UNIVERSITE DE LA ROCHELLE
ECOLE DOCTORALE DE LA ROCHELLE
CENTRE DE RECHERCHE SUR LES ECOSYSTEMES LITTORAUX ANTHROPISES

DEVENIR DES BACTERIES DANS LES RESEAUX TROPHIQUES DES VASIERES INTERTIDALES : LE CAS DE BROUAGE (BAIE DE MARENNES-OLERON)

THESE DE DOCTORAT

Pour l'obtention du grade de docteur de l'université de La Rochelle
Discipline : Océanologie biologique et environnement marin

Par

PIERRE-YVES PASCAL

Soutenue à La Rochelle le 24 janvier 2008 devant le jury composé de :

M. T. MOENS, Professeur, Univ. de Gent, Belgique	Rapporteur
M. F. JORISSEN, Professeur, Univ. d'Angers	Rapporteur
M. J. C. MARQUES, Professeur, Univ. de Coimbra, Portugal	Examinateur
M. A. DINET, D. R. au CNRS, UPMC, Banyuls-sur-mer	Examinateur
Mme N. NIQUIL, Maître de conférences, Univ. La Rochelle	Directrice de thèse
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M. P. RICHARD, D. R. au CNRS, CRELA, La Rochelle	Responsable scientifique

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Pascal PY, Dupuy C, Mallet C, Richard P, Niquil N (Sous presse) Bacterivory by benthic organism in sediment: quantification using ^{15}N enriched bacteria. J Exp Mar Biol Ecol

Pascal PY, Dupuy C, Richard P, Niquil N (Acceptée avec modifications mineures) Which environmental parameters influence bacterivory of foraminifera (*Ammonia tepida*)? J Exp Mar Biol Ecol

Pascal PY, Dupuy C, Richard P, Rzeznik-Orignac J, Niquil N (Soumise) Bacterivory of a mudflat nematode community according to biotic and abiotic factors. Mar Biol

Pascal PY, Dupuy C, Haubois AG, Richard P, Niquil N (Non soumise) Ingestion rate of *Hydrobia ulvae*: comparison with meiofauna.

Pascal PY, Dupuy C, Richard P, Niquil N (Non soumise) Trophic fate of benthic bacteria in an intertidal mudflat: influence of environmental factors.

L'analyse de la diversité bactérienne des échantillons récoltés durant cette thèse devrait permettre la rédaction d'un article supplémentaire en collaboration avec Clarisse Mallet.

Cette thèse a fait l'objet de communications orales lors de congrès internationaux :

Pascal PY, Niquil N, Dupuy C, Richard P, Mallet C, Debenay JP (4-9 June 2006) Bacterivory of foraminifera (*Ammonia tepida*) in intertidal mudflat according to various biotic and abiotic factors. ASLO summer meeting. Victoria (British Columbia, Canada)

Pascal PY, Dupuy N, Richard P, Rzeznik J, Niquil N (July 29 – August 3 2007) Bacterial ingestion by a nematode community from an intertidal mudflat: influence of biotic and abiotic factors. THIRIMCO. Recife (Brazil). Présentation orale primée.

Pascal PY, Dupuy N, Richard P, Mallet, C, Armynot du Chatelet E, Niquil N (4-8 November) Trophic role of benthic bacteria along the year in an intertidal mudflat. ERF. Providence (Rhode Island, USA)

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- Les dénombrements des flagellés ont été réalisés par Christine Dupuy (CRELA, La Rochelle)
- L'analyse granulométrique et les mesures de ratio C/N des échantillons de sédiment prélevés lors du suivi de terrain ont été réalisées par Laurence Debeauvais (UMR Géosystèmes, Lille)
- L'analyse de la teneur en protéines et carbohydrates des échantillons de sédiment prélevés lors du suivi de terrain a été réalisée par Frédérique Bonnemoy (LBP, Aubière)

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La production biologique de la zone côtière représente entre 18 et 33 % de la production océanique mondiale en dépit d'une surface relativement faible représentant 7% de la surface océanique mondiale (Wollast 1991). Parmi les écosystèmes côtiers, les vasières du littoral atlantique et nord européen font partie des systèmes les plus productifs (McLusky 1989).

Caractéristiques des vasières intertidales

Les vasières sont dépourvues de végétation macrophytique et la production phytoplanctonique y est limitée en raison de la turbidité des eaux (McLusky 1989). Ainsi la majorité de la production primaire est réalisée par des microalgues benthiques : le microphytobenthos composé essentiellement de diatomées. A chaque émersion diurne, pour accéder à la ressource lumineuse, les microalgues migrent à la surface du sédiment où elles forment un biofilm microalgal temporaire. Ces algues répondent à un rythme endogène si bien qu'à la fin de la marée basse, le biofilm temporaire disparaît (Colijn 1982, Serôdio et al. 1997). Ce biofilm algal ne mesure que quelques dizaines de microns d'épaisseur et sa forte activité photosynthétique (Blanchard & Guarini 1996, Blanchard et al. 1997a) lui permet de synthétiser l'équivalent de sa masse pendant la durée d'une émersion. Ces productions microphytobenthiques, extrêmement élevées (MacIntyre et al. 1996, Underwood & Kromkamp 1999) servent de nourritures aux organismes vivants sur la vasière qu'ils soient déposivores (e.g. Blanchard et al. 2000) ou suspensivores (e.g. Riera & Richard 1996, 1997). Ainsi, la caractérisation des mécanismes de régulation de la production microphytobenthique et la quantification de son devenir au sein du réseau trophique constituent des enjeux écologiques majeurs et c'est pourquoi ils ont fait l'objet de nombreux travaux. La conversion de la biomasse végétale en biomasse animale permet d'alimenter et d'entretenir une intense activité des producteurs secondaires pour tout ou une partie de leur cycle biologique (McLusky 1989). Les oiseaux, par exemple, utilisent les vasières comme des sites d'hivernage et de halte migratrice et se nourrissent de l'abondante macrofaune benthiques à la base de leur régime alimentaire (van de Kam et al. 2004). Ces milieux jouent également un rôle de nourricerie pour les juvéniles de poissons en fournissant refuge et ressources alimentaires à de nombreuses espèces d'intérêt commercial (Beck et al. 2001). Enfin, ces zones de forte productivité biologique sont propices à l'installation des activités aquacoles et en particulier de la

conchyliculture, ce qui leur donne une dimension socio-économique de première importance (Héral et al. 1989).

Caractéristiques du compartiment bactérien hétérotrophe

Le domaine côtier se caractérise par des apports massifs de matière organique et de nutriments continentaux et océaniques piégés dans les sédiments côtiers. Ainsi, les vasières intertidales présentent de très fortes teneurs en matière organique. Cette matière organique constitue un mélange de matériel dissous et particulaire d'origine allochtone (terrigène et océanique) et autochtone. Les bactéries benthiques jouent un rôle majeur dans la minéralisation de cette matière organique (Blackburn 1988). Les macromolécules dissoutes ou particulaires ne sont pas directement assimilables par les bactéries. Les polymères organiques sont hydrolysés, par des enzymes extracellulaires, en monomères qui sont alors incorporés dans les cellules bactériennes. La dégradation des molécules complexes en molécules de plus en plus simples est réalisée par des bactéries de plus en plus spécialisées (Bianchi et al. 1989). Les bactéries présentent des morphologies relativement simples : en bâtonnets, en sphères ou en filaments d'une taille généralement inférieure à 1 µm. Dans les sédiments intertidaux, les bactéries présentent une grande diversité de métabolismes. Les différents modes de respiration sont distribués verticalement, depuis la surface vers le fond, en fonction des différents accepteurs terminaux d'électrons qui sont l'oxygène, le nitrate, le sulfate et les carbonates. Cette succession s'accompagne d'une réduction chimique du sédiment qui se manifeste par une réduction de son potentiel d'oxydoréduction et par une succession des communautés bactériennes (Bianchi et al. 1989).

L'intérêt manifesté pour le compartiment bactérien est relativement ancien (première moitié du 20^{ième} siècle). Cependant, les avancées majeures, aussi bien techniques que conceptuelles, ne sont significatives que depuis les 25 dernières années (Kirchman 2000). Les mesures d'abondances de bactéries réalisées dans les années 1970 ne permettaient de quantifier qu'une faible partie des bactéries présentes dans le milieu (i.e. bactéries cultivables). Au début des années 1980, l'utilisation de nouvelles techniques telles que la microscopie à épifluorescence et l'utilisation de marqueurs spécifiques de l'ADN tels que l'acridine orange (Watson et al. 1977), le DAPI (Porter & Feig 1980) et le SYBR Green (Noble & Furham 1998) ont largement changé la vision de ce compartiment bactérien. En effet, à l'aide de ces techniques, les abondances trouvées sont généralement très fortes, de l'ordre de 10^9 l^{-1} en milieu planctonique et 10^9 ml^{-1} en milieu benthique (Schmidt et al. 1998). Au sein des sédiments marins, la biomasse

bactérienne représente jusqu'à 4% du carbone organique total (Rublee 1982). Les abondances bactériennes sont corrélées à la teneur en matière organique et à la taille des particules sédimentaires. La production bactérienne a dans un premier temps été déterminée par le dénombrement des cellules en division (Hagström et al. 1979), puis par la mesure de la synthèse d'ADN bactérien grâce à l'utilisation de thymidine marquée (Fuhrman & Azam 1982, Moriarty 1986) ou par mesure de la synthèse protéique par l'incorporation de leucine marquée (Kirchman et al. 1985). La majorité des bactéries benthiques sont en dormance (Luna et al. 2002) mais en dépit de la faible proportion de bactéries actives, la production secondaire est dominée par les bactéries dans la plupart des sédiments (Kemp 1990). De plus, certains auteurs ont observé des taux de production bactérienne benthique excédant les taux de production primaire (Alongi 1988, 1991, Cammen 1991). Outre leur rôle dans la dégradation de la matière organique, les bactéries constituent ainsi une ressource nutritive potentielle pour les échelons trophiques supérieurs.

Rôle des bactéries dans les réseaux trophiques marins

L'étude du fonctionnement des réseaux trophiques microbiens dans les systèmes aquatiques constitue un thème majeur de l'océanographie biologique depuis les vingt dernières années (Ducklow 2000).

En milieu pélagique, le concept de chaîne alimentaire herbivore a dans un premier temps, été établi : une grande partie de biomasse phytoplanctonique est consommée par le zooplancton herbivore qui à son tour est consommé par les poissons (Steele 1974). Il est ensuite apparu que la production primaire n'est pas majoritairement assurée par de grandes cellules phytoplanctoniques mais par des organismes de taille inférieure à 3 µm (cyanobactéries, picoeucaryotes) (Pomeroy 1974, Platt et al. 1983). Une autre avancée majeure dans la description des réseaux trophiques planctoniques à été la mise en évidence de la boucle microbienne : la matière organique dissoute (MOD) produite par le phytoplancton est assimilée par les bactéries, elles-mêmes consommées par les flagellés (Azam et al. 1983). Ainsi, les bactéries jouent un rôle central en rendant accessible une partie de la production primaire sous forme de MOD, d'abord aux flagellés, puis à l'ensemble du réseau trophique. Sans l'intervention des bactéries cette MOD ne serait donc pas utilisée. Ce réseau microbien permet donc un transfert de la production bactérienne au réseau trophique supérieur (Rassoulzadegan 1993). En milieu pélagique, les bactéries sont trop petites pour sédimenter, cependant leur abondance reste relativement constante au sein de la colonne d'eau. Cette constance s'explique par des

mécanismes qui suppriment les bactéries à un taux identique à celui avec lequel elles sont produites. Même si des processus comme la lyse virale peuvent réguler la production bactérienne (synthèse dans Fuhrman & Noble 1995), il est généralement admis qu'en milieu pélagique, la régulation se fait essentiellement par la prédation des protistes (Thingstad 2000). En effet, les protistes, ubiquistes dans le milieu marin pélagique, peuvent se développer et réguler l'abondance des bactéries (Azam et al. 1983).

De nombreux travaux se sont donc appliqués à décrire les réseaux trophiques planctoniques. En revanche, très peu d'études portent sur les systèmes benthiques et le fonctionnement des réseaux microbiens benthiques demeure peu connu (synthèse dans Kemp 1990). Le devenir trophique des bactéries et leur intégration dans le réseau trophique benthique demeure en conséquence peu documenté. Ce manque d'intérêt est probablement dû à la complexité intrinsèque des systèmes sédimentaires et aux difficultés techniques de mesure de la bactérivorie des organismes benthiques. Ces difficultés, moins marquées en milieu planctonique, pourraient en outre être à l'origine des résultats contradictoires obtenus lors des expériences de mesure de bactérivorie benthique (Kemp 1990). En effet, selon les études, la régulation des abondances de bactéries par la macrofaune est inexistante (Bianchi & Levinton 1981, Levinton & Bianchi 1981, Bianchi & Levinton 1984, Kemp 1987), faible (Cammen 1980b, Juniper 1987) ou importante (Hargrave 1970, Moriarty et al. 1985). Les résultats des études portant sur la méiofaune ne sont pas moins contradictoires. Certains auteurs considèrent que la fraction de production bactérienne consommé par la méiofaune est inférieure à 3 % (Epstein & Shiaris 1992a, van Oevelen et al. 2006a) alors que Montagna (1984b) considère que la méiofaune seule peut réguler le compartiment bactérien. Les résultats concernant la bactérivorie de la microfaune sont également tout aussi variables. En considérant l'ensemble des données de la littérature, Kemp (1990) conclut que les protozoaires sont moins abondants dans le sédiment que dans la colonne d'eau et que la fraction de production bactérienne ingérée par les flagellés ne serait significative que quand le ratio entre les abondances de flagellés et de bactéries dépasserait 1 pour 1000.

Actuellement, il est donc relativement délicat d'établir un schéma général de fonctionnement du réseau microbien en milieu benthique comme cela a été réalisé en milieu pélagique. Cette difficulté vient du fait que les recherches dans ce domaine n'en sont qu'a leur début. Par conséquent, l'apport de connaissances sur la bactérivorie des organismes benthiques est susceptible de contribuer à l'élaboration d'un tel schéma.

Cette thèse s'inscrit donc dans une problématique générale de compréhension du fonctionnement des réseaux trophiques microbiens du benthos. Les études se sont focalisées sur le site d'étude de Brouage à Marennes-Oléron car ce site a fait l'objet de nombreuses études qui ont permis, entre autre, d'identifier les consommateurs potentiels de bactéries.

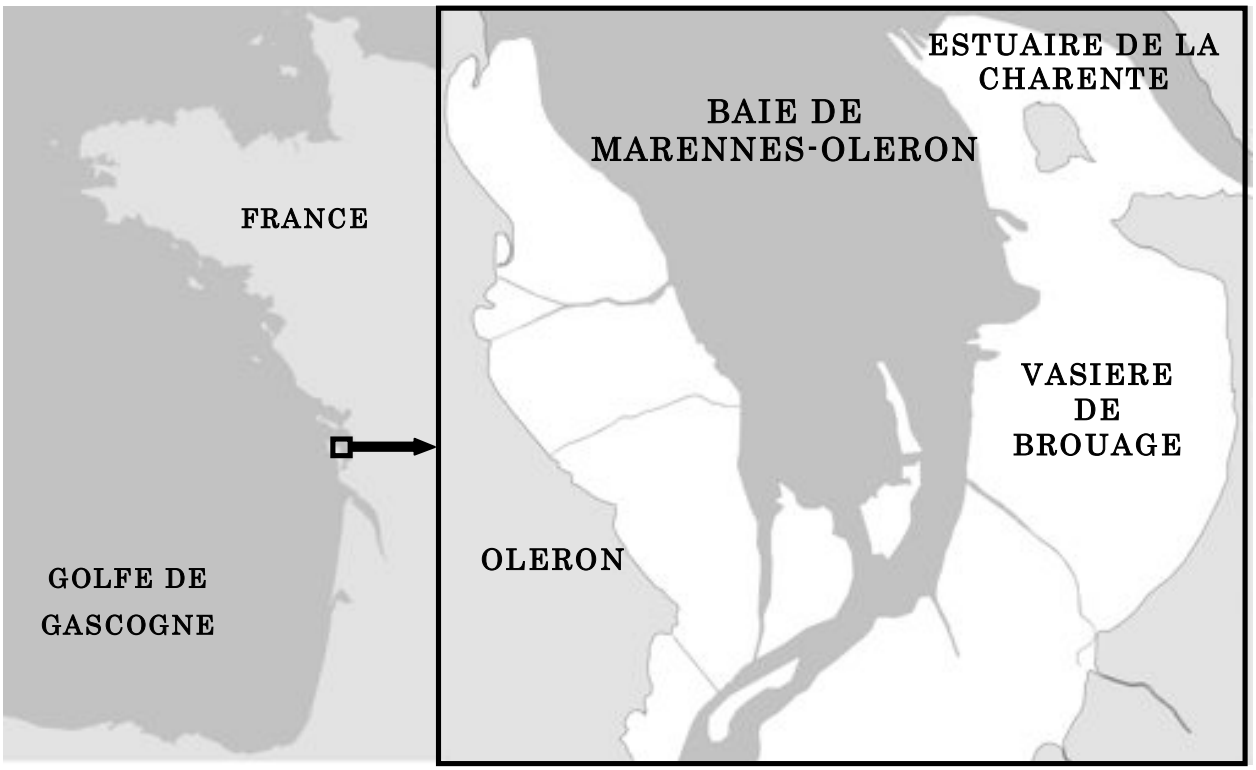


Figure 1. Carte présentant la Baie de Marennes-Oléron et la vasière de Brouage

Le site de Marennes Oléron

La baie de Marennes Oléron, située sur la côte atlantique française, représente une entité de 170 km^2 (Fig. 1). C'est un système macrotidal de faible profondeur moyenne, environ 60% de la surface du bassin est émergée durant la marée basse et la vasière de Montportail-Brouage constitue 30% de cette zone découverte. Cette vasière de pente très faible (1/1000) émerge à marée basse sur 3,5 à 4,5 km selon les coefficients de marées. Les eaux océaniques qui entrent pendant le flot par le Pertuis d'Antioche se dirigent vers le Sud en longeant la côte d'Oléron et repoussent les eaux de la Charente vers la partie Sud-Est du bassin. Ainsi la vasière de Brouage est une zone de moindre hydrodynamisme où se déposent les particules terrigènes. La matière organique contenue dans le sédiment est donc essentiellement d'origine continentale (Vouvé 2000). Les sédiments de cette vasière sont homogènes, composés de vase fine, pure, dont la fraction pélitique (<63 µm) dépasse 85% (Sauriau et al. 1989).

Plusieurs études se sont attachées à décrire les fluctuations de la composition du peuplement microphytobenthique de cette vasière (Carriou-Le Gall & Blanchard 1995, Haubois et al. 2005b), à quantifier la dynamique de sa production à court terme (Blanchard et al. 1996, Blanchard et al. 1997a, Guarini et al. 2000a, Guarini et al. 2000b, Blanchard et al. 2002) et à décrire la structure du biofilm microalgal à micro-échelle (Herlory et al. 2004b). Des biomasses supérieures à 200 mgChla m^{-2} et des productions nettes de 1 gC m^{-2} $jour^{-1}$ ont été mesurées (Blanchard et al. 1998). Ces valeurs sont équivalentes aux plus fortes valeurs consignées dans la littérature (Colijn 1987). Le devenir trophique de cette production primaire a été évalué en mesurant la consommation d'algues par la méiofaune (Blanchard 1991, Rzeznik-Orignac 2004) et le gastéropode *H. ulvae* (Blanchard et al. 2000, Haubois et al. 2005a).

D'autres études ont été menées localement afin de mieux connaître le fonctionnement de cet écosystème composé en majorité de vasières intertidales. Le bassin de Marennes Oléron est le premier site ostréicole européen avec une production de 35 000 tonnes par an (Thomas et al. 1999). Des modèles ont été élaborés pour établir la consommation réalisée par les bivalves cultivés (Struski & Bacher 2006) et estimer la capacité de charge du bassin (Bacher et al. 1998). D'autres études se sont attachées à décrire la distribution, la biomasse et la dynamique de la macrofaune non cultivée (Bacher & Sauriau 1995, Kang 1999, Haubois et al. 2002, Bocher et al. 2006). En terme d'abondance, ce peuplement est dominé par le gastéropode *H. ulvae*. Le bivalve le plus abondant est *Scrobicularia plana*, essentiellement localisé dans la partie haute de la

vasière. La méiofaune est majoritairement composée de nématodes dont le peuplement est dominé par deux groupes trophiques : (i) les suceurs d'épistrate qui se nourrissent principalement en perçant les diatomées et en aspirant leur contenu et (ii) les déposivores non sélectifs qui ingèrent sans distinction sédiment et particules nutritives associées (Rzeznik-Orignac et al. 2003). En terme d'abondance, les copépodes représentent moins de 15% de cette méiofaune (Montagna et al. 1995b, Rzeznik-Orignac et al. 2003). Une étude préliminaire a montré une forte biomasse de foraminifères benthiques avec un peuplement dominé par l'espèce *Ammonia tepida* (Armynot du Châtelet données non publiées). Les abondances et productions des bactéries benthiques de la vasière de Brouage ont été déterminées (Garet 1996), cependant aucune étude portant sur la consommation de ces bactéries benthiques n'a été entreprise à ce jour.

L'ensemble des résultats évoqués ci-dessus, augmenté de données non mentionnées, ont été intégrés dans un modèle par analyse inverse rendant compte du fonctionnement du réseau trophique de la vasière de Brouage (Leguerrier et al. 2003, Leguerrier 2005, Degré et al. 2006). La méthode de l'analyse inverse est particulièrement adaptée à la construction de réseaux trophiques lorsque de nombreux éléments manquent à la détermination des flux reliant les composants du système (Vézina & Platt 1988, Vézina 1989). En effet, cette méthode permet d'obtenir un modèle complet en estimant la valeur des flux inconnus par l'application de contraintes résumant les connaissances préalables des compartiments du réseau et d'un critère final de décision : le principe de parcimonie. L'analyse de sensibilité du modèle de réseau trophique de Brouage a mis en évidence la nécessité d'obtenir des informations complémentaires sur la consommation du compartiment bactérien par les organismes de la vasières (Leguerrier et al. 2003, Leguerrier 2005).

Les consommateurs potentiels de bactéries benthiques peuvent appartenir à la microfaune composée essentiellement de ciliés et de flagellés. Dans les sédiments vaseux comme ceux de Brouage, les ciliés sont connus pour présenter des abondances très faibles (Alongi 1986). En outre, des études préliminaires ont montré une faible abondance des flagellés à Brouage (Dupuy données non publiées). La microfaune aurait donc un impact réduit sur le compartiment bactérien en raison de sa faible abondance. A Brouage, la méiofaune est dominée par les nématodes dont le peuplement a été décrit par Rzeznik-Orignac et al. (2003). A l'exception des nématodes prédateurs, toutes les espèces de nématodes sont susceptibles de consommer des bactéries (Moens & Vincx 1997). Les bactéries peuvent également être consommées par les copépodes benthiques (e.g. Carman & Thistle 1985, Carman 1990a). *Ammonia tepida*, l'espèce dominant le

peuplement de foraminifères de la vasière de Brouage (Armynot du Châtelet, données non publiées) appartient à un genre consommant des bactéries (Goldstein & Corliss 1994, Langezaal et al. 2005). La macrofaune de la vasière de Brouage est dominée par le gastéropode *H. ulvae* (Bocher et al. 2006). Les espèces du genre *Hydrobia* présentent une alimentation composée de microphytobenthos mais aussi de bactéries (Cammen 1980a, Jensen & Siegismund 1980, Bianchi & Levinton 1981, Levinton & Bianchi 1981). Les consommateurs potentiels des bactéries de la vasière de Brouage appartiendraient donc principalement à la méiofaune et à la macrofaune.

Objectifs de la thèse

Différentes méthodes peuvent être utilisées pour quantifier la bactérivorie de la méiofaune et de la macrofaune. La méthode la plus fréquemment utilisée consiste à injecter un marqueur directement dans le sédiment. Celui-ci va être assimilé par les bactéries, elles-mêmes consommées simultanément par divers bactérivores (Montagna 1993). La quantité de marqueur mesurée dans les bactérivores renseigne donc sur la quantité de bactéries marquées ingérées. L'inconvénient principal de cette approche réside dans le fait que le marqueur peut également être ingéré directement par les bactérivores. Une autre approche consiste à marquer les bactéries au préalable et à les mettre en contact avec les bactérivores dans le sédiment.

Le premier objectif de cette thèse est de mettre au point une méthode permettant d'utiliser des bactéries enrichies en isotope stable pour quantifier la consommation de bactéries par des organismes de la méiofaune et de la macrofaune, tout en conservant au mieux différentes caractéristiques rencontrées en milieu naturel et susceptibles d'influencer la prédation.

Les vasières intertidales sont sujettes à des variations de conditions environnementales importantes à trois échelles de temps différentes : à long terme (cycle saisonnier), à moyen terme (cycle lunaire) et à court terme (cycles nycthéméraux et tidaux) (e.g. Guarini et al. 1997). En effet, l'alternance de périodes d'émersion et d'immersion liée au cycle tidal a des conséquences sur les sédiments intertidaux qui subissent de fortes variations de facteurs environnementaux. Entre l'émersion et l'immersion, l'interface sédimentaire évolue rapidement : Variation de température, dessalure en cas de précipitation, variation de luminosité, formation d'un biofilm microphytobenthique, font partie des caractéristiques spécifiques aux sédiments des

vasières intertidales. Ces variations pourraient jouer un rôle important dans la dynamique de la bactérivorie de la vasière.

Le deuxième objectif de cette thèse est de déterminer l'influence de ces variables environnementales sur la consommation de bactéries des principaux bactérivores de la vasière de Brouage.

Les études réalisées par Garet (1996) sur la vasière de Brouage ont montré que la production des bactéries fluctue fortement alors que leur abondance demeure relativement constante au cours de l'année. Il existerait donc un mécanisme de régulation de l'abondance bactérienne, dont l'incidence varierait au cours de l'année, et qui détruirait les bactéries à un taux identique à celui de leur production. Si ce mécanisme est la prédation, il y aurait donc une évolution du niveau de cette prédation au cours de l'année. Ces variations du niveau de bactérivorie pourraient être liées à des fluctuations de comportements alimentaires. En effet, les bactérivores sont susceptibles de présenter des comportements d'acclimatations aux variations des conditions environnementales au cours de l'année.

Le troisième objectif de la thèse est donc de déterminer le devenir de la production bactérienne dans le réseau trophique de la vasière de Brouage au cours de l'année.

Plan de la thèse

Ce manuscrit s'articule en trois chapitres :

Chapitre 1 : Ce chapitre méthodologique, décrit la mise au point d'une méthode permettant la mesure de la consommation de bactéries par la méiofaune et la macrofaune. Des bactéries de culture ont été enrichies en ^{15}N et utilisées comme marqueur durant des expériences de broutage. Ces expériences étant fondées sur l'hypothèse que les bactéries cultivées ont les mêmes caractéristiques que celles du milieu naturel. C'est pourquoi, les caractéristiques de taille, d'activité et de diversité des bactéries enrichies sont décrites et prises en considération dans la discussion.

Chapitre 2 : Ce chapitre relève de la démarche expérimentale *ex situ*, et traite de l'incidence de variations de facteurs abiotiques (température, salinité, luminosité) et biotiques (abondance d'algues et de bactéries) sur la consommation de bactéries par trois bactérivores: le foraminifère *A. tepida*, un peuplement de nématodes et le gastéropode *H. ulvae* tous issus de la vasière de Brouage.

Chapitre 3 : Ce chapitre relève de la démarche expérimentale *in situ*, et traite de la consommation de bactéries pendant un cycle annuel sur la vasière de Brouage. Des expériences de broutage ont été réalisées *in situ* régulièrement pendant un an. Des variables environnementales ont été mesurées simultanément pour déterminer leurs influences sur la consommation de bactéries. Les productions bactériennes ont aussi été mesurées simultanément afin d'apprécier la fraction de production bactérienne consommée et le devenir trophique des bactéries.

Discussion et perspectives : Les résultats obtenus au cours de la thèse sont discutés et les différentes perspectives de recherche sont formulées dans cette dernière partie.

CHAPITRE I

UTILISATION DE BACTERIES PRE-ENRICHIES EN ^{15}N POUR QUANTIFIER LA BACTERIVORIE DE LA MEIOFAUNE ET DE LA MACROFAUNE LORS D'EXPERIENCES DE BROUTAGE

CHAPITRE I

BACTERIVORY BY BENTHIC ORGANISMS IN SEDIMENT: QUANTIFICATION USING ^{15}N ENRICHED BACTERIA

Pierre-Yves Pascal, Christine Dupuy, Clarisse Mallet, Pierre Richard, Nathalie Niquil

Abstract

The fate of benthic bacterial biomass in benthic food webs is a topic of major importance but poorly described. This paper describes an alternative method for evaluation of bacterial grazing rate by meiofauna and macrofauna using bacteria pre-enriched with stable isotopes. Natural bacteria from the sediment of an intertidal mudflat were cultured in a liquid medium enriched with $^{15}NH_4Cl$. Cultured bacteria contained 2.9 % of ^{15}N and were enriched sufficiently to be used as tracers during grazing experiments. Cultured-bacteria presented a biovolume (0.21 μm^3) and a percentage of actively respiring bacteria (10 %) similar to those found in natural communities. The number of Operational Taxon Units found in cultures fluctuated between 56 and 75 % of that found in natural sediment. Despite this change in community composition, the bacterial consortium used for grazing experiments exhibited characteristics of size, activity and diversity more representative of the natural community than usually noticed in many other grazing studies. The bacterial ingestion rates of three different grazers were in the range of literature values resulting from other methods: 1149 ngC ind^{-1} h^{-1} for the mud snail *Hydrobia ulvae*, 0.027 ngC ind^{-1} h^{-1} for the nematode community, and 0.067 ngC ind^{-1} h^{-1} for the foraminifera *Ammonia tepida*. The alternative method described in this paper overcomes some past limitations and it presents interesting advantages such as short time incubation and *in situ* potential utilisation.

Introduction

Development of improved methods for measuring bacterial abundance and production have radically changed the perception of the role of bacteria in pelagic marine ecosystems. Bacteria are known to play a major role in organic matter degradation and regeneration of nutrients. Moreover the "microbial loop" model (e.g. Azam et al. 1983) considers bacteria as a "link" more than a "sink", increasing the ratio of primary production available for higher trophic levels. Therefore, bacteria appear to play a major role in pelagic foods web models (e.g. Vézina & Savenkoff 1999).

Bacterial abundance in marine soft sediments is relatively constant, around 10^9 cells.ml^{-1} porewater (Schmidt et al. 1998), being a thousand times more abundant than in pelagic systems. Moreover, high rates of production have been measured in aquatic sediments (e.g. van Duyl & Kop 1990). These findings have driven a debate on the fate of bacteria in benthic food webs. Due to technical limitations, studies dealing with benthic bacterivory are not as developed as pelagic ones (Kemp 1990).

