (Fig. 13C). In contrast, MPB biomass was distributed heterogeneously on and around shells, with the highest density close to live shells (e.g., white arrow ② in Fig. 13F).

MPB biomass displayed contrasted responses in the dead and live treatments among the different ROIs (Fig. 14A–B). The model incorporating both effects of treatment and ROI on MPB biomass revealed significant effect of both “Treatment” ($p < 0.0001$) and “ROI” ($p < 0.0001$) factors as well as of the interaction “Treatment:ROI” ($p < 0.0001$) (Fig. 14C, Table 2). *Post hoc* multiple comparisons showed that the MPB biomass was not significantly different on the outer bed sediment between dead and live treatments ($p = 0.41$). However, live treatment showed significantly higher MPB biomass than the dead treatment both for inner bed sediment ($p < 0.01$) and for shells ($p < 0.0001$). Moreover, the relative difference in MPB biomass between the dead and live treatments was higher on shells (by 50 %) than on the inner bed sediment (by 12 %). Together, these results showed that the enhancement of the MPB growth by the biological activity of *C.*

forficata progressively decreased with the distance from the shells: while the effect was significant both on shells and on the inner bed sediment, with the former more pronounced than the latter, the effect disappeared on the outer bed sediment.

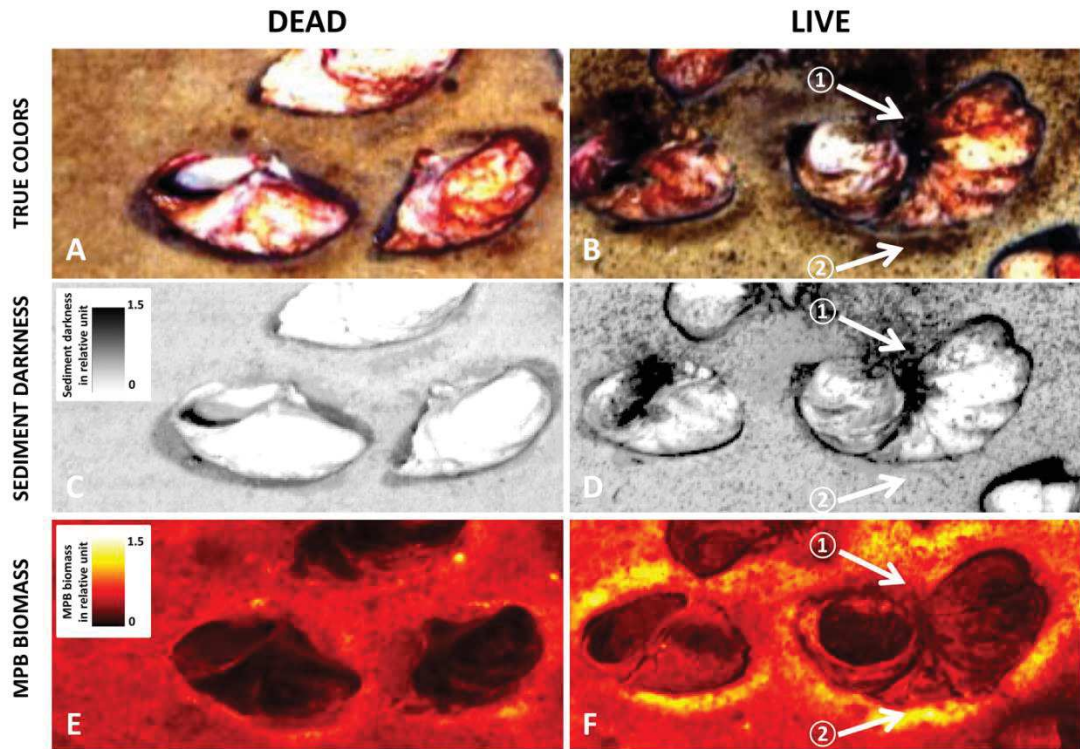


Figure 13: Close-up images of microphytobenthos (MPB) biomass within *Crepidula fornicata* bed in dead and live treatments after 10 days. White arrows indicate regions (1) with freshly deposited pseudofeces and (2) patches of high MPB biomass.

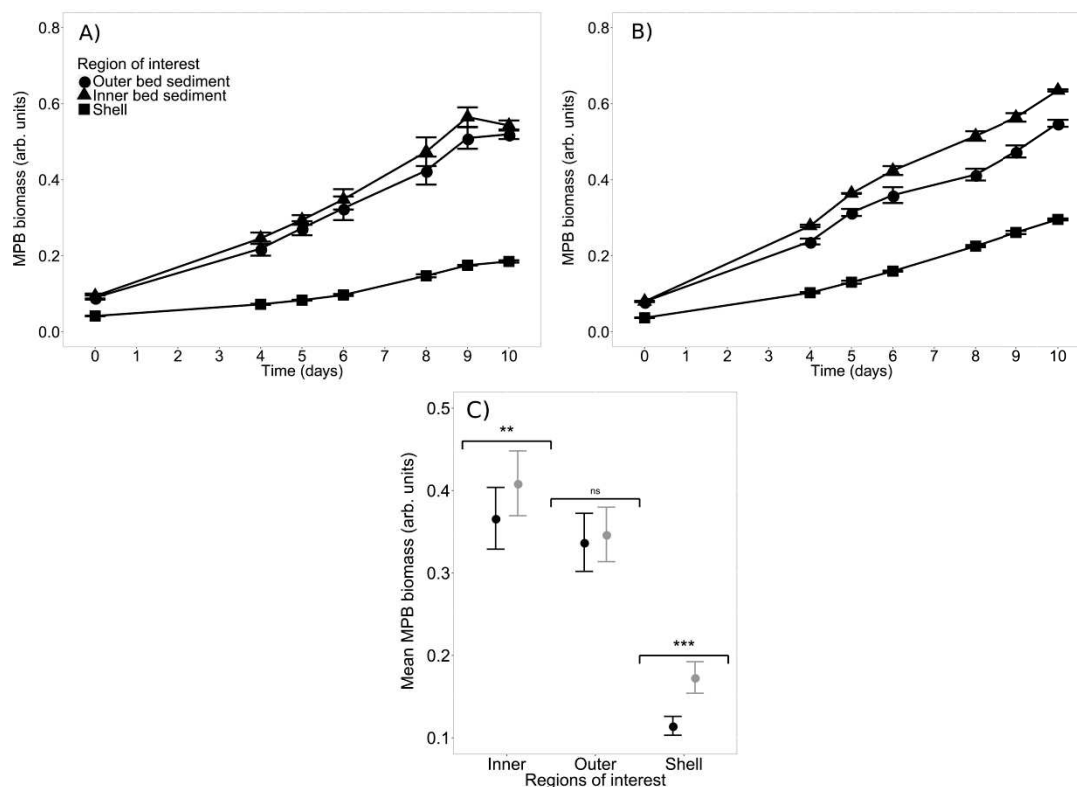


Figure 14: Microphytobenthos (MPB) biomass as a function of time (mean \pm SE, $n = 3$) averaged over the three regions of interest in treatments with dead (A) and live (B) *Crepidula fornicata*, and mean MPB biomass for each combination of treatment and region of interest (C). ns non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2: Results of the RM-ANOVA applied to assess differences in microphytobenthos biomass between two treatments and three regions of interest (ROI).

RM-ANOVA					
		num Df	den Df	approx F	p-value
(Intercept)		1	20	102.5	3×10^{-9}
Treatment		1	20	18.3	4×10^{-4}
ROI		2	19	51.94	8×10^{-10}
Treatment:ROI		2	19	14.97	3×10^{-3}
Post hoc test					
Dead - Live	Value	num Df	den Df	approx F	p-value
Outer	-0.01	1	20	0.72	0.41
Inner	-0.04	1	20	11.09	9×10^{-3}
Shell	-0.06	1	20	51.66	2×10^{-6}

3.3. Evolution of carbohydrates, proteins and heterotrophic prokaryotes

In contrast to the clear differences between treatments and ROIs observed for the MPB biomass at the sediment surface, no significant differences between the beginning and end of the experiment and among the treatments were found for any

of the three additional sediment properties (Fig. 15). Carbohydrates concentrations were not significantly different between conditions ($F = 4.72$, $p = 0.45$). The same conclusion was reached for protein content ($F = 3.93$, $p = 0.56$). Heterotrophic prokaryotes abundances showed significant differences among treatments ($F = 16.76$, $p < 0.01$), however *post hoc* multiple comparisons failed to find any significant differences.

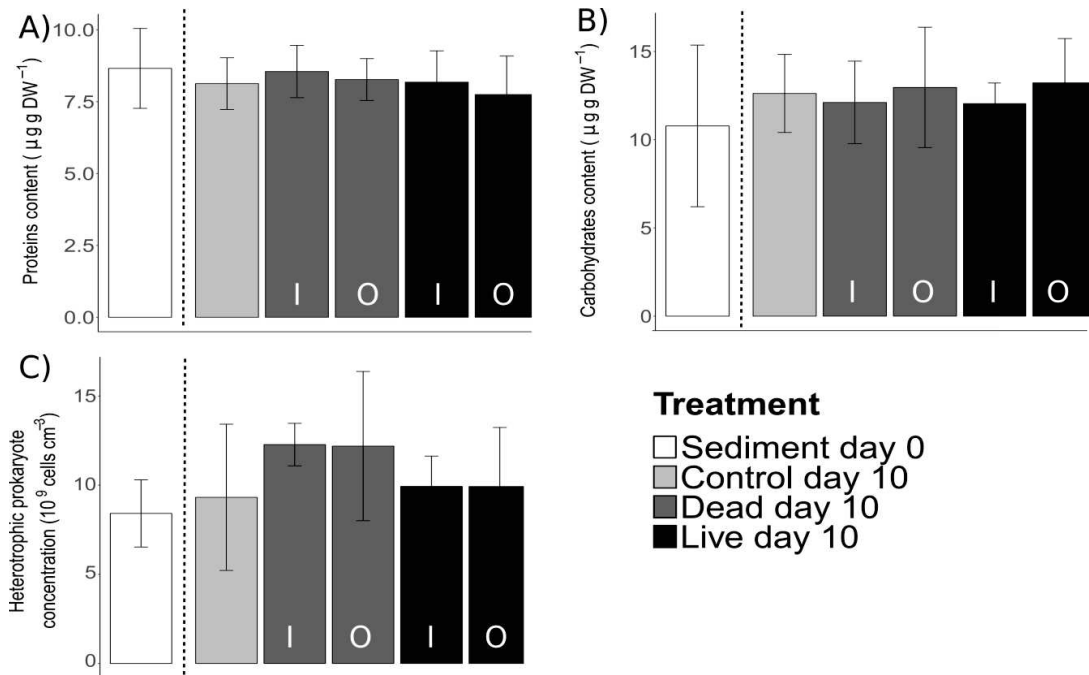


Figure 15: Concentrations (mean \pm SD, $n = 4$) of (A) proteins, (B) carbohydrates and (C) heterotrophic prokaryotes between treatments collected at days 0 and 10 from the inner (I) and outer (O) bed sediment for the dead and live treatments.

4. Discussion

4.1. Effects of *Crepidula fornicata* on subtidal microphytobenthos

Our results showed that at a local scale (i.e., inner bed sediment), and on a time-scale of days, the engineer species *C. fornicata* stimulated subtidal MPB growth, through both physical and biological effects. Moreover, the biological effect was more important than the pure physical one linked to the presence of shells. This result is consistent with the hypothesis that the biological activity of this species promotes MPB growth via enhanced nutrient supply. Thus, it adds to a growing body of evidences showing that ecosystem engineering species have promoting effects on benthic primary production (Chennu et al., 2015; Echappé et al., 2018; Engel et al., 2017). Our data also showed that the enhancement of MPB

growth by the biological activity of *C. fornicata* depends on the distance from the individuals; while MPB growth increased on shells and inner bed sediment, no effect was observed outside the *C. fornicata* bed. This spatial heterogeneity may reflect different processes involved in the enhancement of nutrient supply, as discussed below.

Regarding the physical effect, *C. fornicata* influences hydrodynamics in the overlying water by increasing rugosity of the benthic boundary layer as shell's density increases (Moulin et al., 2007). The resulting sheltering effect against erosion has been suggested to explain enhanced MPB growth conditions in natural mollusk beds (Engel et al., 2017). Although the hydrodynamics was lower in our experiment than in natural conditions, it is possible that this sheltering effect also played a role in our mesocosms. However, another possible explanation is that, similar to what occurs in permeable sediments with ripples (Precht et al., 2004), nutrient efflux at the sediment-water interface around *C. fornicata* shells was increased by a small but significant advective porewater outflow induced by the complex interactions between the overlying water flow and the seabed microtopography, ultimately leading to enhanced MPB growth. This explanation is supported by our observation that, in addition to the inner bed sediment, the enhanced MPB biomass growth was also observed close to the water inlets in our mesocosms, where a locally increased advective efflux of porewater nutrients induced by over-pressurization of porewater relative to the overlying water (due to a locally faster flow) is plausible.

Regarding the biological effect, our data suggest that excretion of dissolved metabolic products is likely the main factor promoting MPB growth in *Crepidula* beds on time-scale of days. Although we have not measured nutrient concentrations directly, this hypothesis is supported by the interpretation of our hyper-spectral data. Specifically, if nutrients supporting the growth of MPB on shells were delivered only via transport from the sediment next to the shells, their concentrations at the shell-water interface would be, due to dilution, lower than at the sediment-water interface. This would imply that, when comparing the life vs. dead treatment, their stimulation of MPB growth on shells should not exceed the stimulation on the sediment. However, the relative difference in MPB growth between our live and dead treatments was larger on shells (50 %) than on the sediment (12 %). This is only possible if the shells of live *C. fornicata* individuals

acted as an additional nutrient source, even stronger than the sediment around the shells. The most likely mechanism behind such a nutrient source is direct excretion of dissolved metabolic products by *C. fornicata*. However, more experiments involving direct nutrient measurements are necessary to further test this hypothesis. Nutrient supply from mollusk metabolism has been shown to be a significant process in primary production fertilization (Asmus and Asmus, 1991; Gardner et al., 1995; Newell et al., 2005). Our data combined with the observation that *C. fornicata* catabolism accounts for 85 % of the benthic community excretion in natural *Crepidula* beds (Martin et al., 2006) suggest that excretion of dissolved metabolic products plays potentially an important role in benthic primary production in these habitats, as previously suggested by (Ní Longphuirt et al., 2007).

However, there are additional mechanisms that might enhance nutrient supply in *Crepidula* beds (Fig. 16). First, *C. fornicata* is a suspension-feeder that significantly contributes to organic enrichment of the sediment through biodeposition (Ehrhold et al., 1998; Manac'h, 1995). Inorganic nitrogen produced by the mineralization of biodeposits can be a significant nutrient source for primary producers (Newell, 2004; van Broekhoven et al., 2015). After *C. fornicata* produced pseudofeces at the beginning of the incubation phase, a slow but stable remineralisation process probably occurred over the course of our experiment.

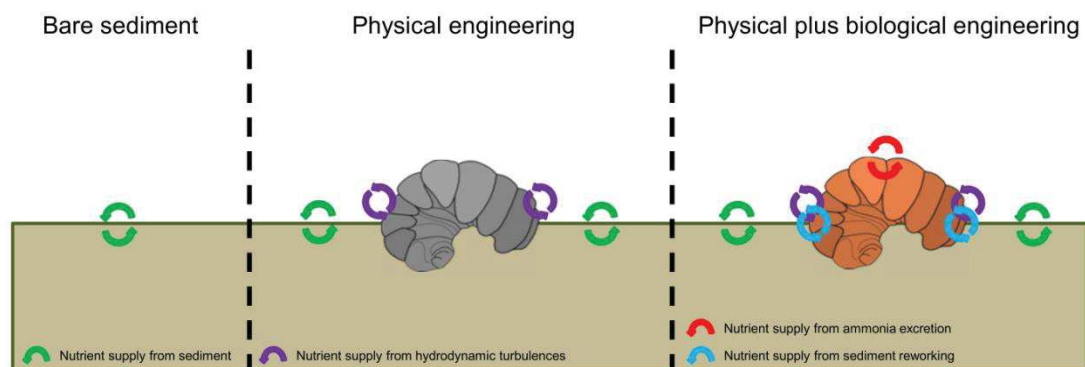


Figure 16: Schematic diagram of the effects of *Crepidula fornicata* on microphytobenthos growth, discriminating between the effects of dead (physical engineering) and live (physical plus biological engineering) animals.

However, the fact that the highest MPB biomass recorded in the vicinity of live shells did not overlap with the area covered by pseudofeces (Fig. 13D and 13F) indicates that pseudofeces were not the main cause of MPB fertilization during our short-term experiment. Because pseudofeces tend to accumulate on the substrate,

this process probably plays a significant role in medium-to-long-term stimulation of MPB development in natural *Crepidula* beds (Laruelle et al., 2009). A second possibility, consistent with the fact that the highest MPB biomass was observed in the close vicinity of the live individuals' shells, is linked to postural changes of *C. fornicata* (Powell-Jennings and Callaway, 2018). As suggested by (Loomis and van Nieuwenhuyze, 1985), movements of *C. fornicata* individuals in direct contact with muddy sediment destabilize the sediment-water interface. This bioturbation can promote MPB growth by increasing nutrient fluxes from underlying pore water. Finally, solubilisation of mucous is also known to promote MPB growth through the supply of dissolved nutrients, as shown for mollusks (Cognie and Barillé, 1999). *C. fornicata* secretes high amounts of mucous during its filter-feeding activity (Shumway et al., 2014), which can also contribute to promote MPB development.

In our experiment the clear increase in the MPB biomass was not accompanied by the corresponding increase in the other biofilm components measured (i.e., proteins, carbohydrates and heterotrophic prokaryotes). This was an unexpected result given that previous studies did observe coupling between these parameters and the MPB growth (Hubas et al., 2010; Orvain et al., 2003; Underwood and Paterson, 2003). The absence of changes in these biofilm parameters could indicate that during our experiment, the fertilization impact of *C. fornicata* was restricted to the very surface of the sediment, and that we did not detect these changes due to an insufficient sampling resolution. Indeed, carbohydrates, proteins and heterotrophic prokaryotes were measured in the top cm, while MPB biomass quantification by the hyper-spectral imaging method is limited to the depth of light penetration (Chennu et al., 2013), which is about 0.3 mm in marine muddy sediments (Kühl et al., 1994). Thus, even if significant changes in carbohydrates, proteins and heterotrophic prokaryotes did occur within the MPB biofilms, they were likely minor compared to their standing stocks in the top cm of the sediment, and thus not detected. Another possibility is that the conditions employed in our experiment were not optimal to maintain a clear link between the MPB biomass and the concentrations of the other parameters. For instance, exopolysaccharides (EPS) produced by MPB (mixture of proteins and carbohydrates) could have been removed by the overlying water through solubilisation and/or hydrolysis (Orvain et al., 2003; Underwood and Paterson, 2003). Additionally, their production is driven by photosynthesis and thus strongly

depends on irradiance and temperature (Perkins et al., 2001; Smith and Underwood, 2001). The rather low temperature and irradiance, combined with the continuous water flow in the mesocosms, likely limited EPS accumulation, and thus its colonization by heterotrophic prokaryotes, in the MPB biofilms in our experiment.

4.2. Limitations

In our study we did not calibrate our hyper-spectral data against the concentrations of MPB in the sediment, but relied on previous studies that showed that hyper-spectral measurements provide accurate measures of MPB biomass (Barillé et al., 2007; Carrère et al., 2004; Combe et al., 2005; Chennu et al., 2013; Jesus et al., 2014; 2008). Nevertheless, to indicate the range of the actual MPB biomass in our mesocosms, we measured chlorophyll *a* concentrations by HPLC for a limited number of sediment samples (1 cm deep sediment cores). For the study site, the *in situ* chlorophyll *a* concentrations were 17–24 $\mu\text{g (g DW)}^{-1}$ ($n = 2$), which is similar to the value reported previously for a subtidal muddy sediment in the bay of Brest in winter (18 $\mu\text{g (g DW)}^{-1}$; Riaux-Gobin et al., 1987). With respect to the experiment, chlorophyll *a* concentrations in the mesocosms increased from 26–29 $\mu\text{g (g DW)}^{-1}$ ($n = 4$) to 60–136 $\mu\text{g (g DW)}^{-1}$ ($n = 10$) between days 0 and 10, which is an increase by a factor of 2–5 (3.5 on average). Taking into account the substantially larger sampling depth of this HPLC-based pigment analysis (1 cm), this increase was consistent with the relative increase in the MPB biomass derived from our hyper-spectral measurements (by a factor of 5–6; Fig. 12).

While our experimental design was able to detect significant effects of *C. fornicata* on the MPB growth rate, its duration was not adequate for quantifying differences in the biotic capacity of the studied system (Orvain et al., 2003). Indeed, at the end of the experiment, although MPB growth appeared to slow down in the dead treatment, MPB was still growing at roughly a constant rate in the live treatment, irrespective of the ROI (Fig. 14A–B). The duration of our experiment was designed according to previous experiments investigating similar ecological processes (Agogu e et al., 2014; Chennu et al., 2015; Jauffrais et al., 2015; Orvain et al., 2003). Based on these reports we expected the MPB to follow a logistic growth model and reach a plateau within ~10 days (Chennu et al., 2015; Jauffrais et al., 2015; Orvain et al., 2003). However, the abiotic conditions chosen for our experiment (e.g., winter temperature and low light) were probably too harsh for the

MPB to approach the biotic capacity of the substrate in such a short time. We suggest that future experiments studying short-term effects of altered nutrient supply on MPB growth should employ slightly longer duration of the experimental treatments (e.g., 2–3 weeks) to allow a more complete understanding of the growth dynamics.

5. Conclusions

Overall, we provided evidence that *Crepidula fornicata* enhances the growth of microphytobenthic biofilms. Our experiment disentangled two potential effects of this engineer species (physical vs. biological), and revealed that live *Crepidula* beds supported faster MPB growth than dead ones. Dissolved metabolic products excreted by *C. fornicata* were likely the main factor stimulating MPB growth in our short term experiment. While fertilization of MPB by engineer species such as mussels (Engel et al., 2017), oysters (Echappé et al., 2018) or lugworms (Chennu et al., 2015) has been documented for intertidal sediments, our study is the first to quantify this phenomenon in a subtidal habitat.

Considering the large geographical extent of this invasive species in shallow European waters (Blanchard, 2009), the potential contribution of *C. fornicata* to subtidal benthic primary production and its fate in higher trophic levels need to be further investigated. Despite emerging evidence of its trophic significance (Evrard et al., 2012; Grall et al., 2006; Rigolet et al., 2014), subtidal MPB remains difficult to sample and hence is usually disregarded as a potential source of labile organic matter in benthic food-web studies. The trophic role of subtidal MPB is likely to be significant in *C. fornicata* habitats, where the biomass of primary consumers (i.e., deposit- and suspension-feeders) is high (de Montaudoin et al., 1999; Ní Longphuirt et al., 2007). Because it has no predators in its introduction area, *C. fornicata* is considered to be a trophic dead-end (Arbach Leloup et al., 2008). Nevertheless, the intense filtering activity and the low assimilation efficiency of this species (Shumway et al., 2014) suggest that *C. fornicata* may redirect a large amount of energy and material from the water column to the benthic compartment not only through biodeposition (Chauvaud et al., 2000), but also through stimulation of benthic primary production.

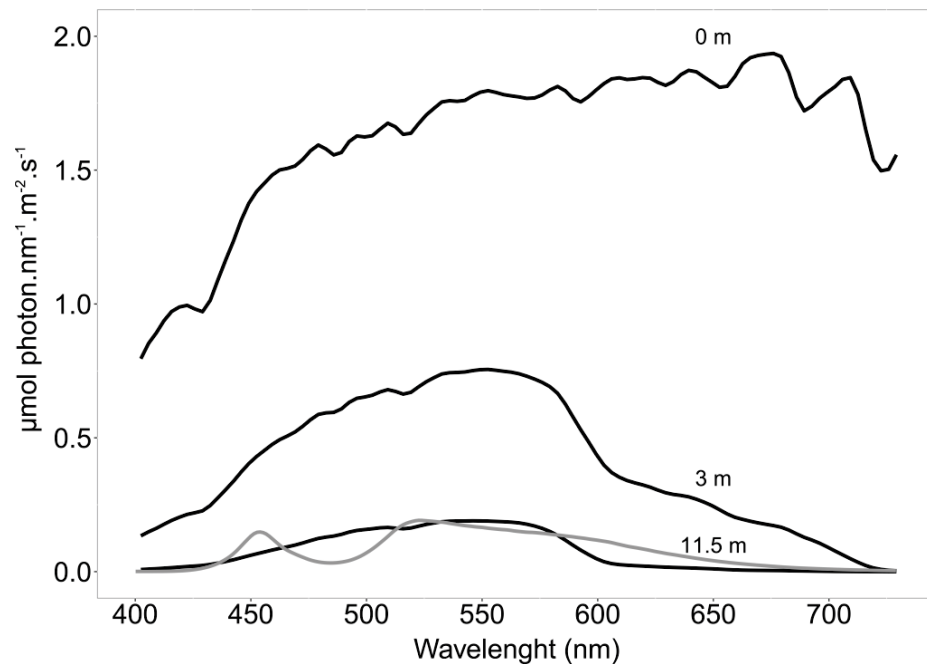
Despite invasive species being generally considered as a threat for ecosystems, their engineering effects have the potential to enhance ecosystem

functioning and compensate for the loss of native habitats (Ramus et al., 2017; Romić and Nakajima, 2017). In the case of *C. fornicata*, both its physical (e.g., habitat-forming heterogeneity and niche provisioning) and biological (e.g., stimulation of MPB growth) engineering effects could offset the negative ecological impacts usually attributed to this species.

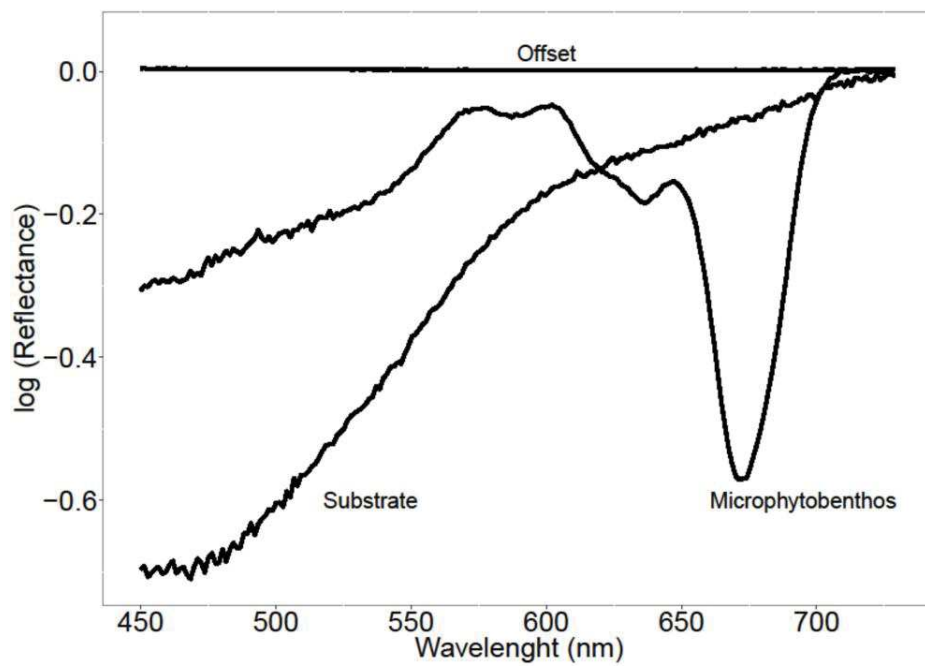
6. Acknowledgements

We thank JM. Pédrón for providing access to aquaculture facility, C. Cordier and X. Caisey for the technical support during field sampling and experiment, D. Morigeon and F. Quéré for their kind assistance on board of the R/V Albert Lucas, G. Delebecq for providing *in situ* light spectra, and A. Jones and B. Taormina for helpful discussions. Prokaryotes analyses were performed using the cytometry and imaging platform of the LIENSs laboratory in La Rochelle, and we thank M. Bréret and L. Beaugard for the technical assistance. This study is part of the TRECH project funded by the Total Foundation for the Biodiversity and the Sea. We acknowledge IFREMER, LabexMER and Region Bretagne for co-funding T. Androuin's PhD and L. Polerecky's collaboration visits.

7. Supplementary materials



Supplementary Figure 17: *In situ* light spectra recorded at 0, 3 and 11.5 meter depth in the bay of Brest. The artificial light spectrum provided during the experiment is shown as a grey line.



Supplementary Figure 18: End-member spectra used to quantify microphytobenthos biomass from the log-transformed spectral reflectance data obtained from the experimental mesocosm using hyper-spectral imaging.

CHAPITRE II : Écologie
trophique de *Crepidula fornicata*

Méthode 2 : Isotopes stables

Les isotopes stables d'un élément chimique sont naturellement présents dans l'environnement, et diffèrent par leur nombre de neutrons. Par exemple, l'atome de carbone possède deux isotopes stables, le carbone 12 et le carbone 13, comprenant respectivement 12 et 13 neutrons. L'assimilation du carbone par les organismes se fait via le carbone inorganique dissout de l'atmosphère ou du milieu marin pour les producteurs primaires et via l'ingestion de matière organique pour les consommateurs. Le carbone lourd étant plus difficilement mobilisable lors des réactions biochimiques, il a tendance à être moins utilisé et donc à s'accumuler dans l'organisme (voir Fry, 2006 pour une synthèse sur l'utilisation des isotopes stables en écologie). Ceci aura pour effet d'augmenter le rapport $^{13}\text{C}/^{12}\text{C}$ dans les tissus d'un consommateur. Ce rapport sera ensuite corrigé par un standard naturel et international, il est noté $\delta^{13}\text{C}$ et est exprimé en ‰.

“We are what we eat, plus a few per mil” (DeNiro and Epstein, 1978)

Leur utilisation en écologie trophique repose donc sur le fait qu'un consommateur incorpore la signature de sa source de nourriture, en s'enrichissant en isotopes lourds de façon prévisible. Cette différence entre une source et un tissu est appelé fractionnement isotopique. De nombreux ratios isotopiques sont utilisés en écologie pour répondre à diverses problématiques telles que la nutrition, la migration, etc. Par exemple, le $\delta^{13}\text{C}$ est utilisé pour caractériser différents producteurs primaires (phytoplancton, macroalgues, herbiers, microphytobenthos), et leur assimilation par des consommateurs. En effet, l'accès à leur source de carbone inorganique ainsi que l'enzyme à l'origine de la fixation du CO_2 va conditionner leur $\delta^{13}\text{C}$. Le second ratio isotopique souvent utilisé est le $\delta^{15}\text{N}$, qui mesure le ratio entre l'azote 15 et l'azote 14. Du fait d'un fractionnement plus important entre une source et son consommateur, cet isotope est utilisé comme indicateur du niveau trophique d'un organisme calculé à partir de la source à la base de la chaîne trophique (Figure 19).

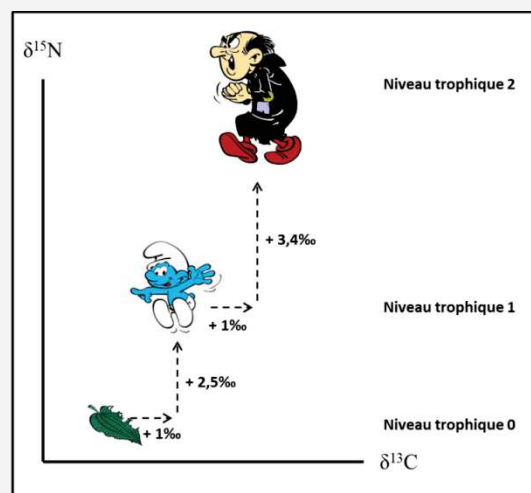


Figure 19 : Diagramme isotopique conceptuel illustrant l'enrichissement attendu entre un producteur primaire (Salsepareille), un consommateur primaire (Schtroumpf) et un consommateur secondaire (Gargamel). Les valeurs de fractionnement sont tirées de McCutchan et al., 2003

Article 2 : The dark side of soft tissue: unexpected carbonates in the invasive slipper limpet *Crepidula fornicata* and its implications for stable isotope interpretations

Thibault Androuin ¹, Stanislas F. Dubois ¹, Priscilla Decottignies ², Ewan Pelleter ³, Antoine Carlier ¹.

¹ DYNECO-LEBCO, IFREMER, Plouzané, France

² Lab. Mer Molécules Santé EA 2160, University of Nantes, Nantes, France

³ REM-UGM-LGM, IFREMER, Plouzané, France

Abstract

Stable isotopic analysis is extensively used in trophic ecology. Inorganic carbonates, usually originating from shell fragments, are routinely removed from samples using an acid treatment because they affect $\delta^{13}\text{C}$ values. However, acid treatment can also change $\delta^{15}\text{N}$. For some taxa such as molluscs, the general assumption is that acid pre-treatment is not necessary since their shell is easily dissected from soft tissues and represents the only source of inorganic carbonates. However, other sources of non-dietary carbon (e.g., intracellular inorganic carbon) enriched in ^{13}C thus get overlooked. Soft tissues (foot) of the invasive gastropod *Crepidula fornicata* of different size classes were analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with and without acid pre-treatment using isotope ratio mass spectrometry. *In toto* microscopic investigations coupled with acid treatment, scanning electron microscopy and energy dispersive spectroscopy were used to highlight the presence of inorganic carbonate. A correction model was derived and applied to existing stable isotope data for *C. fornicata*. We used both seasonal variations in $\delta^{13}\text{C}$ signatures and mixing model outputs to assess the error $\delta^{13}\text{C}$ values. Acid pre-treatment had a significant effect on stable isotope compositions of *C. fornicata* foot tissue, especially on $\delta^{13}\text{C}$ values: isotopic differences increased with size, up to 3 ‰ for large females. No effect was detected for small (below ~20 mm) and motile males. *In toto* microscopic analysis revealed the presence of small spherules of inorganic carbonate, hence explaining the differences in $\delta^{13}\text{C}$ values. Mixing model outputs and seasonal variation of $\delta^{13}\text{C}$ values showed that untreated samples can lead to large misinterpretations about diet proportions and degree of trophic niche overlap, respectively. Spherules of inorganic carbonate in *C. fornicata* soft tissues are likely to be linked with motility of this species and their mucus production. We recommend assessing the presence of inorganic carbonate in soft tissue of sessile gastropods.

Keywords: Stable isotope, sample preparation, *Crepidula fornicata*, inorganic carbonate, mixing model

1. Introduction

Stable isotope (SI) analysis has been increasingly used in ecology for several decades (Fry and Sherr, 1984; Herman et al., 2000), especially to infer organic matter pathways in ecosystems, trophic positions of species, or animal movement (Fry, 2006). Among the isotopes used in this way, $\delta^{13}\text{C}$ is one of the most frequently assessed isotopic ratios in marine trophic ecology. Indeed, the small fractionation (~ 1 ‰) between a consumer and its diet makes it possible to trace the origin and fate of organic carbon (Boecklen et al., 2011; DeNiro and Epstein, 1978; Fry, 2006). SI analysis has been popularized worldwide by an increasing number of stable isotope facilities and a steadily falling analytical cost. As a result, sample preparation is now done routinely and may suffer from a lack of care and attention. Two major well-documented biases can occur when preparing samples for SI analysis: a high storage lipid content (Post et al., 2007) and the presence of non-dietary carbon, such as inorganic carbonates (Schlacher and Connolly, 2014). In the animal kingdom, some hard structures are composed of inorganic carbonates, which are isotopically ‘heavier’ (i.e., enriched in ^{13}C) relative to dietary organic carbon. To cope with potential carbonate contamination, scientists usually use an acid treatment prior to SI analysis (Guerin et al., 2013; Jacob et al., 2005; Jaschinski et al., 2008; Kennedy et al., 2005; Lorrain et al., 2003; Mateo et al., 2008).

For some taxa such as molluscs, ecologists take the easy approach of carefully removing external/internal shells and other hard structures (operculum, calcified radula). In many cases, the muscular part of the body (i.e., the foot and adductor muscles) is thus dissected out to avoid using acid treatment. Supposedly, dissection saves time over acid treatment, which poses a potential risk of affecting isotopic ratios (Guerin et al., 2013; Mateo et al., 2008). Choosing this common method makes the assumption that soft tissues do not contain inorganic carbonates. However, inorganic carbonates in the form of calcium spherules have been shown to be present in different organs, e.g., in the foot muscle, arteries and digestive glands of terrestrial gastropods (Burton, 1972; Little, 1965; Tompa and Watabe, 1977), also in gills, mantle and digestive glands of freshwater bivalves (Istin and Girard, 1970; Pynnönen et al., 1987). To our knowledge, only five species of marine gastropods have been studied for their calcium spherules (Mason and Nott, 1981; Nott and Nicolaidou, 1989).

Among marine gastropods, the slipper limpet *Crepidula fornicata* is a model species in many scientific fields (Henry and Lyons, 2016). This gregarious animal lives in stacks of several individuals and forms characteristic subtidal banks (Barnes et al., 1973). *C. fornicata* is a hermaphroditic species that begins its benthic life as a male with low motility and then becomes a sessile female. Because of its introduction to many parts of the world and potential cascading effect on food web functioning, numerous studies have used SI analysis to investigate its diet and to infer inter-specific trophic overlap. These include its potential trophic competition with native suspension feeders such as the scallop *Pecten maximus* (Richard, 2005), or cultivated species such as the blue mussel *Mytilus edulis* or Pacific oyster *Magallena gigas* (formerly *Crassostrea gigas*) (Decottignies et al., 2007ab; Lefebvre et al., 2009; Riera et al., 2002). Recent personal observations revealed unexpected and repeated bubbling occurred from *C. fornicata* foot dissected and dropped into a 1N hydrochloric acid solution. This gas emission strongly suggests overlooked inorganic carbonates in the soft tissues of *C. fornicata*.

Here, we investigated the effect of acid pre-treatment on carbon and nitrogen SI compositions in soft tissue (foot) of *C. fornicata*. We looked for inorganic carbonates in the foot using *in toto* microscopic investigation. The relationship between the $\delta^{13}\text{C}$ deviation and individual size was quantified and a correction model proposed to correct for potential bias. We also assessed potential ecological pitfalls and misinterpretations between corrected and uncorrected data using both mixing model outputs and seasonal variations in ^{13}C signatures.

2. Materials and methods

2.1. Sample collection and preparation for stable isotope analysis

Forty-one *Crepidula fornicata* individuals ranging from 10.4 to 39.8 mm in total length were collected in the bay of Brest, France, in June 2015 (48° 23.36068' N, 004° 23.86901' W). For each individual, the soft tissues were carefully removed from the shell to avoid breaking small pieces of the septa (thin calcareous part of the shell). The foot was then dissected out and cut into two equal parts, one was rinsed with MilliQ (Merck Millipore®, France) water and the second was sliced into small pieces, placed in a beaker containing 1N HCl until the bubbling stopped,

and then rinsed with MilliQ water. All the samples were freeze-dried and ground into homogenous powder using a ball mill (Retsch MM400, France).

2.2. Stable isotope analysis

Samples of approximately 400 µg of powder per animal were weighed in tin capsules. SI analysis was performed on a Thermo Scientific Flash EA 2000 elemental analyser (Thermo Electron, Bremen, Germany) coupled to a Delta V Plus mass spectrometer in continuous flow (Thermo Electron, Bremen, Germany) at the *Pôle de Spectrométrie Océan* (PSO, Plouzané, France). Organic carbon was expressed as percentage of the total organic matter, and stable isotope ratios in standard δ notation based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$) according to the equation:

$$\delta = (R_{\text{sample}}/R_{\text{standard}}) - 1 \text{ (in ‰)}$$

where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. International isotopic standards of known $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were used: IAEA-600 caffeine, IAEA-CH-6 sucrose, and IAEA-N-1 and IAEA-N-2 ammonium sulphate. The experimental precision was estimated using the standard deviation of an internal standard (Thermo Acetanilide, n = 8), which indicated an analytical precision of ± 0.11 ‰ and ± 0.07 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively.

2.3. *In toto* microscopic investigation

For microscopic investigations, we sampled small transverse sections (~1 mm) of foot tissue from thirty adult *C. fornicata* individuals (mean length = 35 ± 5 mm). After observations under an optical microscope to find structures (i.e., spherules), we added one drop of 1N HCl between the slide and the plate. The presence of inorganic carbon was verified by observing dissolutions of the spherules and the accompanying gas emission. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) analyses were also performed to investigate the foot tissue of adult *C. fornicata*. Whereas SEM provides information about the surface topography of a sample, EDS analysis gives a qualitative elemental analysis. Small pieces of dried samples (50°C for 48 h) were mounted on pin stubs and coated with carbon or gold. SEM and EDS secondary electron images were then obtained with a FEI Quanta 200 coupled with an OXFORD X-MAXN

Silicon Drift Detector (detector size: 80 mm²) at the *Laboratoire de Géochimie et Métallogénie* of IFREMER (Plouzané, France).

2.4. Data analysis

Since the treated and non-treated samples were non-independent, and the values of pair-differences normally distributed (Shapiro-Wilk, p -value > 0.2), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value differences between acid-treated and non-treated samples were tested using paired t-tests. The relationships between isotopic deviations due to acid treatment with individual size were investigated using both linear and exponential models. The best fit was chosen based on the coefficient of determination. Differences in paired $\delta^{13}\text{C}$ values (i.e., $\delta^{13}\text{C}$ of treated and untreated halves of a given sample) were plotted on individual size. The relationship thus obtained was used as a correction model. Correction consists in replacing the unknown parameter “x” in the equation with the size of the individual. This correction factor y should be subtracted from any ^{13}C value obtained from non-acidified samples. Percentage of organic carbon was compared between treatments and categories of *C. fornicata* (i.e., motile males, sessile males and sessile females) using a mixed-effect model to take into account repeated measurement in the two-factor analysis of variance. This model allows the incorporation of a random effect term in a linear model, which produces a correlation structure between measurements from the same individual (Mangiafico, 2015; Zuur et al., 2009). Normality and homogeneity of residual variance were checked visually and *post hoc* comparisons were made using Tukey tests.

An isotopic mixing model allows the calculation of contributions of potential food sources to the diet of a species (Phillips et al., 2014). In order to assess the influence of the carbonated spherules (which is neutralized by acid treatment) in the computation of dietary proportions of *C. fornicata* individuals of varying sizes, a mixing model was run using $\delta^{15}\text{N}$ values of untreated consumer samples and $\delta^{13}\text{C}$ values of either treated or untreated consumer samples. We considered three potential food sources (marine suspended particulate organic matter, benthic diatoms and an association of macroalgae/C4 plants) and used realistic food source isotopic ratios and trophic discrimination factors previously published by Decottignies et al. (Decottignies et al., 2007a) (Supplementary Table 3). Although these authors considered a fourth potential food source (marine

angiosperms *Halimione* sp. and *Salicornia* sp.), we did not include this in our mixing model due to its very low contribution to the diet of filter feeders in their case study (Decottignies et al., 2007b). The dual element ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), three-source, mass-balance, linear mixing model can be described by the following equations:

$$\delta^{13}\text{C}_m = f_1 \delta^{13}\text{C}_1 + f_2 \delta^{13}\text{C}_2 + f_3 \delta^{13}\text{C}_3$$

$$\delta^{15}\text{N}_m = f_1 \delta^{15}\text{N}_1 + f_2 \delta^{15}\text{N}_2 + f_3 \delta^{15}\text{N}_3$$

$$1 = f_1 + f_2 + f_3,$$

where f_1 , f_2 and f_3 are proportions of the three potential food sources. Because mixing models use a Bayesian approach that integrates uncertainty such as diet-tissue discrimination factors (Phillips et al., 2014), classic statistical tests to assess mean or distribution differences are not relevant (White et al., 2014). We used the interquartile range (IQR), which is a measure of statistical dispersion (Upton and Cook, 1996). The difference between the third and the first quartile is defined as an index of overlap between dietary proportions. In this study, we considered that two distributions of dietary proportions were different if the two corresponding IQR were non-overlapping.

We extracted $\delta^{13}\text{C}$ values from (Decottignies et al., 2007a) to infer interspecific relationships between *C. fornicata* and *Magallena gigas*. The trophic niche overlap between species was investigated using seasonal $\delta^{13}\text{C}$ values for both species. Differences between species were analysed using non-parametric Mann-Whitney tests since data were not normally distributed. We focused on two specific seasons (winter-early summer and late summer) during which changes in trophic relationships are known to occur. All statistical analysis were computed with the free software R (R Core Team, 2012) using the *ggplot2* (Wickham, 2016), *nlme* (Pinheiro and Bates, 2010), *rcompanion* (Mangiafico, 2015) and *simmr* packages (Parnell et al., 2013).

3. Results

3.1. Effect of acid treatment on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

The $\delta^{15}\text{N}$ values of foot tissue increased linearly with individual size for both untreated and acid-treated samples, with a steeper slope for acid-treated samples (Fig. 20A). The $\delta^{13}\text{C}$ values increased exponentially with individual size,

with a higher slope for untreated samples (Fig. 20B). Differences between untreated and acid-treated samples were significant for both nitrogen and carbon isotope ratios (paired t-test; $T = 4.63$, $p\text{-value} = 0.0001$ and $T = 3.87$, $p\text{-value} = 0.0009$, respectively). To formalize isotopic deviation in untreated samples, a correction model was computed from the $\delta^{13}\text{C}$ values using a three-order polynomial regression (Fig. 20C).

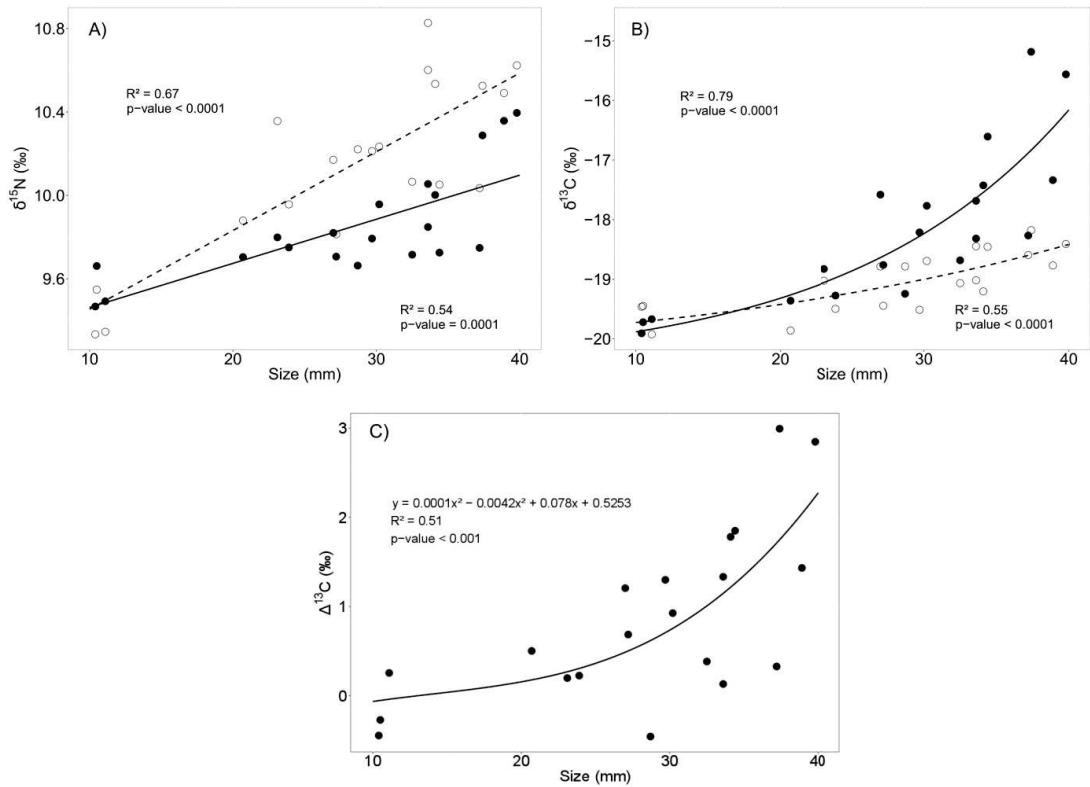


Figure 20: Relationships between (A) nitrogen or (B) carbon stable isotope ratios (‰) measured in the foot of *Crepidula fornicata* and individual size for both untreated (filled circles) and acid-treated (open circles) samples. (C) Correction model showing the differences of $\delta^{13}\text{C}$ value between acid-treated and untreated samples, according to individual size.

The organic carbon content of the foot tissue was dependent on both treatment and *C. fornicata* development stage (significant interaction between treatment \times stage: $F = 9.22$, $p\text{-value} < 0.001$, Fig. 21). Significantly lower organic carbon content was observed for both untreated sessile males and females (Fig. 21).

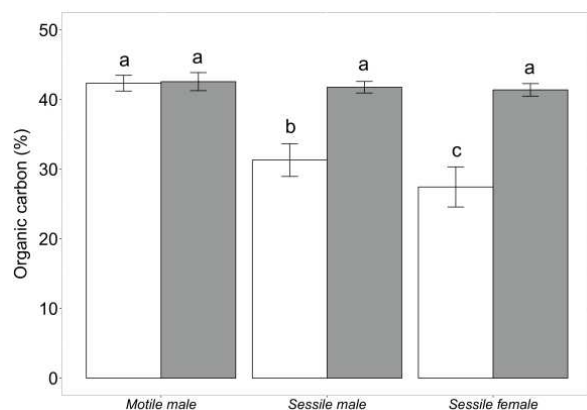


Figure 21: Organic carbon content (%) in foot of *Crepidula fornicata* in untreated (white) and acid-treated (grey) samples. Significantly different groups are shown with different letters.

3.2 *In toto* microscopic investigation

Numerous spherules of ~30 μm size consisting of granular material were observed in the foot tissue of all examined individuals ($n = 30$) (Fig. 22A). The addition of one drop of 1N HCl to the preparation triggered gas bubbles and the dissolution of the spherules (Fig. 22B, C). After less than 3 minutes, all the spherules had dissolved (Fig. 22D).

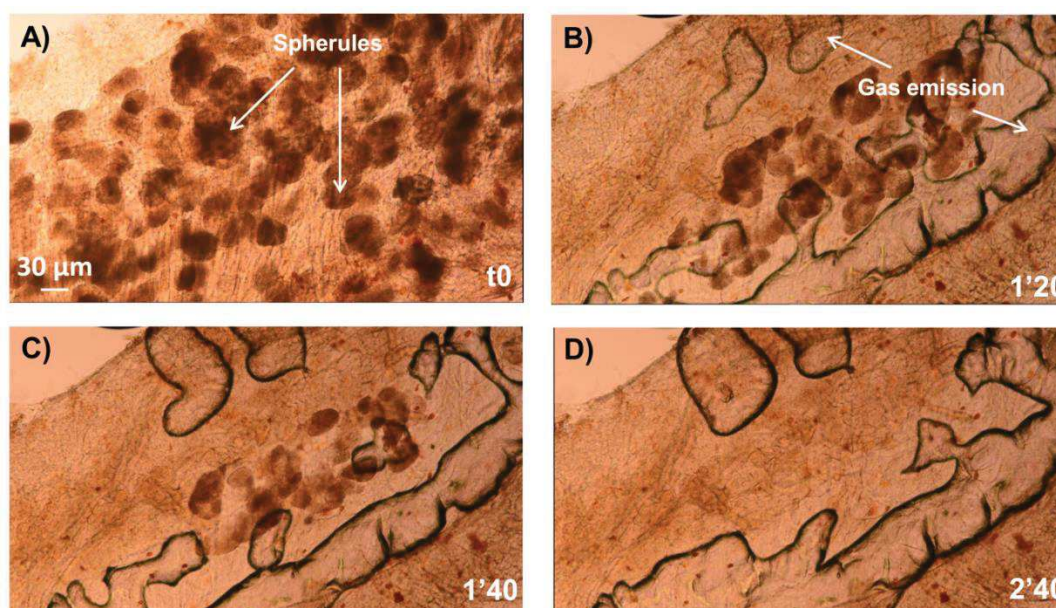


Figure 22: Example of an *in toto* histological cut of the slipper limpet *Crepidula fornicata* foot (A) before acidification (t0) and (B) 1'20, (C) 1'40, (D) and 2'40 after 1 M HCl acidification. Note the production of gas accompanied by the dissolution of calcareous spherules. All images are at the same scale.

We also performed the 'limewater' test to check for CO_2 in the emitted gas. Fresh tissue of *C. fornicata* (foot) was crushed and put in an Erlenmeyer connected by a pipe to another filled with distilled water saturated in lime. 20 ml of 1N hydrochloric acid was added to the tissue and this Erlenmeyer then hermetically

closed. After 1 minute, bubbles came through the pipe into the limewater, which became cloudy thus indicating the presence of CO₂. Further observations revealed the presence of similar spherules in the entire animal, but less abundant than in the foot tissue. Spherules were clearly visible under the surface of the skin of dehydrated foot tissue (Fig. 23A-B). Close-up images of a broken part of this tissue sample highlighted the internal composition of the spherules. These consist of a multitude of tiny balls of about 1 µm diameter (Fig. 23C). EDS qualitative analysis indicated that these spherules are composed of carbon, oxygen, calcium and magnesium.

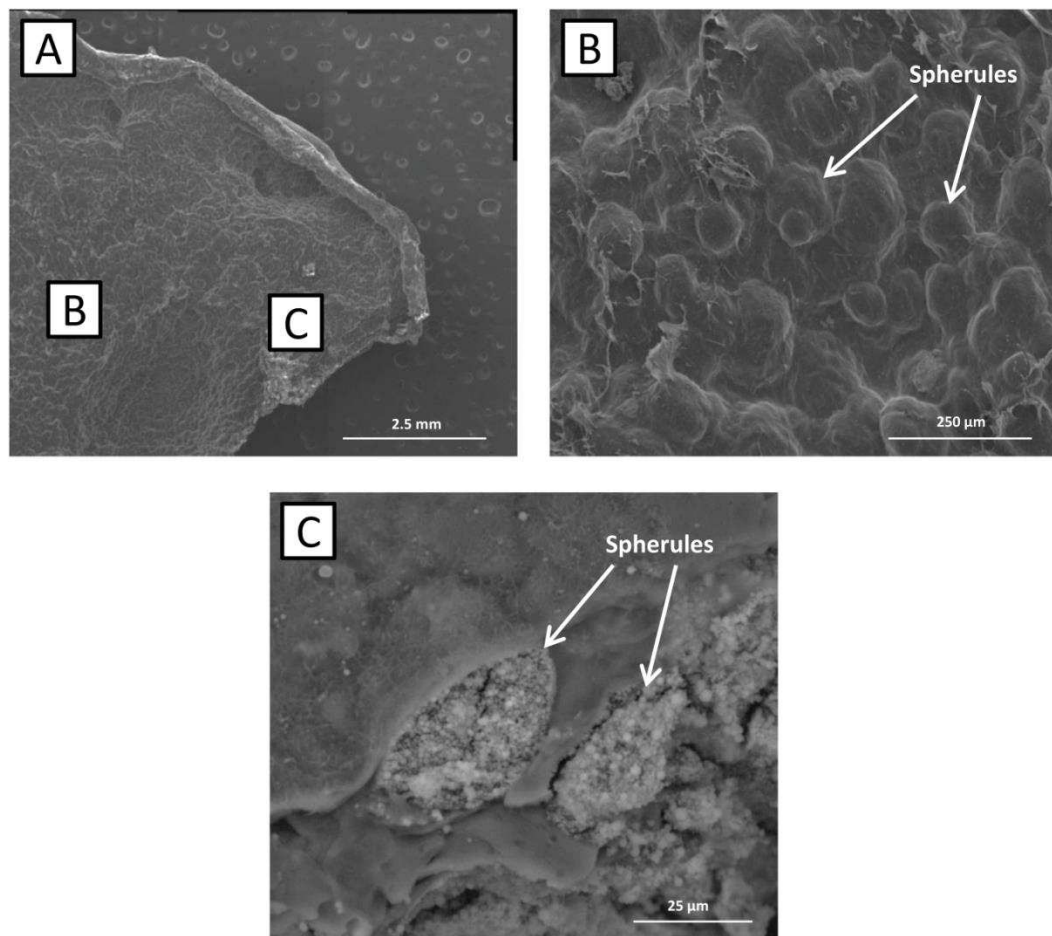


Figure 23: Scanning electron microscopy of the foot of adult of *Crepidula fornicata* at different magnification levels.

3.3. Presence of spherules in relation to the substrate of *C. fornicata*

When removed from underlying shell substrates, *C. fornicata* individuals leave a hard, white (more or less pronounced) deposit, which perfectly matches their foot imprint (Fig. 24A). This deposit also reacts with acid and produces gas, but was not observed for five supplementary individuals inhabiting non-calcareous

substrates (Fig. 24B). Nevertheless, these individuals all showed the same pattern of calcareous spherule distribution in their bodies (Fig. 24C) with comparable amounts in the foot (Fig. 24D).

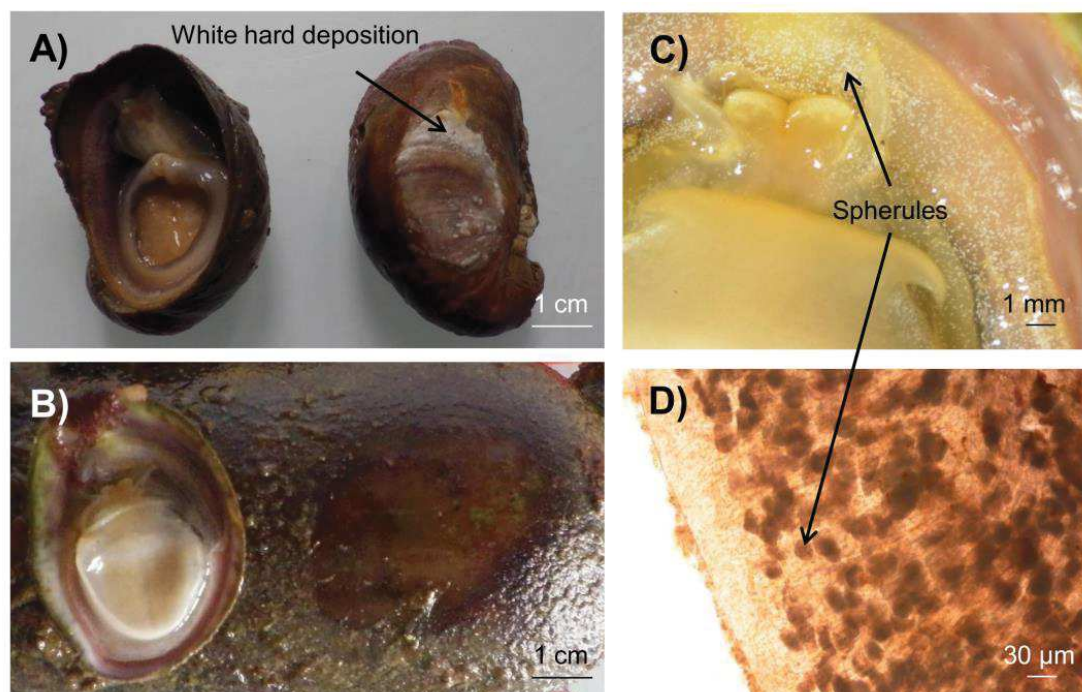


Figure 24: Images of the slipper limpet *Crepidula fornicata* inhabiting (A) a calcareous substrate and (B) a non-calcareous substrate. (C) Calcareous concretions in *Crepidula fornicata* soft tissue fixed on a non-calcareous substrate and (D) close-up of a foot tissue sample from the same individual.

3.4. Implications for diet composition assessment of *C. fornicata*

The estimation of the contributions of potential food sources to the diet of *C. fornicata* (i.e., Marine POM, Benthic diatoms and Macroalgae and C4 plants) and the extent of change greatly varied according to *C. fornicata* size classes and sources (Fig. 25), whether corrected or uncorrected $\delta^{13}\text{C}$ values were used. Due to an overall small dietary contribution, the proportion of benthic diatoms remained little impacted by acid treatment, except for the largest size class 36–40 mm (Fig. 25A). The dietary proportions of macroalgae and C4 plants showed clear differences for individuals larger than 30 mm (Fig. 25B). Finally, marine POM contributions showed the highest differences between uncorrected and corrected samples, with increasing ranges from 25 mm and above (Fig. 25C). For the largest individuals (36–40 mm), corrected $\delta^{13}\text{C}$ values revealed that contributions were overestimated for benthic diatoms (53 %) and macroalgae/plants (22 %) but underestimated marine POM (43 %), when comparing with uncorrected values.

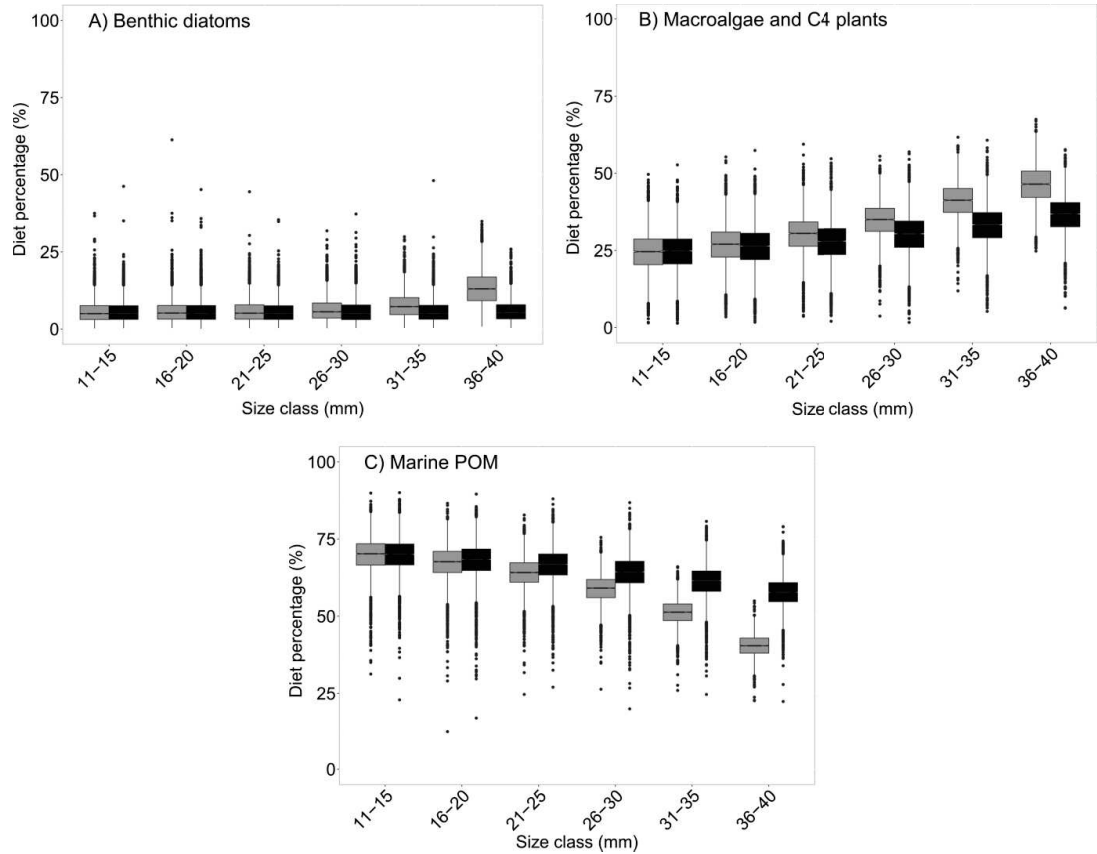


Figure 25: Relative contributions of (A) benthic diatoms, (B) macroalgae and C4 plants, and (C) marine POM to the diet of *C. fornicata* of six size classes calculated with uncorrected (grey) and corrected (black) $\delta^{13}\text{C}$ values (data extracted from Decottignies et al., 2007a).

3.5. Implications in interspecific trophic overlap assessment

Inter-specific isotopic differences between the two suspension-feeding species *C. fornicata* and *M. gigas* showed opposite seasonal patterns when comparing corrected and uncorrected $\delta^{13}\text{C}$ values (Fig. 26). For instance, during late summer, $\delta^{13}\text{C}$ value differences between oysters and slipper limpets were non-significant when considering uncorrected values of *C. fornicata*, but were significant after correction. Conversely, from winter to early summer, significant differences became non-significant after correction.