Pelagic bacteria are mainly grazed by protozoa (e.g. Sherr & Sherr 1994) and a similar pattern was expected in benthic systems (van Duyl & Kop 1990, Bak et al. 1991, Hondeveld et al. 1994). Nevertheless, numerous authors consider the bacterial grazing by benthic ciliates and flagellates as insignificant (Alongi 1986, Kemp 1988, Epstein & Shiaris 1992a, Epstein 1997a). Depending on the studies, meiofauna grazing is considered either as (i) high enough to structure microbial communities (Montagna 1984b), (ii) using 3 % of bacterial production (van Oevelen et al. 2006a), or (iii) negligible (Epstein & Shiaris 1992a). Data on macrofaunal grazing rates are not less variable than on meiofaunal ones. In a synthesis, Kemp (1990) asserted that bacteria density is not high enough to play a major role in macrobenthos diet. These contrasting conclusions probably reflect the use of different methods. In conclusion, it appears that drawing a general view of the role played by microfauna, meiofauna and macrofauna in bacterial grazing is presently difficult (Kemp 1990).

Most of the benthic studies on trophic process employ tracers. Labels can be added directly to sediment. In such a situation, bacteria incorporate labels and are simultaneously grazed by predators (Montagna 1995, van Oevelen et al. 2006a, van Oevelen et al. 2006b). This technique minimizes disturbance of the spatial distribution and metabolism of grazers and bacteria (Carman et al. 1989). Nevertheless, only a small part of the bacterial assemblages takes up detectable quantities of labels (Carman 1990b). Moreover, the main drawback to this method is that a large part (up to 83 % in

Montagna & Bauer (1988)) of the total labels uptake may be attributable to processes other than grazing. Grazers may become labeled by absorption and adsorption of dissolved organic matter (DOM) (Montagna 1984a) or by uptake of labels by non-prey microorganisms associated with grazers (e.g. epicuticular or gut microorganisms) (Carman 1990a).

To reduce this bias, microbial prey can be prelabeled with fluorescent products, or isotopes either stable or radioactive. Fluorescent Labeled Bacteria (FLB) with monodispersed FLB or whole-sediment staining methods are used mainly to assess grazing activity of small predators like flagellates and ciliates (Novitsky 1990, Epstein & Shiaris 1992a, Hondeveld et al. 1992, Starink et al. 1994, Hamels et al. 2001). Meiofauna studies using FLB are seldom because FLB detection is difficult and time consuming in large sized grazers. Consequently, only a small number of specimens can be examined preventing detection of inter individual variations in grazing rate. Therefore, grazers like nematodes are able to discharge various digestive enzyme to realise extracorporeal hydrolyse of food (Riemann & Helmke 2002). In such a case, pre-digested FLB ingested are impossible to detect in grazers. Nevertheless bacterivory levels by foraminifera (Langezaal et al. 2005) and nematodes (Epstein 1997a) were assessed using FLB.

Bacterivory assessment by the way of adding radioactive or stable isotope to sediment was performed on meiofauna and macrofauna (e. g. Montagna 1984b, Sundbäck et al. 1996b, van Oevelen et al. 2006a, van Oevelen et al. 2006b).

Bacterivory assessment using prelabeled bacteria was performed with radioactive isotopes (Rieper 1978, Carman & Thistle 1985). To our knowledge, the use of stable isotopes on prelabeled bacteria has never been performed until present. Compared to radioactive isotopes, bacteria enriched with stable isotopes are more convenient to use, since they can be used *in situ* without negative environmental effects and legal restrictions. This method will help investigators who are limited by radioactive material prohibition.

The aim of this paper is to describe a method using ^{15}N stable isotope to prelabel bacteria in the view to assess bacterivory of large size benthic organisms (meiofauna and macrofauna). Experiments were performed mainly to assess the validity of this method, taking in consideration size, diversity, and activity of the prelabeled bacteria in order to be close to natural population parameters. The method was applied to 3 grazers from an intertidal mudflat in order to appreciate its potential generalization: one mollusc *Hydrobia ulvae*, a nematode community and the foraminifera *Ammonia tepida*.

Material and methods

Bacterial culture

Superficial sediment (1 cm) was collected from the Brouage intertidal mudflat located in the eastern part of Marennes-Oléron Bay (45°55N, 1°06W) on the Atlantic Coast of France. One ml of this sediment was added to 20 ml of bacterial liquid culture medium and kept in the dark at 13°C during 24 hours. The liquid bacterial culture medium was composed of: peptone 3 $g.l^{-1}$ (BioRad), yeast extract 1 $g.l^{-1}$ (BioRad), $^{15}NH_4Cl$ 1 $g.l^{-1}$ (99 % ^{15}N-enriched NH_4Cl CortecNet); sodium glycerophosphate 0.025 $g.l^{-1}$ and sequestren Fe 6 $g.l^{-1}$. It was completed with 0.2 µm filtered distilled water (500 ml) and 0.2 µm filtered sea water (500 ml) at pH 7.4. The first culture was subcultured during 24 hours under the same incubation conditions in the view to reach approximately 2×10^9 $cells.ml^{-1}$. Bacteria were rinsed (i.e. separated from culture medium) by the means of 3 centrifugations (3500 g, 10 min, 20°C) in 0.2 µm filtered sea water, then frozen in liquid nitrogen and kept frozen (-80°C) until grazing experiments.

The bacteria $\delta^{15}N$ was measured on an Eurovector Elemental Analyser coupled with an Isotope Mass Ratio Spectrometer (Isoprime, Micromass). Nitrogen isotope composition is expressed in the delta notation ($\delta^{15}N$) relative to air N_2: $\delta^{15}N = [(^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{reference})-1] \times 1000$. Rinsing efficiencies were tested using bacteria cultured in the medium previously described with non-enriched NH_4Cl. These bacteria were killed by formalin (2 %), placed in the ^{15}N-enriched culture medium previously described, harvested by the means of 3 centrifugations (3500 g, 10 min, 20°C) before isotope ratio measurement.

Cultured bacteria size

Size of bacteria from original sediment and cultures were measured. For sediment samples, particle-associated bacteria were detached by pyrophosphate (0.01M) and sonication. Bacteria were stained using 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) (2500 $\mu g.l^{-1}$) and filtered onto 0.2 µm Nucleopore black filters (Porter & Feig 1980).

Length (L) and width (2r) of each bacteria was determined by a computer-assisted image analysis (AxioVision Release 4.3) with an epifluorescence microscope (AxioSkop 2 mot plus – Zeiss) equipped with a charge-coupled device camera (AxioCam MRc5 – Zeiss). Bacterial biovolumes (V) were calculated for cultured bacteria (N = 1981) and natural bacteria (N = 1806) as follows: $V = \pi r^2.(L-2/3\ r)$ (Fuhrman 1981).

Cultured bacteria activity

Frozen aliquots of cultured bacteria were thawed and immediately incubated with 5-cyano-2,3-ditoyl tetrazolium chloride (CTC) (final concentration of 5 mM). After 2, 3 and 5 hours of incubation, experiments were stopped with formalin (2%) and stored at 4°C. Bacterial samples were processed as described above for the DAPI staining in order to simultaneously count total cells (UV excitation) and active cells (green excitation) on same slide.

Cultured bacteria diversity

The bacterial diversity of original sediment and culture aliquots was assessed. The DNA was extracted using an Ultraclean Soil DNA Kit (MO BIO, Oxyme) for sediment samples and a QIAamp DNA Mini Kit (Qiagen) for cultures. Bacterial 16S rRNA gene fragments of about 520 bp (the V6-V8 regions of 16r DNA (Gelsomino et al. 1999)) were amplified by PCR using primers 968fGC (5'-AACGCGAAGAACCTTAC-3'[with GC clamp 5'] and 1401r (5'-CGGTGTGTACAAGGCCC-3').

PCR products (300 ng) were loaded onto polyacrilamide gel (8% w/v, 7M urea) in TAE 1 X buffer. Electrophoresis was processed under a constant voltage of 68 V, during 17 h, with a thermal gradient from 66 to 69.7°C increasing at the rate of 0.2°C h^{-1} (Dcode™System: Biorad). The gel was stained with 0.5 µg.ml^{-1} Gelstar (BMA) in 1.25× TAE buffer during 30 min and checked through a UV transilluminator system (Versa Doc (Bio-Rad)) equipped with a camera. Temperature Gradient Gel Electrophoresis (TGGE) banding patterns were automatically calculated by the Bionumerix software (Applied Biomaths, Koutrai, Belgium) using the Dice coefficient (DC), without band weighing by both the complete linkage and unweighted pair group method with arithmetic mean (UPGMA) algorithms (threshold of 1%).

Grazing experiments

The first centimetre of sediment was collected from a square meter patch during ebb tide from the Brouage intertidal mudflat (France) on March 13, 2006. It was sieved on 500 µm, 200 µm and 50 µm in order to concentrate respectively *H. ulvae*, *A. tepida* and nematodes. Choice of these organisms was driven by their high natural abundance in the study area. Each type of grazer was placed in individual microcosms. Seventeen handpicked specimens of *H. ulvae* were placed in polypropylene Petri dishes (diameter 9 cm). For the foraminifera and nematode experiments, 1 ml of the fraction remaining on the 200 µm and on the 50 µm mesh sieves respectively were placed in 100 ml Pyrex

beakers. Each experiment was carried out in triplicate, along with triplicate controls. Control samples were frozen (-80°C) during 12 hours in order to kill grazers before thawing.

Sediment that passed through the 50 µm mesh was mixed with ^{15}N enriched bacteria. Abundance of sediment and cultured bacteria were counted using the methods previously described. This slurry containing 1.05×10^9 bacteria ml^{-1} and ^{15}N enriched bacteria were twice as abundant as natural ones. Seventeen ml of this slurry were placed in *H. ulvae* microcosms and 4 ml were placed in nematode and foraminifera microcosms. Grazing incubations were run in the dark at 20°C. Incubations were stopped by freezing the microcosms at -80°C.

Hydrobia ulvae were separated by hand from their shell and all specimens of each microcosm were pooled and homogenised using a Potter-Eveljhem. Nematodes were extracted from sediment using Ludox HS40 TM (Heip et al. 1985). Approximately 700 nematodes were randomly handpicked from each sample. Foraminifera were stained with rose Bengal in order to identify living specimens. As Rose Bengal is an organic compound, it could affect isotopic composition but control experiments were also stained in order to take this bias into account. For each sample, 150 specimens of *A. tepida* were picked individually and cleaned of any adhering particles.

$\delta^{15}N$ of grazers was determined using the technique described above. Incorporation of ^{15}N is defined as excess (above background) ^{15}N and is expressed in terms of specific uptake (I) (gN ind^{-1}). I was calculated as the product of excess ^{15}N (E) and biomass of N per grazer. E is the difference between the fraction ^{15}N in the background ($F_{background}$) and in the sample (F_{sample}): $E = F_{sample} - F_{background}$, where $F = {}^{15}N / ({}^{15}N + {}^{14}N) = R / (R + 2)$ and R = the nitrogen isotope ratio. For the $F_{background}$, we used control values measured with killed grazers (frozen). R was derived from the measured $\delta^{15}N$ values as: $R = ((\delta^{15}N/1000)+1) \times R_{airN2}$ with $R_{airN2} = 7.35293 \times 10^{-3}$ (Mariotti 1982). The uptake of bacteria (gC ind^{-1} h^{-1}) was calculated as Uptake = ($I \times$ (% $C_{enriched\ bacteria}$ / % $N_{enriched\ bacteria}$)) / ($F_{enriched\ bacteria} \times$ incubation time). This uptake was multiplied by the ratio between the abundance of total and enriched bacteria, determined from DAPI counts. Uptake ($gC_{bacteria}/h/gC_{grazer}$) were obtained by dividing uptake of bacteria (gC/ind/h) by grazer mean weight (gC / ind).

Table 1. Calculation of ingestions rates of three different grazers

	Enriched Bacteria	Gastropoda *H. ulvae*	Nematod community	Foraminifera *A. tepida*
% C by dry weight	35.2	33.6	38.2	5.8
% N by dry weight	10.2	8.0	7.4	0.8
Weight (g/ind)		5.4E-04	3.0E-07	1.8E-05
$\delta^{15}N$ living grazers	7068.2	95.0	20.6	20.0
$\delta^{15}N$ dead grazers		10.7	11.1	16.7
Incubation time (h)		2	5	5
Ratio (enriched/non enriched bacteria) = 1.5				
Ingestion rate (ngC/ind/h)		1149.16	0.03	0.07
Ingestion rate (10^{-3} $gC_{bacteria}$ / gC_{grazer}/h)		6.43	0.23	0.06

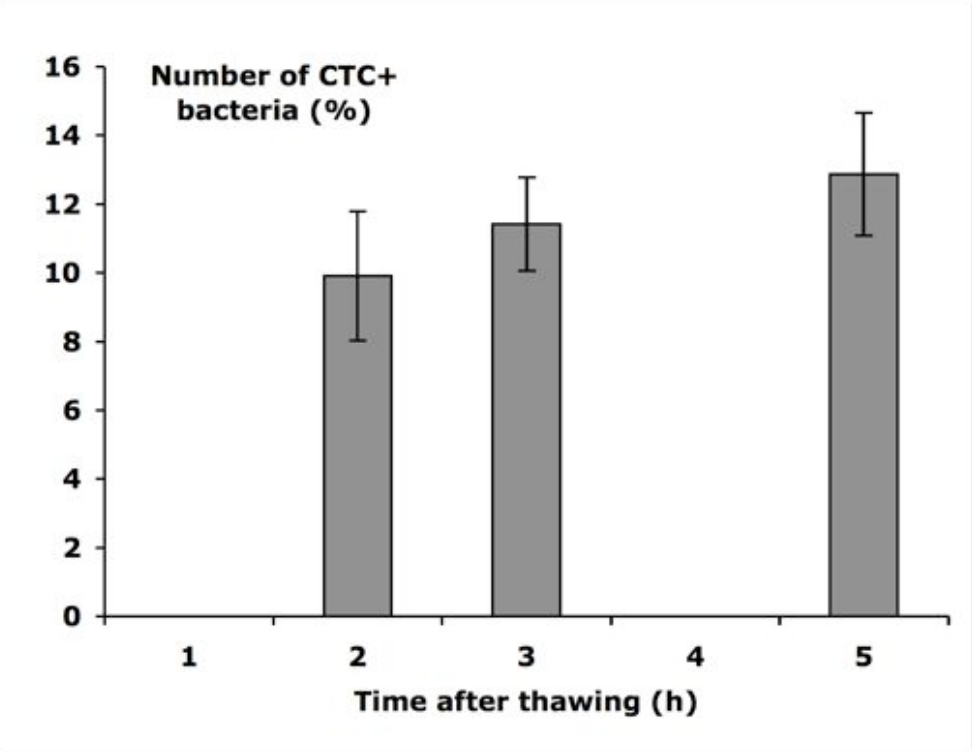


Fig 1. Evolution of the percentage of CTC + cells related to total bacteria after thawing cultured bacteria. Bars indicate standard deviation (n = 3).

Results

Characteristics of enriched bacteria

Bacteria cultured in a liquid medium with a 18 mM $^{15}NH_4Cl$ subsequently centrifuged to remove unincorporated label were found to contain 2.88 ± 0.03 % ^{15}N. Bacteria killed by formaldehyde before being placed in the same culture medium and centrifuged, contained 0.028 % ^{15}N. Cultured bacteria were enriched enough to allow their detection in the three studied grazers (Tab. 1). Bacterial abundance was not affected by the liquid nitrogen freezing process.

Cell volume of cultured bacteria (0.21 μm^3 ± 0.26) was not significantly (bilateral unpairwise student test; $p = 0.07$) different from cell volume of natural bacteria (0.23 μm^3 ± 0.62).

The ratio between active and non active bacteria increased significantly with time in cultured bacteria (Analyse of variance, $p < 0.01$) and evolved from 9.9 to 12.9 % during the first five hours after thawing (Fig. 1).

The number of Operational Taxon Units (OTU) found in cultures fluctuated between 56 and 75 % of that found in natural sediment (Fig. 2). The resulting dendrogram of TGGE patterns for cultured and natural sediment samples displayed two clusters. These clusters, of similar community composition, were defined by 49 % pattern similarity. Subculturing does not seem to affect community composition to a great extent (75 % of similarity) and did not change bacterial diversity. Freezing process induced a decline of 25 % in the diversity of bacteria and slightly affected the bacterial community composition (84 % of similarity).

Bacterial ingestion rates

After grazing experiments with pre-labeled bacteria, frozen grazers (control) were systematically less ^{15}N enriched than living ones for the three grazers types under study (Tab. 1). ^{15}N concentration increased linearly in grazers according to incubation time (Fig. 3). This linearity pointed that ingestion rates were constant during incubation period studied: 2 hours for *H. ulvae* and 5 hours for the nematode community and *A. tepida*. Raw data used for ingestion rates calculations are presented in Tab. 1. The mud snail *H. ulvae* grazed 1149 (± 0.285) ngC ind^{-1} h^{-1}, each nematode in the community grazed 0.027 (± 0.005) ngC ind^{-1} h^{-1} and the foraminifera *A. tepida* grazed 0.067 (± 0.013) ngC ind^{-1} h^{-1}.

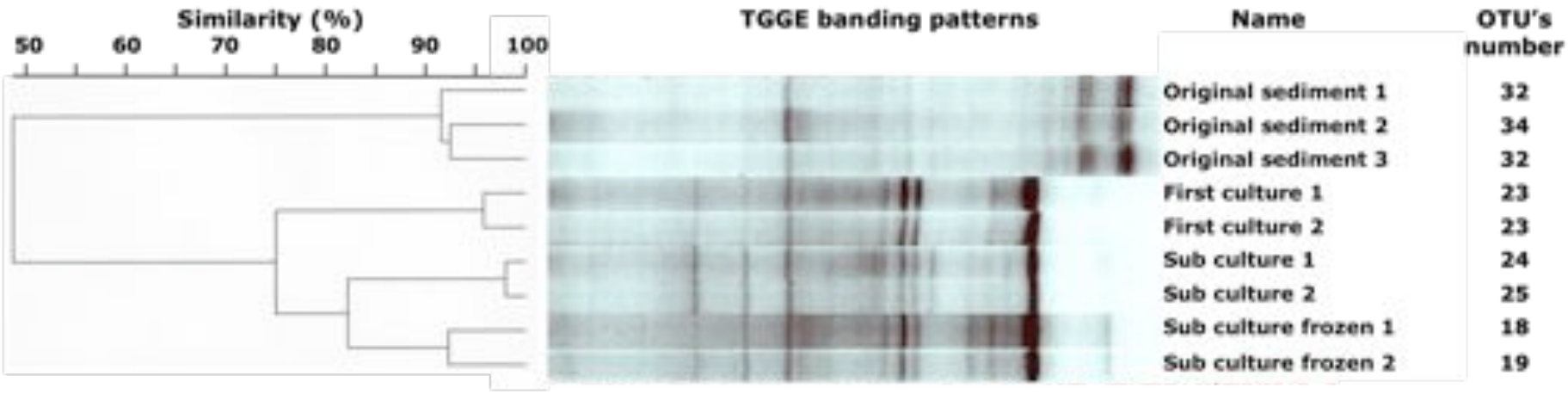


Fig 2. TGGE analyses of natural sediment, first culture, subculture from the first culture and subculture from the first frozen culture. The right panel shows the relating band similarity (%) of bacterial communities.

Discussion

Discussion on methodology

• Success of enrichment

Rinsing efficiency was tested by placing non enriched killed bacteria in enriched medium and by separating them from this medium by centrifugations. Those bacteria were poorly enriched in ^{15}N, showing that the bacterial rinsing centrifugation process was efficient. Thus, ^{15}N enrichment of bacteria was due to a bacterial assimilation and not to culture medium remaining between bacterial cells. This high bacterial rinsing efficiency is essential, since some grazers are able to consume directly DOM (Montagna & Bauer 1988) from the culture medium.

There is one disadvantage in using ^{15}N instead of ^{13}C enriched bacteria. As grazers contain more C than N, more biomass is required for isotopic measurements. However, the use of ^{15}N avoids the decalcification step required by ^{13}C and bias associated with this decalcification (Jacob et al. 2005). Moreover, grazing experiences are based on the assumption that isotopic composition of bacteria remains constant during the incubation period. The isotopic composition of ^{13}C enriched bacteria will vary quickly due principally to respiration loss and to a lower degree to production of DOM (Ogawa et al. 2001, Kawasaki & Benner 2006). The use of ^{15}N permits to limit this respiration loss bias so isotopic composition of bacteria remains more stable during incubation.

• Size of cultured bacteria

Discrimination of prey by grazers on the basis of size can influence the estimate of total bacterivory. Bacterial selection according to size has been well documented in planktonic protozoa (Pérez-Uz 1996, Hahn & Höfle 1999). Most protists graze preferentially on medium-sized bacterial cells, grazing being less efficient with smaller and larger cells (review in Hahn & Höfle 2001). The soil nematode, *Caenorhabditis elegans* feeds on bacteria suspended in liquid and smaller bacteria are better food sources than larger ones for this species (Avery & Shtonda 2003). Since, in our study, cultured and natural bacteria presented a similar average size, it can be inferred that there is only a small bias if any due to cell size selection by grazers.

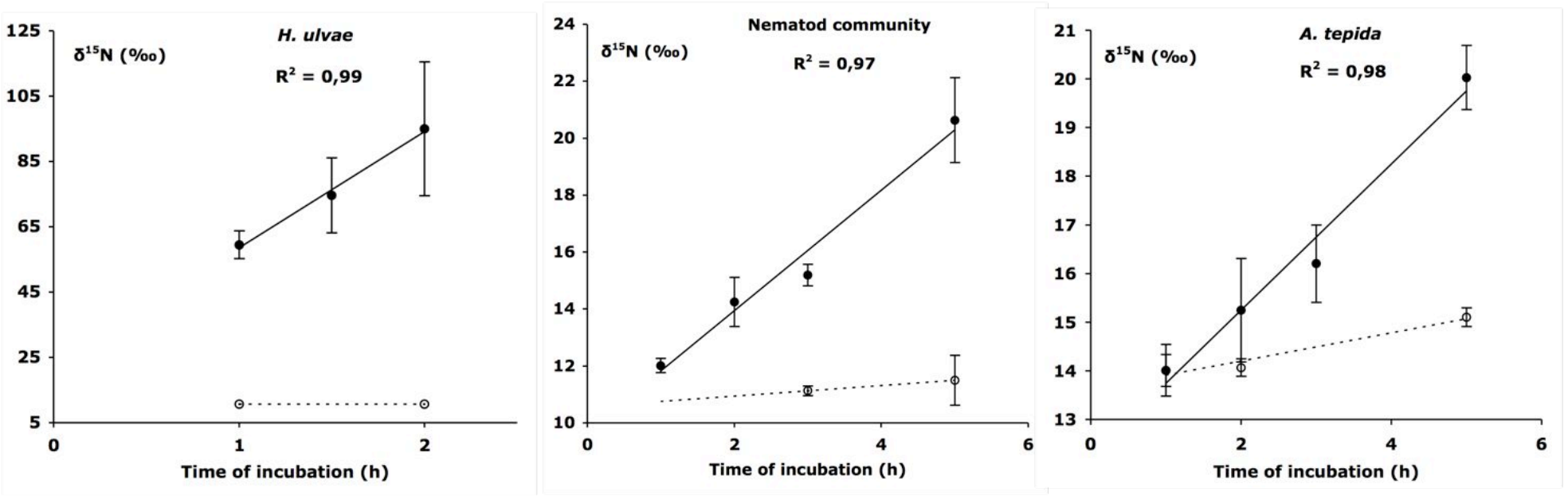


Fig 3. Evolution of isotopic composition of three types of living (●) and dead (○) grazers placed in contact with ^{15}N enriched bacteria. Bars indicate standard deviation (n = 3).

• Activity of cultured bacteria

Few data are available on CTC activities of natural benthic bacteria for comparison with our results. In superficial sediments from intertidal mudflats, 4 to 20% of benthic bacteria were found to be active (van Duyl et al. 1999). Proctor and Souza (2001) found 9 to 10 % active cells in river sediments and 25 % in intertidal sediments in the Gulf of Mexico. Halgund *et al.* (2002) detected 46 % active bacteria in lake sediments.

Enriched bacteria with activity levels different from those of the natural community may induce a bias if grazers select bacteria according to prey activity. Nematodes can discriminate bacteria exhibiting different physiological or nutritional states (Grewal & Wright 1992). *Pellioditis marina* do not assimilate heat-killed bacteria even though it feeds on live cells at high rates (Moens 1999). In contrast, *Diplolaimelloides meyli* is more attracted by killed than by live bacteria (Moens et al. 1999a). The foraminifera *Ammonia beccarii* collects dead and living stained bacteria without discrimination (Langezaal et al. 2005).

Many existing methods to quantify bacterivory use bacterial communities with activity levels different from those of the natural communities. For instance, labels directly added to the sediment are only incorporated by the active fraction of the bacterial community: 100 % of labeled bacteria are active. In contrast, prelabeled bacteria such as standard FLB are generally dead (heat-killed): 0 % of labeled bacteria are active. With the method described in our study, 10 % of labeled bacteria are active after thawing of frozen cultures (Fig. 1). This activity is included in range found in natural environments (van Duyl et al. 1999, Proctor & Souza 2001, Haglund et al. 2002). With the present method, grazers have the opportunity to pick up active or inactive bacteria according to their preference like in the natural environment.

• Diversity in the cultured bacteria

Subculturing of bacteria produces a final culture free of sediment. Freezing allows storage of aliquots that may be enriched under standardised conditions at any time. The freezing step induces small variations in the diversity of the bacterial community that must be nevertheless kept in mind when using this method.

Grazers may be highly selective of prey species. To our knowledge, selection of bacteria has never been observed for macrofauna but demonstrated for nematodes (Moens et al. 1999a) and foraminifera (Lee et al. 1966, Bernhard & Bowser 1992, Langezaal et al. 2005).

Grazers	Grazing rate (10^{-3} $gC_{bacteria}$ h^{-1} gC_{grazer})	Grazing rate (ngC h^{-1} ind^{-1})	Labeling methode	Reference
Gastropoda				
Hydrobia ulvae	6.43	1149.16	Stable isotope pre-enriched bacteria	Present study
Hydrobia ulvae		40-2080	Radioactive prelabeled algae	(Haubois et al. 2005a)
Hydrobia ulvae		896-1064	Radioactive prelabeled algae	(Blanchard et al. 2000)
Hydrobia truncata		506-2873	Radioactive prelabelled algae	(Forbes & Lopez 1989)
Nematode				
Mudflat Nematode community	0.23	0.03	Stable isotope pre-enriched bacteria	Present study
Mudflat Nematode community	2.59-3.66		In situ radioactive labeled bacteria	(Montagna 1984b)
Subtidal Nematode community	0.01		In situ radioactive labeled bacteria	(Montagna et al. 1995a)
Plectrus palustris		10.54*	Radioactive prelabeled bacteria	(Duncan et al. 1974)
Monhystera disjuncta		0.15-0.49 *	Radioactive prelabeled bacteria	(Herman & Vranken 1988)
Diplolaimelloides meeyli		11-17	Radioactive prelabeled bacteria	(Moens & Vincx 2000b)
Pellioditis marina		55-60	Radioactive prelabeled bacteria	(Moens & Vincx 2000b)
Mudflat Nematode community		0.02 *	Fluorescent prelabeled bacteria	(Epstein & Shiaris 1992a)
Foraminifera				
Ammonia tepida	0.06	0.07	Stable isotope pre-enriched bacteria	Present study
Ammonia tepida	2.18	2.18	Stable isotope pre-enriched algae	(Moodley et al. 2000)
Ammonia beccarii		$0.5\ 10^{-4}$ - $1,6\ 10^{-7}$ *	Fluorescent prelabeled bacteria	(Langezaal et al. 2005)

Table 2. Ingestion rate of bacteria observed in this study and compared with data from the literature concerning bacterivory and herbivory. * ingestion rate converted with a bacterial biomass of 35 fgC $cell^{-1}$ (Theil-Nielsen & Søndergaard 1998).

Nematodes used to be considered as generalist feeders, but they were recently shown to be selective feeders exhibiting various preferences for algal and microbial prey. Their reproduction rates differ according to the ingested strain of bacteria (Venette & Ferris 1998, Blanc et al. 2006). Moens *et al.* (1999a) show that monhysterid nematodes are able to select bacterial strain. Selection can be due to the bacterial size: filamentous bacteria escape uptake by nematodes with small buccal cavities (Blanc et al. 2006). Moens *et al.* (1999a) consider that the chemotaxic responses of nematodes to their bacterial prey may be due more to chemical cues produced by the bacteria than to bacterial cell-wall structure that determine their palatability. Nematodes are also able to significantly modify the composition of a bacterial community by their species-specific bacterial food preferences (De Mesel et al. 2004).

The foraminifera *A. beccarii* distinguishes food and non-food particles during collection (Langezaal et al. 2005). Two allogromiidae species (Foraminifera) have been shown to be non selective grazers, actively harvesting bacterial biofilm from 3 different inocula (Bernhard & Bowser 1992). Lee *et al.* (1966) found that most species of bacteria do not serve as food for foraminifera whereas selected species of bacteria are consumed in large quantity.

Each bacterial species presents characteristics such as cell surface, nutritional quality or chemical cues which may influence bacterial grazer behaviour. These differences have not been evaluated between cultured versus natural bacteria in the present study. However, estimation of total community composition and diversity gives us an approximate idea of these differences. This molecular approach has the advantage to target dominant community members. The cultured community presents 49 % of similarity with the natural bacterial community. Although cultivation of natural bacteria induces a shift in community composition (Fig. 2), this bacterial consortium seems more representative of the natural community than that of many other grazing studies. The majority of experiments that use FLB are done with monospecific bacteria or with a really limited number of bacterial species. Even if natural and cultured community are not strictly identical, the probability for grazers to find and ingest their preferred bacterial species is higher in the supplied bacterial consortium than with monospecific bacteria.

• Characteristics of grazing experiment

All various methods developed and applied to measure bacterivory in natural communities possess methodological shortcomings that make interpretation of the

resulting data problematic. The method presented in this study, using ^{15}N pre-enriched bacteria also presents bias. Sieving the sediment changes the bacterial availability for predators, bacteria being not attached to particle as in natural situation. The best way to minimize this artefact is to add the label directly to sediment in order to label bacteria while they are being grazed. This method is problematic as a high fraction of label found in grazers is due to processes other than grazing as underlined before. This requires control of incubations with a prokaryote activity inhibitor and the effectiveness of this inhibitor has to be tested for each grazer. The pre-enriched bacteria technique does not require the use of such inhibitors and only necessitate one control to determine adsorption of enriched bacteria on grazers.