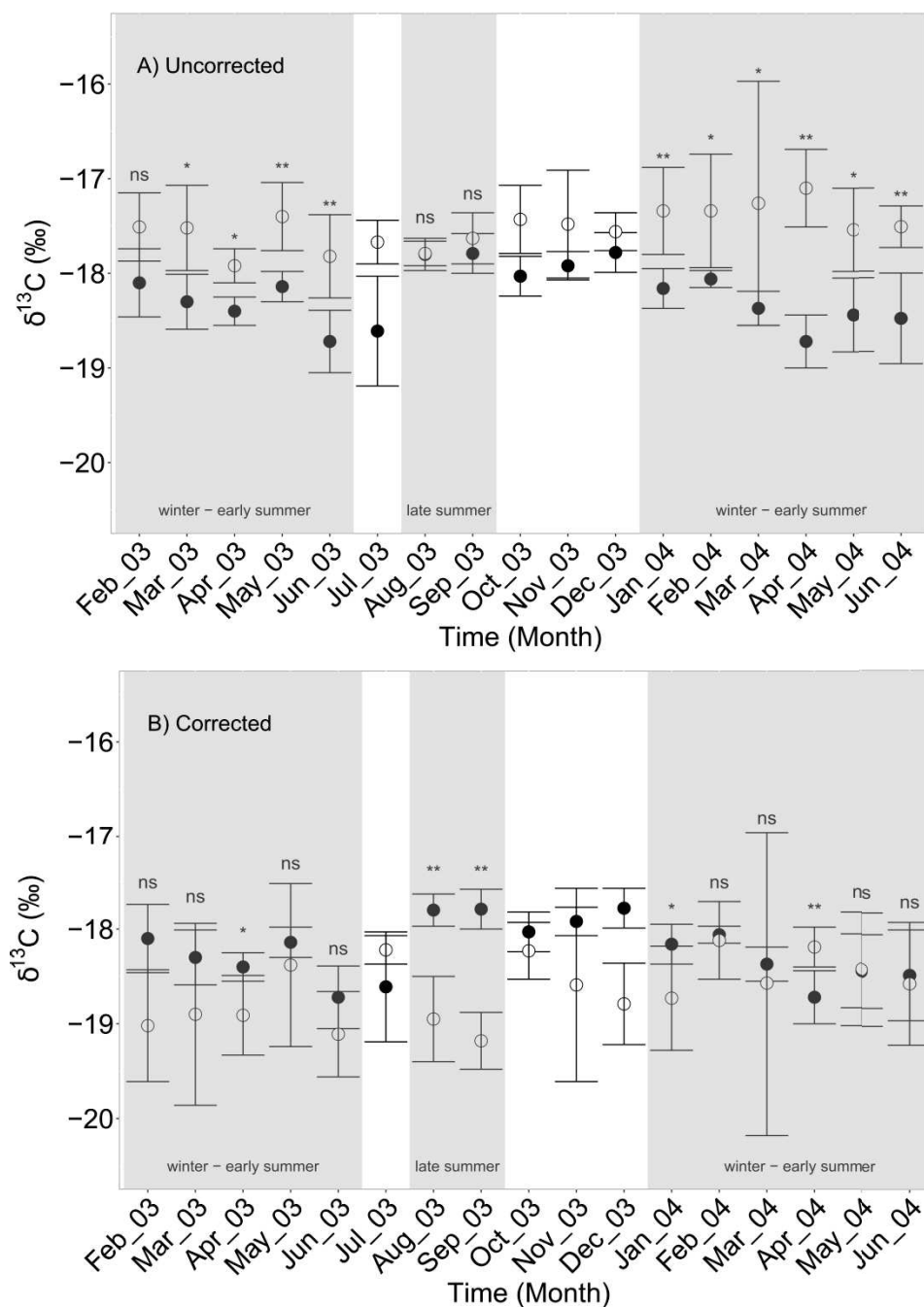


Figure 26: Seasonal variations of $\delta^{13}\text{C}$ measured for *Crepidula fornicata* (open circles) and *Magallena gigas* (filled circles) (mean \pm SD; n = 5). Interspecific $\delta^{13}\text{C}$ comparisons are shown with (A) uncorrected and (B) corrected (data extracted from Decottignies et al., 2007a). Asterisks show significant interspecific differences. ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$.

4. Discussion

The present study aimed to investigate the effect of acidification of soft tissues (foot) on carbon and nitrogen stable isotope ratios in slipper limpet *C. fornicata*, a common and widespread invasive gastropod. We coupled isotopic

compositions and microscopic observations to quantify the effect of the acid pre-treatment on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

4.1. The need for acid treatment in $\delta^{13}\text{C}$ value assessments in *C. fornicata*

Acid pre-treatment had no effect on either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values for small sizes of *C. fornicata*. For individual sizes above ~20 mm, the difference between treated and untreated samples increased linearly up to 0.6 ‰ for $\delta^{15}\text{N}$, and exponentially up to 3 ‰ for $\delta^{13}\text{C}$. These values are higher than those found in other investigations on acid treatments in gastropods. As reviewed in (Schlacher and Connolly, 2014), $\delta^{13}\text{C}$ values in untreated gastropods had a mean deviation of 0 ‰ (± 0.09) compared with acidified samples, whereas $\delta^{15}\text{N}$ values were slightly higher, by 0.14 ‰ (± 0.09). Although a low concentration solution of hydrochloric acid (1 N) is not supposed to significantly transform organic matter (by chemical reactions or loss of organic matter) or fractionate stable isotope ratios (Schlacher and Connolly, 2014), we cannot exclude that acid treatment may cause slight deviations of stable isotope ratios. Potential reactions include solubilisation of acid-soluble organic carbon (Brodie et al., 2011), break-up of complex compounds such as amino acids and subsequent loss in rinses (Mateo et al., 2008), and volatilization (Lohse et al., 2000) or fractionation of organic carbon (Kennedy et al., 2005). This is why many authors have stressed the need for a common sample preparation protocol in stable isotope studies (McCutchan et al., 2003).

The largest individuals in this study were 40 mm in length, but *C. fornicata* can reach 50 mm (Richard et al., 2006). As a result, one could expect even higher differences in $\delta^{13}\text{C}$ values for larger adults, although extrapolating the correction beyond 40 mm has not been validated here. Acidified samples have higher organic carbon content per unit weight compared with non-acidified ones ($27 \pm 3\%$ vs. $41 \pm 1\%$ for sessile female), indicating that organic and/or inorganic carbon reacts with acid. Gas releases and decreases in inorganic carbon during acidification suggest that *C. fornicata* foot tissue contains inorganic carbonates in higher concentrations in sessile individuals compared with motile ones.

When dealing with molluscs in general and gastropods in particular, inorganic carbon content is expected to originate from shell fragments. Isotopic investigations on *C. fornicata* have been carried out either on undissected animals rinsed with 1 N HCl (Decottignies et al., 2007ab; Lefebvre et al., 2009; Riera et al.,

2002), or dissected animals without acid treatment (Guérin, 2004; Richard, 2005). In six out of seven studies, inorganic carbonates in the soft tissues were not dissolved by acid, hence generating measures falsely-enriched in ^{13}C in these studies (Supplementary Table 4). We found only one study in the literature likely to obtain comparable results for $\delta^{13}\text{C}$: (Dubois et al., 2014) used freeze-dried powder with 1.2 N HCl solution directly in silver cups as an acidification process. This treatment allows a total dissolution of inorganic carbonates from the sample, giving a mean $\delta^{13}\text{C}$ value of -19.7 ‰, which is in the same range as our acid-treated samples. As a result, we recommend analysing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in two different ways: untreated samples for nitrogen and acidified samples for carbon. In order to allow perfect penetration of acid and avoid any loss of organic carbon during rinsing, we also encourage using in-cup acidification (Guerin et al., 2013).

4.2. Origins of inorganic carbonates in *Crepidula fornicata* soft tissue

In gastropod taxa, the foot is the organ of movement and/or fixation onto the substrate. It is therefore in direct contact with the environment. For freshwater gastropods, calcium is a limiting element taken up through the foot and stored as spherules in the soft tissues (Fournié and Chétail, 1984). Because mucus secreted by the foot combines with calcium (Rao and Goldberg, 1954), it has been suggested that calcium is absorbed percutaneously from the substrate and stored in the foot (Kapur and Gibson, 1968; Rao and Goldberg, 1954; Tanaka and Hatano, 1955). In freshwater gastropods, calcium deposits are mostly known to play roles in pH homeostasis (Sminia et al., 1977), and to a lesser extent in hemocyte reactions to particle or toxic invasions (Hinzmann et al., 2015). Unlike freshwater, seawater is not calcium limited and marine molluscs are not expected to use calcium storage processes for homeostasis regulation. In the few studies focusing on calcium deposits in marine gastropods, spherules were investigated for their metal detoxification capacities (Mason and Nott, 1981; Nott and Nicolaidou, 1989). Interestingly, an investigation of feeding processes and the use of mucus in particle processing in *C. fornicata* by (Shumway et al., 2014) clearly showed white dots on the ventral surface (see Fig. 1 in (Shumway et al., 2014)). Even though this previous study was not designed to investigate calcium spherules, high quality micrographs of living animals are a good way of revealing these spherules.

Ecological traits, and specifically the loss of motility of developing *C. fornicata*, could provide an explanation for the presence of calcareous spherules in soft tissues. Ontogenic histological changes have already been described for another *Crepidula* species (Chaparro et al., 1998). A specific type of mucocyte is indeed preferentially associated with females and linked to their greater adhesive capacity on the substrate. Interestingly, these authors mentioned a homogenous granular material in two kinds of mucocytes of the epithelial and sub-epithelial tissue. Since calcium binds with mucous to form calcium carbonates (Rao and Goldberg, 1954), it is likely that this granular material corresponds to inorganic carbonates, as confirmed by the EDS analysis of the present study. Interestingly, we also detected a large amount of magnesium in our samples (Magnesium/Calcium ratio ~5). While this was beyond the scope of our present study, future experiments should investigate the origin of such great quantity of magnesium in the soft tissue of *C. fornicata*. The nature of the substrate should also be examined. Adhesive capacity of mucus increases with viscosity and acidity (Grenon and Walker, 1978; Hunt, 1973). Because *C. fornicata* spends its entire life (~10 years) on the same substrate, it is expected to show high mucus acidity (i.e., high adhesive capacity). A calcareous substrate associated with mucus acidity also potentially explains the calcareous concretion on the underlying shell and increases the percutaneous uptake of carbonates by the foot (Fig. 24A). However, qualitatively comparable amounts of spherules were also found in *C. fornicata* inhabiting non-calcareous substrates (Fig. 24B-D), which suggests that the accumulation of inorganic carbonates is linked to intrinsic physiological processes rather than the type of substrate. In our study we differentiated motile and sessile males because males larger than 20 mm are found in their final position, on top of female shells, and become sessile even though they are still male. Because motility is not strictly related to sexual differentiation, we also suggest that the increasing adhesive capacity of the species in relation to its gregarious behaviour explains the concentration of inorganic carbonates in its soft tissues.

Similar histological observations were made for individuals of *C. fornicata* collected in the bays of Mont-Saint-Michel and Bourgneuf (France), suggesting again that they are the result of an intrinsic physiological process rather than local conditions. Because the presence of inorganic carbonates is related to biological traits of *C. fornicata* (protandry, motility and mucus production), local

environmental conditions could enhance or lower this physiological process and ultimately slightly affect the correction factor provided here. The amplitude of the deviation in ^{13}C might therefore need to be characterized by replicating the same protocol in additional study sites. Preliminary results also showed that other Calyptraeidae species – such as *Calyptraea chinensis* – have similar biological traits (i.e., sessile and suspension-feeding) but should be processed with caution as the correction factor provided here cannot readily be applied to other species.

4.3. Trophic implications

Differences in isotopic composition between treated and untreated samples potentially indicate large ecological differences and could therefore have consequences for data interpretation. Results from the literature have commonly shown *C. fornicata* to be higher in ^{13}C than other filter-feeder species, suggesting a higher proportion of ^{13}C -enriched food sources, such as benthic microalgae, in their diet. Here, we showed using published data (Decottignies et al., 2007a) that the contribution of microalgae to the diet of *C. fornicata* has generally been over-estimated (up to 53 %), while, conversely, the contribution of marine POM has been under-estimated (up to 43 %). Interpretations in benthic-pelagic coupling regarding allochthonous *versus* autochthonous food sources are therefore compromised. Mixing models computed with corrected $\delta^{13}\text{C}$ values also showed higher similarities with other suspension-feeding species such as the Pacific oyster *Magallana gigas*, and a potentially higher trophic competition when food is limited. The two species were thought to compete for food in winter and spring (Decottignies et al., 2007a), but corrected contributions of food sources show that their overlap in diet is higher than previously estimated. Conversely, they do not seem to overlap in late summer. It is thus possible that the invasive slipper limpet *C. fornicata* could be a stronger trophic competitor than previously reported. It is worth noting that we used a correction model based on foot tissue only, whereas (Decottignies et al., 2007a) based their analysis on the whole body. However, because microscopic observations revealed the presence of calcareous concretions in several types of tissue (Fig. 24C) and because the foot represents 55 to 65 % of the body mass of *C. fornicata* (Richard, 2005), this difference should not weaken our conclusions.

5. Conclusions and recommendations

Crepidula fornicata contains a significant proportion of inorganic carbonates in its soft tissue. Even though SI is now commonly used in trophic ecology studies, we still need to be careful about sample preparation, which should be species-specific. We recommend that researchers using SI take into consideration that “soft tissue” does not necessarily mean “without inorganic carbonate”, e.g., by applying the quick and easy ‘Champagne test’ (Schlacher and Connolly, 2014) on a small piece of sample. This test could make it possible to target specific parts of the tissue of interest, which could be dissected without risk of ‘carbonate pollution’, thus avoiding serious misinterpretations when inferring conclusions from SI analysis.

6. Acknowledgements

The authors are grateful to the staff of the stable isotope facility (*Pole de Spectrometrie Ocean*) for technical help and Gilles Chazot for fruitful discussion about spherule composition. TA was funded by an Ifremer-Region Bretagne PhD grant. AC and SFD received a grant from the national EC2CO-DRIL initiative. We would also like to thank three anonymous reviewers for their pertinent comments that greatly improved the manuscript.

7. Supplementary materials

Supplementary Table 3: Food source and trophic fractionation values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of organic matter sources used in the mixing model.

Organic matter sources	Food source values		Trophic fractionation values	
	$\delta^{13}\text{C}$ (mean \pm sd)	$\delta^{15}\text{N}$ (mean \pm sd)	$\delta^{13}\text{C}$ (mean \pm sd)	$\delta^{15}\text{N}$ (mean \pm sd)
Marine POM	-22.8 \pm 1.4	3.6 \pm 1.4	1.63 \pm 0.63	3.54 \pm 0.74
Benthic diatoms	-13.2 \pm 2	5.2 \pm 0.6	1.63 \pm 0.63	3.54 \pm 0.74
Macroalgae and C4 plants	-15.4 \pm 1.4	8.6 \pm 1.6	1.63 \pm 0.63	3.54 \pm 0.74

Supplementary Table 4: $\delta^{13}\text{C}$ measured for *Crepidula fornicata* according to isotopic sample preparation in seven previous studies.

References	Dissection	Acidification	$\delta^{13}\text{C}$ (mean \pm SD)
Decottignies et al., 2007ab	No	Yes	-17.8 ± 0.5
Dubois et al., 2014a	Yes	Yes	-19.7 ± 0.6
Guérin, 2005	Yes	No	-16 ± 0.6
Lefebvre et al., 2009	No	Yes	-18.4 ± 0.4
Richard, 2005	Yes	No	-15.8 ± 0.6
Riera et al., 2002	No	Yes	-16.3 ± 0.4

Méthode 3 : Pigments photosynthétiques

Les pigments photosynthétiques sont des molécules capables de capter l'énergie lumineuse pour la transformer en énergie métabolisable par l'organisme. Ils sont très largement représentés par les organismes autotrophes (plantes et algues majoritairement) qui tirent leur source d'énergie de la photosynthèse et qui sont à la base des réseaux trophiques. À l'image des acides gras, la diversité des pigments permet elle aussi de discriminer certains groupes de producteurs primaires (Table 5). Cependant, la dégradation suite à leur assimilation dans les tissus des consommateurs ainsi que des problèmes de quantification n'en font pas un outil très utilisé en écologie trophique. La quantification de leur biomasse n'en reste pas moins un bon indicateur de l'identité des sources de matière organique en présence, et donc de l'environnement trophique disponible pour un consommateur.

Il faut également noter que certains de ces pigments, en plus d'être des biomarqueurs, sont aussi impliqués dans des mécanismes physiologiques de photo-protection. En dissipant l'excédent d'énergie lumineuse, ces pigments évitent ainsi le stress oxydatif des centres réactionnels de la photosynthèse et les dégâts physiologiques qu'il engendre. C'est par exemple le cas du cycle des xanthophylles chez les algues vertes, où la violaxanthine est convertie en antheraxanthine, puis en zeaxanthine, l'équivalent pour les diatomées étant la conversion de la diadinoxanthine en diatoxanthine. Enfin, l'eau absorbe les radiations lumineuses et limite la pénétration des grandes longueurs d'ondes (> 650 nm). Certains pigments, comme la fucoxanthine, sont capables de capter l'énergie lumineuse à de faibles longueurs d'onde (460 nm), ce qui peut révéler une photo-acclimatation à des profondeurs où seule la lumière bleue est disponible. Il est donc important de tenir compte de l'environnement lumineux pour l'utilisation de ces biomarqueurs.

Table 5 : Exemples de biomarqueurs utilisés pour le microphytobenthos (tirés de Méléder, 2003). ++ : pigments majeurs (> 10 %), + : pigments mineurs (< 10 %), - : traces (< 1 %).

Pigments	Chlorophycées (algues vertes)	Cyanobactéries	Bacillariophycées (Diatomées)	Dinophytes (Dinoflagellées)	Euglénophytes
Chlorophylle <i>a</i>	++	++	++	++	++
Chlorophylle <i>b</i>	++				++
Chlorophylle <i>c</i>			++	++	
α carotène	-				
β carotène	+	+	-	-	+
Antheraxanthine	-				-
Diadinoxanthine			++	++	++
Diatoxanthine			-	-	-
Dinoxanthine				+	
Fucoxanthine			++		
Lutéine	++				
Néoxanthine	++				-
Péridine				++	
Zéaxanthine	+	++			

Méthode 4 : Acides gras

Les lipides, les glucides et les protides sont les nutriments essentiels à la vie. Ils sont impliqués dans la structure, le métabolisme et la physiologie de tout organisme vivant. Parmi eux, les lipides sont des molécules composées de nombreuses classes intervenant dans la structure des membranes cellulaires (phospholipides, stéroïdes), comme précurseurs de messagers cellulaires (stéroïdes, diacylglycérols) ou comme réserve d'énergie (triacylglycérols). Les triacylglycérols sont composés d'acide gras (AG), c'est-à-dire une chaîne carbonée possédant un groupe méthyle à une extrémité et un groupe acide de l'autre, et sont tirés de la nourriture assimilée. Les AG sont identifiés selon leur nombre de carbone (4 à 40), le nombre et la position des insaturations (nombre de doubles liaisons entre 2 carbones) :

$$X : Y_n - Z$$

où X représente le nombre de carbone, Y le nombre d'insaturation et Z le carbone où se situe la première insaturation en partant de la fin de la chaîne. Leur utilisation en écologie trophique repose sur le fait que leur incorporation dans les tissus du consommateur se fait sans modifications majeures. De plus, la synthèse *de novo* de certains AG est connue mais reste limitée chez de nombreux organismes. Sans capacité de synthèse de certains AG qui sont dit essentiels, les consommateurs sont contraints de les trouver dans leur alimentation. Le second avantage de ces AG est leur spécificité à être synthétisé par un ou plusieurs groupes de producteurs primaires, et donc à être des biomarqueurs trophiques (Table 6).

Table 6 : Exemples de biomarqueurs couramment utilisés en écologie trophique. AGB : AG branchés, AGSLC : acides gras (AG) saturés à longue chaîne (≥ 22 carbones), AGS : AG saturés, AGPI : AG polyinsaturés.

Acide gras	Indicateurs de	Références
14:0; 16:1n-7, 20:5n-3	Diatomées	Dunstan et al., 1994; Napolitano et al., 1997
20:5n-3/22:6n-3	Dominance de diatomées sur flagellés	Budge and Parrish, 1998; Lavaud et al., 2018
18:1n-9	Flagellés, matériel animal	Napolitano et al., 1997; Dalsgaard et al., 2003
Σ AGB	Bactéries	Perry et al., 1979; Haack et al., 1994
Σ AGSLC	Plantes terrestres	Canuel; 1991; Budge et al., 2001; Meziane et al., 1997
Σ AGPI/ Σ AGS	Matière organique labiles vs. detritique	Biandolino et al., 2008; Pommier et al., 2010
18:3n-3; 18:2n-6	Plantes terrestres, macroalgues vertes	Meziane et al., 1997; Kelly and Scheibling, 2012
18:4n-3	Macroalgues vertes, flagellés	Fleurence et al., 1994; Kelly and Scheibling, 2012

Article 3 : Sharing is caring: ontogenic trophic niche differentiation in the invasive gregarious species *Crepidula fornicata*

Thibault Androuin ¹, Stanislas F. Dubois ¹, Cédric Hubas ³, Gwendoline Lefebvre ², Fabienne Legrand ², Gauthier Schaal ², Antoine Carlier ¹.

¹ DYNECO-LEBCO, IFREMER, Plouzané, France

² UMR CNRS 6539, LEMAR-IUEM-UBO, Plouzané, France

³ UMR BOREA, Muséum National d'Histoire Naturelle, Concarneau, France

Abstract

Crepidula fornicata is a common and widespread invasive species along the European coast. Among its life-history traits, ontogenic changes of behavior (i.e., motile male to sessile female) suggest potential shift in feeding strategy during its early life stage. Considering the ecological significance of this species in colonized areas and its gregarious way of life, understanding how conspecifics share the trophic resource is crucial. Using complementary trophic markers, we conducted a field survey between winter and spring to investigate the availability of potential food sources and the trophic niche of *C. fornicata* regarding three ontogenic classes, bearing different sexual (male/female) and motility (motile/sessile) traits. Potential trophic sources were well discriminated by their pigments, fatty acids (FA) as well as stable isotopes (SI) compositions over the study period. Interestingly, the biofilm covering *C. fornicata* shells showed higher contribution and biomass of saturated FA (e.g., 16:0 and 18:0), chlorophytes and diatoms than the surrounding sediment. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signals suggested higher trophic plasticity in motile males than in sessile males and females, attributed to its motile behavior. FA compositions also highlighted a trophic differentiation between motile and sessile classes of *C. fornicata*. The high contribution of saturated FA to the diet of motile males suggested they were grazing on the biofilm during winter when food is less available. Conversely, FA and SI were complementary to indicate that adults of *C. fornicata* mainly relied on diatoms, dinoflagellates and bacteria, coming from re-suspended particulate organic matter. While physiological processes such as sexual maturation or growth rate may hamper SI and FA interpretations when looking at ontogenic changes, our results suggest that facilitation processes through biofilm stimulation as well as trophic shift during early life stages may contribute to the invasive success of *C. fornicata*. Our results also suggest that microphytobenthos enter the diet of *C. fornicata* in our study site, confirming its potentially high trophic significance in subtidal coastal habitats.

Key words: *Crepidula fornicata*, trophic niche, ontogenic shift, fatty acids, stable isotopes, pigments

1. Introduction

In trophic ecology of coastal ecosystems, most of studies have addressed issues of spatio-temporal changes at the species/community scale by only considering the adult phase of analysed individuals (Carlier et al., 2007; Dubois and Colombo, 2014; Rigolet et al., 2014). Intraspecific variability of feeding behaviour has often been overlooked whereas this factor can affect population dynamics, interspecific interaction and ultimately ecosystem functioning (Bolnick et al., 2011; Miner et al., 2005; Turcotte and Levin 2016). Among intraspecific biological processes, ontogenic shift diet can influence the structure and composition of benthic population (Hentschel, 1998) and should be taken into account when depicting trophic relationships (Rossi et al., 2004). Whereas size-specific competition for food resource may limit population densities (Hentschel and Jumars, 1994), ontogenic shift of diet is a competitive advantage and a way to limit conspecific trophic competition (Olson, 1996; Schellekens et al., 2010), potentially increasing invasive success (Carlsson and Brönmark, 2006).

The slipper limpet *Crepidula fornicata* is a non-indigenous species originating from the East coast of the US (Blanchard, 1997). This invasive species has extensively colonized soft bottoms in Europe, from Norway to the Mediterranean Sea (Blanchard, 1997). Because of its introduction in many parts of the world and its potential cascading effect on food web functioning (Arbach Leloup et al., 2008; Chauvaud et al., 2000; Cugier et al., 2010), several studies have investigated its diet to infer potential trophic overlap with co-occurring species (Blanchard et al., 2008; Decottignies et al., 2007ab; Lefebvre et al., 2009; Riera 2002; 2007). *C. fornicata* is a hermaphroditic and gregarious species, which begins its benthic life as a low-motile male to become a sessile female. Adults are sessile and form stacks of several individuals, while juveniles and small males (~10 mm) are motile on the substratum. *C. fornicata* is known to be a suspension-feeders, but contrary to bivalves which select the food collected using gills and labial palps, adult females form a food cord in a groove at the distal end of their gill filaments and ultimately catch this cord with their radula before ingesting it (Beninger et al., 2007; Shumway et al., 2014). Substrate has already been shown to be of primary importance in the feeding ecology of *C. fornicata* juveniles after metamorphosis (Eyster and Pechenik, 1988; Pechenik et al., 2015). For instance, newly settled

individuals of *Crepidula fecunda* first adopt a grazing feeding mechanism and gradually shift it to a filtering behaviour once their gill are fully developed (Montiel et al., 2005). Young individuals are even able to use both feeding mechanisms (i.e., grazing and filter-feeding) during the motile phase of their life cycle (size < 28 mm), whereas females are exclusive filter-feeders (Chaparro et al., 2002; Navarro and Chaparro, 2002). Such information about the grazing feeding of young motile males is unknown in *C. fornicata* while the species exhibit the same ontogenic changes of behavior than *C. fecunda* (i.e., motile male to sessile female). Since *C. fornicata* often occurs in large accumulations (up to 2000 ind m⁻²) on the sea floor of invaded ecosystems (Guérin, 2004; Martin et al., 2006) with all ontogenic classes grouped in the same stacks, one can expect high intraspecific competition for food.

Different kinds of trophic markers have long been used to investigate the trophic niche of benthic species (Blanchet-Aurigny et al., 2015; Cresson et al., 2016; Dubois and Colombo, 2014) as well as describing the origin of particulate organic matter (OM) (Ke et al., 2017; Lavaud et al., 2018; Lienart et al., 2017). Among these tools, SI compositions are common to infer trophic niche of consumers (Fry and Sheer, 1984; Layman, 2012). Classically, nitrogen isotope ratio informs about the trophic position of a species and carbon isotope ratio reflects the origin of assimilated food sources (e.g., continental *vs.* oceanic). SI have been used several times to assess the diet of *C. fornicata* during its adult phase (Decottignies et al., 2007ab; Lefebvre et al., 2009; Riera et al., 2002; 2007; Sauriau et al., 2002). However, a recent study highlighted the presence of inorganic carbonates in *C. fornicata* soft tissues, which leads to overestimated $\delta^{13}\text{C}$ ratios and misinterpretations of food sources' contributions to the diet of this species, raising the need for re-evaluating the trophic niche of this invasive species (Androuin et al., *article 2*). Moreover, in coastal ecosystems, the pool of particulate OM, which composed the diet of most of benthic primary consumers, is a mixture of OM from various origins (phytoplankton, continental detritus, zooplankton, etc) which are often difficult to disentangle based on the isotopes of only two elements (e.g., carbon and nitrogen). This diversity of food source thus implies that complementary trophic markers are used together with SI. For instance, pigment analyses have been widely used to study community composition of microscopic primary producers in the water column or in the sediment, since some pigments are specific of clades of algae (Brotas and Plante-Cuny, 2003). To a lesser extent, fatty acid compositions

can be also specific of group of organisms, such as diatoms, bacteria, copepods, vascular plants, etc (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Very recently, the combined use of SI, FA and pigments have proved to increase our understanding of trophic pathways from the sources of particulate OM to primary consumers (Lavaud et al., 2018; Majdi et al, 2018).

In this study, we conducted a field survey from late winter to early summer to re-assess the trophic niche of *C. fornicata* and to investigate a potential trophic shift along with ontogenic changes of behavior (i.e., motile male to sessile female). According to previous experimental study on stimulated microphytobenthic biofilm in *Crepidula* beds (Androuin et al., *article 1*), we hypothesized that i) *C. fornicata* rely on benthic food sources such as microphytobenthos, and ii) ontogenic changes of this species allow food partitioning within stacks. OM food sources were characterized using SI, FA and pigments compositions and their potential assimilation by *C. fornicata* was inferred using both SI and FA compositions.

2. Materials and methods

2.1. Sampling and dissecting protocol

The bay of Brest (Britany, France) is a semi-enclosed marine ecosystem of 180 km². The sampling site is located near the Elorn estuary (48°23'N, 4°23', average depth: 10 m) in a dense *Crepidula* beds (~2000 ind m⁻²) (Guérin 2004). Four sampling dates (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April) during spring were chosen to encompass time period with potential contrasted OM sources availability (e.g., spring blooms). OM sources and *C. fornicata* individuals were collected by SCUBA divers at around mid and rising-tide to ensure oceanic seawater.

Pelagic particulate organic matter (PPOM) was sampled using two 8 L Niskin bottles at 50 cm above the sediment-water interface, immediately filtered on board on a 200 µm nylon mesh to remove large zooplankton and particles. In the laboratory, between 1 L and 1.5 L was filtered on pre-combusted (450°C during 5 hours) GF/F filters (0.7 µm). Three replicates for each of the three analyses (SI, FA and pigment analyses) were filtered. Re-suspended particulate organic matter (RPOM) was sampled from three cores of 15 cm diameter and 15 cm depth. In the laboratory, the sediment-water interface was re-suspended by flushing seawater

with a 30 ml syringe. 60 ml of RPOM was pre-filtered on a 200 μm nylon mesh to being consistent with RPOM samples, and filtered on pre-combusted (450°C during 5 hours) GF/F filters (0.7 μm). Three replicates for each of the three analyses were filtered. Biofilm from *C. fornicata* stacks was scrapped off using a toothbrush and suspended in 600 ml of filtered seawater (0.7 μm). 200 ml of the suspended solution was filtered on pre-combusted (450°C during 5 hours) on GF/F filters (0.7 μm). Three replicates for each of the three analyses were filtered. Filters for FA analysis were put in glass tubes containing 6 ml of chloroform-methanol solution (2:1, v:v) and stored at -80°C before analysis, whereas filters for pigment and SI analysis were immediately stored at -80°C.

Females of *C. fornicata* were sampled at the basis of the stacks (mean shell length 33 ± 6 mm), fixed on a dead *C. fornicata* shell. Sessile and motile males were sampled if they had a penis and a mean shell length of 20 ± 8 mm and 10 ± 1 mm, respectively. We used digestive gland as trophic integrator because it has a higher turnover rate than muscle tissue and because it is an energy storage organ enriched in lipids (McCutchan et al., 2003). However, digestive gland and gonad are fused in one organ in *C. fornicata*; therefore we used both tissues together. The whole body of motile male were used to ensure sufficient lipid concentration.

2.2. Pigment analysis

The photosynthetic communities of RPOM, biofilm and PPOM have been analyzed by the quantification of pigments by High Performance Liquid Chromatography (HPLC) according to Brotas and Plante-Cuny (2003). Filters were crushed and extracted in 3 ml of 95 % cold buffered methanol (2 % ammonium acetate) for 20 min at -20°C in the dark. Samples were centrifuged for 3 minutes at 3000 RCF after the extraction period. Extracts were then filtered with Whatman membrane filters (0.2 μm) immediately before HPLC analysis. Pigment extracts were analysed using an Agilent 1260 Infinity HPLC composed of a quaternary pump (VL 400 bar), a UV-VIS photodiode array detector (DAD 1260 VL, 190–950 nm), and a 100 μl sample manual injection loop (overfilled with 250 μl). Chromatographic separation was carried out using a C18 column for reverse phase chromatography (Supelcosil, 25 cm long, 4.6 mm inner diameter). The solvents used were A: 0.5 M ammonium acetate in methanol and water (85:15, v:v), B: acetonitrile and water (90:10, v:v), and C: 100 % ethyl acetate. The solvent gradient

followed the Brotas and Plante-Cuny method (2003), with a flow rate of 0.5 mL min⁻¹. Identification and calibration of the HPLC peaks were performed with chlorophyll a, β -carotene, chlorophyll c2, diatoxanthin, diadinoxanthin and fucoxanthin standards. All peaks detected were identified by their absorption spectra and relative retention times using the Open Lab CDS software (ChemStation Edition for LC/MS Systems, Agilent Technologies). Quantification was performed by repeated injections of standards over a range of dilutions to establish a standard curve of concentrations. The relative abundance of each pigment (%) was calculated from their respective surface and volume ($\mu\text{g}\cdot\text{cm}^{-2}$ for biofilm and RPOM, and $\mu\text{g L}^{-1}$ for PPOM). We measured the mean surface of three stacks of *C. fornicata* to standardize surfaces.

2.3. Stable isotope analysis

Two separate analyses were done for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for both OM sources and *C. fornicata* tissues. Filters were freeze-dried and half of the filter was scrapped off and weighed in tin capsules for $\delta^{15}\text{N}$ analysis. The second half was decarbonated using acid-flume (10 N hydrochloric acid solution) during 7 hours (Lorrain et al. 2003). Filters were then dried at 40 °C for 12 h, scrapped off and weighed in tin capsules for $\delta^{13}\text{C}$ analysis. After freeze-drying, *C. fornicata* samples were ground into homogenous powder using a mortar and pestle. Approximately 400 μg of powder was weighed in tin capsules for $\delta^{15}\text{N}$ analysis. Because both lipids content and inorganic carbonates can influence SI ratio of $\delta^{13}\text{C}$ (Androuin et al. *article 2*), approximately 400 μg of powder was added to 1 ml of cyclohexane in Eppendorf tubes. After 15 second of vortex, tubes were centrifuged at 3000 g during 5 min. The supernatant was discarded and the tubes dried at 40°C during 12 h. If the supernatant was still coloured, the sample was re-processed. Lipid-free tissues were then weighed in tin silver capsules and in-cup decarbonated using 1N HCl. Each capsule were visually checked, dried at 40°C during 1 h, and closed. Samples were analysed by continuous flow on a Thermo Scientific Flash EA 2000 elemental analyser coupled to a Delta V Plus mass spectrometer at the Pôle de Spectrométrie Océan (PSO, Plouzané, France). Results are expressed in standard δ notation based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$) following the equation:

$$\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 \text{ (in ‰)}, \text{ where R is } ^{13}\text{C}/^{12}\text{C} \text{ or } ^{15}\text{N}/^{14}\text{N}.$$

International isotopic standards of known $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were used: IAEA-600 Caffeine, IAEA-CH-6 Sucrose, IAEA-N-1 and IAEA-N-2 Ammonium Sulphate. The experimental precision was estimated using the standard deviation of an internal standard (Thermo Acetanilide, $n = 8$). It indicated an analytical precision of ± 0.11 ‰ and ± 0.07 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively.

2.4. Fatty acids analysis

2.4.1. Lipid extraction

Immediately after freeze-drying, samples of *C. fornicata* were ground into homogenous powder using a mortar and pestle. Samples (between 2 and 20 mg depending on species) were immediately put in glass tubes previously heated for 6 h at 450°C and containing 6 mL of a chloroform/methanol mixture (2:1, v:v), and extracted with a Dounce homogenizer. OM sources and *C. fornicata* samples were sonicated during 10 min and kept at -20°C until further analysis.

2.4.2. Separation

OM source were analyzed on the total lipid fraction, therefore without separation. However, only the FA in the neutral lipids of the animals were analyzed because they reflect the FA profiles of food assimilated, whereas the polar lipids are less sensitive to dietary changes (Dalsgaard et al., 2003). The neutral lipids of *C. fornicata* samples were isolated following the method following Le Grand et al., (2014). An aliquot of total lipid extract (between 1 and 6 mL depending on species) was evaporated to dryness under nitrogen, recovered with three 0.5 mL washings of chloroform/methanol (98:2, v:v) and deposited at the top of a silica gel micro-column (Pasteur pipette of 40 mm \times 5 mm i.d., plugged with glass wool and filled with silica gel 60, which were both previously heated for 6 h at 450°C and deactivated with 6 % water by weight). Only the neutral lipids (NL), including triglycerides, free fatty acids and sterols, were eluted with 10 mL of chloroform/methanol (98:2, v:v) and collected in 20 mL glass vials. After evaporation to a dryness under nitrogen, the NL fraction was recovered and transferred to 7 mL vials with three 1 mL washings of chloroform/methanol (98:2, v:v).

2.4.3. Transesterification and Gas chromatographer analysis

After the addition of tricosanoic acid as an internal standard and evaporation to dryness under nitrogen, the fatty acid methyl esters (FAME) were obtained using a method modified from Le Grand et al. (2014). A total of 0.8 mL of a sulphuric acid solution (3.8 % in methanol) was added, vortexed and heated for 10 min at 100°C. Before GC analysis, 0.8 mL of hexane was added and the organic phase containing FAME was washed three times with 1.5 mL of hexane-saturated distilled water. The organic phase was finally transferred to tapering vials and stored at -20°C. FAMEs were analyzed in a Varian CP 8400 gas chromatograph (GC) equipped with a split/splitless injector and a flame-ionization detector. FAMEs were identified using two different capillary columns (ZBWAX 30 m × 0.25 mm i.d., 0.25 µm thickness, Phenomenex; and ZB-5HT 30 m × 0.25 mm i.d., 0.25 µm thickness, Phenomenex) by means of a standard 37 component FAME mix (Sigma) and other known standard mixtures. FAs were presented using the common expressed as the molar percentage of the total FA content.

2.5. Statistical analyses

Pigments and FA compositions of OM sources were represented together using a multiple factorial analysis (MFA) (Escofier and Pagès, 1984) whereas principal component analysis (PCA) were used for FA compositions of ontogenic classes of *C. fornicata*. Homogeneity of the data was tested using the permutation analysis of multivariate dispersion (PERMDISP) (Anderson, 2001). Statistical analyses on OM sources (pigments and FA) and *C. fornicata* (FA) were conducted using a non-parametric distanced-based permutation multivariate analysis of variance (PERMANOVA) based on a Euclidean distance. Analyses were performed using two variables: OM sources or ontogenic classes (3 level factors) and sampling dates (4 level factors). Following significant PERMANOVA results, *post hoc* tests were carried out using multiple pairwise comparisons with Bonferroni correction to identify differences among factors (Martinez Arbizu, 2017). However, the number of samples at each sampling date ($3 < n < 5$) was not sufficient to allow significant differences among the two factor levels, because of lack of statistical power when using Bonferroni correction in too many multiple comparisons. Temporal variations and differences in pigment and SI ratios between OM sources at each sampling date

were assessed using non-parametric Kruskal-Wallis test. For the same reason as above, no pairwise comparisons were calculated between OM sources for each sampling date, but over the whole period. Moreover, because concentrations in PPOM were not comparable with biofilm and RPOM (surface *vs.* volume), only RPOM and biofilm concentrations were compared together using a non-parametric Mann-Whitney test at each sampling date. Two-way factorial analyses of variance (ANOVA) were used to assess differences in SI ratios and FA classes/ratios between ontogenic classes of *C. fornicata* and sampling date. When significant, *post hoc* multiple comparisons were carried out using Tukey HSD. Normality and homogeneity of residuals were graphically assessed. Isotopic niche of ontogenic classes of *C. fornicata* was quantified using standard ellipse areas corrected for small samples (Jackson et al., 2011). Statistical analyses were performed in R version 3.3.0 (R Core Team, 2012) using packages ‘vegan’, ‘plyr’, ‘FactoMiner’, ‘SIBER’ and ‘ggplot’.

3. Results

3.1. Organic matter sources

3.1.1. Pigment and fatty acid compositions

Multiple factorial analysis (MFA) combined datasets of pigments and FA (Fig. 27, Table 7 and Table 8). Type of OM sources and sampling dates explained 37 % and 17 % of the inertia in the MFA, respectively. Altogether, these two factors explained 78 % of the inertia. OM sources were well discriminated along the first axis and showed significant difference in FA and pigment compositions (PERMANOVA, $p < 0.001$; pairwise tests, all $p < 0.01$). Although both sampling date and the interaction term were significant (PERMANOVA, $p < 0.001$ and $p < 0.001$, respectively), no differences were detected during *post hoc* pairwise comparisons due to lack statistical power. Biofilm was mainly characterized by four pigments (chlorophyll *b*, neoxanthin, UK 6 and UK 7) and one saturated fatty acids (18:0). The FA 18:2n-6, ant17:0 and 20:4n-6 contributed to a lesser extent to the biofilm. Re-suspended particulate organic matter (RPOM) was mainly characterized by three pigments (lutein, pheophorbide and pheophytin) and three fatty acids, including two SFA (20:0 and 22:0) and one odd-branched FA (ant15:0). The MUFA (16:1n-7) contributed to this source in a lesser extent. Considering the

positions of the two MUFA 18:1n-7 and 18:1n-9 and of the PUFA 20:5n-3, they all contributed to both RPOM and biofilm. PPOM was separated from the two other sources by the second axis and its composition was more variable in time than those of biofilm and RPOM. Temporal variation showed more similarity with RPOM at sampling dates S1 and S2 than at S3 and S4. Overall, PPOM was characterized by four pigments (fucoxanthin, alloxanthin, violaxanthin and diadinoxanthin) and four fatty acids, two PUFA (20:4n-3, 22:5n-6), one SFA (14:0) and one odd-branched FA (iso17:0). 16:0 and chlorophyll *a* contributed to both PPOM and biofilm whereas chlorophyll *c* was present in both PPOM and RPOM.

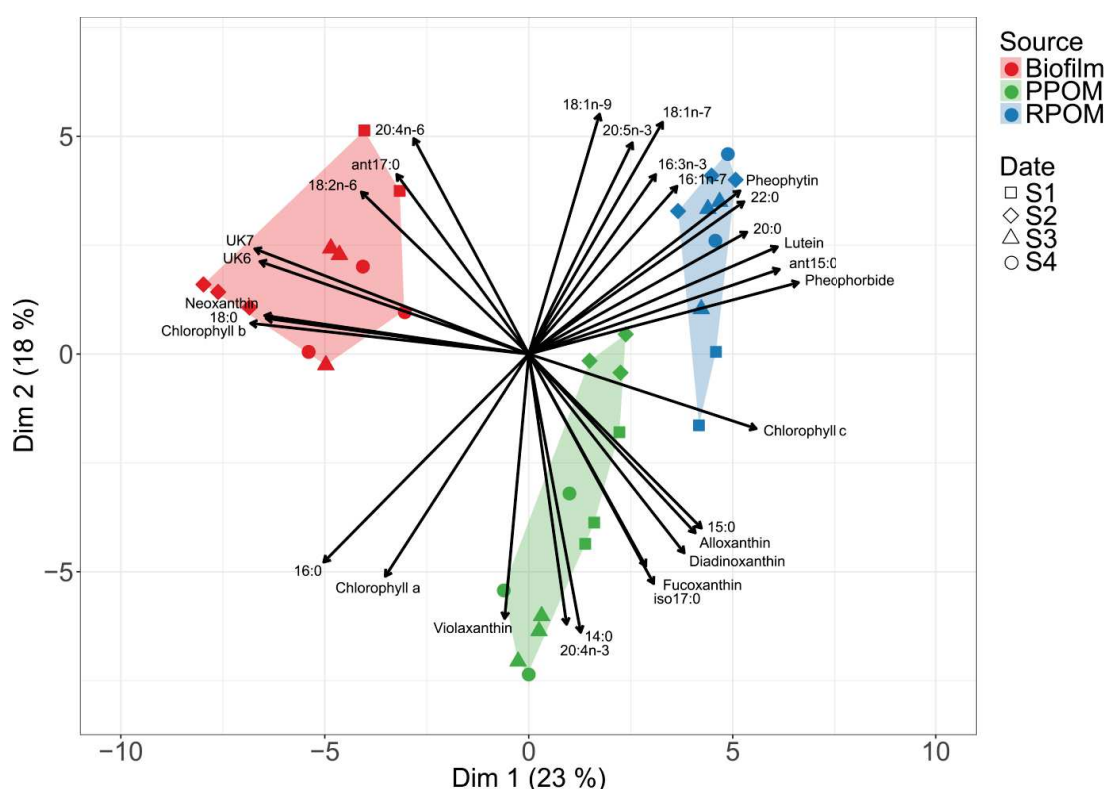


Figure 27: Multiple factorial analysis on the total pigment and fatty acid compositions between organic matter sources (Biofilm, pelagic particulate organic matter (PPOM), re-suspended particulate organic matter (RPOM)) and sampling period (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April). Ordination is along the two first axes and only the 30 most contributing variables are shown. UK : unknown pigments.

Table 7: Pigment composition of organic matter sources (Biofilm, pelagic particulate organic matter (PPOM), re-suspended particulate organic matter (RPOM)) and sampling period (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April) (% of total pigment concentration, mean \pm SD, n = 3). UK: unknown pigments. Chl : chlorophyll.

Pigments	S1			S2			S3			S4		
	Biofilm	PPOM	RPOM	Biofilm	PPOM	RPOM	Biofilm	PPOM	RPOM	Biofilm	PPOM	RPOM
Chlorophyll a	29.6 \pm 3.8	44.3 \pm 5.1	7.6 \pm 1.9	28.5 \pm 1.9	38.1 \pm 2.6	2.7 \pm 1.3	28.7 \pm 2.2	42.4 \pm 0.6	3.9 \pm 0.6	30.9 \pm 3.5	38.6 \pm 2.4	3 \pm 1.1
Chlorophyll b	9.2 \pm 7.9	4.7 \pm 0.2	0.3 \pm 0.2	18.6 \pm 2	3.1 \pm 0.2	0.3 \pm 0.1	15.6 \pm 0.4	4.3 \pm 0	0.3 \pm 0.1	13.5 \pm 1.9	2.7 \pm 0.3	0.2 \pm 0.1
Chlorophyll c	0.8 \pm 0.5	2.9 \pm 0.4	3.6 \pm 0.1	0 \pm 0	8.2 \pm 1.4	6.2 \pm 0.3	0.9 \pm 0.2	9.3 \pm 0.1	6.9 \pm 0.7	0.9 \pm 0.2	4.2 \pm 0.6	7.8 \pm 1.4
Chlorophyllide	1.9 \pm 0.5	0 \pm 0	0.5 \pm 0.2	2.3 \pm 2.3	0.5 \pm 0.9	0.7 \pm 0.2	2.8 \pm 0.7	1.3 \pm 0.2	0.7 \pm 0.1	3.7 \pm 3	2.1 \pm 0.4	0.9 \pm 0.1
Fucoxanthin	9 \pm 1.4	15.2 \pm 2.2	10.5 \pm 0.6	8.7 \pm 1.5	16.4 \pm 1.4	10.2 \pm 0.6	9.7 \pm 2	14.2 \pm 0.2	10.7 \pm 1.3	10.4 \pm 0.4	16 \pm 0.7	12 \pm 0.1
Alloxanthin	2 \pm 1.9	7.1 \pm 1.1	2.9 \pm 1.1	0.8 \pm 0.5	4.7 \pm 1	2.9 \pm 0.8	1.3 \pm 0.8	5.7 \pm 0.2	3.1 \pm 0.3	1.4 \pm 0.2	3.2 \pm 0.4	4 \pm 0.3
Diadinoxanthin	0.6 \pm 0.1	1.6 \pm 0.4	1.1 \pm 0.1	0.4 \pm 0.1	2.2 \pm 0.2	1 \pm 0.1	0.5 \pm 0	2 \pm 0	0.9 \pm 0.2	0.5 \pm 0.1	1.3 \pm 0	1.2 \pm 0
β caroten	0.9 \pm 0.7	0 \pm 0	0.5 \pm 0.1	0.1 \pm 0	0.2 \pm 0.2	0 \pm 0	0.3 \pm 0.1	0.4 \pm 0.1	0 \pm 0	1.1 \pm 0.6	2 \pm 2.1	0.1 \pm 0.1
Neoxanthin	4.3 \pm 2	1 \pm 0.1	0 \pm 0	10.7 \pm 0.8	1.8 \pm 0.2	0 \pm 0	9.3 \pm 0.5	3.1 \pm 0.2	0 \pm 0	4.2 \pm 3	1.3 \pm 0.1	2.8 \pm 0.1
Lutein	1.6 \pm 0.9	5.1 \pm 0.7	9.4 \pm 7.3	1.1 \pm 0.4	4.9 \pm 0.5	11.4 \pm 2.3	1.6 \pm 0.3	1.7 \pm 0.2	10.2 \pm 1	0.9 \pm 0.2	2.8 \pm 0.4	8 \pm 1.5
Violaxanthin	0.3 \pm 0.3	0.8 \pm 0.2	0 \pm 0	0 \pm 0	0.5 \pm 0.5	0 \pm 0	0.6 \pm 0	2 \pm 0.3	0 \pm 0	0.2 \pm 0.4	1.1 \pm 1.1	0 \pm 0
Xanthophyll	0.7 \pm 0.2	1.5 \pm 0.2	7.9 \pm 6.2	0 \pm 0	1.2 \pm 1.1	2.8 \pm 0.7	0.4 \pm 0.1	1.2 \pm 0.1	3.2 \pm 0.3	0.5 \pm 0.2	1.3 \pm 0.1	3 \pm 0.2
Zeaxanthin	6.7 \pm 9.2	3.1 \pm 0.3	3.1 \pm 3	0.6 \pm 0.2	1.7 \pm 0.2	4.6 \pm 1.1	1.2 \pm 0	1 \pm 0.1	4 \pm 0.3	0.8 \pm 0	2 \pm 0.2	3.6 \pm 0.6
Pheophorbide	8.3 \pm 3.7	12 \pm 1.5	30.6 \pm 2	4.8 \pm 1.6	15.5 \pm 1.5	24.5 \pm 1.4	6.3 \pm 1	9.6 \pm 0.3	25.8 \pm 1.5	9.1 \pm 2.3	14.2 \pm 1.5	23.5 \pm 0.3
Pheophytin	2.1 \pm 2.3	0 \pm 0	21.2 \pm 0.4	0.7 \pm 0.6	0.5 \pm 0.9	31.9 \pm 2.1	2.1 \pm 0.4	0.8 \pm 0	29.8 \pm 1.6	5.5 \pm 1	6.1 \pm 3.8	29 \pm 2.1
UK3	0.8 \pm 0.1	0.5 \pm 0	0.7 \pm 0.1	1.2 \pm 0.2	0.6 \pm 0.3	0.7 \pm 0.1	0.8 \pm 0	0.9 \pm 0.1	0.6 \pm 0	0.7 \pm 0.2	0.5 \pm 0.3	1 \pm 0.2
UK6	8.5 \pm 6.2	0 \pm 0	0 \pm 0	19.4 \pm 4	0 \pm 0	0 \pm 0	16.5 \pm 0.6	0 \pm 0	0 \pm 0	14.1 \pm 2.6	0 \pm 0	0 \pm 0
UK7	0.8 \pm 0.1	0 \pm 0	0 \pm 0	1.2 \pm 0	0 \pm 0	0 \pm 0	0.8 \pm 0.3	0 \pm 0	0 \pm 0	1.1 \pm 0.1	0 \pm 0	0 \pm 0
UK8	11.9 \pm 19.9	0 \pm 0	0 \pm 0	0.7 \pm 0.4	0 \pm 0	0 \pm 0	0.7 \pm 0.6	0 \pm 0	0 \pm 0	0.5 \pm 0.4	0 \pm 0	0 \pm 0
UK9	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0.5 \pm 0.2	0 \pm 0
Chl b/a	0.3 \pm 0.3	0.1 \pm 0	0 \pm 0	0.7 \pm 0.1	0.1 \pm 0	0.1 \pm 0.1	0.5 \pm 0.1	0.1 \pm 0	0.1 \pm 0	0.4 \pm 0.1	0.1 \pm 0	0.1 \pm 0
Chl c/a	0 \pm 0	0.1 \pm 0	0.5 \pm 0.1	NA \pm NA	0.2 \pm 0.1	2.8 \pm 1.8	0 \pm 0	0.2 \pm 0	1.8 \pm 0.1	0 \pm 0	0.1 \pm 0	2.9 \pm 1.5
Fucoxanthin / chl a	0.3 \pm 0	0.4 \pm 0.1	1.4 \pm 0.3	0.3 \pm 0.1	0.4 \pm 0.1	4.6 \pm 2.6	0.3 \pm 0	0.3 \pm 0	2.8 \pm 0.5	0.3 \pm 0	0.4 \pm 0	4.3 \pm 1.6

Table 8: Fatty acids (FA) composition of organic matter sources (Biofilm, pelagic particulate organic matter (PPOM), re-suspended particulate organic matter (RPOM)) and sampling period (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April) (% of total FA concentration, mean \pm SD, n = 5). BFA: Branched FA; SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; UK: unknown FA.

Fatty acids	S1			S2			S3			S4		
	Biofilm	PPOM	RPOM	Biofilm	PPOM	RPOM	Biofilm	PPOM	RPOM	Biofilm	PPOM	RPOM
ant15:0	0.6 \pm 0.1	1.2 \pm 0.1	3.7 \pm 0.7	0.3 \pm 0.1	1 \pm 0.1	2.9 \pm 0.6	0.8 \pm 0.2	1 \pm 0.1	3 \pm 0.4	0.5 \pm 0.2	1.2 \pm 0.2	3.2 \pm 0.3
ant17:0	2.5 \pm 2.4	0.3 \pm 0.3	0.9 \pm 0.1	0.9 \pm 0.6	1.2 \pm 0.1	1.1 \pm 0.1	2.6 \pm 0.8	0 \pm 0	0.6 \pm 0	1.6 \pm 0.5	0 \pm 0	0.2 \pm 0.2
iso15:0	1.8 \pm 0.7	1 \pm 0.2	2.7 \pm 0.4	0.6 \pm 0.5	1 \pm 0.1	1.9 \pm 0.8	1.3 \pm 0.9	1.1 \pm 0.1	2 \pm 0.1	1.2 \pm 0.4	1.3 \pm 0.2	2.5 \pm 0.2
iso16:0	0.8 \pm 0.2	0.9 \pm 0.2	2.3 \pm 0.4	0.2 \pm 0	0.5 \pm 0	0.9 \pm 0.1	0.6 \pm 0.2	0.5 \pm 0	0.8 \pm 0.2	0.5 \pm 0.2	0.5 \pm 0.1	0.9 \pm 0.1
iso17:0	0.9 \pm 0.3	1.7 \pm 0.3	1.4 \pm 0.2	0.1 \pm 0.1	0.3 \pm 0.1	1.2 \pm 0.1	0.5 \pm 0.4	2.8 \pm 0.1	1.2 \pm 0.1	0.8 \pm 0.5	2.7 \pm 0.2	1.6 \pm 0.1
iso18:0	0.1 \pm 0.1	1.2 \pm 0.9	1.1 \pm 0.2	0.1 \pm 0.1	0.4 \pm 0	0.6 \pm 0.1	1.1 \pm 0.2	0.9 \pm 0.2	0.4 \pm 0.1	0.4 \pm 0.3	1 \pm 0.3	0.7 \pm 0.1
Σ Branched	6.8 \pm 2.4	6.3 \pm 0.3	12.1 \pm 1.4	2.2 \pm 0.2	4.4 \pm 0.1	8.5 \pm 1.6	6.8 \pm 0.8	6.2 \pm 0.4	8 \pm 0.7	5 \pm 1.3	6.6 \pm 0.9	9 \pm 0.4
14:0	3.1 \pm 0.3	7.3 \pm 1.3	4.9 \pm 0.8	1.5 \pm 0.4	5.2 \pm 0.5	3.5 \pm 0	4.2 \pm 0.6	12 \pm 0.8	4.1 \pm 0.2	6.1 \pm 2.1	8.6 \pm 1	4.1 \pm 0.3
15:0	0.9 \pm 0.3	2.6 \pm 0.4	1.5 \pm 0.2	0.2 \pm 0.1	1.9 \pm 0.2	1.2 \pm 0.1	0.9 \pm 0.2	1.7 \pm 0.1	1 \pm 0.1	0.8 \pm 0.1	1.4 \pm 0.2	1.2 \pm 0.1
16:0	21.5 \pm 3.4	33 \pm 4.9	22.4 \pm 3.2	38.6 \pm 0.7	26.3 \pm 2.3	17.6 \pm 1.6	29.5 \pm 4.3	32.7 \pm 1.9	14.8 \pm 1.4	30.5 \pm 4.5	34.1 \pm 2.3	18.2 \pm 0.2
17:0	1.6 \pm 0.7	1.6 \pm 0.3	1.3 \pm 0.2	0.8 \pm 0.8	0.7 \pm 0	0.9 \pm 0.1	0.8 \pm 0.3	0.9 \pm 0.1	0.8 \pm 0.1	0.7 \pm 0.1	0.9 \pm 0.1	1 \pm 0.1
18:0	16.5 \pm 1.3	11.8 \pm 1.7	9.6 \pm 1.3	42.3 \pm 2.6	13.3 \pm 1.5	10.9 \pm 2.5	21.5 \pm 4.8	9.2 \pm 0.7	6.8 \pm 1	21.9 \pm 3.8	14.9 \pm 5.3	7.6 \pm 0.3
20:0	0.6 \pm 0.1	1.7 \pm 0.1	1.9 \pm 1.7	0.6 \pm 0	1.3 \pm 0	2.8 \pm 0.3	0.8 \pm 0.2	0.9 \pm 0	2.6 \pm 0.3	0.9 \pm 0.2	0.4 \pm 0.7	3 \pm 0.2
22:0	1 \pm 0.1	1.4 \pm 1.2	3.2 \pm 3	0.3 \pm 0.2	1.6 \pm 0	4.4 \pm 0.7	1.2 \pm 0.3	0.7 \pm 0.6	4.5 \pm 0.5	1.4 \pm 0.4	1.4 \pm 0.3	5.4 \pm 0.3
24:0	0.1 \pm 0	0 \pm 0	0.5 \pm 0.4	0 \pm 0	0 \pm 0	0.2 \pm 0.3	0.7 \pm 0.3	4.4 \pm 2	6.1 \pm 5.2	0.1 \pm 0.1	0.1 \pm 0.2	0.3 \pm 0.3
Σ SFA	45.2 \pm 4.7	59.4 \pm 7.6	45.4 \pm 3.3	84.2 \pm 2.1	50.3 \pm 3.9	41.4 \pm 3.3	59.6 \pm 9.9	62.4 \pm 1.1	40.8 \pm 7.3	62.3 \pm 7.4	61.9 \pm 5.9	40.7 \pm 0.5
14:1n-5	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0.1 \pm 0.1	0 \pm 0	0.5 \pm 0.1	0.2 \pm 0.2	0 \pm 0	0 \pm 0
16:1n-5	0.5 \pm 0.1	0.8 \pm 0.1	0.4 \pm 0.4	0.1 \pm 0	0.7 \pm 0.1	0.7 \pm 0.1	0.4 \pm 0.1	0.5 \pm 0.4	0.8 \pm 0.2	0.3 \pm 0.1	0.4 \pm 0.1	1.2 \pm 0.3
16:1n-7	3.8 \pm 0.1	1.9 \pm 1.5	5.6 \pm 3	0.8 \pm 0.4	4.5 \pm 0.5	8.8 \pm 0.1	2.7 \pm 1.8	0.9 \pm 0.2	7.3 \pm 2.4	2.5 \pm 1.1	1.6 \pm 0.7	0 \pm 0
16:1n-9	0.9 \pm 0.1	1.5 \pm 0.9	0.3 \pm 0.5	0.2 \pm 0.2	2 \pm 0.2	1.2 \pm 0.2	0.6 \pm 0.3	0.4 \pm 0.4	0.7 \pm 0.2	0.9 \pm 0.2	0.5 \pm 0.1	0.9 \pm 0.1
16:2n-4	0.2 \pm 0.2	0 \pm 0	0.2 \pm 0.4	0.2 \pm 0.2	0.1 \pm 0.2	0.7 \pm 0.1	2.1 \pm 0.7	0 \pm 0	0.4 \pm 0.5	0.9 \pm 1.1	0.3 \pm 0.3	0.2 \pm 0.2
16:3n-3	0.2 \pm 0.1	0 \pm 0	0.2 \pm 0.3	0.1 \pm 0.1	1 \pm 0.1	1.2 \pm 0.3	0.6 \pm 0.5	0 \pm 0	1.2 \pm 0.9	0.4 \pm 0.2	0.3 \pm 0.3	0.9 \pm 0.2
16:3n-4	0.6 \pm 0.1	0.3 \pm 0.5	0.7 \pm 0.6	0.1 \pm 0.1	1.2 \pm 0.1	1.3 \pm 0.1	0 \pm 0	0 \pm 0	0 \pm 0	0.5 \pm 0.1	0 \pm 0	1 \pm 0.9
16:3n-6	0.4 \pm 0.2	0.2 \pm 0.3	0 \pm 0	0.1 \pm 0.1	0 \pm 0	0.3 \pm 0.3	0.4 \pm 0.3	0 \pm 0	0.2 \pm 0.4	0.3 \pm 0.1	0.1 \pm 0.2	1.1 \pm 0.2
16:4n-3	0.3 \pm 0.1	1.3 \pm 1.1	1.2 \pm 0.2	0.3 \pm 0.2	1.3 \pm 0.1	0.8 \pm 0.1	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
17:1n-7	0.1 \pm 0.2	0.4 \pm 0.1	0.8 \pm 0.3	0.2 \pm 0.1	0.4 \pm 0.1	0.6 \pm 0.1	0.6 \pm 0.3	0.8 \pm 0.1	0.3 \pm 0.2	0.7 \pm 0.3	0.8 \pm 0.1	0.7 \pm 0.2
18:1n-7	2 \pm 0.2	0 \pm 0	0 \pm 0	1.1 \pm 0.3	2.7 \pm 0.5	5.8 \pm 0.3	2.1 \pm 1.3	0.5 \pm 0.5	4.2 \pm 1.5	2 \pm 0.8	0.6 \pm 0.7	6.9 \pm 1.4
18:1n-9	1.6 \pm 0.7	0.3 \pm 0.5	1.3 \pm 2.3	1 \pm 0.7	2.5 \pm 0.7	2.9 \pm 0.3	0.9 \pm 0.8	0 \pm 0	1.9 \pm 1	2.2 \pm 0.9	0.2 \pm 0.3	3.1 \pm 1.1
20:1n-7	0.6 \pm 0.5	0 \pm 0	0 \pm 0	0.1 \pm 0	0 \pm 0	0 \pm 0	0.2 \pm 0.1	0 \pm 0	0 \pm 0	0.2 \pm 0.1	0 \pm 0	0.1 \pm 0.2
20:1n-9	0.3 \pm 0	0.3 \pm 0.5	0.3 \pm 0.4	0.1 \pm 0	0 \pm 0	0.3 \pm 0.3	0.3 \pm 0.2	0 \pm 0	0.4 \pm 0.8	0.3 \pm 0	0.5 \pm 0.4	0.1 \pm 0.2
20:1n-11	1.2 \pm 0.4	1.1 \pm 1.5	0.3 \pm 0.5	0.2 \pm 0.1	3.6 \pm 0.6	0.5 \pm 0.4	0.5 \pm 0.4	1.2 \pm 1	0.6 \pm 0.6	0.6 \pm 0.3	0.4 \pm 0.7	0.8 \pm 0.1
22:1n-9	0.2 \pm 0.1	0 \pm 0	0.3 \pm 0.6	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
Σ MUFA	11.3 \pm 1.3	6.4 \pm 4.3	9.2 \pm 6.4	3.6 \pm 0.7	16.4 \pm 2.5	20.7 \pm 0.6	8.3 \pm 4.7	4.3 \pm 2.4	16.7 \pm 6.3	9.9 \pm 2.4	5.2 \pm 1.9	13.8 \pm 3.4
18:2n-6	4.2 \pm 2.1	0.9 \pm 0.8	0.7 \pm 1	1.9 \pm 0.4	1.2 \pm 0.2	1.1 \pm 0.3	3 \pm 1.8	0.9 \pm 0.1	1.2 \pm 0.3	2.3 \pm 0.9	0.9 \pm 0.2	1.4 \pm 0.3
18:3n-3	0.7 \pm 0.3	0.6 \pm 0.7	0.3 \pm 0.5	0.2 \pm 0.1	1.3 \pm 0.2	0.7 \pm 0	0.5 \pm 0.2	0.2 \pm 0.3	0.4 \pm 0.4	0.4 \pm 0.2	0.4 \pm 0.4	0.7 \pm 0.1
18:3n-4	0.6 \pm 0.1	1.4 \pm 0.5	5.9 \pm 1.7	0.1 \pm 0	1.2 \pm 0.4	1.6 \pm 0.1	0.4 \pm 0	0.8 \pm 0.3	2.5 \pm 1.7	0.2 \pm 0	0.7 \pm 0.3	0.7 \pm 0.1
18:3n-6	0.2 \pm 0	0.2 \pm 0.3	0.4 \pm 0.7	0.1 \pm 0	0.4 \pm 0	0 \pm 0	0.3 \pm 0	0 \pm 0	0.2 \pm 0.2	0.2 \pm 0.2	0.1 \pm 0.2	0.4 \pm 0.1
18:4n-3	1.1 \pm 0.9	2.7 \pm 0.3	1.2 \pm 0.2	0.2 \pm 0.1	3.8 \pm 0.5	1.6 \pm 0.2	1 \pm 0.4	2.5 \pm 0.5	2.2 \pm 0.6	0.8 \pm 0.3	1.9 \pm 0.6	2.5 \pm 0.5
20:2n-6	0.6 \pm 0.2	1.3 \pm 0.2	0.9 \pm 0.2	0.2 \pm 0	0.1 \pm 0.2	0.2 \pm 0.3	0.3 \pm 0	0 \pm 0	0.4 \pm 0.7	0.4 \pm 0.4	1.1 \pm 0.2	1.3 \pm 0.1
20:3n-3	0.2 \pm 0	0.5 \pm 0.9	0 \pm 0	0.1 \pm 0	0 \pm 0	0.3 \pm 0.2	0 \pm 0	0 \pm 0	0 \pm 0	0.2 \pm 0.1	0 \pm 0	0.4 \pm 0
20:3n-6	0.2 \pm 0	0.5 \pm 0.1	0 \pm 0	0.1 \pm 0	0.5 \pm 0	0.5 \pm 0.1	0.2 \pm 0.1	0 \pm 0	0.4 \pm 0.1	0.3 \pm 0.2	0.3 \pm 0.6	0.4 \pm 0
20:4n-3	0.4 \pm 0.2	1.6 \pm 0.2	1.1 \pm 0.2	0.1 \pm 0	0.7 \pm 0	0.7 \pm 0.1	0.8 \pm 0.3	2.3 \pm 0.5	0.6 \pm 0.1	1.3 \pm 0.5	2.2 \pm 0.9	0.5 \pm 0.4
20:4n-6	6.3 \pm 0.9	0 \pm 0	0.7 \pm 0.7	1.6 \pm 0.2	0.2 \pm 0.2	1.5 \pm 0.1	2.8 \pm 1.7	0.2 \pm 0.3	1.2 \pm 0.4	2 \pm 0.7	0.1 \pm 0.2	2.2 \pm 0.4
20:5n-3	8.2 \pm 2.1	2.2 \pm 1.3	4.6 \pm 1	1.4 \pm 0.7	4.5 \pm 0.6	6.3 \pm 1.2	3.4 \pm 2.5	0.8 \pm 0.3	3 \pm 3	2.2 \pm 0.6	1.7 \pm 1	9.7 \pm 2.3
21:5n-3	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	3 \pm 5.3	2.3 \pm 3.4	0 \pm 0	0 \pm 0
22:2n-6	0.3 \pm 0.1	0.4 \pm 0	0.9 \pm 0.1	0.3 \pm 0	0.9 \pm 0	1.3 \pm 0.2	2.2 \pm 1.5	7.3 \pm 1	2.8 \pm 2	1.5 \pm 0.8	9.1 \pm 3.3	3 \pm 2.5
22:5n-3	0.7 \pm 0.8	0 \pm 0	0 \pm 0	0.1 \pm 0	0.1 \pm 0.2	0 \pm 0	0.7 \pm 0.1	2.1 \pm 0.6	2.1 \pm 1.7	0.6 \pm 0.5	2.2 \pm 0.5	2.3 \pm 0.7
22:5n-6	0.											

concentration increased over time for both PPOM and RPOM ($p < 0.05$ and $p < 0.05$, respectively, Fig. 28c).