Nematodes (Gerlach 1978), foraminifera from the genera *Ammonia* (Chandler 1989) and *H. ulvae* secrete mucus. During experiments with pre-enriched bacteria, controls must be performed to determine abundance of enriched bacteria stuck in the mucus secreted by grazers. Stuck bacteria modify the isotopic composition of grazers and controls are required to evaluate this bias due to non-grazing processes. In this study, freeze-killed grazer controls were used to determine this adsorption assuming that mucus post-freezing and mucus never frozen absorb bacteria at the same rate.

During grazing experiments, prey egestion from grazers may occur when chemical preservatives are used and this leads to underestimating grazing rates. Nematodes can egest a significant part of their gut contents when killed with formaldehyde (Moens et al. 1999c). In this study, grazers were frozen at -80°C to reduce this bias.

Prior to grazing experiments, enriched bacteria were frozen in liquid nitrogen to prevent spontaneous and enzymatic degradation and maintain their viability. Bacteria are commonly cryopreserved during long-term storage in liquid nitrogen. Some agents like glycerol and methanol can be used to enhance cryopreservation, but in the case of grazing experiments, they can be toxic to grazers. Low-temperature storage enables standardised cultures, helping to ensure reproducible results in a series of experiments. This storage is really useful when monitoring over long periods is considered.

The method used allows short incubations that limit bias due to recycling. Bacterial ingestion is detectable after 2 hours of incubation for 3 grazers (Fig. 3). During incubation, labeled bacteria may be first ingested by grazers that are themselves preyed by studied grazers. In such a situation, it is impossible to determine the part of label present in studied grazers that is provided respectively by bacteria and first grazers. Even if a short incubation time does not prevent this type of bias, it reduces it substantially.

Demonstration of applicability

Data from literature to compare with our values are scarce. First, these predator species have not been systematically studied. Secondly, herbivory is more commonly studied than bacterivory. Thirdly, the manner in which to report grazing rates depends on the aim of the study. Studies dealing with carbon flow generally report values on a biomass basis (e.g. ngC. ind^{-1} h^{-1}). When the aim of the study is the impact of grazers on microbial community, grazing rates are generally reported as rate constants (e.g. h^{-1}) (Montagna 1995).

While meiofaunal grazers are adapted to pick out specific microbial particles, macrofaunal deposit feeders process large volumes of sediment. Mud snails of the genus *Hydrobia* assimilate epipelic diatoms and attached bacteria (Newell 1965, Kofoed 1975, Lopez & Levinton 1978, Jensen & Siegismund 1980) contained in the ingested sediment. To our knowledge bacterial ingestion rates have never been determined but data is available concerning algal ingestion rates. The bacterial ingestion rate found in our study is in the same range as algal ingestion rates found by Forbes and Lopez (1989), Blanchard *et al.* (2000) and Haubois *et al.*, (2005a) (Tab. 2).

In literature, grazing rates of nematodes are strongly variable with a range of fluctuations of more than two orders of magnitude (Tab. 2). Thus, comparison of our data with literature is difficult. Those discrepancies may arise from a lot of reasons such as the use of different techniques or the experimental conditions. When grazing experiments are performed in monoxenical conditions, nematodes are in an environment constituted by water (or agar) and bacteria. Nematodes would probably present higher grazing rates in such conditions than during grazing experiments where bacterial food is mixed with minerals and refractory organic matter and therefore is less available. However, when our results are compared to values resulting from experiments using nematodes from mudflat grazing on labeled bacteria mixed with sediment, it appeared that they are in the range of grazing rates found by Epstein & Shiaris (1992a) but more than ten times lower than those found by Montagna (1984b).

Algal ingestion rates by *A. tepida* are higher (Moodley et al. 2000) than bacterial ingestion rates found in our study (Tab. 2). Langezaal *et al.* (2005) used FLB in simplified microcosms with one specimen of *A. beccarii* in a reduced volume of water. Their bacterial grazing rate is lower than ours. This may be linked to the bacterial concentration used in microcosms ($1.4\ 10^{3}$cell ml^{-1}), which is considerably lower than benthic bacterial abundance in the natural environment (c.a. 10^{9}cell ml^{-1}) and in the present study.

Conclusion

The fate of benthic bacterial biomass is a topic of major importance in microbial ecology and in food web studies. All various methods developed and applied to measure bacterivory in natural communities possess artefacts and difficulties that make interpretation of the resulting data problematic. Our experimental approach is not an exception and also presents shortcomings. These bias are due principally to sediment manipulation. Labeled bacteria are not available to bacterivores in the same manner as bacteria in unmanipulated sediment.

However, grazing experiments with ^{15}N pre-enriched bacteria also present several advantages: (i) they can be performed *in situ* without environmental consequences, (ii) they do not require long incubations, so bias due to recycling is minimized, (iii) they require quite simple control tests with freezing of enriched prey, (iv) they can be performed at different times under standardised conditions, (v) they can be extended to other types of sediment or soil and (vi) they can be used in double-labeling experiments with ^{13}C enriched algae, in order to simultaneously measure bacterial and algal ingestion rates.

CHAPITRE II

INFLUENCE DE FACTEURS BIOTIQUES ET ABIOTIQUES SUR L'INGESTION DE BACTERIES PAR LES BACTERIVORES

CHAPITRE II A

BACTERIVORY IN THE COMMON FORAMINIFER *AMMONIA TEPIDA*: ISOTOPE TRACER EXPERIMENT AND THE CONTROLLING FACTORS

Pierre-Yves Pascal, Christine Dupuy, Pierre Richard, Nathalie Niquil

Abstract

The majority of sediment dweller foraminifera are deposit feeders. They use their pseudopodia to gather sediment with associated algae, organic detritus and bacteria. Uptake of bacteria by foraminifera have been observed but rarely quantified. We measured uptake of bacteria by the common foraminifera *Ammonia tepida* using ^{15}N pre-enriched bacteria as tracers. In intertidal flats, seasonal, tidal and circadian cycles induce strong variations in environmental parameters. Grazing experiments were performed in order to measure effects of abiotic (temperature, salinity and irradiance) and biotic (bacterial and algal abundances) factors on uptake rates of bacteria. In mean conditions, *A. tepida* grazed 78 pgC ind^{-1} h^{-1} during the first eight hours of incubation, after which this uptake rate decreased. Uptake of bacteria was optimal at 30°C, decreased with salinity and was unaffected by light. Above 7×10^8 bacteria ml wt sed^{-1}, uptake of bacteria remained unchanged when bacterial abundance increased. Algal abundance strongly affected algal uptake but did not affect uptake of bacteria. As uptake of bacteria represented 8 to 19% of microbes (algae plus bacteria) uptake, *Ammonia* seemed to be mainly dependant on algal resource.

Introduction

Benthic foraminifera are heterotrophic protozoa that have the morphological characteristics of pseudopodia and a test with one or more chambers. Since the Cambrian era, they have been present in a wide range of environments, from shallow brackish waters to deepest oceans. They are used as proxies for paleoecological studies because they are wide spread, numerous and well preserved. In recent times, foraminifera increasingly appeared as dominant members of benthic communities in both shallow and deep-sea environments (Snider et al. 1984; Alongi 1992; Gooday et al. 1992; Moodley et al. 1998; Moodley et al. 2000), suggesting that they may play an important role in food webs (Altenbach 1992; Linke et al. 1995).

Foraminifera exhibit a wide range of trophic behaviours: dissolved organic matter (DOM) uptake, herbivory, carnivory, suspension feeding and most commonly, deposit feeding (Lipps 1983). Deposit feeders are omnivorous, using their pseudopodia to gather fine-grained sediment with associated bacteria, organic detritus and, if available, algal cells. As a large part of organic detritus is indigestible, it must be cycled by bacteria before becoming available to deposit feeders (Levinton 1979). Benthic bacteria are highly abundant and productive in benthic sediments. Due to their high nutritional value they are suspected to be an important resource for sediment dwelling fauna.

Bacteria could play a major role or be an obligatory item in foraminiferal nutrition. Several littoral benthic foraminifera require bacteria to reproduce (Muller and Lee 1969) and have been shown to selectively ingest bacteria according to strain (Lee et al. 1966; Lee and Muller 1973). Some epiphytic foraminifera show a farming strategy. They produce nutrient-rich substrate for bacteria and then ingest cultured bacteria (Langer and Gehring 1993). Foraminifera are also able to feed actively on bacterial biofilms (Bernhard and Bowser 1992). Bacteria may also play a symbiotic role in bathial species of foraminifera (Bernhard 2003). Uptake of bacteria by *Ammonia* has been displayed using direct food vacuole observation (Goldstein and Corliss 1994) and bacteria labelled with fluorescent dyes (Langezaal et al. 2005). Nevertheless, those studies do not give access to quantitative data concerning the uptake rate of bacteria, and the precise role that bacteria play in foraminiferal nutrition remains elusive. Assessing grazing rate on bacteria remains a major point that must be documented to determine the role that foraminifera play in benthic food webs.

Ammonia is one of the most common genera of benthic foraminifera with a worldwide distribution in inner shelf, estuarine, and salt marsh environments (Murray

1991). One remarkable characteristic of this genus is its ability to survive over a broad range of temperatures, salinities, and seasonal regimes (Bradshaw 1961; Walton and Sloan 1990).

The aim of this study is to assess experimentally in different controlled conditions uptake rates of bacteria by *Ammonia* from an intertidal mudflat habitat (Marennes-Oléron, France). ^{15}N enriched bacteria were used as tracers to determine uptake rate of bacteria (Chapter I). This habitat is subject to large and quick changes in many environmental features. Three relevant time scales drive these environmental variations: long-term (seasonal cycle), medium-term (lunar cycle) and short-term (solar and tidal cycles) (Guarini et al. 1997). Since these variations may influence foraminiferal feeding behaviour, grazing experiments were performed in order to evaluate effects of abiotic (temperature, salinity and irradiance) and biotic (bacterial and algal abundances) factors on uptake rates of bacteria.

Experimental procedure

Study site

The Brouage intertidal mudflat is located in the eastern part of the Marennes-Oléron Bay (Atlantic coast of France). Meteorological conditions exhibit a strong seasonality typical of a temperate climate. Temperature and salinity of emerged sediments are more extreme during summer tidal cycles (Guarini et al. 1997). Minimum and maximum mud temperatures are 5°C and 34°C respectively. The maximum daily range of mud temperature due to emersion and immersion cycle reaches 18°C (Guarini et al. 1997). Salinity of overlaying water is controlled by the river Charente freshwater input, ranging from 25 to 35‰ over the year (Héral et al. 1982). Salinity of the upper layers of sediment may also decrease with rainfall. The sediment surface irradiance shifts from dark during submersion and night emersions to high levels of incident light during daytime emersions. This irradiance can reach 2000 µM of photons m^{-2} s^{-1} (Underwood and Kromkamp 2000). Details of numerous benthic organisms and processes are available concerning this intertidal zone (gathered in Leguerrier et al. 2003; Leguerrier et al. 2004; Degré et al. 2006).

Preparation of ^{15}N enriched bacteria

Superficial sediment (1 cm depth) was collected on the Brouage mudflat (45,55,074 N; 1,06,086 W). One ml of the collected sediment was added to 20 ml of bacterial liquid culture medium and kept in darkness during 24 hours at 13°C. The composition of this culture medium were previously described in Chapter I. This primary culture was then subcultured during 24 hours under the same conditions to get approximately 2×10^9 cells ml^{-1}. Finally, bacteria were collected in 0.2 µm filtered seawater after 3 centrifugations (3500 g, 10 mn, 20°C), frozen in liquid nitrogen and kept frozen at -80°C until grazing experiments.

Preparation of ^{13}C enriched algae

An axenic clone of the diatom *Navicula phyllepta* (CCY 9804, Netherlands Institute of Ecology NIOO-KNAW, Yerseke, The Netherlands), the most abundant diatom species in the study area (Haubois et al. 2005), was cultured in medium described by Antia and Cheng (1970) and containing $NaH^{13}CO_3$ (4 mM). Diatoms were concentrated by centrifugation (1500 g, 10 mn, 20 °C), washed three times to remove the ^{13}C-bicarbonate, and freeze-dried.

Quantification of bacteria and algae abundance

In order to determine the ratio between enriched and non-enriched preys in microcosms, abundances of bacteria and algae were assessed. To separate bacteria from sediment particles, incubation in pyrophosphate (0.01M during at least 30 min) and sonication (60 W) were performed. Bacteria from both sediment and culture were labelled using 4.6-diamidino-2-phenylindole dihydrochloride (DAPI) (2500 µg l^{-1}), filtered onto 0.2 µm Nucleopore black filter (Porter and Feig 1980) and then counted by microscopy. We check the absence of ciliate and flagellate in bacterial culture during this microscope observation step. Abundance of diatom in sediment was assessed using Chl *a* as a proxy, measured using fluorometry (Lorenzen 1966).

Grazing experiments

Incubation of enriched bacteria and algae with foraminifera were performed in Petri dishes (4.5 cm diameter). Experiments were done in standardized condition similar to field ones: temperature (20°C), irradiance (darkness), salinity (31‰), bacterial abundance (10.5×10^8 cells ml wt sed^{-1}) and algal abundance (15 µgChla g dry sed^{-1}). For

each type of experiment, one environmental incubation factor was modified in order to determine its impact on foraminifera grazing activity. Each experiment was carried out in triplicate, along with triplicate controls. Control samples were frozen (-80°C) in order to kill foraminifera.

During the ebb tide of 13th of March 2006, one sample of the first centimetre of sediment was collected from the Brouage intertidal mudflat (France). First, the sediment was sieved on a 500 µm mesh in order to remove macrofauna. Then, it was sieved on a 200 µm mesh to extract large foraminifera. One ml of the sediment remaining on the mesh was put in each microcosm. Sediment that passed through the 200 µm mesh was sieved through a 50 µm mesh. Fraction passing through the mesh was mixed with ^{15}N enriched bacteria. This slurry contained 10.5×10^8 cells ml of wet sediment^{-1} with a ratio of total and enriched bacteria of 1.5. Four ml of this slurry were put in each microcosm.

First, for the calculation of grazing rates, a kinetic study was realised to validate the linear or hyperbolic uptake kinetics. Incubations for this kinetic study were run during variable times (1 to 12 hours). As all other experiments were run for 5 hours, this first step is necessary to check the linear uptake during the first five hours of incubation. For each type of experiment one environmental incubation factor was modified. Light effect was tested with one irradiance (83 µM of photons m^{-2} s^{-1}). In order to decrease salinity, cultured bacteria were rinsed with 0.2 µm filtered sea-water diluted with 0.2 µm filtered fresh water (final salinity of 18‰). Bacterial abundance was modified adding various quantities of bacteria enriched in ^{15}N. Bacterial abundances (total enriched and non-enriched) tested were 4, 7 and 17 cells ml wt sed^{-1} with respectively the following ratio between abundance of total and enriched bacteria: 6.1, 2.0 and 1.3. Algal abundance was modified adding various quantities of cultured *N. phyllepta* enriched in ^{13}C while bacterial abundances (total enriched and non-enriched) were kept constant at 10×10^8 cell ml^{-1}. Algal abundance (total enriched and non-enriched) were 26, 64 and 114 µgChla g dry sed $^{-1}$ with respectively the following ratio between abundance of total and enriched algae: 2.4, 1.3 and 1.2.

Incubations were stopped by freezing the microcosms at -80°C. Samples were thawed and stained with rose Bengal in order to identify freshly dead foraminifera. For each sample, 150 specimens of the species *A. tepida* were picked up individually and cleaned of any adhering particles. Samples from experiments with ^{13}C enriched *N. phyllepta* were processed with HCl 0.1 M in silver boats to remove any inorganic carbon.

Isotope analysis and calculations

$\delta^{15}N$ and $\delta^{13}C$ of prey (bacteria and algae) and grazers (*A. tepida*) were measured using an EA-IRMS (Isoprime, Micromass, UK). Nitrogen isotope composition is expressed in the delta notation ($\delta^{15}N$) relative to air N_2: $\delta^{15}N = [(^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{reference})-1] \times 1000$. Carbon isotope composition is expressed in the delta notation ($\delta^{13}C$) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = [(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{reference})-1] \times 1000$.

Incorporation of ^{15}N is defined as excess above background ^{15}N (control experiment) and is expressed in terms of specific uptake (*I*). *I* was calculated as the product of excess ^{15}N (*E*) and biomass of N per grazer. *I* was converted in bacterial carbon grazed using C/N ratio of bacteria. *E* is the difference between the background ($F_{background}$) and the sample (F_{sample}) ^{15}N fraction: $E = F_{sample} - F_{background}$, with $F = {}^{15}N / ({}^{15}N + {}^{14}N) = R / (R + 2)$ and *R* = the nitrogen isotope ratio. For the $F_{background}$, we used control values measured with killed grazers (frozen). *R* was derived from the measured $\delta^{15}N$ values as: $R = ((\delta^{15}N/1000)+1) \times R\text{air}N_2$ where $R\text{air}N_2 = 7.35293 \times 10^{-3}$ (Mariotti 1982). The uptake of bacteria was calculated as Uptake = ($I \times$ (% $C_{enriched\ bacteria}$ / % $N_{enriched\ bacteria}$) / ($F_{enriched\ bacteria} \times$ incubation time). This uptake was multiplied by the ratio between the abundance of total and enriched bacteria determined by DAPI counts.

Incorporation of ^{13}C was calculated analogously, with $F = {}^{13}C / ({}^{13}C + {}^{12}C) = R / (R + 1)$, $R\text{air}N_2$ is replaced by $R_{VPDB} = 0.0112372$ and Uptake = I / ($F_{enriched\ bacteria} \times$ incubation time). The uptake measured was multiplied by the ratio between the abundance of total and enriched diatom, determined from fluorometrical measurements.

Enriched *N. phyllepta* carbon consisted of 22.95 ± 0.54% ^{13}C. The C/N ratio of enriched bacteria was 3.49 and bacterial nitrogen consisted of 2.88 ± 0.03% ^{15}N. The average weight of *A. tepida* used was 18.1 ± 3 µg DW (n = 115 samples of 150 specimens each). Decalcified specimens of *A. tepida* were composed on average of 1.03 ± 0.23 µg of C and 0.15 ± 0.03 µg of N. Uptake expressed as $gC_{bacteria}\ h^{-1}\ gC_{Ammonia}^{-1}$ was obtained by dividing uptake of bacteria ($gC\ ind^{-1}\ h^{-1}$) by *A. tepida* decalcified mean weight ($gC\ ind^{-1}$). Variations of uptake rates according to salinity and irradiance were tested using bilateral independent-samples t-tests. One-way analyses of variance (ANOVA) were used in order to test the impact of temperature and algal and bacterial abundance on uptake rates of bacteria and algae. The Tukey test was used for post-hoc comparisons.

Table 1. Foraminiferal isotopic compositions ($\Delta^{15}N$ and $\Delta^{13}C$ means ± SD, N = 3) and bacterial and algal uptake rates calculated.

	$\Delta^{15}N$		Bacteria uptake	$\Delta^{13}C$		Algae uptake
	Control	Normal	(pg C ind^{-1} h^{-1})	Control	Normal	(pg C ind^{-1} h^{-1})
Kinetics (hours)						
1	13.21 ± 0.33	13.40 ± 0.33	19.07 ± 33.03			
2	13.27 ± 0.18	14.50 ± 1.06	61.29 ± 52.57			
3	14.83 ± 0.12	17.02 ± 0.79	72.35 ± 26.24			
5	16.70 ± 0.19	20.08 ± 0.66	66.96 ± 13.00			
8	16.45 ± 0.21	22.33 ± 1.37	72.83 ± 16.97			
12	17.23 ± 0.48	23.67 ± 1.91	53.20 ± 15.80			
Temperature (°C)						
5	15.89 ± 0.45	16.01 ± 0.23	2.44 ± 4.54			
10	15.11 ± 0.34	16.68 ± 0.69	31.24 ± 13.63			
30	16.71 ± 0.63	22.44 ± 1.15	113.44 ± 22.77			
40	18.53 ± 0.24	21.28 ± 0.39	54.42 ± 7.65			
Irradiance						
Light	18.07 ± 0.19	21.69 ± 0.79	71.65 ± 15.73			
Salinity						
18	16.24 ± 0.48	17.87 ± 0.55	32.22 ± 10.82			
Bacterial abundance (10^8 cells ml wt sed^{-1})						
4.2	12.83 ± 0.10	12.82 ± 0.11	0.84 ± 7.78			
7.0	14.10 ± 0.10	16.70 ± 0.57	69.00 ± 15.16			
17.4	20.96 ± 1.67	24.86 ± 0.86	64.17 ± 14.20			
Algal abundance (µg Chla g dry sed^{-1})						
25.6	16.70 ± 0.19	20.55 ± 0.57	76.29 ± 11.35	-3.63 ± 1.99	9.86 ± 1.55	328,80 ± 37.77
64.3	16.94 ± 0.08	22.03 ± 0.29	100.97 ± 5.73	-11.37 ± 0.27	36.31 ± 2.39	598,90 ± 30.05
113.7	17.48 ± 0.60	21.63 ± 1.84	82.15 ± 36.55	-8.21 ± 0.50	75.73 ± 5.93	971.45 ± 68.58

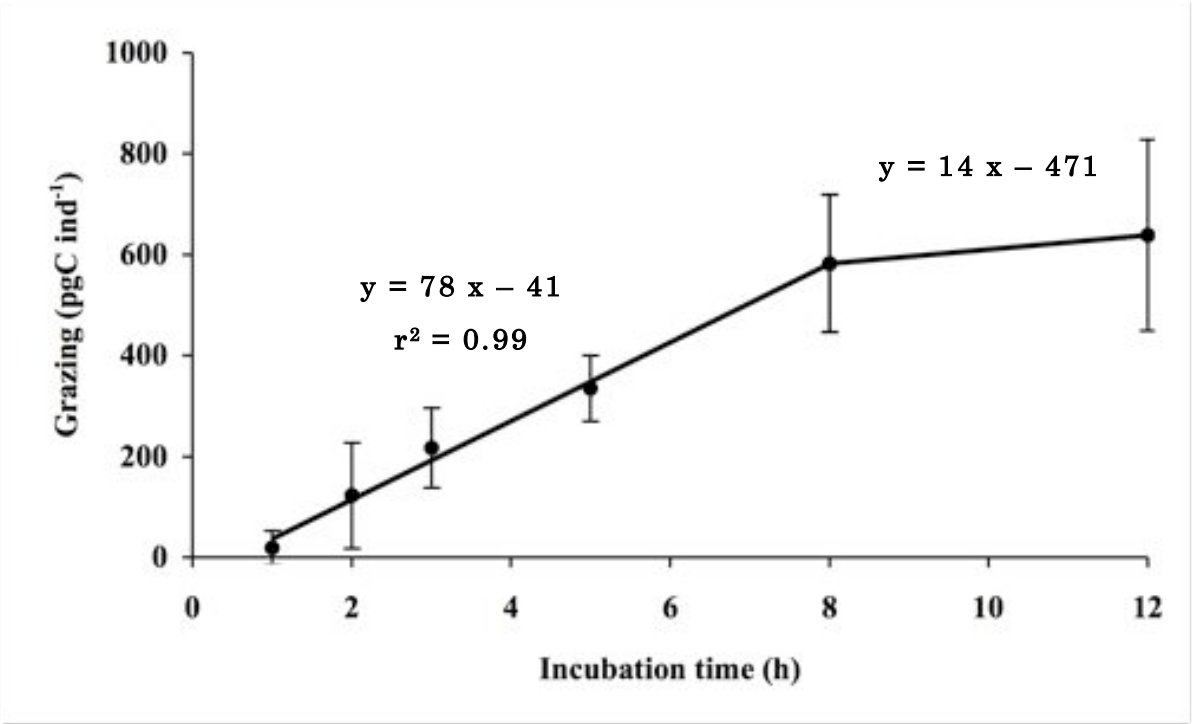


Figure 1. Bacteria uptake (mean ± SD, N = 3) as function of incubation time (h).

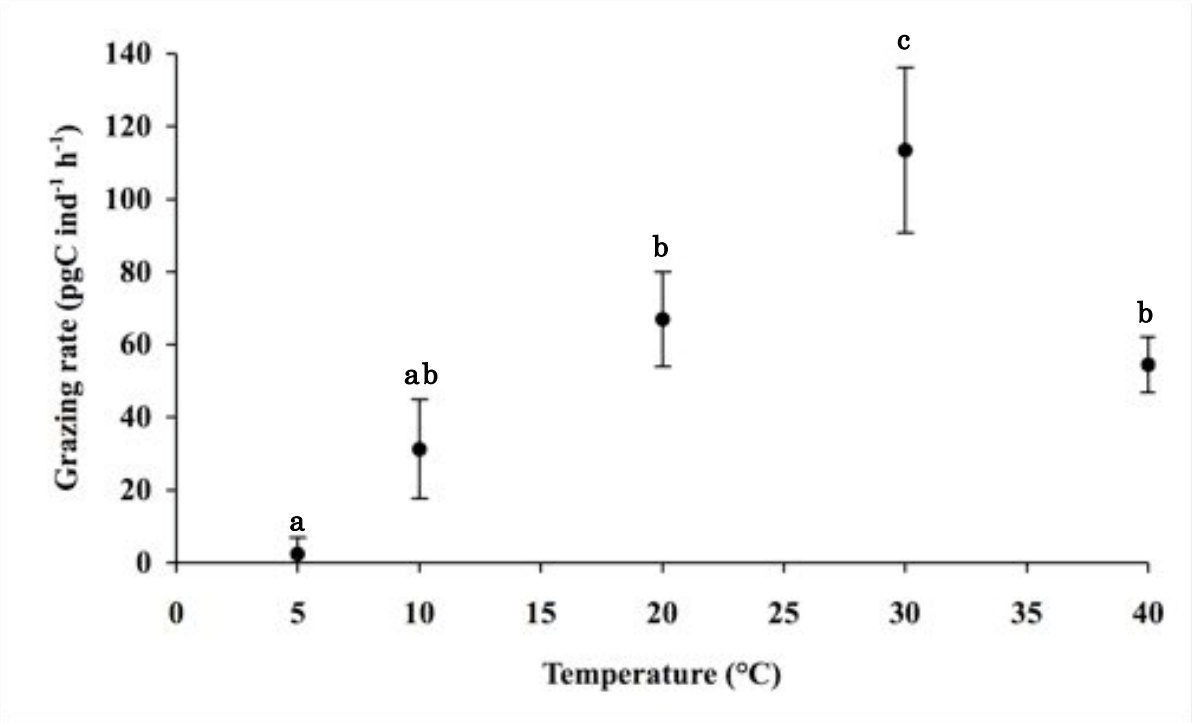


Figure 2. Bacteria uptake rate (mean ± SD, N = 3) as function of temperature (°C). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

Variations of uptake rates according to salinity and irradiance were tested using bilateral independent-samples t-tests. One-way analyses of variance (ANOVA) were used in order to test the impact of temperature and algal and bacterial abundance on uptake rates of bacteria and algae. The Tukey test was used for post-hoc comparisons.

Results

Foraminiferal isotopic compositions and rates of bacterial and algal uptakes rates are presented in Table 1.During the kinetic experiment, uptake of bacteria by *A. tepida* increased linearly during the first eight hours of incubation and then levelled off (Fig. 1). The linear regression slope for the first eight hours suggested an uptake rate of 78 pgC ind^{-1} h^{-1} equivalent to 75 × 10^{-6} $gC_{bacteria}$ $gC_{Ammonia}^{-1}$ h^{-1} (r^2 = 0.99). The linear regression slope between eight and twelve hours was more than five times lower than for the first eight hours and suggested an uptake rate of 14 pgC ind^{-1} h^{-1}.

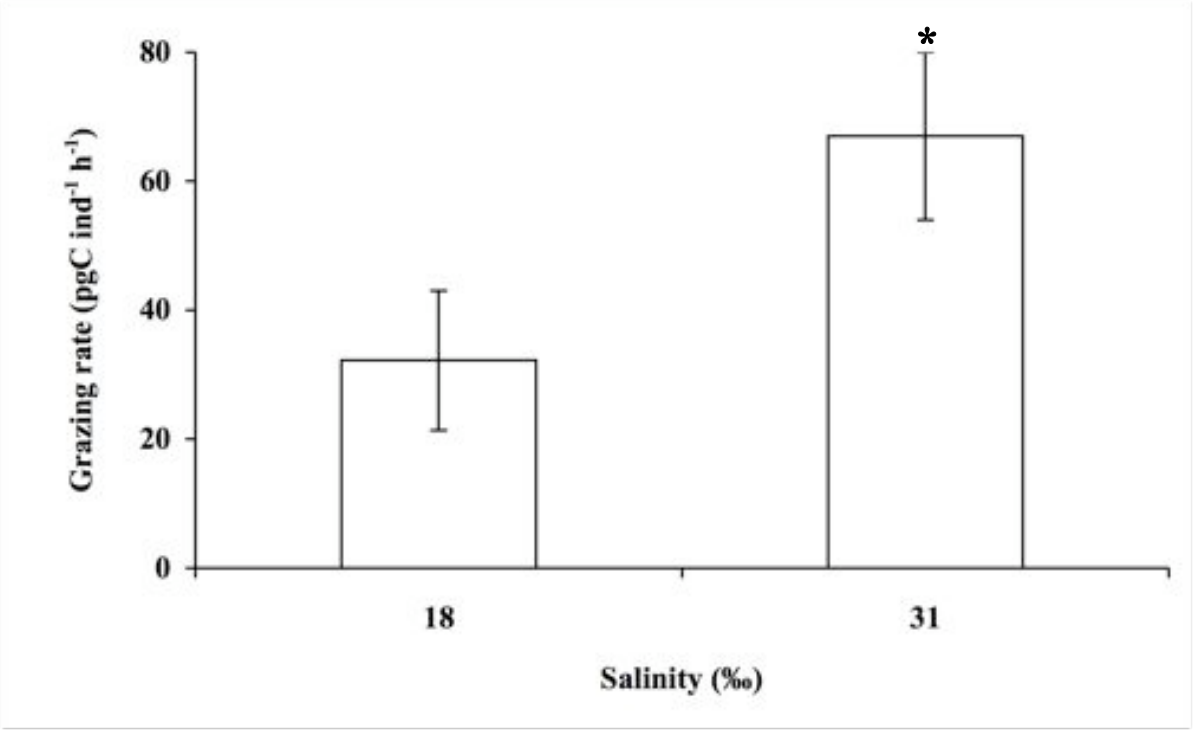
Temperature had a significant effect on *Ammonia* uptake rate of bacteria (F = 27; $p<0.001$). Temperatures tested fluctuated between 5 and 40°C and were in the range of those found in the study area (Guarini et al. 1997). Uptake of bacteria was almost null at 5°C, then increased with temperature. It reached an optimum at around 30°C and then decreased (Fig. 2). Uptake rates measured at 10, 20 and 40 °C were not significantly different. Maximum uptake rate of bacteria (30°C) reached 113 pgC ind^{-1} h^{-1} and was significantly different from others.

Uptake rate of bacteria by *Ammonia* decreased significantly from 67 to 32 pgC ind^{-1} h^{-1} when salinity dropped down from 31 to 18‰ (bilateral t-test; $p<0.05$) (Fig. 3). In the study area, salinity of overlaying water fluctuates between 25 to 35‰ (Héral et al. 1982) but salinity of sediment can be reduced by rainfall.

The sediment surface irradiance shifts from dark during submersion and night emersions to high levels of incident light during daytime emersions. Irradiance tested (83 µM of photons m^{-2} s^{-1}) corresponds to a low irradiance. Light did not affect foraminifera feeding activity: uptake rates of bacteria were similar under light and darkness (bilateral t-test; $p = 0.71$) (Fig. 3).

Ingestion of bacteria was significantly linked with abundance of bacteria in microcosm (F = 32; $p<0.001$) (Fig. 4). Four different bacterial concentrations were tested: 4, 7, 10 and 17 × 10^8 cells ml wt sed^{-1}. There was no uptake when the bacterial abundance was 4 × 10^8 cells ml wt sed^{-1} (Fig. 5), uptake rate of bacteria remained constant around 67 pgC ind^{-1} h^{-1} when bacterial concentrations increased from 7 to 17 × 10^8 cells ml wt sed^{-1} (Fig. 4).

a



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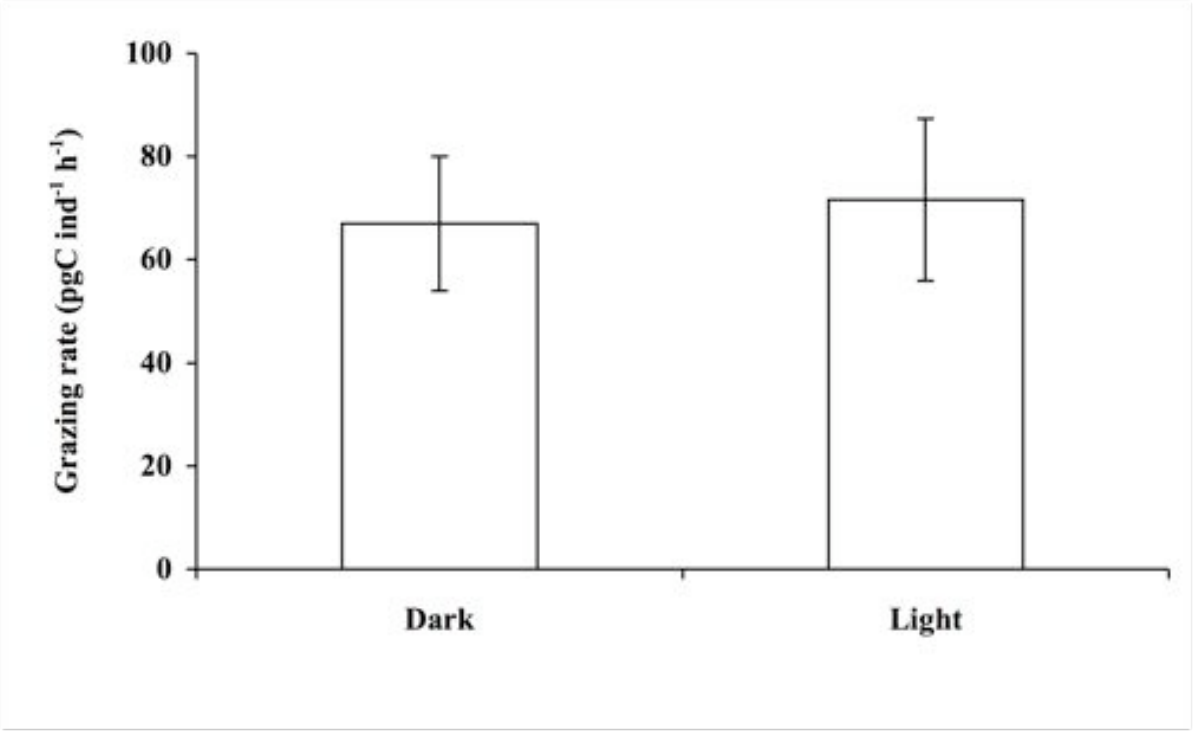

Figure 3a & 3b. Bacteria uptake rate (mean ± SD, N = 3) under low versus high salinity (a) and dark versus light incubation (b). * indicate significant difference (t-test).

When algal concentration increased from 15 to 114 µgChla g dry sed^{-1} with constant bacterial abundance (10.5 × 10^8 cell ml^{-1}), the uptake rate of bacteria remained constant (F = 1.4; p = 0.29) (Fig. 5). The uptake rate of algae increased from 329 to 971 pgC ind^{-1} h^{-1} linearly when algal abundance increased (r^2 = 0.99; p<0.001) (Fig. 7). When algal abundance increased, the fraction of algae in the diet of foraminifera increased. The fraction of bacteria decreased from 18.8 to 14.4 and 7.8% of microbes (algae plus bacteria) taken up when algal concentration was equal to 25.6, 64.3 and 113.7 µgChla g dry sed^{-1} respectively.

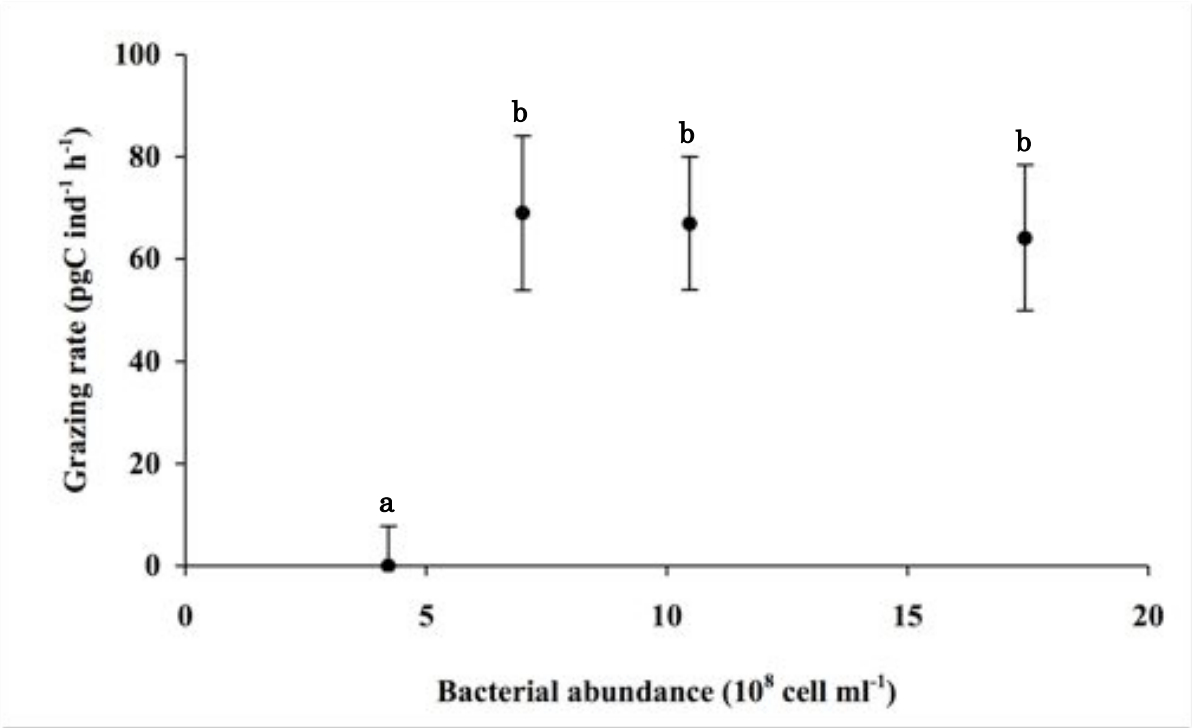

Figure 4. Bacteria uptake rate (mean ± SD, N = 3) as function of bacteria abundance (10^8 cell ml wt sed^{-1}). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

Discussion

Experimental procedure

Like all various methods previously developed and applied to measure bacterivory, the method used in the present study presents methodological shortcomings that make interpretation of the resulting problematic. For instance, sieving the sediment changes the bacterial availability for foraminifera, bacteria being not attached to particle as in natural situation. Foraminifera are known to selectively graze different bacterial strains (Lee et al. 1966; Bernhard and Bowser 1992). As grazing experiments are based on the hypothesis that grazers take up ^{15}N enriched bacteria and natural sediment bacteria at the same rate, the cultured bacteria community has to present characteristics roughly similar to the natural one. Despite the fact that culture modified the specific composition of the natural bacterial community, characteristics of size, activity and diversity of the cultured bacterial consortium in our experiments would be more representative of the natural community than in most previous grazing experiments (Chapter I). As enriched algae (monospecific and freeze-dried) may present characteristics different from natural algal community, bias due to selective ingestion of algae may exist. Control experiments were always performed in similar conditions to assess bias due to bacterial or algal cell adhesion on foraminiferal test. ^{13}C enriched freeze dried algae are potential source of enriched DOM and transfer to bacteria drive to formation of ^{13}C enriched bacteria but as incubations were short-term, we consider as negligible this bias due to recycling.

The kinetic of bacterial uptake

Foraminifera use pseudopodia in order to form a long and extensive network for trapping food particles (Travis and Bowser 1991). Actively feeding specimens are characterized by feeding cysts. Aggregates of particles are firmly attached around the test apertures and they may encompass the entire test. Collected material is partitioned into small fractions before ingestion. The possibility of extracellular digestion (Meyer-Reil and Köster 1991) and reticulopodial digestion (Lee et al. 1991) has been suggested. Food vacuoles contain large amounts of sediment, organic detritus, algal cells and bacteria (Goldstein and Corliss 1994). Those vacuoles are most abundant in the terminal chamber, but occur throughout the last four chambers as well. The digestion of bacteria seems to occur in the terminal chamber of *Ammonia* (Goldstein and Corliss 1994; Langezaal et al. 2005). This genus ingests bacteria and readily digests them, implying

that bacteria are more probably used as food source than as symbionts (Langezaal et al. 2005).

Langezaal et al. (2005) found that *Ammonia beccarii* grazed 90 bacteria during a 20 h period. Converting their uptake rate into bacterial biomass (Norland et al. 1995), gives a grazing rate of 1.7 pgC ind^{-1} h^{-1}. This rate is lower than the rate found in the present study (78 pgC ind^{-1} h^{-1}). This may be linked to the bacterial concentration used by Langezaal et al. (2005) in their microcosms (1.4×10^3 cells ml^{-1}), which was substantially lower than benthic bacterial abundance in natural environments (c.a. 10^9 cells ml^{-1}) and in the present study.

In present experiments, uptake of bacteria by *Ammonia* was rapid and detectable after 2 hours of incubation (Fig. 1). This is in accordance with Moodley et al. (2000) who observed a detectable uptake of algal carbon by *Ammonia* after three hours of incubation. Uptake of bacteria increased linearly during the first eight hours and then levelled off (Fig. 1). A similar pattern was observed for uptake of algae by *Ammonia* (Moodley et al. 2000). This levelling off may reflect satiation or more likely excretion, effective after eight hours. Foraminifera have been reported to assimilate ingested algal carbon within 12 h (Rivkin and De Laca 1990) and *Ammonia* was found to assimilate carbon from phytodetritus in 12 h (Moodley et al. 2000). A simplified energy budget can be calculated with following parameters, $C = A + FU$ (C = food uptake, A = assimilation of metabolisable energy, FU = loss by faeces and urinary wastes) (Klekowski et al. 1979; Schiemer 1982). We suggest that during the first eight hours of incubation, *Ammonia* takes up and assimilates bacteria. Then, after eight hours, uptake and assimilation still occur but excretion begins. By assuming, that uptake is constant during all the grazing experiments, assimilation and excretion rates can be assessed. The first slope from the origin to eight hours would correspond to the uptake rate while the second slope would correspond to the assimilation rate. *Ammonia* retains five times more tracer during the first eight hours than after. As a result, 17% of ingested bacteria would be assimilated and 83% would be rejected. This result is disputable because the uptake rate constancy was not determined and the uptake rate after eight hours is determined from only two data points, however, this result fits well with assimilation rate of bacteria by nematodes (25%) (Herman and Vranken 1988) and polychaetes (26%) (Clough and Lopez 1993).

Effects of abiotic factors

Studies on the influence of environmental factors on *Ammonia* are limited. However, Bradshaw (1957; 1961) determined the influence of temperature and salinity on *Ammonia* reproductive activity, growth rate and survival under experimental conditions.

In the present experiments, temperature had a similar effect on uptake rate of bacteria to the one shown by Bradshaw on growth and reproductive rates. We found no uptake of bacteria at 5°C (Fig. 2), in accordance with Bradshaw, showing that foraminifera metabolism is very low at low temperatures. Under a temperature less than 10°C, *Ammonia* fail to grow and reproduce and individuals appear to live an indefinitely long period (Bradshaw 1957). The optimal grazing temperature appeared at 30°C (Fig. 2) as in Bradshaw's experiments (Bradshaw 1961). When temperature exceeded 30°C in our microcosms, grazing rate decreased. Similarly, no growth was observed in Bradshaw's experiments, and specimens lived less than one day at 35°C (Bradshaw 1957). Those physiological characteristics have implications for foraminiferal abundances at the seasonal time scale. Limited activity during winter prevents reproduction and limits abundance, while in summer, high temperature can lead to mortality, in particular in the intertidal habitat subject to a wide range of rhythmically and rapidly varying temperature due to tidal cycles.

Uptake rate of bacteria declined when salinity was reduced to 18‰ (Fig. 3). This result is also in accordance with those of Bradshaw (1957; 1961). According to this author, normal growth and reproduction of *Ammonia* occur when salinity fluctuates between 20 and 40‰, and *Ammonia* failed to grow below 13‰. *Ammonia* is an euryhaline genus found from brackish (Debenay and Guillou 2002) to hypersaline environments (Almogi-Labin et al. 1992). However, conditions of brackish environments would not be optimal for *Ammonia*. Foraminifera use a network of pseudopoda to gather and ingest food particles. Each pseudopoda contains an elongated cytoskeleton primarily composed of microtubules. Modifications of salinity induce a decrease of the number of pseudopodal microtubules (Koury et al. 1985), that may lead to a lower pseudopodal efficiency. This could explain the lower uptake rate observed at low salinity, considering that sediment salinity is under control of tidal cycles and weather conditions, like rainfall, which induces a strong decrease of salinity during low tide.

Light did not affect uptake rate of *Ammonia* (Fig. 3). Although foraminifera frequently form symbiosis with algae, *Ammonia* is not known as an algal-bearing foraminifera (Lee and Anderson 1991). Consequently, irradiance would not influence feeding behaviour of

Ammonia. This is confirmed by our grazing experiment results with a low irradiance rate. Owing to this, *Ammonia* seems not influenced by nycthemeral cycles and seems able to graze in superficial sediment exposed to sun in the temperature range that allows grazing. This suggests that *Ammonia* is able to feed actively in environment affected by irradiance variations.

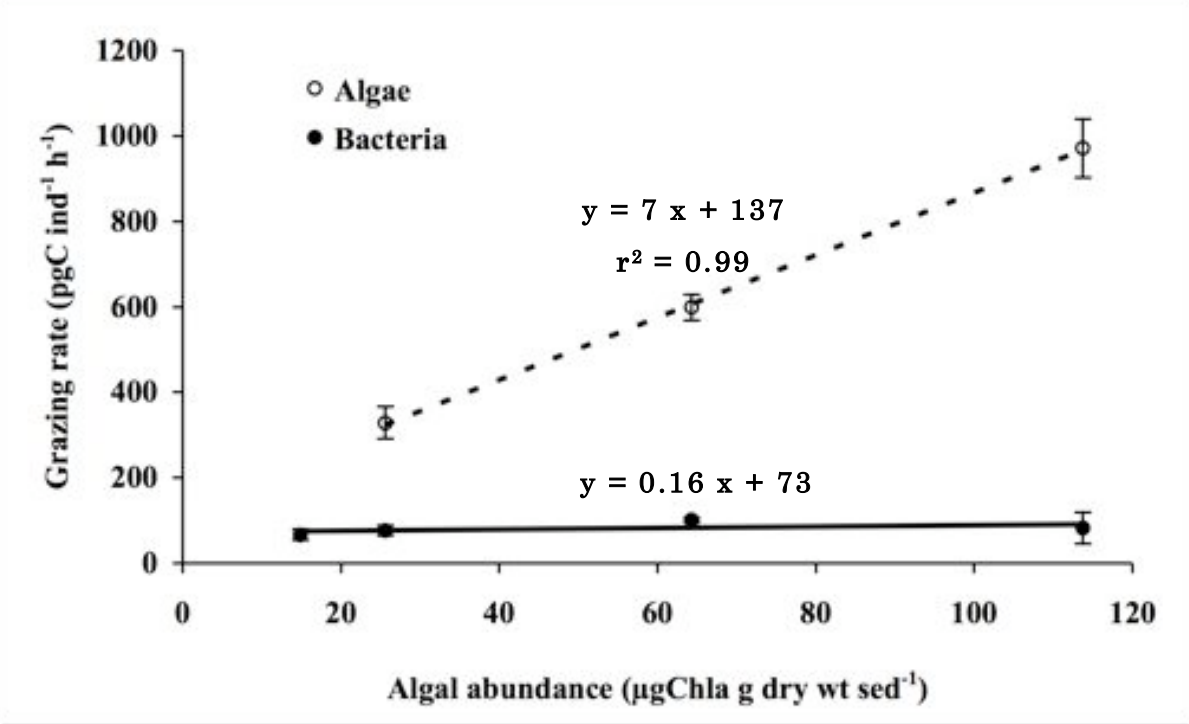

Figure 5. Algae uptake rate ○ (mean ± SD, N = 3) and bacteria uptake rate ● (mean ± SD, N = 3) as function of algal abundance (µgChla g dry wt sed^{-1}) under constant bacteria abundance (10.5×10^8 cell ml wt sed^{-1}).

Effects of biotics factors

Sedimented organic carbon from the photic zone can represent a major food source for deep-sea benthic foraminifera (Gooday 1988). Many studies report that the abundance of benthic foraminifera assemblages is strongly correlated with surface ocean productivity (e. g. Altenbach et al. 1999; Fontanier et al. 2002). Quick uptake of phytodetritus was observed within the deep sea but also within shallow water dwellers (Middelburg et al. 2000; Moodley et al. 2000; Moodley et al. 2002; Moodley et al. 2005; Nomaki et al. 2005a; Nomaki et al. 2005b). This high reactivity to food pulses may imply that uptake rates are strongly linked with food abundance and type of food.

Theoretically, food uptake by a grazer increases with abundance of food. However, above a threshold value of prey concentration, uptake rate remains constant (Holling's

prey-dependent type II functional response (Holling 1959)). Uptake of bacteria is not detectable at the lowest bacterial concentration of 4.2×10^8 cells ml wt sed^{-1} (Fig. 4). This lack of uptake may occur for different reasons. One possibility could be that foraminifera does not feed at low bacterial concentrations. Most probably, the ratio between enriched and non-enriched bacteria used in our experiments was not high enough to allow uptake detection at low concentration. Above 7×10^8 cells ml wt sed^{-1}, the rate of uptake of bacteria remained constant, despite the increased of bacterial abundance (Fig. 5). The threshold value of prey abundance may have been overshot, which would mean that uptake by *Ammonia* would seldom be higher than those measured. Bacterial abundance in superficial marine sediment is relatively constant around 10^9 cells ml wt sed^{-1} and seldom lower than 7×10^8 cells ml wt sed^{-1} (Schmidt et al. 1998). According to these data, bacterial abundance in natural environment would always satisfy the *Ammonia* optimal uptake rate and would never be limiting factor for uptake.

Algal uptake rate increased linearly with algal abundance reaching 971 pgC ind^{-1} h^{-1} without levelling off for the tested values (Fig. 5). Algal abundances used during this experiment (15 to 114 µgChla g dry sed^{-1}) are not high enough to reach the maximum algal uptake rate; indeed *Ammonia* was found to graze at a higher rate on *Chlorella* (2180 pgC ind^{-1} h^{-1}) (Moodley et al. 2000). In natural conditions, chlorophyll *a* content of the first centimetre of sediment varies between 0 and 50 µgChla g dry sed^{-1} (review in MacIntyre et al. 1996). However, through vertical migration, benthic microalgae concentrate near the surface during diurnal low tides producing a biofilm. In this algal mat, concentration of chlorophyll *a* can reach 150 µgChla g dry sed^{-1} (Serôdio et al. 1997) and even 300 µgChla g dry sed^{-1} (Kelly et al. 2001). *Ammonia* feeding on the algal biofilm would then present a higher uptake rate than in the present study.

The use of differential labelling of bacterial food (^{15}N) and algal food (^{13}C) allows to access simultaneous uptake rates of bacteria and algae, thus permitting to determine the preferred item according to their availability. While algal uptake increased with algal abundance, uptake of bacteria remained constant (Fig. 5). *Ammonia* still ingested bacteria when other food resources were available. Bacteria might be a source of essential compounds for deposit feeders (Lopez and Levinton 1987). This assumption is in accordance with Muller and Lee (1969) who suggested that some foraminifera reproduce only when bacteria are present as food source. Then, uptake of bacteria would be essential for *Ammonia*.

Uptake of bacteria by *Ammonia* never represented more than 19% of microbial biomass (bacteria plus algae) taken up. This low contribution of bacteria to food uptake

was also observed with algal concentrations comparable with sediment natural conditions of 25 µg Chla g dry sed^{-1} (MacIntyre et al. 1996). Muller (1975) suggest that shallow water dwelling species mainly depend on algal resources. In their study, van Oevelen et al. (2006) found that bacterial carbon constitutes only 9% of total needs of hard-shelled foraminifera of an intertidal mudflat community.

In intertidal areas, algal abundances vary seasonally (e. g. Haubois et al. 2005). In addition, benthic microalgae of intertidal sediments vertically migrate with rhythms associated with diurnal and tidal cycles (Blanchard et al. 2001). During day time, at low tide, algal cells concentrate near the surface of sediment and form a mat (Herlory et al. 2004). According to our results, *Ammonia* seems to depend principally on algal feeding resource. For this reason, this species may feed on the mat of microphybenthos when it is formed in order to maximize its rate of energy gain. This feeding behaviour would imply that *Ammonia* dwells at the surface of the sediment during low tide. Thus, *Ammonia* would be subject to all of the fast and large environmental variations that are typical of the intertidal habitat, especially at the air-sediment interface during low tide. Though *Ammonia* is considered as one of the most tolerant genus of foraminifera to temperature and salinity variations (Bradshaw 1961; Walton and Sloan 1990), we showed that variations of these parameters influence uptake of bacteria (Fig. 2 and 3). Vertical migration from the food-rich surface into deeper layers is a possible mechanism for foraminifera to avoid unfavourable conditions (Groß 2002). In this deeper layer, bacteria would constitute a large part of the diet of *Ammonia*. When temperature and salinity allow *Ammonia* to migrate to the surface sediment, *Ammonia* would principally graze on the microphytobenthic mat.

In conclusion, bacteria appeared to be quantitatively of minor importance in the nutrition of foraminifera compared to algae. The present work demonstrates that, at the tidal scale, grazing rate of bacteria is affected by abiotic (temperature and salinity) whereas it would not be affected by biotic (algal and bacterial abundances) factors. *A. tepida* may further respond to environmental changes at a seasonal scale, by physiological adjustment and shifting of its optimum conditions. However, the present study does not permit to evaluate this acclimation capacity and more efforts need to be made to take it into account.

CHAPITRE II B

BACTERIVORY OF A MUDFLAT NEMATODE COMMUNITY UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Pierre-Yves Pascal, Christine Dupuy, Pierre Richard,
Jadwiga Rzeznik-Orignac, Nathalie Niquil

Abstract

The fate of the benthic bacterial biomass is a topic of major importance in understanding how soft-bottom environments function. Because of their high abundance, production and nutritional value, benthic bacteria may constitute an important food resource for benthic fauna. The trophic role of bacteria for a nematode community on the Brouage mudflat (Marennes-Oléron-France), dominated by three species: *Chromadora macrolaima* (64% of the abundance), *Daptonema oxycerca* (15%) and *Ptycholaimellus jacobi* (8%), was determined in grazing experiments using ^{15}N pre-enriched bacteria. On intertidal flats, seasonal, tidal and circadian cycles induce strong variations in environmental conditions. Grazing experiments were performed in order to measure the effects of abiotic (temperature, salinity and luminosity) and biotic (bacterial and algal abundances) factors on assimilation rates of bacteria by nematodes. In order to assess simultaneously bacteria and algal assimilation rates, algal abundances were modified adding ^{13}C pre-enriched *Navicula phyllepta*. Assimilation rate was significantly lower at 5°C, moreover general trend show a prominent temperature effect with an optimum around 30°C. Assimilation at salinity 18 was not significantly different from the assimilation at salinity 31. Assimilation was higher under light condition than in the dark. Above 10^9 bacteria ml^{-1}, assimilation of bacteria remained unaffected by bacterial abundance. However, assimilation of algae increased with the algal concentration. Nematode kept feeding under conditions of stress which are typical of the surficial sediment habitat and they appeared to be principally dependent on the algal resource.

Introduction

Bacteria play a major role in cycling organic matter in marine sediments through the remineralization of nutrients and organic matter and production of particulate and dissolved carbon (e.g. Legendre and Rassoulzadegan 1996; Rivkin et al. 1996). Although bacterial abundance remains stable, around 10^9 cells ml^{-1} (review in Schmidt et al. 1998), bacterial production rates vary greatly (Sander and Kalff 1993). This discrepancy between abundance and production may be induced by bottom-up, biochemical and top-down control (van Oevelen et al. 2006a). In the top-down control situation, bacterial carbon is assumed to be regulated by higher trophic levels of benthic food webs.

The majority of animals feeding on sedimentary deposits are more dependent on attached bacteria than on nonliving organic debris (e. g. Fenchel 1972). Models of benthic ecosystems emphasize the role of bacteria and their immediate grazers as a major route through which organic material is processed (e. g. Kuipers et al. 1981). Consequently, quantitative data dealing with the link between bacteria and benthic fauna are essential for understanding the extent to which this trophic link structures energy and material fluxes in the communities.

Results concerning the impact of meiofaunal grazing on bacteria are conflicting (Kemp 1990). Montagna (1984b) suggested that meiofaunal grazing pressure (principally through polychaetes) represents a significant stimulatory effect on the microbial community and may be important in sandy sediments (Montagna and Bauer 1988). However, quantitative studies on meiobenthos bacterivory are sparse.

Although they are small and inconspicuous, nematodes are consistently the most abundant meiobenthic taxon in mudflat sediments. Their average densities of 10^6 ind m^{-2} represents a biomass of roughly 0.2 to 2 gC m^{-2} and some authors have suggested that their ecological significance is great in terms of food-web relationships (review in Platt and Warwick 1980; Heip et al. 1985).

Benthic bacteria can constitute a significant food source for nematodes (Lopez et al. 1979; Tietjen 1980; Montagna 1984b; Montagna 1995; Moens et al. 1999b). According to Wieser (1960), there are four different feeding groups of nematodes: selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A) and omnivore-predators (2B). Nematodes of each feeding groups are potential bacterivores, even predacious may benefit directly from bacterial carbon (Moens et al. 1999b). Deposit and epigrowth feeders feed on bacteria and unicellular eukaryotes in different ways. Deposit-feeding species have no teeth and generally swallow the food whole and undamaged.

They feed predominantly on bacteria associated with detritus. The epigrowth feeding species puncture the cell membrane with their teeth and ingest only the cell contents (juice feeders). Diatoms and other benthic microalgae are known to be important trophic sources for many epigrowth feeders but the importance of bacteria as a food source remains poorly documented (Moens and Vincx 1997). The aim of this study was to experimentally assess rates of bacteria uptake by a nematode community from an intertidal mudflat using ^{15}N enriched bacteria as tracers. The intertidal habitat studied is subject to a wide range of environmental varying factors. Three relevant time scales drive these environmental variations: long-term (seasonal cycle), medium-term (lunar cycle) and short-term (solar and tidal cycles) (Guarini et al. 1997). Variations concern both biotic (i.e. temperature, salinity and luminosity) and abiotic factors (i.e. bacterial and algal abundances). As those variations may influence the feeding behavior of nematodes, one aim of the present study is to determine if nematodes bacterivory is constant in the mudflat or influenced by environmental factors. Other aim is to describe feeding behavior of nematodes when an alternative algal resource is available.

For this purpose, a mudflat nematode community from surficial sediment was put in microcosms, in contact with labeled preys: a bacterial community and one algal species. Grazing experiments were performed in order to evaluate effects of abiotic (temperature, salinity and luminosity) and biotic (bacterial and algal abundances) factors on rates of prey uptake. We focused on the surficial mudflat sediment nematode community because the surficial sediment (i) has high bacterial production, (ii) contains the highest nematodes densities and (iii) undergoes faster and more wide-ranging changes in environmental factors than do the deeper layers.

Material and methods

Study site

The Brouage intertidal mudflat is located in the eastern part of the Marennes-Oléron Bay (Atlantic coast of France). Meteorological conditions exhibit a strong seasonality typical of a temperate climate. Temperature and salinity of emerged sediments are more extreme during summer tidal cycles (Guarini et al. 1997). Minimum and maximum mud temperatures are 5°C and 34°C respectively. The maximum daily range of mud temperature due to emersion and immersion cycle reaches 18°C (Guarini et al. 1997). Salinity of overlaying water is controlled by the river Charente freshwater input, ranging from 25 to 35 over the year (Héral et al. 1982). Salinity of the upper

layers of sediment may also decrease with rainfall. The sediment surface irradiance shifts from dark during submersion and night emersions to high levels of incident light during daytime emersions. This irradiance can reach 2000 µM of photons m^{-2} s^{-1} (Underwood and Kromkamp 2000). Details of numerous benthic organisms and processes are available concerning this intertidal zone (gathered in Leguerrier et al. 2003; Leguerrier et al. 2004; Degré et al. 2006).

^{15}N enriched bacteria and ^{13}C enriched algae as tracers

The method used was described in Chapter I. This method is based on the assumption that grazers ingest unselectively enriched and natural bacteria. Briefly, one centimeter-depth of surficial sediment was sampled during ebb tide in the Brouage mudflat (45°55N, 1°06W) (Fig. 1). Bacteria from surficial sediment were cultured in a liquid bacterial culture medium containing $^{15}NH_4Cl$ 1 g l^{-1} (99% ^{15}N-enriched NH_4Cl CortecNet), rinsed by centrifugation and frozen until the grazing experiments. An axenic clone of the *Navicula phyllepta* diatom (CCY 9804, Netherlands Institute of Ecology NIOO-KNAW, Yerseke, The Netherlands) was cultured in a liquid medium containing $NaH^{13}CO_3$, then rinsed and freeze-dried until the grazing experiments. Isotopic composition of enriched prey was assessed using mass spectrometer. For these experiments, labeled preys were mixed with sediment from the Brouage mudflat that had been previously sieved through a 50 µm mesh. The abundance of bacteria and algae in the slurry was estimated in order to determine the ratio between enriched and unenriched prey.