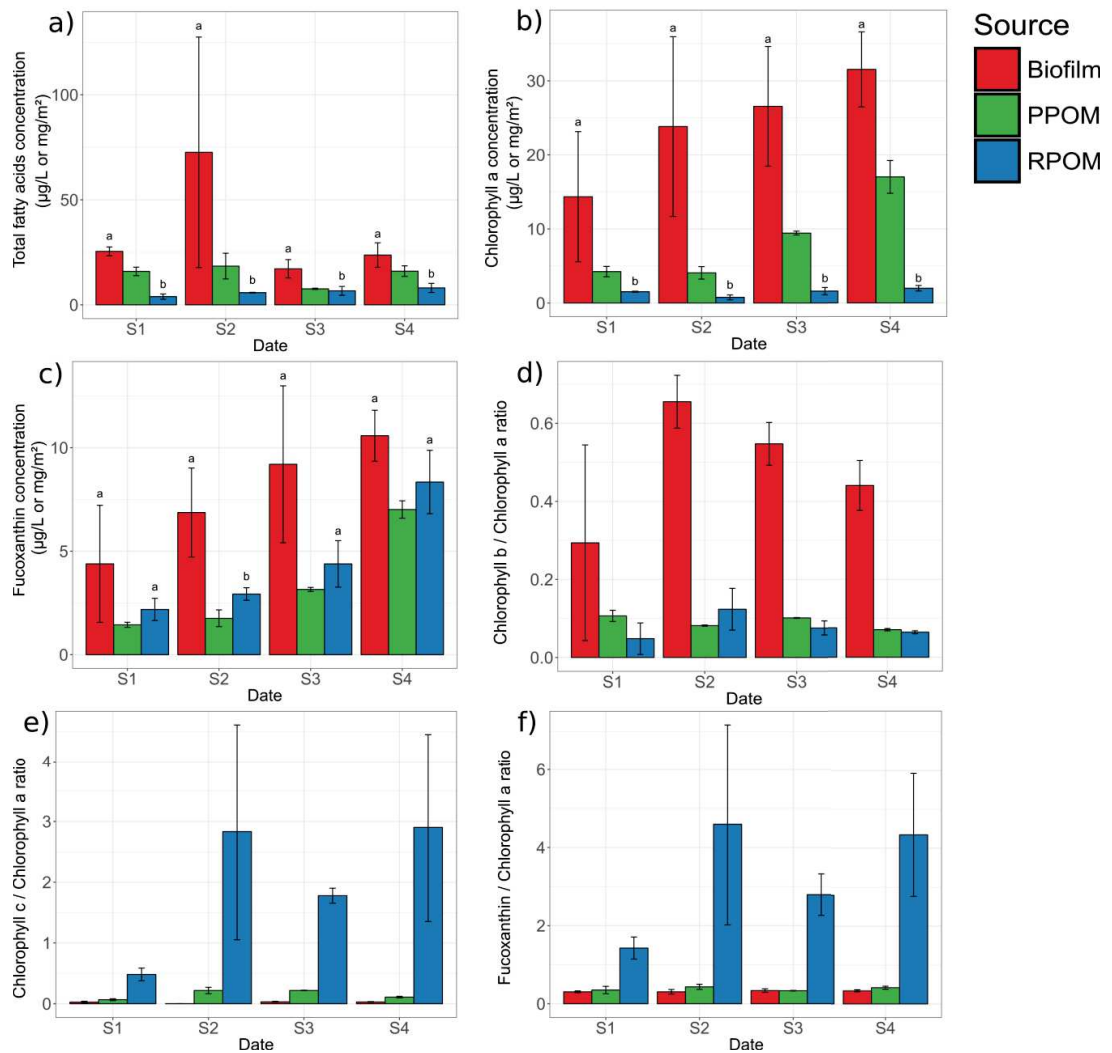


Figure 28: (a) Total fatty acids, (b) chlorophyll *a* and (c) fucoxanthin concentrations (mean \pm SD, $n = 3$), and ratios between (d) chlorophyll *b*, (e) chlorophyll *c*, and (f) fucoxanthin over chlorophyll *a* for the potential organic matter sources. S1 to S4 corresponds to sampling periods (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April). Letters correspond to significant between-sources differences at each sampling date ($p < 0.05$).

For the same reason as above (i.e., variability), biofilm did not change over time, and was significantly higher than RPOM only at sampling date S2. Chlorophyll *b* over chlorophyll *a* ratio showed significant change over time in biofilm ($p < 0.05$) and PPOM ($p < 0.05$) (Fig. 28d). Over the study period, this ratio was significantly higher for biofilm than for RPOM ($p < 0.001$) and PPOM ($p < 0.001$), whereas it did not differ between RPOM and PPOM. Fucoxanthin and chlorophyll *c* over chlorophyll *a* ratios did not show significant changes over time for any OM sources, despite lower values at S1 for RPOM (Fig. 28e and Fig. 28f, respectively). However, over the study period, ratios were significantly higher in RPOM than in

PPOM ($p < 0.001$ and 0.001 respectively) and biofilm ($p < 0.001$ and 0.001 respectively). PPOM ratios were also significantly higher than for the biofilm ($p < 0.05$ and 0.001 respectively).

3.1.2. Stable isotopes composition

No significant temporal variations were observed in the SI composition of OM sources over the studied period (Fig. 29a and 29b). However, OM sources were well discriminated according to their carbon and nitrogen SI compositions (Fig. 29c). Biofilm was significantly enriched in ^{15}N (9.5 ± 0.6 ‰) compared to PPOM (6.8 ± 0.5 ‰, $p < 0.001$) and RPOM (7 ± 0.3 ‰, $p < 0.001$), and PPOM was significantly depleted in ^{13}C (-25.2 ± 0.4 ‰) compared to RPOM (-22.7 ± 0.2 ‰, $p < 0.001$) and biofilm (-22.7 ± 0.7 ‰, $p < 0.001$). Biofilm and RPOM respectively showed the highest and lowest temporal variations over the sampling period.

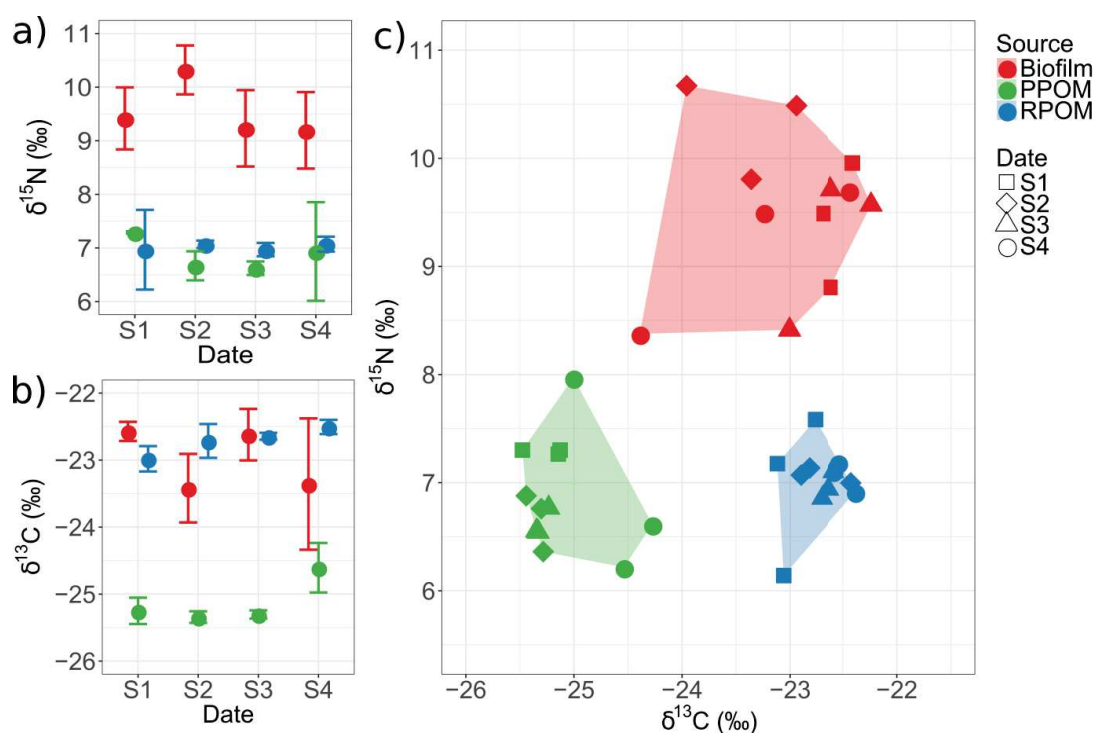


Figure 29: (a) $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ isotopic compositions (mean \pm SD, $n = 3$), and overall isotopic (c) biplot for organic matter source (Biofilm, pelagic particulate organic matter (PPOM), re-suspended particulate organic matter (RPOM)) and sampling period (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April).

3.2. *Crepidula fornicata*

3.2.1. Fatty acids composition

Ontogenic classes and sampling dates explained 23 % and 17 % of the inertia in the PCA, respectively (Fig. 30). Altogether, these two factors explained 56 % of the inertia. Ontogenic classes were well separated along the first axis and showed significant difference in FA compositions (PERMANOVA, $p < 0.001$), with no overlap between motile males and sessile females. FA composition differed with sampling dates (PERMANOVA, $p < 0.001$), which were discriminated along the second axis by the two FA 16:1n-5 and 18:4n-3 (Table 9). Particularly, sampling date S1 was significantly different from S3 and S4 (pairwise tests, all $p < 0.05$). The interaction between the two factors was significant (PERMANOVA, $p < 0.001$) with more marked variations for motile males than for sessile females. However, *post hoc* test failed to find significant differences due to lack statistical power. We also observed higher intra-class variability for motile males than for sessile females, without any overlap. This is illustrated by the convex hull area.

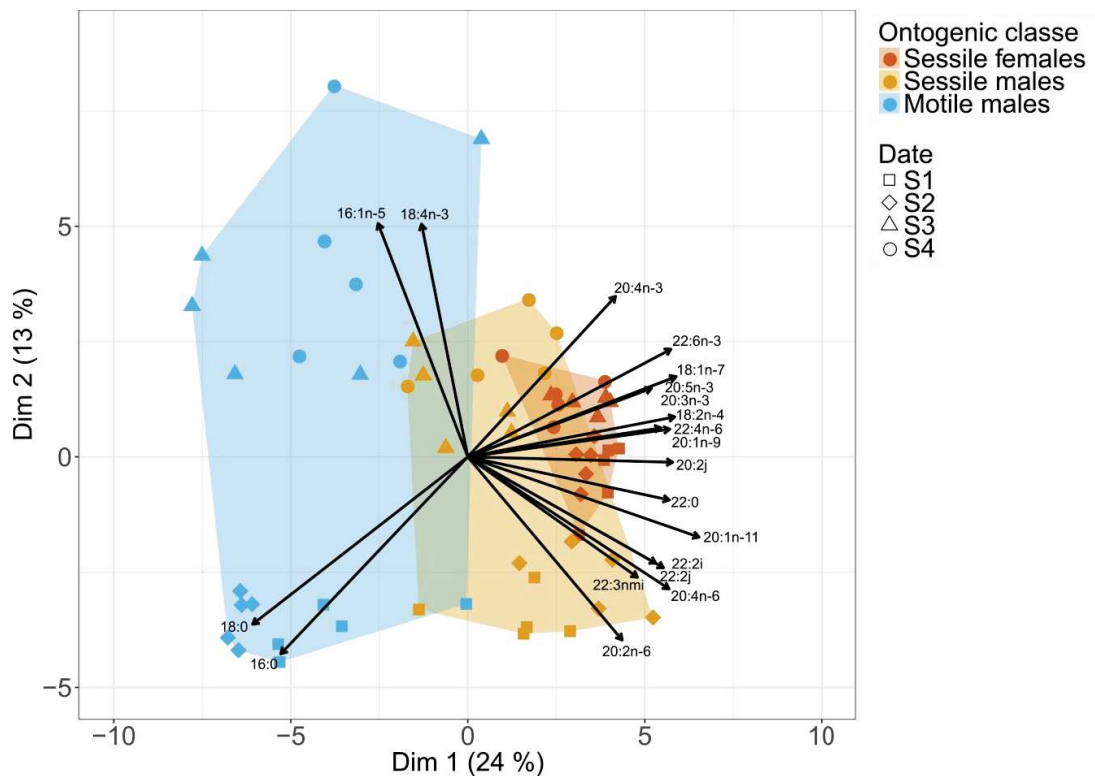


Figure 30: Principal component analysis based on total fatty acids (FA) compositions of ontogenic classes of *Crepidula fornicata* (motile males, sessile males, sessile females) and sampling periods (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April). Ordination is along the two first axes and only the 20 most contributing FA are shown.

Motile males at S1 and S2 were characterized by a high contribution of the two SFA 16:0 and 18:0 and a low inter-individual variability of FA profiles whereas the S3 and S4 period was characterized by a higher contribution of 18:4n-3 and 16:1n-5 and a high inter-individual variability of FA profiles (Table 9). When considering sessile males, the S1-S2 period was characterized by a higher contribution of C₂₀ FA (20:4n-6, 20:2n-6 and 20:1n-11) as well as non-methyl-interrupted (NMI) FA whereas the S3-S4 period was mostly characterized by a higher contribution of 20:4n-3. Sessile female showed the lowest variability of the three ontogenic classes and were characterized by higher contribution of PUFA (22:6n-3, 20:5n-3, 20:3n-3, 18:2n-4 and 22:4n-6) and MUFA (18:1n-7 and 20:1n-9).

Table 9: Fatty acids (FA) composition of the ontogenic classes of *Crepidula fornicata* (motile males, sessile males, sessile females) at the four sampling date (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April) (% of total FA concentration, mean \pm SD, n = 5). BFA: Branched FA; SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; NMI FA: non-methyl-interrupted FA; DMA: Dimethyl acetals FA.

Fatty acids	S1			S2			S3			S4		
	Motile male	Sessile male	Sessile female	Motile male	Sessile male	Sessile female	Motile male	Sessile male	Sessile female	Motile male	Sessile male	Sessile female
TMTD	0.8 ± 0.6	2.1 ± 1.1	1.3 ± 0.3	0.5 ± 0.4	0 ± 0.1	1.3 ± 0.2	0.9 ± 1.2	2.5 ± 0.4	1.3 ± 0.7	2.5 ± 1	2.9 ± 0.8	2.1 ± 1
ant15:0	0 ± 0	0 ± 0	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.1	0.3 ± 0.5	0 ± 0	0.1 ± 0.1
ant17:0	2.4 ± 0.6	3.2 ± 0.7	3.6 ± 1.6	1.5 ± 0.3	3.5 ± 1	2.1 ± 0.5	3 ± 1	3.1 ± 0.4	2.8 ± 0.7	2.5 ± 0.3	1.8 ± 0.2	2.4 ± 0.7
iso16:0	1.1 ± 0.1	1.1 ± 0.3	1.3 ± 0.5	0.1 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 1	1 ± 0.1	0.9 ± 0.1	0.6 ± 0.4	0.5 ± 0.1	0.8 ± 0.2
iso17:0	3.4 ± 0.5	4.1 ± 1	5.4 ± 1.8	1.1 ± 1	1.7 ± 2.4	3.1 ± 1.8	2.5 ± 1.1	2.8 ± 1.4	3.5 ± 2	3 ± 0.3	2.4 ± 0.1	3.8 ± 1
iso18:0	0.2 ± 0.4	0.6 ± 0.4	0.7 ± 0.4	0 ± 0	0.2 ± 0.3	0.3 ± 0.3	0.2 ± 0.4	0.9 ± 0.2	0.5 ± 0.5	2.1 ± 2.9	0.7 ± 0.1	0.4 ± 0.6
Σ BFA	7.1 ± 1.3	8.9 ± 2.1	11 ± 4.3	2.7 ± 1.1	6.3 ± 2.6	6.2 ± 2.4	6.3 ± 2.2	7.8 ± 1.8	7.7 ± 2.7	8.6 ± 3.6	5.5 ± 0.3	7.4 ± 2.3
14:0	1.4 ± 0.4	1.3 ± 0.7	1.5 ± 0.3	1.6 ± 0.1	3 ± 0.9	1.3 ± 0.2	0.8 ± 0.8	1.4 ± 0.3	1.3 ± 0.4	1.9 ± 0.7	2.3 ± 0.4	2 ± 0.5
15:0	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.4	0.5 ± 0.1	0.4 ± 0	0.1 ± 0.3	0.4 ± 0	0.5 ± 0.1	0.6 ± 0	0.5 ± 0.1	0.5 ± 0.1
16:0	25.2 ± 3.5	16.7 ± 3.1	11.9 ± 0.8	33.7 ± 1.7	11.6 ± 3.3	11.7 ± 1	12.3 ± 2.3	13 ± 0.9	10.9 ± 1	14.2 ± 2.1	12.6 ± 3.1	12 ± 0.4
17:0	1 ± 0.1	1.2 ± 0.3	1.5 ± 0.1	0.7 ± 0.4	1.2 ± 0.1	1.4 ± 0.1	0.4 ± 0.5	0.8 ± 0.1	1 ± 0.5	1 ± 0.1	0.8 ± 0.1	0.9 ± 0.5
18:0	27.5 ± 4.2	17.7 ± 7.3	6.3 ± 0.9	35.6 ± 0.9	9.2 ± 3.9	6.8 ± 0.7	14.8 ± 4.5	10.4 ± 1.3	6.6 ± 0.9	14.3 ± 3.1	9.1 ± 5.6	6.9 ± 0.4
20:0	0.5 ± 0.3	0.7 ± 0.2	0.5 ± 0.1	0.6 ± 0.3	0.2 ± 0.2	0.5 ± 0.3	0 ± 0	0.2 ± 0.3	0.2 ± 0	0.1 ± 0.2	0.2 ± 0.1	0.2 ± 0
22:0	0.1 ± 0.2	0.3 ± 0.2	0.5 ± 0.1	0 ± 0	0.2 ± 0	0.4 ± 0.1	0 ± 0	0.1 ± 0.1	0.4 ± 0.1	0 ± 0	0.2 ± 0.1	0.3 ± 0.1
24:0	0.6 ± 0.4	0.6 ± 0.4	0.4 ± 0.1	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0	1.6 ± 1.8	0.1 ± 0.1	0.3 ± 0.2	1.2 ± 0.5	0.3 ± 0.1	0.4 ± 0.1
Σ SFA	57 ± 8.2	39.2 ± 9.6	23.1 ± 1.6	73.5 ± 2.4	26 ± 7.1	22.8 ± 1.5	30.1 ± 5.9	26.4 ± 1.7	21.2 ± 1.1	33.3 ± 4.6	25.8 ± 8.6	23.1 ± 1
14:1n-5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1	0.2 ± 0.5	0.1 ± 0.1	0 ± 0
16:1n-11	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.4	0.4 ± 0.1	1.1 ± 1	0.2 ± 0.1	0.1 ± 0.1
16:1n-5	0 ± 0	0 ± 0	0.4 ± 0.4	0 ± 0	0.1 ± 0.1	0.3 ± 0.1	1.4 ± 1.4	0.8 ± 0.4	0.3 ± 0.1	1.8 ± 1.2	0.4 ± 0.2	0.5 ± 0.2
16:1n-7	0.8 ± 0.3	0.9 ± 0.2	1.4 ± 0.6	0.3 ± 0.4	1.3 ± 0.4	1.7 ± 0.2	2.6 ± 1.3	1.2 ± 0.4	1.5 ± 0.3	2.1 ± 0.3	2.9 ± 0.8	2.3 ± 0.6
16:1n-9	0.3 ± 0.3	0.5 ± 0.3	0.7 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.7 ± 0.2	0.4 ± 1	0.7 ± 0.5	0.5 ± 0.1	0.7 ± 0.3	0.6 ± 0.1	0.5 ± 0
17:1n-7	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0.1 ± 0.3	0.4 ± 0.5	0.2 ± 0.3	0.1 ± 0.1	0.3 ± 0.4
18:1n-5	0.1 ± 0.2	0.2 ± 0.2	0.4 ± 0	0.2 ± 0.4	0.4 ± 0	0.4 ± 0	0.3 ± 0.4	0.2 ± 0.2	0.4 ± 0	0.5 ± 0.3	0.3 ± 0.1	0.3 ± 0
18:1n-7	1.4 ± 0.2	1.8 ± 0.3	2.5 ± 0.2	1 ± 0.1	2.1 ± 0.3	2.8 ± 0.3	0.7 ± 1.1	1.7 ± 0.3	2.5 ± 0.2	2 ± 0.9	2.3 ± 0.5	2.3 ± 0.3
18:1n-9	5.1 ± 2.3	0 ± 0	0 ± 0.1	4 ± 0.8	2.1 ± 0.2	2.1 ± 0.3	1.6 ± 1	2.3 ± 0.1	2 ± 0.2	1.8 ± 0.4	2 ± 0.1	2 ± 0.2
18:1n-11	0.1 ± 0.3	0.4 ± 0.1	0.5 ± 0.2	0 ± 0	0.4 ± 0.1	0.5 ± 0.1	1.6 ± 1	0.9 ± 0.2	0.5 ± 0.2	1.4 ± 1	0.5 ± 0.1	0.8 ± 0.2
20:1n-7	2.4 ± 0.8	3.9 ± 0.6	4.7 ± 0.5	1.3 ± 0.2	5.2 ± 0.9	4.6 ± 0.2	5.9 ± 4.3	3.5 ± 0.8	4.6 ± 0.4	4 ± 1	4.2 ± 1.1	4.2 ± 0.3
20:1n-9	0.5 ± 0.4	1 ± 0.2	0.9 ± 0.1	0.1 ± 0.2	1.2 ± 0.1	0.8 ± 0	0.4 ± 0.5	1 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
20:1n-11	3.2 ± 0.7	5.3 ± 0.7	5.8 ± 0.5	1.9 ± 0.5	6.4 ± 0.9	6.1 ± 0.8	2.3 ± 1.5	4 ± 0.9	5.6 ± 0.6	2.3 ± 0.6	4.1 ± 1	5.2 ± 0.9
22:1n-7	0 ± 0	0 ± 0	0.2 ± 0.1	0 ± 0	0.1 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0.1 ± 0.1
22:1n-9	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	0.2 ± 0.1	0 ± 0	0.1 ± 0.1	0.2 ± 0.2
Σ MUFA	13.9 ± 2.5	14 ± 2.1	17.7 ± 1.1	8.8 ± 0.7	19.4 ± 1.5	20.2 ± 0.6	17.2 ± 1.5	17.2 ± 1.5	19.9 ± 1	18.9 ± 2.8	18.8 ± 2.9	19.7 ± 0.9
16:2n-4	0 ± 0	0.1 ± 0.1	0.4 ± 0.5	0.2 ± 0.5	0.5 ± 0.4	0.2 ± 0	0.2 ± 0.4	1 ± 0.9	0.7 ± 0.3	0.1 ± 0.3	0.7 ± 0.3	0.4 ± 0.2
16:2n-7	0 ± 0	0 ± 0	0 ± 0	0.7 ± 1	2.6 ± 2.5	0.7 ± 1.5	0 ± 0	0 ± 0	0.8 ± 1.7	0 ± 0	0 ± 0	0 ± 0
16:3n-3	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0.1	0.1 ± 0.1	0.5 ± 0.7	0.2 ± 0.2	0.1 ± 0.1	1.8 ± 0.7	0.2 ± 0.1	0.3 ± 0.1
16:3n-4	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0.1	0.1 ± 0.1	0 ± 0	0.1 ± 0.1	0 ± 0.1	0.3 ± 0.3	0.3 ± 0.2	0.1 ± 0.2
16:3n-6	0.2 ± 0.3	0.6 ± 0.2	0.4 ± 0.3	0 ± 0	0.1 ± 0.2	0.5 ± 0.1	0 ± 0	0.1 ± 0.3	0.1 ± 0.2	0 ± 0	0.2 ± 0.1	0.5 ± 0.2
16:4n-3	0 ± 0	0.2 ± 0.2	0.3 ± 0.2	0.1 ± 0.2	0.6 ± 0.5	0.3 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
18:2n-4	0 ± 0	0.2 ± 0.2	0.4 ± 0.1	0 ± 0	0.3 ± 0.1	0.5 ± 0.1	0 ± 0	0.4 ± 0	0.5 ± 0	0 ± 0	0.3 ± 0.1	0.4 ± 0.1
18:2n-6	1.6 ± 0.3	1.4 ± 0.3	1.3 ± 0.2	0.7 ± 0.4	0.9 ± 0.2	1.2 ± 0.2	0.9 ± 0.6	1.5 ± 0.2	1.1 ± 0.1	1.6 ± 0.5	1.4 ± 0.2	1.3 ± 0.1
18:3n-3	0 ± 0	0.2 ± 0.2	0.9 ± 0.2	1.7 ± 1	1 ± 0.8	0.9 ± 0.2	2.1 ± 3.3	1.2 ± 0.3	0.9 ± 0.1	0.7 ± 0.4	1.6 ± 0.4	1.1 ± 0.2
18:3n-4	3.1 ± 1.7	2.7 ± 1.2	1 ± 0.5	0 ± 0	0.4 ± 0.5	0.4 ± 0.3	1.3 ± 1.3	3.5 ± 1.4	1.1 ± 0.3	2.4 ± 0.8	0.6 ± 0.3	1 ± 0.4
18:3n-6	0 ± 0	0 ± 0	0.1 ± 0	0 ± 0	0.1 ± 0.2	0.1 ± 0	0 ± 0	0 ± 0.1	0.2 ± 0	0 ± 0	0.1 ± 0.1	0.1 ± 0.1
18:4n-1	0 ± 0	0 ± 0	0.1 ± 0	0 ± 0	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 1	0 ± 0	0 ± 0.1	0 ± 0	0 ± 0	0 ± 0
18:4n-3	0.6 ± 0.6	0 ± 0	0.7 ± 0.6	2.3 ± 0.2	1.4 ± 0.5	1.8 ± 0.4	2.9 ± 0.7	3 ± 0.6	1.7 ± 0.3	3.2 ± 0.8	4.4 ± 0.8	3.1 ± 1
20:2n-6	1.1 ± 0.2	2.2 ± 0.5	1.2 ± 0.1	0.9 ± 0.2	2.1 ± 0.6	1.2 ± 0.2	0.4 ± 0.5	1.1 ± 0.2	1.1 ± 0.2	0.5 ± 0.4	1 ± 0.1	1 ± 0.2
20:3n-3	0 ± 0	0 ± 0.1	0.4 ± 0.1	0 ± 0	0.2 ± 0.1	0.4 ± 0.1	0 ± 0	0.3 ± 0.3	0.4 ± 0.1	0 ± 0	0.4 ± 0.1	0.4 ± 0.1
20:3n-6	0 ± 0	0 ± 0	0.1 ± 0	0 ± 0	0 ± 0.1	0.1 ± 0.1	0.3 ± 0.6	0 ± 0	0.2 ± 0.1	0.1 ± 0.3	0 ± 0.1	0.1 ± 0.1
20:4n-3	0.3 ± 0.3	0.3 ± 0.2	0.6 ± 0.1	0 ± 0	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.8	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.3	0.8 ± 0.2	0.6 ± 0.1
20:4n-6	2.3 ± 0.6	3.5 ± 0.5	2.7 ± 0.3	1.2 ± 0.1	3.5 ± 0.3	2.7 ± 0.4	0.8 ± 1.2	2.6 ± 0.5	2.6 ± 0.6	1.4 ± 0.4	1.7 ± 0.4	2.5 ± 0.4
20:5n-3	3.3 ± 0.7	5.7 ± 1.2	10.5 ± 2.6	2.5 ± 0.5	8.3 ± 1.3	12.1 ± 1.3	3.3 ± 1.7	6.9 ± 0.9	11.1 ± 1.8	5.8 ± 1.9	11.1 ± 2.6	11.4 ± 1.8
21:5n-3	0.1 ± 0.3	0.3 ± 0.2	0.4 ± 0	0 ± 0	0.6 ± 0.1	0.5 ± 0.1	0 ± 0	0.7 ± 0.2	1.5 ± 0.6	0.1 ± 0.2	0.8 ± 0.2	0.5 ± 0.1
22:4n-6	0.1 ± 0.2	0.4 ± 0.1	0.6 ± 0.2	0 ± 0	0.4 ± 0.1	0.5 ± 0.3	0 ± 0	0.2 ± 0.2	0.6 ± 0.1	0.4 ± 0.4	0.2 ± 0.1	0.6 ± 0.2
22:5n-3	0.5 ± 0.5	1.2 ± 0.3	2 ± 0.5	0.4 ± 0.9	1.2 ± 0.2	2.4 ± 0.3	2.1 ± 2.2	1.4 ± 0.4	2.2 ± 1.3	2.8 ± 1.8	2.8 ± 2.2	2.2 ± 0.5
22:5n-6	0.1 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0 ± 0	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 1	0.4 ± 0.4	0.4 ± 0.1	0.3 ± 0.3	0.4 ± 0.1	0.4 ± 0.1
22:6n-3	2.6 ± 0.5	4.9 ± 0.9	9.7 ± 2.1	1.9 ± 0.5	6 ± 0.6	11.1 ± 1.4	4.5 ± 2.4	7 ± 0.7	10.3 ± 1.6	5.4 ± 1.5	7.8 ± 1.5	9.4 ± 2
Σ PUFA	15.7 ± 2.5	24.2 ± 4.5	34.4 ± 5.1	12.6 ± 1.7	31 ± 2.5	38.7 ± 4.6	20.8 ± 10.4	32.1 ± 2.3	38 ± 3.7	27.2 ± 5.9	36.6 ± 4	37.3 ± 3.4
20:2i	0.7 ± 0.5	1.1 ± 0.2	1.1 ± 0.3	0 ± 0	1.6 ± 0.6	0.9 ± 0.2	1.4 ± 2.8	0.8 ± 0.3	0.9 ± 0.2	0.1 ± 0.3	1 ± 0.3	0.9 ± 0.2
20:2j	0.2 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0 ± 0	0.6 ± 0.1	0.4 ± 0	0.2 ± 0.5	0.3 ± 0.3	0.4 ± 0.1	0 ± 0	0.4 ± 0.1	0.3 ± 0.1
22:2i	1.3 ± 0.7	2.6 ± 0.5	1.9 ± 0.3	0.1 ± 0.2	3.8 ± 1.5	1.6 ± 0.5	0.3 ± 0.6	1.9 ± 0.6	1.7 ± 0.4	0.8 ± 0.3	1.7 ± 0.8	1.5 ± 0.3
22:2j	3.2 ± 2	6 ± 0.6	5.4 ± 0.8	1.4 ± 0.3	8.7 ± 3.2	5.1 ± 1.1	1.8 ± 1.7	4.3 ± 1.4	4.2 ± 0.7	2 ± 0.7	4.5 ± 1.9	4.6 ± 0.8
22:3nmi	0.1 ± 0.2	1.1 ± 0.3	0.5 ± 0.2	0 ± 0	1.4 ± 0.6	0.5 ± 0.2	0 ± 0	0 ± 0	0.5 ± 0.2	0 ± 0	0.6 ± 0.3	0.5 ± 0.1
Σ NMI	5.4 ± 3.3	11.3 ± 1.2	9.4 ± 1.4	1.5 ± 0.3	16 ± 5.6	8.4 ± 1.8	3.7 ± 3.4	7.3 ± 2.4	7.8 ± 1.3	3 ± 1.1	8.2 ± 3.1	7.8 ± 1.4
16:0DMA	0 ± 0	0.3 ± 0.2	0.1 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0.4 ± 0.5	0.4 ± 0.2	0.2 ± 0.2	1.8 ± 2.1	0.5 ± 0.2	0.5 ± 0.2
18:0DMA	0 ± 0	0 ± 0	1 ± 0.5	0 ± 0	0.1 ± 0.1	1.2 ± 0.4	13.5 ± 5.8	4 ± 1.9	1.7 ± 1	2.7 ± 2	0.8 ± 0.6	0.8 ± 0.4
Σ DMA	0 ± 0	0.3 ± 0.2	1.1 ± 0.5	0 ± 0	0.2 ± 0.1	1.3 ± 0.5	13.8 ± 5.9	4.4 ± 2	1.9 ± 0.8	4.5 ± 1.1	1.3 ± 0.8	1.3 ± 0.6
n-3	7.4 ± 2.2	12.8 ± 2.3	25.6 ± 6.1	8.9 ± 1.9	19.6 ± 2.4	30.2 ± 3.5	16 ± 5.8	21.2 ± 1.4	28.6 ± 2.2	20.1 ± 6	29.7 ± 3.3	29 ± 4
n-6	5.3 ± 1.2	8.										

temporally with lower values at S1 or S2 compared to S4 (all $p < 0.05$). PUFA relative abundances were significantly higher in females than in motile males at S1, S2 and S4 (all $p < 0.05$) (Fig. 31c), whereas sessile males showed intermediate values. PUFA did not vary temporally in females whereas they globally increased over the study period both for sessile and motile males, with lower values at S1 compared to S3 and S4 for sessile males (all $p < 0.05$) and lower values at S2 compared to S4 for motile males ($p < 0.05$). The abundance of BFA did not differ between ontogenic stages whatever the sampling date, except for S2 where motile males had lower values than sessile males and females (Fig. 31d). Only motile males showed significant temporal variations with lower relative abundances at S2 ($p < 0.01$). No temporal changes were observed in the relative abundances of NMI FA whatever the ontogenic classe (Fig. 31e). However, motile males showed significantly lower values than sessile males and females at S2 ($p < 0.05$), and than females at S4 ($p < 0.05$). The n-3/n-6 ratio showed no temporal variation for sessile females but increased significantly over time both for sessile ($p < 0.001$) and motile males ($p < 0.01$) (Fig. 31f).

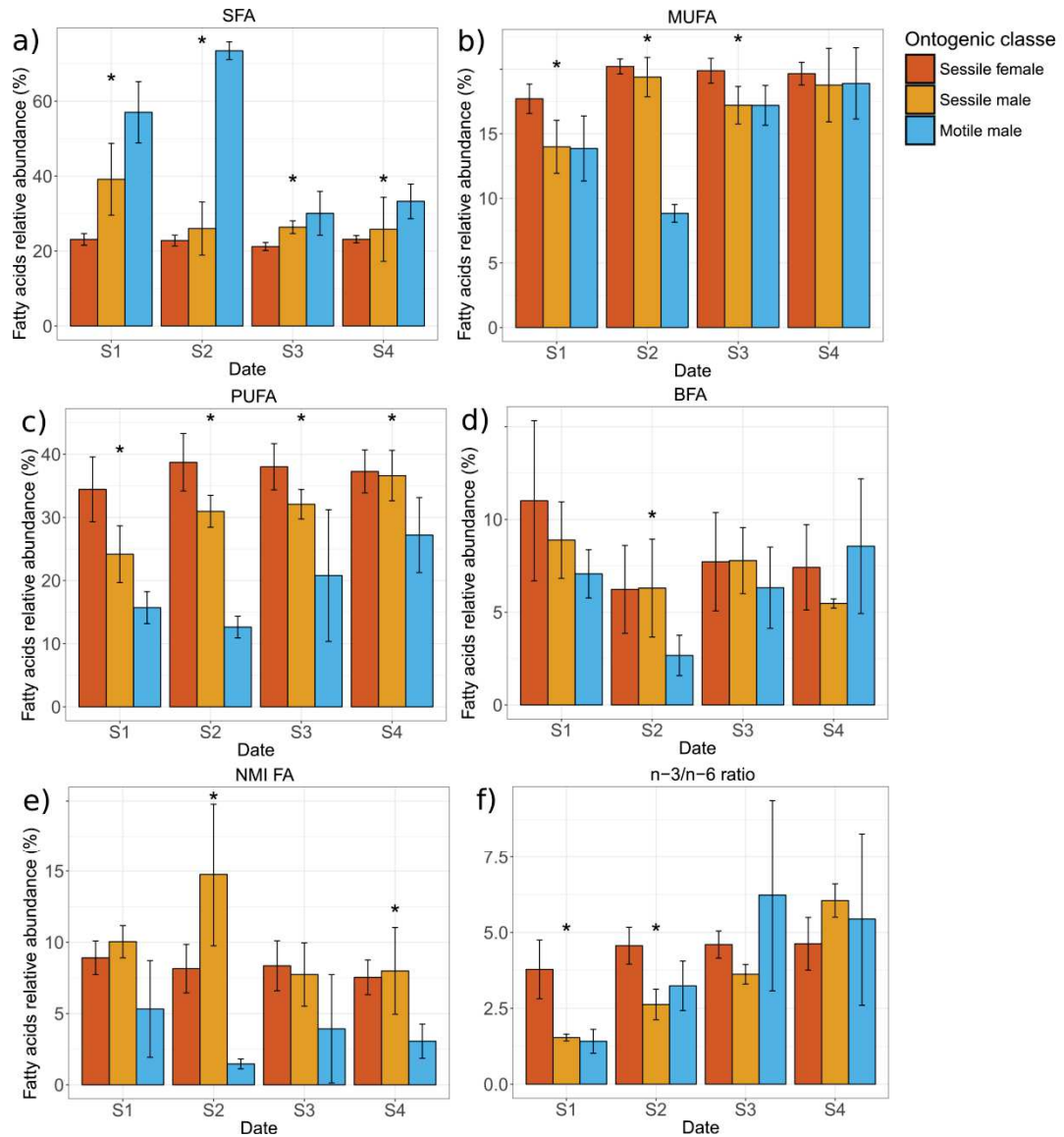


Figure 31: Relative abundance of fatty acids (FA) classes in the three ontogenic classes of *Crepidula fornicata* (motile males, sessile males, sessile females) (mean \pm SD, $n = 5$): (a) Saturated FA, (b) Monounsaturated FA, (c) Polyunsaturated FA, (d) Branched FA, (e) Non-Methyl Interrupted FA and (f) n-3/n-6 ratio. S1 to S4 corresponds to sampling periods (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April). * corresponds to significant differences between ontogenic classes for each sampling date ($p < 0.05$).

3.2.1. Stable isotope compositions

The three ontogenic classes exhibited similar isotopic patterns with lower $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for motile males compared to sessile males and females. No significant temporal variations in SI composition were observed for any ontogenic class except for the $\delta^{15}\text{N}$ of sessile females between S3 and S4 ($p < 0.05$) (Fig. 32a and 32b). The only significant inter-classes differences were observed between sessile females and motile males for $\delta^{15}\text{N}$ at S3 ($p < 0.05$). Isotopic niche calculations showed higher overlap of SEAc between sessile females and males (49

%) than between sessile and motile males (39 %) or between sessile females and motile males (18 %) (Fig. 32c).

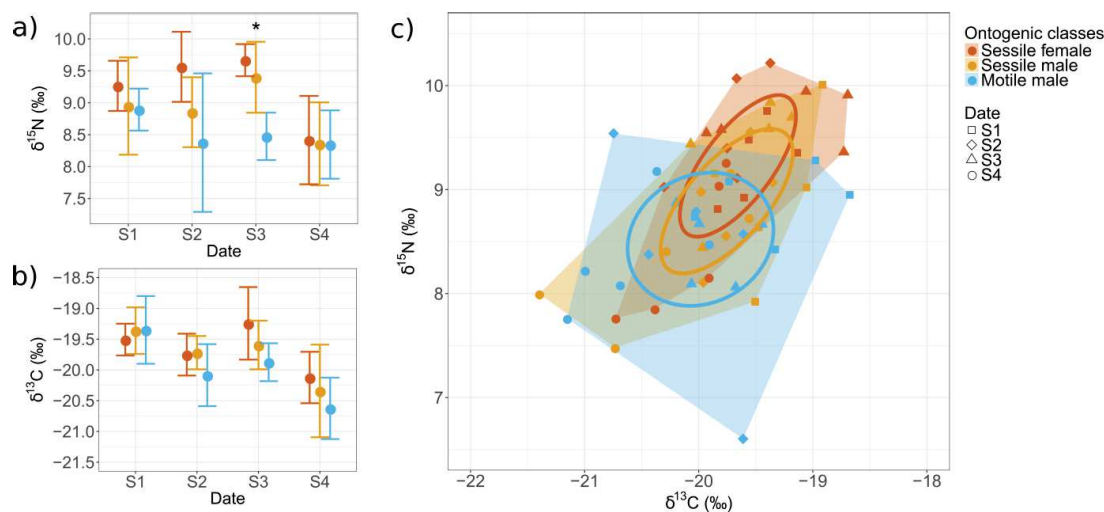


Figure 32: (a) $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ isotopic compositions (mean \pm SD, $n = 5$), and overall isotopic (c) biplot for three ontogenic classes of *Crepidula fornicata* (motile males, sessile males, sessile females). S1 to S4 corresponds to sampling periods (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April). Ellipses enclosed the standard ellipse area corrected for small sample size (SEAc).

4. Discussion

The proliferation of *C. fornicata* often results in large biomass accumulation on the colonised sea floors. Being gregarious, all ontogenic classes are grouping in the same stacks, therefore questioning a possible intraspecific competition for food. However, the biological traits of young individuals suggest niche differentiation between these classes, as already experienced for other species of *Crepidula* (Chaparro et al., 2002; Navarro and Chaparro, 2002). Using two different trophic markers, we examined if intraspecific food partitioning exists within *C. fornicata* stacks and if it could be related to ontogenic changes of behavior (shift from motile male to sessile females).

4.1. Characterization and availability of potential food sources

Pelagic and re-suspended particulate organic matter as well as biofilm associated with *C. fornicata* shells were well discriminated according to their pigment, fatty acid and stable isotope compositions, whatever the sampling date. The $\delta^{13}\text{C}$ of PPOM (-25.4 ± 0.7 ‰) compared to marine (-22.3 ± 1.2 ‰ over the same period in the bay of Brest) (French Coastal Monitoring Network SOMLIT; <http://somlit.epoc.u-bordeaux1.fr/fr/>) and terrestrial one (-27.5 ± 1.2 ‰ in the upper Elorn estuary) (Nerot, 2011) suggests a terrestrial influence, as already suggested by

Marchais et al. (2013). Moreover, we recorded high biomass of chlorophyll *a* (5-18 $\mu\text{g L}^{-1}$) in the PPOM over the study period, whereas marine phytoplankton blooms occurring in the rest of the bay of Brest commonly reach 4-6 $\mu\text{g L}^{-1}$ (Chatterjee et al., 2013). Again, primary productivity in the study area is likely to be influenced by terrestrial inputs such as nutrient (Khalil et al., 2013).

RPOM appeared as a complex mixture of low and high quality OM. On one hand, it was characterized by i) pheophorbide and pheophytin, which are both degradation products of pigments, ii) odd branched FA (such as ant15:0) and the MUFA 18:1n-7 which suggested the presence of bacteria (Jaschinski et al, 2011; Meziane et al., 1997) and iii) the long chain saturated fatty acids 22:0 which confirms a terrestrial contribution in this environment (Canuel, 2001). Moreover, RPOM was the less variable of the three organic matter sources over time, which is often the case for detritic pool of OM. On the other hand, RPOM exhibited the highest PUFA/SFA ratio (Table 2), suggesting higher quality/lability compared to PPOM and biofilm (Biandolino et al., 2008; Pommier et al., 2010). It was confirmed by Fucoxanthin / Chlorophyll *a* and Chlorophyll *c* / Chlorophyll *a* ratios which indicated a higher contribution of diatoms in RPOM compared to PPOM and biofilm (Gieskes et al., 1991; Méléder, 2003). This high concentration of fucoxanthin, which is a blue-light absorbing pigment, and the very low light irradiance of our study site (0.01 %, Supplementary Fig. 33), strongly suggested that low light acclimated diatoms contributed to the RPOM (McGee et al., 2008).

Biofilm scrapped on shells of *C. fornicata* showed high biomass of chlorophytes, as suggested by the stronger presence of chlorophyll *b* and neoxanthin (Brotas and Plante-Cuny, 2003), compared to the surrounding RPOM. However, FA characterizing macrochlorophytes (such as 18:3n-3 and 18:4n-3 found in *Ulva* spp.; Fleurence et al., 1994) were not found in the biofilm. The major FA were saturated FA, which were especially high during the first two sampling dates, with the highest concentration of 16:0 and 18:0 (42 % and 39 %, respectively) recorded at sampling date S2. While considered as ubiquitous FA (Kelly and Scheibling, 2012), 16:0 and 18:0 likely characterized the biofilm of *C. fornicata*'s shells in our study site. The high concentration of fucoxanthin also suggests the presence of diatoms on these shells (Ní Longphuirt et al., 2007). As already suggested by Androuin et al., (*article 1*), lived *C. fornicata* may harbour high biomass of primary producers on its shell. For instance, the biomass in biofilm

was ~10 times higher than in the surrounding RPOM. The biofilm was also ^{15}N -enriched by ~2.5 ‰ compared to RPOM, which may indicate that primary producers present in the biofilm use *C. fornicata*'s dissolved excretion as a nitrogen source. Indeed, even if the excretion product (i.e., ammonium) is expected to be ^{15}N -depleted relative to *C. fornicata*'s tissues (DeNiro and Epstein, 1981), it could still be enriched compared to the available dissolved nitrogen in the environment (Cifuentes et al., 1989). This could indicate that nutrients are not a limiting factor for primary producers inhabiting *C. fornicata* shells, making them available through the year when sufficient light reach the sea floor.

4.2. Ontogenic trophic shift in *Crepidula fornicata*

FA compositions and to a lesser extend SI ratios of the three ontogenic classes of *C. fornicata* highlighted a trophic shift that co-occurred with the change of behavioural (i.e., motility) and sex (i.e., protandry) of this species. Those results suggest that young motile males grazed on the biofilm during period with less food availability (i.e., S1 and S2 sampling period), whereas sessile individuals were mostly filtering and relying on re-suspended particulate organic matter (RPOM). Overall, SI ratios showed that sessile males and females had similar trophic niches (high overlap between their ranges of isotopic signatures), whereas motile males had a more distinct and broader niche (larger convex hull and standard ellipse area and lower overlap with those of sessile individuals). Considering the classical diet-consumer enrichment factors of ~1 ‰ for carbon and ~2.5 ‰ for nitrogen (McCutchan et al., 2003), one could expect that all ontogenic classes mostly relied on RPOM. However, inferring diet shift using only SI ratios may be hampered by physiological changes occurring during ontogeny such as gonadal maturation or differential tissue growth between young and adult individuals. Such physiological processes can modify the isotope signal of tissues even without significant change of diet (Blanchet-Aurigny et al., 2012b, Hentschel, 1998, Rossi et al., 2004). The speed of isotopic enrichment through time also depends on tissue turnover rate (McCutchan et al., 2003; Vander Zanden et al., 2015). Indeed, motile males are small and more active than adults (Hoagland, 1978; Walne, 1956), so the rapid production of new tissue during their growth may reduce fractionation processes, resulting in lower SI ratios with respect to older individuals (Gannes et al., 1997; Lefebvre and Dubois, 2016). For the largest adults (i.e. females), the net production

of tissue tends to zero and the maintenance metabolism (through respiration and excretion) results in relatively higher SI signature in their tissues (McCutchan et al., 2003; Minagawa and Wada, 1984).

Neutral lipids are essential energy reserves for sustaining early life stages and play a key role in settlement, habitat selectivity and recruitment in marine invertebrates (Pernet and Tremblay, 2004; Pernet et al., 2005; Tremblay et al., 2007). FA compositions of the three ontogenic classes of *C. fornicata* showed low overlap, especially between motile males and sessile females, and suggesting a trophic shift between motile and sessile individuals. According to these biomarkers, young individuals exhibited a larger temporal change in their diet than old individuals. In particular, we observed a clear temporal shift after the second sampling date (S2) for motile males. The two first dates, characterized by very low PUFA contributions, likely corresponded to a period that had still integrated the trophic signal of the winter season's food sources. During this period, motile males were characterized by higher proportions of saturated fatty acids such as 16:0 (25-34 %) and 18:0 (27-36 %). Although these FA are usually common in animal and do not necessarily reflect a specific diet (Dalsgaard et al., 2003; Kelly and Scheibling, 2012), we found high amount of these two FA in the biofilm, both in terms of contribution and concentration. As already suggested for larvae of *C. fornicata*, the high amount of saturated FA may result from a diet enriched in SFA (Leroy et al., 2013). In our case, such FA composition could originate from the biofilm covering *C. fornicata*'s shells. A low proportion of energy-storage lipids (i.e., PUFA) also suggest a regular food supply (Prato et al., 2012), a hypothesis supported by the higher availability of the biofilm compared to those of PPOM and RPOM. During the two last sampling dates, the PUFA/SFA ratio increased in motile males, suggesting more PUFA in its diet. Considering the greater availability of PPOM and RPOM at the end of our study period, motile males probably have increasingly relied on these two food sources using both filter-feeding mechanisms.

Sessile individuals showed higher contribution of PUFA to their diet than motile ones. As suggest above by SI, FA confirmed that RPOM was the main food source of sessile individuals, which probably exploit this resource during regular resuspension events linked to tidal currents (Beudin et al., 2014). In this pool of OM, the slipper limpet likely fed on diatoms (16:1n-7 and 20:5n-3; Dunstan et al., 1994; Napolitano et al., 1997; Passarelli et al., 2012), dinoflagellates (22:6n-3;

Lavaud et al., 2018; Zhukova and Aizdaicher, 1995) and bacteria (Branched FA, 18:1n-7; Perry et al., 1979; Haack et al., 1994; Zhukova et al., 1992), which is in agreement with the conclusions of previous studies (Dubois et al., 2014; Leroy et al., 2013). In sediment, bacteria are often associated with detritus and are therefore not considered as a high quality food source (Dalsgaard et al., 2003). This is confirmed by the PUFA/SFA ratio, a biomarker of fresh vs. detritic OM (Biandolino et al., 2008), which was lower in the slipper limpet than in others filter-feeding species of the bay of Brest, such as *Pecten maximus* (Lavaud et al., 2018) or *Ophiotrix fragilis* (Blanchet-Aurigny et al., 2015). The fact that adults of *C. fornicata* do not have organs of selection is likely to explain their opportunistic trophic behaviour based on both fresh and detritic organic matter (Beninger et al., 2007). Interestingly, we also found large contribution of non-methylene-interrupted (NMI) FA in the neutral lipids of sessile *C. fornicata* (~8 %). *De novo* synthesis of such FA is well documented in mollusks (Barnathan, 2009; Zhukova, 1991) and is therefore not expected to be linked to a specific diet. However, the fact that they are preferentially incorporated in polar than in neutral lipids suggest a higher proportion in polar lipids of *C. fornicata*, thus confirming their potential importance for this species. The unusual unsaturation pattern of these FA confers to cell membranes a higher resistance to oxidative processes and microbial lipases than the common PUFA (Barnathan, 2009). Considering the unusual way of life of this species, it could represent a biochemical adapting feature linked to the sessile behavior of adults of *C. fornicata* which showed ontogenic histological changes in their food part (Chapparo et al., 1998; Androuin et al., *article 2*). Besides, the FA 18:4n-3 showed an increasing contribution over time for sessile as well as for motile males. According to the literature, this FA may be associated with different primary producers such as dinoflagellates (Budge and Parrish, 1998) or green macroalgae (Fleurence et al., 1994; Kelly and Scheibling, 2012). Considering the low temporal variation of another dinoflagellate biomarker (22:6n-3) in the OM sources and the seasonal accumulation of green macroalgae near our study site (Study Centre for Algal Promotion, <http://www.ceva.fr>; Ragueneau et al., 2018), we can expect a seasonal importance of these green macroalgae in the diet of *C. fornicata* in our study site. The FA 20:1n-11 was found in relative high abundance in *C. fornicata* (4-6 %). Although it is known to be a biomarker of copepod in tropical estuaries (Bachok et al., 2003), the ¹⁵N-enrichment of *C. fornicata* compared to PPOM

wasn't high enough to suggest a significant contribution of zooplankton in its diet (Kopp et al., 2015). The same finding was reported from another study in the bay of Brest for two species of ophiurid (Blanchet-Aurigny et al., 2015), evidencing that this FA was not a good biomarker of zooplankton for the species considered.

As with SI, FA composition of *C. fornicata* showed decreasing inter-individual and temporal variability with the ontogenic development. As suggested above, a lower growth rate associated with a lower metabolism may explain the lower variability in sessile females. This difference can also be explained by processes related to two biological traits, namely the movement capacity of young males (behaviour) and the sexual development (physiology). Thanks to their mobility, young males have access to a greater diversity of food sources than sessile adults, e.g. by grazing on the substrate (e.g., microalgae, bacteria, crustose macroalgae, detritus, etc). The ability to switch between grazing and filtering strategies may increase the variability of their FA compositions. As we used both digestive gland and gonad for neutral FA analysis, the state of lipid storage can also depend on the sexual development stage. Indeed, females allocate more energy than males in the reproduction due to gametogenesis (Desloup-Paolis, 1986; Leroy et al., 2013) and may store more lipids than males. The n-6/n-3 ratio increased over time for both sessile and motile males, and the unchanged ratio for females is probably linked to a preferential allocation of n-3 to early embryos, which showed an increase over the reproductive period of the slipper limpet (Leroy et al., 2013).

4.3. Ecological implications

The recruitment phase is of primary importance in the success of all species and especially for invasive ones (Stachowicz and Byrnes, 2006). Specific studies emphasized the key role of adults in facilitating larval recruitment (Cahill, 2015; Pechenik and Gee, 1993; Woods, 1989) as well as the substrate in the settlement and in the feeding ecology of *C. fornicata* recruit (Cahill, 2015; Pechenik et al., 2015). In addition, shells of lived *C. fornicata* harbors significant amount of microphytobenthic biofilm (Androuin et al., *article 1*; this study; Ní Longphuirt et al., 2007). As demonstrated here, the use of this biofilm by motile males individuals strongly suggest a facilitation process of adult toward newly recruiting individuals, at least during some period of the year with potential harsh food condition (i.e., winter season). Given that *C. fornicata* often proliferates on muddy and turbid

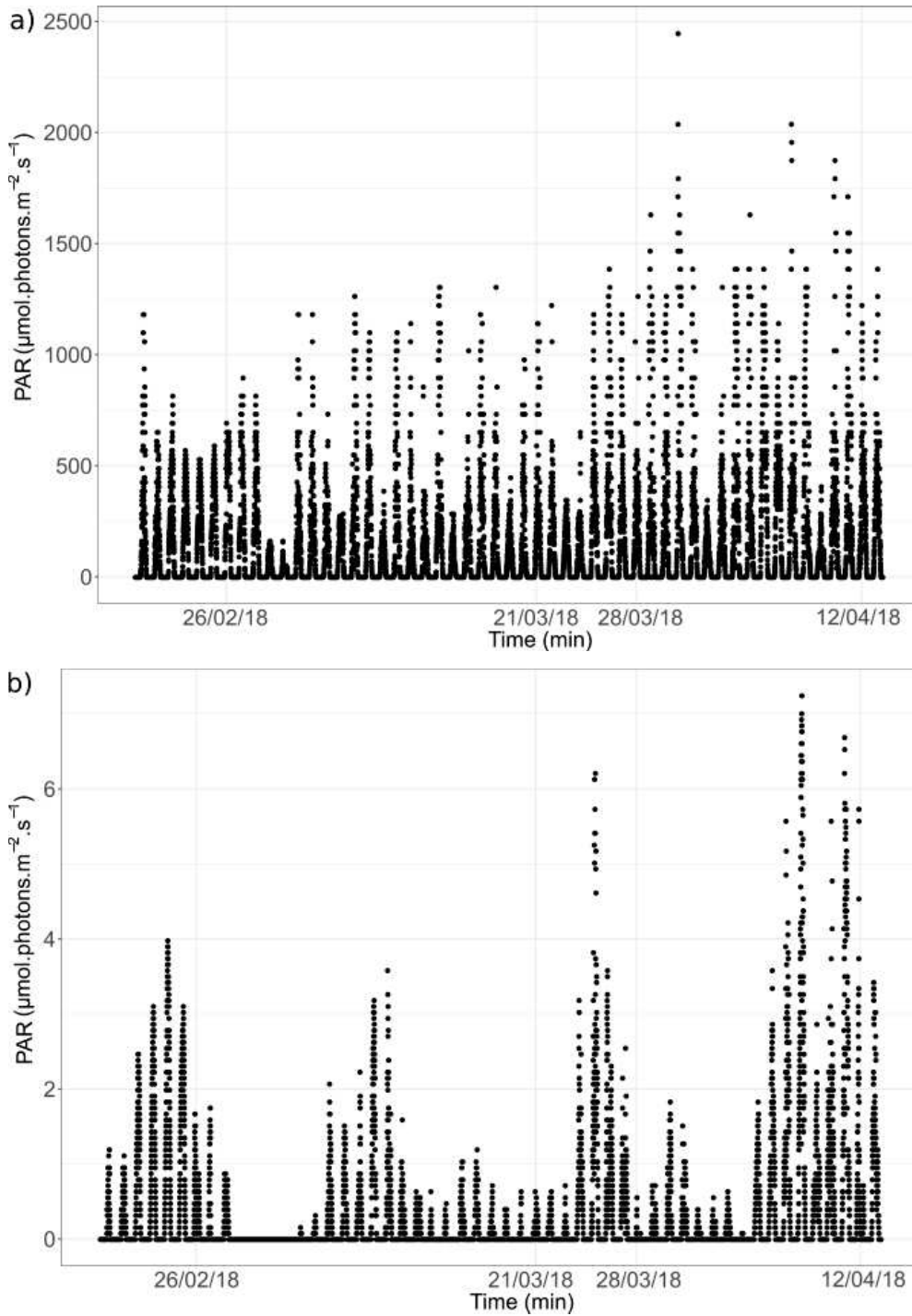
habitats with high suspended inorganic load, grazing behavior of motile males could also prevent the overloading of their digestive tract with inert matter of low nutritional quality (Navarro et Chaparro, 2002). Grazing of motile males could be therefore another advantage in the invasive success of this species, as already shown for another aquatic invasive gastropod (Carlsson and Brönmark, 2006).

As complementary tools, pigments, SI and FA compositions highlighted the importance of diatoms at the surface of sediment, as well as in the diet of *C. fornicata*. This study confirms previous experimental work on subtidal *Crepidula* habitat where microphytobenthos was shown to be stimulated by the slipper limpet itself (Androuin et al., *article 1*). Altogether, these study suggest that the slipper limpet can promote microphytobenthos, both on sediment and shells, and use it as a food source, thus confirming the “gardening” hypothesis demonstrated for other engineer species (Chennu et al. 2015, Hylleberg, 1975). In addition to having direct consequences for the invasive success of this species at different ontogenic stages, microphytobenthos may contribute to the trophic ecology of surrounding species. Its fate at multiple scales (i.e., trophic levels and community) needs to be further investigated.

5. Acknowledgements

We thank the PSO and LIPIDOCEAN platforms for stable isotope and fatty acid facilities, respectively. We are grateful to Aline Blanchet-Aurigny for commenting upon preliminary versions of this manuscript. We also thank the LEBCO diving team (A. Curd, X. Caisey, S. Dubois and A. Tancray) for providing biological samples. TA was funded by an IFREMER, LabexMER and Region Bretagne PhD grant. This work was funded by the Politique de site initiative and by the TOTAL foundation for biodiversity.

6. Supplementary materials



Supplementary Figure 33 : Photosynthetic available radiations (PAR) (a) at the surface and (b) at the bottom in the study site.

**CHAPITRE III : Interactions
trophiques interspécifiques multi-
échelles**

Article 4 : Enemies together in close quarters: food partitioning between suspension feeders in an engineered benthic habitat

Thibault Androuin ¹, Stanislas F. Dubois ¹, Gwendoline Lefebvre ², Fabienne Legrand ², Gauthier Schaal ², Antoine Carlier ¹.

¹ DYNECO-LEBCO, IFREMER, Plouzané, France

² UMR CNRS 6539, LEMAR-IUEM-UBO, Plouzané, France

Abstract

The invasive slipper limpet *Crepidula fornicata* is a suspension-feeding gastropod which is a potential trophic competitor for other suspension-feeders when present in large densities. However, this habitat-forming species facilitates the installation of some suspension-feeders thanks to the presence of its shell. In this study, we investigated the degree of trophic niche overlap between the slipper limpet and four co-occurring suspension-feeders in a *C. fornicata* bed in the Bay of Brest (France). Trophic niches were assessed for a late winter-early spring period using both stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and fatty acid compositions and were discussed taking into account the preferred micro-habitat, degree of motility and particle sorting capacity. Whereas the two bivalves *Chlamys varia* and *Ostrea edulis* relied on high quality food sources (diatoms, green macroalgae, dinoflagellates), the motility and the larger size-spectrum allowed higher trophic specialization for the scallop, which showed the smaller niche width. Conversely the calyptraeid gastropods *C. fornicata* and *Calyptraea chinensis*, exhibiting limited movement and no selection capacity, likely fed on lower quality food sources (detritic organic matter, bacteria). Although living very close to each other, the micro-habitat of *C. chinensis* in the dead shells at the base of *C. fornicata* stacks seems to provide a different nitrogen food source for this species. Alternatively, barnacles, which are sessile without sorting capacity but with a broad particle size spectrum, showed more temporal variability than the other species, feeding on animal material and detritus in winter and shifting their diet to diatoms in early spring. This study provides new evidence that *C. fornicata* does not necessary compete for food with co-occurring species, and that food partitioning explains the coexistence of suspension-feeding species, even on a small spatial scale.

Key words: trophic niche, suspension feeders, stable isotope, fatty acids, *Crepidula fornicata*, feeding mechanisms

1. Introduction

Suspension-feeders often dominate hard and soft bottom habitats and represent the main part of the biomass in benthic coastal areas (Gili and Coma, 1998; Little et al., 2009). They provide ecological functions thanks to their filtering activity, which stimulate the benthic-pelagic coupling (Chauvaud et al., 2000; Cloern, 1982). They are also involved in nutrient cycling, either directly through the excretion of dissolved nutrient (Newell et al., 2005; Yamamura and Koike, 1993) or indirectly through the microbial activity associated with the remineralization of their feces/pseudofeces (Dame 2001; Ragueneau et al., 2005; van Broekhoven 2014).

The suspension-feeding trophic mode is present in a diverse array of marine taxa (Jørgensen, 1996; Riisgård and Larsen, 2010; Ward and Shumway, 2004), and represents an advantage in terms of energy costs (Coma et al., 2001; Gili and Coma, 1998; Riisgård and Larsen, 2000). Because they rely on suspended particulate organic matter (POM), suspension feeders are adapted to a wide spectrum of particle types, sizes and nutritive quality. Species can be passive, active, exclusive or facultative suspension feeders, increasing the number of trophic niches in this group (Coma et al., 2001; Jørgensen, 1996). Whereas competition for space has long been considered as a limiting factor for these species (Connell, 1961; Dayton, 1971; Keough, 1984), competition for food is also of significant importance for an interspecific relationship (Svensson and Marshall, 2015). Moreover, food partitioning and trophic plasticity are common strategies used by syntopic species in order to limit the negative impact of trophic competition (Dubois and Colombo, 2014; Dubois et al., 2007; Lacoste et al. 2016; Lefebvre et al. 2009; Lesser et al., 1992; Richoux et al. 2014ab; Yakovis et al., 2012). For instance, bivalves such as mussels have significant food selection capacities thanks to their labial palps, whereas cirriped crustaceans mostly select according to the inter-setae space on their cirri (Riisgård and Larsen, 2010).

Non-native species can dramatically affect trophic relationships and food webs properties (Ehrenfeld, 2010; Gallardo et al., 2015; Walsh et al., 2016). The impact is particularly strong when introduced species are dominant in terms of biomass, such as proliferating and habitat-forming species (Crooks et al., 2002), and

can have consequences among and across trophic levels (Arbach Leloup et al., 2008; Ozersky et al., 2012).

Along the European coast, *Crepidula fornicata* is one of the most invasive marine species (Blanchard, 2009). This species was first recorded in 1880 in England (Orton, 1912) and since then is present and has spread in most of coastal bays, occurring in various types of soft bottoms and reaching several kg m⁻² in the most impacted areas (Blanchard, 1997; 2009). Being gregarious and sessile, this species is an ecosystem engineer, providing structural complexity on the substrate (Barnes et al., 1973; de Montaudouin et al., 1999) and modifying sediment characteristics (Ehrhold et al., 1998; Martin et al., 2007). It therefore acts as a facilitative species for some suspension feeders through provision of hard substrate. *C. fornicata* may also play a role in the structuration of benthic food webs, by modifying key aspects of trophic structure such as redundancy and trophic plasticity (Androuin et al., *article 5*). A recent study suggests that it may stimulate the microphytobenthic biofilm in subtidal colonized area, providing nutrient conditions for microphytobenthic growth, on both hard substrate (i.e., the surface of living *C. fornicata* shells) and soft sediment (Androuin et al., *article 1*; *article 3*). As a non-selective filter-feeder, *C. fornicata* is expected to interact with local suspension-feeding species (Barnes, 1973; Blanchard, 1997; Thouzeau et al., 2000). While trophic studies based on stable isotope ratios (SIR) showed little trophic overlap between *C. fornicata* and other suspension-feeders (Decottignies et al. 2007ab; Lefebvre et al., 2009; Riera et al., 2002; Sauriau et al., 2002), such bivariate data ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) often failed to accurately discriminate origin of microscopic organic matter such as POM (Lienart et al., 2016). Moreover, a recent study demonstrated an analytical bias in SIR of *C. fornicata* due to non-expected inorganic carbonates in its soft tissues (Androuin et al., *article 2*) and raised the need to re-evaluate its trophic impact on co-occurring filter-feeding species, using complementary tools. Whereas bulk SIR enables discrimination between benthic and pelagic food (Fry and Sherr, 1984), fatty acids are particularly accurate to deal with POM which is composed of a complex mixture of microscopic organisms and detritus exhibiting a large array of size, nutritive quality or assimilation properties (Canuel, 2001; Androuin et al., *article 3*).