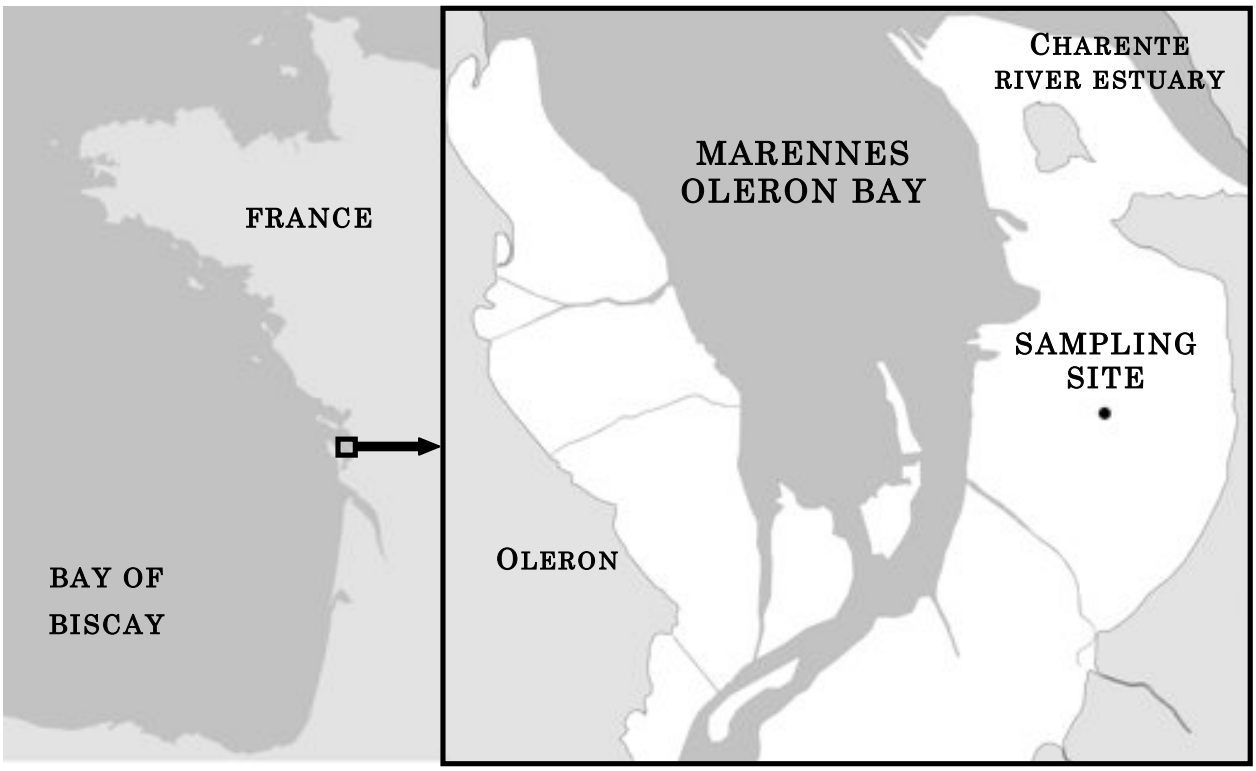

Figure 1. Map of the study site.

Grazing experiments

The top centimeter of sediment was collected on March 13, 2006. At the time of sampling, sediment presented a temperature of 7°C and a salinity of 29. Sediment sampled was first sieved through a 500 µm mesh to remove macrofauna, then through a 50 µm mesh to extract meiofauna. One ml of the sediment remaining in the mesh was put into each microcosm (Pyrex beakers, ø = 4.5 cm) and the fraction which passed through the mesh was mixed with ^{15}N enriched bacteria. This slurry contained 10.5×10^8 bacteria ml^{-1}, with the ^{15}N enriched bacteria being twice as abundant as unenriched ones. Four ml of this slurry were put into each microcosm. Each experiment was carried out in triplicate. Control samples were frozen (-80°C) in order to kill any nematodes.

A kinetic study was performed to validate its linear or hyperbolic shape in order to calculate the grazing rate. Incubations for this kinetic study were run for 1 to 12 hours, including the 3 hours run that was used for all other experiments. Incubations were made under the following standardized conditions that were close to the year-round mean values recorded on the study site: temperature (20°C), salinity (31), luminosity (darkness), bacterial abundance (10.5×10^8 bacterial cell ml^{-1}) and algal abundance (15 µgChla g^{-1}).

For each other experiment one environmental incubation factor was modified. In order to decrease salinity, cultured bacteria were rinsed with 0.2 µm filtered-sea-water diluted with 0.2 µm filtered water (final salinity of 18). The light effect was tested with a light intensity of 83 µM photons m^{-2} s^{-1}. Bacterial abundances (total enriched and non-enriched) tested were 4, 7 and 17 cells ml wt sed-1 with respectively the following ratio between abundance of total and enriched bacteria: 6.1, 2.0 and 1.3. Algal abundance was modified by adding various quantities of cultured *N. phyllepta* enriched in ^{13}C while bacterial abundances (total enriched and non-enriched) were kept constant at 10×10^8 cells ml^{-1}. Using two isotopes for labeling bacterial (^{15}N) and algal food (^{13}C) offers the opportunity to assess bacterial and algal ingestion rates simultaneously. Algal abundance (total enriched and non-enriched) were 26, 64 and 114 µgChla g dry sed^{-1} with respectively the following ratio between abundance of total and enriched algae: 2.4, 1.3 and 1.2.

Incubations were halted by freezing the microcosms at -80°C. Samples were thawed and nematodes were extracted from the sediment using Ludox TM (Heip et al. 1985). For each sample, at least 700 nematode specimens were picked up randomly and

individually with Pasteur pipette, rinsed twice in Milli-Q water to remove adhering particle and transferred in aluminium cup.

Isotope analysis and calculations

$\delta^{15}N$ and $\delta^{13}C$ of prey (bacteria and algae) and grazers (nematodes) were measured using an EA-IRMS (Isoprime, Micromass, UK). Nitrogen isotope composition is expressed in the delta notation ($\delta^{15}N$) relative to air N_2: $\delta^{15}N = [(^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{reference})\text{-}1] \times 1000$. Carbon isotope composition is expressed in the delta notation ($\delta^{13}C$) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = [(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{reference})\text{-}1] \times 1000$.

The ^{15}N incorporated was defined as excess (above the background level) ^{15}N and is expressed in terms of specific uptake (I). I was calculated as the product of excess ^{15}N (E) and the biomass of N per grazer. E is the difference between the background ($F_{background}$) and the sample (F_{sample}) ^{15}N fraction: $E = F_{sample} - F_{background}$, with $F = {}^{15}N / ({}^{15}N + {}^{14}N) = R / (R + 2)$ and R = the nitrogen isotope ratio. For the $F_{background}$, we used highest control values ($\Delta^{15}N = 11.78$ and $\Delta^{13}C = -16.34$) measured using killed (frozen) grazers. R was derived from the measured $\delta^{15}N$ values as: $R = ((\delta^{15}N/1000)+1) \times R\text{air}N_2$ where $R\text{air}N_2 = 7.35293\ 10^{-3}$ (Mariotti 1982). The uptake of bacterial carbon was calculated as Uptake = ($I \times$ (% $C_{enriched\ bacteria}$ / % $N_{enriched\ bacteria}$) / ($F_{enriched\ bacteria} \times$ incubation time). This uptake was multiplied by the ratio between the abundance of total and enriched bacteria determined by DAPI counts.

Incorporation of ^{13}C was calculated analogously, with $F = {}^{13}C / ({}^{13}C + {}^{12}C) = R / (R + 1)$, $R\text{air}N_2$ is replaced by $R_{VPDB} = 0.0112372$ and Uptake = I / ($F_{enriched\ bacteria} \times$ incubation time). The uptake measured was multiplied by the ratio between the abundance of total and enriched diatom, determined from fluorometrical measurements.

Enriched *N. phyllepta*-produced carbon consisted of 22.95 ± 0.54% ^{13}C. The C/N ratio of enriched bacteria was 3.49 and bacterial nitrogen consisted of 2.88 ± 0.03% ^{15}N. The average weight of nematodes was 0.33 ± 0.18 µg DW and each nematode was composed on average of 0.11 ± 0.05 µgC and 22.28 ± 5.82 ngN (N = 72 samples of at least 700 specimens each). Uptake expressed as $gC_{bacteria}\ gC_{nematode}^{-1}\ h^{-1}$ was obtained by dividing the uptake of bacteria ($gC\ ind^{-1}\ h^{-1}$) by the mean nematode weight ($gC\ ind^{-1}$).

Variations of assimilation rates with respect to the salinity and luminosity were tested using two-tailed test. One-way analysis of variance (ANOVA) was used in order to test the impact of temperature and algal and bacterial abundance on the uptake rates of bacteria and algae. The Tukey test was used for post-hoc comparisons.

Nematode community composition

Nematode communities used in the grazing experiments were extracted from sediment with Ludox TM (Heip et al. 1985). In order to determine the taxonomic composition of the community studied, 303 nematodes were collected at random, determined to species or generic level under the microscope and sorted by feeding group as indicated by Wieser (1953; 1960).

Results

Composition of the nematode community

In the sample collected for the taxonomy of the nematode community, 19 species belonging to 18 genera were observed (Tab. 1). Three species made up more than 87 % of the community: *Chromadora macrolaima* (64%), *Daptonema oxycerca* (15%) and *Ptycholaimellus jacobi* (8%). The other species were much less abundant, 11 representing less than 1 %.

The community was dominated by epigrowth feeders 2A (75%) due to high abundances of *C. macrolaima* and *P. jacobi*. Non-selective deposit feeders 1B (21%) were the second most abundant trophic group due to high abundance of *D. oxycerca*. Selective deposit feeders 1A (2%) and omnivores/predators 2B (1%) exhibited marginal abundances in the community studied.

Table 1. List of species ranked by dominance (feeding types according to Wieser (1953, 1960)). 1A: selective deposit feeders; 1B: non-selective deposit feeders; 2A: epigrowth feeders; 2B: omnivores/predators

Genera species	Feeding type	Abundance relative (%)
Chromadora macrolaima	2A	64.2
Daptonema oxycerca	1B	15.2
Ptycholaimellus jacobi	2A	7.9
Sabatieria pulchra	1B	2.6
Axonolaimus paraspinosus	1B	1.7
Praeacanthonchus punctatus	2A	1.7
Halalaimus sp.	1A	1.0
Aegialoalaimus sp.	1A	1.0
Sphaerolaimus gracilis	2B	0.7
Spilophorella sp.	2A	0.7
Metachromadora sp.	2A	0.7
Theristus sp.	1B	0.3
Parodontophora marina	1B	0.3
Tripyloides marinus	1B	0.3
Eleutherolaimus sp.	1B	0.3
Desmolaimus zeelandicus	1B	0.3
Daptonema hirsutum	1B	0.3
Terschellingia sp.	1A	0.3
Viscosia sp.	2B	0.3

Table 2. Nematodes isotopic composition ($\Delta^{15}N$ and $\Delta^{13}C$ mean ± SD, N = 3) and bacterial and algal uptake rates calculated.

	$\Delta^{15}N$		Bacteria uptake	$\Delta^{13}C$		Algae uptake
	Control	Normal	(pg C ind^{-1} h^{-1})	Control	Normal	(pg C ind^{-1} h^{-1})
Kinetics (hours)						
1		12.01 ± 0.25	3.67 ± 4.06			
2		14.24 ± 0.86	20.00 ± 7.03			
3	11.78 ± 0.23	15.20 ± 0.38	18.51 ± 2.05	-15.4 ± 1.32		
5		20.63 ± 1.49	28.80 ± 4.84			
8		27.72 ± 1.55	32.41 ± 3.15			
12	11.56 ± 0.87	32.66 ± 4.91	28.31 ± 6.65	-15.8 ± 0.77		
Temperature (°C)						
5		12.41 ± 0.53	3.38 ± 2.87			
10		14.60 ± 0.50	15.26 ± 2.70			
30		16.38 ± 0.79	24.92 ± 4.27			
40		15.18 ± 1.73	18.42 ± 9.38			
Irradiance						
Light		19.42 ± 2.01	41.43 ± 10.88			
Salinity						
18		15.03 ± 0.66	17.62 ± 3.57			
Bacterial abundance (10^8 cells ml wt sed^{-1})						
4.2		11.75 ± 0.20	-0.65 ± 4.39			
7.0		12.25 ± 0.40	3.34 ± 2.93			
17.4		15.86 ± 0.10	18.34 ± 0.45			
Algal abundance (µg Chla g dry sed^{-1})						
25.6		15.39 ± 0.59	19.58 ± 3.19		-4.44 ± 6.53	58.94 ± 32.35
64.3		15.58 ± 0.51	20.59 ± 2.78		25.60 ± 4.79	107.11 ± 12.24
113.7	11.50 ± 0.17	14.34 ± 0.61	13.87 ± 3.33	-16.34 ± 0.53	52.14 ± 33.29	161.20 ± 78.30

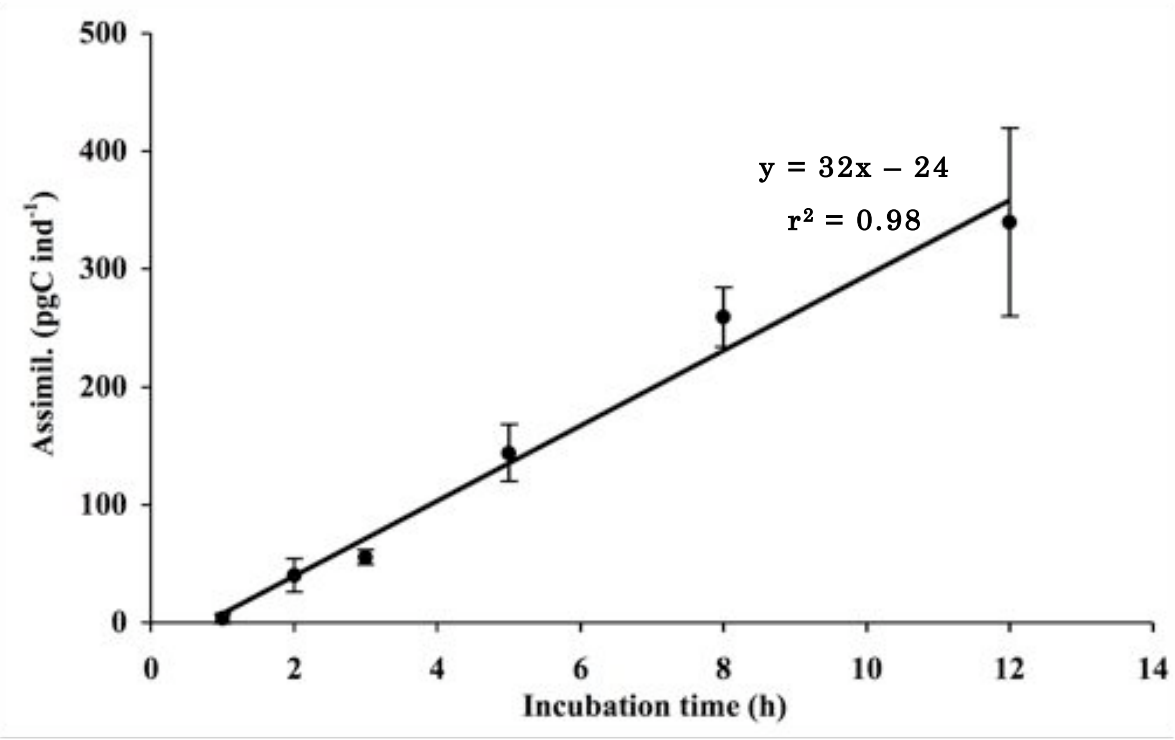

Figure 2. Assimilation of bacterial carbon (mean ± SD, N =3) as function of incubation time (h).

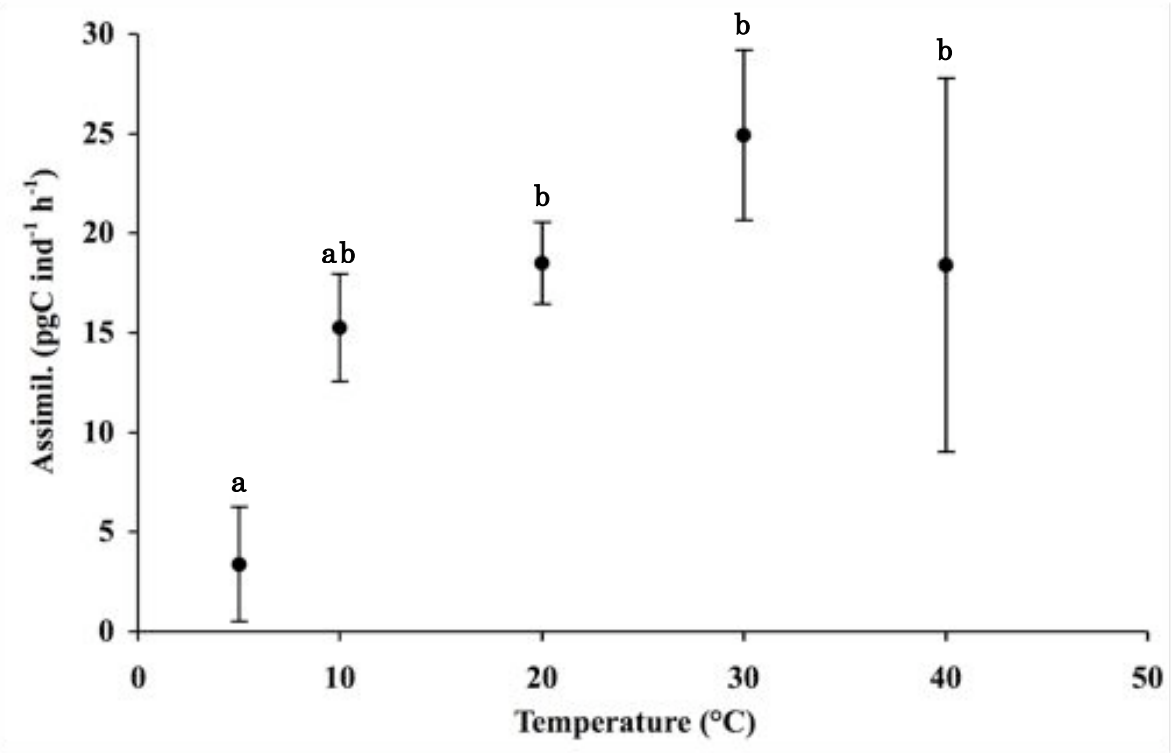

Figure 3. Assimilation rate of bacterial carbon (mean ± SD, N =3) as function of temperature (°C). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

Uptake of microbes by nematodes

Nematodes isotopic compositions and rates of bacterial ad algal uptakes are presented in Table 2. The kinetic experiment showed that bacterial uptake by the nematode community increased linearly during the twelve hours of incubation (Fig. 2). The linear regression slope suggested an assimilation rate of bacteria of 32 pgC ind^{-1} h^{-1} equivalent to 25×10^{-5} $gC_{bacteria}$ $gC_{nematode}^{-1}$ h^{-1} ($r^2 = 0.98$).

Temperature had a significant effect on the assimilation rate of bacteria ($F = 7.5$, $p<0.005$). Temperature tested fluctuated between 5 and 40°C and were in the range of those found in the study area (Guarini et al. 1997). Uptake of bacteria was reduced at 5°C, then increased with temperature to reach an optimum at around 30°C and then decreased (Fig. 3). This rate increased from 3 pgC ind^{-1} h^{-1} to 25 pgC ind^{-1} h^{-1} when the temperature rose from 5°C to 30°C and then decreased, reaching 18 pgC ind^{-1} h^{-1} at 40°C. Overall trend is showing a prominent temperature effect but assimilation rates observed at 10, 20, 30 and 40°C were nevertheless not significantly different from each other but were significantly different from those observed at 5°C.

The assimilation rate at a salinity of 31 (19 ± 2 pgC ind^{-1} h^{-1}) was similar (two-tailed test, $p = 0.72$) to that found for a salinity of 18 (18 ± 4 pgC ind^{-1} h^{-1}) (data not shown).

Light significantly affects the feeding activity of nematodes (two-tailed test, $p<0.05$). Assimilation rates were more than twice as high (41 ± 11 pgC ind^{-1} h^{-1}) under light (83 µM of photons m^{-2} s^{-1}) than in darkness (19 ± 2 pgC ind^{-1} h^{-1}) (data not shown).

Assimilation rates were significantly linked with bacterial abundance ($F = 52$, $p<0.001$) (Fig. 4). Its value was null for the lowest tested abundance (4×10^8 cells ml^{-1}), was amplified more than fourfold when abundance increased from 7×10^8 cells ml^{-1} to 10×10^8 cells ml^{-1} and remained stable between this last value and 17×10^8 cells ml^{-1}.

Using two isotopes to label bacterial (15N) and algal food (13C) offers the opportunity to assess bacterial and algal uptake rates simultaneously. Assimilation of algae increased linearly when algal abundance increased ($r2 = 0.99$, $p<0.05$). Bacteria represented 25, 16 and 8 % of algal plus bacterial intake when algal concentrations were respectively 26, 64 and 114 µgChla g-1, with bacterial abundance remaining constant (Fig. 6). Plotted data indicated that assimilation of bacteria declines when the algal concentration is high (114 µgChla g-1) and the bacterial concentration remains constant (Fig. 5). Nevertheless, these differences were not significant and assimilation of bacteria was unaffected by algal abundance ($F = 3.24$, $p = 0.11$).

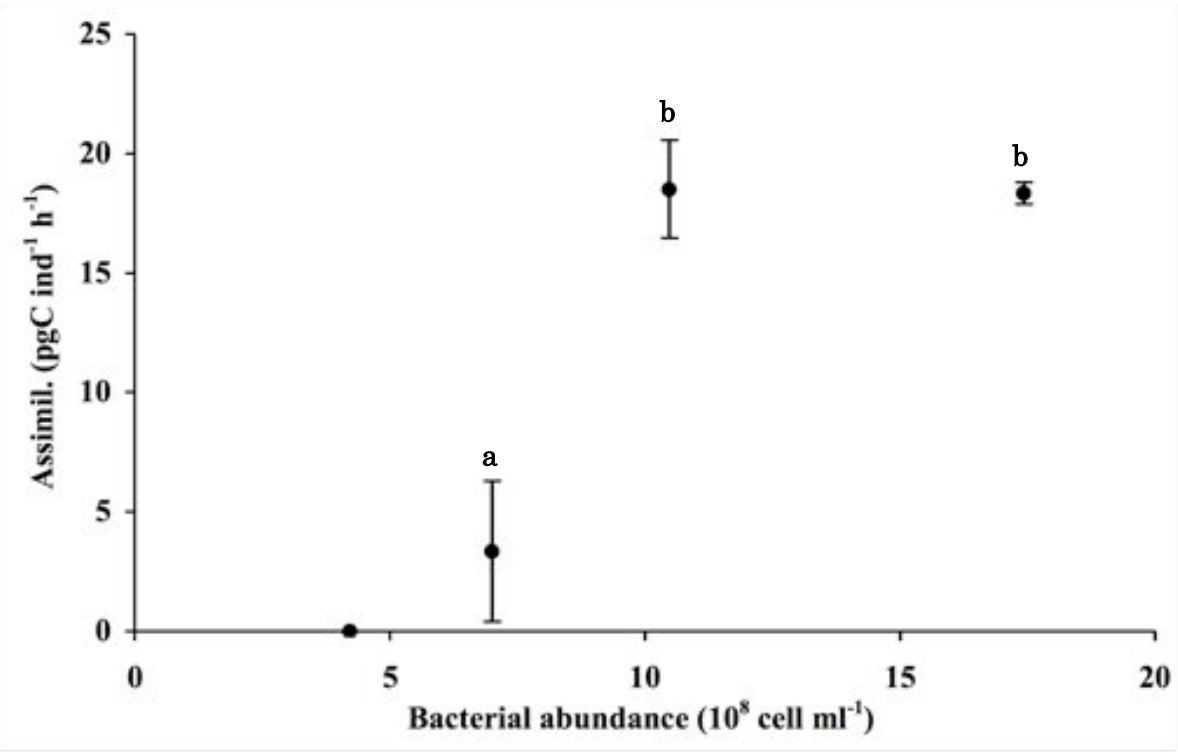

Figure 4. Assimilation rate of bacterial carbon (mean ± SD) as function of bacterial abundance (10^8 cell.ml^{-1}). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

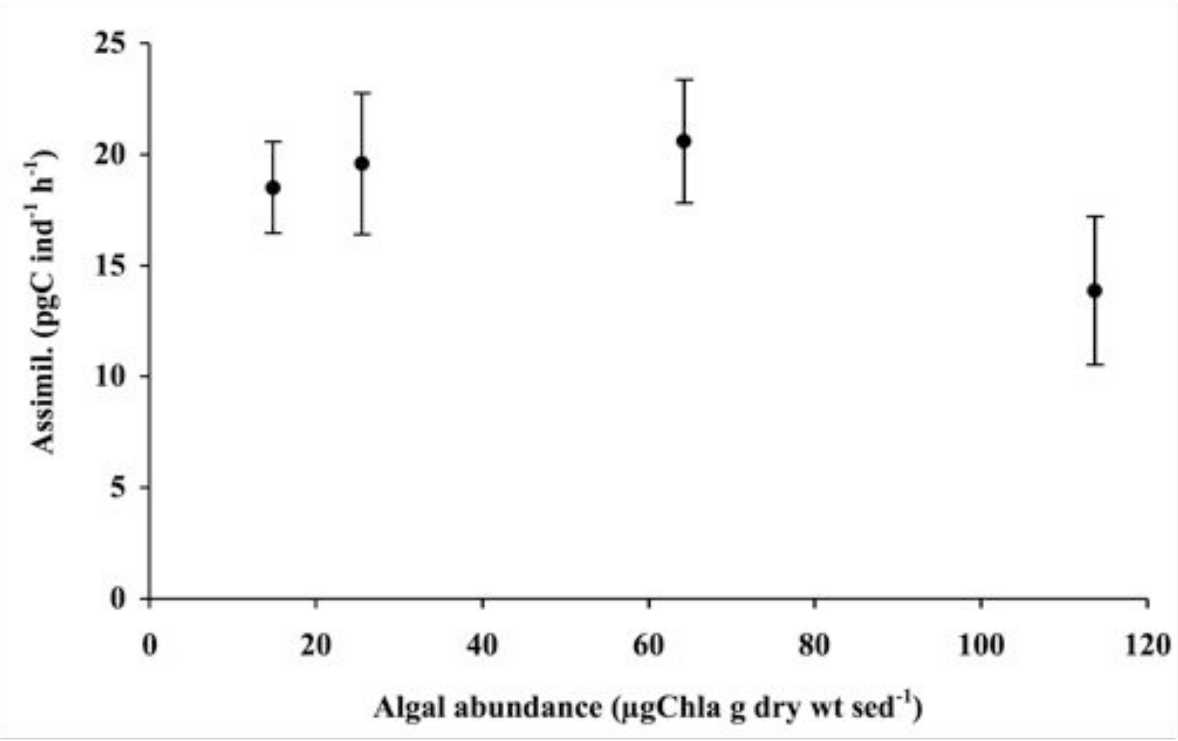

Figure 5. Assimilation rate of bacterial carbon (mean ± SD) as function of algal abundance (µgChla.g^{-1}).

Discussion

Nematode community

Rzeznik-Orignac *et al.* (2003) studied the Brouage mudflat nematode community and showed that in the lower part of the mudflat and over four seasons (2000-2001), six dominant species represented 45% of the community: *Metachromadoroides remanei* (11.5%), *Terschellingia longicaudata* (11.2%), *P. jacobi* (8.6%), *C. macrolaima* (8.6%), *Sabatiera pulchra* (5.2%) and *D. oxycerca* (0.7%). In our study, performed in March 2006, only three species, although all mentioned in the Rzeznik-Orignac *et al.* study, represented 87% of the community (Tab. 1). As the sampling site was the same, difference in nematode community composition may be due to inter-seasons or inter-years fluctuations. More probably, major difference has to be put into relation with the sampling method used in each of these studies. In the present one, only the top centimeter of sediment was sampled, whereas the top 5 cm were sampled in the Rzeznik-Orignac *et al.* (2003) study. As nematodes exhibit a strong vertical distribution of species in sediment (e. g. Platt 1977; Steyaert et al. 2001), the community in the present study is not representative of the total Brouage mudflat nematode community. Moreover, the three major species of the community under study belong to the *Daptonema* and *Ptycholaimellus* genera that were mainly found in the 5 mm surficial layer of sediment (Steyaert et al. 2003) and the third one, belonging to the family Chromadoridae is a typical surface-dwelling epigrowth feeder (Platt 1977).

Feeding habits on diatoms of some genera close to *Chromadara* are known from culture experiments: *Chromadorita tenuis* (Jensen 1987) and *Chromadora macrolaimoides* (Tietjen and Lee 1973). *Chromadora macrolaima* and *Ptycholaimellus jacobi* break or pierce the frustule of diatoms to suck out their contents whereas *Daptonema oxycerca* swallow the whole diatom cell (Wieser 1953; Wieser 1960). Our nematode community is mainly composed by three dominant species known or suspected to feed predominantly on diatoms. The entire nematode community presents however higher abundances of bacterial grazers (Rzeznik-Orignac et al. 2003). As a result, extrapolation of the present grazing results to the rest of the community or to other communities must be realized with caution.

Grazing experiments

Nematode grazing can be highly dependant on their bacterial prey's activity, size and species (Tietjen et al. 1970, Tietjen & Lee 1973, Romeyn & Bouwman 1983, Grewal & Wright 1992, Moens 1999, Moens et al. 1999a). One major hypothesis in grazing experiments is that grazers ingest ^{15}N enriched bacteria at the same rate as the natural ones in the sediment. Consequently, the cultured bacteria community must present characteristics which are roughly similar to the natural one. Despite the fact that our culturing process modified the specific composition of the natural bacterial community, the size, activity and diversity of the bacterial consortium used in the present study were closest to those of the natural community than in most of the previous grazing experiments (Chapitre I).

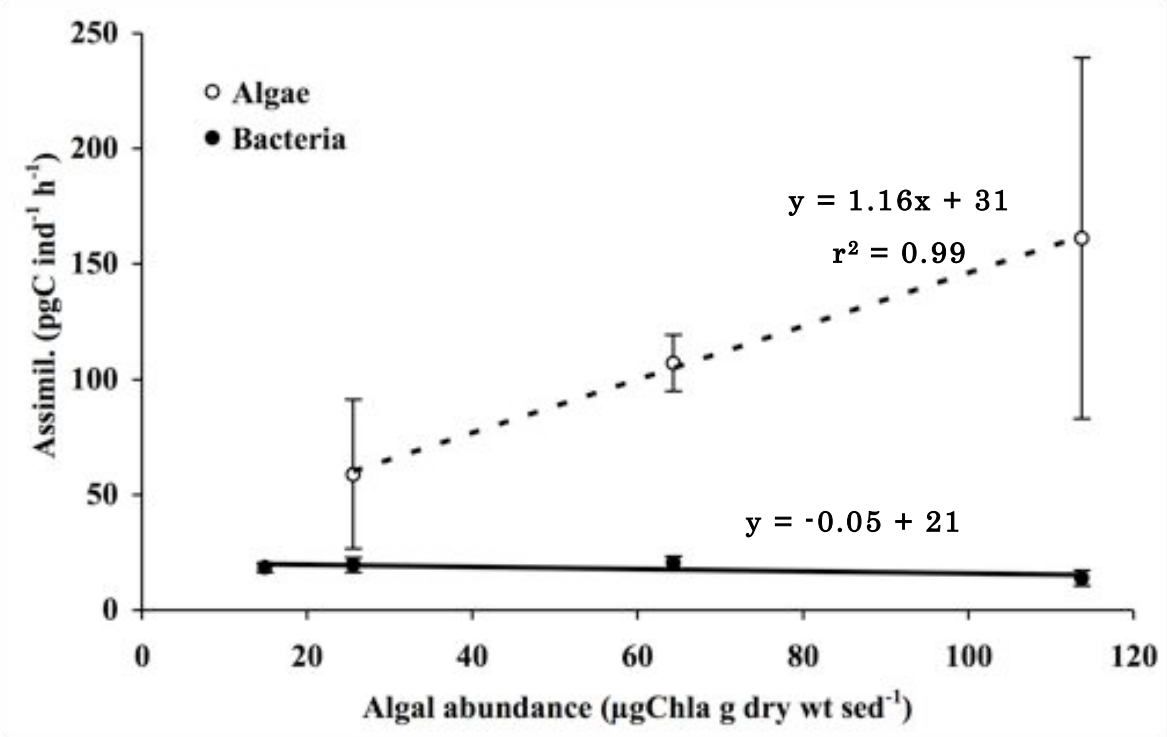

Figure 6. Uptake rate of algal carbon ○ (mean ± SD) and bacterial carbon ● (mean ± SD) as function of algal abundance (µgChla g^{-1}). Bacterial abundance was constant (10.5 × 10^8 cells ml^{-1}).

Bacterial and algal uptake

^{15}N accumulation in nematodes was linear over the 12-hour incubation period (Fig. 2). The constant accumulation of labeled food in nematodes had already been observed with comparable incubation times (Herman and Vranken 1988; Moens et al. 1999c). Since the nematode defecation intervals are very short (e. g. Thomas 1989) and since the gut is emptied completely with each defecation (Duncan et al. 1974), the gut contents would thus be renewed every few minutes. For these reasons, it has been suggested that during long incubation times, the linear accumulation of the label indicates assimilation rather than ingestion (Schiemer 1987; Moens et al. 1999c). The slope of this linear curve gives an assimilation rate of 32 pgC ind^{-1} h^{-1}. Assuming an assimilation rate of 25% (Herman and Vranken 1988; Somerfield et al. 2005), the ingestion rate would be four times higher, at 128 pgC ind^{-1} h^{-1}. As all other grazing experiments were run for 3 hours, they provided the assimilation rate of food.

Abiotic factors

Behavioral responses of plant-parasites and terrestrial nematodes to several stimuli such as electrical, mechanical and chemical stimuli and physical factors such as temperature and light have been well described (review in Croll 1970). However, similar studies concerning the effect of environmental conditions on the feeding behavior of marine or brackish-water nematodes are scarce and limited in scope.

Temperature has received attention as a factor influencing the growth and reproduction of estuarine and marine nematodes (review in Heip et al. 1985). The influence of temperature on feeding has only been studied in the predacious nematode Enoploides (Moens et al. 2000), the epigrowth feeder *Chromadora macrolaimoides* (Tietjen and Lee 1973) and in two brackish-water bacterivorous species: *Pellioditis marina* and *Diplolaimelloides meyli* (Moens et al. 1996; Moens and Vincx 2000). In the present study, nematodes exhibited a classical response which was more or less related to a bell-shaped function also observed by Moens and Vincx (2000). The assimilation rate of bacteria rose when the temperature increased and reached its upper value at 30°C before declining. As assimilation rates recorded for the tested temperatures were statistically different at 5°C, it may be suggested that the nematode grazing rate is lower in winter when the temperature drops to under 5°C. However, nematodes may adjust their optimum temperature conditions along seasons (Gee 1985). Studied nematodes were probably adapted to low temperature as sediment temperature was 7°C at the time of the sampling. Montagna (1984b) did not observe different grazing rates between

winter and summer, but the temperature range in his study area was small (18 and 23°C).

Salinity plays a major role in determining the spatial structure of the nematode community along permanent gradients (Soetaert et al. 1995) or in its seasonal variations (Chatterji et al. 1995). However nematodes can also be affected by short-term variations in salinity (Forster 1998) and intertidal habitats are subject to major changes in interstitial salinity over short time periods. During low tide, raindrops implode into fine sediments, disturbing and mixing the surface sediment of mudflats. Moreover, freshwater run-off passively diffuses into the interstitial pores of the sediment. In their review of marine nematode ecology, Heip et al. (1985) compiled an extensive list of marine and estuarine species with their salinity tolerances. *C. macrolaima*, the most abundant species in the present study, is found in areas with salinities ranging from 35 to 24 whereas *D. oxycerca*, the second most abundant species, is found in area ranging from 35 to 0.9. This species seemed to be able to tolerate gradual changes in salinity but not rapid ones. After exposure to salinity of 3.33 for 10 min and 48 h, Forster (1998) recorded adult mortalities of 10-35% and 70% respectively. This species from the lower level of the intertidal zone is able to osmoregulate but unable to sustain water regulation over long periods (Forster 1998). In the present study, assimilation at salinity 18 was not significantly different from the assimilation at salinity 31. Since the nematodes were not acclimated until the grazing experiment, they seemed to be able to cope with rapid, but limited, osmotic stress. Our conclusions concerning the minor role of salinity in bacterial assimilation by nematodes are in accordance with Moens and Vincx's (2000) results. Among the three factors they investigated (temperature, salinity and food abundance) affecting *P. marina* and *D. meyli* food intake, salinity played a minor role in bacterial assimilation by nematodes.

When exposed to air at low tide, benthic organisms at the sediment's surface are subject to the highest solar and UVB radiation that can be experienced by marine organisms. There are few investigations assessing the effects of luminosity on nematodes. The predacious nematode Enoploides caught approximately twice as many prey nematodes in the dark as in light (Moens et al. 2000). On the other hand, Sundbäck et al. (1996a) concluded that ambient UVBs did not exert any strong selective pressure on the meiofaunal community of a muddy microtidal area. Nozais et al. (1999) observed a deleterious effect of UVBs on the nauplia stages of harpacticoid copepods from a tidal mudflat. However, they did not observe any effect on nematode abundance. In our study, light may not present damaging effect as nematodes fed actively, moreover bacterial

assimilation was enhanced when light increased. Uptake rates of bacteria by foraminifera (Chapter IIa) and the gastropoda *Hydrobia ulvae* (Chapter IIc) that were obtained by grazing experiments performed under the same conditions as in present study did not demonstrate any effect of light. Consequently, the response we observed should not be due to experimental bias and seems to be specific to nematodes. Montagna et al. (1995) observed a significant correlation between algal production and the grazing rate of a nematode community from the Brouage mudflat. In their experience, algal production was increased by increasing the light intensity above microcosms. They concluded that nematode's ingestion rate increases with algal production. In intertidal mudflats, benthic diatoms migrate in the surface sediment along the diurnal cycle (e. g. Serôdio et al. 1997). The nematode community under study was dominated by epigrowth feeders (Tab. 1), with a diet mainly composed of benthic diatoms. Those nematodes may graze when luminosity is high during low tide in order to graze in algal biofilm and maximize their algal intake. This feeding behavior has not been documented for nematodes, but harpaticoid copepods were shown to graze at a higher rate just after the mudflat became exposed (Decho 1988). Buffan-Dubau and Carman (2000) also observed a midday feeding peak by ostracods and harpaticoid copepods. This result suggests that nematodes would have a feeding behavior principally controlled by algae and that bacteria may be taken up accidentally with algae.

Biotic factors

For nematodes, the effects of bacterial concentration on growth, fecundity, population development and feeding rates have been studied (Nicholas et al. 1973; Schiemer et al. 1980; Schiemer 1982a; Schiemer 1982b; Schiemer 1983; Vranken et al. 1988; Moens and Vincx 2000). Nematode ingestion rates are generally proportional to food availability (Nicholas et al. 1973; Schiemer et al. 1980; Moens et al. 1996). However, the relationship between assimilation rate and food concentration is not linear and have been described by a Michaelis-Menten function (Schiemer 1982b). This function is consistent with Holling's prey-dependent type II functional response (Holling 1959). Assimilation rates depend on the amount of ingested food and efficiency with which the material is assimilated. The assimilation efficiency have been found to decrease when food concentration increases: at a high food concentration *Plectus palustris* presented a low assimilation efficiency (12%) (Duncan et al. 1974) whereas at a food concentration one order of magnitude lower, the assimilation efficiency reached 57% (Schiemer et al. 1980). A similar conclusion was drawn by Moens et al. (2006) on the marine bacterivore

Pellioditis marina. Low efficiencies at high concentrations appear to result from short gut retention times, with the gut transit being too fast for effective digestion (Taghon et al. 1978). In the present study, it is not possible to determine if assimilation rates fluctuations are due to variations of ingestion rates or assimilation efficiencies. The number of bacterial concentrations tested was limited, however the response observed could be described by a sigmoid function. This function is consistent with Holling's prey-dependent type III functional response (Holling 1959). The threshold value for significant assimilation appeared to fall between 4 and 7 × 10^8 cells ml^{-1} and the threshold value for constant assimilation appeared to be between 7 and 10 × 10^8 cells ml^{-1}. Those values are in the range of data provided by literature for fast growing opportunistic bacterivores nematodes typical of plant material or root systems: an optimal grazing rate was obtained for *Caenorhabditis briggsae* (Nicholas et al. 1973) and *P. marina* and *D. meyli* (Moens and Vincx 2000) at a bacterial concentration of 5, 25 and 5 × 10^8 cells ml^{-1} respectively. Our study, dealing with tidal flat microalgal grazers community, suggests that the optimal level of bacterial foraging is also reached at high bacterial density, when bacterial concentrations attain 1 × 10^9 cells ml^{-1}. As bacterial concentrations are rarely lower in marine sediments, (Hondeveld et al. 1992; Schmidt et al. 1998; Hamels et al. 2004), bacterial assimilation rates would seldom be lower than 18 pgC ind^{-1} h^{-1}.

The relationship between the assimilation rate of algae and the algal concentration is poorly documented. Montagna et al. (1995) observed a proportional rise in the algal grazing rate with increasing algal concentration. In the present study, algal assimilation increased linearly without reaching a plateau. This function may be consistent with Holling's prey-dependent type I functional response (Holling 1959). Thus, the algal concentration threshold was not reached and maximal algal assimilation may be higher than those we obtained. Under natural conditions, the chlorophyll a content of the first centimeter of sediment varies between 0 and 50 µgChla g^{-1} (review in MacIntyre et al. 1996). However, through vertical migration, benthic microalgae were concentrated near the surface during diurnal low tides and produced a biofilm with an average thickness of 50 µm (Herlory et al. 2004). In this thin layer of algal mat, the concentration of chlorophyll a can reach 150 µgChla g^{-1} (Serôdio et al. 1997) and even 300 µgChla g^{-1} (Kelly et al. 2001). Thus, the higher algae abundance used in the present study (114 µgChla g^{-1}) is not representative of conditions occurring in the natural environment and the nematode community feeding on the algal biofilm could thus present a higher assimilation rate of algae.

In our experiment, algal assimilation by nematodes always represented more than three times the bacterial assimilation. Nematodes are able to select potential food items. *Chromadora macrolaimoides* have a preference for diatoms and chlorophytes whereas they assimilate fewer bacteria (Tietjen and Lee 1973). *D. oxycerca* is able to swallow particles of different sizes and large frustules of diatoms (Boucher 1974). The three dominant species of the community studied, i.e. *C. macrolaima*, *D. oxycerca* and *P. jacobi,* were found to dominate the Brouage nematode community during spring diatom blooms (Rzeznik-Orignac et al. 2003) indicating that they are highly dependent on algal resources. The use of natural stable isotopic analyses led to a similar conclusion elsewhere, that the microphytobenthos constitutes the main food source for the nematode community dwelling in the surficial centimeter of mud in the study area (Riera et al. 1996). Montagna et al. (1984b), looking at the grazing rates of a nematode community from a saltmarsh, found that diatoms are selected 14 more times than bacteria. Algal carbon accounted on average for more than 90% of carbon grazed by a nematode community from a microtidal sandy sediment (Sundbäck et al. 1996b). In the present study, nematodes also ingested algae at a higher rate than bacteria. Depending on the algal concentration, the nematode community assimilates 3 to 11 times more diatoms than bacteria.

Nematode production can be estimated on the basis of the P/B ratio, bearing in mind that the choice of this ratio may be inaccurate (Heip et al. 1982). With an individual biomass of 0.11 µgC ind^{-1}, the estimation of production yielded 0.11 ngC ind^{-1} h^{-1}, given a P/B ratio of 9 as often advocated (Gerlach 1971; Warwick and Price 1979; Bouvy 1988). Using a 10% factor for energy conversion efficiency (Bouvy 1988), a nematode carbon demand of 1.13 ngC ind^{-1} h^{-1} can be calculated. In the present study, the maximum ingestion rate of bacteria measured would represent 4% of this energy demand. On the other hand, the maximum algal ingestion measured would correspond to 15% of this demand. To get 100% of their energy needs, nematodes may graze at a higher rate on the algal compartment, as discussed above. Nematodes may also be dependent on other food sources such as detritus, protozoa, oligochaetes or nematodes (Moens and Vincx 1997). The role of Dissolved Organic Matter in nematode nutrition also remains elusive, although it is likely to be highly relevant (Lopez et al. 1979; Meyer-Reil and Faubel 1980; Montagna 1984a; Jensen 1987). Bacterial carbon was found to constitute 6% of the total carbon requirement of a mudflat nematode community (van Oevelen et al. 2006b). On the other hand, using inverse modeling, Van Oevelen et al. (2006c) suggested that mudflat community of nematodes relied for 50 % on algae and 39

% on bacteria. Variations between studies can be due to differences in methodology and to nematode community composition.

In the brouage mudflat, nematode present a mean densities of 2112 ind 10 cm^{-2} (Rzeznik-Orignac et al. 2003) whereas biomasses of benthic bacteria represent 0.846 gC m^{-2} (Degré et al. 2006). If nematodes grazing rates measured in the present study are representative of those of total community, it would imply that 0.02 % of bacterial biomass is assimilated by nematodes each day. This extrapolation is debatable, as studied nematodes feed predominantly on diatoms whereas total community may be more dependent on bacterial resource, consequently grazing of bacteria is probably underestimated.

Conclusion

Caution must be taken in interpreting our results, since the impact of each environmental factor on the feeding behavior of the nematode community was studied separately, whereas in natural environment all these factors covary greatly. The combination of temperature and salinity factors was found to have a higher impact than each factor taken alone (Tietjen and Lee 1972; Tietjen and Lee 1977). Moreover, nematodes may respond to environmental changes at a seasonal scale, by physiological adjustment and shifting of their optimum conditions (Gee 1985). However, the nematode community studied appeared to have adapted to the highly variable environment constituted by the surficial sediment of intertidal mudflats, except at low temperatures (5°C), and their feeding activity is only slightly decreased by temperature, salinity or light stress. Due to high abundance of bacteria in the marine sediment, nematodes may never be food limited with bacteria. Nematodes kept feeding under conditions of stress which were typical of the surficial sediment habitat, moreover they appeared to be principally dependent on the algal resource. Consequently, the community of nematodes dwelling in the top centimeter of the Brouage mudflat may also have a feeding strategy which is strongly linked to the formation of algal biofilm during the diurnal ebb.

CHAPITRE II C

INFLUENCE OF ENVIRONMENT FACTORS ON BACTERIAL INGESTION RATE OF THE DEPOSIT-FEEDER *HYDROBIA ULVAE* AND COMPARISON WITH MEIOFAUNA

Pierre-Yves Pascal, Christine Dupuy, Pierre Richard,
Anne-Gaelle Haubois, Nathalie Niquil

Abstract

Deposit feeders are able to process a considerable volume of sediment, containing large quantities of associated bacteria. However, conclusions concerning the trophic role played by benthic bacteria in marine sediments are still not fully elucidated. This study deals with bacterivory by the gastropod *Hydrobia ulvae*, one of the most abundant deposit-feeding species in intertidal mudflats in Western Europe. Ingestion rates of bacteria were determined during grazing experiments using ^{15}N pre-enriched bacteria. Grazing experiments were performed in order to measure effects of abiotic (temperature, salinity and luminosity) and biotic (bacterial and algal abundances) factors on ingestion rates of bacteria by *H. ulvae* of an intertidal mudflat (Brouage, Marennes-Oléron, France). The mean ingestion rate of bacteria by *H. ulvae* was 1149 ngC $ind^{-1}h^{-1}$. The general trend shows a temperature effect with an optimum around 30°C, and the assimilation rate was significantly lower at 5°C. Bacterial assimilation did not significantly differ between salinity 18 and salinity 31. Ingestion was the same in light and in dark conditions. Results are compared with those of other grazing experiments conducted simultaneously in similar conditions with two other grazers with different size and feeding modes: the foraminifera *Ammonia tepida* and a nematode community from the superficial sediment of the Brouage mudflat. *Hydrobia ulvae* and nematodes presented a feeding behavior less influenced by environmental changes than *A. tepida*. *Hydrobia ulvae* ingested bacteria at a higher rate than smaller meiofaunal grazers and seemed to have a lower ability to selectively ingest diatoms than meiofaunal grazers.

Introduction

In pelagic environments, bacteria are heavily grazed and consequently play a major role in food webs (Azam et al. 1983). In the benthic environment, bacteria are generally 1000 times more abundant than in pelagic systems, reaching abundances of about 10^9 cells ml^{-1} (Schmidt et al. 1998). However, microbial food web research in sediment is in its infancy and the trophic significance of benthic bacteria remains elusive (Review in Kemp 1986).

The deposit-feeder *Hydrobia ulvae* is one of the most abundant species of macrofauna inhabiting intertidal mudflats in Western Europe (Bachelet and Yacine-Kassab 1987; Barnes 1990; Sola 1996). Deposit feeders typically process at least one body weight of sediment daily (Lopez and Levinton 1987). This sediment includes highly digestible and nutritious microphytobenthos and bacteria, less digestible plant debris and completely indigestible refractory detritus (Rice and Rhoads 1989). Diatoms have been found to be a major source of nutrition for *H. ulvae* (Fenchel et al. 1975; Jensen and Siegismund 1980; Lopez and Cheng 1983a; Bianchi and Levinton 1984; Haubois et al. 2005a). However bacteria have also been found as food for *Hydrobia* species (Cammen 1980; Jensen and Siegismund 1980; Bianchi and Levinton 1981; Levinton and Bianchi 1981). Due to high abundances of *H. ulvae* in intertidal mudflats, carbon flow from bacteria to snails may be a significant pathway in this type of environment.

The objective of the present study was to quantify the bacterial ingestion rate of *H. ulvae* and to investigate how this rate varies with abiotic (temperature, salinity and luminosity) and biotic (bacterial and algal abundance) factors. Intertidal mudflats are subject to large and quick changes in many environmental factors at short time scales (circadian and tidal cycles) (Guarini et al. 1997) and these variations may significantly influence snail feeding behaviour. Bacterivory of *H. ulvae* was then compared to that of other grazers of different sizes and feeding modes, the foraminifera *Ammonia tepida* and a nematode community from surficial sediment of an intertidal mudflat (Brouage-Marennes Oléron-France). Bacterial ingestion of both grazers have been previously described (Chapter I; IIa; IIb). All grazing experiments were performed simultaneously in similar conditions using stable isotope enriched prey (^{13}C enriched algae and ^{15}N enriched bacteria).

Experimental procedure

Study Site

The Brouage intertidal mudflat is located in the eastern part of the Marennes-Oléron Bay (Atlantic coast of France). Meteorological conditions exhibit a strong seasonality typical of a temperate climate. Range of temperature and salinity in emerged sediments are more extreme during summer tidal cycles (Guarini et al. 1997). Minimum and maximum mud temperatures are 5°C and 34°C respectively. The maximum daily range of mud temperature due to emersion and immersion cycle reaches 18°C (Guarini et al. 1997). Salinity of overlaying water is controlled by the Charente River freshwater input, ranging from 25 to 35 over the year (Héral et al. 1982). Salinity of the upper layers of sediment may also decrease with rainfall. The sediment surface irradiance shifts from dark during submersion and night emersions to high levels of incident light during daytime emersions. This irradiance can reach 2000 µM of photons m^{-2} s^{-1} (Underwood and Kromkamp 2000). Details of numerous benthic organisms and processes are available concerning this intertidal zone (gathered in Leguerrier et al. 2003; Leguerrier et al. 2004; Degré et al. 2006).

Preparation of ^{15}N enriched bacteria

Superficial sediment (1 cm depth) was collected on the Brouage mudflat (45,55,074 N; 1,06,086 W). One ml of the collected sediment was added to 20 ml of bacterial liquid culture medium and kept in darkness for 24 hours at 13°C. The composition of this culture medium was previously described in Chapter I. This primary culture was then subcultured for 24 hours under the same conditions to get approximately 2×10^9 cells ml^{-1}. Finally, bacteria were collected in 0.2 µm filtered seawater after 3 centrifugations (3500 g, 10 mn, 20°C), frozen in liquid nitrogen and kept frozen at -80°C until grazing experiments.

Preparation of ^{13}C enriched algae

An axenic clone of the diatom *Navicula phyllepta* (CCY 9804, Netherlands Institute of Ecology NIOO-KNAW, Yerseke, The Netherlands), the most abundant diatom species in the study area (Haubois et al. 2005b), was cultured in medium described by Antia and Cheng (1970) and containing $NaH^{13}CO_3$ (4 mM). Diatoms were

concentrated by centrifugation (1500 g, 10 mn, 20 °C), washed three times to remove the ^{13}C-bicarbonate, and freeze-dried.

Quantification of bacteria and algae abundance

In order to determine the ratio between enriched and non-enriched preys in microcosms, abundances of bacteria and algae were assessed. To separate bacteria from sediment particles, incubation in pyrophosphate (0.01M for at least 30 min) and sonication (60 W) were performed. Bacteria from both sediment and culture were labelled using 4.6-diamidino-2-phenylindole dihydrochloride (DAPI) (2500 µg l^{-1}), filtered onto 0.2 µm Nucleopore black filter (Porter and Feig 1980) and then counted by microscopy. We verified the absence of ciliates and flagellates in the bacterial culture during this microscope observation step. The abundance of diatoms in the sediment was assessed using Chl *a* as a proxy, measured using fluorometry (Lorenzen 1966).

Grazing experiments

The top centimeter of sediment was collected during ebb tide from the same study area at midday on March 13, 2006. It was sieved on 500 µm, 200 µm and 50 µm in order to concentrate respectively *H. ulvae*, *A. tepida* and nematodes. Before sieving, snails were placed on natural sediment and kept for 24 h at 20°C in the dark. Time between sieving and the start of the grazing experiment never exceeded two hours in order to avoid starvation bias in feeding behavior (Calow 1975). Seventeen handpicked specimens of *H. ulvae* were placed in polypropylene Petri dishes (ø = 9 cm). This density was chosen in order to avoid a density-dependence effect on the individual ingestion due to space limitation (Blanchard et al. 2000). A fraction of the sediment passing through the 50 µm mesh was mixed with the ^{15}N enriched bacteria. This slurry contained 10.5×10^8 bacteria ml^{-1}, ^{15}N enriched bacteria being twice as abundant as non-enriched ones. Four ml of this slurry were put into each microcosm. Each experiment was carried out in triplicate, along with at least one control. Control samples were frozen (-80°C) in order to kill any grazers.

The calculation of bacterial ingestion rate relies on the assumption that enriched preys accumulate in snail's gut at a constant rate, and that no egestion of labelled materials occurs during incubation time. A kinetic study was run for 1 to 12 hours including the 2 hour run that was used for all other experiments. Incubations were made under the following standardized conditions that were close to the mean values recorded on the study site: temperature (20°C), salinity (31), luminosity (darkness), bacterial

abundance (10.5×10^8 bacterial cells ml^{-1}) and algal abundance (15 µgChla g dry $sediment^{-1}$). For each experiment to determine the effects of environmental factors, only one incubation factor was modified so as to determine its effect on *H. ulvae*'s grazing activity. After the sieving step, snails were transferred without acclimation into different microcosms to simulate short-term changes of environmental factors. To test the effect of temperature, the snails were placed at 5°C, 15°C, 30°C and 40°C: these temperatures are in the range of those measured in the study area (Guarini et al. 1997). The effect of salinity was investigated by placing *Hydrobia* in microcosms with a salinity of 18. To decrease salinity, cultured bacteria were rinsed with 0.2 µm filtered-sea-water diluted with 0.2 µm filtered distilled water. Such decrease in salinity can occur in field conditions when sediment is exposed to heavy rainfall. The light effect was tested with a light intensity of 83 µM of photons m^{-2} s^{-1}. Bacterial abundance was modified adding various quantities of bacteria enriched in ^{15}N. Bacterial abundances (total enriched and non-enriched) tested were 4, 7 and 17 cells ml wt sed^{-1} with respectively the following ratio between abundance of total and enriched bacteria: 6.1, 2.0 and 1.3. Algal abundance was modified by adding various quantities of cultured *N. phyllepta* enriched in ^{13}C while bacterial abundances (total enriched and non-enriched) were kept constant at 10×10^8 cells ml^{-1}. Algal abundance (total enriched and non-enriched) were 26, 64 and 114 µgChla g dry sed^{-1} with respectively the following ratio between abundance of total and enriched algae: 2.4, 1.3 and 1.2.

Incubations were stopped by freezing microcosms at -80°C. Samples were thawed and *H. ulvae* were separated by hand from their shell and the 17 specimens of each microcom were pooled and homogenized using a Potter-Eveljhem.

Isotope analysis and calculations

$\delta^{15}N$ and $\delta^{13}C$ of prey (bacteria and algae) and grazers were measured using an EA-IRMS (Isoprime, Micromass, UK). Nitrogen isotope composition is expressed in the delta notation ($\delta^{15}N$) relative to air N_2: $\delta^{15}N = [((^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{reference})-1] \times 1000$. Carbon isotope composition is expressed in the delta notation ($\delta^{13}C$) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = [((^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{reference})-1] \times 1000$.

Incorporation of ^{15}N is defined as excess above background ^{15}N (control experiment) and is expressed in terms of specific uptake (*I*). *I* was calculated as the product of excess ^{15}N (*E*) and biomass of N per grazer. *I* was converted to bacterial carbon grazed using the C/N ratio of bacteria. *E* is the difference between the background ($F_{background}$) and the sample (F_{sample}) ^{15}N fraction: $E = F_{sample} - F_{background}$, with $F = {}^{15}N / ({}^{15}N + {}^{14}N) = R / (R + 2)$

and R = the nitrogen isotope ratio. For the $F_{background}$, we used control values measured with killed grazers (frozen). For *H. ulvae* we used the highest value measured in control ($\delta^{15}N$ = 12.42 and $\delta^{13}C$ = -13.72). R was derived from the measured $\delta^{15}N$ values as: $R = ((\delta^{15}N/1000)+1) \times R\text{air}N_2$ where $R\text{air}N_2 = 7.35293 \times 10^{-3}$ (Mariotti 1982). The uptake of bacteria was calculated as Uptake = ($I \times$ (% $C_{enriched\ bacteria}$ / % $N_{enriched\ bacteria}$)) / ($F_{enriched\ bacteria} \times$ incubation time). This uptake was multiplied by the ratio between the abundance of total and enriched bacteria determined by DAPI counts.

Incorporation of ^{13}C was calculated analogously, with $F = {}^{13}C / ({}^{13}C + {}^{12}C) = R / (R + 1)$, $R\text{air}N_2$ is replaced by R_{VPDB} = 0.0112372 and Uptake = I / ($F_{enriched\ bacteria} \times$ incubation time). The uptake measured was multiplied by the ratio between the abundance of total and enriched diatom, determined from fluorometrical measurements.

Enriched *N. phyllepta* carbon consisted of 22.95 ± 0.54% ^{13}C. The C/N ratio of enriched bacteria was 3.49 and bacterial nitrogen consisted of 2.88 ± 0.03% ^{15}N. The individual average weight of *H. ulvae* was 0.54 ± 0.08 mg and each specimen was composed on average of 184 ± 19 µgC and 43 ± 4 µgN (N = 72 samples of at least 17 specimens each). Ingestion rate as $gC_{bacteria}\ gC_{H.\ ulvae}^{-1}\ h^{-1}$ was obtained by dividing ingestion rate of bacteria ($gC\ ind^{-1}\ h^{-1}$) by *H. ulvae* mean weight ($gC\ ind^{-1}$).

Variations of uptake rates according to salinity and irradiance were tested using bilateral independent-samples two-tailed tests. One-way analyses of variance (ANOVA) were used in order to test the impact of temperature and algal and bacterial abundance on uptake rates of bacteria and algae. The Tukey test was used for post-hoc comparisons.