In the bay of Brest, with a long past history of *C. fornicata* invasion (Ragueneau et al., 2018), many suspension-feeders are found within *Crepidula*

beds. Some are fixed epibionts more or less associated with *C. fornicata* shells (e.g., the barnacle *Austrominius modestus* and the flat oyster *Ostrea edulis*), others are specifically associated with empty shells of *C. fornicata* (e.g., *Calyptraea chinensis*) and showed low motility, and yet others are relatively motile (e.g., *Chlamys varia*) mostly hiding in dead shells of *C. fornicata* and *O. edulis*. These four species constitute a significant part of the macrofaunal biomass of this habitat (Barnes et al., 1973; Guérin, 2004). In this study, we used *Crepidula fornicata* as a model to investigate the effect of an invasive habitat-forming species on the degree of segregation between the trophic niches of suspension-feeders. We coupled two types of trophic markers (fatty acids and SI compositions) to describe the diet of *C. fornicata* and four co-occurring suspension-feeders (*A. modestus*, *C. chinensis*, *O. edulis*, *C. varia*). We investigated the trophic relationships between these species in relation to their biological traits such as their food retention ability, movement or preferred habitat. We hypothesised that the invasive slipper limpet has a specific diet and does not overlap with co-occurring species.

2. Materials and methods

2.1. Sampling and dissecting protocol

The bay of Brest (Britany, France) is a semi-enclosed marine ecosystem of 180 km². The sampling site is located near the Elorn estuary (48°23'N, 4°23', average depth: 10 m) in dense *Crepidula* beds (~2000 ind m⁻²) (Guérin, 2004). Four sampling dates (26th February, 21th March, 28th March, 12th April) were chosen to encompass a period of time with potentially contrasted availability of OM sources (e.g., spring blooms). Details about organic matter sources (Biofilm on *C. fornicata* shells, particulate OM from the water column and from the re-suspended sediment) sampling and results are provide elsewhere (Androuin et al., *article 3*). Consumers from the five species were collected by SCUBA divers. *C. fornicata* individuals were sampled at the base of the stacks (mean shell length 33 ± 3 mm), fixed on a dead *C. fornicata* shell. Individuals of *C. chinensis* (mean shell length 10 ± 2 mm) were removed from the dead shell below live *C. fornicata*. *Austrominius modestus* (mean shell length 3 ± 1 mm) were sampled at the top of *C. fornicata* shells. *O. edulis* (mean shell length 60 ± 10 mm) and *C. varia* (mean shell length 42 ± 3 mm) were sampled from the central part of the *C. fornicata* bed. For each species,

individuals of similar size were selected to avoid potential confusing effects of ontogenic changes of trophic markers (Androuin et al., *article 3*; Rossi et al., 2004). All individuals were kept overnight in filtered seawater in the laboratory to allow evacuation of their gut content. The trophic markers were analysed in tissue that i) rapidly integrates any change in the assimilation of food sources (high turnover rate) and ii) is a lipid-rich organ that stores energy (McCutchan et al., 2003). Since both the digestive gland and gonads are fused in a single organ in Calyptreidae, we therefore used both tissues together in all species, with the exception of the barnacle *A. modestus* for which we used the entire body. Due to their small body size, several individuals of *A. modestus* and *C. chinensis* were pooled together to get enough material for subsequent analysis. A total of five individuals of *O. edulis*, *C. varia* and *C. fornicata*, three pools of three individuals of *C. chinensis* and three pools of thirty individuals of *A. modestus* were dissected, frozen at -80°C and freeze-dried.

2.2. Fatty acids analyses

2.2.1. Lipids extraction

Immediately after freeze-drying, samples were ground into homogenous powder using a mortar and pestle. Samples (representing 2 and 20 mg of dry weight, depending on species) were immediately put in glass vials (previously burned for 6 h at 450°C) containing 6 mL of a chloroform/methanol mixture (2:1, v:v), and extracted with a Dounce homogenizer. They were then sonicated for 10 min, flushed with nitrogen and stored at -20°C before analysis.

2.2.1. Separation

The neutral lipids (NL) were isolated following the method described by Le Grand et al., (2014). An aliquot (between 1 and 6 mL depending on species) of total lipid extract was evaporated to dryness under nitrogen, recovered with three 0.5 mL washings of chloroform/methanol (98:2, v:v) and deposited at the top of a silica gel micro-column (Pasteur pipette of 40 mm × 5 mm i.d., plugged with glass wool and filled with silica gel 60, which were both previously heated for 6 h at 450°C and deactivated with 6 % water by weight). Only NL, including triglycerides, free fatty acids and sterols, were eluted with 10 mL of chloroform/methanol (98:2, v:v) and collected in 20-mL glass vials. After evaporation to dry state using nitrogen, the NL

fraction was recovered and transferred to 7-mL vials with three 1-mL washings of chloroform/methanol (98:2, v:v).

2.2.1. Transesterification and Gas chromatographer analysis

After the addition of tricosanoic acid as an internal standard and evaporation to dry state using nitrogen, the fatty acid methyl esters (FAME) were obtained using a modified method of Le Grand et al. (2014). A total of 0.8 mL of a sulphuric acid solution (3.8 % in methanol) was added, vortexed and heated for 10 min at 100°C. Before GC analysis, 0.8 mL of hexane was added and the organic phase containing FAME was washed three times with 1.5 mL of hexane-saturated distilled water. The organic phase was finally transferred to tapering vials and stored at -20°C. FAMES were analyzed in a Varian CP 8400 gas chromatograph (GC) equipped with a split/splitless injector and a flame-ionization detector. FAMES were identified using two capillary columns with different polarity (ZBWAX -30 m × 0.25 mm i.d., 0.25- μ m thickness, Phenomenex; and ZB-5HT -30 m × 0.25 mm i.d., 0.25- μ m thickness, Phenomenex) by means of a standard 37- component FAME mix (Sigma) and other known standard mixtures. The FAs were expressed as the molar percentage of the total FA content.

2.3. Stable isotopic ratios analyses

Two separate analyses were done for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analyses. Approximately 400 μg of freeze-dried powder were weighed in tin capsules for $\delta^{15}\text{N}$ analysis. Because both lipid content and inorganic carbonates can influence $\delta^{13}\text{C}$ values (McCutchan et al., 2003; Androuin et al., *article 2*), approximately 400 μg of powder was added to 1 ml of cyclohexane in Eppendorf tubes, to extract lipids. After 15 seconds of vortex, tubes were centrifuged at 3000 g for 5 min. The supernatant was discarded and the tubes dried at 40 °C for 12 h. If the supernatant was still coloured, the sample was re-processed (three times minimum). Lipid-free tissues were then weighed in silver capsules and in-cup decarbonated using 1N HCl. Each capsule was visually checked, dried at 40°C for 1 h, and sealed. Samples were analysed by continuous flow on a Thermo Scientific Flash EA 2000 elemental analyser coupled to a Delta V Plus mass spectrometer at the Pôle de Spectrométrie Océan (PSO, Plouzané, France). Results are expressed in standard δ notation based

on international standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$) following the equation:

$$\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 \text{ (in ‰)}, \text{ where } R \text{ is } ^{13}\text{C}/^{12}\text{C} \text{ or } ^{15}\text{N}/^{14}\text{N}.$$

International isotopic standards of known $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were used: IAEA-600 Caffeine, IAEA-CH-6 Sucrose, IAEA-N-1 and IAEA-N-2 Ammonium Sulphate. The experimental precision was estimated using the standard deviation of an internal standard (Thermo Acetanilide, $n = 8$). It indicated an analytical precision of $\pm 0.11 \text{ ‰}$ and $\pm 0.07 \text{ ‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively.

2.4. Statistical analyses

FA compositions were represented using a principal component analysis (PCA). Homogeneity of the data was tested using the permutation analysis of multivariate dispersion (PERMDISP) (Anderson, 2001). Statistical analyses on species FA were conducted using a non-parametric distanced based permutation multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities. Analyses were performed using two variables: species (fixed factor with 5 levels) and sampling dates (fixed factor with 4 levels). Sampling date was considered as a fixed variable since all measured individuals were considered as independent. Following significant PERMANOVA results, *post hoc* tests were carried out using multiple pairwise comparisons to identify differences among factors (Martinez Arbizu, 2017). Two-way factorial analyses of variance (ANOVA) were used to assess differences in SIR ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and FA biomarkers (Table 10) between species and sampling date. When significant, *post hoc* multiple comparisons were carried out using Tukey HSD. Normality and homogeneity of residuals were graphically assessed. The isotopic niche of each species was assessed using bivariate standard ellipse area (SEA) calculated with values from all sampling dates and corrected for small sample size (Jackson et al., 2011). Statistical analyses were performed in R version 3.3.0 (R Core Team, 2012) using packages ‘vegan’, ‘plyr’, ‘FactoMiner’, ‘SIBER’ and ‘ggplot’.

Table 10: Fatty acids (FA) used as dietary biomarkers in this study. BFA: branched FA, PUFA: polyunsaturated FA, SFA: saturated FA, LCSFA: long chain saturated FA (≥ 22 carbons).

Fatty acids (FA)	Indicators for	References
14:0; 16:1n-7, 20:5n-3	Diatoms	Dunstan et al., 1994; Napolitano et al., 1997
20:5n-3/22:6n-3	Diatoms to flagellates dominance	Budge and Parrish, 1998; Lavaud et al., 2018
18:1n-9	Flagellates, animal material	Napolitano et al., 1997; Dalsgaard et al., 2003
Σ BFA	Bacteria	Perry et al., 1979; Haack et al., 1994
Σ LCSFA (22:0, 24:0)	Terrestrial plants	Canuel; 1991; Budge et al., 2001; Meziane et al., 1997
Σ PUFA/ Σ SFA	Fresh organic matter vs. detritic	Biandolino et al., 2008; Pommier et al., 2010
18:3n-3; 18:2n-6	Green, macroalgae, Terrestrial plant	Meziane et al., 1997; Kelly and Scheibling, 2012
18:4n-3	Green macroalgae, flagellates	Fleurence et al., 1994; Kelly and Scheibling, 2012

3. Results

3.1. Stable isotopes composition

Overall, values spanned over 4 ‰ and 9 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Fig. 34, Table 11). Significant differences were found between species and sampling dates both for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Table 13). Pairwise comparisons showed that all species had significantly different SIR except for *O. edulis* and *C. varia* ($p = 0.47$ and 0.73 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively). These two bivalves were the most ^{13}C -depleted, whereas *A. modestus* was the most ^{13}C - and ^{15}N -enriched species, and *C. chinensis* the most ^{15}N -depleted species. We observed weak temporal variations both for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Only the sampling date S4 showed significantly lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared to S1, S2 and S3 (all $p < 0.05$). SEAc were small for *A. modestus* (0.66 ‰^2), large for *C. chinensis* (1.91 ‰^2) and intermediate and similar for the three species *C. fornicata*, *O. edulis* and *C. varia* (0.82 ‰^2 , 1.14 ‰^2 and 0.05 ‰^2 , respectively), the highest overlap being between *O. edulis* and *C. varia* (Fig. 34).

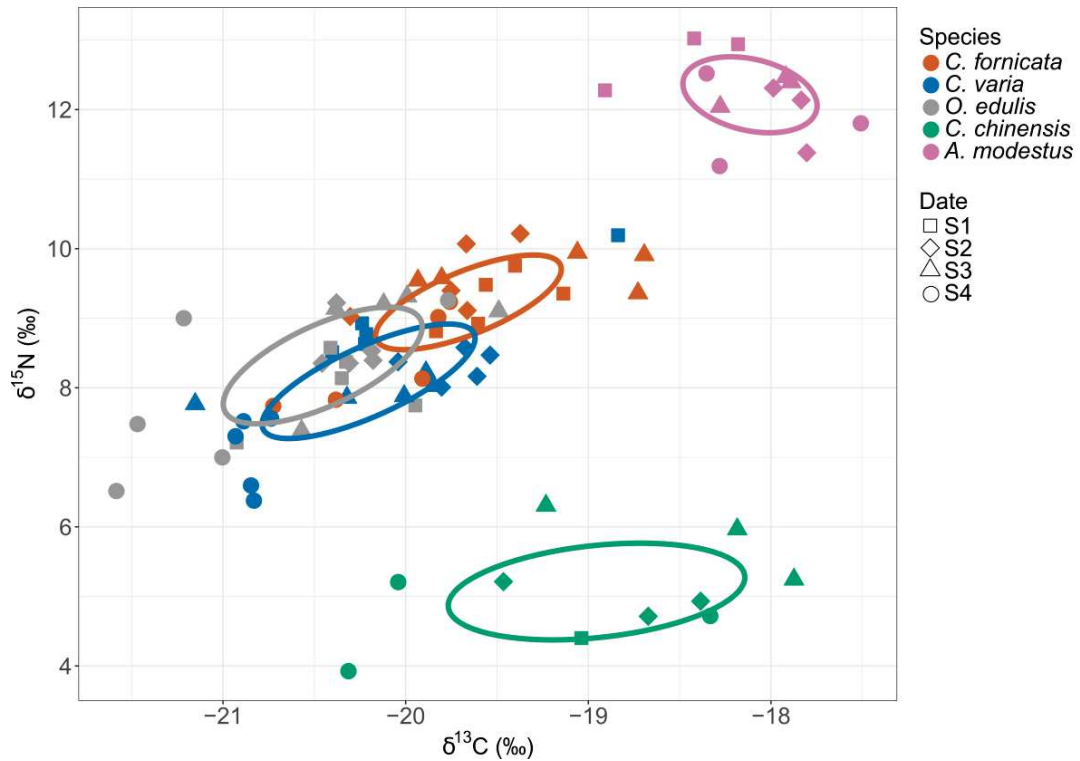


Figure 34: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ compositions of suspension-feeding species (*Crepidula fornicata*, *Chlamys varia*, *Ostrea edulis*, *Calyptrea chinensis*, *Austrominius modestus*) and sampling dates (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April). Ellipses enclosed the standard ellipse area corrected for small sample size (SEAc).

Table 11: $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ compositions (‰, mean \pm SD, $n \geq 3$) of the five suspension-feeders (*Crepidula fornicata*, *Chlamys varia*, *Ostrea edulis*, *Calyptrea chinensis*, *Austrominius modestus*) at the four sampling date (S1, S2, S3, S4).

Species	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$			
	S1	S2	S3	S4	S1	S2	S3	S4
<i>A. modestus</i>	-18.5 \pm 0.4	-17.9 \pm 0.1	-18 \pm 0.2	-18.1 \pm 0.5	12.7 \pm 0.4	11.9 \pm 0.5	12.3 \pm 0.2	11.9 \pm 0.7
<i>C. chinensis</i>	-19 \pm 0	-18.8 \pm 0.6	-18.4 \pm 0.7	-19.6 \pm 1.1	4.4 \pm 0	5 \pm 0.2	5.8 \pm 0.5	4.6 \pm 0.6
<i>C. fornicata</i>	-19.5 \pm 0.3	-19.8 \pm 0.3	-19.2 \pm 0.6	-20.1 \pm 0.4	9.3 \pm 0.4	9.6 \pm 0.5	9.7 \pm 0.3	8.4 \pm 0.7
<i>C. varia</i>	-20 \pm 0.6	-19.7 \pm 0.2	-20.2 \pm 0.5	-20.9 \pm 0.1	9 \pm 0.7	8.3 \pm 0.2	8 \pm 0.2	7.1 \pm 0.5
<i>O. edulis</i>	-20.4 \pm 0.3	-20.3 \pm 0.1	-20.1 \pm 0.4	-21 \pm 0.7	8 \pm 0.5	8.6 \pm 0.4	8.8 \pm 0.8	7.9 \pm 1.2

3.2. Fatty acids composition

Species and sampling dates explained 43 % and 7 % of the inertia of the PCA, respectively (Fig. 35). Altogether, these two factors explained 64 % of the inertia. The 5 species were well separated along the two first axes and showed significant difference in FA compositions (PERMANOVA, $p = 0.001$), species being all significantly different from each other (all $p < 0.01$). Sampling dates and the interaction between the two factors were also significant (PERMANOVA, $p <$

0.01 and $p < 0.01$, respectively). However, none of the pairwise comparisons were significant following Bonferroni correction due to lack of statistical power ($3 < n < 5$). Along the first axis, *C. fornicata* and *C. chinensis* were characterized by much higher contributions of branched FA (iso16:0, iso17:0 and ant17:0), non-methyl interrupted FA (20:2i, 22:2i and 22:2j) and 20:1n-11 than the three other species (Table 12). Overall, these FA were slightly less abundant for *C. chinensis* than for *C. fornicata*. On the other side of the first axis, *C. varia* showed lower inter-individual variability and higher contributions of the C₁₈ FA (18:2n-6, 18:3n-3 and 18:4n-3), the 14:0 and the 16:1n-7 FA than the other species (Table 13). *A. modestus* was characterized by high contributions of 18:1n-9 and 20:5n-3, and to a lesser extent of saturated FA (16:0, 18:0, 22:0) (Table 12). Being in the middle of the ordination, *O. edulis* exhibited a mixed FA composition, with the highest inter-individual variability (Table 12).

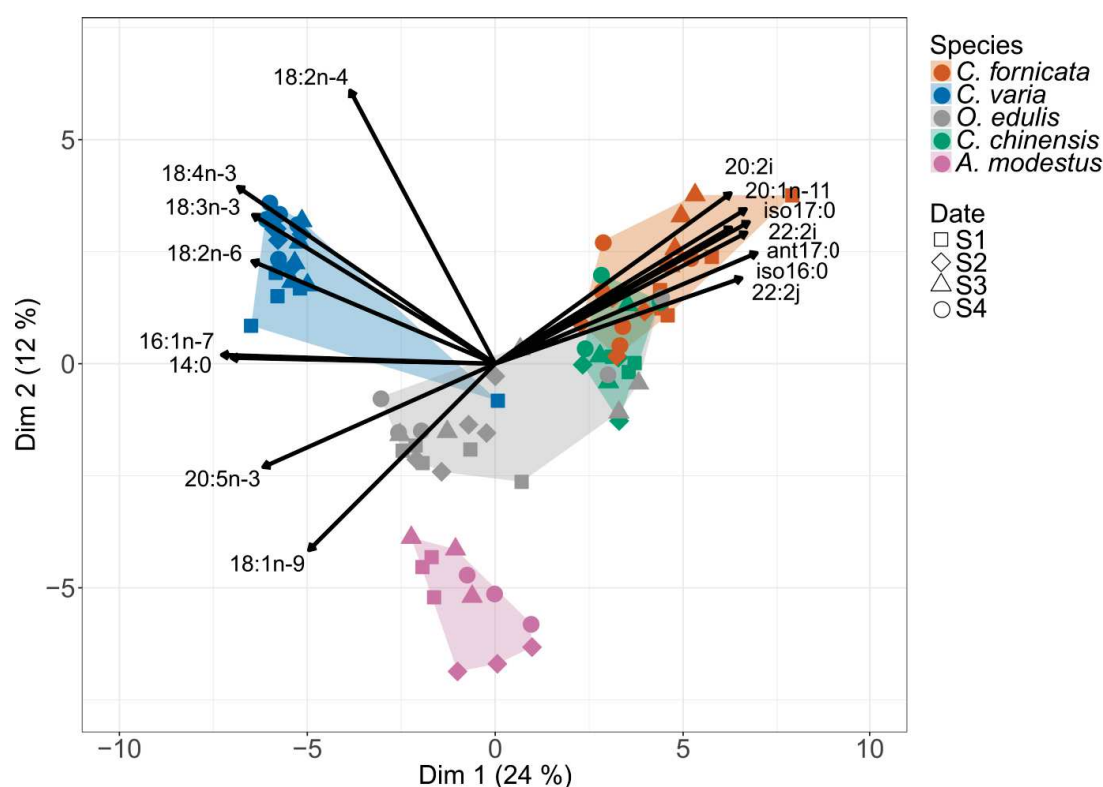


Figure 35: Principal component analysis based on the total fatty acid compositions of suspension-feeding species (*Crepidula fornicata*, *Chlamys varia*, *Ostrea edulis*, *Calyptraea chinensis*, *Austrominius modestus*) and sampling dates (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April). Ordination is along the two first axes and only the 15 most contributing FA are shown.

Table 12: Fatty acids (FA) composition of the five suspension-feeders (*Crepidula fornicata*, *Chlamys varia*, *Ostrea edulis*, *Calyptrea chinensis*, *Austrominius modestus*) on the four sampling dates (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April) (% of total FA concentration, mean ± SD, n ≥ 3). BFA: branched FA; SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA; NMI FA: non-methyl-interrupted FA; DMA: imethyl acetals FA.

Fatty acids	S1					S2				
	<i>A. modestus</i>	<i>C. chinensis</i>	<i>C. fornicata</i>	<i>M. varia</i>	<i>O. edulis</i>	<i>A. modestus</i>	<i>C. chinensis</i>	<i>C. fornicata</i>	<i>M. varia</i>	<i>O. edulis</i>
TMTD	0 ± 0	1.9 ± 0.3	1.3 ± 0.3	1.4 ± 0.4	0.3 ± 0.2	0 ± 0	2.7 ± 1.1	1.2 ± 0.2	1.8 ± 0.2	0.5 ± 0.3
ant15:0	0.2 ± 0	0.1 ± 0	0.1 ± 0	0 ± 0	0.1 ± 0	0.2 ± 0.1	0.1 ± 0.2	0 ± 0	0 ± 0	0.1 ± 0
iso16:0	0.3 ± 0	1 ± 0	1.3 ± 0.5	0.2 ± 0.1	0.3 ± 0	0.2 ± 0.1	1 ± 0.1	0.7 ± 0.1	0.1 ± 0	0.3 ± 0
ant17:0	0.8 ± 0.1	2.1 ± 0.1	3.5 ± 1.6	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.3	1.9 ± 0	2.1 ± 0.5	0.3 ± 0	0.5 ± 0.2
iso17:0	0.6 ± 0	3.6 ± 0.4	5.4 ± 1.8	0.4 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	1 ± 1.7	3.1 ± 1.8	0.5 ± 0	0.8 ± 0.1
iso18:0	0.1 ± 0.1	0.2 ± 0.2	0.7 ± 0.4	0.1 ± 0	0.1 ± 0	0 ± 0	0.1 ± 0.2	0.3 ± 0.3	0.1 ± 0	0.3 ± 0.2
∑ BFA	1.9 ± 0.2	7 ± 0.3	11 ± 4.3	1.1 ± 0.2	1.7 ± 0.1	1.2 ± 0.7	4.1 ± 1.7	6.2 ± 2.4	1 ± 0.1	2 ± 0.4
14:0	2.2 ± 0	1 ± 0.1	1.5 ± 0.3	3.6 ± 1.6	2.7 ± 1.1	2.2 ± 0.2	1.2 ± 0.3	1.3 ± 0.2	3.2 ± 0.3	2.6 ± 0.7
15:0	0.5 ± 0	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.6 ± 0	0.5 ± 0.1	0.4 ± 0	0.3 ± 0	0.7 ± 0.2
16:0	17.5 ± 0.9	14.5 ± 2.2	11.9 ± 0.8	14.7 ± 1.9	16.3 ± 2.7	17 ± 1.9	13.1 ± 3.6	11.7 ± 1	12.3 ± 1.1	16 ± 3.4
17:0	0.9 ± 0	1.2 ± 0.1	1.5 ± 0.1	0.8 ± 0.4	1.7 ± 0.2	1.2 ± 0.1	1.3 ± 0	1.4 ± 0.1	0.6 ± 0	1.6 ± 0.1
18:0	13.5 ± 1.2	10.9 ± 3.5	6.3 ± 0.9	3.4 ± 1.1	4 ± 2.3	12.6 ± 2.9	10.5 ± 4.8	6.8 ± 0.7	2.8 ± 0.5	4.6 ± 0.6
20:0	0.4 ± 0	0.2 ± 0.2	0.4 ± 0.1	0.2 ± 0.3	0.2 ± 0	0.4 ± 0.4	0.3 ± 0.1	0.5 ± 0.3	0.1 ± 0	0.1 ± 0
22:0	0.9 ± 0.4	0.2 ± 0	0.5 ± 0.1	0.2 ± 0.3	0.1 ± 0	1.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0 ± 0	0.1 ± 0
24:0	0.4 ± 0.1	0.5 ± 0.6	0.4 ± 0.1	0.1 ± 0.3	0.1 ± 0	1.9 ± 1.7	0.4 ± 0.2	0.2 ± 0	0 ± 0	0.2 ± 0.2
∑ SFA	36.3 ± 1.8	29.1 ± 5.1	23.1 ± 1.6	23.3 ± 2.3	25.8 ± 2.9	37.3 ± 3	27.6 ± 9	22.8 ± 1.5	19.2 ± 1.5	25.9 ± 4.8
14:1n-5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
16:1n-5	0.3 ± 0	0.3 ± 0.2	0.4 ± 0.4	0.2 ± 0	0.6 ± 0.4	0.5 ± 0.4	1.1 ± 0.6	0.3 ± 0.1	0.2 ± 0	1 ± 1.3
16:1n-7	4.1 ± 0.2	1.4 ± 0.3	1.4 ± 0.6	6.5 ± 2.4	2.9 ± 1	2.8 ± 1.8	1.5 ± 0.1	1.7 ± 0.2	5.4 ± 0.6	2.6 ± 0.6
16:1n-9	0.3 ± 0	0.6 ± 0	0.7 ± 0.2	0.3 ± 0.2	0 ± 0	1.3 ± 1.7	0.5 ± 0.1	0.7 ± 0.2	0.4 ± 0	0.2 ± 0.1
16:1n-11	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
17:1n-7	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0 ± 0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2
18:1n-5	0.3 ± 0	0.3 ± 0	0.4 ± 0	0.2 ± 0	0.2 ± 0	0 ± 0	0.3 ± 0.1	0.3 ± 0	0.3 ± 0	0.2 ± 0
18:1n-7	4.9 ± 0.1	2.7 ± 0.1	2.5 ± 0.2	6.2 ± 1.2	1.6 ± 0.8	4.9 ± 0.3	2.7 ± 0.4	2.8 ± 0.3	6 ± 0.9	2.1 ± 0.3
18:1n-9	9.4 ± 0.5	1.8 ± 0	0 ± 0.1	4.6 ± 1.1	4.2 ± 1.2	5.6 ± 0.3	2 ± 0.8	2.1 ± 0.3	4.5 ± 0.6	4.1 ± 0.2
18:1n-11	0.5 ± 0	0.8 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0 ± 0	0.3 ± 0	1.1 ± 0.5	0.5 ± 0.1	0.2 ± 0.1	0.1 ± 0
20:1n-7	1.1 ± 0.2	5.7 ± 0.3	4.7 ± 0.5	0.5 ± 0.3	4.6 ± 1.3	1.2 ± 0.2	6.5 ± 1.2	4.6 ± 0.2	0.4 ± 0.1	4.2 ± 0.4
20:1n-9	0.5 ± 0	1.1 ± 0	0.9 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.7 ± 0	1 ± 0.1	0.8 ± 0	0.4 ± 0	0.5 ± 0.1
20:1n-11	0.9 ± 0.1	3.1 ± 0.1	5.8 ± 0.5	0.9 ± 0.4	1 ± 0.2	0.6 ± 0.3	2.9 ± 0.5	6.1 ± 0.8	0.9 ± 0.1	1.1 ± 0.2
22:1n-7	0.5 ± 0.1	0.1 ± 0.1	0.2 ± 0	0 ± 0	0.1 ± 0	0.5 ± 0	0.2 ± 0.1	0.1 ± 0.1	0 ± 0	0.1 ± 0
22:1n-9	0 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0.2 ± 0.1	0 ± 0	0 ± 0	0.2 ± 0.2	0 ± 0	0.2 ± 0
∑ MUFA	22.8 ± 0.6	18 ± 0.6	17.7 ± 1.1	20.2 ± 3.3	16 ± 0.5	18.4 ± 1.2	20.1 ± 0.8	20.2 ± 0.6	18.9 ± 1.3	16.7 ± 1.1
16:2n-4	0.3 ± 0.2	0.2 ± 0.1	0.4 ± 0.5	0.5 ± 0.1	0.2 ± 0.1	0.7 ± 0.1	0.4 ± 0.4	0.2 ± 0	0.7 ± 0.1	0.3 ± 0
16:2n-7	0 ± 0	0.2 ± 0	0 ± 0	0.4 ± 0.6	0 ± 0	0.1 ± 0.2	2.8 ± 2.4	0.7 ± 1.5	0.2 ± 0	0 ± 0.1
16:3n-3	0.2 ± 0.1	0.1 ± 0	0.1 ± 0.1	0.3 ± 0.1	0.8 ± 1.2	0.1 ± 0.1	0.3 ± 0.3	0.1 ± 0.1	0.3 ± 0.1	0.5 ± 0.2
16:3n-4	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.8 ± 0.2	0.2 ± 0.1
16:3n-6	0.1 ± 0.2	0.4 ± 0	0.4 ± 0.3	0.1 ± 0	0.1 ± 0	0 ± 0	0.3 ± 0.2	0.5 ± 0.1	0.1 ± 0	0.3 ± 0.2
16:4n-3	0.1 ± 0.1	0.2 ± 0	0.3 ± 0.2	0.4 ± 0.2	0.1 ± 0	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.1	0.5 ± 0	0.1 ± 0.1
18:2n-4	0.3 ± 0	0.2 ± 0	0.4 ± 0.1	0.5 ± 0	0.2 ± 0	0.2 ± 0.2	0.3 ± 0	0.4 ± 0.1	0.8 ± 0.1	0.2 ± 0
18:2n-6	1.5 ± 0.2	1.1 ± 0	1.3 ± 0.2	1.9 ± 0.5	1.5 ± 0.3	1.1 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	1.8 ± 0.1	1.5 ± 0.2
18:3n-3	0.5 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	3.5 ± 1.1	2.8 ± 0.8	0.5 ± 0.2	0.8 ± 0.3	0.9 ± 0.2	3.9 ± 0.1	2.7 ± 0.8
18:3n-4	0.4 ± 0.1	0.6 ± 0.3	1 ± 0.5	0.1 ± 0.1	0.2 ± 0	0.9 ± 0.5	0.7 ± 0.3	0.4 ± 0.3	0.3 ± 0.1	0.1 ± 0
18:3n-6	0 ± 0	0.1 ± 0.1	0.1 ± 0	0.2 ± 0.1	0.1 ± 0	0 ± 0	0.1 ± 0.1	0.1 ± 0	0.2 ± 0	0.2 ± 0.1
18:4n-1	0.1 ± 0.2	0.2 ± 0.1	0.1 ± 0	0.1 ± 0.1	0 ± 0	1.3 ± 1.1	0.3 ± 0.4	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0
18:4n-3	1.2 ± 0.2	1.9 ± 0.5	0.7 ± 0.6	9.2 ± 3.1	3.8 ± 1.1	0.7 ± 0.4	1.6 ± 0.5	1.8 ± 0.4	13.8 ± 1.5	3.8 ± 1.1
20:2n-6	0.2 ± 0.2	1.3 ± 0.3	1.2 ± 0.1	0.7 ± 0.3	0.3 ± 0.1	0.8 ± 0.1	1.2 ± 0.3	1.2 ± 0.2	0.7 ± 0	0.3 ± 0.1
20:3n-3	0 ± 0	0.3 ± 0	0.4 ± 0.1	0.6 ± 0.1	0.1 ± 0	0 ± 0	0.2 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.4 ± 0.3
20:3n-6	0 ± 0	0.1 ± 0	0.1 ± 0	0.3 ± 0.1	0.2 ± 0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0	0.1 ± 0.1
20:4n-3	0.5 ± 0	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.2 ± 0.2	0.6 ± 0.4	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
20:4n-6	3 ± 0.2	2.5 ± 0.2	2.7 ± 0.3	3 ± 0.7	4.4 ± 1.4	2.4 ± 0.1	2.2 ± 0.5	2.7 ± 0.4	1.5 ± 0.7	3.7 ± 1.6
20:5n-3	15.2 ± 0.8	11.9 ± 1.6	10.5 ± 2.6	15.6 ± 2.7	17.1 ± 2.1	16.9 ± 0.6	12.5 ± 4.8	12.1 ± 1.3	17.2 ± 1.3	15 ± 2.4
21:5n-3	0.7 ± 0.1	0.4 ± 0.1	0.4 ± 0	1 ± 0.1	0.7 ± 0.1	0 ± 0	0.4 ± 0	0.5 ± 0.1	1.3 ± 0.1	0.6 ± 0.2
22:4n-6	0.3 ± 0	0.3 ± 0	0.6 ± 0.2	0.4 ± 0.4	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.3	0.2 ± 0	0.5 ± 0.1
22:5n-3	3 ± 0.5	2 ± 0.5	2 ± 0.5	0.7 ± 0.3	1.1 ± 0.2	2.9 ± 0.6	1.6 ± 0.6	2.4 ± 0.3	0.6 ± 0.1	1.1 ± 0.2
22:5n-6	0.2 ± 0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.3	0.5 ± 0.1	0.8 ± 1.1	0.3 ± 0.3	0.4 ± 0.1	0.2 ± 0	0.8 ± 0.4
22:6n-3	9.6 ± 0.9	10.5 ± 2	9.7 ± 2.1	10.7 ± 7.3	13.1 ± 1.7	9 ± 0.4	10.3 ± 4.5	11.1 ± 1.4	9.2 ± 0.6	13.4 ± 1.2
∑ PUFA	37.5 ± 1.4	36.3 ± 4.3	34.4 ± 5.1	51.7 ± 3.7	48.5 ± 3.6	39.7 ± 0.7	38.6 ± 13.7	38.7 ± 4.5	56.3 ± 1.2	46.6 ± 3.4
20:2i	0 ± 0	0.4 ± 0.1	1.1 ± 0.3	0.2 ± 0.1	0.3 ± 0.2	0 ± 0	0.4 ± 0.1	0.9 ± 0.2	0.2 ± 0	0.4 ± 0.2
20:2j	0 ± 0	0.5 ± 0.2	0.5 ± 0.1	0.1 ± 0.1	0.1 ± 0	0 ± 0	0.3 ± 0.2	0.4 ± 0	0.1 ± 0	0.1 ± 0
22:2i	0.2 ± 0.3	0.5 ± 0.1	1.9 ± 0.3	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.5 ± 0.2	1.6 ± 0.5	0.1 ± 0	0.6 ± 0.2
22:2j	0.1 ± 0.1	3.8 ± 0.6	5.4 ± 0.8	0.2 ± 0.2	2.5 ± 1.1	0.3 ± 0.2	3.9 ± 1.8	5.1 ± 1.1	0.1 ± 0	3.4 ± 1.3
22:3nmi	0 ± 0	0.2 ± 0	0.5 ± 0.2	0.1 ± 0.2	0.3 ± 0.2	1 ± 0.1	0.1 ± 0.1	0.5 ± 0.2	0.1 ± 0	0.1 ± 0.1
∑ NMI	0.3 ± 0.1	5.4 ± 1	9.4 ± 1.4	0.8 ± 0.6	3.6 ± 1.5	1.5 ± 0.4	5.1 ± 2	8.4 ± 1.8	0.6 ± 0.1	4.5 ± 1.5
16:0DMA	0.1 ± 0.1	0 ± 0	0.1 ± 0	0.1 ± 0.2	0.1 ± 0.1	0.6 ± 0.1	0.7 ± 1.1	0 ± 0	0.1 ± 0	0.2 ± 0.1
18:0DMA	0.4 ± 0	1.3 ± 0.4	1 ± 0.5	0.8 ± 0.3	3.1 ± 2.3	0.3 ± 0.2	0.4 ± 0.4	1.2 ± 0.4	1.6 ± 0.4	2.9 ± 2.1
∑ DMA	0.5 ± 0.2	1.3 ± 0.4	1.1 ± 0.5	0.9 ± 0.2	3.2 ± 2.3	0.9 ± 0.2	1.1 ± 0.8	1.3 ± 0.5	1.7 ± 0.4	3.1 ± 2.2
n-3	30.8 ± 1.6	28.6 ± 4.4	25.6 ± 6.1	42.6 ± 3.1	40.2 ± 2.7	30.7 ± 0.6	28.6 ± 10.9	30.2 ± 3.5	48.2 ± 1.5	38.3 ± 1.9
n-6	5.3 ± 0.5	6.3 ± 0.4	6.8 ± 0.3	6.8 ± 1.1	7.5 ± 1.2	5.7 ± 1	5.5 ± 0.8	6.6 ± 0.3	4.9 ± 0.8	7.3 ± 1.9
n-3/n-6	5.8 ± 0.8	4.6 ± 1.1	3.8 ± 1	6.3 ± 1	5.4 ± 0.6	5.5 ± 0.9	5.1 ± 1.9	4.6 ± 0.6	10.1 ± 1.7	5.4 ± 1.1
PUFA/SFA	1 ± 0.1	1.3 ± 0.4	1.5 ± 0.3	2.2 ± 0.3	1.9 ± 0.4	1.1 ± 0.1	1.6 ± 0.9	1.7 ± 0.3	2.9 ± 0.2	1.9 ± 0.5
20:5n-3/22:6n-3	1.6 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.9 ± 0.9	1.3 ± 0.2	1.9 ± 0	1.2 ± 0.1	1.1 ± 0.1	1.9 ± 0.2	1.1 ± 0.2
∑ LCFA (22:0 + 24:0)	1.3 ± 0.5	0.7 ± 0.6	0.9 ± 0.1	0.3 ± 0.6	0.2 ± 0.1	3.3 ± 1.6	0.6 ± 0.3	0.6 ± 0.1	0 ± 0	0.3 ± 0.2
∑ (18:2n-6 + 18:3n-3)	2 ± 0.2	1.9 ± 0.1	2.3 ± 0.1	5.4 ± 1.6	4.3 ± 1.1	1.7 ± 0.1	1.9 ± 0.2	2.1 ± 0.2	5.6 ± 0.2	4.1 ± 1
∑ (14:0 + 16:1n-7)	6.3 ± 0.2	2.5 ± 0.3	2.9 ± 0.8	10.2 ± 4	5.7 ± 2	5 ± 2	2.8 ± 0.4	3 ± 0.4	8.5 ± 0.8	5.2 ± 1.3

CHAPITRE III : Interactions trophiques interspécifiques multi-échelles

Fatty acids	S3					S4				
	<i>A. modestus</i>	<i>C. chinensis</i>	<i>C. fornicata</i>	<i>M. varia</i>	<i>O. edulis</i>	<i>A. modestus</i>	<i>C. chinensis</i>	<i>C. fornicata</i>	<i>M. varia</i>	<i>O. edulis</i>
TMTD	0 ± 0	2.3 ± 0.3	1.3 ± 0.7	1.5 ± 0.2	0.3 ± 0.2	0 ± 0	2.8 ± 0.1	2.1 ± 1	1.3 ± 0.1	0.9 ± 1.2
ant15:0	0 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0
iso16:0	0 ± 0	0.9 ± 0.1	0.9 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	0.1 ± 0	0.3 ± 0
ant17:0	0.8 ± 0.3	1.8 ± 0.2	2.8 ± 0.7	0.2 ± 0	0.5 ± 0.1	0.6 ± 0.1	2.1 ± 0.2	2.4 ± 0.7	0.2 ± 0	0.3 ± 0.2
iso17:0	0.4 ± 0.1	2.8 ± 0.3	3.5 ± 2	0.4 ± 0.2	0.7 ± 0.1	1.1 ± 0.2	3.2 ± 0.1	3.8 ± 1	0.5 ± 0.1	0.7 ± 0.2
iso18:0	0.1 ± 0.1	0.6 ± 0.1	0.5 ± 0.5	0.6 ± 0.2	0.2 ± 0.1	0.8 ± 0.4	0.4 ± 0.4	0.3 ± 0.6	0.6 ± 0.1	0.4 ± 0.2
Σ BFA	1.3 ± 0.5	6.2 ± 0.3	7.7 ± 2.6	1.3 ± 0.3	1.7 ± 0.2	2.7 ± 0.4	6.8 ± 0.4	7.4 ± 2.3	1.5 ± 0.1	1.7 ± 0.3
14:0	1.7 ± 0.4	0.9 ± 0.1	1.3 ± 0.4	3.2 ± 0.5	2.2 ± 1.2	2.4 ± 0.8	1.1 ± 0.1	2 ± 0.5	3.1 ± 0.3	2.2 ± 1.3
15:0	0.4 ± 0.1	0.4 ± 0	0.4 ± 0	0.3 ± 0	0.5 ± 0.2	0.5 ± 0	0.4 ± 0	0.5 ± 0.1	0.3 ± 0	0.7 ± 0.2
16:0	16 ± 1.8	14.3 ± 0.8	10.9 ± 1	11.7 ± 1.2	11.9 ± 3.8	16 ± 0.7	11.3 ± 1.2	12 ± 0.4	12.6 ± 0.5	12.8 ± 5.2
17:0	0.7 ± 0.1	0.9 ± 0.1	1 ± 0.5	1 ± 0.2	1.3 ± 0.4	1.3 ± 1.2	1.1 ± 0.1	0.9 ± 0.4	0.5 ± 0.1	1.4 ± 0.2
18:0	14.5 ± 2.8	11.8 ± 1.1	6.6 ± 0.9	2 ± 0.3	4.8 ± 1.3	9.5 ± 3	7.4 ± 1	6.9 ± 0.4	2.2 ± 0.2	5.5 ± 1.6
20:0	0.4 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.2 ± 0.1	0.4 ± 0.1	1.1 ± 1.6	0.2 ± 0	0.1 ± 0	0.8 ± 0.9
22:0	0.5 ± 0	0.2 ± 0	0.4 ± 0.1	0 ± 0	0.2 ± 0.2	0.6 ± 0.3	0.1 ± 0.1	0.3 ± 0.1	0 ± 0	0.1 ± 0.1
24:0	1.1 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0 ± 0	0.3 ± 0.2	1.5 ± 0.6	0.3 ± 0	0.4 ± 0.1	0 ± 0	0.3 ± 0.2
Σ SFA	35.2 ± 4.6	29 ± 1.7	21.2 ± 1.1	18.3 ± 1.4	21.4 ± 5.9	32.1 ± 3.6	22.8 ± 3.4	23.1 ± 1	18.8 ± 0.7	23.8 ± 4.3
14:1n-5	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0.1
16:1n-5	0.1 ± 0.1	0.2 ± 0	0.3 ± 0.1	0.2 ± 0	0.2 ± 0.3	0.3 ± 0.3	0.5 ± 0.3	0.5 ± 0.2	0.2 ± 0	0.5 ± 0.2
16:1n-7	3.6 ± 1.1	1.4 ± 0	1.5 ± 0.3	5.6 ± 1.1	2 ± 1.1	5.4 ± 2	1.7 ± 0.1	2.3 ± 0.6	5.1 ± 0.6	2.2 ± 0.8
16:1n-9	0.2 ± 0.2	0.5 ± 0	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.5 ± 0	0.5 ± 0	0.4 ± 0.1	0.3 ± 0.1
16:1n-11	0 ± 0	0 ± 0.1	0.4 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0
17:1n-7	0.1 ± 0.2	0.1 ± 0	0.4 ± 0.5	0.2 ± 0.2	0.1 ± 0.1	0 ± 0	0.2 ± 0	0.3 ± 0.4	0.2 ± 0	0.2 ± 0
18:1n-5	0.3 ± 0.1	0.3 ± 0	0.3 ± 0	0.2 ± 0	0.1 ± 0.1	0.4 ± 0.2	0.3 ± 0	0.3 ± 0	0.2 ± 0	0.3 ± 0.3
18:1n-7	4.6 ± 0.3	2.6 ± 0.1	2.5 ± 0.2	4.9 ± 1	2.2 ± 0.6	3.6 ± 0.1	2.8 ± 0.2	2.3 ± 0.3	4.5 ± 0.3	1.6 ± 1.4
18:1n-9	4.7 ± 0.2	1.9 ± 0.2	2 ± 0.2	4 ± 0.7	5.3 ± 1.9	3.9 ± 0.7	1.7 ± 0.3	2 ± 0.2	3.9 ± 0.3	2.9 ± 1.4
18:1n-11	0.4 ± 0.1	0.9 ± 0.1	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.6 ± 0.1	1.4 ± 0.4	0.7 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
20:1n-7	0.8 ± 0.1	5 ± 0.4	4.6 ± 0.4	0.3 ± 0	6.6 ± 3.2	1 ± 0.1	4.8 ± 3.6	4.2 ± 0.3	0.4 ± 0	2.5 ± 0.8
20:1n-9	0.6 ± 0.1	1 ± 0.1	0.8 ± 0.2	0.4 ± 0.2	0.5 ± 0.4	0.5 ± 0	0.9 ± 0.6	0.8 ± 0.1	0.4 ± 0	0.3 ± 0.1
20:1n-11	0.4 ± 0.1	3.2 ± 0.6	5.6 ± 0.6	0.9 ± 0.5	1.8 ± 1.1	0.7 ± 0.1	4.5 ± 2.5	5.2 ± 0.9	1 ± 0.1	3.7 ± 3.9
22:1n-7	0.2 ± 0.3	0.3 ± 0.1	0.1 ± 0.1	0 ± 0	0.1 ± 0.2	0.8 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0 ± 0	0 ± 0
22:1n-9	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0 ± 0	0.4 ± 0.5	0.7 ± 0.1	0 ± 0.1	0.2 ± 0.2	0 ± 0	0.1 ± 0.1
Σ MUFA	16.3 ± 1.3	17.6 ± 1.3	19.9 ± 1	17.4 ± 2.6	19.7 ± 3.7	18 ± 1.5	19.6 ± 2.4	19.7 ± 0.9	16.4 ± 0.8	14.9 ± 0.9
16:2n-4	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0.3	0.7 ± 0.1	0.2 ± 0.2	0.9 ± 0.1	0.6 ± 0.2	0.4 ± 0.2	0.6 ± 0.1	0.2 ± 0.1
16:2n-7	0 ± 0	0 ± 0	0.8 ± 1.7	0.1 ± 0.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
16:3n-3	0.5 ± 0.2	0.8 ± 0.6	0.1 ± 0.1	0.1 ± 0	0.6 ± 0.5	0.9 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0	0.4 ± 0.1
16:3n-4	0.1 ± 0.1	0 ± 0	0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.1	0.1 ± 0.2	0.9 ± 0.1	0.1 ± 0.1
16:3n-6	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.9 ± 1.5	0 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
16:4n-3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
18:2n-4	0.2 ± 0.1	0.3 ± 0	0.5 ± 0	0.7 ± 0.1	0.2 ± 0.1	0 ± 0	0.2 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.2 ± 0.1
18:2n-6	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.9 ± 0.1	1.3 ± 0.4	1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.9 ± 0.1	1.4 ± 0.8
18:3n-3	0.5 ± 0.1	1 ± 0.2	0.9 ± 0.1	4.6 ± 0.3	3 ± 1.5	0.4 ± 0.3	1.3 ± 1.6	1.1 ± 0.2	4.9 ± 0.2	3.5 ± 0.9
18:3n-4	0.3 ± 0.1	1.1 ± 0.2	1.1 ± 0.3	0.3 ± 0.1	0.5 ± 0.3	1 ± 0.6	1.5 ± 0.7	1 ± 0.4	0.3 ± 0	0.5 ± 0.4
18:3n-6	0.2 ± 0.1	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0.1	0 ± 0	0.8 ± 0.9	0.1 ± 0.1	0.2 ± 0	0.6 ± 0.6
18:4n-1	0 ± 0	0 ± 0	0 ± 0.1	0.3 ± 0.1	0.2 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.1	0 ± 0
18:4n-3	2.4 ± 0.3	3.3 ± 0.7	1.7 ± 0.3	16.9 ± 2.9	4.8 ± 2.4	3.3 ± 0.6	1.9 ± 1.7	3.1 ± 1	19.1 ± 1.1	4.3 ± 4.1
20:2n-6	0.2 ± 0.1	0.9 ± 0.2	1.1 ± 0.2	0.5 ± 0.1	0.5 ± 0.4	0.4 ± 0.1	0.9 ± 0.1	1 ± 0.2	0.6 ± 0.1	0.2 ± 0.1
20:3n-3	0 ± 0	0.2 ± 0	0.4 ± 0.1	0.4 ± 0.4	0.3 ± 0.4	0 ± 0	0.2 ± 0	0.4 ± 0.1	0.8 ± 0.2	0.2 ± 0
20:3n-6	0 ± 0	0.4 ± 0.1	0.2 ± 0.1	0.4 ± 0.3	0.5 ± 0.5	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0	0.1 ± 0.1
20:4n-3	0.5 ± 0	0.6 ± 0.2	0.5 ± 0.1	1 ± 0.3	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	1.1 ± 0.2	0.5 ± 0.3
20:4n-6	2.4 ± 0.8	2.1 ± 0.4	2.6 ± 0.6	1.4 ± 0.7	3.9 ± 1.9	1.5 ± 0.2	1.9 ± 0.4	2.4 ± 0.4	0.8 ± 0.2	4.1 ± 2.8
20:5n-3	18.1 ± 4	11.3 ± 0.8	11.1 ± 1.8	17.1 ± 0.9	12.2 ± 4.5	14.6 ± 1.9	14 ± 2.2	11.4 ± 1.8	14.9 ± 0.5	13.3 ± 2.1
21:5n-3	0.7 ± 0.6	0.4 ± 0	1.4 ± 0.6	1.4 ± 0	0.5 ± 0.3	0.7 ± 0	0.4 ± 0.1	0.5 ± 0.1	1.2 ± 0	1.4 ± 0.4
22:4n-6	0.5 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.2 ± 0	0.7 ± 0.5	0.3 ± 0.3	0.3 ± 0	0.6 ± 0.2	0.2 ± 0	0.6 ± 0.5
22:5n-3	3.8 ± 0.7	1.4 ± 0.4	2.2 ± 1.3	0.5 ± 0.1	1.1 ± 0.3	9.9 ± 2.1	1.6 ± 0.2	2.2 ± 0.5	0.5 ± 0.1	1.3 ± 0.4
22:5n-6	0.2 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0	0.5 ± 0.2
22:6n-3	10.6 ± 1.5	10.2 ± 1.3	10.3 ± 1.6	9.7 ± 1	11.8 ± 2.1	7.7 ± 1.4	11.1 ± 2.3	9.3 ± 2	10.4 ± 0.6	14.7 ± 1.2
Σ PUFA	42.9 ± 5.8	36.7 ± 1.3	38 ± 3.7	59.1 ± 3.3	43.8 ± 5.3	44.5 ± 2.8	39.7 ± 3.1	37.3 ± 3.4	60 ± 1.6	48.2 ± 3.6
20:2i	0.1 ± 0.1	0.5 ± 0.1	0.9 ± 0.2	0.1 ± 0.1	0.7 ± 0.5	0 ± 0	0.3 ± 0.3	0.9 ± 0.2	0.1 ± 0	0.3 ± 0.1
20:2j	0 ± 0	0.5 ± 0	0.4 ± 0	0.1 ± 0.1	0.8 ± 1.6	0 ± 0	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0	0.1 ± 0.1
22:2i	0.1 ± 0.1	0.6 ± 0.1	1.7 ± 0.4	0.1 ± 0	0.9 ± 0.5	0 ± 0	0.5 ± 0	1.5 ± 0.3	0.1 ± 0	0.8 ± 0.7
22:2j	0.3 ± 0.3	3.8 ± 0.6	4.2 ± 0.7	0.1 ± 0	5.1 ± 3.4	0 ± 0	3.8 ± 0.7	4.6 ± 0.8	0.1 ± 0	5.4 ± 5.1
22:3nmi	0 ± 0	0.1 ± 0.1	0.5 ± 0.2	0 ± 0	0.6 ± 0.5	0 ± 0	0 ± 0.1	0.5 ± 0.1	0 ± 0	0 ± 0
Σ NMI	0.4 ± 0.5	5.5 ± 0.8	7.8 ± 1.3	0.4 ± 0.1	8.1 ± 4.8	0 ± 0	5 ± 1.2	7.8 ± 1.4	0.4 ± 0.1	6.6 ± 5.9
16:0DMA	1.6 ± 0.8	0.1 ± 0	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.9 ± 0.4	0.3 ± 0.1	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
18:0DMA	1.7 ± 0.9	1.6 ± 0.1	1.7 ± 1	1.6 ± 0.4	3.4 ± 1.7	1.2 ± 0.7	1.9 ± 1.3	0.8 ± 0.4	1.1 ± 0.7	3 ± 1.6
Σ DMA	3.4 ± 1.7	1.7 ± 0.1	1.9 ± 0.8	1.7 ± 0.4	3.6 ± 1.7	2.1 ± 1.1	2.2 ± 1.3	1.3 ± 0.6	1.2 ± 0.6	3.1 ± 1.6
n-3	37.1 ± 4.7	29.3 ± 1.3	28.6 ± 2.2	51.7 ± 3.1	34.9 ± 6	38 ± 1.1	31.4 ± 4	29 ± 4	53.1 ± 1.6	39.6 ± 6.6
n-6	4.8 ± 1	5.4 ± 0.4	6.3 ± 0.9	5.1 ± 0.5	7.7 ± 2	4.4 ± 1.7	5.7 ± 0.5	6.3 ± 0.7	4.2 ± 0.3	7.6 ± 3.4
n-3/n-6	7.7 ± 0.7	5.4 ± 0.3	4.6 ± 0.4	10.2 ± 1.3	4.8 ± 1.5	9.4 ± 2.8	5.5 ± 0.8	4.6 ± 0.9	12.6 ± 1	6.3 ± 3.1
PUFA/SFA	1.2 ± 0.3	1.3 ± 0.1	1.8 ± 0.2	3.3 ± 0.4	2.2 ± 0.8	1.4 ± 0.2	1.8 ± 0.3	1.6 ± 0.2	3.2 ± 0.2	2.1 ± 0.3
20:5n-3/22:6n-3	1.7 ± 0.3	1.1 ± 0.1	1.1 ± 0.1	1.8 ± 0.2	1 ± 0.3	2 ± 0.6	1.3 ± 0.2	1.2 ± 0.2	1.4 ± 0.1	0.9 ± 0.2
Σ LCFA (22:0 + 24:0)	1.5 ± 0.2	0.5 ± 0.1	0.7 ± 0.2	0 ± 0	0.5 ± 0.3	2.1 ± 0.9	0.4 ± 0.1	0.7 ± 0.1	0 ± 0	0.4 ± 0.1
Σ (18:2n-6 + 18:3n-3)	1.6 ± 0.2	2.1 ± 0.3	1.9 ± 0.2	6.5 ± 0.4	4.3 ± 1.6	1.4 ± 0.4	2.5 ± 1.5	2.4 ± 0.3	6.8 ± 0.3	4.9 ± 1.6
Σ (14:0 + 16:1n-7)	5.3 ± 1.4	2.3 ± 0.1	2.9 ± 0.7	8.8 ± 1.4	4.2 ± 2.3	7.8 ± 2.8	2.9 ± 0.3	4.3 ± 1	8.2 ± 0.9	4.5 ± 2.1

The sum of 14:0 and 16:1n-7 ranged from 2.5 % to 10 % and differed significantly among species ($p < 0.001$, Table 13). *C. varia* showed the highest values, *C. fornicata* and *C. chinensis* grouped together and exhibited the lowest values and *A. modestus* and *O. edulis* also grouped together and showed intermediate values. The 20:5n-3/22:6n-3 ratio also varied between species ($p < 0.001$, Table 13) and discriminated two groups, *C. varia* and *A. modestus* having the highest ratios (1.4 to 1.9) and *C. chinensis*, *C. fornicata* and *O. edulis* the lowest (0.9 to 1.3). The sum of 18:2n-6 and 18:3n-3 differed significantly according to species ($p < 0.001$, Table 13) and formed three different groups. *C. chinensis*, *C. fornicata* and *A. modestus* were characterized by lower values (1.4 to 2.4 %) than *O. edulis* (4.1 to 4.9 %) which had also lower values than *C. varia* (5.4 to 6.8 %).

The sum of branched FA showed significant differences among species and sampling dates, with a significant interaction between these two factors (all $p < 0.05$, Table 13). The contribution of this marker was higher for *C. fornicata* (7.5 – 11 %) than for *C. chinensis* (4 – 7 %) and than for the three species *C. varia*, *O. edulis* and *A. modestus* (< 2.5 %). This marker showed significant temporal variation only for *C. fornicata* and *C. chinensis*, due to very high values at S1 (11 % and 7 %, respectively).

The ratio between the sum of PUFA and the sum of SFA showed significant difference among species ($p < 0.001$, Table 13). *C. varia* had significantly higher values (~ 2.9) than *O. edulis* (~ 2). *A. modestus*, *C. chinensis* and *C. fornicata* formed a third group with significantly lower values (< 1.7) than the two bivalves. This marker showed significant temporal variation ($p < 0.01$, Table 13), with lower ratios at S1 than at S3 and S4.

Table 13: Results of Factorial two way ANOVA showing the effects of species (*C. fornicata*, *C. varia*, *O. edulis*, *C. chinensis*, *A. modestus*) and sampling dates (S1 = 26th February, S2 = 21th March, S3 = 28th March and S4 = 12th April). Significant threshold: $p < 0.05$.

Source of variation	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	14:0 + 16:1n-7	20:5n-3 /22:6n-3	Σ Branch FA	18:2n-6 + 18:3n-3	18:4n-3	18:1n-9	Σ LCFA	Σ PUFA / Σ SFA
Species	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Date	< 0.001	< 0.001	ns	ns	< 0.05	ns	< 0.001	< 0.01	ns	< 0.01
Species \times Date	ns	< 0.05	ns	ns	< 0.05	ns	< 0.001	< 0.001	< 0.001	ns

The sum of long chain saturated FA (22:0 + 24:0) differed significantly between species ($p < 0.001$, Table 13), *A. modestus* showing higher values (1.5 to

3.3 %) than all other species (< 1 %). The interaction between species and sampling dates was also significant, due to high values of *A. modestus* (3.3 %) at S2. The FA 18:1n-9 showed significant differences among species and sampling dates, with a significant interaction between these two factors (all $p < 0.01$, Table 13). Overall, this FA contributed significantly more in *A. modestus* than in *C. varia* and *O. edulis*. *C. chinensis* and *C. fornicata* showed similar values which were significantly lower than those of the three previous species. The contribution of 18:1n-9 decreased over time for all species with significantly lower values at S4. This temporal variation was stronger for *A. modestus* (9 % at S1 to 3 % at S4) and *O. edulis* (4.2 % at S1 to 2.9 % at S4) than for other species.

The FA 18:4n-3 showed significant difference among species and sampling dates, with a significant interaction between these two factors (all $p < 0.001$, Table 13). Overall, the contribution of this FA was significantly higher in *C. varia* than in *O. edulis*. *C. chinensis*, *C. fornicata* and *A. modestus* showed similar values which were significantly lower than those of the bivalves. The contribution of 18:4n-3 increased over time for all species but showed a marked seasonality for *C. varia* (9 % at S1 to 19 % at S4), *C. fornicata* (0.7 % at S1 to 3.1 % at S4) and *A. modestus* (1.2 % at S1 to 3.3 % at S4).

4. Discussion

Benthic suspension-feeding species are mostly sessile and have to deal with a potentially high interspecific competition for space and food (Connell, 1961; Svensson and Marshall, 2015). For comparable reason, the introduction of non-native suspension-feeders may have large consequences for natives ones. In this study, we investigated how the invasive species *Crepidula fornicata* interacts in terms of degree of food partitioning with co-occurring species within its habitat. We focused on four species commonly associated with *C. fornicata*, belonging to different taxa and closely associated with the *Crepidula* beds, namely the Calyptraeidae *Calyptraea chinensis*, the barnacle *Austrominius modestus*, the scallop *Chlamys varia* and the oyster *Ostrea edulis*. Trophic niches were assessed using both SIR and FA compositions, the latter being particularly relevant when dealing with the complex origin of particulate organic matter (POM). Indeed, POM in coastal waters is composed of mixture of microscopic organisms and detritus

exhibiting a large array of size, nutritive quality or assimilation properties (Androuin et al., *article 3*; Lienart et al., 2016; 2017).

4.1. Linking biomarkers to the trophic niches

The combined use of SIR and FA markers enabled a clear discrimination of trophic niches of the five studied species, confirming our hypothesis that the invasive slipper limpet does not overlap with the co-occurring species. Whereas bivalve species *C. varia* and *O. edulis* relied more on higher food source quality such as diatoms or green macroalgae, the Calyptraeid's species *C. fornicata* and *C. chinensis* likely fed on lower quality food sources due to more bacteria in their diet. The barnacles showed more temporal variability with higher food source quality in spring (diatoms) than in winter (detritus and animal material).

As already mentioned by Androuin et al. (*article 3*), stable isotopes suggested that *C. fornicata* relied mainly on diatoms, dinoflagellates and bacteria from re-suspended POM (RPOM). Indeed, this food source was not only composed of fresh (diatoms) but also bacteria and detritic (pheopigments, and vascular plants) organic matter. Fatty acids suggest a lower food quality of *C. fornicata* due to the higher contribution of branched FA (bacterial biomarker), the lower ratio of polyunsaturated/saturated FA (biomarker of fresh organic matter) as well as the lower contribution of diatoms biomarkers than for the other species. It is therefore possible that *C. fornicata* may have only access to a fraction of RPOM with lower food quality (Dubois et al., 2014).

Overall, the FA compositions of *C. chinensis* and *C. fornicata* were similar, FA biomarkers indicating a diet based on a rather low food quality compared to bivalves and barnacles. However, these two Calyptraeid's species had very different SIR. *C. chinensis* was slightly enriched in ^{13}C but especially depleted in ^{15}N . Such depleted values have been recorded in anoxic sediment due to the activity of denitrifying bacteria which add ^{15}N depleted biomass to the residual material. $\delta^{15}\text{N}$ values of this potential food can then drop down by 3-5 ‰ compared to freshly RPOM (Lehmann et al., 2002), which is in agreement with the organic matter food sources in our study site (Androuin et al., *article 3*). Since *C. fornicata* and *C. chinensis* are taxonomically (i.e., family), biologically (i.e., feeding mode) and physiologically (i.e., protandric hermaphroditism) very close, it is unlikely that such difference in $\delta^{15}\text{N}$ was due to different diet-fractionation processes (Gannes and al.,

1997; Schlacher and Connelly, 2014). The standard ellipse area and the path direction are also informative (Jackson et al., 2011). In the case of *C. chinensis*, the ellipse was wider and flatter than the one of *C. fornicata*, which followed the same trend as those of the two bivalves (Fig. 34). This suggests more variation in carbon sources but also less temporal variation in the nitrogen food source of *C. chinensis*, which is consistent with our microhabitat hypothesis.

The fact that the scallop *C. varia* exhibited high ratio of PUFA/SFA, and did not show branched FA (bacterial biomarkers), suggests its diet was based on fresher and/or more labile sources of organic matter than that of the other species (Blanchet-Aurigny et al., 2015; Pernet et al., 2012). Over the study period, this bivalve species also showed the highest contribution of the well correlated two diatom markers (14:0 and 16:1n-7) (Budge and Parrish, 1998) which originate from RPOM and biofilm for 16:1n-7 and from pelagic POM (PPOM) for 14:0 (Androuin et al., *article 3*). These results mean that *C. varia* could feed both on phytoplankton and microphytobenthos. Interestingly, this species exhibited a sharp increase of the contribution of the 18:4n-3 at the end of the sampling period (from 8 % at S1 to 19 % at S4), a FA which may originate from different food sources. According to previous studies, it could be a biomarker of either dinoflagellates (Budge and Parrish, 1998; Dalsgaard et al., 2003), or green macroalgae (Fleurence et al., 1994). Accumulations of green macroalgae are recorded almost every year near our study site (Study Centre for Algal Promotion, <http://www.ceva.fr>; Ragueneau et al., 2018) and FA measurements performed with two species of green macroalgae from the same area revealed high amounts of 18:3n-3 (10 %) and 18:4n-3 (13 %), as well as 18:1n-7 (8 %) and 18:2n-6 (2 %) (Supplementary Table 15). These FA have already been measured for the same genera (*Ulva* spp.) in the study area (Fleurence et al., 1994) and well characterized *C. varia*. 18:3n-3, when present together with 18:2n-6, are biomarkers of green macroalgae or terrestrial vascular plants (Kelly et Scheibling, 2012; Parrish et al., 1995). However, *C. varia* did not show any contribution of long chain saturated FA (22:0 and 24:0) which also characterized terrestrial vascular plants (Budge et al., 2001; Canuel, 1991). Our results strongly suggest therefore that green macroalgae significantly contribute to the diet of *C. varia* in our study site.

O. edulis exhibited an intermediate FA composition between those found in other species. The low PUFA/SFA ratio as well as the low contributions of diatom's

and green macroalgae's biomarkers suggest that *O. edulis* rely less on high quality food sources than *C. varia*. Besides that, *O. edulis* showed the lowest 20:5n-3/22:6n-3 ratio among all investigated species, indicating more dinoflagellates than diatoms in its diet. This species also showed the highest inter-individual variability, some individuals having FA composition more close to Calyptraeid's species and some others being more close to *C. varia* and *A. modestus* (see also discussion below). Given the isotopic signatures found for the potential food sources (Androuin et al., *article 3*) and the classical diet-consumer enrichment factors (+1 ‰ for carbon and +2.5 ‰ for nitrogen) (McCutchan et al., 2003), both *C. varia* and *O. edulis* were likely to rely on RPOM (-22.7 ± 0.2 ‰ for $\delta^{13}\text{C}$ and 7 ± 0.3 ‰ $\delta^{15}\text{N}$). POM from the water column (PPOM) was influenced by riverine input and has been shown to not contribute to the diet *Magallena gigas* (Marchais et al., 2013). The low contribution of PPOM is in agreement with FA composition since most of the biomarkers found in these bivalves were also present in RPOM, which showed the highest PUFA/SFA ratio (Androuin et al., *article 3*).

The FA compositions obtained for *A. modestus* over the study period showed a strong temporal variation in its diet. Although only 10 days separated sampling dates S2 and S3, we observed marked difference in the corresponding FA profiles, suggesting a high tissue turnover rate linked to its small size and its high cirral activity (Crips and Southward, 1961; McCutchan et al., 2003). The shift after the second sampling date can also be attributed to an increased availability of high quality benthic food sources such as diatoms (16:1n-7 and 20:5n-3) present in both biofilm and RPOM. High $\delta^{15}\text{N}$ values of barnacles with respect to other co-occurring suspension-feeders have already been evidenced in several ecosystems and have been explained by a diet based on zooplankton and/or ^{15}N -enriched green macroalgae (Dubois et al., 2007; 2014; Richoux et al., 2014a; Schaal et al., 2010). In our case, the 18:1n-9 which is often used as biomarker for dinoflagellates (Kelly and Scheibling, 2012; Nerot et al., 2015) or animal material (Cook et al., 2010, Dalsgaard et al., 2003 and reference therein; Jaschiniski et al., 2011), was significantly correlated with $\delta^{15}\text{N}$ (Pearson's $r = 0.6$, $p < 0.05$). Considering the temporal variation of this FA and other dinoflagellate biomarkers such as 22:6n-3, it is more likely that 18:1n-9 reflected a contribution of animal's material in the diet of *A. modestus* during the winter period, when food in the water column is no longer abundant (Androuin et al., *article 3*). Moreover, 18:1n-9 was found to be

associated with RPOM and biofilm, the latter showing higher $\delta^{15}\text{N}$ values than the other sources, so a significant contribution of the biofilm is possible. During the winter period, *A. modestus* may have also relied on detritus, as suggested by the presence of LCSFA (Canuel, 1991).

4.2. Linking the trophic niche to biological traits

According to their SIR and FA compositions, all five species seem to rely mainly on benthic food sources rather than food from the water column. Nevertheless, they exhibited distinct trophic niches which can result from their relative abilities to qualitatively sort particles and to move and their preferred micro-habitat within the *Crepidula* bed (Table 14).

Table 14: Biological traits associated with the five species encountered in this study, with a special reference to their trophic niche.

Species	Scallop <i>Chlamys varia</i>	Oyster <i>Ostrea edulis</i>	Slipper limpet <i>Crepidula fornicata</i>	Chinese hat <i>Calyptrea chinensis</i>	Barnacles <i>Austrominius modestus</i>
Class	Bivalvia	Bivalvia	Gastropoda	Gastropoda	Crustacea
Preferred habitat	Dead shells	None	Lives shells	Dead shells	Lives shells
Movement capacity	High	None	None	Low	None
Main collecting structure	Gills	Gills	Gills	Gills	Cirral branches
Gill plasticity	High (regulation of ingestion volume)	None	None	None	None
Sorting organs	Labial palps	Labial palps	None	None	None
Particles sorting capacity	High	High	None	None	None
Particles Retention efficiency (100%) Lower size limit	5-7 μm	4 μm	2-4 μm	NA	~ 10 μm
Particles Retention efficiency Upper size limit	350-950 μm	70 μm	NA	NA	300-1000 μm
References	Beninger Le Pennec, 2016; Shumway et al., 1987	Mohlenberg and Riisgard, 1978	Beninger et al., 2007; Barillé et al., 2006	Expert knowledges	Crisp and Southward, 1961; Rainbow and Wang, 2001

Both oysters and scallops showed high capacity for qualitative selection, which occurs at two levels: first on the gills and second at the labial palps (Cognie et al., 2001; Beninger et al., 2004). However, in contrast to the oyster gills, the width and the plasticity of the scallop principal gill filaments allow the ingestion of large particles in their stomach, with size probably above 500 μm (Shumway et al., 1987). The range of size selection is therefore much larger than for oysters (Cognie

et al., 2001; Beninger and Le Penneec, 2016), representing a potential advantage under the typically high seston concentrations encountered on soft-sediment habitats (Beninger and Le Penneec, 2016).

This may explain the larger proportion of macroalgae found in the diet of *C. varia* compared to those of *O. edulis*. The other major trait difference between these two species is their movement capacity. The scallop *C. varia* lives fixed on the substrate (preferentially hidden in *C. fornicata*'s or *O. edulis* dead shells, depending on its size) but can easily swim away and settle in another more suitable place (Cranford et al., 2005; Napolitano et al., 1993, Shumway et al., 1987). It is not the case for *O. edulis* which can be found either closed to the sediment-water interface (SWI) or with full access to the water column, depending on its substrate. This variability of position within the *Crepidula* bed is well reflected by the dispersion of obtained FA compositions where individuals have FA profiles close to those of *C. varia*, whereas others are closer to those of *C. chinensis*, two species showing very distinct diets.

However, *C. fornicata* is not able to qualitatively sort particles (Beninger et al., 2007) and its retention capacity (4 μm) is comparable to that of oysters (Barillé et al., 2006) and mussels (Lesser et al., 1992). Considering their FA composition and their biological similarity, we can argue that *C. chinensis* probably exhibited the same particle retention efficiency as *C. fornicata*. Therefore, these two species likely strongly depend on their local food environment, considering their absence of (or low) motility (Orton, 1912; Powell-Jennings and Callaway, 2018). However, their distinct $\delta^{15}\text{N}$ values indicate they probably do not share the same diet, which can be explained by the context of their preferred micro-habitat. More specifically, *C. chinensis* is found preferentially on the concave side of dead shells, at the base of *C. fornicata* stacks. This micro-habitat could be associated with specific biogeochemical conditions since *C. fornicata* is known to significantly modify the nutrient cycle at the SWI (Martin et al., 2006) as well as the composition of the bacterial community (Thouzeau et al., 2011). As suggested earlier, *C. chinensis* could assimilate ^{15}N -depleted organic matter which may result from the activity of denitrifying bacteria in the sediment or within the dead shells.

Finally, since barnacles do not have pre-ingestive selection organs, all particles collected through the mesh created by their articulated appendages (cirral fans) are brought to the mouthparts and ingested without further processing

(Riisgård and Larsen, 2010). Although they are not able to sort particles, they showed the broadest food size-spectrum, allowing them to feed on a wide range of food sources. Because barnacles are totally sessile, the quality of their diet is mainly driven by temporal variation in food source availability.

4.3. Summary and conclusive remarks

In this study, we provide new evidence that suspension feeders are optimal foragers. Feeding on a wide range of food sources, they limit competition, even on a very small spatial scale. This conclusion could not have been achieved with only one of our trophic markers, so we strongly recommend coupling different markers when dealing with the trophic ecology of consumers feeding on complex food sources such as suspension-feeders (Lavaud et al., 2018; Majdi et al., 2018). Using five species belonging to different taxa and having distinct preferred habitats, ability to sort particles and movement capacity, we found five well discriminated trophic niches. Species with a higher capacity to select and sort particles showed the lowest temporal variations and the smallest trophic niche width (Dubois and Colombo, 2014). This was the case for *C. varia* which can not only select its food, but also its micro-habitat. The selectivity of both preferred habitat and food source was reflected by a diet rich in PUFA mainly based on diatoms and green macroalgae. Being able to sort particles but unable to select its micro-habitat (i.e., mix of mud and hard substrate), the oyster *O. edulis* exhibited a more variable diet, as shown by its FA composition, and having individuals overlapping with *C. varia* and *C. chinensis*. The lower contribution of green macroalgae to the diet of *O. edulis* may also result from its low ability to ingest large particles (Cognie et al., 2001; Beninger et al., 2004). Regarding the two Calyptraeid species, the sessile *C. fornicata* and the low motile *C. chinensis*, the fact that they do not select their food and live very close to the SWI obliges them to feed on mostly decaying organic matter, poor in PUFA and rich in bacterial FA (Dubois et al., 2014). Moreover, the micro-habitat of *C. chinensis* probably allows an access to a specific ^{15}N -depleted food source. Finally, the barnacle *A. modestus*, being sessile on the upper side of *C. fornicata* stacks, showed a diet largely influenced by temporal variation of RPOM, with a winter period based on omnivory with both detritus and animal material, and a spring period based on a shift towards diatoms.

Although the invasive species *C. fornicata* is a suspension-feeder, its specific trophic niche indicates that it does not trophically out-compete syntopic suspension feeders. Moreover, the slipper limpet provides a suitable habitat for them. For species of commercial interest such as *O. edulis* and *C. varia*, the *Crepidula* bed could even act as a bivalve nursery, as already suggested by Barbier et al. (2017). Finally, considering its promoting effect of on microphytobenthos, *C. fornicata* could well provide an additional available food source for other primary consumers of the *Crepidula* bed.

5. Acknowledgements

We thank the PSO and LIPIDOCEAN platforms for stable isotope and fatty acid facilities, respectively. We are grateful to Aline Blanchet-Aurigny for commenting upon preliminary versions of this manuscript. We also thank the LEBCO diving team (A. Curd, X. Caisey and A. Tancray) for providing biological samples. TA was funded by an IFREMER, LabexMER and Region Bretagne PhD grant. This work was funded by the IFREMER's 'Politique de site' initiative and the TOTAL Foundation for Biodiversity.

6. Supplementary materials

Supplementary Table 15: Relative abundance of the main fatty acids (% of total FA concentration) of individuals green macroalgae sampled in May 2016 at the study site (protocol described in Androuin et al., article 3).

Fatty acid	<i>Enteromorpha</i> sp.	<i>Enteromorpha</i> sp.	<i>Enteromorpha</i> sp.	<i>Ulva</i> sp.
16:0	23.107	19.909	21.382	23.012
16:4n-3	1.634	6.714	9.44	10.301
18:1n-7	5.838	8.309	6.082	8.64
18:2n-6	2.092	1.789	2.364	2.856
18:3n-3	11.26	9.768	12.244	14.296
18:4n-3	12.102	11.147	14.262	10.616

Méthode 5 : Analyse des traits biologiques

Une communauté faunistique peut être analysée par le biais de sa diversité structurale (DS) qui se base sur l'identité taxonomique des espèces. Au contraire la diversité fonctionnelle (DF) se définit comme le nombre d'espèces ou groupes d'espèces fonctionnellement différents, c'est-à-dire réalisant des fonctions biologiques différentes. La diversité fonctionnelle considère les espèces comme des éléments des fonctions assurées par un écosystème et considère que leurs rôles fonctionnels sont caractérisés par leurs traits biologiques. La DF peut donc être évaluée par l'analyse des traits biologiques des espèces qui sont des caractéristiques morphologiques, physiologiques ou phénologiques susceptibles d'influencer directement ou indirectement les processus écologiques (Figure 36). Ainsi, l'étude de la DF, comme analyse complémentaire de la DS, permet d'avoir une approche plus intégrée pour décrire les relations complexes au sein d'un écosystème. Une bonne estimation de la DF dépend donc principalement du choix des traits biologiques pour relier la composition d'un peuplement aux processus écologiques qu'il assure.

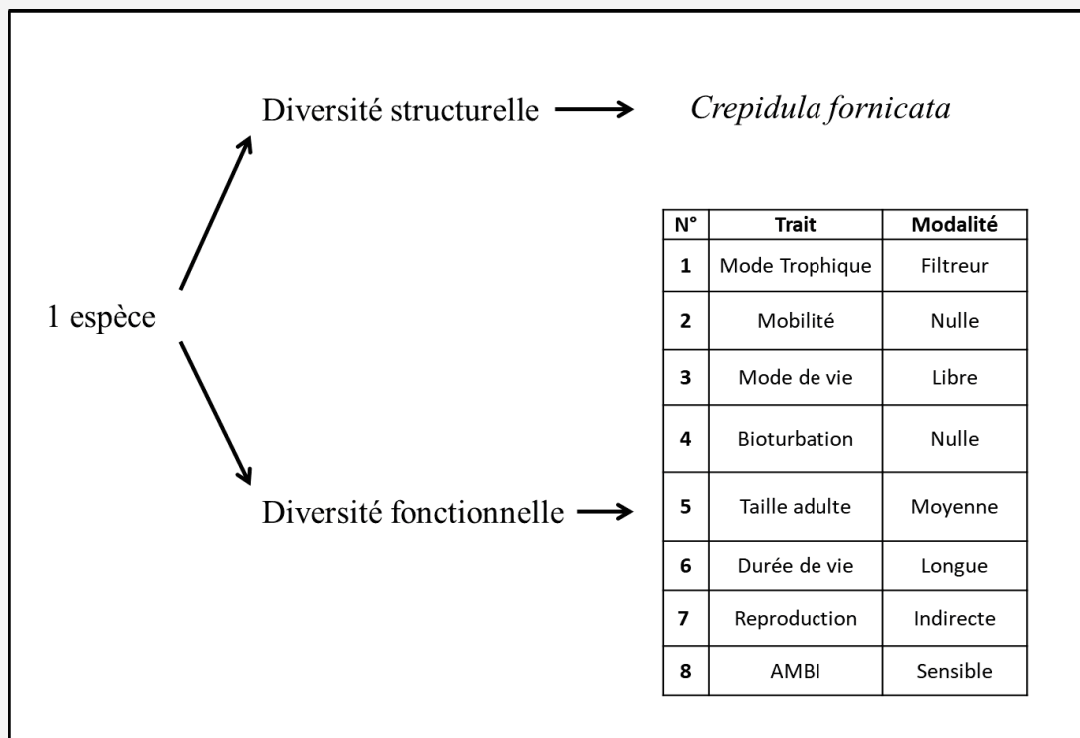


Figure 36 : Illustration de l'analyse par traits biologiques avec l'exemple de *Crepidula fornicata*

Article 5 : *Crepidula* beds, a legacy for complex food web ?

Thibault Androuin ¹, Stanislas F. Dubois ¹, Antoine Carlier ¹.

¹ DYNECO-LEBCO, IFREMER, Plouzané, France

Abstract

Crepidula fornicata is an invasive species which has massively proliferated along the European coasts. This stack-forming engineer gastropod has become a dominant species both in terms of density and biomass, with potential cascading effects on food web. In this study, we investigated the effects of *C. fornicata* on benthic food web structure by combining both biological traits analysis and isotopic metrics. We built tropho-functional (TF) groups which gather similar biological traits compositions linked to their trophic ecology (i.e., feeding mode, living habit, mobility and reworking activity). We then quantified the trophic structure using several metrics based on isotopic signatures of consumers both at community and TF scales. The biological effect of *C. fornicata* (i.e., organic enrichment through biodeposition) was assessed by comparing a dead and a live *Crepidula* bed. Its physical effect (i.e., habitat structuration through shells accumulation) was assessed by comparing a dead *Crepidula* bed and a maerl bed, since maerl is a physical engineer species known to harbor high diversity and complex food web. We found that, in summer, live *C. fornicata* acted as an environmental filter for the colonized community through its intense filtering and biodeposition activities, which tends to homogenize the entire food web. The extent of this effect was stronger for the TF grouping secondary consumers (lower trophic niche width and trophic diversity, and higher redundancy) and weaker for the TF group composed by consumers relying on detritic organic matter. In the TF group of fixed suspension-feeders and grazers, the trophic niche diversity was affected but not the trophic evenness, probably due to widespread mechanisms of food selection. We also found that dead *Crepidula* bed and maerl bed exhibited the same trophic structure, suggesting that an invasive ecosystem engineer, even dead, may provide as much food web complexity as the emblematic high biodiversity maerl bed.

Key words: *Crepidula fornicata*, engineer species, food web, trophic niche, isotopic metrics, biological traits

1. Introduction

The rise of invasive species (IS) is one of the main threats for biodiversity (Bax et al., 2003; Vitousek et al., 1997). IS impact ecosystem functioning through direct biotic interactions (predation, grazing, competition...) and indirect changes in the abiotic conditions of the host ecosystem (Ehrenfeld et al., 2010; Gallardo et al., 2015), which in turn alter ecosystem services (Walsh et al., 2016). Marine ecosystems are particularly sensitive to biological invasion due to seawater associated vectors such as ballast waters, shellfish farming or boat hull fouling (Bax et al., 2003; Padilla et al., 2011; Stiger-Pouvreau and Thouzeau, 2015). In the Atlantic coast of Europe, more than 100 species are recorded as invasive (Gouletquer et al., 2002; Katsanevakis et al., 2014). The Atlantic slipper limpet *Crepidula fornicata* is one of those and it results in highly significant impacts and side-effects on economic coastal activities (Gouletquer et al., 2002; Thieltges et al., 2003).

Native from the East coast of America, this species has spread in Europe following the introduction of the pacific oyster *Magallena gigas* used to shellfish farming (Blanchard, 1997). It is now conspicuous all along European coasts, from Norway to Portugal, including a few places in the Mediterranean Sea (Blanchard, 1997). This engineer gregarious gastropod lives in stacks of several individuals and forms extended beds in shallow subtidal areas. At large densities, this habitat-forming species (Woods, 1989) builds complex 3D benthic habitats, and constitutes an interesting biological model to assess the effects of a colonization by an engineering species on the ecosystem (Jones et al., 2010; Sax et al., 2007). *C. fornicata* modifies its surrounding environment in two different ways. First, their calcareous shells increase heterogeneity and topographic complexity of soft bottoms sediment (Gutiérrez et al., 2003), which enhances the local species diversity of both macrofauna and megafauna (Barnes et al., 1973; de Montaudouin et al., 2017; de Montaudouin and Sauriau, 1999). Secondly, its intense filtering activity and its low assimilation efficiency (Shumway et al., 2014) redirect energy and material from the water column to the benthic compartment both through biodeposition (Ehrhold et al., 1998) and stimulation of benthic primary production (Androuin et al., *article 1*). Moreover, *C. fornicata* has no predators and is considered as a trophic dead end

(Arbach Leloup et al., 2008) and thus may limit the trophic transfer through higher trophic levels in the ecosystems.

Trophic complexity and energy flows are key components of ecosystem functioning (Duffy et al., 2007) and can be assessed through carbon and nitrogen stable isotope ratios (SIR). Whereas carbon SIR is used to infer food source assimilation (Fry and Sherr, 1984; Herman et al., 2000), trophic position of species can be estimated by nitrogen SIR (Post, 2002). Together, they give clues on trophic pathways in ecosystems (Boecklen et al., 2011; Fry, 2006). Considering the turnover rate of tissues, SIR provide an integrated measure of functional traits such as feeding behaviour, habitat preferences, organic matter availability, etc (Newsome et al., 2007). Because SIR are a measure of the way of life of species, they correspond to “response traits”. Those ratios respond to environmental factors such as resources or disturbance (Lavorel and Garnier, 2002). For the same reason, they can be seen as a proxy of the realized niche of a species, trophic group, and to a larger extent, of a community (Newsome et al., 2007). As they integrate several functional traits, it can be sometimes difficult to disentangle the factor affecting a species niche or a group of species (e.g., change in basal resource, ontogenic trophic shift ...). Decomposing the functional role of species can be addressed by the biological trait-based approaches which link species biological traits (e.g. morphological, behavioural, phenological) and ecosystem functionality (rem mineralization, secondary production, etc.) (Petchey and Gaston, 2006). Contrary to “response traits”, “effect traits” determine an ecosystem function or process (Lavorel and Garnier, 2002) and provide a proxy of the fundamental niche of a species/group of species (Devictor et al., 2010). Combining both SIR (realized niche) and biological traits analysis (fundamental niche) should therefore allow better understanding on the relationship between the realized niche of a species and its functional traits (Fitzerald et al., 2017). Using this approach, Zhao et al., (2014) showed that ontogenic change in the trophic position of the largemouth bass was significantly correlated with locomotion attributes, which is linked to the foraging behaviour and the mobility of prey encountered during ontogeny.

To quantitatively characterize community-wide aspects of trophic structure, metrics based on SIR have been developed in the last decade (Jackson et al., 2011; Layman et al., 2007). Among them, trophic plasticity (isotopic space occupation), redundancy (neighborhood distance) or evenness (intra-group variability), have

been useful to address ecological questions relative to habitat-forming species (Chaalali et al., 2017; Rezek et al., 2017) or disturbance such as eutrophication (Quillien et al., 2016) or IS (Guzzo et al., 2013; Jackson et al., 2012).

This study took place in the bay of Brest and the invasive species *C. fornicata* was used as a model to investigate the effect of engineer species on benthic food webs structure. Using natural contrasting *Crepidula* beds in terms of biomasses (live or dead), the biological activity of this species (i.e., organic enrichment through biodeposition) on trophic structure was tested. Its physical effect (i.e., habitat structuration) was also tested using dead *Crepidula* bed and a local maerl bed. This calcareous red algae was chosen since it also act as a physical engineer species (Grall et al., 2006). To answer those questions, we first used a biological trait-based approach to define tropho-functional (TF) relevant groups, and calculated trophic niche metric based on SIR at both community and TF level.

2. Materials and methods

2.1. Study site

The bay of Brest (France) is a semi-enclosed marine ecosystem of 180 km² connected to shelf waters (Iroise Sea) by a narrow (2 km wide) and deep (40 m) strait. This shallow basin (average depth 8 m), is characterized with two main watersheds responsible for freshwater inputs, the Aulne (1842 km²) and the Elorn (402 km²) rivers. The macro tidal regime caused by the high hydrodynamic mixing with adjacent marine waters induces short-term variability in hydrological factors and limits the spatial variability of phytoplankton (Le Pape et al., 1996, Chauvaud et al., 2000; Laruelle et al., 2009). Moreover, isotopic gradient showed that the bay was under marine water influence, even close to the two main rivers (Mortillaro et al., 2014). After its first observation in the Bay of Brest in 1949 (Blanchard, 1997), the slipper limpet became the main benthic suspension feeder within the megafauna during the early 1990's (Chauvaud et al., 2000), which biomass reached more than 120 000 tones (Guérin, 2004). However, a recent survey has revealed a strong decrease in abundance between 2000 and 2013 in the south part of the bay (Blanchet-Aurigny et al., 2012), without clear explanation. The resulting consequence is the formation of large dead shells accumulation on the sea floor (Fig. 37C).

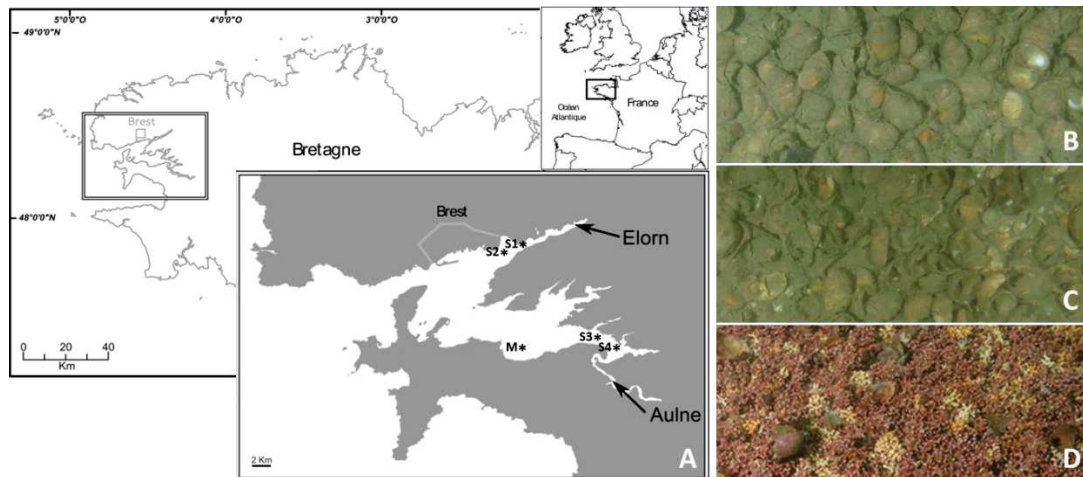


Figure 37: (A) Sampling stations in the bay of Brest. (B) S1 - S2: live *Crepidula* beds, (C) S3 - S4: dead *Crepidula* beds, (D) M: maerl beds.

2.2. Sampling protocol

Sampling was carried out in February 2014 (winter) and June 2015 (summer) at four locations of the Bay of Brest. Stations 1 and 2 were located near the Elorn estuary and stations 3 and 4 were located near the Aulne estuary (Fig. 37A). Sampling locations were selected according to a previous benthic survey (REBENT, French national benthic survey, 2013) which highlighted contrasted biomasses of live *C. fornicata* over the bay. Benthic macrofauna and megafauna were sampled using a dredge (two hauls of ~50 m per station) and a Day-grab ($2 \times 0.1 \text{ m}^2$ per station) in order to capture both epi- and endofaunal assemblages, with a homogeneous sampling effort at each station. All invertebrate species with size $> 1 \text{ mm}$ were selected. Individuals were sorted and held in filtered sea water for 24 hours in the laboratory to allow evacuation of their gut content. They were then dissected (calcareous parts removed) and kept frozen (-20°C) until further processing. Data for maerl bed were retrieved from Grall et al. (2006), sampled in the south part of the bay of Brest in October 2001 (autumn) (Fig. 37D).

2.3. Stable isotope analysis

Isotopic analyses were performed on muscle tissue samples of megafauna or whole-body without digestive gut. Small species were pooled together to reach sufficient biomass for stable isotope analysis. All samples were acidified to prevent any inorganic carbonates contamination and then rinsed with milliQ water. Samples were freeze dried and ground into homogenous powder using a mortar and pestle.

Approximately 400 µg of powder was weighed in tin capsules for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analysis. Samples were analysed by continuous flow on a Thermo Scientific Flash EA 2000 elemental analyser coupled to a Delta V Plus mass spectrometer at the Pôle de Spectrométrie Océan (PSO, Plouzané, France). Results are expressed in standard δ notation based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$) following the equation:

$$\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 \text{ (in ‰)}, \text{ where R is } ^{13}\text{C}/^{12}\text{C} \text{ or } ^{15}\text{N}/^{14}\text{N}$$

International isotopic standards of known $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were used: IAEA-600 Caffeine, IAEA-CH-6 Sucrose, IAEA-N-1 and IAEA-N-2 Ammonium Sulphate. The experimental precision was estimated using the standard deviation of an internal standard (Thermo Acetanilide, $n = 8$). It indicated an analytical precision of ± 0.11 ‰ and ± 0.07 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively.

2.4. Building tropho-functional groups

In most of food web studies, trophic groups are only based on the feeding mode of species. However, it is clear that SIR encompass a wide variety of biological traits related to trophic mode but also habitat preferences, mobility, organic matter availability, etc. (Boecklen et al., 2011; Newsome et al., 2007). In this study, we selected four meaningful biological traits considering what is integrating in SIR, avoiding correlated traits, and those for which knowledges were scarce. Traits, modalities as well as the ecological meaning in terms of trophic niche behind these traits are figured in Table 16. Taxa were assigned to one or several modalities in each traits using a fuzzy coding approach (Chevenet et al., 1994), with a 0 to 3 scale where 0 indicating no affinity to 3 indicating high affinity for the modality. This technique allows the implementation of intraspecific variability within each taxa. Trait information's were gathered from several sources. Whereas information on feeding mode and living habit were picked from data base such as the Biological Traits Information Catalogue (<http://www.marlin.ac.uk/biotic/>) or Polytraits database (<http://polytraits.lifewatchgreece.eu/>), taxonomic text books, scientific publications as well as expert knowledges. Informations on mobility and reworking type were nearly all picked from the publication of Queiros et al. (2013), which provide a classification for more than 1000 macroinvertebrate species

inhabiting soft sediment. It is noteworthy that the fuzzy coding approach allows missing information about taxa traits.

Table 16: Biological traits, modalities and rationale behind the traits selection.

Traits	Modalities	Rationale behind the trait selection
Feeding mode	Carnivorous - omnivorous (co)	Linked to prey availability, secondary production, organic matter enrichment
	Surface deposit feeder (sdf)	
	Subsurface deposit feeder (ssdf)	
	Grazer (gr)	
	Filter-feeders (ff)	
Mobility	Fixed (fix)	Linked to feeding behaviour, space occupation and availability
	Limited movement (lim)	
	Slow, free movement in/on the sediment matrix (fmx)	
	Free movement on the sediment or through burrow system (fbr)	
Living habit	Epibenthic (epi)	Linked to space occupation and availability
	Endobenthic (endo)	
	Supra benthic (supra)	
	None (no)	
Reworking type	Surfical modifiers (sm)	Linked to organic matter recycling, primary productivity
	Upward - downward conveyor (cvr)	
	Biodiffusors (bio)	

Once the matrix data constructed, we ran a fuzzy correspondence analysis (Chevenet et al., 1994) which provide an ordination of taxa according to their modalities. An agglomerative hierarchical clustering analysis was performed to define groups, using Ward aggregation criterion (Ward, 1963) and Euclidean distance (Usseglio-Polatera et al., 2000). The more parsimonious number of groups was calculated using the sum of the within-cluster inertia, the suggested partition being the one with the higher relative loss of inertia.

2.5. Trophic web structure analysis

Due to recent observations on the dynamic of *Crepidula fornicata* in the bay of Brest (REBENT, French national benthic survey, 2013), we compared two population parameters: size structure and *C. fornicata* biomass, and shell weight in the four stations in winter. The size frequency distributions between pairs of stations were compared with a Kolmogorov-Smirnoff test (supplementary Fig. 44 and Table 17), biomasses and shells weight with a non-parametric test of Kruskal-Wallis (Supplementary Table 18). These results highlighted a clear pattern between the north and the south part of the Bay. Stations S1 and S2 were characterized by high *C. fornicata* biomasses (300-600 g per 0.1 m⁻²) and a size structure showing 75 % of the population higher than 20 mm. On the contrary, stations S3 and S4 were characterized by very low biomasses (4-28 g per 0.1 m⁻²) and a size structure showing 75 % of the population lower 10 mm. However, *C. fornicata* shells weight

was not different between stations but the high values (1300-1600 g per 0.1 m⁻²) confirmed that each station was lying on a *Crepidula* beds. According to these results and to gain more insight in the trophic structure of the benthic communities, we thus decided to merge stations S1 and S2 referred as live *Crepidula* bed, and stations S3 and S4 referred as dead *Crepidula* bed. Dead *Crepidula* bed was compared to maerl bed only in summer because we think that the trophic environment would be more comparable with summer than with winter due to both allochthonous supplies through the water column (spring and autumn bloom).

We tested whether stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and trophic niche position differ between groups using respectively, Kruskal-Wallis test and PERMANOVA (Anderson et al., 2001). A first PERMANOVA test was performed between dead and live *Crepidula* beds using bed (2 modalities), season (2 modalities) and tropho-functional group (3 modalities) as factors. A second PERMANOVA test was performed between dead *Crepidula* bed and maerl bed in summer using bed (2 modalities) and tropho-functional group (3 modalities) as factors. Following significant PERMANOVA results, *post hoc* tests were carried out using multiple pairwise comparisons to identify differences among factors (Martinez Arbizu, 2017). When Kruskal-Wallis test was significant, pairwise comparisons were achieved using Mann-Whitney tests with a Bonferroni correction.

Based on the mean consumer SIR, we calculated several community-wide metrics within each tropho-functional group to quantify aspects of trophic niches, primarily developed by Layman et al. (2007). Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ range were defined as the distance between the two species with the most enriched and most depleted δ values. The $\delta^{15}\text{N}$ range (NR) represents the vertical structure of the niche, a larger value suggesting more trophic levels and thus a greater degree of trophic diversity. Conversely, the $\delta^{13}\text{C}$ range (CR) is expected to increase with higher basal resources available to consumer. The mean distance to centroid (CD) provides a measure of the average degree of trophic average of trophic diversity within a group of species, higher values meaning higher space occupation and higher trophic diversity. The redundancy within group was assessed by the mean nearest neighbor distance (NND) which measures the overall density of species packing in the isotopic space. Smaller NND characterized food webs with a large proportion of species with similar trophic ecologies (i.e., redundancy). Standard

deviation of this measure (SDNND) estimates the evenness of species packing in the isotopic space, low values suggesting more even distribution of trophic niches. Finally, the size of the trophic niches was estimated through standard ellipse area corrected for small sample size (SEAc) (Jackson et al., 2011). Stable isotope metrics are known to be sensitive to a low number of species (Brind'Amour and Dubois, 2013) with an indicated threshold of 20 species. Since the maerl bed has only 38 isotopic signatures, we only compared dead *Crepidula* and maerl bed at the community level. Moreover, as we did not know the sampling effort for the maerl bed, they were compared using the same number of isotopic signatures. Therefore we re-sampled multiple times ($n = 10$) in the pool of the 68 isotopic signatures to calculate the metrics on 38, corresponding to the sampling effort in the maerl bed. A key issue when dealing with stable isotope metric is the basal source, as it can hamper the calculations of those metric (Hoeinghaus and Zeug, 2008). In this study we considered that the main allochthonous source (i.e., phytoplankton) was the same for each habitat (i.e., dead/live *Crepidula* bed and maerl bed). However, we allow for autochthonous additional resource (e.g., microphytobenthos, bacterial mat) which would be habitat specific and discussed. Because we were interested in potential difference in basal habitat within each habitat, we did not scale SIR (Cucherousset and Vileger, 2015). To account for intra-group isotope variability, 95 % confidence intervals in isotopic metrics were generated using the leave-one-out Jackknife estimation (Efron and Tibshirani, 1993). Comparisons in which 95 % confidence intervals were not overlapping were considered as significantly different. Statistical analyses were performed in R version 3.3.0 (R Core Team, 2012) using packages 'plyr', 'SIBER', 'SIAR', 'bootstrap' and 'ggplot'.

3. Results

3.1. Building tropho-functional groups

162 taxa were used to build the tropho-functional (TF) groups. The clustering analysis revealed three significant TF groups of taxa sharing similar association of biological traits (Fig. 38a). Detailed profiles can be retrieved from the Fig. 38b. The first TF group was characterized by carnivorous-omnivorous taxa with various capacity of mobility but receiving all supra benthic taxa. Exclusively free in the environment, they reworked sediment through biodiffusion. The second

one gathered surface or subsurface deposit feeders, which were almost all endobenthic with limited movement. Their reworking activity was represented by conveyors and surficial modifiers taxa. The last TF group was largely defined by epibenthic species with low or without movement capacity. They were filter-feeders or grazers and not involved in reworking processes.

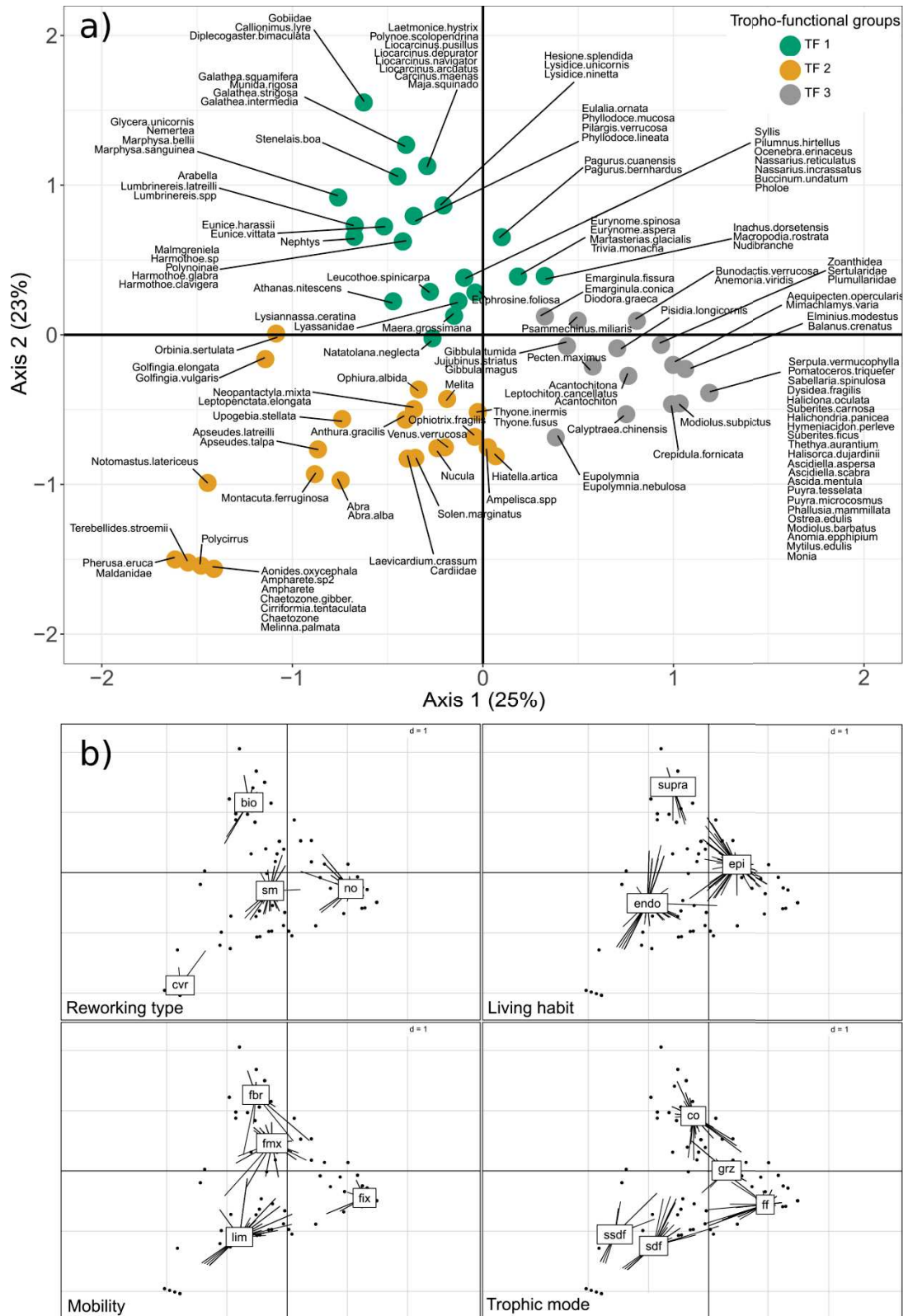


Figure 38: Ordination of taxa and biological traits by fuzzy correspondence analysis. Distribution of taxa in clustered (a) tropho-functional (TF) group and (b) modalities of the four biological traits on the two first factorial plane of the analysis. Each modality was located at the weighted average of taxa positions which are representing this modality.

3.2. Food web structure

3.2.1. Dead vs. live Crepidula bed comparison

Comparing dead and live *Crepidula* bed (Fig. 39), change in trophic niche position was only significant for beds (PERMANOVA, $p < 0.05$), season (PERMANOVA, $p < 0.01$) and TF group (PERMANOVA, $p < 0.01$) without any significant interactions. Beds differed due to higher $\delta^{15}\text{N}$ values (11.9 ± 1.8 ‰) in a dead than in a live *Crepidula* bed (11.3 ± 1.7 ‰) (KW, $p < 0.05$). Season was different because of more depleted carbon values in summer (-19.4 ± 1.6 ‰) than in winter (-18.7 ± 1.5 ‰). Between TF groups, the TF group 1 was significantly different from group 2 and 3 (pairwise, all $p < 0.01$) but TF 2 and 3 did not differ. This difference was due to both higher carbon (-18.3 ± 1.5 ‰, -19.4 ± 1.5 ‰ and -19.6 ± 1.5 ‰ for TF group 1, 2 and 3, respectively) and nitrogen values (13 ± 1.3 ‰, 10.9 ± 1.2 ‰ and 10.5 ± 1.5 ‰ for TF group 1, 2 and 3, respectively) in the TF 1 (MW, all $p < 0.001$).

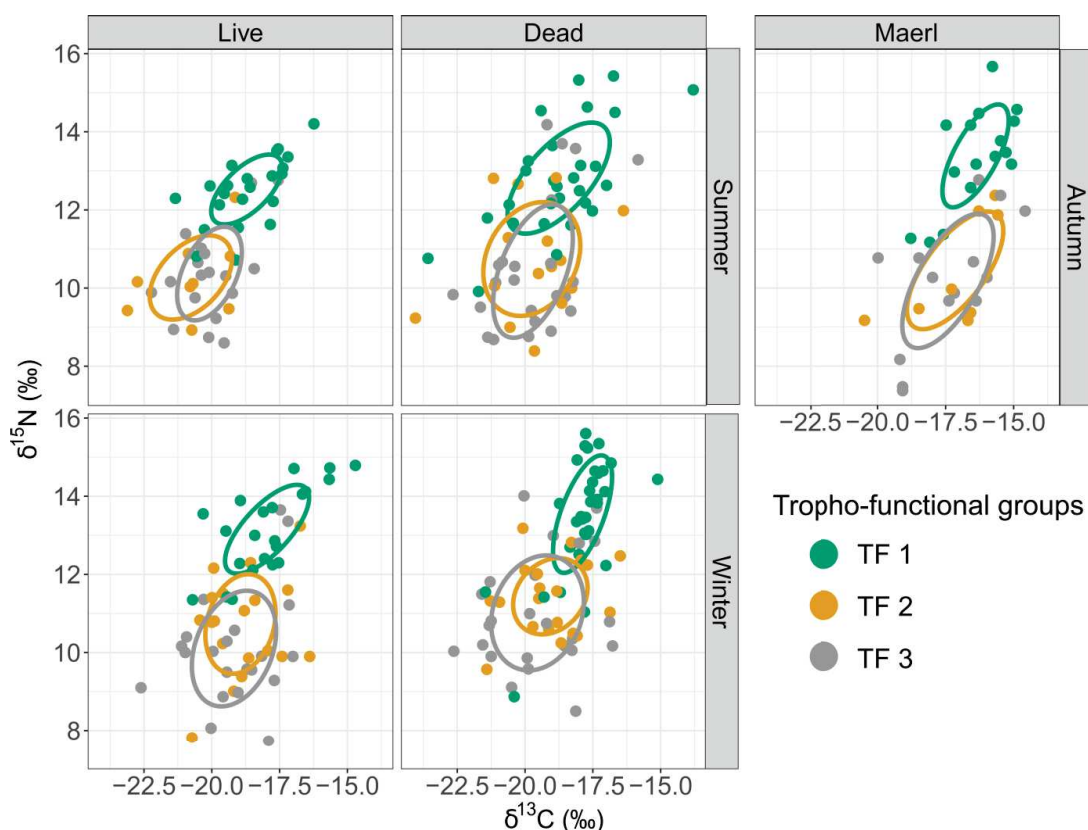


Figure 39: Biplot of mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of taxa sampled in dead and live *Crepidula* beds in summer and winter. Isotopic data of the maerl bed were taken from Grall et al., (2006). Ellipses enclosed the standard ellipse areas built for each of the three tropho-functional (TF) groups and corrected for small sample size.

3.2.2. Dead vs. live Crepidula bed comparison

Comparing dead Crepidula bed and maerl bed in summer and autumn, respectively (Fig. 39), trophic niche position significantly differed between beds (PERMANOVA, $p < 0.01$) and TF groups (PERMANOVA, $p < 0.01$). The TF group 1 was significantly different from group 2 and 3 (pairwise, all $p < 0.01$) but TF 2 and 3 did not differ. Specifically, $\delta^{13}\text{C}$ values ($-19.2 \pm 1.7 \text{ ‰}$ and $-16.9 \pm 1.5 \text{ ‰}$ for dead Crepidula and maerl bed, respectively) significantly differed between bed (KW, $p < 0.001$) whereas $\delta^{15}\text{N}$ values ($11.5 \pm 1.9 \text{ ‰}$ and $11.6 \pm 2.1 \text{ ‰}$ for dead Crepidula and maerl bed, respectively) did not change. According to the difference between TF groups, $\delta^{15}\text{N}$ values ($13 \pm 1.4 \text{ ‰}$, $10.6 \pm 1.4 \text{ ‰}$ and $10.3 \pm 1.6 \text{ ‰}$ for TF group 1, 2 and 3, respectively) significantly differed between TF group (KW, $p < 0.001$), with higher values of TF 1 than TF 2 and TF 3 (MW, all $p < 0.001$). Assimilated carbon slightly but significantly changed with TF group as $\delta^{13}\text{C}$ values ($-17.9 \pm 2 \text{ ‰}$, $-18.8 \pm 2.1 \text{ ‰}$ and $-18.9 \pm 1.8 \text{ ‰}$ for TF group 1, 2 and 3, respectively) differed significantly between TF group (KW, $p < 0.05$) but pairwise comparisons indicated that only TF 1 and TF 3 were different (MW, $p < 0.05$).

3.3. Isotopic niche metrics

3.3.1. Dead vs. live Crepidula bed comparison

3.3.1.1. Community level

At the community level, no significant difference was observed in winter except for the mean NND with higher values in live Crepidula bed (Fig. 40e). In summer, all metrics showed significant higher values in dead than in live Crepidula bed (Fig. 40abcdf) except for the mean NND (Fig. 40e).

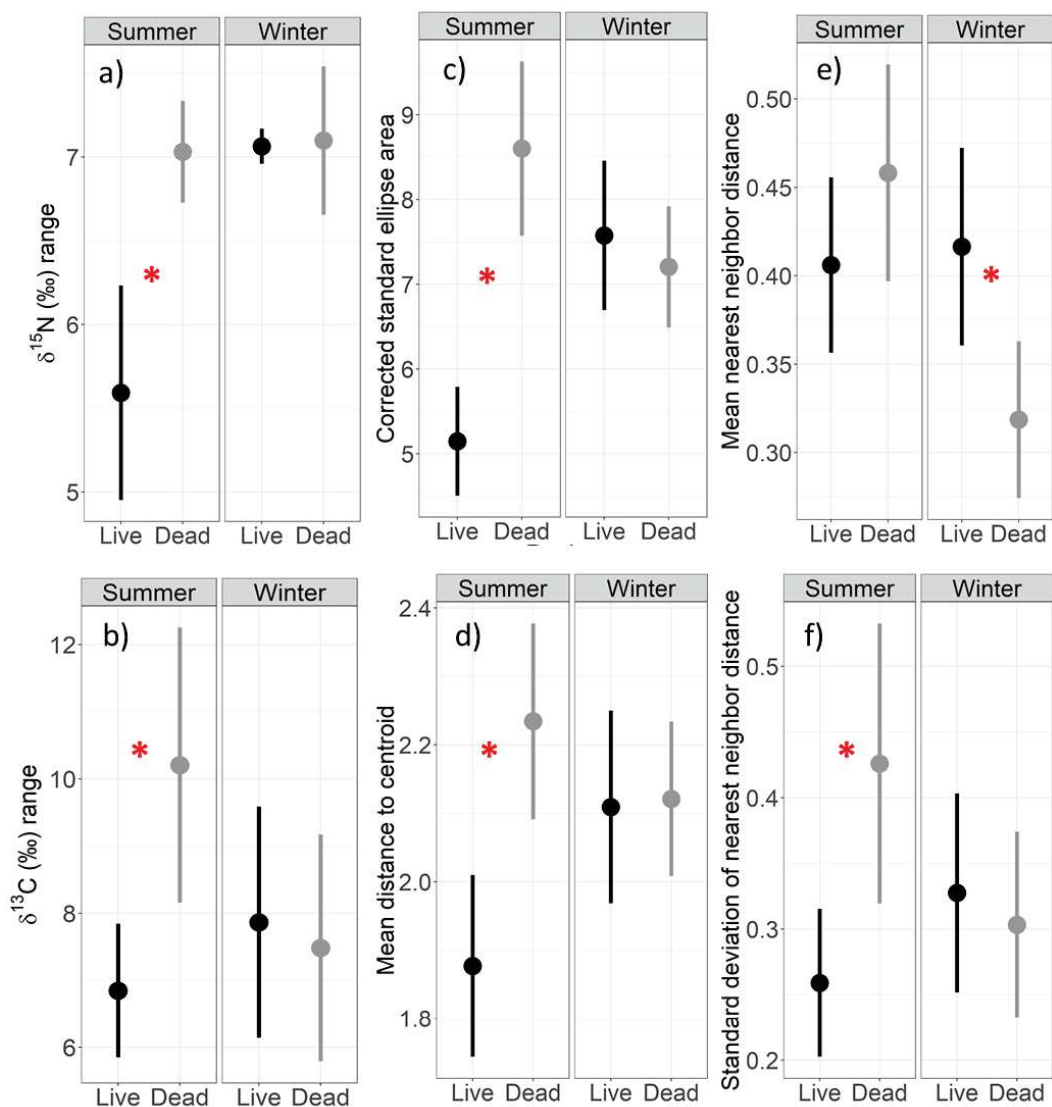


Figure 40: Comparison of six isotopic metrics calculated for the community in live (black) and dead (grey) *Crepidula* beds in winter and summer period, with 95 % confidence intervals based on Jackknife re-sampling estimations. Significant differences between beds were indicated with an *asterisk*.

3.3.1.2. Tropho-functional group level

Overall, no significant differences were observed in winter, except for nitrogen range (Fig. 41a) and sdNND in TF group 1 (Fig. 41f). This group also exhibited the highest difference between *Crepidula* beds in summer. All metrics were significantly higher in the dead *Crepidula* than in the live one. Conversely, the second TF group only showed significantly higher carbon range in the dead compared to the live *Crepidula* bed (Fig. 41b). Metrics relative to the trophic diversity (SEAc, CD) did not differ whereas being higher in the dead than in the live *Crepidula* bed (Fig. 41cd). Alternatively, the TF group 3 showed significant higher values for NR, SEAc and CD in the dead *Crepidula* bed compared to the live one (Fig. 41acd).

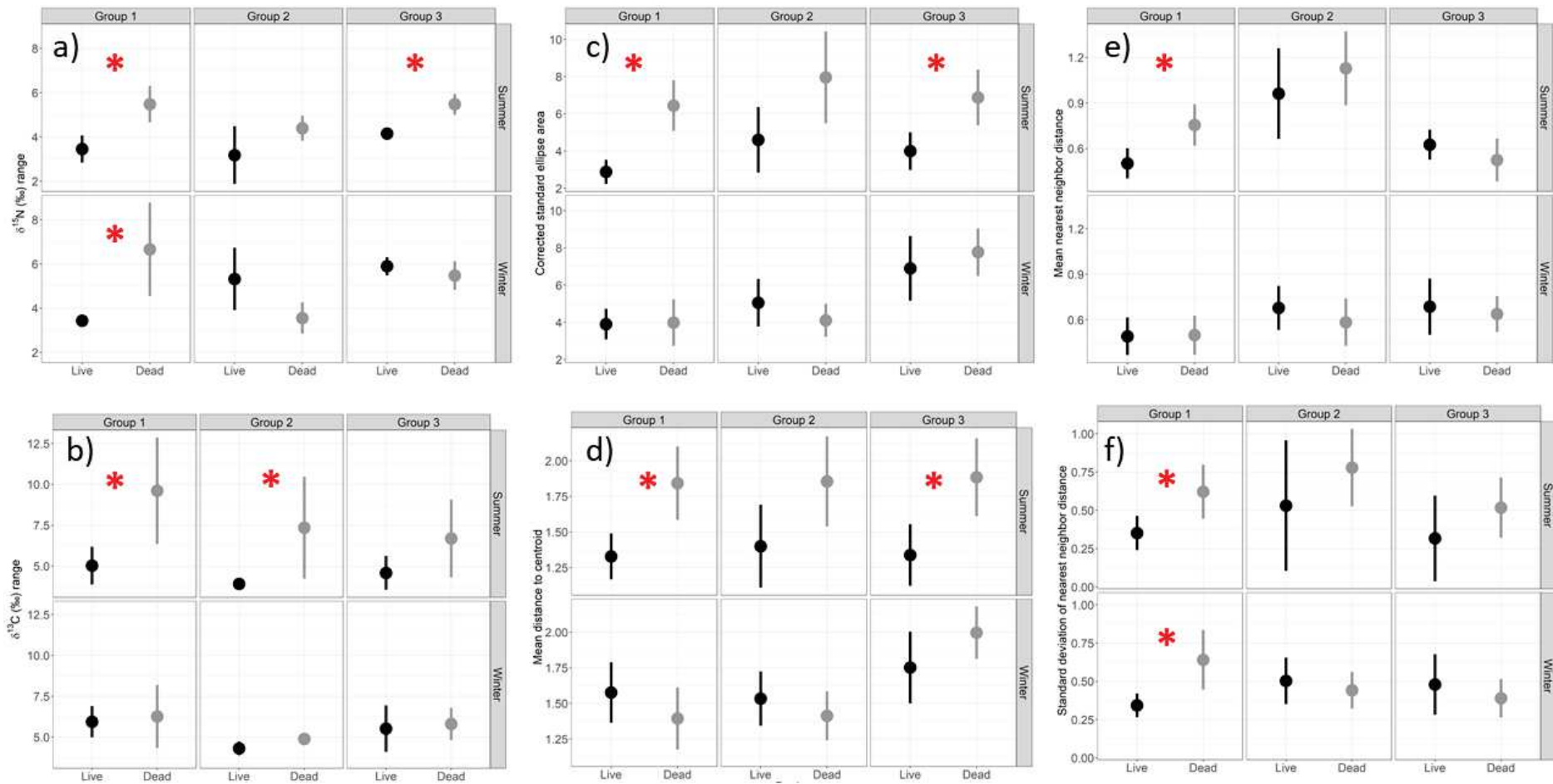


Figure 41: Comparisons of six isotopic metrics calculated for the three tropho-functional groups in live (black) and dead (grey) *Crepidula* beds, in winter and summer periods, with 95 % confidence intervals based on Jackknife re-sampling estimations. Significant differences between beds were indicated with an *asterisk*.

3.3.2. Dead *Crepidula* bed vs. maerl comparison

3.3.2.1. Community level

Comparing indices, only nitrogen and carbon range were significantly different between dead *Crepidula* and maerl bed, with higher value of nitrogen range in the maerl bed (Fig. 42a) but lower value of carbon range (Fig. 42b).

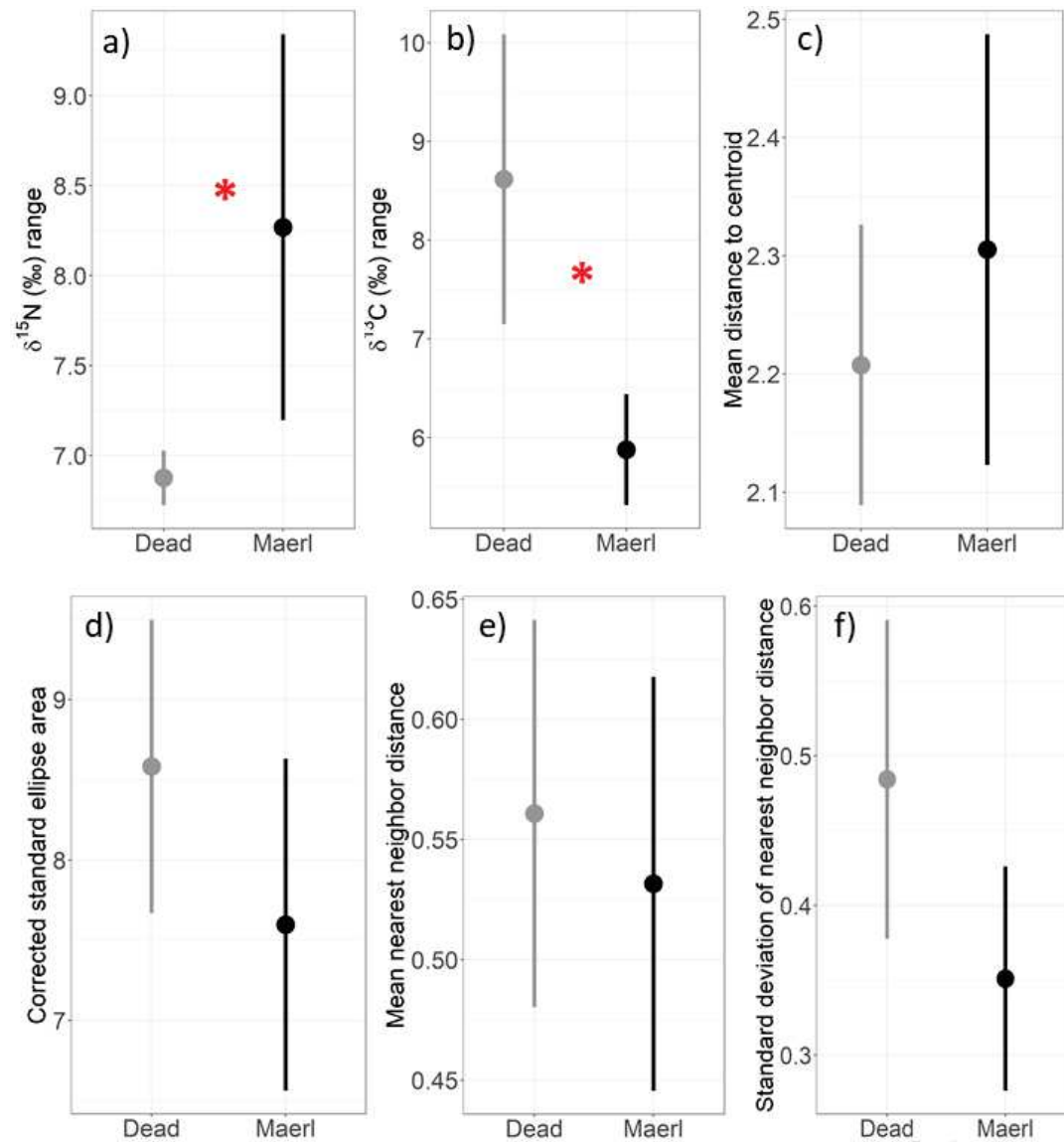


Figure 42: Comparisons of six isotopic metrics calculated for the community in dead *Crepidula* bed (black) and maerl bed (grey), with 95 % confidence intervals based on Jackknife re-sampling estimations. Significant differences between beds were indicated with an *asterisk*.

4. Discussion

Understanding how ecosystem engineers, and especially invasive ones, affect ecosystem functioning requires the integration of trophic complexity assessed within and across trophic levels (Duffy et al., 2007). In this study, we used biological traits analysis to gather taxa of *Crepidula* bed in functional groups which focus on their trophic ecology (i.e., feeding mode, living habit, mobility and reworking activity) and are therefore called tropho-functional (TF) groups. We then quantified several aspects of the trophic niche on both TF and whole benthic community scales using isotopic metrics. Our objective was to investigate the effects of the invasive ecosystem engineer *C. fornicata* on the benthic trophic structure using two kinds of comparison. First, the biological effect of *C. fornicata* (i.e., organic enrichment) was deduced by comparing dead and live *Crepidula* beds. Second, its physical effect (i.e., habitat structuration) was assessed by comparing a dead *Crepidula* bed and a maerl bed, which is a local engineer species supporting a high diversity and a complex food web.

4.1. Dead vs. live *Crepidula* bed comparison

By comparing dead and live *Crepidula* beds, we showed that the biological activity of the ecosystem engineer *Crepidula fornicata* impacts the trophic structure at community and tropho-functional group levels, depending on season. During summer, lower carbon and nitrogen ranges in live bed suggests a homogenization of the food web at the community level, with smaller food chain length (nitrogen range), smaller TF niche width (SEAc), lower trophic diversity (CD) and higher evenness (sdNND). We proposed that *C. fornicata* acts as an environmental filter that redirects large amounts of (re)suspended organic matter towards the sediment (Ehrhold et al., 1998; Shumway et al., 2014), thus modifying basal resources for the entire food web. This homogenization is likely linked to the input of allochthone food from the water column after the spring bloom (Chlorophyll *a* concentration of $6.3 \mu\text{g L}^{-1}$ measured two months before the sampling date). In winter, the similarity observed between live and dead beds for all metrics and all TF is likely linked to the low metabolism and food availability. Indeed, invertebrate species showed minimal activity and a low secondary production during low temperature period

(Coma et al., 2000), and the absence of food supply from the water column probably lowered the filtering activity of *C. fornicata*.

The extent of change observed between dead and live *Crepidula* beds in summer varied according to the tropho-functional group. The largest effect was found for the first group which is mainly represented by vagile secondary consumers, living in and on the sediment (Fig. 43). In live bed, this group showed lower trophic diversity, higher redundancy and higher evenness than in a dead bed. Both direct and indirect effects of *C. fornicata* may have impaired the trophic niche of TF 1.

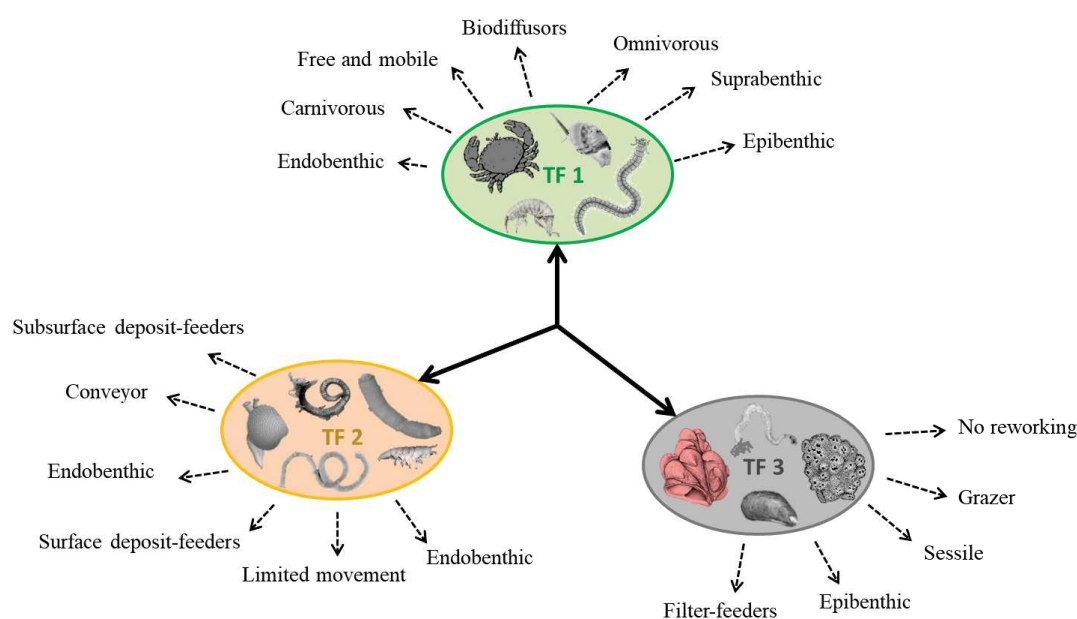


Figure 43: Summary of tropho-functional groups considered in this study, schematized by a few illustrative species. *Crepidula fornicata* is figured out in red.

It seems that the homogenization of the base of the food web and the primary consumers' trophic niche has also been reflected to higher trophic levels. Such cascading homogenization affecting the whole food chain has already been demonstrated by changes in the basal resource following fish farming organic enrichment (Nordstrom and Bonsdorff, 2017). These authors observed a lower species richness in the group of secondary consumers but with no change in their proportion regarding the rest of the community, which was also the case in our study (Live and dead *Crepidula* beds exhibited 18 and 28 taxa in TF 1, representing 41 % and 44 % of the community richness, respectively). However, the approach by Nordstrom and Bonsdorff (2017) was theoretical and did not highlight any change in the realized trophic niche of this functional group. Here, we went further in the

description of the actual trophic web structure (i.e., realized niche), showing that modifications at the basis of the food web would also affect the trophic ecology of secondary consumers. Such cascading effect following toward higher trophic levels has also been demonstrated following green macroalgae accumulations on sandy beaches community (Quillien et al., 2016). A direct influence on TF 1 would be a sheltering effect of live stacks of *C. fornicata*. At high biomass, *C. fornicata* covers almost 100 % of the substrate and forms a thicker 3D structure than in dead bed. This potentially limits the access to certain type of prey for large epifaunal predators such as crustaceans. Such sheltering effect limiting predation has already been demonstrated for other habitat forming species such as mussel beds (Beekey et al., 2004; Stewart et al., 1999). Finally, the group TF 1 was also characterized by biodiffusive species (Fig. 8). Those species are key component in soft bottoms as their reworking activity largely contributes to the irrigation and oxygenation of the sediment (Kristensen, 2000; Volkenborn et al., 2007). Live *Crepidula* bed can encounter temporary hypo/anoxia events due to organic matter enrichment (Khalil et al., 2013; Martin et al., 2006; Thouzeau et al., 2011). Change in the trophic ecology of this TF group such as biodiffusive bioturbation, may affect ecosystem function such as organic matter recycling, leading to more anoxic sediment (Dauwe et al., 1998).

The second group (TF 2) was mostly composed with endofaunal deposit feeders with limited movement (Fig. 43). It should be specified that the number of taxa belonging to TF 2 was less than 20 in summer (9 vs. 15 taxa in live and dead *Crepidula* beds, respectively), which is the threshold recommended by Brind'Amour et Dubois (2013) for an accurate use of their isotopic metrics. Therefore, the interpretation of the metrics should be considered with caution. Nevertheless, we argue that our sampling effort was comparable between live and dead beds so less taxa in a community is expected to reflect natural community, and metrics can be applicable. Although non-significant, we observed a slightly lower trophic diversity in the live bed in summer, reflected by a lower carbon range. We explain this small homogenization effect by the fact that this group is composed by both surface and sub-surface deposit feeders. While species relying on surface organic matter would be directly impacted by live *C. fornicata*, it is likely that species feeding on organic matter below the surface would rely on organic matter which is already processed and homogeneous. Deposit feeders are often non-

selective species, especially for ones living in the sediment with low movement capacity (Jumars et al., 2015). This may explain the similarity in terms of trophic redundancy and evenness between dead and live *Crepidula* beds.