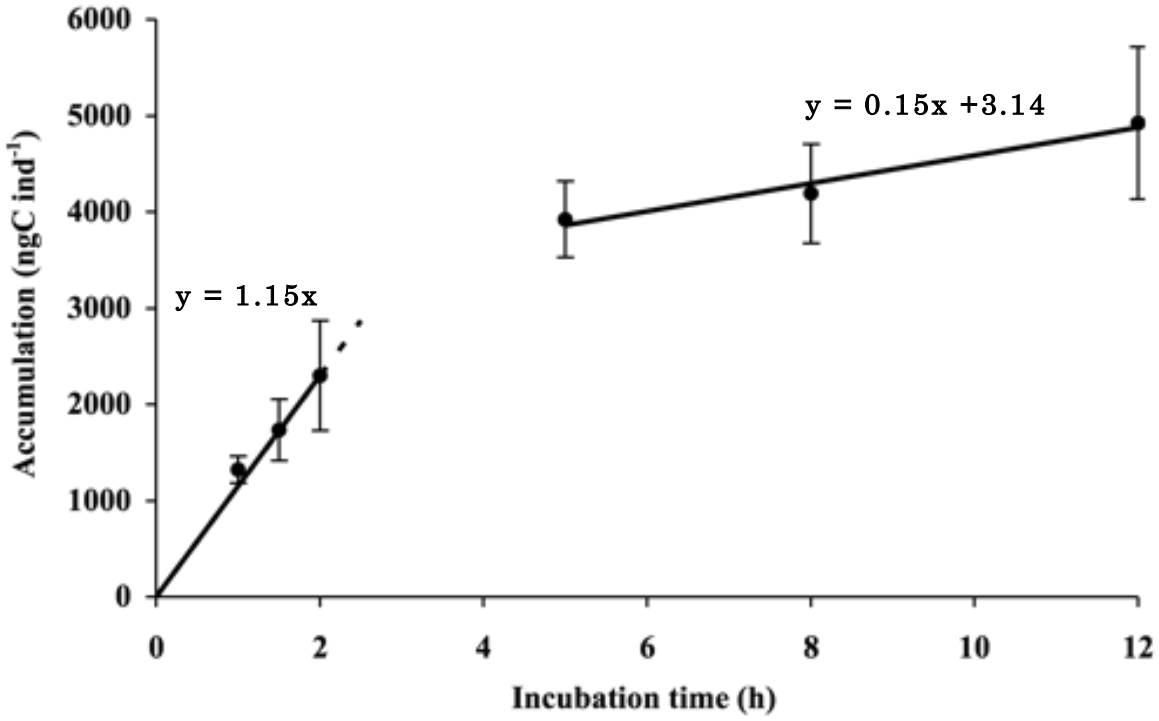

Figure 1. Bacteria uptake by *H. ulvae* (mean ± SD, N =3) as function of incubation time (h).

Results

The kinetic experiment showed that accumulation of bacteria in *H. ulvae* increased linearly during the first two hours of incubation and then levelled off (Fig. 1). The linear regression slope for the first two hours indicates an uptake rate of 1149 ngC ind^{-1} h^{-1} equivalent to 6.43 10^{-3} $gC_{bacteria}$ $gC_{H.\ ulvae}^{-1}$ h^{-1} (r^2 = 0.98). The linear regression slope between five and twelve hours is more than seven times lower than for the two first hours and indicates an uptake rate of 145 µgC ind^{-1} h^{-1} equivalent to 0.81 10^{-3} $gC_{bacteria}$ $gC_{H.\ ulvae}^{-1}$ h^{-1} (r^2 = 0.98).

Ingestion of bacteria increased from 462 to 1277 ngC ind^{-1} h^{-1} when temperature increased from 5°C to 30°C, and then decreased reaching 1059 µgC ind^{-1} h^{-1} at 40°C (Fig. 2). Ingestion rate of bacteria by *H. ulvae* was significantly decreased at 5°C (F = 10; $p<0.01$), but ingestion rates observed at 10, 20, 30 and 40°C were not significantly different.

The ingestion rate measured for a salinity of 31 (1149 ± 285 ngC ind^{-1} h^{-1}) was similar to the one measured for a salinity of 18 (1085 ± 58 ngC ind^{-1} h^{-1}) (two-tailed test, $p = 0.20$).

The ingestion rate observed under light conditions (1478 ± 246 ngC ind^{-1} h^{-1}) was similar to the one observed in darkness (1149 ± 285 ngC ind^{-1} h^{-1}) (two-tailed test, $p = 0.72$).

Ingestion rates of bacteria were significantly linked to bacterial abundance in microcosms (F = 38; $p<0.001$) (Fig. 3). Ingestion rate increased linearly from 38 ± 13 to 1117 ± 93 ngC ind^{-1} h^{-1}) when bacterial concentrations increased from 4 to 10 × 10^8 cells ml^{-1} and increased, though not significantly to 1604 ± 366 ngC ind^{-1} h^{-1} for a bacterial concentration of 17 × 10^8 cells ml^{-1}.

Dual labeling of prey allows simultaneous assessment of the ingestion of bacteria and algae. When algal concentration increased from 15 to 114 µgChla g^{-1} with constant bacterial abundance (10.5 × 10^8 cells ml^{-1}), the ingestion rate of algae remained constant ($F = 3.3$; $p = 0.11$) (Fig. 4). However, ingestion rate of bacteria remained constant for algal concentration in the sediment between 15 and 64 µgChla g dry wt sed^{-1} but significantly decreased at the highest algal concentration of 114 µgChla g dry wt sed^{-1} ($F = 4.4$; $p < 0.05$). As a result, when algal abundance increased, the fraction of algae in the diet of *H. ulvae* increased.

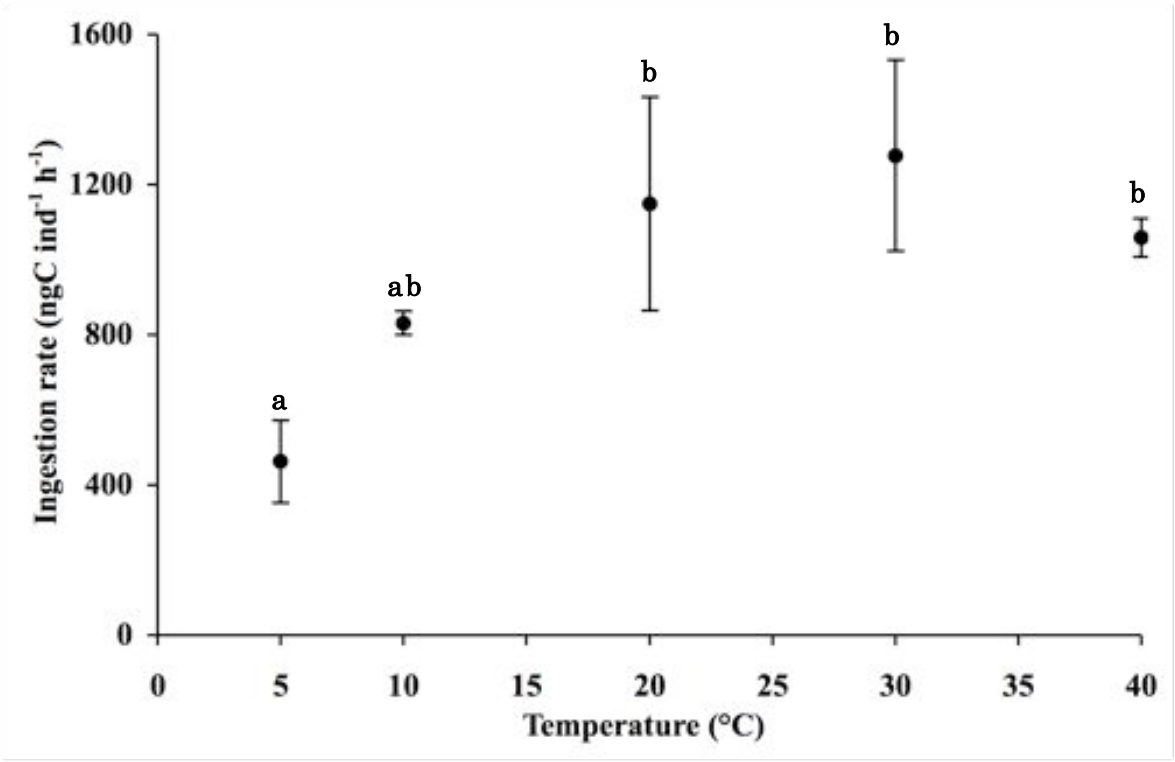

Figure 2. Bacterial ingestion rate by *H. ulvae* (mean ± SD, N =3) as function of temperature (°C). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

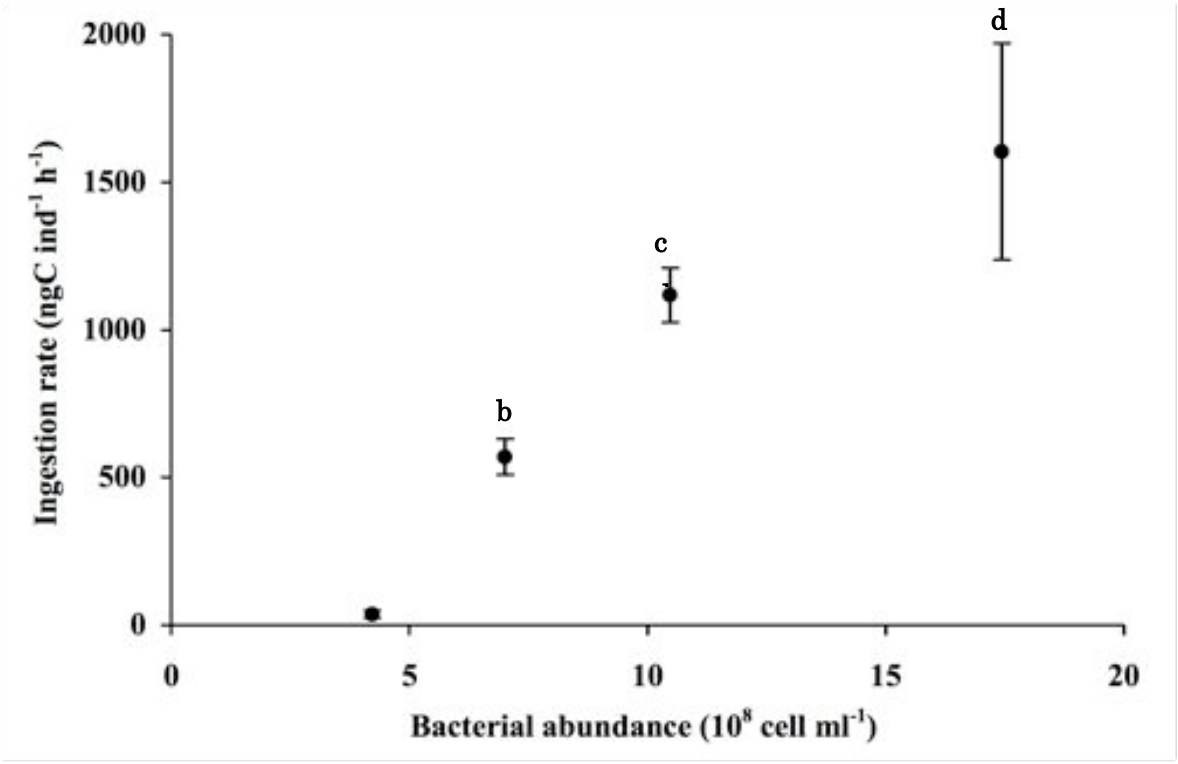

Figure 3. Bacterial ingestion rate by *H. ulvae* (mean ± SD, N =3) as function of bacterial abundance (10^8 cells ml^{-1}). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

Grazing experiments were simultaneously performed with two other grazers: the foraminifera *A. tepida* and a nematode community. Feeding behaviour of *A. tepida* (Chapter IIa) and nematodes (Chapter IIb) has been previously described. All grazing incubations were conducted at the same time and in similar conditions, making comparisons between grazers possible. The effects of environmental factors (temperature, salinity and luminosity) on ingestion rates of bacteria are summarized in Table 1. Among the different grazers tested, *A. tepida* was the most affected by salinity and temperature. Light only affected nematodes and increased their feeding activity. Classification of grazers according to their maximal ingestion rates of bacteria and algae reported per grazer weight gave the following list arranged in ascending order: *A. tepida*, nematode community and *H. ulvae* (Table 1). For the dual labelling experiment, ratios between algae and bacteria ingested were measured for each grazer, at each algal concentration. Comparison of those ratios between grazers reflects their respective ability to discriminate between food sources (*i.e.* algae and bacteria). At the three algal concentrations tested, *A. tepida* and nematodes showed a higher ratio of algae:bacteria ingested than *H. ulvae*, suggesting a better ability to preferentially ingest algae over bacteria compared to the gastropod (Fig. 5).

Discussion

Kinetic experiment

During the 12 hour incubation, *H. ulvae* first accumulated the enriched bacteria linearly over 2 hours (Fig. 1). This suggests that ingestion rate of bacteria was constant and that no egestion of recently ingested labelled material occurred during this period. After 4 hours of incubation, the accumulation rate of bacteria by *H. ulvae* decreased. This may have two nonexclusive origins: egestion of labelled bacteria and a decrease in feeding activity over time. As all other grazing experiments were performed during two hours, they consequently reflect the ingestion rates of *H. ulvae*.

Linear accumulation of labelled diatom or bacteria by *H. ulvae* had been previously recorded from 45 min (Fenchel et al. 1975) to 2 hours (Blanchard et al. 2000; Haubois et al. 2005a). Molluscs have complex digestive tracts allowing partitioning of food particles within the gut. Relatively indigestible material passes quickly to the intestine and is subjected to extracellular digestion. More nutritious material like bacteria and algae is diverted to the digestive gland where it undergoes intracellular digestion. For *Hydrobia totteni*, gut residence time is 30-40 min (Lopez and Cheng 1983b) whereas digestive

gland residence time is 5 hours (Kofoed et al. 1989). Assuming a similar situation for *H. ulvae*, the absence of egestion during the two hour long incubations would mean that all bacteria are diverted to the digestive gland. In their grazing experiment with enriched diatoms, Sokolowski et al. (2005) observed that during the first 4 h phase of experiment, accumulation rate was 3 times higher than during the last 12 h phase. We observed exactly the same ratio between accumulation rates found during the grazing periods 0-2 h and 0-12 h. Those similar results suggest that digestive processes for bacteria and algae may be similar.

Range of ingestion rates

To our knowledge, there is no data dealing with the ingestion rate of bacteria by *Hydrobia* to compare with our values. However, concerning algal ingestion our data are consistent with those previously measured. In the present study, ingestion rates fluctuated between 1.2-1.8 µgC ind^{-1} h^{-1}. In the literature, ingestion rates of snails fed with diatoms are 0.5-2.9 µgC ind^{-1} h^{-1} for *Hydrobia truncata* (Forbes and Lopez 1989) and 1.2 (Sokolowski et al. 2005), 1.12-1.33 (Blanchard et al. 2000) and 0.04-2.08 (Haubois et al. 2005a) for *H. ulvae*.

The maximal ingestion rates of algae and bacteria by *Hydrobia ulvae* were higher than meiofaunal rates. Nevertheless, individual weight of *H. ulvae* is more than one hundred and one thousand times higher than *A. tepida* and nematodes respectively (Table 1). Body size is an important determinant of many physiological processes and maximal ingestion rate is generally inversely correlated to body size (e. g. Moloney and Field 1989). The present study focuses only on two potential prey, bacteria and algae, although other food sources are available in sediment. For instance Dissolved Organic Material may constitute an important food supply for nematodes (Lopez et al. 1979; Meyer-Reil and Faubel 1980; Montagna 1984; Jensen 1987) and foraminifera (Schwab and Hofer 1979). Nematodes and foraminifera may be principally dependent on those other resources and present consequently low ingestion rates of bacteria and algae.

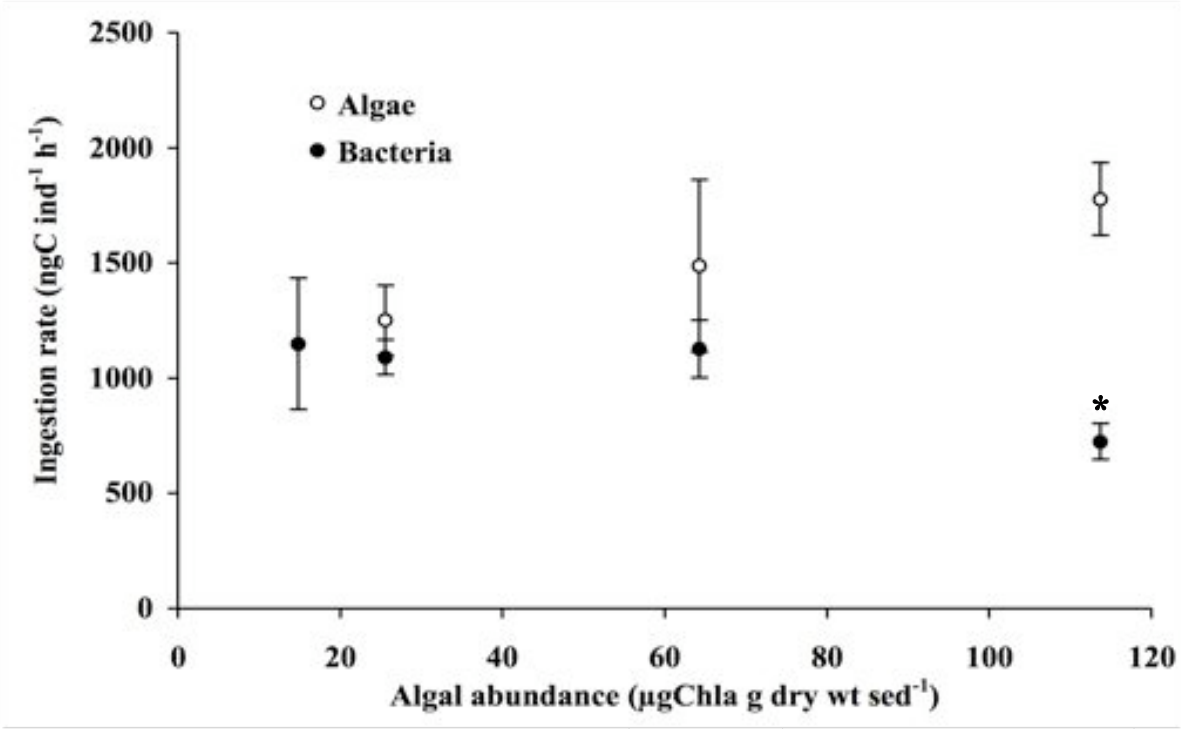

Figure 4. Ingestion rates of algal carbon ○ (mean ± SD) and bacterial carbon ● (mean ± SD) by *H. ulvae* (ngC ind^{-1} h^{-1}) as a function of algal abundance (µgChla g dry wt sed^{-1}). Bacterial abundance was kept constant (1.05 × 10^9 cells ml^{-1}). * above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

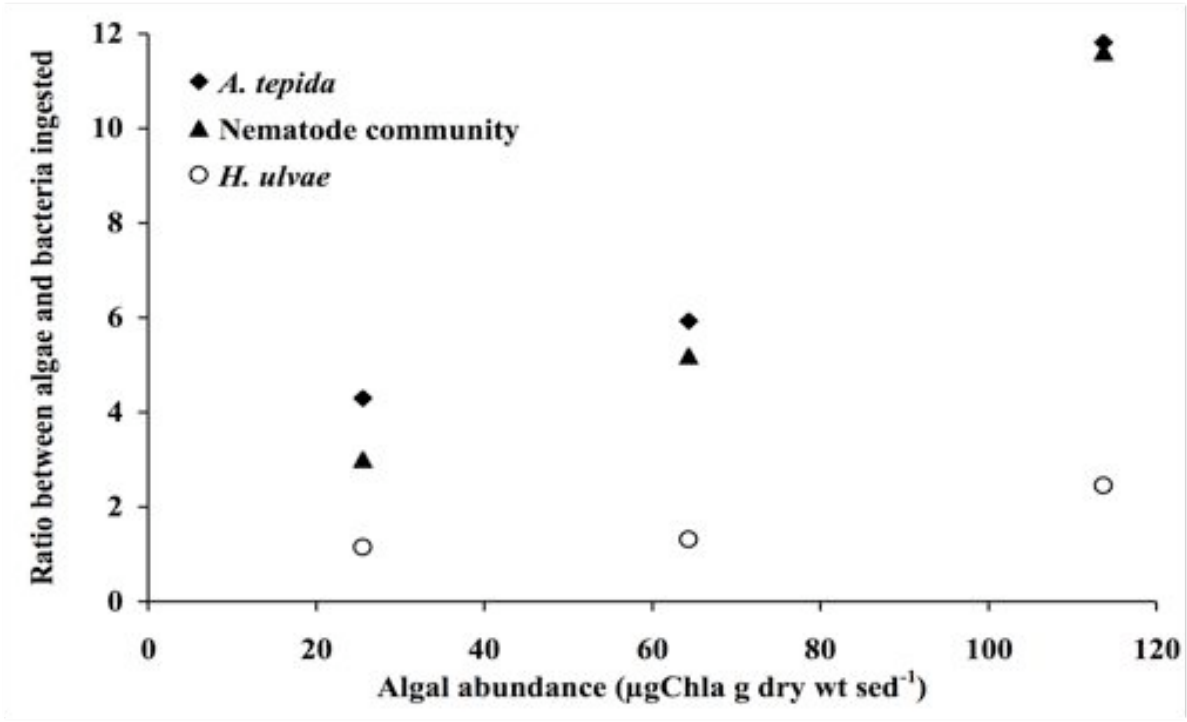

Figure 5. Ratio between algae and bacteria taken up by three different grazers (the foraminifera *A. tepida*, Brouage mudflat nematode community and the gastropod *H. ulvae*) as a function of algal abundance (µgChla g dry wt sed^{-1}). Bacterial abundance was kept constant (1.05 × 10^9 cells ml^{-1}).

Table 1. Comparison of the feeding activity of three different grazers (the foraminifera *A. tepida*, the Brouage mudflat nematode community and the gastropod *H. ulvae*).

	Meiofauna		Macrofauna
	Ammonia tepida	Nematode community	*Hydrobia ulvae*
Individual weight ($gC_{organic}$ ind^{-1})	1.0×10^{-6}	1.3×10^{-7}	1.8×10^{-4}
	Effect of environmental factors on ingestion rate of bacteria		
Range of fluctuation of ingestion rates of bacteria between 10 and 30°C	3.6	1.6	1.5
Effect of salinity (18 against 31 ‰)	Negative	None	None
Effect of luminosity (Darkness against 83 µM of photons m^{-2} s^{-1})	None	Positive	None
	Maximal ingestion rates of bacteria and algae		
Maximal ingestion rate of bacteria (10^{-3} $gC_{bacteria}^{-1}$ gC_{grazer}^{-1} h^{-1})	0.06	0.92	7.45
Maximal ingestion rate of algae (10^{-3} gC_{algae}^{-1} gC_{grazer}^{-1} h^{-1})	0.94	5.08	9.56

Effect of abiotic factors on bacterial ingestion rate

The general trend shows a temperature effect with an optimum around 30°C. However, except at the lowest temperature (5°C), differences between feeding rates of *H. ulvae* observed in the present study are not significant, indicating a limited influence of temperature. In a similar manner, Barnes (2006) did not detect changes in feeding activity of *H. ulvae* during *in situ* experiments with the same range of temperature. Ingestion rates observed at salinities of 18 and 31 were not significantly different in the present study. Grudeno & André (2001) also observed that shell growth of juvenile. *H. ulvae* was unaffected by salinity in the range of 15-30. Light did not affect the ingestion of bacteria by *H. ulvae*. The literature gives conflicting results concerning the effect of light. Barnes (1986) found that feeding activity of snails was higher in darkness whereas Orvain & Sauriau (2002) observed an increase of *H. ulvae* crawling activity with light. However in Orvain & Sauriau's experiment, light may have induced formation of algal biofilm, affecting microphytobenthic distribution and thus indirectly affecting snail activity levels.

In intertidal mudflats, the surficial centimeter of sediment is subjected to fast and large environmental variations. The ability of a grazer to sustain feeding activity when environmental conditions fluctuate can be interpreted as an adaptation to this habitat. All compared grazers in the present study came from the top centimeter of sediment of the same study area and grazing incubations were performed in similar conditions. Compared to other grazers, the feeding response of the foraminifera *A. tepida* presented the largest ranges of variation indicating that they may present a low adaptation to rapid environmental changes (Table 1). Nevertheless, *A. tepida* is considered as one of the most tolerant species of foraminifera to temperature and salinity variations (Bradshaw 1961; Walton and Sloan 1990) and more generally to environmental changes (Samir 2000; Armynot du Chatelet et al. 2004; Bouchet et al. 2007). *A. tepida* may be able to survive starvation when the environment is unfavourable and may await optimal conditions to feed and develop. An alternate explanation could be that when conditions are hostile, foraminifera move from the top layers of sediment to deeper layers (Severin and Erskian 1981; Severin 1987; Groß 2002). Feeding activity of nematodes and *H. ulvae* appeared to be more independent of environmental variables. The nematode community was mainly composed of three species, *Chromadora macrolaima*, *Daptonema oxycerca* and *Ptycholaimellus jacobi* (Chapter IIb), known to dwell and feed in surface sediment. Moreover, *H. ulvae* is considered the most environmentally tolerant of Northwest

European *Hydrobia* (Hylleberg 1975; Lassen and Kristensen 1978). However caution must be taken in interpretation of our results because the effects of each environmental factor on feeding behaviour of snails were studied independently whereas in natural environment, all these factors covary. Moreover, seasonal acclimatising capacity was not taken into account (Barnes 2006).

Feeding response to bacterial and algal abundances

In fine grained environments, *Hydrobia* ingests mouthfuls of sediment containing organic food source, including bacteria and microalgae (Kofoed 1975; Lopez and Cheng 1983b; Levinton et al. 1984). Due to high size and feeding mode of *H. ulvae*, the snail probably has a very low ability to ingest selectively very small preys such as bacterial cells. In opposition, larger preys such as diatom cells can be selectively ingested from sediment by *Hydrobia* (Fenchel 1975; Lopez and Levinton 1978; Lopez and Kofoed 1980). Indiscriminant ingestion of bacteria by *H. ulvae* implies that bacterial ingestion is exclusively dependent on ingestion rate of sediment and the concentration of bacteria in sediment. This type of feeding indicates that relationships between bacterial ingestion and bacterial concentration in sediment can be described by (i) a power law relation, (ii) a linear increase or (iii) null or decreasing relation. Those relations mean that when prey concentration increases, ingestion rate of sediment respectively (i) increases, (ii) remains constant or (iii) decreases. In the present study, the ingestion rate of bacteria first increased linearly with bacterial concentration and then levelled off at the highest concentration (Fig. 3). This relation may involve a constant ingestion rate of sediment at the lowest concentration of bacteria and a decrease of ingested sediment at the highest bacterial concentration.

Ingestion of algae by snails was not influenced by algal concentration in the range of concentration tested (Fig. 4). Contrarily, *Hydrobia* had been found previously to have increasing algal ingestion rate when algal concentration increased in a similar range of values (Forbes and Lopez 1986; Forbes and Lopez 1989; Haubois et al. 2005a). The ability of a deposit feeder to alter its ingestion rate depends on its sensory capacity to recognize food quality (Taghon 1982). This perception may differ between the freeze-dried diatoms in the present study and live diatoms in other studies, which could explain the different responses observed.

The ratio between algae and bacteria ingested denotes grazer ability to select diatoms from the sediment/bacteria aggregate. At each algal concentration, this ratio was always higher for *A. tepida* and nematodes, indicating higher algal selection

efficiency. The nematode community was composed mainly of epigrowth feeders (75%) and non selective deposit feeders (21%). Epigrowth feeders puncture diatom cells with their teeth to ingest cell contents. Consequently, they are mainly dependant on algal resources (Jensen 1987). *A. tepida* uses a network of pseudopodia to gather and ingest food particles. This feeding mode allows foraminifera to be highly selective in ingested food (Lee et al. 1966; Lee and Muller 1973). *Ammonia* may also greatly depend on algal resources, as this foraminifera was found to ingest rapidly and with high efficiency fresh algal deposits (Moodley et al. 2000). Montagna and Yoon (1991) also observed that nematodes demonstrate a high efficiency in selective ingestion of algae in comparison with other meiofaunal groups. *H. ulvae* appeared less proficient in algal selection than meiofaunal grazers. *N. phyllepta,* the algal species used in the present study may have been too small (<30 µm) to allow selective ingestion by *H. ulvae*, but this hypothesis can reasonably be rejected as *Hydrobia* is not able to select diatoms according to the cell size (Levinton 1987; Haubois et al. 2005a). In sandy sediment, *Hydrobia* presents epipsammic browsing activity by taking particles into the buccal cavity, scraping off attached microorganisms and then spitting out the particles (Lopez and Kofoed 1980). As a result, gut contents and even fecal pellets of *Hydrobia* can contain more diatoms than the offered sandy sediment (Fenchel et al. 1975; Lopez and Levinton 1978). Conversely, in fine grained sediment, *Hydrobia* ingests mouthfuls of sediment containing organic food, including microalgae (Kofoed 1975; Lopez and Cheng 1983b; Levinton et al. 1984). Results of the present study also suggest that *H. ulvae* feeding on muddy sediment present a limited ability to discriminate between algae and the sediment/bacteria aggregate. Taghon and Jumars (1984) pointed out that for animals having limited particle selection ability, foraging strategies are mainly a function of ingestion and digestion processes. Indeed, in the present study *H. ulvae* appeared to decrease the rate of ingested sediment at high algal and bacterial concentrations. In the present study, labelled prey were distributed homogeneously in sediment. Feeding rates and feeding behaviour of *H. ulvae* may be different when algae are condensed in biofilm.

In the Brouage mudflat, *H. ulvae* and meiofauna are present, on average, in similar biomasses throughout the year (Degré et al. 2006). The present study suggests that *H. ulvae* ingests bacteria at a higher rate than meiofaunal do. In the study area, benthic bacteria would therefore be grazed to a higher extent by macrofauna than by meiofauna.

CHAPITRE III

SUIVI ANNUEL DU DEVENIR DES BACTERIES BENTHIQUES DANS LE RESEAU TROPHIQUE DE LA VASIERE DE BROUAGE (MARENNES-OLERON)

CHAPITRE III

TROPHIC FATE OF BENTHIC BACTERIA IN AN INTERTIDAL MUDFLAT: INFLUENCE OF ENVIRONMENTAL FACTORS

Pierre-Yves Pascal, Christine Dupuy, Pierre Richard,
Clarisse Mallet, Eric Armynot du Chatelet, Nathalie Niquil

Abstract

Despite their high abundance and production in mudflat sediments, the role of bacteria in benthic food webs remains elusive. The objectives of the present study were to identify environmental parameters influencing uptake of bacteria by grazers and to evaluate the trophic fate of benthic bacteria in an intertidal mudflat (Brouage, Marennes-Oléron, France). Variations of grazing rates of the most abundant organisms of meiofauna and macrofauna were estimated using ^{15}N pre-enriched bacteria at different temporal scales and on a transect over the mudflat. Grazing incubations were performed in situ in microcosms with freshly collected grazers. In the study area, environmental factors varied more at seasonal scale than at daily scale or between sampling stations along the transect. Bacterial uptake by grazers appears poorly influenced by abiotic factors and not linked to bacterial abundances. Algal abundance was negatively correlated with bacterivory of both the nematode community and foraminifera *Ammonia tepida*. This trend suggests that bacteria constitute an alternative resource, consumed when algae are less abundant. Bacteria were mainly ingested by the mudsnail *Hydrobia ulvae* and secondly by the nematode community. The grazing rate of the copepod community and *A. tepida* were considerably lower. In the upper and middle part of the mudflat, the estimated grazing represented 7 and 28% respectively of bacterial production. In the lower mudflat, daily grazing never represented more than 3% of bacterial production throughout the year. Consequently, grazing appears to be a minor factor in the regulation of bacterial production in the studied intertidal mudflat.