The third group, composed with sessile/low mobile epifauna and a majority of filter-feeders and grazers (Fig. 43), showed a lower trophic diversity and niche width within live *Crepidula* bed in summer. It indicated that *C. fornicata* also impacts species that belongs to its TF group. Using both SIR and fatty acids trophic markers, it has been shown that re-suspended POM from the sediment-water interface contribute to the diet of *C. fornicata* and four co-occurring common filter-feeders, showing that filter-feeders can actually be impacted by biodeposition of *C. fornicata* (Androuin et al., *article 4*). While this study highlighted the diversity of trophic niche in winter and early spring before spring bloom, it is likely that the homogenization effect on basal resources by *C. fornicata* in summer affect the trophic diversity of this TF group. No effect on redundancy or evenness was found, which is likely due to the great ability of filter-feeders to be optimal foragers (Dubois and Colombo, 2014; Androuin et al., *article 4*). Using diverse sorting mechanisms, some species are able to select their food, hence maintaining a degree of specialization.

4.2. Dead *Crepidula* bed vs. maerl bed comparison

When comparing a dead *Crepidula* bed with a maerl bed, which both are physical ecosystem engineers, no difference in the trophic structure was found. This means that both species exhibited complex food web and that the habitat structuration rather than the identity of the species may explain web structure. However, these two beds were likely different in their trophic functioning. The nitrogen range was significantly higher in the maerl bed whereas the carbon range was significantly higher in the dead *Crepidula* bed. Moreover, a clear ^{13}C enrichment in the maerl bed community was noticeable compared to the dead *Crepidula* bed ($\sim 2\text{‰}$). This can be attributed to the greater importance of benthic microalgae attached to the maerl thalli (Qui-Minet et al., 2018), since maerl bed is shallower than *Crepidula* bed and receives therefore a higher irradiance (Ní Longphuirt et al., 2007). The trophic importance of this microphytobenthos has already been suggested for this maerl bed (Grall et al., 2006). The higher trophic

role of this benthic food source in the maerl bed could lead to lower carbon range observed for the consumers.

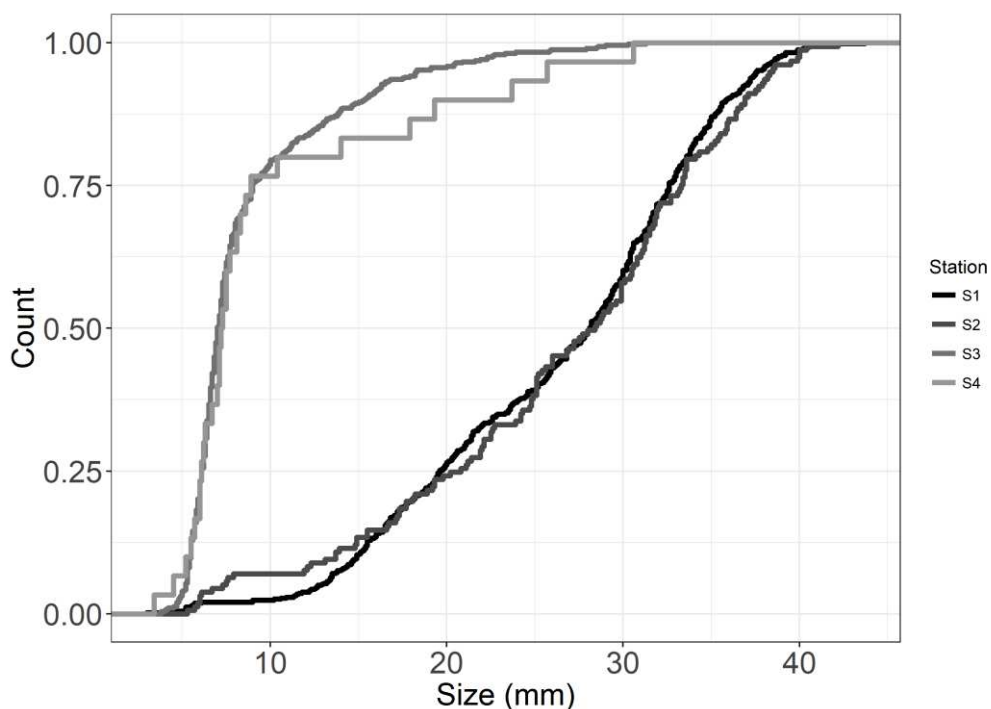
5. Conclusion

According to the trophic position hypothesis, invasive species may alter food web structures differently depending on their trophic position (Byrnes et al., 2007; Thomsen et al., 2014). The increase of dominance of those species can lead to the homogenization of the ecosystem's structure by reducing food-web complexity (Ozersky et al., 2012). In the same way, a large amount of organic matter provided by a source can lead to food web homogenization (Nordstrom and Bonsdorff, 2017, Quillien et al., 2016). In the case of *Crepidula fornicata*, its large dominance as well as its intense filtering activity and its low assimilation efficiency (Shumway et al., 2014) likely redirect a large amount of energy and material from the water column to the benthic compartment (Chauvaud et al., 2000; Ehrhold et al., 1998). The functional groups are impacted by this flux of organic matter differently according to their functional traits. Due to the cascading effect across trophic levels, secondary consumers were the most impacted, with lower trophic niche width, lower trophic diversity, higher redundancy and more trophic evenness. Conversely, endofauna, which relies on surface and subsurface organic matter, was less impacted due to the fact that their trophic requirements are usually already-processed organic matter. Alternatively, filter-feeders and grazers, although having limited movement, were able to cope with the homogenization effect of *C. fornicata* thanks to their food sorting mechanisms. However, this effect is likely to be seasonal due high organic matter supply during spring bloom, since we did not see major changes during winter. Benthic coastal habitats play a key role in nursery function (Able, 2005; Beck et al., 2005; Kritzer et al., 2016). As suggested by Arbach Leloup et al. (2008), *C. fornicata* is a trophic dead-end and drastically limits energy transfer towards higher trophic levels. Through food web homogenization, the slipper limpet may affect the trophic niche of higher consumers not sampled in this study such as demersal fishes (Le Pape et al., 2004, Quillien et al., 2016).

As it is well recognized that maerl beds promote benthic invertebrate diversity and harbor complex food web structure (Grall, 2002; Grall et al., 2006), it is surprising that an invasive species, at a 'dead' state, exhibits similar food web complexity. These results showed that biogenic structure can shape food web

structure, whatever the identity of the ecosystem engineer, and suggest that habitat structuration of *C. fornicata* shells may limit the detrimental effect through organic enrichment of food web structure. Considering the population dynamic of *C. fornicata* in the bay of Brest and its recent decline, the effect of shell accumulations raised the question about the long term effect of dead *Crepidula* bed for the ecosystem functioning of the bay of Brest.

6. Supplementary materials



Supplementary Figure 44: Size frequency distribution of *Crepidula fornicata* in the four sampled stations.

Supplementary Table 17: *p*-value from Kolmogorov-Smirnoff tests between pairs of the four sampled stations.

	S1	S2	S3
S1			
S2	<i>ns</i>		
S3	$p < 0.0001$	$p < 0.0001$	
S4	$p < 0.0001$	$p < 0.0001$	<i>ns</i>

Supplementary Table 18: Crepidula biomass and Crepidula shell weight in the four sampled stations (mean \pm SD g per 0.1m⁻², n = 3). KW represents result from Kruskal-Wallis test.

Station	Crepidula biomass	Crepidula shell weight
S1	626 \pm 124	1307 \pm 188
S2	300 \pm 117	1377 \pm 232
S3	28 \pm 9	1628 \pm 60
S4	4 \pm 4	1387 \pm 267
KW	$p < 0.05$	ns

SYNTHÈSE & DISCUSSION GÉNÉRALE

L'objectif de cette thèse était d'approfondir les connaissances sur l'écologie trophique de l'espèce *Crepidula fornicata* et de déterminer si sa niche trophique pouvait contribuer au succès de cette espèce invasive en lui conférant un avantage par rapport aux autres suspensivores. Il s'agissait en particulier de quantifier l'importance trophique d'un compartiment négligé en milieu côtier subtidal : le microphytobenthos (MPB). En choisissant comme modèle la crépidule et l'habitat particulier qu'elle crée, nous voulions examiner les interactions potentielles avec ce MPB subtidal. Ce travail de thèse met donc en avant autant la qualité d'ingénieur de l'écosystème de la crépidule que son statut d'espèce invasive. Cette thèse était par ailleurs motivée par le fait que des données isotopiques préliminaires, obtenues pour la crépidule en rade de Brest, suggéraient une contribution potentiellement importante du MPB au régime alimentaire de cette espèce. La science n'étant pas avare en surprise, nous avons ensuite constaté que la présence insoupçonnée de carbonates dans le pied de la crépidule engendrait un biais analytique sur les mesures isotopiques et par conséquent une surestimation du rôle trophique du MPB. Utilisant une double approche d'expérimentation *ex situ* et de prélèvements *in situ*, ainsi que des outils complémentaires d'écologie trophique tels que l'imagerie hyper-spectrale, les acides gras, les pigments, les isotopes stables ou encore les traits biologiques des espèces, nous avons contribué à redéfinir la niche trophique de cette espèce et à mieux comprendre le fonctionnement de cet habitat. La synthèse de ces résultats, leur analyse critique ainsi que les perspectives de ce travail sont présentées dans cette dernière partie.

I – Crépidule, carbonates et questionnements

Au cours du siècle dernier, certaines espèces, par leur place dans l'arbre du vivant ou par leur biologie, ont pris une place considérable en qualité de modèle biologique. C'est le cas de la crépidule, qui constitue un modèle pour l'étude du développement des Spiralia (Henry et al., 2016), des traits d'histoire de vie (Padilla et al., 2011), ou encore de l'hermaphrodisme successif (Broquet et al., 2015). En outre, par sa propension à proliférer, elle est devenue une espèce invasive emblématique, dont bon nombre d'impact associés ont été étudiés (Thielges et al., 2003 ; Stiger-Pouvreau et Thouzeau, 2015). Le propre des modèles biologiques est néanmoins de regorger d'inconnues. Pour preuve, la mise au jour de la présence de

sphérules carbonatées dans les tissus de la crépidule a modifié le cheminement de cette thèse.

Spécificités des tissus de crépidule

De l'ordre de 30 μm de diamètre, ces sphérules sont visibles à l'œil nu, semblent présentes dans tout l'organisme, et sont particulièrement abondantes dans le pied. Ces sphérules sont elles-mêmes constituées de plus petites billes d'environ 1 μm de diamètre (Figure 45C et 45D). Une analyse au microscope électronique à balayage élémentaire a permis de mettre en évidence la composition chimique de ces sphérules. Elles sont composées de carbonates de calcium et de magnésium (Figure 45A-D). Le rapport entre ces deux éléments est de 0,2 (Figure 45E) et montre donc une nette dominance du magnésium. Cependant, le diffractogramme réalisé par rayons X semble lui ne pas détecter la minéralogie de ces sphérules (Figure 45F). Après plusieurs essais infructueux, il semble que les quantités analysées soient trop faibles comparées à la masse de tissu, ou que la minéralogie soit trop fine.

Cette présence de sphérules en abondance est d'autant plus inattendue qu'elle semble faire exception chez les gastéropodes marins. En effet, la rétention du calcium par les gastéropodes est bien connue en milieu terrestre pour faire face à la rareté de cet élément dans le milieu (Fournier et al., 1984 ; Kapur et Gibson, 1968), ce qui n'est pas le cas en milieu marin. Quelques rares études ont montré la présence de sphérules carbonatées chez cinq espèces de gastéropode, mais elles sont situées plutôt dans la glande digestive (Mason et Nott, 1981 ; Nott et Nicolaidou, 1989). L'hypothèse avancée dans ces études est la détoxification contre les métaux lourds, ce qui ne semble pas aberrant pour notre site d'étude lorsqu'on sait que la rade de Brest présente un des plus fort taux de métaux lourds des côtes françaises (Réseau d'Observation de la Contamination Chimique : <https://wwz.ifremer.fr/lerpc/Activites-et-Missions/Surveillance/ROCCH>).

Cependant, le fait que nous observons ces sphérules dans les crépidules de plusieurs façades maritimes (rade de Brest, baie de Bourgneuf, baie du Mont Saint-Michel) ainsi que sur plusieurs photographies de *C. fornicata* provenant d'autres régions (publiées dans des articles scientifiques ; par ex : Schumway et al., 2014), nous laisse penser que la détoxification n'est pas la cause première.

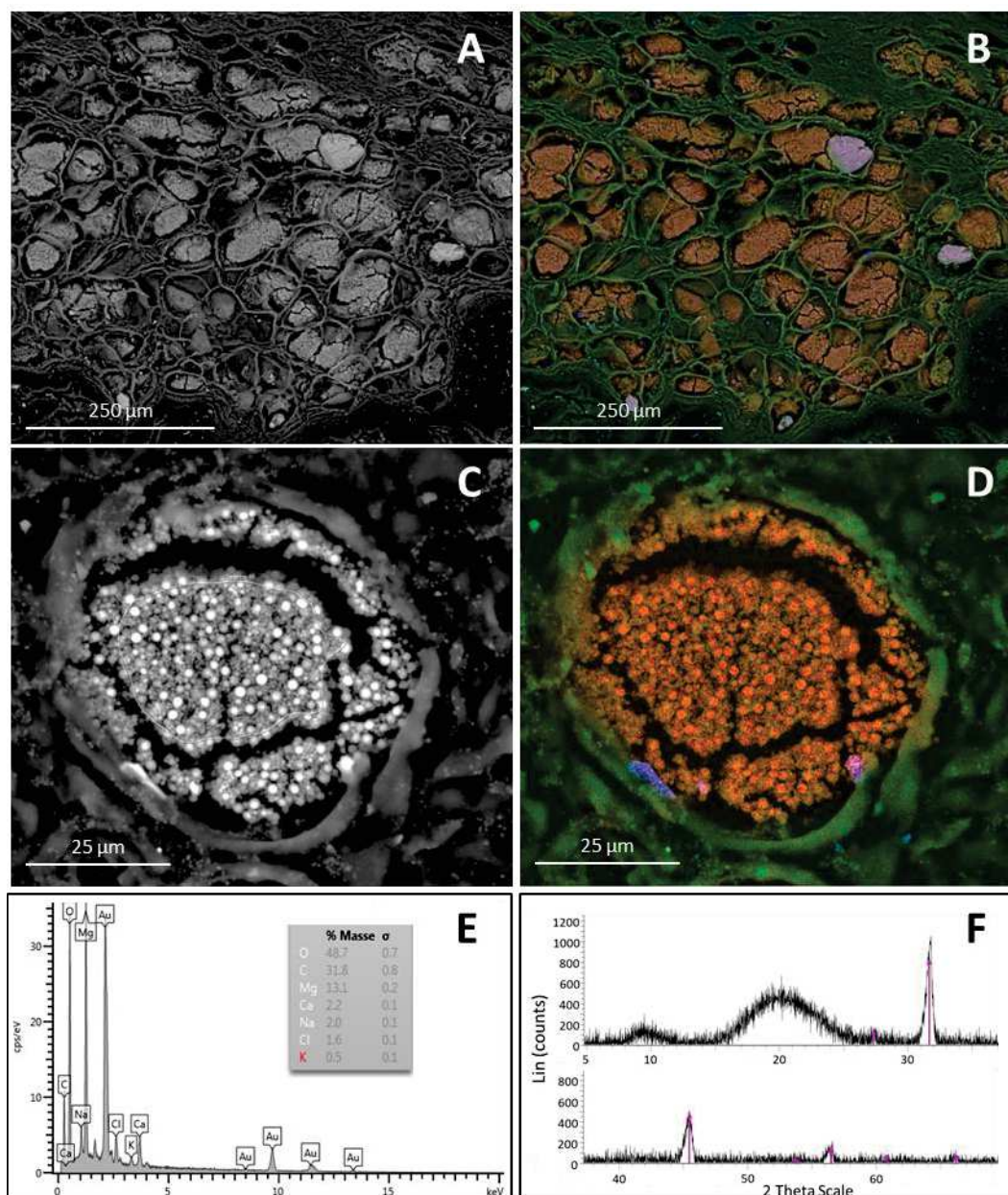


Figure 45 : Microscopie électronique à balayage élémentaire à l'échelle du tissu (A-B) et à l'échelle d'une sphérule (C-D). Spectre élémentaire montrant la composition atomique des sphérules (E). Diffractogramme à rayon X pour analyser la cristallographie des sphérules (F).

Nous avons plutôt attribué la présence de ces sphérules au comportement grégaire de l'espèce et à son changement de mobilité ontogénique. Une étude antérieure sur une autre espèce de crépidule (*Crepidula fecunda*) a montré un changement histologique dans le pied (Chapparo et al., 1998). Parallèlement au changement de mobilité se développait un nouveau type de mucocyte dans le pied des individus analysés. Plus intéressant encore, les auteurs font mention d'une masse granulaire à l'intérieur de ces cellules. Sachant que le calcium est un produit impliqué dans la production de mucus (Rao et Golbert, 1968), et que la crépidule produit de fortes

quantités de mucus pour se nourrir et pour se fixer, l'hypothèse la plus probable est que ces sphérules résultent de son mode de vie et sa production de mucus. Enfin, nous avons aussi mis en évidence que la production de mucus au niveau du pied de cette espèce avait un effet sur la coquille support, qui se matérialise par un dépôt de calcaire nettement visible au niveau de l'empreinte du pied. Il est clair que la présence de ces carbonates pose question et mérite de plus amples investigations. Etant constituées de calcium mais également de magnésium, ces sphérules sont-elles bien liées à la synthèse de mucus ? Si oui, est-ce un simple déchet du métabolisme ? Ont-elles aussi un rôle dans la détoxification ou la production coquillière ? Une autre question qui vient à l'idée est : si le mode de vie particulier de la crépidule explique en partie la présence de ces sphérules, sont-elles présentes chez des espèces qui adoptent un comportement similaire ? Pour l'avoir observé sur des coupes histologiques, il semble que *Calyptrea chinensis* (espèce de la même famille que la crépidule) possède elle aussi des sphérules, alors que la patelle *Patella vulgata* en est dépourvue.

Cela nous amène à la conclusion méthodologique de ce chapitre, c'est-à-dire notre mise en garde concernant la préparation des échantillons de tissus en vue d'analyses isotopiques. La dissection reste une étape importante qui doit se faire avec une connaissance suffisante de l'histologie des organismes étudiés. Il est recommandé d'effectuer de simples tests préalables, tel que le « champagne test » (c-à-d l'apparition d'une effervescence dans une solution d'acide chlorhydrique) préconisé par Schlacher et Connelly (2014), pour éviter la contamination d'échantillons par des carbonates. Qu'ils soient associés au calcium ou au magnésium, les carbonates proviennent du carbone inorganique dissout du milieu environnant et n'ont donc aucun lien avec la nourriture de la crépidule (Schlacher et Connelly, 2014). La valeur de $\delta^{13}\text{C}$ des carbonates étant proche de 0 ‰ (Sackett et Moore, 1966), même une légère fraction biaise considérablement le $\delta^{13}\text{C}$ du tissu, un des traceurs de matière organique les plus utilisés en écologie trophique (McCutchan et al., 2003, encadré méthode n°2). Dans le cas de la crépidule, la différence entre un échantillon avec et sans carbonates intra-tissulaire est en moyenne de 2 ‰ pour des individus adultes. La plupart des études publiées ayant utilisé le $\delta^{13}\text{C}$ de la crépidule se base sur des individus adultes et ne mentionne pas que ces carbonates ont été retirés ; c'est pourquoi l'hypothèse de l'assimilation

d'une source enrichie en ^{13}C , comme le microphytobenthos, par la crépidule s'est posée.

Implications pour le cheminement de la thèse

La mise au jour de ces sphérules, et du biais qu'elles engendrent en termes d'analyse isotopique a donc nécessité un recadrage partiel de la thèse, modifiant notre hypothèse de départ sur l'assimilation du MPB par la crépidule. En effet, l'idée selon laquelle un organisme est enrichi en ^{13}C est liée à une source qui serait elle aussi enrichie en cet isotope lourd. En milieu côtier, où les processus isotopiques régissant les signatures de la matière organique et des producteurs primaires sont bien connus (Fry, 2006), un enrichissement en ^{13}C pour un organisme filtreur est souvent associé à l'assimilation de microalgues benthiques (France, 1995 ; Miller et al., 1996 ; Riera et Richard, 1996). La signature de la crépidule plus enrichie que celle des autres suspensivores, à l'exception de certains bivalves tels que la coquille Saint-Jacques (Richard, 2005), est justement ce qui a motivé ce travail de thèse. Sachant que des crépidules intertidales dont le régime alimentaire était constitué en partie de diatomées benthiques montraient le même enrichissement en ^{13}C , il était donc probable que les crépidules subtidales assimilent également du MPB. Ceci s'ajoute à des études récentes qui montrent ou suggèrent une contribution sous-estimée du MPB pour le fonctionnement des réseaux trophiques subtidaux (Evrard et al., 2010 ; 2012 ; Grall et al., 2006 ; Rigolet et al., 2014). Enfin, les travaux réalisés en rade de Brest sur ce compartiment microphytobenthique offraient une base de travail intéressante. Le biais isotopique associé à ces carbonates correspond à environ 2 ‰ pour des individus adultes couramment échantillonnés (longueur droite ~30 mm). Une fois corrigée, la signature de la crépidule la replace parmi de nombreuses espèces de suspensivores se nourrissant *a priori* principalement du phytoplancton de la colonne d'eau (Decottignies et al., 2007a) et la fait apparaître à nouveau comme un compétiteur potentiel vis-à-vis de ces autres suspensivores. La proportion de microphytobenthos dans le régime alimentaire de la crépidule devenait donc *a priori* beaucoup moins importante. En corrigeant les valeurs isotopiques des données de la figure 6 (introduction), les crépidules ont désormais des valeurs de $\delta^{13}\text{C}$ plus proches de celle de la MOP, ce qui suggère en effet une contribution moins importante du MPB

dans leur régime alimentaire (Figure 46). Seules deux études n'ont pas été corrigées car les échantillons avaient fait l'objet d'une acidification, et présentaient donc des valeurs correctes. Cet artefact pris en compte, il convenait donc de redéfinir la niche de cette espèce, ainsi que son lien présumé avec le MPB subtidal.

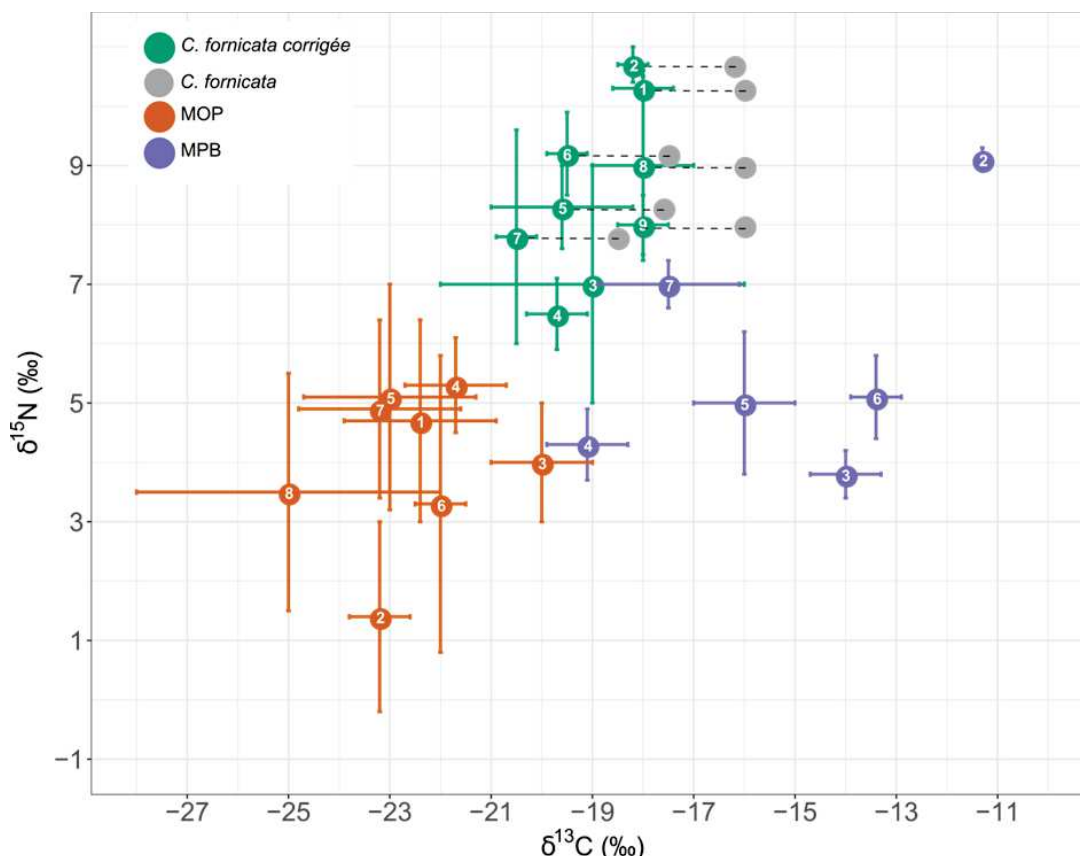


Figure 46 : Valeurs isotopiques de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ de *Crepidula fornicata* et deux sources potentielles de matière organique (matière organique particulaire (MOP) et microphytobenthos (MPB)) issues de la littérature. 1 : Grall et al., 2006 ; 2 : Riera et al., 2002 ; 3 : Riera, 2007 ; 4 : Dubois et al., 2014 ; 5 : Sauriau et al., 2002 ; 6 : Decottignies et al., 2007a ; 7 : Lefebvre et al., 2009 ; 8 : Richard, 2005 ; 9 : Guérin, 2004. Pour les références 1, 2, 5, 6, 7, 8, 9, une correction a été appliquée à l'aide du modèle proposé dans le chapitre 2 sur les données obtenues sans acidification.

II – Écologie trophique associée aux bancs de crépidules

Analyse expérimentale de la stimulation du microphytobenthos subtidal

Le MPB rassemble l'ensemble des microorganismes photosynthétiques qui se développent à la surface d'un substrat. Il est étroitement associé à des bactéries et des protistes hétérotrophes, le tout étant englobé dans une matrice d'exopolysaccharides (EPS). Tout ce microcosme compose le biofilm. Le chapitre 1 de cette thèse visait à comprendre comment la crépidule, ingénieur de son

écosystème, influait sur le MPB en milieu subtidal. L'objectif était de découpler l'effet autogénique (physique) et l'effet allogénique (biologique) de la crépidule. L'hypothèse de ce travail était que l'activité biologique de la crépidule, par l'intermédiaire d'une augmentation des flux de nutriments à l'interface eau-sédiment, conduisait à une augmentation de la croissance du MPB.

Pour tester cette hypothèse de fertilisation, une expérience en milieu contrôlé a été mise en place. Du sédiment collecté *in situ*, tamisé et homogénéisé a servi de substrat pour chaque mésocosme, et un banc miniature de crépidules (mortes ou vivantes) a été recréé au centre de chacun d'entre eux. La biomasse de MPB dans chaque mésocosme a été mesurée à 7 occasions durant les 10 jours d'expérimentation à l'aide d'une caméra hyper-spectrale. Des prélèvements de sédiment ont été réalisés à t_0 et t_{final} pour quantifier les carbohydrates, les protéines et les bactéries hétérotrophes du sédiment. Dans une première partie, la comparaison entre un sédiment témoin, un banc mort et un banc vivant de crépidule a permis de montrer un effet physique sur la stimulation du MPB du sédiment à l'intérieur du banc de crépidule (control vs. banc mort). Cet effet était néanmoins deux fois moins important que l'effet biologique de la crépidule seul (banc mort vs. banc vivant). Nous n'avons pas noté de synergie entre les effets physique et biologique de la crépidule (control vs. banc vivant). Dans une seconde partie, l'étendue spatiale de l'effet biologique a été évaluée en comparant un banc mort et un banc vivant sur trois zones d'intérêts (la surface des coquilles de crépidule, l'intérieur et l'extérieur du banc). Cette partie a mis en évidence un effet plus fort de l'activité biologique de la crépidule sur les coquilles, un effet nul à l'extérieur du banc, et un effet intermédiaire au sein du banc. Compte tenu de la stimulation plus forte sur les coquilles, et de la distribution du MPB autour des chaînes de crépidules, nous avons suggéré que la source de stimulation était probablement davantage liée à l'excrétion dissoute (ammonium) des crépidules plutôt qu'à l'excrétion particulaire (biodéposition). L'origine de la stimulation (dissoute vs. particulaire) et l'importance relative de ces deux processus est susceptible de varier en fonction de l'échelle de temps considérée. En effet, les crépidules n'ont pas été nourries pendant les 10 jours de notre expérimentation et n'ont donc produit des biodépôts que durant les 2-3 premiers jours. Il est donc probable que l'apport de nutriments lié à la reminéralisation des biodépôts soit sous-estimé sur une échelle de temps si courte. Il est également important de noter que d'autres processus sont

susceptibles d'influencer le MPB à proximité des chaînes de crépidule tels que la bioturbation des individus sur la chaîne ou encore la production de mucus associée à la filtration. Enfin, l'analyse des carbohydrates, protéines et bactéries hétérotrophes n'a pas montré de différence entre les traitements. Ceci peut s'expliquer par la technique d'échantillonnage. Alors que l'imagerie hyper-spectrale échantillonne les 300 premiers μm du sédiment, les carottages effectués pour les 3 paramètres l'ont été sur 1 cm de profondeur. Il est donc vraisemblable qu'un éventuel effet de la crépidule sur un ou plusieurs de ces 3 paramètres limité à la surface du sédiment ait pu être dilué par la quantité de sédiment échantillonnée.

Dans ce chapitre, nous avons donc montré que la crépidule pouvait stimuler le MPB subtidal, avec un effet biologique plus fort que l'effet physique seul. De plus, nous avons mis en évidence plusieurs indices suggérant l'importance de l'excrétion dissoute dans ce processus de fertilisation à l'échelle de notre expérimentation.

Quantification *in situ* du microphytobenthos subtidal

Dans le but de confirmer nos résultats expérimentaux, un suivi *in situ* a été réalisé au printemps (février - avril) pour quantifier la biomasse et la composition de la matière organique particulaire (MOP) sur un banc de crépidules vivantes. Trois marqueurs ont été utilisés (pigments, isotopes stables et acides gras) sur trois compartiments : le biofilm présent sur les chaînes de crépidules vivantes, le sédiment de surface pouvant être remis en suspension, et la colonne d'eau au-dessus du banc de crépidules. Ces résultats sont présentés dans l'article 3 de cette thèse.

L'utilisation conjointe des trois biomarqueurs a permis de bien discriminer ces 3 sources de MOP. Les faibles valeurs de $\delta^{13}\text{C}$ (- 25 ‰) indiquent que la MOP pélagique est influencée par les apports de l'Elorn. En effet, la signature moyenne de la MOP marine de la rade est de -22.3 ± 1.2 ‰ et celle de la MOP de l'Elorn en amont est de 27.5 ± 1.2 ‰. Les biomasses de chlorophylle *a* mesurées sur notre site d'étude sont étonnamment élevées ($5\text{-}18 \mu\text{g L}^{-1}$) comparé avec la MOP marine en période de bloom ($4\text{-}6 \mu\text{g L}^{-1}$) et suggèrent un apport de nutriment élevé par ce fleuve. La MOP re-suspendue est composée de matière organique de diverses origines. La présence de pheopigments, de bactéries et de traces de plantes vasculaires suggère une quantité importante de matière détritique. Cependant, les

forts ratios d'acides gras (AG) polyinsaturés sur saturés indiquent que cette matière organique possède une bonne qualité nutritive, avec la présence notable d'AG biomarqueurs de diatomées. Bien que les biomasses de chlorophylle *a* y soit faibles (1-2 mg m⁻²), la concentration de fucoxanthine est plus élevée (2-8 mg m⁻²) et le ratio fucoxanthine sur chlorophylle *a* indique une nette dominance de diatomées composant la communauté comparé à la MOP de la colonne d'eau et au biofilm. Ces forts taux de fucoxanthine, laquelle est connue pour être un pigment absorbant les faibles longueurs d'ondes, indiquent également une forte photo-acclimatation de ces diatomées, ce qui est justifié par l'intensité lumineuse très faible relevée sur la zone (0.01 % de la lumière incidente). Enfin, le biofilm montre une signature très spécifique, que ce soit en termes d'AG, d'isotopes et de pigments. En effet, les marqueurs lipidiques indiquent une très forte concentration d'AG saturés (spécifiquement 16:0 et 18:0). Les AG et les pigments indiquent une communauté photosynthétique également présente en forte concentration, appartenant aux algues vertes en majorité et aux diatomées. Enfin, le biofilm se différencie aussi par un fort enrichissement en ¹⁵N, supérieur de 2,5 ‰ par rapport aux deux autres compartiments.

Ces résultats montrent que le MPB est bien présent sur un banc de crépidules vivantes, avec une forte photo-acclimatation dans cet habitat turbide. Les fortes valeurs de MPB à la surface des coquilles de crépidule comparées au sédiment adjacent confirment nos résultats expérimentaux, à savoir une meilleure croissance du MPB sur les coquilles de crépidules vivantes. Ceci peut être relié aux fortes valeurs de $\delta^{15}\text{N}$ mesurées sur les coquilles, qui suggèrent que le MPB pourrait assimiler une source d'azote spécifique provenant de l'activité biologique de la crépidule (par exemple les produits d'excrétion dissouts). Une fois l'environnement trophique de la crépidule caractérisé, il s'agissait de déterminer la contribution du MPB et des autres sources de matière organique au régime de cette espèce.

Relations trophiques intraspécifiques chez la crépidule

Comme détaillé en introduction, la crépidule est une espèce au mode de vie particulier, dont les traits d'histoire de vie diffèrent de ceux de la majorité des espèces d'invertébrés benthiques. Au cours de la vie de la crépidule, un changement de sexe s'effectue parallèlement à un changement de mobilité : les jeunes mâles

mobiles se sédentarisant devenant des mâles sessiles puis des femelles sessiles. Sans capacité de déplacement, les adultes sessiles filtrent l'eau pour se nourrir. A l'inverse, les individus mobiles sur un substrat - à l'image de la majorité des gastéropodes consommateurs primaires - peuvent potentiellement brouter le substrat tout en maintenant une capacité de filtration. Ce comportement a été mis en évidence chez *C. fecunda*, autre espèce de crépidule présentant les mêmes changements ontogéniques (Navarro et Chaparro, 2002). Comprendre la ségrégation de niche chez la crépidule entre les différents stades ontogéniques semblait pertinent au regard du grégarisme et des fortes biomasses de l'espèce dans les habitats colonisés (jusqu'à 6 kg m⁻², en poids frais). Par ailleurs, suite aux résultats du chapitre 2, il était important de reconsidérer la niche des adultes, dont les mesures isotopiques passées avaient pu conduire à des interprétations biaisées. L'hypothèse testée dans le chapitre 3 porte ainsi sur les changements ontogéniques de la crépidule et leur conséquences sur une différenciation du régime trophique des individus mâles et mobiles capables de brouter le substrat et des individus sessiles filtrant la MOP en suspension.

La double approche isotopes – AG a été conclusive dans ce chapitre. Alors que les isotopes n'ont pas permis de mettre en évidence un changement de régime trophique pour des raisons liées à la physiologie des stades étudiés (c-à-d., différence d'incorporation et de fractionnement en fonction du taux de croissance), ils ont tout de même permis de montrer une plus grande variabilité des sources assimilées pour le stade mâle mobile que pour les stades sessiles (mâle ou femelle). Cette niche trophique élargie chez les individus jeunes est interprétée comme le résultat d'une diversité de sources plus importante, que l'on peut expliquer par la mobilité de ces individus. Les AG montrent une plus grande différenciation entre les stades ontogéniques. En plus d'une variabilité inter-individus plus élevée, on observe également une plus grande variabilité temporelle entre les stades qu'avec les isotopes. Parmi les trois stades, les mâles mobiles montrent la plus grande variabilité, les femelles sessiles la plus petite et les mâles sessiles une variabilité intermédiaire. Cette variabilité traduit d'importants changements physiologiques d'allocations des ressources. Alors que les jeunes mâles encore immatures utilisent la majorité de leur réserve pour la croissance somatique et pour leur déplacement, les mâles adultes partagent leurs ressources entre la fabrication de gamètes et la croissance somatique. Les femelles adultes accordent une part importante de leur

réserve à la gamétogenèse, au développement des embryons et à leur incubation. Malgré ces variations liées à des mécanismes physiologiques, il reste possible de tirer des conclusions sur le régime trophique sachant que les lipides neutres sont représentatifs de la nourriture assimilée, et que les compositions en AG des sources de matière organique potentiellement assimilées ont été mesurées. Les individus sessiles, mâle et femelles, montre un régime trophique basé sur de la matière organique particulaire du sédiment remise en suspension. Au sein de ce *pool* composé à la fois de détritus, de bactéries mais également de diatomées, la crépidule apparaît comme un filtreur opportuniste assimilant du matériel détritique, des bactéries, des diatomées et des flagellés. Les individus mâles et mobiles montrent un découplage temporel dans leur composition en AG. La contribution majoritaire d'AG saturés explique la singularité des deux premières dates d'échantillonnage. Ce type d'AG est souvent considéré comme une source peu nutritive mais à forte valeur énergétique, et peut refléter un régime alimentaire de faible qualité. Cependant, les sources échantillonnées, et notamment le biofilm, montrent les plus fortes concentrations d'AG et une composition faite notamment d'AG saturés. Les individus mobiles ont accès à cette ressource et il est donc probable qu'ils assimilent du biofilm en broutant le substrat. Pour les deux dernières dates, on note une augmentation de la contribution des AG polyinsaturés au régime alimentaire des jeunes mâles. Celle-ci peut être liée à l'augmentation de la contribution en PUFA dans les ressources consommées, à une accumulation avec le développement sexuel, ou à un régime trophique utilisant le broutage et la filtration, en lien avec la diversification des sources de nourriture.

Ce chapitre a donc permis de mettre en évidence le broutage potentiel des jeunes mâles mobiles sur un biofilm présent sur le dos des adultes, avec une intensité plus marquée en hiver. Deuxièmement, il a permis de reconsidérer la niche trophique de la crépidule adulte, ainsi qualifiée d'opportuniste car elle assimile majoritairement de la matière organique particulaire benthique remise en suspension constituée de bactéries, de diatomées et de flagellés. La caractérisation des interactions trophiques de la crépidule avec ses compétiteurs potentiels au sein de son habitat devient dès lors possible.

Relations trophiques interspécifiques au sein des suspensivores

La diversité au sein d'un mode trophique est appelée diversité horizontale, par opposition à la diversité verticale qui s'intéresse à la diversité entre les niveaux trophiques. Comme l'a montré le chapitre 4, les modes de vie et d'alimentation des organismes suspensivores sont très variés. En effet, les 5 espèces étudiées possèdent chacune une niche trophique distincte (Figure 47). Encore une fois, il est intéressant de noter que l'utilisation conjointe de deux marqueurs trophiques (isotopes et acides gras) a permis de mettre en évidence le non-recoupement des niches trophiques : alors que les isotopes et les acides gras permettent de discriminer respectivement 3 ou 4 niches trophiques lorsqu'ils sont considérés indépendamment, l'utilisation conjointe des deux marqueurs a révélé en réalité 5 niches trophiques distinctes. En nous intéressant aux traits biologiques des espèces spécifiquement impliqués dans leur niche trophique (habitat privilégié, capacité de rétention, organes de sélection, capacité de déplacement sur le fond), il s'est avéré que ces traits expliquaient de manière cohérente ce que l'on pouvait déduire des marqueurs trophiques.



Figure 47 : Habitat à crépidules illustrant les espèces de suspensivores étudiés.

En effet, les espèces avec les plus fortes capacités de sélection montrent les plus faibles variations temporelles et les niches trophiques les plus étroites, comme cela a pu être montré pour d'autres assemblages de suspensivores (Dubois et Colombo, 2014). C'est le cas du pétoncle noir, *Chlamys varia*, qui est non seulement capable de sélectionner sa nourriture mais aussi son micro-habitat. Cette sélection se reflète par un régime trophique riche en AG polyinsaturés basé sur des diatomées et des résidus de macroalgues vertes. Comparativement, l'huître plate, *Ostrea edulis*, présente également un fort taux d'AG polyinsaturés mais une plus grande variation inter-individuelle et une contribution moins élevée d'AG biomarqueurs de macroalgues vertes et de diatomées. Ceci s'explique par son incapacité à se mouvoir et à sélectionner un micro-habitat. Une huître peut par exemple être partiellement enfouie dans le sédiment sans pouvoir modifier cette position. De plus, ces organes de sélection ne permettent pas de sélection des particules de grandes tailles, tels que des fragments de macroalgues qui sont écartés lors d'un tri pré-ingestif (Beninger et al., 2004 ; Cognie et al., 2001). *C. varia* et *O. edulis* ont par ailleurs des signatures isotopiques très proches qui suggèrent une alimentation constituée essentiellement de matière organique du sédiment remise en suspension. En ce qui concerne les deux espèces de Calyptraeidae, *Crepidula fornicata* et *Calyptraea chinensis*, les AG indiquent une nourriture globalement similaire basée sur des bactéries, des diatomées et des flagellés. En lien avec le compartiment bactérien, ces deux espèces montrent des rapports entre AG polyinsaturés et AG saturés plus faibles que les autres espèces, indiquant une nourriture de moins bonne qualité. Ceci s'explique par leur proximité avec l'interface eau-sédiment et par leur capacité de mobilité, nulle pour la crépidule et faible pour *C. chinensis*. De plus, ces espèces n'ont pas ou très peu de capacité de sélection des particules ; elles sont donc opportunistes et assimilent ce qui est à leur portée. Néanmoins, *C. chinensis* se différencie par un net appauvrissement en ^{15}N comparé à la crépidule (de près de 4 ‰), ce qui dépasse les valeurs attendues pour un niveau trophique. Encore une fois, cela peut s'expliquer par le micro-habitat de cette espèce. En effet, *C. chinensis* affectionne l'intérieur des coquilles mortes de *C. fornicata* qui est très peu exposé à l'hydrodynamisme. Les individus sont donc proches de la couche limite de fond, où des conditions biogéochimiques particulières peuvent exister. En effet, les crépidules modifient les flux biogéochimiques à l'interface eau-sédiment, modifient et stimulent la respiration

des microorganismes et favorisent l'anoxie du sédiment. Dans ces conditions, il est probable que des bactéries dénitrifiantes, connues pour appauvrir le sédiment en ^{15}N , puisse participer à la source d'azote assimilée par *C. chinensis*. Pour finir, le cirripède *Austrominius modestus* est un crustacé sessile qui vit également de manière associée aux crépidules dans cet habitat (c-à-d., fixé sur la face convexe des coquilles, exposée au courant). Cette espèce montre une forte variabilité temporelle, passant de l'omnivorie en hiver avec une forte contribution de matériel détritique, à un régime composé de diatomées au début du printemps. Ceci s'explique par des organes d'alimentation incapables de sélection et de tri : son régime alimentaire reflète donc ce qui est disponible dans son environnement. Ses valeurs enrichies en ^{13}C et en ^{15}N semblent corrélées à l'omnivorie de cette espèce.

Une conclusion majeure de ce chapitre est que la crépidule n'est pas un compétiteur potentiel pour les autres espèces suspensivores représentatives de cet habitat. Au contraire, les sources de matières organiques disponibles permettent une forte diversification des régimes trophiques, potentiellement sous l'influence de la crépidule. Ce qui est vrai à l'échelle d'un groupe trophique ne l'est pas toujours à l'échelle d'une communauté benthique entière. L'étape suivante était donc d'analyser la structure du réseau trophique associé aux crépidules à l'échelle de la communauté, prenant en compte les autres consommateurs primaires (brouteurs, déposivores de surface et de sub-surface) et les consommateurs secondaires (carnivores, omnivores, nécrophages).

Relations trophiques interspécifiques au sein de la communauté

Les différences observées entre les niches trophiques de la crépidule et celles des principales espèces de suspensivores associées invitent à l'examen de l'ensemble du réseau trophique. Les fortes biomasses de crépidules induisent une biodéposition importante, ce qui enrichit le milieu en matière organique et qui peut être à l'origine d'une simplification du réseau trophique. De plus, la présence de coquilles, qui persistent après la mort des individus, est également susceptible de modifier la structure du réseau trophique. Ces questions sont abordées dans le chapitre 5.

L'effet biologique de la crépidule sur le réseau trophique a été étudié en comparant un banc mort de crépidules (effet autogénique) à un banc vivant (effet auto et allogénique). Un échantillonnage exhaustif de la communauté associée à ces deux types de bancs a été réalisé. Deux saisons contrastées en termes d'apport de matière organique par la colonne d'eau ont été choisies, hiver (faible) et été (fort). Des indices calculés sur la base des signatures isotopiques des espèces présentes ont permis de quantifier certains aspects de la structure du réseau trophique (étendue, diversité, redondance et régularité trophique au sein de la niche). Pour comprendre l'effet de la crépidule à différents niveaux dans le réseau trophique, les espèces ont été regroupées en fonction de traits fonctionnels se rapportant à leur écologie trophique (mode trophique, habitat privilégié, mobilité, bioturbation). Les indices ont donc été calculés à deux niveaux : communauté et groupe tropho-fonctionnel. L'analyse à l'échelle de la communauté a montré une influence de la crépidule vivante en été seulement. Les résultats indiquent que la diversité et la taille de la niche trophique de la communauté diminue en présence de crépidules vivantes, alors que la régularité augmente. Ces résultats suggèrent que la crépidule agit comme un filtre environnemental sur le fonctionnement trophique de son habitat. Lors d'une période de fort apport en matière organique allochtone (ex : efflorescence printanière), la crépidule intensifie le couplage benthopelagique, ce qui tend à enrichir l'habitat en matière organique. Ceci tend à homogénéiser les sources à la base du réseau trophique. L'effet de cette homogénéisation est néanmoins variable selon les 3 groupes tropho-fonctionnels définis. Le groupe composé par les consommateurs primaires endogés, défini par des capacités de mouvements limitées et se nourrissant de matière organique sur/dans le sédiment sont peu impactés par l'effet de la crépidule. Ceci s'explique par leur nourriture qui se compose d'une matière qui a déjà subi des processus de reminéralisation et qui montre donc moins de variation temporelle. Les suspensivores et les brouteurs qui composent le deuxième groupe, ont peu/pas de capacités de mouvement et appartiennent à l'épifaune. Ce groupe est affecté dans une moindre mesure car il présente une taille de niche et une diversité trophique moins importante sur un banc vivant de crépidules, mais pas de différence de redondance avec un banc mort. L'impact de la crépidule sur ce groupe est atténué grâce aux capacités de sélection et de tri de ce groupe d'espèce. Enfin, le dernier groupe tropho-fonctionnel regroupe les consommateurs secondaires, libres et mobiles. Ce groupe est plus

affecté par la modification à la base du réseau trophique. L'effet cascade le long de la chaîne trophique entraîne une diminution de la taille de la niche de ce groupe, une diminution de la diversité et une augmentation de la redondance.

La seconde comparaison de ce chapitre, entre un banc de maërl et un banc de crépidules mortes, visait à comprendre l'effet physique spécifique d'une espèce ingénier sur la structure du réseau trophique. En effet, les coquilles de crépidule ainsi que le maërl sont considérés comme des ingénieurs autogéniques de l'écosystème. Pour le maërl, les données proviennent de la littérature et en particulier d'une étude menée en rade de Brest, avec toutefois un effort d'échantillonnage moins important (Grall et al., 2006) ; c'est pourquoi seule l'analyse à l'échelle de la communauté a été réalisée. Les résultats ne montrent pas de différence en termes de taille de niche, de diversité ou encore de redondance trophique. Bien que similaire d'un point de vue structure, le fonctionnement semble différent. En effet, les sources d'azote et de carbone sont significativement différentes entre les deux types de bancs, avec plus de diversité dans les sources de carbone pour le banc de crépidules mortes et plus de diversité dans les sources d'azote pour le banc de maërl.

Par cette double comparaison, ce chapitre a montré que la crépidule tendait à homogénéiser le réseau trophique lorsque les apports en matière organique allochtone sont importants (ex : efflorescence printanière). L'effet est néanmoins différent selon le groupe trophique des espèces, avec un effet particulièrement fort pour les consommateurs secondaires. D'autres part, nous avons montré que bien que différent en terme de fonctionnement, un banc de crépidule mort montre une structure de réseau trophique aussi complexe que celle d'un banc de maërl, démontrant l'effet structurant de la crépidule même en phase de déclin.

III – Le MPB subtidal : un jardin encore secret

Des conditions du milieu particulières

« Réalisme » de l'approche expérimentale

Dans le chapitre 1 de cette thèse, nous souhaitons démontrer expérimentalement comment la crépidule, en tant qu'ingénieur de l'écosystème, pouvait stimuler la croissance des producteurs primaires benthiques

microscopiques, notamment le microphytobenthos (MPB). L'intérêt de l'approche expérimentale *ex situ* réside dans le contrôle d'un ou de plusieurs facteurs de manière à quantifier leur importance relative ainsi que les relations qui les lient (ex : synergie, inhibition). Il est néanmoins important de considérer le potentiel effet des facteurs non pris en compte. Lors de notre expérience, les paramètres abiotiques de température, lumière et hydrodynamisme n'ont pas varié. La température et la lumière reproduisaient des conditions hivernales retrouvées en rade de Brest sur notre site de suivi (estuaire de l'Elorn) et étaient donc potentiellement limitantes pour le MPB. Une température froide, en agissant sur le métabolisme, tend à ralentir la croissance des microalgues (Bernard et Rémond, 2012). La lumière est un élément particulièrement critique pour les organismes photosynthétiques, tant en quantité qu'en qualité. En travaillant sur un habitat subtidal, il était nécessaire de prendre en compte une lumière réaliste pour le développement du MPB. Nous avons donc mis en place un éclairage reproduisant l'intensité et le spectre lumineux qui caractérise notre site d'étude à 10 m de profondeur. Pour une raison technique, il s'est avéré qu'une plage de longueur d'onde autour de 480 nm était quasi absente du spectre fourni par l'éclairage (Supplementary Figure 17). Néanmoins, cela ne semble pas avoir influencé nos résultats pour deux raisons. La première est que les pics d'absorption des pigments majoritaires étaient présents sur le spectre pour la profondeur donnée (fucoxanthine : 450-60 nm, chlorophylle *a* : 665-675 nm). Deuxièmement, la plage de longueur d'onde située autour de 480 nm ne correspond pas à un pic d'absorbance pour le MPB intertidal (Méléder, 2003). L'hydrodynamisme est un facteur primordial, car il contribue à déstructurer le biofilm à l'interface eau-sédiment. Bien que le milieu subtidal soit considéré comme plus stable et moins stressant d'un point de vue abiotique que le milieu intertidal (Little et al., 2009 ; Moran, 1999), les forts courants de marée dans la rade de Brest entraînent une remise en suspension des fonds meubles à chaque marée (Beudin, 2014).

Pour ce qui est des facteurs biotiques, le sédiment a été tamisé sur 500 µm. Le but était d'enlever la macrofaune susceptible d'induire une variabilité importante et non contrôlable sur la biomasse de MPB, par l'intermédiaire de deux processus antagonistes ; le broutage, qui diminue la biomasse (Miller et al., 1996), et la bioturbation du sédiment, qui peut stimuler la production primaire (Kristensen et al., 2012). En modifiant l'interface eau-sédiment par leurs terriers, leurs

mouvements dans la matrice sédimentaire ou la fabrication de tubes, la macrofaune modifie les flux de nutriments qui seront utilisés par le MPB (Braeckman et al., 2010 ; Lohrer et al., 2004). La méiofaune (63 μm – 1 mm) joue également ce rôle dans le sédiment (Bonaglia et al., 2014) mais il n'est pas possible de la supprimer (par exemple en la congelant ou en la brûlant) sans dénaturer complètement le substrat. En supprimant la macrofaune seule, on autorise donc que ces processus se réalisent par le seul biais de la méiofaune. Cependant, la petite taille et les fortes abondances d'individus de ce compartiment permettent une répartition homogène au sein des mésocosmes en début d'expérimentation. Il est donc peu probable que la méiofaune ait induit une forte variabilité dans notre expérience, nous permettant ainsi de mesurer l'effet de la crépidule seule lors de cette expérimentation. Examiner l'effet de la crépidule sur le compartiment méiofaunique était l'un des objectifs de ce travail. Nous souhaitions relier l'évolution de la distribution spatiale de la méiofaune avec celle de la biomasse microphytobenthique (Blanchard, 1990 ; Danovaro et al., 2001). Par manque de temps, ces échantillons n'ont pas été traités. L'étude de la diversité de la méiofaune étant très chronophage, avec une résolution taxonomique faible du fait d'un manque de spécialistes, ce compartiment reste souvent sous-estimé alors que son rôle n'est pourtant plus à démontrer (Schratzberger et Ingels, 2018).

Les faibles températures et irradiances utilisées (représentatives de conditions hivernales), ainsi que l'absence de bioturbation ont pu être des conditions limitant le développement du MPB dans notre expérience. Elles sont donc susceptibles d'avoir sous-estimé le potentiel de stimulation du MPB par la crépidule. Cependant, il est probable que l'absence d'autres facteurs tels que l'hydrodynamisme ou le broutage ait au contraire surestimé ce potentiel de stimulation. Bien que les mesures présentées dans le chapitre 3 confirment l'importance du métabolisme de la crépidule sur le biofilm des coquilles, une comparaison *in situ* des biomasses de MPB entre un banc vivant de crépidule et un sédiment comparable sans crépidules permettrait de valider notre hypothèse de stimulation.

Une autre question relative à notre expérimentation est la représentativité de notre condition « contrôle ». En effet, le sédiment collecté *in situ* a été échantillonné dans un banc de crépidule très dense ($> 2000 \text{ ind m}^{-2}$) et riche en matière organique ($\sim 10\%$). Lors de la préparation du sédiment, il est possible que l'homogénéisation,

la dilution et l'oxygénation aient modifié sa biogéochimie, avec pour effet l'augmentation des flux de nutriments venant du sédiment (Na et al., 2008). Bien que nous n'ayons pas mesuré ces flux, la croissance homogène du MPB dans notre condition « contrôle » (c.-à-d. sans crépidules) supporte l'hypothèse d'un apport en nutriments. On peut donc considérer que le sédiment utilisé dans notre condition « contrôle » était déjà influencé par la présence de crépidules depuis un certain temps (plus de 30 ans de colonisation à cet endroit). En effet, la crépidule est connue pour homogénéiser le sédiment sur lequel elle se développe, et le rendre plus riche en vase et en matière organique (Ehrhold et al., 1998). Nos conclusions ne sont donc valables que pour un stade invasif avancé, où la crépidule a déjà transformé son habitat. Il aurait donc été intéressant de tester un autre sédiment « contrôle » représentant un substrat à un stade précoce d'invasion de la crépidule (par exemple les fonds sableux de la baie de Saint-Brieuc, en bordure de l'habitat à crépidule). Les sédiments sableux ont généralement des teneurs en matière organique plus faibles que les sédiments vaseux et une communauté microphytobenthique différente. Il est donc probable que l'influence de la crépidule soit dépendante du substrat présent.

Influence de la lumière sur l'estimation des biomasses de MPB

Le MPB des milieux meubles subtidaux est beaucoup moins étudié que son homologue intertidal. La colonne d'eau permanente ne permet pas d'échantillonner ni d'isoler de manière précise le MPB (Laviale et al., 2015), et son rôle écologique est probablement sous-estimé (Cahoon, 1999 ; Cahoon, 2017 ; MacIntyre et al., 1996). Il est donc intéressant de confronter nos résultats à ceux obtenus en zone intertidale. Au cours du suivi *in situ* des sources de matière organique présenté dans le chapitre 3, nous avons mesuré la biomasse des pigments par HPLC. La matière remise en suspension, qui représente une fraction du sédiment de surface, présentait des teneurs en chlorophylle *a* de l'ordre de 2-4 mg m⁻², et le biofilm des valeurs de 5-15 mg m⁻², ce qui paraît assez faible comparé aux valeurs de 30-50 mg m⁻² observées sur une vasière intertidale à la même saison (Méléder, 2003). La lumière est un élément fondamental pour le développement du MPB. La quantité mais également la qualité de la lumière influencent le contenu pigmentaire des cellules (Barnett, 2013 ; Serôdio et al., 2012 ; Van Leeuwe et al., 2008) et donc l'estimation de leur biomasse. Nous avons montré sur notre site d'étude que malgré une intensité

lumineuse très faible, correspondant à moins de 0.1 % de la lumière incidente (Figure 33), le MPB était présent et montrait une forte photo-acclimatation grâce à la présence de pigments accessoires tels que la fucoxanthine. Ce pigment permettant de capter l'énergie lumineuse à de faibles longueurs d'ondes (Post et al., 1984), présentait des biomasses 4 à 8 fois supérieures à celle de la chlorophylle *a*. Le ratio fucoxanthine / chlorophylle *a* variait de 2 à 4 dans le MPB du sédiment sur notre site d'étude, ce qui est 10 fois supérieur au ratio mesuré sur le MPB intertidal (Méléder, 2003). Comparer des milieux avec des conditions de lumière très différentes sur la base de la seule chlorophylle *a* peut donc engendrer une sous-estimation de la biomasse de MPB, que ce soit pour des comparaisons intertidale-subtidale, inter-sites subtidaux (Riau-Gobin et al., 1998, Ní Longphuirt et al., 2007) ou sédiment-colonne d'eau (Chatterjee et al., 2013). À l'image des fortes différences entre le MPB des coquilles de crépidules vivantes et le sédiment dans notre étude, la non prise en compte du type de substrat biogénique (ex : mollusques, brin de maërl) peut également conduire à une sous-estimation de la biomasse du MPB. Ceci pourrait expliquer que lors d'une évaluation spatiale de la biomasse de MPB dans les sédiments de la rade de Brest, les valeurs les plus faibles aient été mesurées sur les habitats à crépidules alors que ces coquilles abritent un biofilm important de diatomées benthiques (Ní Longphuirt et al., 2007 ; chapitres 1 et 3) (Figure 48).

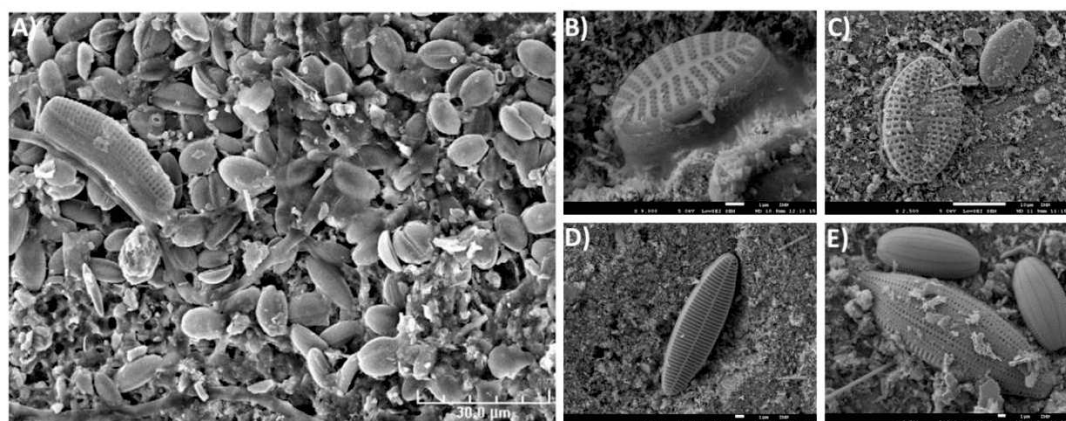


Figure 48 : Photographies au microscope électronique à balayage de diatomées épiphytes présentes à la surface d'une coquille de *Crepidula fornicata* échantillonnée à 5 m de profondeur dans la rade de Brest (A) (Ní Longphuirt et al., 2007) et à la surface d'une coquille de *Crepidula fornicata* provenant de notre expérimentation *ex situ* (B-E).

Quid de la mixotrophie en milieu subtidal ?

Il semble qu'un autre processus soit sous-évalué dans ces milieux faiblement lumineux : la mixotrophie. La mixotrophie est la capacité d'un organisme à effectuer soit l'autotrophie (synthèse de matière organique par photosynthèse ou chimiosynthèse) soit l'hétérotrophie (synthèse de matière organique à partir de l'oxydation de matière organique). Lorsque la lumière ne permet plus l'autotrophie (limite inférieure de la zone photique fixée à 1 % de la lumière incidente), l'utilisation de substrat organique devient une source de synthèse de matière organique. Cette mixotrophie est bien connue chez le plancton et notamment chez les dinoflagellés, mais les connaissances actuelles laissent supposer qu'un grand nombre de clades d'algues en sont potentiellement capables (Burkholder et al., 2008). Les diatomées en général, et les diatomées benthiques plus particulièrement, ont déjà fait l'objet d'études à ce sujet (Glud et al., 2002 ; Levin, 1976 ; 1978 ; Sundbäck et Grinéli, 1988). Il a été démontré qu'en absence de lumière, les souches testées répondaient à l'ajout de matière organique dissoute pour leur croissance (Tuchman et al., 2006), et que les souches les plus aptes à l'hétérotrophie provenaient de substrats vaseux riches en matière organique (Admiraal et al., 1979). Bien que de récentes études commencent à démontrer l'importance de ce phénomène, celles-ci se limitent encore au milieu intertidal (Jauffrais et al., 2015). Même si ce type de processus n'a pas été testé dans notre approche expérimentale, il est clair qu'à des intensités lumineuses si faibles et avec des taux de matière organique élevés, la mixotrophie est probable (Rigolet et al., 2014).

Rôle trophique du MPB subtidal

Portée des résultats

Les mesures effectuées durant cette thèse (chapitres 3 et 4) ont été réalisées en période de pré-éfflorescence phytoplanctonique. Elles n'ont pas permises d'observer toute la dynamique saisonnière du régime trophique de la crépidule et des autres suspensivores. Ci-dessous sont présentés des résultats préliminaires d'un suivi spatio-temporel effectué en 2016 dans la rade de Brest (Figure 49). Deux sites d'étude (Elorn/Lanvéoc, voir Figure 7) à deux dates contrastées en termes de production primaire (pré/post efflorescence phytoplanctonique printanière) ont été échantillonnées. L'objectif était de caractériser le régime trophique de la crépidule, du pétoncle noir (*C. varia*) et d'un crustacé cirripède (*A. modestus*), en lien avec les

sources de matière organique (matière organique particulaire (MOP) de la colonne d'eau et biofilm de substrats artificiels). Les sources et les consommateurs ont été analysés selon le protocole décrit au chapitre 4.

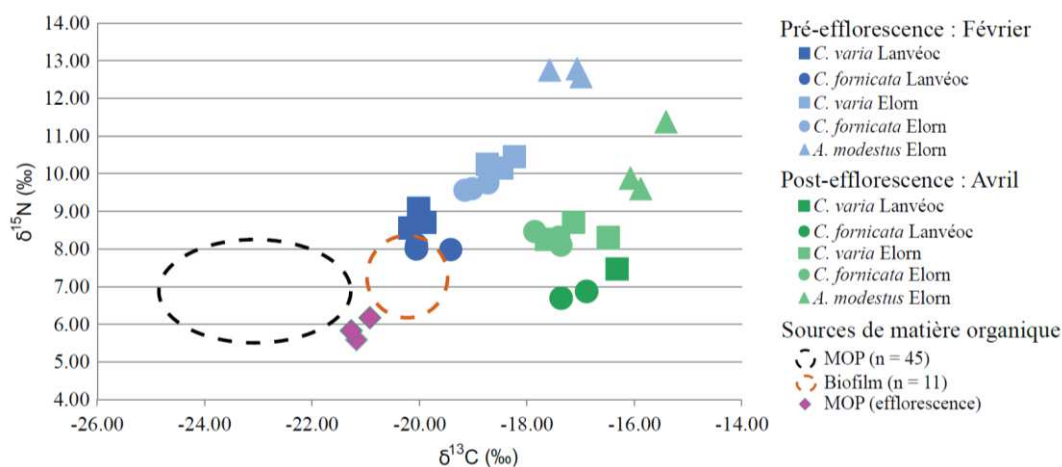


Figure 49 : Valeurs de $\delta^{13}\text{C}$ et $\delta^{15}\text{N}$ obtenues pour trois suspensives à deux saisons (pré/post-éfflorescence) et pour deux sites (Elorn/Lanvéoc). Deux sources de matière organique sont représentées d'après leur surface d'ellipse (Jackson et al., 2011). La matière organique particulaire (MOP) prélevée lors de l'efflorescence (Mars) est également représentée.

Ces résultats montrent que les sources de matière organique sont bien différenciées. La MOP est appauvrie en ^{13}C et ses valeurs de $\delta^{13}\text{C}$ ne recoupent pas celles du biofilm récolté sur les substrats artificiels, qui est par ailleurs dominé par des diatomées benthiques (Marchais, 2014 ; pers. obs.). La matière organique sédimentée n'a pas pu être mesurée pour des raisons techniques lors de l'échantillonnage (prélèvements à la benne). La MOP figurée en violet a été échantillonnée le jour où le maximum de chlorophylle *a* a été mesuré ($3,2 \mu\text{L}^{-1}$, SOMLIT, <http://somlit.epoc.u-bordeaux1.fr/>). L'enrichissement observé en $\delta^{13}\text{C}$ durant une efflorescence est classique et dû à la compétition des cellules phytoplanctoniques pour le carbone inorganique dissous (Rau et al., 1989 ; Gu et al., 2006). La différence de $\delta^{13}\text{C}$ entre le MPB subtidal et intertidal (Figure 6) peut également s'expliquer par leur source de carbone inorganique dissous. En effet, il est probable que la limitation en zone intertidale soit beaucoup plus importante car le MPB est régulièrement émergé et il n'y a alors pas de renouvellement par l'eau sus-jacente (Vieira et al., 2016). Ces résultats semblent en accord avec ceux de Marchais (2014) obtenus sur un biofilm sur le même type de plaques immergées dans la rade de Brest. En ce qui concerne les consommateurs, les valeurs isotopiques des crépidules et des pétoncles sont supérieures de 3-5 ‰ par rapport à celles de la MOP et de 1-2 ‰ par rapport à celle du MPB. Ces espèces sont donc

susceptibles d'assimiler une part non négligeable de MPB, comme démontré au chapitre 4. Les valeurs de $\delta^{15}\text{N}$ chez le crustacé cirripède sont plus élevées que celles des autres suspensivores, appuyant les résultats du chapitre 4. En avril, on observe un enrichissement de 2 à 1 ‰ en ^{13}C en fonction des espèces. Ce décalage semble suivre celui de la MOP après l'efflorescence phytoplanctonique qui a lieu 1 mois plus tôt. Ceci montre que le phytoplancton est bien assimilé par les consommateurs benthiques lorsque les biomasses sont fortes dans la colonne d'eau. Enfin, la comparaison Elorn – Lanvéoc laisse supposer que l'enrichissement est plus fort sur le site Lanvéoc suite à l'efflorescence. Bien que faible et avec un échantillonnage restreint, cette différence pourrait être la conséquence d'une contribution benthique moins importante sur ce site comparé au site Elorn.

“There is no harm in having too much” (Majdi et al., 2018)

Dans ce travail de thèse, il a été possible d'apporter plusieurs réponses grâce à la complémentarité des marqueurs utilisés (isotopes stables, acides gras, pigments). Dans une synthèse récente sur l'écologie trophique, Majdi et al. (2018) font le point sur les outils à la disposition des chercheurs dans cette discipline. Ils arrivent à la conclusion qu'en dépit du grand nombre d'outils (moléculaires, biochimiques, visuels, numériques, ...), l'utilisation conjointe de plusieurs d'entre eux reste assez rare, alors qu'elle permettrait une meilleure compréhension des systèmes étudiés. Ici, l'objectif a souvent été de mettre en évidence le régime trophique d'espèces microphages, la MOP étant la source de nourriture privilégiée des espèces suspensivores, comme la crépidule. Le fait qu'elle soit diffuse dans le milieu, microscopique, et composée d'organismes appartenant à des règnes et à des embranchements différents complique sérieusement sa caractérisation dans le régime alimentaire d'une espèce. Bien que nous ayons tenté au cours de cette thèse de coupler plusieurs outils, il est important d'en rappeler ici les principales limites et de réfléchir aux améliorations possibles.

Isotopes stables

Comme présenté succinctement dans l'encadré « méthode n°2 », le principe de l'utilisation des isotopes stables en écologie repose sur le fait qu'un fractionnement prévisible s'opère entre une source et son consommateur (DeNiro et Epstein, 1978 ; McCutchan et al., 2003). Ce caractère prévisible du fractionnement

est aussi un problème récurrent en écologie trophique (Martinez del Rio et al., 2009). A grande échelle, en comparant différents milieux (aquatique, terrestre, marin), les valeurs montrent une certaine constance chez les consommateurs primaires ($1,3 \pm 0,3 \text{ ‰}$ pour le $\delta^{13}\text{C}$ et $2,2 \pm 0,3 \text{ ‰}$ pour le $\delta^{15}\text{N}$, McCutchan et al., 2003). Cependant, des études plus spécifiques montrent aussi de fortes variations en fonction de l'espèce, de la source utilisée, du tissu analysé, de la croissance, ou encore de la maturation sexuelle (Yokohama et al., 2008 ; Blanchet-Aurigny et al., 2012b ; Lefebvre et Dubois, 2016). C'est probablement ces facteurs physiologiques qui expliquent en grande partie les valeurs isotopiques mesurées pour les différents stades ontogéniques de la crépidule dans le chapitre 3 (voir discussion). La spécificité des données expérimentales -et en particulier le fait que la nourriture fournie est souvent de haute qualité nutritive (ex : phytoplancton de culture) alors que dans le milieu naturel, les espèces tirent une part de leur énergie de matière moins labile- posent le problème de l'application directe des mesures de laboratoire au contexte du terrain. Une dizaine d'année après que Gannes et al., (1997) aient incité les chercheurs à plus d'expérimentations en laboratoire pour comprendre les mécanismes à l'œuvre derrière l'incorporation et le fractionnement isotopique, Martinez del Rio et al., (2009) constataient que seulement 7 % des études étaient réalisées en laboratoire par rapport aux applications terrain (Martinez del Rio et al., 2009). Ils notaient également que beaucoup restait à faire malgré les progrès accomplis, et indiquaient les voies à suivre suivantes : 1) une meilleure prise en compte de l'acheminement et du remodelage des molécules et des isotopes qu'elles contiennent ("*isotopic routing*"), 2) le développement de modèles à plusieurs compartiments (ex : tissus de structures vs. tissus de réserves) pour comprendre la dynamique de l'incorporation des isotopes, et 3) une meilleure compréhension du fractionnement isotopique, que ce soit pour l'estimation du niveau trophique ou l'utilisation des modèles de mélange.

Depuis, certains ont tenté de lier l'isotopie au métabolisme, couplant mesure de croissance et théorie métabolique (« *Dynamic Energy Budget theory* ») pour mieux comprendre les mécanismes qui déterminent le taux d'incorporation isotopique et le fractionnement dans les tissus (Emmery et al., 2011 ; Lefebvre et Dubois, 2016 ; Pecquerie et al., 2010). Malheureusement, ces résultats sont encore peu utilisés, d'abord parce que ces approches nécessitent des données supplémentaires liées à la croissance ou la physiologie des organismes, et ensuite

parce qu'elles font appel à des compétences numériques spécifiques. D'autres ont amélioré l'utilisation des modèles de mélanges en incorporant une variabilité supplémentaire dans la définition des sources potentielles, dans l'estimation du fractionnement, dans l'implémentation de nouveaux modèles statistiques de type bayésien, et en proposant des règles de bonnes conduites sur leur utilisation (Jackson et al., 2011 ; Phillips et al., 2014). Cependant, ces efforts ne règlent pas le problème de fond qu'est la caractérisation des sources pures et le fractionnement isotopique. D'autres enfin, bousculent les croyances et les dogmes établis. Ainsi, Nadon et al. (2006) montrent que des organismes, prélevés dans une zone côtière avec des valeurs de $\delta^{13}\text{C}$ élevées, et donc censés avoir assimilés une source enrichie en ^{13}C (source benthique) gardaient ce même enrichissement après avoir passé plusieurs mois dans la colonne d'eau, au large, sans aucune contribution benthique possible. Cette étude n'est pas isolée et d'autres sont résumées par Grippo et al. (2011). Souvent associées à des profondeurs où la production benthique est *a priori* négligeable, ces études observent un enrichissement en ^{13}C de 2-6 ‰ des invertébrés benthiques comparé à la MOP de la colonne d'eau (Kharlamenko et al., 2008 ; Le Loc'h et al., 2008 ; Sherwood et Rose, 2005). Une des raisons serait la dégradation du phytoplancton le long de la colonne d'eau ainsi que des processus de reminéralisation de la matière à l'interface eau-sédiment. Dans ce travail de thèse, nous avons quantifié la biomasse des producteurs primaires benthiques, et montré que celle-ci n'était pas négligeable et qu'elle contribuait au régime trophique de certaines espèces de suspensivores. De plus, la hauteur de la colonne d'eau étant très faible sur notre site d'étude (~10 m), l'hypothèse d'un enrichissement du phytoplancton pendant sa sédimentation semble très peu probable. Il est cependant très clair que notre connaissance des processus d'enrichissement de la matière organique et des consommateurs benthiques mérite plus d'investigations. Ceci met également en évidence le manque de références de sources pures sur le terrain en ce qui concerne la MOP pour confirmer certaines interprétations. Par exemple, les mesures de microphytobenthos intertidal s'étendent de -11 à -19 ‰ pour le $\delta^{13}\text{C}$ et de 4 à 9 ‰ pour le $\delta^{15}\text{N}$ sur seulement 6 études réalisées sur la façade Manche – Atlantique (Figure 6). Le niveau de « pureté » de l'échantillon de MPB, la condition physiologique du MPB et la valeur isotopique du carbone inorganique dissout ou même les différences interspécifiques qui pourraient exister à l'intérieur même du MPB sont probablement responsables de ces variations isotopiques. Les études qui

s'intéressent à caractériser finement la MOP sont rares (Lammers et al., 2017 ; Lienart et al., 2017 ; 2018 ; Savoye et al., 2003), et sont surtout minoritaires comparé aux études qui les utilisent. Bien que des patrons isotopiques puissent être observés à grandes échelles, la plupart des études ont lieu à l'échelle d'une baie ou d'un estuaire et sont donc soumises à ces variations. Dans ces conditions, il reste toujours délicat d'utiliser les valeurs de sources d'autres études pour par exemple implémenter un modèle de mélange.

Une alternative au problème de fractionnement est l'analyse isotopique de composés spécifiques (Larsen et al., 2009 ; Mac Mahon et al., 2013). Par exemple, certains acides aminés essentiels ne sont théoriquement pas sujets au fractionnement, et il est possible d'utiliser plusieurs d'entre eux conjointement pour renforcer la contribution d'une source trophique à un consommateur (analyse discriminante). Encore très peu utilisé en France, cet outil est pourtant utilisé en routine aux Etats-Unis depuis une petite dizaine d'année et semble prometteuse. Au cours de la thèse, nous avons pu profiter d'un développement méthodologique de la plateforme d'analyse isotopique et lipidique (Mathieu-Resuge, 2018) pour effectuer des tests d'analyses isotopiques de composés spécifiques, non pas sur des acides aminés mais sur des acides gras. Les données sont actuellement en cours d'analyse et n'ont pas été intégrées à ce manuscrit. Bien que certaines études aient démontré l'intérêt de cette approche pour caractériser par exemple des diatomées d'origines benthiques et pélagiques sur la base du $\delta^{13}\text{C}$ d'un AG biomarqueur de diatomées (Budge et al., 2008), il semble néanmoins que le fractionnement très variable des AG pose problème lors de l'interprétation.

Enfin, expliquer des processus aussi complexes que ceux à l'œuvre dans un réseau trophique en utilisant seulement une donnée bi-variée ($\delta^{13}\text{C}$ et $\delta^{15}\text{N}$) est une approche intégratrice certes, mais elle réduit parfois aussi les interprétations écologiques. En revanche, ce n'est pas le cas des acides gras qui montrent une forte diversité mais dont l'interprétation est parfois délicate.

Acides gras

L'utilisation des acides gras (AG) en écologie trophique repose sur deux constats. Le premier est que la synthèse des AG est limitée chez les consommateurs, surtout en ce qui concerne les AG dits essentiels (AG polyinsaturés à longue

chaîne). Le second est que certains d'entre eux peuvent être spécifiques d'un groupe d'organismes. Les limites de ces deux constats sont rappelées ici.

Parmi la grande diversité d'AG identifiables, certains sont ubiquistes et se retrouvent chez tous les êtres vivants, et d'autres sont plus spécifiques. Ces derniers sont donc utilisés comme biomarqueurs dans les consommateurs. Cependant, la spécificité d'un biomarqueur peut dépendre de la zone d'étude (tropicale, tempérée, polaire), de la présence ou non d'autres AG, des sources de matière organique disponibles dans le milieu (macroalgues, terrestres), etc. Dans le cas de la MOP, pour les mêmes raisons évoquées précédemment pour l'isotopie, il est difficile de la caractériser avec certitude sans coupler différents types de biomarqueurs. En effet, certains AG peuvent avoir plusieurs assignations. Par exemple, le 18:4n-3 est présenté comme un biomarqueur de prymnesiophytes (Zhukova et Aizdaicher, 1995), de dinoflagellés (Napolitano et al., 1997) mais également de macroalgues vertes (Fleurence et al., 1994) et brunes (Fleurence et al., 1994, Kelly et Schleibling, 2012). Le 18:1n-9 peut être considéré comme un biomarqueur de dinoflagellés (Napolitano et al., 1997), de copépodes ou plus vaguement de matériel animal (Dalsgaard et al., 2003). Le 18:1n-7, souvent présent chez les bactéries (Zhukova et al., 1992), est également très abondant chez certaines macroalgues vertes (Fleurence et al., 1994). Le 18:2n-6 et le 18:3n-3, quant à eux, sont des biomarqueurs de macroalgues vertes mais également de plantes vasculaires (Kelly et Schleibling, 2012). Le 16:1n-7, qui est un biomarqueur typique de diatomées (Dunstan et al., 1994 ; Napolitano et al., 1997) peut aussi être présent chez des bactéries (Haack et al., 1994). Le 22:6n-3 est caractéristique des dinoflagellés (Zhukova et Aizdaicher, 1995), mais aussi des cryptophytes (Parrish et al., 1995, Zhukova et Aizdaicher, 1995). Par conséquent, il paraît évident que dans les écosystèmes côtiers où toutes ces sources de matière organique sont susceptibles de se retrouver dans la MOP, il est important i) de multiplier les biomarqueurs (ex : isotopes, pigments, stéroïdes, etc.) pour identifier correctement ces sources et ii) de procéder à des vérifications de terrain telles que l'observation microscopique (Parrish et al., 1995 ; Napolitano et al., 1997, Lavaud et al., 2018) ou les analyses moléculaires (Majdi et al., 2018).

Imagerie hyper-spectrale

L'imagerie hyper-spectrale nous a permis de quantifier la biomasse de MPB dans l'espace et dans le temps, en condition expérimentale. Très peu utilisée en écologie trophique, cette technique mérite d'être explorée, par exemple pour étudier les relations trophiques entre gastéropodes brouteurs de biofilm et d'algues encroûtantes. Grâce à cet outil, il est possible de quantifier la biomasse et la surface broutée à une échelle submillimétrique, mais aussi de déterminer la nature de l'algue broutée (algue verte, brune ou rouge). Coupler ces données à des mesures de croissance des brouteurs, d'analyses biométriques de radula (type, taille) ou encore d'occupation de l'espace donnerait probablement de nouvelles informations sur les relations trophiques entre sources potentielles et brouteurs. A l'image des ingénieurs de l'écosystème qui stimulent le MPB, il a été démontré que le mucus laissé par les brouteurs stimulait le biofilm et les algues encroûtantes (Peduzzi et Herndl, 1991 ; Davies et Beckwith, 1999). Dans le cas de la patelle, qui montre un comportement singulier de « *homing* » (Cook et al., 1969), la spatialisation de la biomasse des producteurs primaires benthiques pourrait aider à la compréhension sur son utilisation de l'habitat et des ressources. Cette technique d'imagerie est non invasive et fournit de grandes quantités de données qui peuvent être exploitées avec un traitement automatisé, autorisant une grande réplication. Pour des raisons techniques et pratiques, son déploiement est plus aisé à l'échelle d'un mésocosme *ex situ*, mais des initiatives *in situ* commencent à apparaître (Chennu et al., 2013 ; 2017).

Quantification directe du MPB dans le régime trophique de la crépidule

Nous avons montré grâce aux isotopes stables et aux acides gras que le MPB participait au régime trophique de la crépidule ainsi qu'à plusieurs espèces de suspensivores partageant son habitat. En revanche, ces biomarqueurs trophiques représentent une preuve indirecte de l'assimilation du MPB. Un moyen efficace direct pour démontrer qualitativement qu'une source est assimilée par un consommateur est le marquage isotopique (Middleburg et al., 2000 ; Oakes et al., 2012 ; 2016). En effet, modifier le ratio isotopique d'une source par ajout d'un isotope lourd en surabondance dans le milieu permet de suivre son assimilation quelques heures après son ingestion (Hermann et al., 2000). Cependant, supposer qu'un filtreur puisse avoir accès à du MPB qui par définition vit à la surface du sédiment implique nécessairement une remise en suspension. Cela implique deux

choses : reproduire l'hydrodynamisme *in situ*, et reproduire un biofilm ayant les mêmes qualités cohésives. La remise en suspension étant intimement liée à la cohésion du sédiment, les deux paramètres doivent être réalistes. Widdows et al. (1998) ont mis au point un modèle réduit de canal hydrodynamique faisant varier la force du courant et utilisant des carottes de sédiment prélevées *in situ*, ce qui permet de ne pas déstructurer le biofilm. En utilisant un isotope marqué auquel le microphytobenthos aurait accès, il serait donc possible de vérifier notre hypothèse sur son assimilation par la crépidule. Un double marquage du MPB des crépidules et du MPB du sédiment permettrait de confirmer à la fois le broutage des mâles mobiles, et l'utilisation du MPB du sédiment remis en suspension par toutes les classes d'âges grâce à la filtration. Cette expérimentation a été envisagée mais n'a pas pu être réalisée faute de temps.

IV – Impact à long terme sur l'écosystème de la rade de Brest

La crépidule en voie de disparition ?

Le dernier suivi du réseau benthique (www.rebent.org, données non publiées) sectoriel effectué en 2013 a montré que la crépidule avait nettement régressé dans tout le bassin sud de la rade de Brest, de la baie de Roscanvel au chenal de l'Aulne. Bien que de nombreuses explications soient possibles (maladie, prédation, densité-dépendance, pollution), le fait que seul le bassin sud soit touché et que cet effet semble plus important dans l'embouchure de l'Aulne suggère un effet des apports de cette rivière. Pour expliquer certaines différences phénotypiques (taille, fécondité, indice de condition) des crépidules au sud-est de la rade, Richard (2005) évoquait des conditions du milieu plus contraignantes et voyaient ces différences phénotypiques comme une plasticité de cette espèce invasive pour coloniser tous types d'environnements. Néanmoins, on sait d'après Coum (1979) que l'installation des crépidules s'est faite initialement dans l'Aulne. Il semble donc peu probable que l'espèce ait choisi comme site d'implantation une zone où les conditions ne seraient pas des plus favorables. Pour vérifier cela, il serait intéressant de comparer la structure de taille des crépidules pour le secteur de l'Aulne au cours de sa colonisation grâce aux données recueillies successivement

par Coum (1979), Guérin (2004) et Richard (2005). Une autre hypothèse brièvement développée dans le travail de thèse de Richard (2005) est la possible contamination par des métaux lourds provenant d'anciennes mines de plomb argentifère présentes sur le bassin versant de l'Aulne. La difficulté pour établir un tel lien réside dans la grande spécificité de l'effet d'un métal sur un organisme. Par exemple, l'huître *C. gigas* et le pétoncle *M. varia* accumulent l'argent contrairement à la moule *M. edulis*, en revanche seul *M. varia* est négativement affecté (Meteyer et al., 1990). Il faut donc des études très spécifiques pour répondre à ce type d'interrogation. On peut néanmoins mentionner que l'argent est un élément connu pour avoir des effets négatifs sur la reproduction et la croissance de la crépidule (Calabrese, 1981 ; Nelson et al., 1983). Le plomb, quant à lui, est connu pour être toxique quelque soit la dose et affecte toutes les espèces (Zhang et al., 2010). Le saturnisme, effet bien connu de l'intoxication au plomb, résulte en une taille anormalement petite des organismes et à des troubles de la digestion. Ces effets peuvent corroborer le phénotype décrit par Richard (2005) sur les crépidules du sud-est de la rade, c'est-à-dire des individus de tailles réduites, des indices de conditions et une fécondité plus faible. De plus, le suivi annuel du ROCCH (Réseau d'Observation de la Contamination Chimique) montre une augmentation drastique de la teneur en plomb entre 2000 et 2014 dans les tissus des huîtres aux abords de l'Aulne, comparées aux huîtres de l'Elorn, ce qui montre bien une modification du milieu depuis une quinzaine d'année dans cette zone. Bien que la mine soit fermée depuis près de 100 ans, de fortes pluies conjuguées à des changements de pratiques du sol sur le bassin versant peuvent conduire à un relargage de métaux lourds et à un lessivage dans le bassin versant de l'Aulne. Egalement, des travaux sur cette rivière tels que des levées de barrages, dragages ou modifications d'écluses peuvent contribuer à remobiliser des polluants jusqu'alors séquestrés dans des sédiments anciens. Ménesguen et al. (2018a) ont montré à l'aide d'un modèle hydrodynamique, qu'un traceur tel qu'un polluant métallique provenant de l'Aulne auquel une mortalité sur la crépidule était appliquée conduisait à une distribution spatiale proche de celle actuellement observée dans la rade. Enfin, la sclérochronologie couplée à des analyses fines de métaux traces a permis de suivre l'incorporation de ces derniers, et notamment le plomb, dans des coquilles d'huître plates provenant du sud-est de la rade de Brest (Cariou et al., 2017). De surcroît, il semble que cette incorporation soit corrélée positivement avec le débit de l'Aulne,

ce qui suggère fortement que le plomb provienne de cette rivière et que de fortes crues augmentent sa concentration dans l'eau et les sédiments. En remontant le temps grâce aux archives sur les débits de l'Aulne, on pourrait espérer apporter des éléments de réponse sur la présence de plomb en plus ou moins grande concentration dans le milieu durant les années d'expansion de la crépidules. Des projets sont en cours dans la rade de Brest (par exemple MODELISM) pour mieux évaluer la biodisponibilité des métaux lourds dans les différents compartiments (faune benthique, eau, sédiment) ainsi que les potentielles conséquences pour l'écosystème de la rade de Brest.

L'envahisseur part, les coquilles restent

Le résultat du déclin massif de cette espèce ingénier entraîne le retour à un sédiment plus sableux et potentiellement moins riche en matière organique, mais qui garde une structure biogénique grâce aux amoncellements de coquilles mortes. A l'échelle du réseau trophique, son impact biologique (par exemple l'enrichissement en matière organique) semble avoir un effet uniquement lors d'une arrivée allochtone de matière organique (efflorescence de phytoplancton). Au contraire, la comparaison de la structure trophique d'un banc de maërl à celui d'un banc de crépidules mortes suggère que la diversité des niches trophiques sont comparables. Cette observation est d'autant plus intéressante que le maërl est considéré comme un habitat remarquable abritant une macrofaune benthique très diversifiée et un réseau trophique complexe (Grall 2002 ; Grall et al., 2006).

Une étude parallèle aux travaux présentés dans ce manuscrit a démontré qu'un banc mort de crépidules abritait une diversité spécifique significativement plus importante qu'un banc vivant (index de Shannon), et une forte abondance et dominance d'espèces opportunistes associée aux crépidules vivantes (index de Piélou). La richesse spécifique (nombre d'espèces) présentait des valeurs élevées (29-59 espèces pour 0,1 m⁻²), sans qu'aucune différence significative n'ait été observée entre les deux types de bancs. D'autres études ont également rapporté une augmentation de la richesse spécifique en présence de crépidules (mortes et/ou vivantes) comparé à un substrat nu (Barnes et al., 1973 ; de Montaudoin et Sauriau, 1999 ; REBENT sectoriel 2013, données non publiées ; Reynaud, 2013).

La structure fonctionnelle, analysée ici par le biais des traits biologiques de la faune associée (Figure 50) montre également des différences contrastées entre les effets physiques et biologiques du caractère ingénieur de la crépidule. Le long du gradient de quantité de crépidules (vivantes ou mortes), la plus forte équitabilité entre les fréquences des 4 groupes fonctionnels coïncide avec un niveau de complexité de l'habitat caractérisé par une grande abondance de coquilles mortes (effet physique) et une quasi-absence de crépidule vivante (effet biologique et enrichissement en MO en particulier). La diversité fonctionnelle semble donc maximisée sur un banc mort dense comparé à un banc vivant de crépidule. Ces résultats semblent confirmer nos résultats du chapitre 5 où l'enrichissement saisonnier en matière organique diminue la diversité trophique au sein du réseau trophique. Il semble donc que la crépidule, à l'échelle de la communauté, affecte à la fois la diversité structurelle et le fonctionnement trophique.

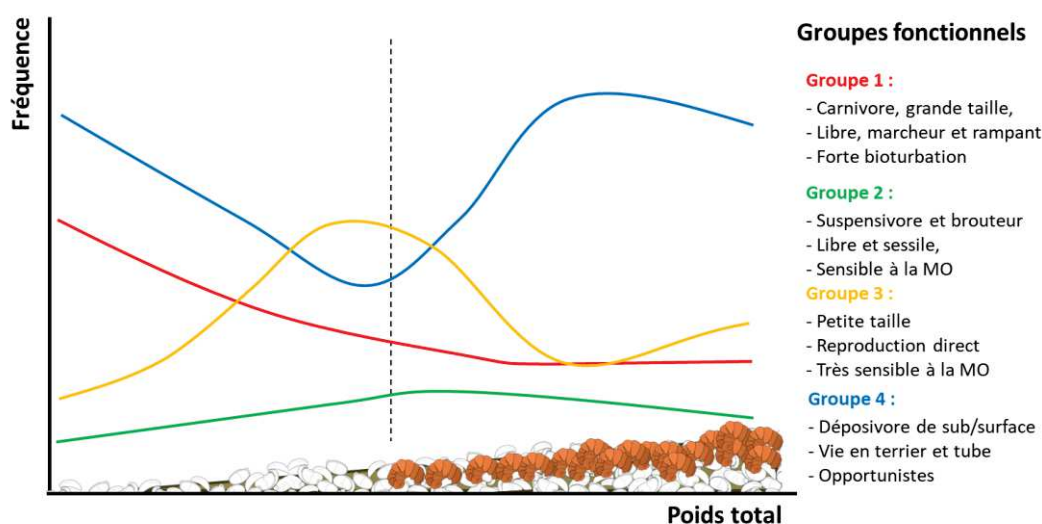


Figure 50 : Evolution des fréquences de 4 groupes fonctionnels en fonction du poids total de crépidules (mortes + vivantes), schématisée d'après les données de Vallée (2016). La part des crépidules vivantes est représentée de manière qualitative sur l'axe des abscisses. L'axe pointillé matérialise la séparation entre les échantillons ne contenant que des crépidules mortes (coquilles vides) et ceux contenant à la fois des crépidules vivantes et mortes. Le profil de traits biologiques des groupes fonctionnels est synthétisé dans la légende. MO : matière organique.

V – Espèces invasives et ingénieurs, pour ou contre ?

Une vision anthropocentrée de la nature

Les espèces invasives sont des moteurs de changement de diversité et de composition d'assemblages d'espèces (Bax et al., 2003 ; Molnar et al., 2008 ; Sax et al., 2007). Il est en pratique impossible d'éradiquer une espèce invasive installée,

surtout en milieu marin où la dispersion est facilitée par rapport au milieu terrestre. Néanmoins, il semble que l'impact négatif de ces espèces en milieu marin ne soit pas si évident (Thomsen et al., 2011). Les qualificatifs de « positif » ou « négatif » sont d'ailleurs assez souvent subjectifs car ils dépendent du point de vue de celui qui les exprime (scientifiques, gestionnaires, pêcheurs, ONG environnementale, etc). Dans une revue sur les impacts des espèces invasives en milieu marin en Europe, Katsanekavis et al. (2014) montrent que sur 87 espèces, 17 seulement ont un impact négatif avéré, 7 un impact seulement positif et 63 un double impact. Sur ces 87 espèces, 49 étaient des ingénieurs de l'écosystème. Les auteurs pointent le fait que ces espèces souffrent d'une mauvaise perception, au-delà du fait qu'elles pourraient avoir des effets positifs et fournir un moyen de compréhension supplémentaire d'un écosystème (Brown et Sax, 2004).

La facilitation comme aide à l'interprétation

A cause de cette perception négative des espèces invasives, certains concepts écologiques ont tardés à être intégrés dans les études d'« impact » de ces espèces, limitant notre compréhension des mécanismes qui lient une espèce invasive au fonctionnement de l'écosystème dans lequel elle se développe (Rodriguez, 2006). La facilitation prévoit que la présence d'une espèce peu améliorer l'existence d'une autre, sans qu'il n'y ait de bénéfice réciproque (Bertness et al., 1997 ; Bruno et al., 2003). Il s'applique particulièrement aux ingénieurs de l'écosystème présents dans des milieux contraignants d'un point de vue abiotique (ex : zone intertidale) ou biotique (ex : forte compétition) (Bertness et al., 1999 ; Jones, 2017 ; Wright et al., 2017 ; 2018). On peut définir trois processus par lesquels une espèce invasive peut faciliter une espèce native : facilitation nouvelle, de substitution, et indirect (Rodriguez, 2006). Dans le premier cas, l'espèce invasive fournit une ressource non présente par ailleurs pour une espèce native : c'est le cas par exemple de l'espèce invasive *Gracillaria vermiculophylla* qui va complexifier les vasières, permettre l'installation d'une épifaune non présente par ailleurs, et alimenter le réseaux trophique indirectement par la voie détritique (Davoult et al., 2017). Dans le second cas, l'espèce invasive remplace une espèce native mais joue le même rôle fonctionnel : c'est le cas de l'huître invasive *Magallena gigas* qui montre les mêmes densités et diversités d'organismes que

l'habitat fournit par l'espèce native *Ostrea edulis* (Zwerchke et al., 2018). Enfin dans un dernier cas, l'espèce invasive facilite une espèce native en modifiant une relation trophique. C'est le cas de la crépidule qui par exemple diminue la prédation des moules par les étoiles de mer (Thielges et al., 2006).

Une décision difficile

Le concept de facilitation appliqué aux espèces invasives et ingénieurs a des implications importantes en termes de gestion des écosystèmes, laquelle débouche parfois sur des tentatives d'éradication de ces espèces et de restauration des espèces natives (Rodriguez, 2006). Bien que le contexte environnemental et les différences fonctionnelles entre espèces invasives et espèces natives doivent être prises en compte, certaines de ces espèces peuvent avoir un rôle à jouer dans nos écosystèmes (Bouma et al., 2009 ; Padilla, 2010). Des questions relatives à la restauration d'écosystèmes dégradés et d'utilisation d'espèces ingénieur mais invasives comme facilitateur font actuellement débat au sein de la communauté scientifique. Par exemple le cas de l'algue rouge invasive *Gracillaria vermiculophylla*. Alors que certains auteurs montrent une amélioration de plusieurs fonction de l'écosystème par cette espèce (Ramus et al., 2017 ; Thomsen et al., 2018), certains mettent en garde contre la promotion de telle espèce (Byers et Sotka, 2018 ; Sotka et Byers, 2018).

Les mêmes questions se sont posées pour la crépidule. Au départ, cette espèce était uniquement vue comme un envahisseur à combattre, un compétiteur spatial et trophique pour les espèces suspensivores autochtones exploitées (à commencer par la coquille Saint-Jacques). L'espèce était impopulaire auprès d'une majorité d'acteurs du milieu marin et a fait l'objet de plusieurs tentatives d'éradication. Puis, face aux efflorescences de microalgues toxiques (des dinoflagellés en particulier) en rade de Brest, les scientifiques ont suggéré au contraire de maintenir cette espèce, en avançant que son rôle dans la pompe biologique à silice contrôlait ces efflorescences (Ragueneau et al., 2002). Aujourd'hui nous montrons que cet habitat semble propice à certaines espèces d'intérêt commercial comme le pétoncle et l'huître plate, qui font par ailleurs l'objet de tentatives de réimplantation dans la rade. Toute aussi importante, la communauté de macrofaune associée aux habitats à crépidule est diversifiée et supporte un réseau trophique complexe hors période d'efflorescence, où la production primaire benthique pourrait être stimulée. Enfin, la crépidule laisse

après sa mort un habitat coquillé complexe qui montre également une forte diversité structurelle et un fonctionnement trophique proche d'un habitat remarquable comme le maërl. Longtemps décrié, également mal comprise, la crépidule a-t-elle aujourd'hui sa place dans les habitats benthiques ? Une fois la dynamique de prolifération enraillée, ne constitue-t-elle pas un habitat de choix ?

**RÉFÉRENCES
BIBLIOGRAPHIQUES**

RÉFÉRENCES BIBLIOGRAPHIQUES

- Able, K. W. (2005). A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. *Estuarine, Coastal and Shelf Science* 64, 5–17.
- Admiraal, W., and Peletier, H. (1979). Influence of organic compounds and light limitation on the growth rate of estuarine benthic diatoms. *British Phycological Journal* 14, 197–206.
- Agogué, H., Mallet, C., Orvain, F., De Crignis, M., Mornet, F., and Dupuy, C. (2014). Bacterial dynamics in a microphytobenthic biofilm: A tidal mesocosm approach. *Journal of Sea Research* 92, 36–45.
- Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance: non-parametric MANOVA for ecology. *Austral Ecology* 26, 32–46.
- Arbach Leloup, F., Desroy, N., Le Mao, P., Pauly, D., and Le Pape, O. (2008). Interactions between a natural food web, shellfish farming and exotic species: The case of the Bay of Mont Saint Michel (France). *Estuarine, Coastal and Shelf Science* 76, 111–120.
- Arnott, D. L., and Vanni, M. J. (1996). Nitrogen and phosphorus recycling by the zebra mussel (*Dreissena polymorpha*) in the western basin of Lake Erie. 53, 14.
- Asmus, R. M., and Asmus, H. (1991). Mussel beds: limiting or promoting phytoplankton? *Journal of Experimental Marine Biology and Ecology* 148, 215–232.
- Azovsky, A. I., Chertoprood, E. S., Saburova, M. A., and Polikarpov, I. G. (2004). Spatio-temporal variability of micro- and meiobenthic communities in a White Sea intertidal sandflat. *Estuarine, Coastal and Shelf Science* 60, 663–671.
- Bachok, Z., Mfilinge, P. L., and Tsuchiya, M. (2003). The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. *Journal of Experimental Marine Biology and Ecology* 292, 187–197.
- Bajjouk, T., Guillaumont, B., Michez, N., Thouin, B., Croguennec, C., Populus, J., et al. (2015). Traduction française des habitats benthiques des régions Atlantique et Méditerranée. 231 p.
- Barbier, P., Meziane, T., Forêt, M., Tremblay, R., Robert, R., and Olivier, F. (2017). Nursery function of coastal temperate benthic habitats: New insight from the bivalve recruitment perspective. *Journal of Sea Research* 121, 11–23.
- Barillé, L., Cognie, B., Beninger, P., Decottignies, P., and Rincé, Y. (2006). Feeding responses of the gastropod *Crepidula fornicata* to changes in seston concentration. *Marine Ecology Progress Series* 322, 169–178.
- Barillé, L., Le Bris, A., Méléder, V., Launeau, P., Robin, M., Louvrou, I., et al. (2017). Photosynthetic epibionts and endobionts of Pacific oyster shells from oyster reefs in rocky versus mudflat shores. *PLoS ONE* 12, 1–22.

- Barillé, L., Méléder, V., Combe, J.-P., Launeau, P., Rincé, Y., Carrère, V., et al. (2007). Comparative analysis of field and laboratory spectral reflectances of benthic diatoms with a modified Gaussian model approach. *Journal of Experimental Marine Biology and Ecology* 343, 197–209.
- Barnathan, G. (2009). Non-methylene-interrupted fatty acids from marine invertebrates: occurrence, characterization and biological properties. *Biochimie* 91, 671–678.
- Barnes, R. S. K., Goughlan, J., and Holmes, N. J. (1973). A preliminary survey of the macroscopic bottom fauna of the Solent, with particular reference to *Crepidula fornicata* and *Ostrea edulis*. *Proceedings of the Malacological Society of London* 40, 253–275.
- Barnett, A., (2013). Régulation de l'activité photosynthétique du microphytobenthos et conséquence sur la dynamique temporelle de la production primaire dans les vasières intertidales de la côte atlantique de l'Europe de l'Ouest. Thèse de doctorat, Université de la Rochelle. 336 p.
- Barranguet, C., van Beusekom, S., Veuger, B., Neu, T., Manders, E., Sinke, J., et al. (2004). Studying undisturbed autotrophic biofilms: still a technical challenge. *Aquatic Microbial Ecology* 34, 1–9.
- Bax, N., Williamson, A., Agüero, M., Gonzalez, E., and Geeves, W. (2003). Marine invasive alien species: a threat to global biodiversity. *Marine Policy* 27, 313–323.
- Beck, M. W., Heck, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., et al. The role of nearshore ecosystems as fish and shellfish nurseries. *Issues in Ecology*, 1–12.
- Beekey, M. A., McCabe, D. J., and Marsden, J. E. (2004). Zebra mussels affect benthic predator foraging success and habitat choice on soft sediments. *Oecologia* 141, 164–170.
- Beninger, P., Decottignies, P., Guiheneuf, F., Barillé, L., and Rincé, Y. (2007). Comparison of particle processing by two introduced suspension feeders: selection in *Crepidula fornicata* and *Crassostrea gigas*. *Marine Ecology Progress Series* 334, 165–177.
- Beninger, P., Decottignies, P., and Rincé, Y. (2004). Localization of qualitative particle selection sites in the heterorhabdic filibranch *Pecten maximus* (Bivalvia: Pectinidae). *Marine Ecology Progress Series* 275, 163–173.
- Beninger, P. G., and Le Pennec, M. (2016). “Scallop, structure and function,” in *Developments in Aquaculture and Fisheries Science* (Elsevier), 85–159.
- Beninger, P. G., Valdizan, A., and Pennec, G. L. (2016). The seminal receptacle and implications for reproductive processes in the invasive gastropod *Crepidula fornicata*. *Zoology* 119, 4–10.
- Bernard, O., and Rémond, B. (2012). Validation of a simple model accounting for light and temperature effect on microalgal growth. *Bioresource Technology* 123, 520–527.

- Bertness, M. D., and Leonard, G. H. (1997). The role of positive interactions in communities: lessons from intertidal habitats. *Ecology* 78, 1976–1989.
- Bertness, M. D., Leonard, G. H., Levine, J. M., Schmidt, P. R., and Ingraham, A. O. (1999). Testing the relative contribution of positive and negative interactions in rocky intertidal communities. *Ecology* 80, 2711–2726.
- Beudin, A., (2014). Dynamique et échanges sédimentaires en rade de Brest impactés par l'invasion de crépidules. Thèse de doctorat, Université de Bretagne Occidentale, 224 p.
- Beudin, A., Chapalain, G., and Guillou, N. (2014). Modelling dynamics and exchanges of fine sediments in the bay of Brest. *La Houille Blanche* 6, 47–53.
- Biandolino, F., Prato, E., and Caroppo, C. (2008). Preliminary investigation on the phytoplankton contribution to the mussel diet on the basis of fatty acids analysis. *Journal of the Marine Biological Association of the UK* 88, 1009-1017.
- Blanchard, G. (1990). Overlapping microscale dispersion patterns of meiofauna and microphytobenthos. *Marine Ecology Progress Series* 68, 101–111.
- Blanchard, M. (1997). Spread of the slipper limpet *Crepidula fornicata* (L. 1758) in Europe. Current state and consequences. *Scientia Marina* 61, 109–118.
- Blanchard, M. (2009). Recent expansion of the slipper limpet population (*Crepidula fornicata*) in the Bay of Mont-Saint-Michel (Western Channel, France). *Aquatic Living Resources* 22, 11–19.
- Blanchard, M., Pechenik, J. A., Giudicelli, E., Connan, J.-P., and Robert, R. (2008). Competition for food in the larvae of two marine molluscs, *Crepidula fornicata* and *Crassostrea gigas*. *Aquatic Living Resources* 21, 197–205.
- Blanchet-Aurigny, A., Dubois, S., Hily, C., Rochette, S., Le Goaster, E., and Guillou, M. (2012a). Multi-decadal changes in two co-occurring ophiuroid populations. *Marine Ecology Progress Series* 460, 79–90.
- Blanchet-Aurigny, A., Dubois, S., Quéré, C., Guillou, M., and Pernet, F. (2015). Trophic niche of two co-occurring ophiuroid species in impacted coastal systems, derived from fatty acid and stable isotope analyses. *Marine Ecology Progress Series* 525, 127–141.
- Blanchet-Aurigny, A., Guillou, M., Pernet, F., Gaffet, J.-D., and Dubois, S. F. (2012b). Tissue-diet discrimination factors of isotopic ratios ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) in two brittle star species: Effect of reproductive state, diet and tissue composition. *Journal of Experimental Marine Biology and Ecology* 426, 68–77.
- Boecklen, W. J., Yarnes, C. T., Cook, B. A., and James, A. C. (2011). On the Use of Stable Isotopes in Trophic Ecology. *Annual Review of Ecology, Evolution, and Systematics* 42, 411–440.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Bohn, A. K. (2012). The distribution and potential northwards spread of the non-native gastropod *Crepidula fornicata* in welsh coastal waters. PhD thesis, Bangor University, 178p.
- Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., et al. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution* 26, 183–192.
- Bonaglia, S., Nascimento, F. J. A., Bartoli, M., Klawonn, I., and Brüchert, V. (2014). Meiofauna increases bacterial denitrification in marine sediments. *Nature Communications* 5:5133.
- Botts, P. S., Patterson, B. A., and Schloesser, D. W. (1996). Zebra mussel effects on benthic invertebrates: physical or biotic? *Journal of the North American Benthological Society* 15, 179–184.
- Bouma, T. J., De Vries, M. B., Low, E., Peralta, G., Tánzos, I. C., van de Koppel, J., et al. (2005). Trade-offs related to ecosystem engineering: a case study on stiffness of emerging macrophytes. *Ecology* 86, 2187–2199.
- Bouma, T. J., Olenin, S., Reise, K., and Ysebaert, T. (2009). Ecosystem engineering and biodiversity in coastal sediments: posing hypotheses. *Helgoland Marine Research* 63, 95–106.
- Braeckman, U., Provoost, P., Gribsholt, B., Van Gansbeke, D., Middelburg, J., Soetaert, K., et al. (2010). Role of macrofauna functional traits and density in biogeochemical fluxes and bioturbation. *Marine Ecology Progress Series* 399, 173–186.
- Brind'Amour, A., and Dubois, S. F. (2013). Isotopic diversity indices: how sensitive to food web structure? *PLoS ONE* 8, e84198.
- Brodie, C. R., Leng, M. J., Casford, J. S. L., Kendrick, C. P., Lloyd, J. M., Yongqiang, Z., et al. (2011). Evidence for bias in C and N concentrations and $\delta^{13}\text{C}$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods. *Chemical Geology* 282, 67–83.
- Broquet, T., Barranger, A., Billard, E., Bestin, A., Berger, R., Honnaert, G., et al. (2015). The size advantage model of sex allocation in the protandrous sex-changer *Crepidula fornicata*: role of the mating system, sperm storage, and male mobility. *The American Naturalist* 186, 404–420.
- Brotas, V., and Plante-Cuny, M.-R. (2003). The use of HPLC pigment analysis to study microphytobenthos communities. *Acta Oecologica* 24, S109–S115.
- Brown, J. H., and Sax, D. F. (2004). An assay on some topics concerning invasive species: an essay on invasive species. *Austral Ecology* 29, 530–536.
- Bruno, J. F., Stachowicz, J. J., and Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution* 18, 119–125.

- Budge, S. M., Parrish, C. C., and McKenzie, C. (2001). Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. *Marine Chemistry* 76, 285–303.
- Budge, S. M., and Parrish, C. C. (1998). Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. *Organic Geochemistry* 29, 1547–1559.
- Budge, S. M., Wooller, M. J., Springer, A. M., Iverson, S. J., McRoy, C. P., and Divoky, G. J. (2008). Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia* 157, 117–129.
- Burkholder, J. M., Glibert, P. M., and Skelton, H. M. (2008). Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* 8, 77–93.
- Burton, R. F. (1972). The storage of calcium and magnesium phosphates and of calcite in the digestive glands of the pulmonata (Gastropoda). *Comparative Biochemistry and Physiology Part A: Physiology* 43, 655–663.
- Byers, J. E., and Sotka, E. E. (2018). Promoting invasive species to enhance multifunctionality in a native ecosystem still requires strong(er) scrutiny. *Biological Invasions*. Letter to the editor.
- Byrnes, J. E., Reynolds, P. L., and Stachowicz, J. J. (2007). Invasions and Extinctions Reshape Coastal Marine Food Webs. *PLoS ONE* 2, e295.
- Cahill, A. E. (2015). Adult density affects larval recruitment in the calyptraeid gastropod *Crepidula fornicata*. *Journal of Experimental Marine Biology and Ecology* 465, 77–82.
- Cahoon, L. B. (1999). The role of benthic microalgae in neretic ecosystems. *Oceanography and Marine Biology: an Annual Review* 37, 47–86.
- Cahoon, L. B. (2017). “The Importance of Benthic Habitats for Coastal Fisheries” (Kritzer et al. 2016): Soft Bottoms Are Biologically Productive, Not “Abiotic.” Letter.
- Calabrese, A. (1981). “Ecological testing with marine molluscs,” in *Ecological testing for the marine environment* (Persoone, Jaspers and Claus), 455–477.
- Canuel, E. A. (2001). Relations between river flow, primary production and fatty acid composition of particulate organic matter in San Francisco and Chesapeake Bays: a multivariate approach. *Organic Geochemistry*, 21.
- Cariou, E., Guivel, C., La, C., Lenta, L., and Elliot, M. (2017). Lead accumulation in oyster shells, a potential tool for environmental monitoring. *Marine Pollution Bulletin* 125, 19–29.
- Carlier, A., Riera, P., Amouroux, J.-M., Bodiou, J.-Y., and Grémare, A. (2007). Benthic trophic network in the bay of Banyuls-sur-Mer (northwest Mediterranean, France): An assessment based on stable carbon and nitrogen isotopes analysis. *Estuarine, Coastal and Shelf Science* 72, 1–15.

- Carlsson, N. O. L., and Bronmark, C. (2006). Size-dependent effects of an invasive herbivorous snail (*Pomacea canaliculata*) on macrophytes and periphyton in Asian wetlands. *Freshwater Biology* 51, 695–704.
- Carrère, V., Spilmont, N., and Davoult, D. (2004). Comparison of simple techniques for estimating chlorophyll a concentration in the intertidal zone using high spectral-resolution field-spectrometer data. *Marine Ecology Progress Series* 274, 31–40.
- Cartaxana, P., Ruivo, M., Hubas, C., Davidson, I., Serôdio, J., and Jesus, B. (2011). Physiological versus behavioral photoprotection in intertidal epipelagic and epipsammic benthic diatom communities. *Journal of Experimental Marine Biology and Ecology* 405, 120–127.
- Chaalali, A., Brind'Amour, A., Dubois, S. F., and Le Bris, H. (2017). Functional roles of an engineer species for coastal benthic invertebrates and demersal fish. *Ecology and Evolution* 7, 5542–5559.
- Chaparro, O. R., Bahamondes-Rojas, I., Vergara, A. M., and Rivera, A. A. (1998). Histological characteristics of the foot and locomotory activity of *Crepidula dilatata* Lamarck (Gastropoda: Calyptraeidae) in relation to sex changes. *Journal of Experimental Marine Biology and Ecology* 223, 77–91.
- Chaparro, O., Thompson, R., and Pereda, S. (2002). Feeding mechanisms in the gastropod *Crepidula fecunda*. *Marine Ecology Progress Series* 234, 171–181.
- Chatterjee, A., (2014). Rôle des micro-algues benthiques dans la zone côtière : biomasse, biodiversité, productivité. Thèse de doctorat, Université de Bretagne Occidentale. 162 p.
- Chatterjee, A., Klein, C., Naegelen, A., Claquin, P., Masson, A., Legoff, M., et al. (2013). Comparative dynamics of pelagic and benthic micro-algae in a coastal ecosystem. *Estuarine, Coastal and Shelf Science* 133, 67–77.
- Chauvaud, L., (1998). La coquille Saint-Jacques en rade de Brest : un modèle biologique d'étude des réponses de la faune benthique aux fluctuations de l'environnement. Thèse de doctorat, Université de Bretagne Occidentale. 266 p.
- Chauvaud, L., Jean, F., Ragueneau, O., and Thouzeau, G. (2000). Long-term variation of the Bay of Brest ecosystem: benthic-pelagic coupling revisited. *Marine Ecology Progress Series* 200, 35–48.
- Chennu, A., Färber, P., De'ath, G., de Beer, D., and Fabricius, K. E. (2017). A diver-operated hyperspectral imaging and topographic surveying system for automated mapping of benthic habitats. *Scientific Reports* 7 :7122.
- Chennu, A., Färber, P., Volkenborn, N., Al-Najjar, M. A. A., Janssen, F., de Beer, D., et al. (2013). Hyperspectral imaging of the microscale distribution and dynamics of microphytobenthos in intertidal sediments. *Limnology and Oceanography: Methods* 11, 511–528.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Chennu, A., Volkenborn, N., De Beer, D., Wetthey, D. S., Woodin, S. A., and Polerecky, L. (2015). Effects of bioadvection by *Arenicola marina* on microphytobenthos in permeable sediments. *PLoS one* 10, 1–16.
- Chevenet, F., Doleadec, S., and Chessel, D. (1994). A fuzzy coding approach for the analysis of long-term ecological data. *Freshwater Biology* 31, 295–309.
- Cifuentes, L. A., Fogel, M. L., Pennock, J. R., and Sharp, J. H. (1989). Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. *Geochimica et Cosmochimica Acta* 53, 2713–2721.
- Claquin, P., Ní Longphuirt, S., Foullaron, P., Huonnic, P., Ragueneau, O., Klein, C., et al. (2010). Effects of simulated benthic fluxes on phytoplankton dynamic and photosynthetic parameters in a mesocosm experiment (Bay of Brest, France). *Estuarine, Coastal and Shelf Science* 86, 93–101.
- Cloern, J. (1982). Does the benthos control phytoplankton biomass in south San Francisco bay? *Marine Ecology Progress Series* 9, 191–202.
- Cognie, B., and Barille, L. (1999). Does bivalve mucus favour the growth of their main food source, microalgae? *Oceanologica Acta* 22, 441–450.
- Cognie, B., Barillé, L., Rincé, Y., Barille, L., and Rince, Y. (2001). Selective feeding of the oyster *Crassostrea gigas* fed on a natural microphytobenthos assemblage. *Estuaries* 24, 126.
- Cole, H. A. The american slipper limpet (*Crepidula fornicata*) on Cornish beds. *Fish. Invest. Ser II* 17, 1–13.
- Collin, R. (2006). Sex ratio, life-history invariants, and patterns of sex change in a family of protandrous gastropods. *Evolution* 60, 735–745.
- Coma, R., Ribes, M., Gili, J.-M., and Hughes, R. (2001). The ultimate opportunists: consumers of seston. *Marine Ecology Progress Series* 219, 305–308.
- Coma, R., Ribes, M., Gili, J.-M., and Zabala, M. (2000). Seasonality in coastal benthic ecosystems. *Trends in Ecology & Evolution* 15, 448–453.
- Combe, J., Launeau, P., Carrere, V., Despan, D., Meleder, V., Barille, L., et al. (2005). Mapping microphytobenthos biomass by non-linear inversion of visible-infrared hyperspectral images. *Remote Sensing of Environment* 98, 371–387.
- Connell, J. H. (1961). The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology* 42, 710–723.
- Cook, A., Bamford, O. S., Freeman, J. D. B., and Teideman, D. J. (1969). A study of the homing habit of the limpet. *Animal Behaviour* 17, 330–339.
- Cook, E. J., Shucksmith, R., Orr, H., Ashton, G. V., and Berge, J. (2010). Fatty acid composition as a dietary indicator of the invasive caprellid, *Caprella mutica* (Crustacea: Amphipoda). *Marine Biology* 157, 19–27.

- Coum, A., (1979). La population de crépidules *Crepidula fornicata* (L., 1758) en rade de Brest. Thèse de doctorat, Université de Bretagne Occidentale. 146p.
- Cranford, P. J., Armsworthy, S. L., Mikkelsen, O. A., and Milligan, T. G. (2005). Food acquisition responses of the suspension-feeding bivalve *Placopecten magellanicus* to the flocculation and settlement of a phytoplankton bloom. *Journal of Experimental Marine Biology and Ecology* 326, 128–143.
- Cresson, P., Ruitton, S., and Harmelin-Vivien, M. (2016). Feeding strategies of co-occurring suspension feeders in an oligotrophic environment. *Food Webs* 6, 19–28.
- Crisp, D. J., and Southward, A. J. (1961). Different types of cirral activity of barnacles. *Philosophical Transactions of The Royal British Society* 243, 271–307.
- Crooks, J. A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* 97, 153–166.
- Cucherousset, J., and Villéger, S. (2015). Quantifying the multiple facets of isotopic diversity: New metrics for stable isotope ecology. *Ecological Indicators* 56, 152–160.
- Cugier, P., Struski, C., Blanchard, M., Mazurié, J., Pouvreau, S., Olivier, F., et al. (2010). Assessing the role of benthic filter feeders on phytoplankton production in a shellfish farming site: Mont Saint Michel Bay, France. *Journal of Marine Systems* 82, 21–34.
- Dalsgaard, J., John, M., Kattner, G., Müller-Navarra, D., and Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* 46, 225–340.
- Dame, R. F., Bushek, D., and Prins, T. C. (2001). *Benthic suspension feeders as determinants of ecosystem structure and function in shallow coastal waters*. Springer. Berlin, Heidelberg. pp. 11-37.
- D’Andrea, A. F., and DeWitt, T. H. (2009). Geochemical ecosystem engineering by the mud shrimp *Upogebia pugettensis* (Crustacea: Thalassinidae) in Yaquina Bay, Oregon: Density-dependent effects on organic matter remineralization and nutrient cycling. *Limnology and Oceanography* 54, 1911–1932.
- Danovaro, R., Armeni, M., Dell’Anno, A., Fabiano, M., Manini, E., Marrale, D., et al. (2001). Small-scale distribution of bacteria, enzymatic activities, and organic matter in coastal sediments. *Microbial Ecology* 42, 177–185.
- Dauwe, B., Herman, P. M. J., and Heip, C. H. R. Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. *Marine Ecological Progress Series* 173, 67–83.
- Davies, M., and Beckwith, P. (1999). Role of mucus trails and trail-following in the behaviour and nutrition of the periwinkle *Littorina littorea*. *Marine Ecology Progress Series* 179, 247–257.

- Davoult, D., Surget, G., Stiger-Pouvreau, V., Noisette, F., Riera, P., Stagnol, D., et al. (2017). Multiple effects of a *Gracilaria vermiculophylla* invasion on estuarine mudflat functioning and diversity. *Marine Environmental Research* 131, 227–235.
- Dayton, P. K. (1971). Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecological Monographs* 41, 351–389.
- de Brouwer, J. F. C., and Stal, L. J. (2002). Daily fluctuations of exopolymers in cultures of the benthic diatoms *Cylindrotheca closterium* and *Nitzschia* sp. (Bacillariophyceae). *Journal of Phycology* 38, 464–472.
- de Montaudouin, X., Audemard, C., and Labourg, P.-J. (1999). Does the slipper limpet (*Crepidula fornicata*, L.) impair oyster growth and zoobenthos biodiversity? A revisited hypothesis. *Journal of Experimental Marine Biology and Ecology* 235, 105–124.
- de Montaudouin, X., Blanchet, H., and Hippert, B. (2017). Relationship between the invasive slipper limpet *Crepidula fornicata* and benthic megafauna structure and diversity, in Arcachon bay. *Journal of the Marine Biological Association of the United Kingdom*, 1–12.
- de Montaudouin, X., and Sauriau, P. G. (1999). The proliferating Gastropoda *Crepidula fornicata* may stimulate macrozoobenthic diversity. *Journal of the Marine Biological Association of the United Kingdom* 79, 1069–1077.
- Decottignies, P., Beninger, P. G., Rincé, Y., and Riera, P. (2007a). Trophic interactions between two introduced suspension-feeders, *Crepidula fornicata* and *Crassostrea gigas*, are influenced by seasonal effects and qualitative selection capacity. *Journal of Experimental Marine Biology and Ecology* 342, 231–241.
- Decottignies, P., Beninger, P. G., Rincé, Y., Robins, R. J., and Riera, P. (2007b). Exploitation of natural food sources by two sympatric, invasive suspension-feeders: *Crassostrea gigas* and *Crepidula fornicata*. *Marine Ecology Progress Series* 334, 179–192.
- Del Amo, Y., Le Pape, O., Tréguer, P., Quéguiner, B., Ménesguen, A., and Aminot, A. (1997). Impacts of high-nitrate freshwater inputs on macrotidal ecosystems. I. Seasonal evolution of nutrient limitation for the diatom-dominated phytoplankton of the Bay of Brest (France). *Marine Ecology Progress Series* 161, 213–224.
- Deming, J. W., and Baross, J. A. (1993). “The early diagenesis of organic matter: bacterial activity,” in *Organic Geochemistry*, 119–144.
- DeNiro, M. J., and Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42, 495–506.
- Deslous-Paoli, J.-M., and Héral, M. (1986). *Crepidula fornicata* L. (Gastéropode, Calyptraeidae) dans le bassin de Marennes-Oléron: composition et valeur énergétique des individus et des pontes. *Oceanologica Acta* 9, 305–311.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., et al. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology* 47, 15–25.
- Donadi, S., Westra, J., Weerman, E. J., van der Heide, T., van der Zee, E. M., van de Koppel, J., et al. (2013). Non-trophic Interactions Control Benthic Producers on Intertidal Flats. *Ecosystems* 16, 1325–1335.
- Dubois, S., Blanchet, H., Garcia, A., Massé, M., Galois, R., Grémare, A., et al. (2014). Trophic resource use by macrozoobenthic primary consumers within a semi-enclosed coastal ecosystem: stable isotope and fatty acid assessment. *Journal of Sea Research* 88, 87–99.
- Dubois, S. F., and Colombo, F. (2014). How picky can you be? Temporal variations in trophic niches of co-occurring suspension-feeding species. *Food Webs* 1, 1–9.
- Dubois, S., Orvain, F., Marin-Léal, J., Ropert, M., and Lefebvre, S. (2007). Small-scale spatial variability of food partitioning between cultivated oysters and associated suspension-feeding species, as revealed by stable isotopes. *Marine Ecology Progress Series* 336, 151–160.
- Duffy, J. E., Cardinale, B. J., France, K. E., McIntyre, P. B., Thébault, E., and Loreau, M. (2007). The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecology Letters* 10, 522–538.
- Dupont, L. (2004). Invasion des côtes françaises par le mollusque exotique *Crepidula fornicata*: contribution de la dispersion larvaire et du système de reproduction au succès de la colonisation.
- Dunstan, G. A., Volkman, J. K., Barrett, S. M., Leroi, J.-M., and Jeffrey, S. W. (1994). Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). *Phytochemistry* 35, 155–161.
- Dutertre, M., Hamon, D., Chevalier, C., and Ehrhold, A. (2013). The use of the relationships between environmental factors and benthic macrofaunal distribution in the establishment of a baseline for coastal management. *ICES Journal of Marine Science* 70, 294–308.
- Echappé, C., Gernez, P., Méléder, V., Jesus, B., Cognie, B., Decottignies, P., et al. (2018). Satellite remote sensing reveals a positive impact of living oyster reefs on microalgal biofilm development. *Biogeosciences Discussions*, 1–30.
- Eckman, J. E. (1985). Flow disruption by an animal-tube mimic affects sediment bacterial colonization. *Journal of Marine Research* 43, 419–435.
- Efron, B., and Tibshirani, R. (1993). *An introduction to the bootstrap*. Chapman & Hall, Inc. 257 p.
- Ehrenfeld, J. G. (2010). Ecosystem Consequences of Biological Invasions. *Annual Review of Ecology, Evolution, and Systematics* 41, 59–80.

- Ehrhold, A., Blanchard, M., Auffret, J.-P., and Garlan, T. (1998). Conséquences de la prolifération de la crépidule (*Crepidula fornicata*) sur l'évolution sédimentaire de la baie du Mont-Saint-Michel (Manche, France). *Comptes Rendus de l'Académie des Sciences de Paris, Science de la Terre et des Planètes* 327, 583–588.
- Emmery, A., Lefebvre, S., Alunno-Bruscia, M., and Kooijman, S. A. L. M. (2011). Understanding the dynamics of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in soft tissues of the bivalve *Crassostrea gigas* facing environmental fluctuations in the context of Dynamic Energy Budgets (DEB). *Journal of Sea Research* 66, 361–371.
- Engel, F. G., Alegria, J., Andriana, R., Donadi, S., Gusmao, J. B., van Leeuwe, M. A., et al. (2017). Mussel beds are biological power stations on intertidal flats. *Estuarine, Coastal and Shelf Science* 191, 21–27.
- Escofier, B., and Pagès, J. (1994). Multiple factor analysis (AFMULT package). *Computational Statistics & Data Analysis* 18, 121–140.
- Evrard, V., Huettel, M., Cook, P., Soetaert, K., Heip, C., and Middelburg, J. (2012). Importance of phytodetritus and microphytobenthos for heterotrophs in a shallow subtidal sandy sediment. *Marine Ecology Progress Series* 455, 13–31.
- Evrard, V., Soetaert, K., Heip, C., Huettel, M., Xenopoulos, M., and Middelburg, J. (2010). Carbon and nitrogen flows through the benthic food web of a photic subtidal sandy sediment. *Marine Ecology Progress Series* 416, 1–16.
- Eyster, L. S., and Pechenik, J. A. (1988). Comparison of growth, respiration and feeding of juvenile *Crepidula fornicata* (L.) following natural or KCl-triggered metamorphosis. *Journal of Experimental Marine Biology and Ecology* 118, 269–279.
- Fitzgerald, D. B., Winemiller, K. O., Sabaj Pérez, M. H., and Sousa, L. M. (2017). Using trophic structure to reveal patterns of trait-based community assembly across niche dimensions. *Functional Ecology* 31, 1135–1144.
- Fleurence, J., Gutbier, G., Mabeau, S., and Leray, C. (1994). Fatty acids from 11 marine macroalgae of the French Brittany coast. *Journal of Applied Phycology* 6, 527–532.
- Forster, R. M., and Jesus, B. (2006). Field spectroscopy of estuarine intertidal habitats. *International Journal of Remote Sensing* 27, 3657–3669.
- Foullaron, P., Claquin, P., L'Helguen, S., Huonnic, P., Martin-Jézéquel, V., Masson, A., et al. (2007). Response of a phytoplankton community to increased nutrient inputs: a mesocosm experiment in the Bay of Brest (France). *Journal of Experimental Marine Biology and Ecology* 351, 188–198.
- Fournié, J., and Chétail, M. (1984). Calcium Dynamics in Land Gastropods. *American Zoologist* 24, 857–870.
- France, R. (1995). Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 52, 651–656.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Friedrichs, M., and Graf, G. (2009). Characteristic flow patterns generated by macrozoobenthic structures. *Journal of Marine Systems* 75, 348–359.
- Frolund, B., Palmgren, R., Keiding, K., and Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research* 30, 1749–1758.
- Fry, B. (2006). *Stable isotope ecology*. New York, NY: Springer. 308 p.
- Fry, B., and Sherr, E. B. (1984). $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contribution in Marine Science* 27, 13–47.
- Gallardo, B., Clavero, M., Sánchez, M. I., and Vilà, M. (2015). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology* 22, 151–163.
- Gannes, L. Z., O'Brien, D. M., and Martinez del Rio, C. (1997). Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78, 1271–1276.
- Gardner, W., Cavaletto, J., Johengen, T., Johnson, J., Heath, R., and Cotner JB (1995). Effects of the zebra mussels, *Dreissena polymorpha*, on community nitrogen dynamics in Saginaw Bay, Lake Huron. *J. Great Lakes Res.* 21, 529–544.
- Gieskes, W. W. C. (1991). “Algal pigment fingerprints: clue to taxon-specific abundance, productivity and degradation of phytoplankton in seas and oceans,” in *Particle Analysis in Oceanography*, ed. S. Demers (Berlin, Heidelberg: Springer Berlin Heidelberg), 61–99.
- Gili, J.-M., and Coma, R. (1998). Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends in Ecology & Evolution* 13, 316–321.
- Glud, R. N., Kühl, M., Wenzhöfer, F., and Rysgaard, S. (2002). Benthic diatoms of a high Arctic fjord (Young Sound, NE Greenland): importance for ecosystem primary production. *Marine Ecology Progress Series* 238, 15–29.
- Gouletquer, P., Bachelet, G., Sauriau, P. G., and Noel, P. (2002). “Open Atlantic Coast of Europe — A Century of Introduced Species into French Waters,” in *Invasive Aquatic Species of Europe. Distribution, Impacts and Management*, eds. E. Leppäkoski, S. Gollasch, and S. Olenin (Dordrecht: Springer Netherlands), 276–290.
- Grall, J., (2002). Biodiversité spécifique et fonctionnelle du maërl : réponses à la variabilité de l’environnement côtier. Thèse de doctorat, Université de Bretagne Occidentale.
- Grall, J., Le Loc’h, F., Guyonnet, B., and Riera, P. (2006). Community structure and food web based on stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) analysis of a North Eastern Atlantic maërl bed. *Journal of Experimental Marine Biology and Ecology* 338, 1–15.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Grenon, J.-F., and Walker, G. (1978). The histology and histochemistry of the pedal glandular system of two limpets, *Patella vulgata* and *Acmaea tessulata* (Gastropoda: Prosobranchia). *Journal of the Marine Biological Association of the United Kingdom* 58, 803–816.
- Grime, J. P. (1998). Benefits of plants diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology* 86, 902–910.
- Grippo, M. A., Fleeger, J. W., Dubois, S. F., and Condrey, R. (2011). Spatial variation in basal resources supporting benthic food webs revealed for the inner continental shelf. *Limnology and Oceanography* 56, 841–856.
- Gu, B., Chapman, A. D., and Schelske, C. L. (2006). Factors controlling seasonal variations in stable isotope composition of particulate organic matter in a softwater eutrophic lake. *Limnology and Oceanography* 51, 2837–2848.
- Guerin, A. J., Jensen, A. C., and McGill, R. A. R. (2013). Effects of distilled water rinsing on stable isotope ratios of acid-treated marine invertebrate (Paguridae) samples: Distilled water rinsing of acid-treated invertebrate samples. *Rapid Communications in Mass Spectrometry* 27, 2051–2056.
- Guérin, L. (2004). La crépidule en rade de Brest: un modèle biologique d'espèce introduite proliférante en réponse aux fluctuations de l'environnement.
- Gutiérrez, J. L., Jones, C. G., Strayer, D. L., and Iribarne, O. O. (2003). Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos* 101, 79–90.
- Guzzo, M. M., Haffner, G. D., Legler, N. D., Rush, S. A., and Fisk, A. T. (2013). Fifty years later: trophic ecology and niche overlap of a native and non-indigenous fish species in the western basin of Lake Erie. *Biological Invasions* 15, 1695–1711.
- Haack, S. K., Garchow, H., Odelson, D. A., Forney, L. J., and Klug, M. J. (1994). Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. *Applied and Environmental Microbiology* 60, 2483–2493.
- Halpern, B. S., Silliman, B. R., Olden, J. D., Bruno, J. P., and Bertness, M. D. (2007). Incorporating positive interactions in aquatic restoration and conservation. *Frontiers in Ecology and the Environment* 5, 153–160.
- Hastings, A., Byers, J. E., Crooks, J. A., Cuddington, K., Jones, C. G., Lambrinos, J. G., et al. (2007). Ecosystem engineering in space and time. *Ecology Letters* 10, 153–164.
- Henry, J. Q., and Lyons, D. C. (2016). Molluscan models: *Crepidula fornicata*. *Current Opinion in Genetics & Development* 39, 138–148.
- Hentschel, B. T. (1998). Intraspecific variations in $\delta^{13}\text{C}$ indicate ontogenic diet changes in deposit-feeding polychaetes. *Ecology* 79, 1357–1370.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Hentschel, B. T., and Jumars, P. A. (1994). In situ chemical inhibition of benthic diatom growth affects recruitment of competing, permanent and temporary meiofauna. *Limnology and Oceanography* 39, 816–838.
- Herman, P., Middelburg, J., Widdows, J., Lucas, C., and Heip, C. (2000). Stable isotopes as trophic tracers: combining field sampling and manipulative labelling of food resources for macrobenthos. *Marine Ecology Progress Series* 204, 79–92.
- Hernández Fariñas, T., Ribeiro, L., Soudant, D., Belin, C., Bacher, C., Lampert, L., et al. (2017). Contribution of benthic microalgae to the temporal variation in phytoplankton assemblages in a macrotidal system. *Journal of Phycology* 53, 1020–1034.
- Hinzmann, M. F., Lopes-Lima, M., Bobos, I., Ferreira, J., Domingues, B., and Machado, J. (2015). Morphological and chemical characterization of mineral concretions in the freshwater bivalve *Anodonta cygnea* (Unionidae). *Journal of Morphology* 276, 65–76.
- Hoagland, K. E. (1975). Reproductive strategies and evolution in the genus *Crepidula* (Gastropoda: Calyptraeidae).
- Hoagland, K. E. (1978). Protandry and the evolution of environmentally-mediated sex change: a study of the mollusca. *Malacologia* 17, 365–391.
- Hoeinghaus, D. J., and Zeug, S. C. (2008). Can stable isotope ratios provide for community-wide measures of trophic structure? comment. *Ecology* 89, 2353–2357.
- Hubas, C., Sachidhanandam, C., Rybarczyk, H., Lubarsky, H., Rigaux, A., Moens, T., et al. (2010). Bacterivorous nematodes stimulate microbial growth and exopolymer production in marine sediment microcosms. *Marine Ecology Progress Series* 419, 85–94.
- Hunt, S. (1973). Fine structure of the secretory epithelium in the hypobranchial gland of the prosobranch gastropod mollusc *Buccinum undatum* L. *Journal of the Marine Biological Association of the United Kingdom* 53, 59–71.
- Hylleberg, J. (1975). Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and a concept of gardening in lugworms. *Ophelia* 14, 113–137.
- Istin, M., and Girard, J. P. (1970). Carbonic anhydrase and mobilisation of calcium reserves in the mantle of lamellibranchs. *Calcified Tissue Research* 5, 247–260.
- Jackson, A. L., Inger, R., Parnell, A. C., and Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R: Bayesian isotopic niche metrics. *Journal of Animal Ecology* 80, 595–602.
- Jacob, U., Mintenbeck, K., Brey, T., Knust, R., and Beyer, K. (2005). Stable isotope food web studies: a case for standardized sample treatment. *Marine Ecology Progress Series* 287, 251–253.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Jaschinski, S., Brepohl, D., and Sommer, U. (2011). Seasonal variation in carbon sources of mesograzers and small predators in an eelgrass community: stable isotope and fatty acid analyses. *Marine Ecology Progress Series* 431, 69–82.
- Jaschinski, S., Hansen, T., and Sommer, U. (2008). Effects of acidification in multiple stable isotope analyses: Acidification in stable isotope samples. *Limnology and Oceanography: Methods* 6, 12–15.
- Jauffrais, T., Drouet, S., Turpin, V., Méléder, V., Jesus, B., Cognie, B., et al. (2015). Growth and biochemical composition of a microphytobenthic diatom (*Entomoneis paludosa*) exposed to shorebird (*Calidris alpina*) droppings. *Journal of Experimental Marine Biology and Ecology* 469, 83–92.
- Jesus, B., Brotas, V., Marani, M., and Paterson, D. M. (2005). Spatial dynamics of microphytobenthos determined by PAM fluorescence. *Estuarine, Coastal and Shelf Science* 65, 30–42.
- Jesus, B., Mouget, J.-L., and Perkins, R. G. (2008). Detection of diatoms xanthophyll cycle using spectral reflectance. *Journal of Phycology* 44, 1349–1359.
- Jesus, B., Rosa, P., Mouget, J.-L., Méléder, V., Launeau, P., and Barillé, L. (2014). Spectral-radiometric analysis of taxonomically mixed microphytobenthic biofilms. *Remote Sensing of Environment* 140, 196–205.
- Jones, A., (2017). Effect of an engineer species on the diversity and functioning of benthic communities: the *Sabellaria Alveolata* reef habitat. Thèse de doctorat, Université de Bretagne Occidentale. 277 p.
- Jones, C. G., Gutiérrez, J. L., Byers, J. E., Crooks, J. A., Lambrinos, J. G., and Talley, T. S. (2010). A framework for understanding physical ecosystem engineering by organisms. *Oikos* 119, 1862–1869.
- Jones, C. G., Lawton, J. H., and Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos* 69, 373.
- Jones, C. G., Lawton, J. H., and Shachak, M. (1997). Positive and negative effects of organisms as physical ecosystem engineers. *Ecology* 78, 1946–1957.
- Jørgensen, C. B. (1996). Bivalve filter feeding revisited. *Marine Ecology Progress Series* 142, 287–302.
- Jumars, P. A., Dorgan, K. M., and Lindsay, S. M. (2015). Diet of worms emended: An update of polychaete feeding guilds. *Annual Review of Marine Science* 7, 497–520.
- Juneau, P., Barnett, A., Méléder, V., Dupuy, C., and Lavaud, J. (2015). Combined effect of high light and high salinity on the regulation of photosynthesis in three diatom species belonging to the main growth forms of intertidal flat inhabiting microphytobenthos. *Journal of Experimental Marine Biology and Ecology* 463, 95–104.

- Kapur, S. P., and Gibson, M. A. (1968). A histochemical study of calcium storage in the foot of the freshwater gastropod, *Helisoma duryi eudiscus* (Pilsbry). *Canadian Journal of Zoology* 46, 987–990.
- Katsanevakis, S., Wallentinus, I., Zenetos, A., Leppäkoski, E., Çinar, M. E., Oztürk, B., et al. (2014). Impacts of invasive alien marine species on ecosystem services and biodiversity: a pan-European review. *Aquatic Invasions* 9, 391–423.
- Ke, Z., Tan, Y., Huang, L., Zhao, C., and Jiang, X. (2017). Spatial distributions of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and CN ratios in suspended particulate organic matter of a bay under serious anthropogenic influences: Daya Bay, China. *Marine Pollution Bulletin* 114, 183–191.
- Kelly, J., and Scheibling, R. (2012). Fatty acids as dietary tracers in benthic food webs. *Marine Ecology Progress Series* 446, 1–22.
- Kennedy, P., Kennedy, H., and Papadimitriou, S. (2005). The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. *Rapid Communications in Mass Spectrometry* 19, 1063–1068.
- Keough, M. J. (1984). Effects of patch size on the abundance of sessile marine invertebrates. *Ecology* 65, 423–437.
- Khalil, K., Raimonet, M., Laverman, A. M., Yan, C., Andrieux-Loyer, F., Viollier, E., et al. (2013). Spatial and temporal variability of sediment organic matter recycling in two temperate eutrophicated estuaries. *Aquatic Geochemistry* 19, 517–542.
- Kharlamenko, V. I., Kiyashko, S. I., Rodkina, S. A., and Imbs, A. B. (2008). Determination of food sources of marine invertebrates from a subtidal sand community using analyses of fatty acids and stable isotopes. *Russian Journal of Marine Biology* 34, 101–109.
- Kombiadou, K., Ganthly, F., Verney, R., Plus, M., and Sottolichio, A. (2014). Modelling the effects of *Zostera noltei* meadows on sediment dynamics: application to the Arcachon lagoon. *Ocean Dynamics* 64, 1499–1516.
- Kopp, D., Lefebvre, S., Cachera, M., Villanueva, M. C., and Ernande, B. (2015). Reorganization of a marine trophic network along an inshore–offshore gradient due to stronger pelagic–benthic coupling in coastal areas. *Progress in Oceanography* 130, 157–171.
- Kristensen, E. (2000). Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. *Life at Interfaces and Under Extreme Conditions*, 1–24.
- Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C., and Banta, G. (2012). What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Marine Ecology Progress Series* 446, 285–302.

- Kritzer, J. P., DeLucia, M.-B., Greene, E., Shumway, C., Topolski, M. F., Thomas-Blate, J., et al. (2016). The importance of benthic habitats for coastal fisheries. *BioScience* 66, 274–284.
- Kühl, M., Lassen, C., and Jorgensen, B. (1994). Light penetration and light intensity in sandy marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes. *Marine Ecology Progress Series* 105, 139–148.
- Lacoste, É., Raimbault, P., Harmelin-Vivien, M., and Gaertner-Mazouni, N. (2016). Trophic relationships between the farmed pearl oyster *Pinctada margaritifera* and its epibionts revealed by stable isotopes and feeding experiments. *Aquaculture Environment Interactions* 8, 55–66.
- Lammers, J. M., Reichart, G. J., and Middelburg, J. J. (2017). Seasonal variability in phytoplankton stable carbon isotope ratios and bacterial carbon sources in a shallow Dutch lake: *Seasonality in phytoplankton and bacterial $\delta^{13}\text{C}$* . *Limnology and Oceanography* 62, 2773–2787.
- Larsen, T., Taylor, D. L., Leigh, M. B., and O’Brien, D. M. (2009). Stable isotope fingerprinting: a novel method for identifying plant, fungal, or bacterial origins of amino acids. *Ecology* 90, 3526–3535.
- Laruelle, G., Regnier, P., Ragueneau, O., Kempa, M., Moriceau, B., Ní Longphuirt, S., et al. (2009). Benthic–pelagic coupling and the seasonal silica cycle in the Bay of Brest (France): new insights from a coupled physical–biological model. *Marine Ecology Progress Series* 385, 15–32.
- Lavaud, R., Artigaud, S., Le Grand, F., Donval, A., Soudant, P., Flye-Sainte-Marie, J., et al. (2018). New insights into the seasonal feeding ecology of *Pecten maximus* using pigments, fatty acids and sterols analyses. *Marine Ecology Progress Series* 590, 109–129.
- Lavergne, C., Beaugeard, L., Dupuy, C., Courties, C., and Agogué, H. (2014). An efficient and rapid method for the enumeration of heterotrophic prokaryotes in coastal sediments by flow cytometry. *Journal of Microbiological Methods* 105, 31–38.
- Laviale, M., Ezequiel, J., Pais, C., Cartaxana, P., and Serôdio, J. (2015). The “crème brûlée” sampler: A new high-resolution method for the fast vertical sampling of intertidal fine sediments. *Journal of Experimental Marine Biology and Ecology* 468, 37–44.
- Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology* 16, 545–556.
- Layman, C. A., Arrington, D. A., Montaña, C. G., and Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88, 42–48.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., et al. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews* 87, 545–562.
- Le Cam, S., (2009). Grégarité, changement de sexe et polyandrie : modalité de la reproduction chez une espèce invasive *Crepidula fornicata*. Thèse de doctorat, Université Pierre et Marie Curie. 271 p.
- Le Gall, P., (1980). Etude expérimentale de l'association en chaîne et de son influence sur la croissance et la sexualité chez la crépidule *Crepidula fornicata* Linné 1958 (mollusque mésogastéropode). Thèse de doctorat, Université de Caen-Basse Normandie.
- Le Loc'h, F., Hily, C., and Grall, J. (2008). Benthic community and food web structure on the continental shelf of the Bay of Biscay (North Eastern Atlantic) revealed by stable isotopes analysis. *Journal of Marine Systems* 72, 17–34.
- Le Grand, F., Soudant, P., Siah, A., Tremblay, R., Marty, Y., and Kraffe, E. (2014). Disseminated neoplasia in the soft-shell clam *Mya arenaria*: membrane lipid composition and functional parameters of circulating cells. *Lipids* 49, 807–818.
- Le Pape, O., Del Amo, Y., Ménesguen, A., Aminot, A., Quequiner, B., and Treguer, P. (1996). Resistance of a coastal ecosystem to increasing eutrophic conditions: the Bay of Brest (France), a semi-enclosed zone of Western Europe. *Continental Shelf Research* 16, 1885–1907.
- Le Pape, O., Guérault, D., and Désaunay, Y. (2004). Effect of an invasive mollusc, American slipper limpet *Crepidula fornicata*, on habitat suitability for juvenile common sole *Solea solea* in the Bay of Biscay. *Marine Ecology Progress Series* 277, 107–115.
- Lefebvre, S., and Dubois, S. F. (2016). The stony road to understand isotopic enrichment and turnover rates: insight into the metabolic part. *Vie et Milieu - Life and Environment* 66, 305–314.
- Lefebvre, S., Marín Leal, J. C., Dubois, S., Orvain, F., Blin, J.-L., Bataillé, M.-P., et al. (2009). Seasonal dynamics of trophic relationships among co-occurring suspension-feeders in two shellfish culture dominated ecosystems. *Estuarine, Coastal and Shelf Science* 82, 415–425.
- Lehmann, M. F., Bernasconi, S. M., Barbieri, A., and McKenzie, J. A. (2002). Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. *Geochimica et Cosmochimica Acta* 66, 3573–3584.
- Lejart, M., Clavier, J., Chauvaud, L., and Hily, C. (2012). Respiration and calcification of *Crassostrea gigas*: contribution of an intertidal invasive species to coastal ecosystem CO₂ fluxes. *Estuaries and Coasts* 35, 622–632.

- Lejart, M., and Hily, C. (2011). Differential response of benthic macrofauna to the formation of novel oyster reefs (*Crassostrea gigas*, Thunberg) on soft and rocky substrate in the intertidal of the Bay of Brest, France. *Journal of Sea Research* 65, 84–93.
- Leroy, F., Meziane, T., Riera, P., and Comtet, T. (2013). Seasonal variations in maternal provisioning of *Crepidula fornicata* (Gastropoda): fatty acid composition of females, embryos and larvae. *PLoS ONE* 8, e75316.
- Lesser, M. P., Shumway, S. E., Cucci, T., and Smith, J. (1992). Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. *Journal of Experimental Marine Biology and Ecology* 165, 91–102.
- Lewin, J., and Hellebust, J. A. (1976). Heterotrophic nutrition of the marine pennate diatom *Nitzschia angularis* var. *affinis*. *Marine Biology* 36, 313–320.
- Lewin, J., and Hellebust, J. A. (1978). Utilization of glutamate and glucose for heterotrophic growth by the marine pennate diatom *Nitzschia laevis*. *Marine Biology* 47, 1–7.
- Liénart, C., Savoye, N., Bozec, Y., Breton, E., Conan, P., David, V., et al. (2017). Dynamics of particulate organic matter composition in coastal systems: a spatio-temporal study at multi-systems scale. *Progress in Oceanography* 156, 221–239.
- Liénart, C., Savoye, N., David, V., Ramond, P., Rodriguez Tress, P., Hanquiez, V., et al. (2018). Dynamics of particulate organic matter composition in coastal systems: Forcing of spatio-temporal variability at multi-systems scale. *Progress in Oceanography* 162, 271–289.
- Liénart, C., Susperregui, N., Rouaud, V., Cavalheiro, J., David, V., Del Amo, Y., et al. (2016). Dynamics of particulate organic matter in a coastal system characterized by the occurrence of marine mucilage – A stable isotope study. *Journal of Sea Research* 116, 12–22.
- Little, C. (1965). Osmotic and ionic regulation in the prosobranch gastropod mollusc, *Viviparus viviparus* Linn. *Journal of Experimental Biology* 43, 23–37.
- Little, C., Williams, G. A., and Trowbridge, C. D. (2009). *The biology of rocky shores*. Oxford University Press. 352p.
- Lohrer, A. M., Thrush, S. F., and Gibbs, M. M. (2004). Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature* 431, 1092–1095.
- Lohse, L., Kloosterhuis, R. T., de Stigter, H. C., Helder, W., van Raaphorst, W., and van Weering, T. C. (2000). Carbonate removal by acidification causes loss of nitrogenous compounds in continental margin sediments. *Marine Chemistry* 69, 193–201.
- Loomis, S. H., and van Nieuwenhuyze, W. (1985). Sediment correlates to density of *Crepidula fornicata* Linnaeus in the Pantanguanset river, Connecticut. *Veliger* 27, 266–272.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Lorrain, A., Savoye, N., Chauvaud, L., Paulet, Y.-M., and Naulet, N. (2003). Decarbonation and preservation method for the analysis of organic C and N contents and stable isotope ratios of low-carbonated suspended particulate material. *Analytica Chimica Acta* 491, 125–133.
- MacIntyre, H. L., Geider, R. J., and Miller, D. (1996). Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow water marine habitats. I. Distribution, abundance and primary production. *Estuaries* 19, 186–201.
- Madsen, K. N., Nilsson, P., and Sundbäck, K. (1993). The influence of benthic microalgae on the stability of a subtidal sediment. *Journal of experimental marine biology and ecology* 170, 159–177.
- Majdi, N., Hette-Tronquart, N., Auclair, E., Bec, A., Chouvelon, T., Cognie, B., et al. (2018). There’s no harm in having too much: a comprehensive toolbox of methods in trophic ecology. *Food Webs* 17, e00100.
- Manac’h, N. (1995). La biodéposition de la crépidule (*Crepidula fornicata*). Impact sur l’écosystème de la rade de Brest. Rapport de stage. 54 p.
- Mangiafico, S. S. (2015). *An R Companion for the Handbook of Biological Statistics*. Rutgers Cooperative Extension, New Brunswick, NJ.
- Marchais, V., (2014). Relations trophiques entre producteurs primaires et 4 consommateurs primaires benthiques dans un écosystème côtier tempéré. Thèse de doctorat, Université de Bretagne Occidentale. 289 p.
- Marchais, V., Schaal, G., Grall, J., Lorrain, A., Nerot, C., Richard, P., et al. (2013). Spatial variability of stable isotope ratios in oysters (*Crassostrea gigas*) and primary producers along an estuarine gradient (Bay of Brest, France). *Estuaries and Coasts* 36, 808–819.
- Markert, A., Wehrmann, A., and Kröncke, I. (2010). Recently established *Crassostrea*-reefs versus native *Mytilus*-beds: differences in ecosystem engineering affects the macrofaunal communities (Wadden Sea of Lower Saxony, southern German Bight). *Biological Invasions* 12, 15–32.
- Marques da Silva, J., Cruz, S., and Cartaxana, P. (2017). Inorganic carbon availability in benthic diatom communities: photosynthesis and migration. *Philosophical Transactions of The Royal British Society* 372.
- Martinez del Rio, C., Wolf, N., Carleton, S. A., and Gannes, L. Z. (2009). Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84, 91–111.
- Martin, S., (2005). Rôle d’une algue exploitée, le maërl, et d’une espèce invasive, la crépidule, sur les flux à l’interface eau-sédiment en rade de Brest. Thèse de doctorat, Université de Bretagne Occidentale.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Martin, S., Thouzeau, G., Chauvaud, L., Jean, F., Guérin, L., and Clavier, J. (2006). Respiration, calcification, and excretion of the invasive slipper limpet, *Crepidula fornicata* L.: Implications for carbon, carbonate, and nitrogen fluxes in affected areas. *Limnology and Oceanography* 51, 1996–2007.
- Martin, S., Thouzeau, G., Richard, M., Chauvaud, L., Jean, F., and Clavier, J. (2007). Benthic community respiration in areas impacted by the invasive mollusk *Crepidula fornicata*. *Marine Ecology Progress Series* 347, 51–60.
- Martinez Arbizu, P. (2017). pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.0.1.
- Mason, A. Z., and Nott, J. A. (1981). The role of intracellular biomineralized granules in the regulation and detoxification of metal in marine gastropods with special reference to the marine prosobranch *Littorina littorea*. *Aquatic Toxicology* 1, 239–256.
- Mateo, M. A., Serrano, O., Serrano, L., and Michener, R. H. (2008). Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. *Oecologia* 157, 105–115.
- Mathieu-Resuge, M., (2018). Ecophysiologie trophique des bivalves *Nodipecten subnodosus* et *Spondylus crassisquama* soumis à la variabilité environnementale dans la lagune d'Ojo de Liebre (Basse Californie, Mexique). Thèse de doctorat, Université de Bretagne Occidentale. 195 p.
- McCarthy, J. J., Taylor, W. R., and Taft, J. L. (1977). Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. *Limnology and oceanography* 22, 996–1011.
- McCutchan, J. H., Lewis, W. M., Kendall, C., and McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102, 378–390.
- McGee, D., Laws, R., and Cahoon, L. (2008). Live benthic diatoms from the upper continental slope: extending the limits of marine primary production. *Marine Ecology Progress Series* 356, 103–112.
- McMahon, K. W., Hamady, L. L., and Thorrold, S. R. (2013). A review of ecogeochemistry approaches to estimating movements of marine animals. *Limnology and Oceanography* 58, 697–714.
- Méléder, V., (2003). Étude de la structure des peuplements intertidaux du microphytobenthos : apport de la télédétection visible - infrarouge. Thèse de doctorat, Université de Nantes.
- Méléder, V., Launeau, P., Barillé, L., and Rincé, Y. (2003). Cartographie des peuplements du microphytobenthos par télédétection spatiale visible-infrarouge dans un écosystème conchylicole. *Comptes Rendus Biologies* 326, 377–389.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Ménesguen, A., Desmit, X., Dulière, V., Lacroix, G., Thouvenin, B., Thieu, V., et al. (2018b). How to avoid eutrophication in coastal seas? A new approach to derive river-specific combined nitrate and phosphate maximum concentrations. *Science of The Total Environment* 628–629, 400–414.
- Ménesguen, A., and Grégoris, T. (2018a). Modelling benthic invasion by the colonial gastropod *Crepidula fornicata* and its competition with the bivalve *Pecten maximus*. 1. A new 0D model for population dynamics of colony-forming species. *Ecological Modelling* 368, 277–287.
- Menge, B. A. (1976). Organization of the New England rocky Intertidal community: role of predation, competition, and environmental heterogeneity. *Ecological Monographs* 46, 355–393.
- Mermillod-Blondin, F., and Rosenberg, R. (2006). Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. *Aquatic Sciences* 68, 434–442.
- Metayer, C., Amiard-Triquet, C., and Baud, J. P. (1990). Variation inter-spécifiques de la bioaccumulation et de la toxicité de l'argent à l'égard de trois mollusques bivalves marins. *Water Research* 24, 995–1001.
- Meziane, T., Bodineau, L., Retiere, C., and Thoumelin, G. (1997). The use of lipid markers to define sources of organic matter in sediment and food web of the intertidal salt-marsh-flat ecosystem of Mont-Saint-Michel Bay, France. *Journal of Sea Research* 38, 47–58.
- Middelburg, J. J., Barranguet, C., Boschker, H. T. S., Herman, P. M. J., Moens, T., and Heip, C. H. R. (2000). The fate of intertidal microphytobenthos carbon: An in situ ¹³C-labeling study. *Limnology and Oceanography* 45, 1224–1234.
- Miller, D. C., Geii, R., and Macintyre, H. L. (1996). Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. II. Role in sediment stability and shallow-water food webs. *Estuaries* 19, 202–212.
- Miller, W., and DuBAR, J. R. (1988). Community replacement of a Pleistocene *Crepidula* biostrome. *Lethaia* 21, 67–78.
- Minagawa, M., and Wada, E. (1984). Stepwise enrichment of $\delta^{15}\text{N}$ along food web chains, further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 38, 1135–1140.
- Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K., and Relyea, R. A. (2005). Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution* 20, 685–692.
- Møhlenberg, F., and Riisgård, H. U. (1978). Efficiency of particle retention in 13 species of suspension feeding bivalves. *Ophelia* 17, 239–246.
- Molnar, J. L., Gamboa, R. L., Revenga, C., and Spalding, M. D. (2008). Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment* 6, 485–492.

- Montiel, Y. A., Chaparro, O. R., and Segura, C. J. (2005). Changes in feeding mechanisms during early ontogeny in juveniles of *Crepidula fecunda* (Gastropoda, Calyptraeidae). *Marine Biology* 147, 1333–1342.
- Moran, A. L. (1999). Size and performance of juvenile marine invertebrates: potential contrasts between intertidal and subtidal benthic habitats. *American Zoologist* 39, 304–312.
- Mortillaro, J. M., Schaal, G., Grall, J., Nerot, C., Brind'Amour, A., Marchais, V., et al. (2014). Comparative study of isotopic trends in two coastal ecosystems of North Biscay: A multitrophic spatial gradient approach. *Estuarine, Coastal and Shelf Science* 136, 149–156.
- Moulin, F. Y., Guizien, K., Thouzeau, G., Chapalain, G., Mülleners, K., and Bourg, C. (2007). Impact of an invasive species, *Crepidula fornicata*, on the hydrodynamics and transport properties of the benthic boundary layer. *Aquatic Living Resources* 20, 15–31.
- Muñoz, R. C., and Warner, R. R. (2003). A new Version of the size-advantage hypothesis for sex change: incorporating sperm competition and size-fecundity skew. *The American Naturalist* 161, 749–761.
- Na, T., Gribsholt, B., Galaktionov, O. S., Lee, T., and Meysman, F. J. (2008). Influence of advective bio-irrigation on carbon and nitrogen cycling in sandy sediments. *Journal of Marine Research* 66, 691–722.
- Nadon, M.-O., and Himmelman, J. H. (2006). Stable isotopes in subtidal food webs: Have enriched carbon ratios in benthic consumers been misinterpreted? *Limnology and Oceanography* 51, 2828–2836.
- Napolitano, G. E., Ackman, R. G., and Silva-Serra, M. A. (1993). Incorporation of dietary sterols by the sea scallop *Placopecten magellanicus* (Gmelin) fed on microalgae. *Marine Biology* 117, 647–654.
- Napolitano, G. E., Pollero, R. J., Gayoso, A. M., Macdonald, B. A., and Thompson, R. J. (1997). Fatty acids as trophic markers of phytoplankton blooms in the Bahía Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). *Biochemical Systematics and Ecology* 25, 739–755.
- Navarro, J. M., and Chaparro, O. R. (2002). Grazing–filtration as feeding mechanisms in motile specimens of *Crepidula fecunda* (Gastropoda: Calyptraeidae). *Journal of Experimental Marine Biology and Ecology* 270, 111–122.
- Nelson, A., Calabrese, A., Greigl, R. A., Yevich, P. P., and Chang, S. (1983). Long-term silver effects on the marine gastropod *Crepidula fornicata*. *Mar Ecol. Prog. Ser.*, 11.
- Nerot, C., (2011). Invertébrés benthiques et biomarqueurs: témoins du fonctionnement trophique de l'écosystème côtier. Thèse de doctorat, Université de Bretagne Occidentale. 169 p.

- Nerot, C., Meziane, T., Schaal, G., Grall, J., Lorrain, A., Paulet, Y.-M., et al. (2015). Spatial changes in fatty acids signatures of the great scallop *Pecten maximus* across the Bay of Biscay continental shelf. *Continental Shelf Research* 109, 1–9.
- Newell, R., Fisher, T., Holyoke, R., and Cornwell, J. (2005). “Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake bay, USA,” in *The comparative roles of suspension-feeders in ecosystems* Earth and Environmental Sciences. (Netherland: Richard F. Dame and Sergei Olenin), 93–120.
- Newell, R. I. E. (2004). Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *Journal of Shellfish Research* 23, 51–61.
- Newell, R. I. E., Cornwell, J. C., and Owens, M. S. (2002). Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: A laboratory study. *Limnology and Oceanography* 47, 1367–1379.
- Newsome, S. D., Martinez del Rio, C., Bearhop, S., and Phillips, D. L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment* 5, 429–436.
- Ní Longphuirt, S. (2006). Rôle du microphytobenthos dans le cycle du silicium et le fonctionnement d’un écosystème côtier : La rade de Brest.
- Ní Longphuirt, S., Clavier, J., Grall, J., Chauvaud, L., Le Loc’h, F., Le Berre, I., et al. (2007). Primary production and spatial distribution of subtidal microphytobenthos in a temperate coastal system, the Bay of Brest, France. *Estuarine, Coastal and Shelf Science* 74, 367–380.
- Ní Longphuirt, S., Leynaert, A., Guarini, J.-M., Chauvaud, L., Claquin, P., Herlory, O., et al. (2006). Discovery of microphytobenthos migration in the subtidal zone. *Marine Ecology Progress Series* 328, 143–154.
- Noisette, F., (2013). Impacts de l’acidification des océans sur les organismes benthiques calcifiants des milieux côtiers tempérés. Thèse de doctorat, Université Pierre et Marie Curie. 302 p.
- Nordström, M. C., and Bonsdorff, E. (2017). Organic enrichment simplifies marine benthic food web structure: Organic enrichment simplifies food webs. *Limnology and Oceanography* 62, 2179–2188.
- Nott, J. A., and Nicolaidou, A. (1989). The cytology of heavy metal accumulations in the digestive glands of three marine gastropods. *Proceedings of the Royal Society of London* 237, 347–362.
- Oakes, J. M., Eyre, B. D., and Middelburg, J. J. (2012). Transformation and fate of microphytobenthos carbon in subtropical shallow subtidal sands: A ¹³C-labeling study. *Limnology and Oceanography* 57, 1846–1856.
- Oakes, J. M., Rysgaard, S., Glud, R. N., and Eyre, B. D. (2016). The transformation and fate of sub-Arctic microphytobenthos carbon revealed through ¹³C-labeling: MPB carbon fate in the sub-Arctic. *Limnology and Oceanography* 61, 2296–2308.

- Olson, M. H. (1996). Ontogenetic niche shifts in largemouth bass: variability and consequences for first-year growth. *Ecology* 77, 179–190.
- Orton, J. H. (1912). The mode of feeding of *Crepidula*, with an account of the current-producing mechanism in the mantle cavity, and some remarks on the mode of feeding in Gastropods and Lamellibranchs. *Journal of the Marine Biological Association of the United Kingdom* 9, 444–478.
- Orvain, F., Galois, R., Barnard, C., Sylvestre, A., Blanchard, G., and Sauriau, P.-G. (2003). Carbohydrate production in relation to microphytobenthic biofilm development: an integrated approach in a tidal mesocosm. *Microbial Ecology* 45, 237–251.
- Ozersky, T., Evans, D. O., and Barton, D. R. (2012). Invasive mussels alter the littoral food web of a large lake: stable isotopes reveal drastic shifts in sources and flow of energy. *PLoS ONE* 7, 1–11.
- Padilla, D. K. (2010). Context-dependent Impacts of a non-native ecosystem engineer, the Pacific Oyster *Crassostrea gigas*. *Integrative and Comparative Biology* 50, 213–225.
- Padilla, D. K., McCann, M. J., and Shumway, S. E. (2011). “Marine invaders and bivalve aquaculture: sources, impacts, and consequences,” in *Shellfish Aquaculture and the Environment*, ed. S. E. Shumway (Oxford, UK: Wiley-Blackwell), 395–424.
- Paine, R. T. (1969). A note on trophic complexity and community stability. *The American Naturalist* 103, 91–93.
- Parnell, A. C., Phillips, D. L., Bearhop, S., Semmens, B. X., Ward, E. J., Moore, J. W., et al. (2013). Bayesian stable isotope mixing models: BAYESIAN STABLE ISOTOPE MIXING MODELS. *Environmetrics* 24, 387–399.
- Parrish, C., McKenzie, C., MacDonald, B., and Hatfield, E. (1995). Seasonal studies of seston lipids in relation to microplankton species composition and scallop growth in South Broad Cove, Newfoundland. *Marine Ecology Progress Series* 129, 151–164.
- Passarelli, C., Hubas, C., Nicolas Segui, A., Grange, J., and Meziane, T. (2012a). Surface adhesion of microphytobenthic biofilms is enhanced under *Hediste diversicolor* (O.F. Müller) trophic pressure. *Journal of Experimental Marine Biology and Ecology* 438, 52–60.
- Paterson, D. M., Wiltshire, K. H., Miles, A., Blackburn, J., Davidson, I., Yates, M. G., et al. (1998). Microbiological mediation of spectral reflectance from intertidal cohesive sediments. *Limnology and Oceanography* 43, 1207–1221.
- Pechenik, J. A., Diederich, C. M., Chaparro, O. R., Cubillos, V. M., and Mardones-Toledo, D. A. (2015). Role of the Substrate in Feeding and Growth of the Marine Suspension-Feeding Gastropods *Crepidula fornicata* and *Crepidatella peruviana*. *The Biological Bulletin* 229, 289–298.

- Pechenik, J. A., and Gee, C. C. (1993). Onset of metamorphic competence in larvae of the gastropod *Crepidula fornicata* (L.), judged by a natural and an artificial cue. *Journal of Experimental Marine Biology and Ecology* 167, 59–72.
- Pecquerie, L., Nisbet, R. M., Fablet, R., Lorrain, A., and Kooijman, S. A. L. M. (2010). The impact of metabolism on stable isotope dynamics: a theoretical framework. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365, 3455–3468.
- Peduzzi, P., and Herndl, G. (1991). Mucus trails in the rocky intertidal. A highly active microenvironment. *Marine Ecology Progress Series* 75, 267–274.
- Perkins, R. G., Williamson, C. J., Brodie, J., Barillé, L., Launeau, P., Lavaud, J., et al. (2016). Microspatial variability in community structure and photophysiology of calcified macroalgal microbiomes revealed by coupling of hyperspectral and high-resolution fluorescence imaging. *Scientific Reports* 6:22343.
- Perkins, R., Underwood, G., Brotas, V., Snow, G., Jesus, B., and Ribeiro, L. (2001). Responses of microphytobenthos to light: primary production and carbohydrate allocation over an emersion period. *Marine Ecology Progress Series* 223, 101–112.
- Pernet, F., Bricelj, V. M., and Parrish, C. C. (2005). Effect of varying dietary levels of $\omega 6$ polyunsaturated fatty acids during the early ontogeny of the sea scallop, *Placopecten magellanicus*. *Journal of Experimental Marine Biology and Ecology* 327, 115–133.
- Pernet, F., Malet, N., Pastoureaud, A., Vaquer, A., Quéré, C., and Dubroca, L. (2012). Marine diatoms sustain growth of bivalves in a Mediterranean lagoon. *Journal of Sea Research* 68, 20–32.
- Pernet, F., and Tremblay, R. (2004). Effect of varying levels of dietary essential fatty acid during early ontogeny of the sea scallop *Placopecten magellanicus*. *Journal of Experimental Marine Biology and Ecology* 310, 73–86.
- Perry, G. J., Volkman, J. K., Johns, R. B., and Bavor, H. J. (1979). Fatty acids of bacterial origin in contemporary marine sediments. *Geochimica et Cosmochimica Acta* 43, 1715–1725.
- Petchey, O. L., and Gaston, K. J. (2002). Functional diversity (FD), species richness and community composition. *Ecology Letters* 5, 402–411.
- Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., et al. (2014). Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* 92, 823–835.
- Pinheiro, J. C., and Bates, D. M. (2010). *Mixed-effects models in S and S-PLUS*. Springer.
- Polerecky, L., Bissett, A., Al-Najjar, M., Faerber, P., Osmers, H., Suci, P. A., et al. (2009a). Modular spectral imaging system for discrimination of pigments in cells and microbial communities. *Applied and Environmental Microbiology* 75, 758–771.

- Polerecky, L., Klatt, J. M., Al-Najjar, M., and de Beer, D. (2009b). Hyper-spectral imaging of biofilm growth dynamics. in *Hyperspectral Image and Signal Processing: Evolution in Remote Sensing, 2009. WHISPERS'09. First Workshop on* (IEEE), 1–4.
- Pommier, J., Frenette, J., and Glémet, H. (2010). Relating RNA:DNA ratio in *Eurytemora affinis* to seston fatty acids in a highly dynamic environment. *Marine Ecology Progress Series* 400, 143–154.
- Porter, E., Cornwell, J., and Sanford, L. (2004). Effect of oysters *Crassostrea virginica* and bottom shear velocity on benthic-pelagic coupling and estuarine water quality. *Marine Ecology Progress Series* 271, 61–75.
- Porter, E., Mason, R., and Sanford, L. (2013). Effects of shear stress and hard clams on seston, microphytobenthos, and nitrogen dynamics in mesocosms with tidal resuspension. *Marine Ecology Progress Series* 479, 25–46.
- Post, A. F., Dubinsky, Z., Wyman, K., and Falkowski, P. G. (1984). Kinetics of light-intensity adaptation in a marine planktonic diatom. *Marine Biology* 83, 231–238.
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703–718.
- Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., and Montaña, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152, 179–189.
- Powell-Jennings, C., and Callaway, R. (2018). The invasive, non-native slipper limpet *Crepidula fornicata* is poorly adapted to sediment burial. *Marine Pollution Bulletin* 130, 95–104.
- Precht, E., Franke, U., Polerecky, L., and Huettel, M. (2004). Oxygen dynamics in permeable sediments with wave-driven pore water exchange. *Limnology and Oceanography* 49, 693–705.
- Prins, T. C., Smaal, A. C., and Dame, R. F. (1997). A review of the feedbacks between bivalve grazing and ecosystem processes. *Aquatic Ecology* 31, 349–359.
- Pynnönen, K., Holwerda, D. A., and Zandee, D. I. (1987). Occurrence of calcium concretions in various tissues of freshwater mussels, and their capacity for cadmium sequestration. *Aquatic Toxicology* 10, 101–114.
- Queirós, A. M., Birchenough, S. N. R., Bremner, J., Godbold, J. A., Parker, R. E., Romero-Ramirez, A., et al. (2013). A bioturbation classification of European marine infaunal invertebrates. *Ecology and Evolution* 3, 3958–3985.
- Quéguiner, B. (1982). Variations qualitatives et quantitatives du phytoplancton dans un écosystème eutrophe soumis aux effets des marées : la rade de Brest. Thèse de doctorat, Université de Bretagne Occidentale.

- Quillien, N., Nordström, M. C., Schaal, G., Bonsdorff, E., and Grall, J. (2016). Opportunistic basal resource simplifies food web structure and functioning of a highly dynamic marine environment. *Journal of Experimental Marine Biology and Ecology* 477, 92–102.
- Qui-Minet, Z. N., Delaunay, C., Grall, J., Six, C., Cariou, T., Bohner, O., et al. (2018). The role of local environmental changes on maerl and its associated non-calcareous epiphytic flora in the Bay of Brest. *Estuarine, Coastal and Shelf Science* 208, 140–152.
- R Core Team (2012). R: A language and environment for statistical computing. Available at: <http://www.R-project.org/>.
- Ragueneau, O., Chauvaud, L., Leynaert, A., Thouzeau, G., Paulet, Y.-M., Bonnet, S., et al. (2002). Direct evidence of a biologically active coastal silicate pump: ecological implications. *Limnology and Oceanography* 47, 1849–1854.
- Ragueneau, O., Chauvaud, L., Moriceau, B., Leynaert, A., Thouzeau, G., Donval, A., et al. (2005). Biodeposition by an invasive suspension feeder impacts the biogeochemical cycle of Si in a coastal ecosystem (Bay of Brest, France). *Biogeochemistry* 75, 19–41.
- Ragueneau, O., Raimonet, M., Mazé, C., Coston-Guarini, J., Chauvaud, L., Danto, A., et al. (2018). The impossible sustainability of the Bay of Brest? Fifty years of ecosystem changes, interdisciplinary knowledge construction and key questions at the science-policy-community interface. *Frontiers in Marine Science* 5:124.
- Rainbow, P., and Wang, W. (2001). Comparative assimilation of Cd, Cr, Se, and Zn by the barnacle *Elminius modestus* from phytoplankton and zooplankton diets. *Marine Ecology Progress Series* 218, 239–248.
- Ramus, A. P., Silliman, B. R., Thomsen, M. S., and Long, Z. T. (2017). An invasive foundation species enhances multifunctionality in a coastal ecosystem. *Proceedings of the National Academy of Sciences* 114, 8580–8585.
- Rao, K. P., and Goldberg, E. D. (1954). Utilization of dissolved calcium by a pelecypod. *Journal of Cellular and Comparative Physiology* 43, 283–292.
- Rau, G. H., Teyssie, J.-L., Rassoulzadegan, F., and Fowler, S. W. (1990). $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ variations among size-fractionated marine particles: implications for their origin and trophic relationships. *Mar Ecol. Prog. Ser* 59, 33–38.
- Reise, K. (2002). Sediment mediated species interactions in coastal waters. *Journal of Sea Research* 48, 127–141.
- Reise, K., Buschbaum, C., Büttger, H., and Wegner, K. M. (2017). Invading oysters and native mussels: from hostile takeover to compatible bedfellows. *Ecosphere* 8, e01949.

- Reynaud, M., (2013). Influence de la prolifération du mollusque gastéropode *Crepidula fornicata* sur la diversité biologique et la structuration des peuplements d'invertébrés benthiques. Rapport de stage. 35p.
- Rezek, R. J., Lebreton, B., Roark, E. B., Palmer, T. A., and Pollack, J. B. (2017). How does a restored oyster reef develop? An assessment based on stable isotopes and community metrics. *Marine Biology* 54:164.
- Riaux-Gobin, C., Llewellyn, C., and Klein, B. (1987). Microphytobenthos from two subtidal sediments from North Brittany. II. Variations of pigment compositions and concentrations determined by HPLC and conventional techniques. *Marine Ecology Progress Series* 40, 275–283.
- Ricciardi, A., Whoriskey, F. G., and Rasmussen, J. B. (1997). The role of the zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 2596–2608.
- Richard, J., (2005). *Crepidula fornicata* : un modèle biologique pour l'étude du rôle de la variabilité des caractères phénotypiques (reproduction, croissance et nutrition) sur les processus de colonisation en milieu marin. Thèse de doctorat, Université de Bretagne Occidentale. 371 p.
- Richard, J., Huet, M., Thouzeau, G., and Paulet, Y.-M. (2006). Reproduction of the invasive slipper limpet, *Crepidula fornicata*, in the Bay of Brest, France. *Marine Biology* 149, 789–801.
- Richoux, N. B., Vermeulen, I., and Froneman, P. W. (2014b). Stable isotope ratios indicate differential omnivory among syntopic rocky shore suspension-feeders. *Marine Biology* 161, 971–984.
- Richoux, N., Vermeulen, I., and Froneman, P. (2014a). Fatty acid profiles reveal temporal and spatial differentiation in diets within and among syntopic rocky shore suspension-feeders. *Marine Ecology Progress Series* 495, 143–160.
- Riera, P. (2007). Trophic subsidies of *Crassostrea gigas*, *Mytilus edulis* and *Crepidula fornicata* in the Bay of Mont Saint Michel (France): A $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ investigation. *Estuarine, Coastal and Shelf Science* 72, 33–41.
- Riera, P., and Richard, P. (1996). Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine bay of Marennes-Oléron. *Estuarine, Coastal and Shelf Science* 42, 347–360.
- Riera, P., Stal, L., and Nieuwenhuize, J. (2002). $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ of co-occurring molluscs within a community dominated by *Crassostrea gigas* and *Crepidula fornicata* (Oosterschelde, The Netherlands). *Marine Ecology Progress Series* 240, 291–295.
- Rigal, F., (2010). Dynamique spatio-temporelle du nuage larvaire du gastéropode introduit *Crepidula fornicata* au sein d'une baie mégatidale, la baie de Morlaix (France). Thèse de doctorat, Université Pierre et Marie Curie. 443 p.

- Rigolet, C., Thiébaud, E., and Dubois, S. (2014). Food web structures of subtidal benthic muddy habitats: evidence of microphytobenthos contribution supported by an engineer species. *Marine Ecology Progress Series* 500, 25–41.
- Riisgård, H., and Larsen, P. (2010). Particle capture mechanisms in suspension-feeding invertebrates. *Marine Ecology Progress Series* 418, 255–293.
- Riisgård, H. U., and Larsen, P. S. (2000). Comparative ecophysiology of active zoobenthic filter feeding, essence of current knowledge. *Journal of Sea Research*, 25.
- Riquet, F., (2012). Processus micro-évolutifs chez l'espèce marine invasive *Crepidula fornicata*. Thèse de doctorat, Université Pierre et Marie Curie. 277 p.
- Rodriguez, L. F. (2006). Can invasive species facilitate native species? evidence of how, when, and why these impacts occur. *Biological Invasions* 8, 927–939.
- Romić, I., and Nakajima, Y. (2017). Ecosystem engineering as an energy transfer process: a simple agent-based model. *Theoretical Ecology*.
- Rossi, F., Herman, P. M. J., and Middelburg, J. J. (2004). Interspecific and intraspecific variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in deposit- and suspension-feeding bivalves (*Macoma balthica* and *Cerastoderma edule*): Evidence of ontogenetic changes in feeding mode of *Macoma balthica*. *Limnology and Oceanography* 49, 408–414.
- Sackett, W. M., and Moore, W. S. (1966). Isotopic variations of dissolved inorganic carbon. *Chemical Geology* 1, 323–328.
- Sanders, H. L. (1958). Benthic studies in Buzzards bay. I. Animal-sediment relationships. *Limnology and Oceanography* 3, 245–258.
- Sauriau, P.-G., de Montaudouin, X., Gomez, S., Joassard, L., and Bréret, M. (2002). La crépidule : identifier les mécanismes de sa prolifération et caractériser ses effets sur le milieu pour envisager sa gestion (Chantier : Baie de Marennes-Oléron). Rapport Liteau. 61 p.
- Savoye, N., Aminot, A., Tréguer, P., Fontugne, M., Naulet, N., and Kérouel, R. (2003). Dynamics of particulate organic matter $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ during spring phytoplankton blooms in a macrotidal ecosystem (Bay of Seine, France). *Mar Ecol Prog Ser*, 16.
- Sax, D., Stachowicz, J., Brown, J., Bruno, J., Dawson, M., Gaines, S., et al. (2007). Ecological and evolutionary insights from species invasions. *Trends in Ecology & Evolution* 22, 465–471.
- Schaal, G., Riera, P., Leroux, C., and Grall, J. (2010). A seasonal stable isotope survey of the food web associated to a peri-urban rocky shore. *Marine Biology* 157, 283–294.
- Schellekens, T., de Roos, A. M., and Persson, L. (2010). Ontogenetic diet shifts result in niche partitioning between two consumer species irrespective of competitive abilities. *The American Naturalist* 176, 625–637.

- Schlacher, T. A., and Connolly, R. M. (2014). Effects of acid treatment on carbon and nitrogen stable isotope ratios in ecological samples: a review and synthesis. *Methods in Ecology and Evolution* 5, 541–550.
- Schratzberger, M., and Ingels, J. (2018). Meiofauna matters: The roles of meiofauna in benthic ecosystems. *Journal of Experimental Marine Biology and Ecology* 502, 12–25.
- Sellheim, K., Stachowicz, J., and Coates, R. (2010). Effects of a nonnative habitat-forming species on mobile and sessile epifaunal communities. *Marine Ecology Progress Series* 398, 69–80.
- Serôdio, J., Ezequiel, J., Barnett, A., Mouget, J., Méléder, V., Laviale, M., et al. (2012). Efficiency of photoprotection in microphytobenthos: role of vertical migration and the xanthophyll cycle against photoinhibition. *Aquatic Microbial Ecology* 67, 161–175.
- Seuront, L., and Spilmont, N. (2002). Self-organized criticality in intertidal microphytobenthos patch patterns. *Physica A: statistical mechanics and its applications* 313, 513–539.
- Sheehan, E. V., Bridger, D., and Attrill, M. J. (2015). The ecosystem service value of living versus dead biogenic reef. *Estuarine, Coastal and Shelf Science* 154, 248–254.
- Sherwood, G. D., and Rose, G. A. (2005). Stable isotope analysis of some representative fish and invertebrates of the Newfoundland and Labrador continental shelf food web. *Estuarine, Coastal and Shelf Science* 63, 537–549.
- Shumway, S. E., Selvin, R., and Schick, D. F. (1987). Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin, 1791): a qualitative study. *Journal of Shellfish Research* 6, 89–95.
- Shumway, S. E., Ward, J. E., Heupel, E., Holohan, B. A., Heupel, J., Heupel, T., et al. (2014c). Observations of feeding in the common Atlantic slippersnail *Crepidula fornicata* L., with special reference to the “mucus net.” *Journal of Shellfish Research* 33, 279–291.
- Sminia, T., De With, N. D., Bos, J. L., Van Nieuwmegen, M. E., Witter, M. P., and Wondergem, J. (1977). Structure and function of the calcium cells of the freshwater pulmonate snail *Lymnaea stagnalis*. *Netherlan Journal of Zoology* 27, 195–208.
- Smith, D. J., and Underwood, G. J. C. (2001). The production of extracellular carbohydrates by estuarine benthic diatoms: the effects of growth phase and light and dark treatment. *Journal of Phycology* 36, 321–333.
- Sotka, E. E., and Byers, J. E. (2018). Not so fast: promoting invasive species to enhance multifunctionality in a native ecosystem requires strong(er) scrutiny. *Biological Invasions*.

- Stachowicz, J., and Byrnes, J. (2006). Species diversity, invasion success, and ecosystem functioning: disentangling the influence of resource competition, facilitation, and extrinsic factors. *Marine Ecology Progress Series* 311, 251–262.
- Stal, L. J. (2010). Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. *Ecological Engineering* 36, 236–245.
- Stewart, T. W., Gafford, J. C., Miner, J. G., and Lowe, R. L. (1999). Dreissena-shell habitat and antipredator behavior: combined effects on survivorship of snails co-occurring with molluscivorous fish. *Journal of the North American Benthological Society* 18, 274–283.
- Stewart, T. W., Miner, J. G., and Lowe, R. L. (1998). Quantifying mechanisms for zebra mussel effects on benthic macroinvertebrates: organic matter production and shell-generated habitat. *Journal of the North American Benthological Society* 17, 81–94.
- Stiger-Pouvreau, V., and Thouzeau, G. (2015). Marine species introduced on the french channel-atlantic coasts: a review of main biological invasions and impacts. *Open Journal of Ecology* 05, 227–257.
- Sundbäck, K., and Granéli, W. (1988). Influence of microphytobenthos on the nutrient flux between sediment and water: a laboratory study. *Marine Ecology Progress Series* 43, 63–69.
- Svensson, J. R., and Marshall, D. J. (2015). Limiting resources in sessile systems: food enhances diversity and growth of suspension feeders despite available space. *Ecology* 96, 819–827.
- Tanaka, S., and Hatano, H. (1955). Biochemical studies on the pearl oyster, *Pinctada martensii*. IV. On the uptake of radioactive calcium by pearl oyster and its deposition on the pearl and the shell. *J. Chem. Soc. Jap.* 76, 602–605.
- Taylor, I. S., and Paterson, D. M. (1998). Microspatial variation in carbohydrate concentrations with depth in the upper millimetres of intertidal cohesive sediments. *Estuarine, Coastal and Shelf Science* 46, 359–370.
- Thieltges, D. W., Strasser, M., and Reise, K. (2006). How bad are invaders in coastal waters? The case of the American slipper limpet *Crepidula fornicata* in western Europe. *Biological Invasions* 8, 1673–1680.
- Thieltges, D. W., Strasser, M., and Reise, K. (2003). The American slipper limpet *Crepidula fornicata* (L.) in the northern Wadden Sea 70 years after its introduction. *Helgoland Marine Research* 57, 27–33.
- Thomsen, M., Byers, J., Schiel, D., Bruno, J., Olden, J., Wernberg, T., et al. (2014). Impacts of marine invaders on biodiversity depend on trophic position and functional similarity. *Marine Ecology Progress Series* 495, 39–47.
- Thomsen, M. S., Ramus, A. P., Long, Z. T., and Silliman, B. R. (2018). A seaweed increases ecosystem multifunctionality when invading bare mudflats. *Biological Invasions*. Invasion note.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Thomsen, M. S., Wernberg, T., Olden, J. D., Griffin, J. N., and Silliman, B. R. (2011). A framework to study the context-dependent impacts of marine invasions. *Journal of Experimental Marine Biology and Ecology* 400, 322–327.
- Thouzeau, G. (2011). Modifications de la composition et du fonctionnement des communautés microbiennes et macrozoobenthiques associées à la prolifération d'une espèce introduite (*Crepidula fornicata*) dans un écosystème côtier anthropisé : la rade de Brest. Rapport EC2CO. 14 p.
- Thouzeau, G., Chauvaud, L., Grall, J., and Guérin, L. (2000). Rôle des interactions biotiques sur le devenir du pré-recrutement et la croissance de *Pecten maximus* (L.) en rade de Brest. *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie* 323, 815–825.
- Tompa, A. S., and Watabe, N. (1977). Calcified arteries in a gastropod. *Calcified Tissue Research* 22, 159–172.
- Tremblay, R., Olivier, F., Bourget, E., and Rittschof, D. (2007). Physiological condition of *Balanus amphitrite* cyprid larvae determines habitat selection success. *Marine Ecology Progress Series* 340, 1–8.
- Troadec, P., and Le Goff, R. C. (1997). Etat des lieux et des milieux de la rade de Brest et de son bassin versant. Phase préliminaire du Contrat de Baie de la rade de Brest. 335 p.
- Tuchman, N. C., Schollett, M. A., Rier, S. T., and Geddes, P. (2006). Differential heterotrophic utilization of organic compounds by diatoms and bacteria under light and dark conditions. *Hydrobiologia* 561, 167–177.
- Turcotte, M. M., and Levine, J. M. (2016). Phenotypic plasticity and species coexistence. *Trends in Ecology & Evolution* 31, 803–813.
- Underwood, G. J. C. (2005). Microalgal (Microphytobenthic) biofilm in shallow coastal waters: how important are species? *Proceedings of the California Academy of Sciences* 56, 162–169.
- Underwood, G. J. C., and Paterson, D. M. (2003). The importance of extracellular carbohydrate production by marine epipelagic diatoms. *Advances in Botanical Research* 40, 183–240.
- Upton, G., and Cook, I. (1996). *Understanding statistics*. Oxford University Press. Butler & Tanner Ltd, Frome and London.
- Usseglio-Polatera, P., Bournaud, M., Richoux, P., and Tachet, H. (2000). Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. *Freshwater Biology* 43, 175–205.
- Vander Zanden, M. J., Clayton, M. K., Moody, E. K., Solomon, C. T., and Weidel, B. C. (2015). Stable isotope turnover and half-life in animal tissues: a literature synthesis. *PLOS ONE* 10, e0116182.

RÉFÉRENCES BIBLIOGRAPHIQUES

- van Broekhoven, W., Jansen, H., Verdegem, M., Struyf, E., Troost, K., Lindeboom, H., et al. (2015). Nutrient regeneration from feces and pseudofeces of mussel *Mytilus edulis* spat. *Marine Ecology Progress Series* 534, 107–120.
- van Broekhoven, W., Troost, K., Jansen, H., and Smaal, A. (2014). Nutrient regeneration by mussel *Mytilus edulis* spat assemblages in a macrotidal system. *Journal of Sea Research* 88, 36–46.
- van der Zee, E. M., Tielens, E., Holthuijsen, S., Donadi, S., Eriksson, B. K., van der Veer, H. W., et al. (2015). Habitat modification drives benthic trophic diversity in an intertidal soft-bottom ecosystem. *Journal of Experimental Marine Biology and Ecology* 465, 41–48.
- van Leeuwe, M. A., Brotas, V., Consalvey, M., Forster, R. M., Gillespie, D., Jesus, B., et al. (2008). Photoacclimation in microphytobenthos and the role of xanthophyll pigments. *European Journal of Phycology* 43, 123–132.
- Vieira, S., Cartaxana, P., Máguas, C., and Marques da Silva, J. (2016). Photosynthesis in estuarine intertidal microphytobenthos is limited by inorganic carbon availability. *Photosynthesis Research* 128, 85–92.
- Vitousek, P. M. (1990). Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57, 7–13.
- Vitousek, P. M., D'Antonio, C. M., Loope, L. L., Rejmánek, M., and Westbrooks, R. (1997). Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* 21, 1–16.
- Volkenborn, N., Polerecky, L., Hedtkamp, S. I. C., van Beusekom, J. E. E., and de Beer, D. (2007). Bioturbation and bioirrigation extend the open exchange regions in permeable sediments. *Limnology and Oceanography* 52, 1898–1909.
- Walne, P. R. (1956). The biology and distribution of the slipper limpet *Crepidula fornicata* in Essex rivers with notes on the distribution of the larger epi-benthic invertebrates. *Fish. Invest. Ser II* 20, 1–50.
- Walsh, J. R., Carpenter, S. R., and Vander Zanden, M. J. (2016). Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proceedings of the National Academy of Sciences* 113, 4081–4085.
- Ward, J. (1963). Hierarchical Grouping to Optimize an Objective Function. *Journal of the American Statistical Association* 58, 236–244.
- Ward, J. E., and Shumway, S. E. (2004). Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. *Journal of Experimental Marine Biology and Ecology* 300, 83–130.
- White, J. W., Rassweiler, A., Samhouri, J. F., Stier, A. C., and White, C. (2014). Ecologists should not use statistical significance tests to interpret simulation model results. *Oikos* 123, 385–388.

- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer Nature. Springer International Publishing AG Switzerland.
- Widdows, J., Brinsley, M. D., Bowley, N., and Barrett, C. (1998). A Benthic Annular Flume for In Situ Measurement of Suspension Feeding/Biodeposition Rates and Erosion Potential of Intertidal Cohesive Sediments. *Estuarine, Coastal and Shelf Science* 46, 27–38.
- Widdows, J., Pope, N., Brinsley, M., Asmus, H., and Asmus, R. (2008). Effects of seagrass beds (*Zostera noltii* and *Z. marina*) on near-bed hydrodynamics and sediment resuspension. *Marine Ecology Progress Series* 358, 125–136.
- Williamson, M. (1996). *Biological invasion*. Chapman & Hall, London.
- Woods, D. M. (1989). Paleocology from *Crepidula* (Mollusca: Gastropoda) beds, James city formation (Pleistocene), North Carolina. *Tulane studies in geology and paleontology* 22, 69–91.
- Wright, J. T., and Gribben, P. E. (2017). Disturbance-mediated facilitation by an intertidal ecosystem engineer. *Ecology* 98, 2425–2436.
- Wright, J. T., Holmes, Z. C., and Byers, J. E. (2018). Stronger positive association between an invasive crab and a native intertidal ecosystem engineer with increasing wave exposure. *Marine Environmental Research*.
- Wright, S. W., Jeffrey, S. W., Mantoura, R. F. C., Llewellyn, C. A., Bjornland, T., Repeta, D., et al. (1991). Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Marine Ecology Progress Series* 77, 183–196.
- Yakovis, E., Artemieva, A., Fokin, M., and Varfolomeeva, M. (2012). Intraspecific variation in stable isotope signatures indicates no small-scale feeding interference between a horse mussel and an ascidian. *Marine Ecology Progress Series* 467, 113–120.
- Yamamuro, M., and Koike, I. (1993). Nitrogen metabolism of the filter-feeding bivalve *Corbicula japonica* and its significance in primary production of a brackish lake in Japan. *Limnology and Oceanography* 38, 997–1007.
- Yokoyama, H., Ishihi, Y., and Yamamoto, S. (2008). Diet–tissue isotopic fractionation of the Pacific oyster *Crassostrea gigas*. *Marine Ecology Progress Series* 358, 173–179.
- Zaiko, A., Daunys, D., and Olenin, S. (2009). Habitat engineering by the invasive zebra mussel *Dreissena polymorpha* (Pallas) in a boreal coastal lagoon: impact on biodiversity. *Helgoland Marine Research* 63, 85–94.
- Zhang, Y., Song, J., Yuan, H., Xu, Y., He, Z., and Duan, L. (2010). Biomarker responses in the bivalve (*Chlamys farreri*) to exposure of the environmentally relevant concentrations of lead, mercury, copper. *Environmental Toxicology and Pharmacology* 30, 19–25.

RÉFÉRENCES BIBLIOGRAPHIQUES

- Zhao, T., Villéger, S., Lek, S., and Cucherousset, J. (2014). High intraspecific variability in the functional niche of a predator is associated with ontogenetic shift and individual specialization. *Ecology and Evolution* 4, 4649–4657.
- Zhukova, N. V., Aizdaicher, N. A., (1995). Fatty acid composition of 15 species of marine microalgae. *Phytochemistry* 39, 351–356.
- Zhukova, N. V. (1991). The pathway of the biosynthesis of non-methylene-interrupted dienoic fatty acids in molluscs. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 100, 801–804.
- Zhukova, N. V., Kharlamenko, V. I., Svetashev, V. I., and Rodionov, I. A. (1992). Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. *Journal of Experimental Marine Biology and Ecology* 162, 253–263.
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., and Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer Science.
- Zwerschke, N., Emmerson, M. C., Roberts, D., and O'Connor, N. E. (2016). Benthic assemblages associated with native and non-native oysters are similar. *Marine Pollution Bulletin* 111, 305–310.
- Zwerschke, N., Hollyman, P. R., Wild, R., Strigner, R., Turner, J. R., and King, J. W. (2018). Limited impact of an invasive oyster on intertidal assemblage structure and biodiversity: the importance of environmental context and functional equivalency with native species. *Marine Biology* 165:89.

Titre : Ecologie trophique de l'espèce ingénieur *Crepidula fornicata*, et implications pour le fonctionnement de son habitat.

Mots clés : Isotopes stables, acides gras, imagerie hyperspectrale, microphytobenthos, rade de Brest

Résumé : La crépidule (*Crepidula fornicata*) est une espèce invasive des côtes européennes. Hermaphrodite protandre, cette espèce grégaire forme des chaînes d'individus qui s'accumulent en forte densité sur les fonds. Longtemps considérée comme un envahisseur néfaste, la crépidule est aussi un ingénieur de l'écosystème, modifiant physiquement et biologiquement son habitat. Elle constitue un modèle biologique pour étudier comment les espèces invasives et ingénieurs peuvent structurer et modifier l'écosystème qu'elles colonisent. Dans le cadre de cette thèse, ces effets ont été examinés à travers le fonctionnement trophique des habitats à crépidules, en lien notamment avec la production primaire benthique. Il a été mis en évidence expérimentalement une stimulation du microphytobenthos (MPB) subtidal grâce à l'activité biologique de la crépidule. La niche trophique de *C. fornicata* a été redéfinie suite à la découverte de la présence de sphérules de carbonates dans ses tissus, surestimant la contribution du MPB dans son régime alimentaire. L'utilisation de différents marqueurs trophiques (pigments, acides gras, isotopes) a révélé que

les jeunes individus mobiles étaient susceptibles de brouter le MPB associé au biofilm présent sur les coquilles. Les adultes sessiles, sont des filtreurs opportunistes, où la matière détritique mais aussi du MPB contribuent à leur régime alimentaire. L'analyse de différents suspensivores inféodés à cet habitat, a démontré que la crépidule n'était pas un compétiteur trophique pour des espèces commercialement importantes (pétoncle noir et huître plate) dû à leurs mécanismes de sélection trophique. À l'échelle du réseau trophique, la crépidule en très forte densité peut conduire à une homogénéisation du réseau trophique global dû à l'enrichissement en matière organique. Au contraire, un banc mort de crépidules montre une complexité trophique similaire à celle d'un banc de maërl, écosystème à forte biodiversité. Cette thèse, en plus d'avoir caractérisé le fonctionnement trophique des bancs de crépidules, montre qu'une espèce invasive, en facilitant certains compartiments biologiques (MPB, suspensivores), peut contribuer à la richesse d'un écosystème comme celui la rade de Brest.

Title: Trophic ecology of the engineer species *Crepidula fornicata*, and implications for its habitat functioning.

Keywords: stable isotopes, fatty acids, hyperspectral imaging, microphytobenthos, Bay of Brest

Abstract: The slipper limpet (*Crepidula fornicata*) is an invasive species of European coasts. Protandric hermaphrodite, this gregarious species forms individuals' stacks which accumulate in high density on bottom. For a long time considered as a detrimental invader, the slipper limpet is also an ecosystem engineer, modifying its habitat both physically and biologically. It is model to study how invasive and engineer species can structure and modify the ecosystem that they colonize. In this Ph.D. thesis, these effects have been examined through the trophic functioning of habitats colonized by the slipper limpet, with a special reference to the primary benthic production. Experimental stimulation of subtidal microphytobenthos (MPB) has been demonstrated by the biological activity of the slipper limpet. The trophic niche of *C. fornicata* has been redefined following the discovery of the presence of carbonate spherules in its tissues, overestimating the contribution of MPB in its diet. Several trophic markers use (pigments, fatty

acids, isotopes) revealed that young motile individuals were likely to graze the MPB associated to shell biofilm. Sessile adults, were likely opportunistic filter-feeders, where detritus but also MPB contribute to their trophic diet. The analysis of several filter-feeders inhabiting *Crepidula* beds has demonstrated that the slipper limpet was not a trophic competitor for commercially important species (black scallop and flat oyster) due to their trophic sorting mechanisms. At the food web scale, the high density of slipper limpet can lead to a homogenization of the global food web due to organic matter enrichment. Dead *Crepidula* bed showed trophic complexity similar to maerl bed, which is a high biodiverse ecosystem. This thesis, in addition to characterize the trophic functioning of *Crepidula* beds, shows that an invasive species, in facilitating different biological compartments (MPB, filter-feeders), can contribute to the ecosystem richness such as the Bay of Brest.