Introduction

Bacteria constitute the foundation of all of earth's ecosystems through the degradation and regeneration of nutrients. However, bacteria can also play a major role in food webs and determination of this role constitutes a frequent challenge in marine microbial ecology. There is now evidence that a substantial part of bacterial production is grazed in pelagic systems (Azam et al. 1983; Ducklow and Carlson 1992; Sherr et al. 1987). Studies dealing with trophic fate of bacteria in sediments are less common than in pelagic environments (Kemp 1990), due mainly to technical difficulties despite bacterial abundances in marine soft sediment that are generally a thousand times higher than in pelagic systems (Schmidt et al. 1998). By analogy, it has often been hypothesized that grazing is among the most important fate of benthic bacteria (Bak et al. 1991; Hondeveld et al. 1994; Van Duyl and Kop 1990).

Pelagic bacteria are principally consumed by small grazers from nano and microplankton (< 20 μm) (Sherr and Sherr 2000). In benthic systems, sediment bacteria are consumed by microfauna but also by larger grazers from meiofauna and macrofauna (Review in Kemp 1990). One of the simplest approaches to determine trophic links between benthic bacteria and grazers, is the comparison between their respective abundances in natural environments. Interpretation of such results is complex. For instance, Montagna et al. (1987) and Mac Lachlan (1985) found respectively positive and negative relations between grazers and bacterial biomass, and both concluded that trophic links between grazers and bacteria were strong. Because organisms have widely different generation times, hours for bacteria and weeks for grazers, food webs can be considered as system of nested processes with different response times (Thingstad 2000). This approach presents drawbacks, because processes other than grazing such as bioturbation (Alkemade et al. 1992) and mucus secretion (Riemann and Schrage 1978) of grazers can affect bacterial dynamics. Another approach is the simultaneous measurement of bacterial grazing rate and bacterial stock. Such studies reach conflicting conclusions, some authors find a negligible percentage of bacterial production removed by grazers (e.g. Epstein and Shiaris 1992a) whereas others conclude that meiofauna alone can consume the entire bacterial community (Montagna 1984b). Epstein (1997b) suggested that the discrepancy between studies might be due to highly time-dependent grazing rates, thus, when only one or two seasons are sampled, generalizations of findings are not representative of long-term trends. To our knowledge, the trophic fate of bacteria has never been analysed seasonally in intertidal sediment.

The objectives of the present study were (i) to identify environmental parameters influencing uptake of bacteria by grazers in Brouage mudflat (Marennes-Oléron Bay, France) and (ii) to determine the trophic fate of benthic bacteria in this area. ^{15}N pre-enriched bacteria were used as tracer during grazing experiment performed, in situ, in microcosms with freshly collected meiofaunal and macrofaunal grazers.

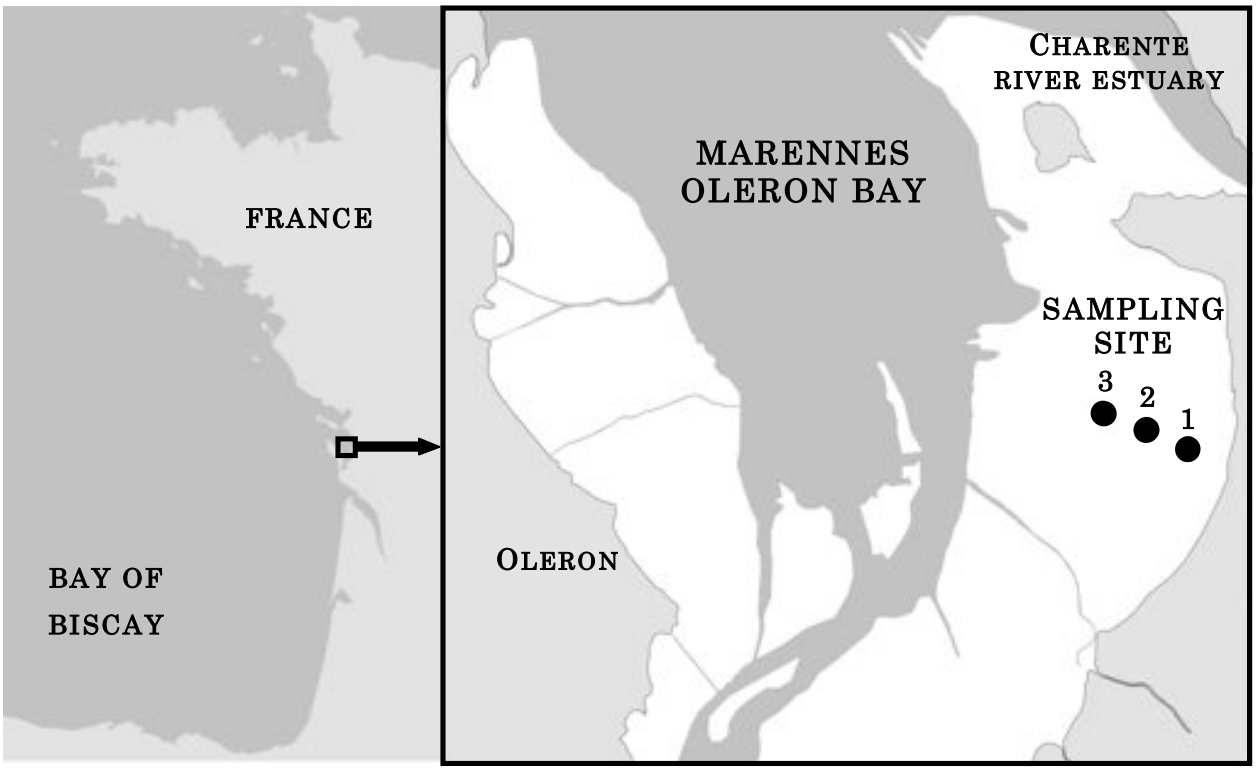

Figure 1. Map of the study area and sampling site.

Experimental procedure

Study site

Sampling was performed in Marennes-Oléron Bay, located between the French Atlantic mainland and Oléron Island. This macrotidal system is influenced by continental inputs mainly from the Charente River and occasionally from the Gironde River. It covers 170 km^2 of which 60 km^2 are intertidal mudflats. Brouage mudflat is more than 4 km wide and the sediment consists of silt and clay particles (95%<63 µm). Samples were collected along a cross shore transect, with the 3 sampled stations located at 0.7, 1.6 and 2.2 km from the coastline (Fig. 1).

Enriched bacteria

The method used for the preparation of enriched bacteria was previously described in Chapitre 1. Briefly, one centimeter-depth of surficial sediment was sampled during ebb tide in station 3 (Fig. 1). Bacteria from surficial sediment were cultured in a liquid bacterial culture medium containing 1 g l^{-1} of $^{15}NH_4Cl$ (99% ^{15}N-enriched NH_4Cl CortecNet), rinsed by centrifugation and frozen in liquid nitrogen until grazing experiments. Bacteria were thawed 20 min before the beginning of each grazing experiment. Enriched bacteria presented an abundance of 10^9 bacteria ml^{-1} and produced bacterial nitrogen comprised 3.85 % ^{15}N.

Sampling

Station 3 was regularly sampled in 2006 (January 31, March 13, April 27, May 24, June 28, July 26, September 21, November 22) and 2007 (February 20). In order to compare the range of fluctuations of measurements at the scale of both years and days, two other sampling campaigns were performed on April 25 and 26, 2006. In order to estimate spatial fluctuations, two other stations (Stations 1 and 2 in Fig. 1) were sampled on April 27, 2006. Each sampling was performed during daylight ebb on spring tides. During each sampling session, three samples of one square meter of sediment (top centimeter) were independently collected in three areas each separated by 2 meters. The pH of the sediment was measured in situ before each sampling. These triplicate sediment samples were independently homogenised and used for grazing experiments and for measurements of biotic parameters. One of this triplicate was used for measurements of abiotic parameters. In order to compare grazing rates in surficial and

deeper sediment, a deeper layer (4-5 cm) was also sampled in April in the three stations of the transect.

Grazing experiment

Grazing experiments are based on the assumption that grazers present inertia in their feeding behavior and that grazing rates remain consequently unchanged between natural environment and microcosms. For each sample, 100 ml of sediment was sieved on a 63 µm mesh to remove fine particles and bacteria, macrofauna was removed by hand. The sediment remaining on the mesh was then mixed with enriched bacteria and placed in 100 ml beakers taped with cellophane. The grazing rate of *Hydrobia ulvae* was measured after adding five snails to the sediment in the beaker. For each session, one control experiment, with five specimens of *H. ulvae*, was performed in similar conditions but the sediment was first frozen in liquid nitrogen for at least 20 min in order to kill grazers. Taped beakers were plunged into muddy sediment and incubated for 5 hours. For technical reasons, the incubation of microcosms was not performed at the sampling station but in muddy sediment nearby the stations. Time between sediment sampling and start of the incubation never exceeded one hour. Incubations were stopped by sieving sediment on 63 µm mesh and freezing the sediment in liquid nitrogen. When the samples were thawed, nematodes and copepods were extracted from sediment using centrifugation after mixing with Ludox HS40 TM (Heip et al. 1985). For each sample, at least 700 nematodes and 130 copepods specimens were picked up individually at random. After centrifugation, the pellet fraction was stained with rose Bengal in order to identify freshly dead foraminifera (Murray and Bowser 2000). *Ammonia tepida* and *Haynesina germanica* were picked up separately and cleaned of any adhering particles. Biomass of *A. tepida* in the higher part of the mudflat (stations 1 and 2) was not high enough to allow isotopic analyses. Biomasses of *H. germanica* were high enough for isotopic analyses only at three times during the year. *H. ulvae* were separated by hand from their shell and all specimens were pooled and homogenized using a Potter-Eveljhem. September samples of *H. ulvae* were lost, thus snail grazing rate data were not available for this period.

Isotope analysis and calculations

$\delta^{15}N$ and $\delta^{13}C$ of bacteria and grazers was measured using an EA-IRMS (Isoprime, Micromass, UK). Nitrogen natural isotope composition is expressed in the delta notation ($\delta^{15}N$) relative to air N_2: $\delta^{15}N = [(^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{reference})-1] \times 1000$. Carbon isotope

composition is expressed in the delta notation ($\delta^{13}C$) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = [(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{reference})-1] \times 1000$. Carbon and nitrogen content were obtained along with isotope ratios during isotope analysis.

Incorporation of ^{15}N is defined as excess (above background) ^{15}N and is expressed in terms of specific uptake (I). I was calculated as the product of excess ^{15}N (E) and biomass of N per grazer. E is the difference between the background ($F_{background}$) and the sample (F_{sample}) ^{15}N fraction: $E = F_{sample} - F_{background}$, with $F = {}^{15}N / ({}^{15}N + {}^{14}N) = R / (R + 2)$ and R = the nitrogen isotope ratio. For the $F_{background}$, we used values measured with the control grazers (frozen). R was derived from the measured $\delta^{15}N$ values as: $R = ((\delta^{15}N/1000)+1) \times R\text{air}N_2$ where $R\text{air}N_2 = 7.35293\ 10^{-3}$ (Mariotti 1982). The uptake of bacteria was calculated as Uptake = ($I \times$ (% $C_{enriched\ bacteria}$ / % $N_{enriched\ bacteria}$) / ($F_{enriched\ bacteria} \times$ incubation time)). In preliminary experiments, we measured that bacterial abundance in 63 µm sieved sediment did not exceed $2.9\ 10^7$ cells ml^{-1}. As this abundance represented less than 3% of the abundance of enriched bacteria added to the microcosm, we considered negligible the fraction of natural bacteria remaining in sieved sediment. Consequently, calculations of grazing rates are based on the assumption that grazers only took up enriched bacteria.

Environmental factors

Flagellates were extracted from sediment using a solution of Percoll-sorbitol (Price et al. 1978), labelled using 4.6-diamidino-2-phenylindole dihydrochloride (DAPI) (2500 µg l^{-1}), filtered onto 3 µm Nucleopore black filter (Porter and Feig 1980). They were then counted and individual length (L) and width (2r) was measured under microscope. Biovolume (V) of individuals was calculated as follows: $V = (4/3)\ \pi\ (L/2)\ r^2$. Biomass (carbon values) was calculated from cell volumes and concentrations using a carbon content of 200 fgC μm^{-3} (Bak and Nieuwland 1989). Copepods and nematodes were extracted using Ludox HS40 TM. For abundance estimations, a Motoda-box was used to split sample (Motoda 1959) and obtain aliquots containing at least 500 individuals. At each sampling, a large number of specimens were extracted and weighed for isotopic analyses. Consequently mean copepod and nematode individual weight and carbon content were determined from those weighing and isotope analyses. Total weight of the foraminifera *A. tepida* was also determined in the same way and a conversion factor was used to determine the organic fraction of foraminifera (organic carbon = 5.8 % of total weight in this area, (Chapter IIb). For each sample, 400 ml of collected sediment were

sieved on 500 µm mesh to determine *H. ulvae* abundance and to recover snails for grazing experiments. Carbon content of snails was determined from isotope analyses.

Algal biomass in sediment was assessed using Chl *a* as a proxy and measured using fluorometry (Lorenzen 1966). Carbon algal biomass was estimated using a ratio between carbon and chlorophyll of 45 (Jonge 1980). To determine bacterial abundance, bacteria were extracted from sediment particles by incubation in pyrophosphate (0.01 M for at least 30 minutes) and sonication. Bacteria were labelled using DAPI (2500 µg l^{-1}), filtered onto 0.2 µm Nucleopore black filters (Porter and Feig 1980) and then counted under an epifluorescence microscope. Individual length (L) and width (2r) was determined by computer-assisted image analysis (Axio Vision Release 4.3) with an epifluorescent microscope (Axioskop 2 mot plus – Zeiss) equipped with a charge-coupled device camera (AxioCam MRc5 – Zeiss). For each sampling, biovolume (V) of at least 500 bacterial cells was calculated as follows : $V = \pi r^2 (L-2/3r)$ (Fuhrman 1981). Biovolume was converted to carbon biomass using a ratio of 220 fgC μm^{-3} (Bratbak and Dundas 1984).

The bacterial production estimated by tritiated thymidine incorporation was measured using the protocol of Garet and Moriarty (1996). Thymidine incorporation was converted to numbers of bacterial cells using a ratio of 5 10^{17} cells mole of $thymidine^{-1}$ (Pollard and Moriarty 1984).

The median grain size of sediment was characterized using a Malvern Mastersizer 2000 (size range 0.02-2000 µm). Elemental analysis (Carbon and Nitrogen) of sediment was determined with a FlashEA 1112 Elemental Analyser (Thermo). The fraction of mineral C contained in carbonate was determined using a Bernard Calcimeter. C abundance used for the calculation of C/N ratio was total C content minus C contained in carbonate. Water content was estimated by weight loss after freeze-drying of 100 ml of sediment. Organic matter content was estimated by weight loss after combustion at 450°C for 24 h of 1 g of freeze-dried sediment. The carbohydrate concentrations were determined using the method of Dubois et al. (1956) as modified by Moal et al. (1985). Particulate proteins were assayed using the method of Lowry et al. (1951). Temperature in microcosms was regularly measured every 30 minutes during the 5 hour long incubation and a mean temperature was calculated.

Table 1. Values of abiotic factors measured in surficial sediment according to space, day and year scales.

	Spatial scale		Day scale			Year scale							
Sampling point	1	2	3			3							
Date	27 Apr-06	27 Apr-06	25 Apr-06	26 Apr-06	27 Apr-06	Jan-06	Mar-06	May-06	Jun-06	Jul-06	Sep-06	Nov-06	Feb-07
pH	7.9	8.0	8.0	8.6	8.1	7.9	7.8	8.4	8.4	7.7	7.4	8.1	7.9
Salinity (‰)	28.7	28.4	28.5	30.8	27.2	31.4	29.3	29.8	28.5	29.9	30.0	27.9	27.0
Temperature (°C)	19.8	19.8	25.9	22.8	19.8	7.2	7.2	19.3	32.9	33.7	23.6	13.8	13.2
Water content (%)	59.8	61.6	66.7	65.2	65.1	62.8	66.6	58.4	57.8	59.4	52.0	60.2	56.3
Median grain (μm)	8.9	8.8	11.9	9.4	12.1	12.3	14.0	9.2	12.8	17.2	16.7	9.4	12.9
Carbohydrate (mg/g dry sed)	2.3	1.8	0.4	1.4	1.0	2.0	1.5	1.8	1.8	2.1	1.7	6.8	9.1
Protein (mg/g dry sed)	52.4	46.8	42.8	35.6	60.5	39.5	20.6	53.2	62.7	51.0	59.1	16.9	22.6
C/N	8.8	7.3	9.1	8.9	8.6	2.3	6.6	9.3	7.7	8.3	10.9	11.9	3.7
Organic Matter (%)	10.7	11.5	11.1	11.2	11.3	10.6	10.7	9.8	10.8	10.2	8.4	8.6	9.1

Table 2. Values of biotic factors measured in surficial sediment according to space, day and year scales (means ± SD).

	Spatial scale		Day scale			Year scale							
Sampling point	1	2	3			3							
Date	27 Apr-06	27 Apr-06	25 Apr-06	26 Apr-06	27 Apr-06	Jan-06	Mar-06	May-06	Jun-06	Jul-06	Sep-06	Nov-06	Feb-07
Algal biomass ($mgC\ m^{-2}$)	3971 (±144)	4624 (±589)	2860 (±203)	2883 (±184)	3067 (±110)	6849 (±422)	5552 (±183)	2804 (±530)	2943 (±141)	2737 (±131)	2776 (±234)	2922 (±203)	2336 (±357)
Bacterial biomass ($mgC\ m^{-2}$)	969 (±144)	1759 (±335)	1013 (±181)	959 (±85)	1085 (±55)	1263 (±317)	937 (±17)	1215 (±122)	1304 (±78)	1544 (±411)	1764 (±73)	1285 (±155)	994 (±208)
Bacterial production ($mgC\ m^{-2}\ d^{-1}$)	828 (±172)	1160 (±266)	808 (±148)	1357 (±176)	756 (±32)	469 (±138)	752 (±401)	2050 (±154)	1172 (±20)	1235 (±169)	992 (±413)	2285 (±523)	994 (±80)
Flagellate biomass ($mgC\ m^{-2}$)	0.25 (±0.03)	1.58 (±1.30)	0.48 (±0.23)	0.45 (±0.21)	0.80 (±0.71)	1.07 (±0.25)	0.53 (±0.12)	0.28 (±0.05)	0.13 (±0.10)	0.35 (±0.12)	0.12 (±0.06)	0.54 (±0.13)	0.34 (±0.21)
Nematode biomass ($mgC\ m^{-2}$)	1521 (±548)	658 (±206)	n. a.	n. a.	230 (±16)	306 (±44)	163 (±38)	242 (±42)	197 (±25)	197 (±56)	271 (±19)	221 (±56)	286 (±8)
Copepod biomass ($mgC\ m^{-2}$)	12.2 (±3.6)	9.9 (±4.3)	n. a.	n. a.	19.3 (±1.4)	21.4 (±1.7)	22.9 (±1.3)	93.2 (±13.2)	21.6 (±5.8)	12.7 (±2.2)	19.0 (±10.1)	30.4 (±7.6)	16.5 (±2.2)
A. tepida biomass ($mgC\ m^{-2}$)	1.0 (±0.1)	0.7 (±0.4)	n. a.	n. a.	23.9 (±2.8)	3.9 (±0.3)	20.9 (±5.6)	7.3 (±2.2)	2.3 (±0.8)	7.4 (±7.5)	2.3 (±0.2)	3.8 (±0.2)	16.8 (±14.7)
H. ulvae biomass ($mgC\ m^{-2}$)	279 (±150)	795 (±234)	n. a.	n. a.	4 (±7)	154 (±55)	31 (±53)	117 (±32)	202 (±50)	113 (±59)	22 (±25)	111 (±40)	19 (±9)

Treatment of data

In order to identify the influence of environmental factors, Principal Component Analysis (PCA) was performed using pH, temperature, water content, median grain size, carbohydrate, protein, C/N, organic matter and algal and bacterial biomasses for all surficial sediment sampling occasions (n = 13). Variations of grazing rates of nematodes according to depth were tested using two-tailed tests. One-way analysis of variance (ANOVA) was used to test variations of grazing rates of meiofauna at yearly and daily scales. The Tukey test was used for post-hoc comparisons. Spearman rank (r_s) correlations were performed to investigate the relationships between grazing rates of meiofauna and environmental parameters. PCA and statistical analyses were run using the statistical software Excel Stat Pro ©.

Results

Environmental factors

Abiotic parameters measured during the monitoring are presented in Table 1 and biotic parameters are presented in Table 2. The F1 and the F2 axes of the PCA together explained 54% of variability (Fig. 2). Data appear separated according to seasons: a summer-autumn group (comprising samples of July, November and September 2006), a winter group (comprising samples of January and March 2006 and February 2007) and a spring group (comprising samples of April, May and June 2007). The sample from February 2007 was slightly distant from winter samples of 2006 indicating possible variability between the two years. During April 2006, spatial and day-to-day variations were considered: two other stations were sampled (Fig. 1) and the station 3 commonly sampled along the year was sampled during three consecutive days. Spatial and day-to-day variations appeared to be lower than seasonal ones as all April samples were pooled in a spring group. Summer-autumn period was characterised by high biomass and production of bacteria and low water content, algal biomass and organic matter (Fig. 2). Winter period was characterised by elevated median grain size and high carbohydrate content of sediment and low temperature, bacterial production and C/N. Moreover January and March 2006 samples were distinguished by higher algal biomass and higher water contents than February 2007 ones. The spring period was characterised by high values of pH and C/N and low values of median grain size and carbohydrates.

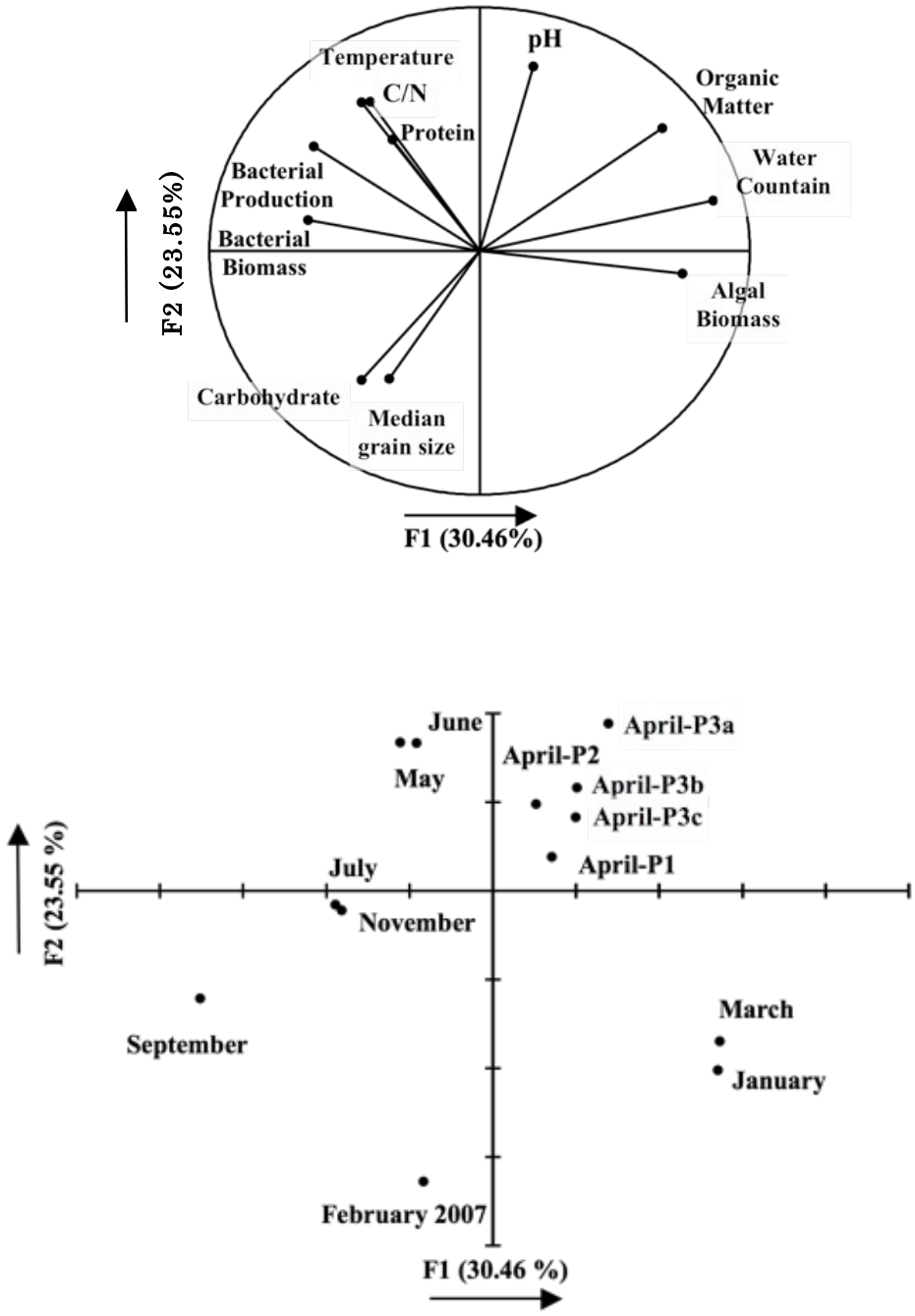

Figure 2. PCA calculated using 13 observations (samples of surficial sediment) and 11 variables (8 abiotic and 3 biotic variables). For the variables, the circle of correlation was reported. For the sampling occasions, the name of the station and the date were reported. Sampling periods were grouped. April 3a, b and c represent sampling performed April, 25, 26 and 27, 2006.

In station 3, mean bacterial and algal biomasses were 1215 ± 259 and 3430 ± 1412 mgC m^{-2} respectively, and mean flagellate biomass was 0.5 ± 0.3 mgC m^{-2} (Table 2). Nematodes dominated the meiofauna in biomass along the year in surficial sediment at station 3 (234 ± 42 mgC m^{-2}). Mean biomass of copepods was 28 ± 24 mgC m^{-2} and mean biomass of foraminifera was 8.1 ± 7.0 mgC m^{-2} (Table 2). During September and November 2006 and February 2007, the community of foraminifera was dominated by *H. germanica*. The rest of the year, *A. tepida* represented 92 ± 5 % of total foraminiferal biomass. The mudsnail *H. ulvae* had an annual mean biomass of 71 ± 70 mgC m^{-2}. In April 2006, station 1 was characterised by a high biomass of nematodes (1521 ± 548 mgC m^{-2}), whereas station 2 was characterised by a high biomass of *H. ulvae* (795 ± 235 mgC m^{-2}).

During April 2006, a deeper layer (2-5cm) of sediment was sampled in 3 stations of the transect. At stations 1, 2 and 3, bacterial biomass of this layer represented respectively 5537 ± 1346 mgC m^{-2}, 5671 ± 258 mgC m^{-2} and 5931 ± 305 mgC m^{-2} whereas nematode biomasses were 88 ± 31 mgC m^{-2}, 65 ± 18 mgC m^{-2} and 18 ± 3 mgC m^{-2}.

In the lower part of the mudflat, the range of fluctuation of grazing rate measured in pgC ind^{-1} h^{-1} was 8-36 for nematodes, 44-228 for copepods, 35-308 for *A. tepida*, 24-123 for *H. germanica* and 883-2339 for *H. ulvae* (Fig. 3 and 4). Along the year, there were significant differences in grazing rates of nematodes ($F = 5.3$; $p < 0.01$), copepods ($F = 6.7$; $p < 0.001$) and *A. tepida* ($F = 5.8$; $p < 0.01$). Samples did not allow measurement of bacterivory of *A. tepida* at short-term and spatial scales. At a daily scale, there were no differences in bacterivory of nematodes ($F = 1.7$; n.s.) and copepods ($F = 7.09$; n.s.) (Fig. 3). Nematode grazing rate was identical at the three stations sampled ($F = 2.6$; n.s.) (Table 3). By contrast copepod grazing rate was significantly higher at station 1 than at stations 2 and 3 ($F = 9.5$; $p < 0.05$).

Low biomasses of copepods and foraminifera in deeper layers of sediment did not allow measurements of their grazing rates. Individual grazing rates on bacteria were identical for nematodes from surficial (0-1 cm) and deeper (1-5 cm) layers of sediment at stations 1 ($t = 0.8$; n.s.) and 2 ($t = 0.8$; n.s.). At station 3, nematodes dwelling in deeper sediment grazed bacteria at a higher rate than surficial nematodes ($t = 3.7$; $p < 0.05$) (Table 3).

Grazing rates	Sampling point 1	2	3
Nematode in surface (pgC ind^{-1} h^{-1})	11.7 (± 3.3)	8 (± 3.6)	18.2 (± 5.3)
Nematode in deep layer (pgC ind^{-1} h^{-1})	13.8 (± 3.3)	9.4 (± 3.6)	30.1 (± 7.1)
Copepod (pgC ind^{-1} h^{-1})	227.6 (± 84.4)	59.9 (± 8.5)	73 (± 5.1)
Foraminifera (pgC ind^{-1} h^{-1})	n. a.	n. a.	125 (± 63)

Table 3. Grazing rates of meiofauna in surficial sediment and grazing rates of nematodes in the deeper layer of sediment along the transect (means ± SD).

As *H. ulvae* can demonstrate large-scale movements in the studied mudflat (Haubois et al. 2002), specimens collected in an area are not necessarily adapted to local conditions of this area. Consequently, we did not study effects of environmental factors on grazing rates of *H. ulvae*. Meiofaunal grazing was poorly affected by abiotic factors (Table 4) with the exception of nematode grazing, negatively influenced by water content of sediment and positively by median grain size. Nematodes and *A. tepida* presented a higher grazing rate of bacteria when biomasses of algae were low. Moreover, grazing rate of nematodes was also negatively correlated with flagellate biomass. The grazing rate of foraminifera *A. tepida* was positively correlated with bacterial production. None of the grazers was affected by grazing activity of other grazers.

At station 3, the range of bacteria removed by studied grazers along the year fluctuated between 0.4 and 34.5 mgC m^{-2} d^{-1}. On average, macrofauna removed 11.5 ± 10.2 times more bacteria than meiofauna. This daily grazing pressure represented 0.15-2.25% of bacterial stock and 0.12-3.41% of bacterial production. Except in April, *H. ulvae* grazed at a higher rate than meiofauna with values ranging from 0.9 to 34.0 mgC m^{-2} d^{-1} compared to ranges of 0.36 to 1.51 mgC m^{-2}d^{-1} for meiofauna (Fig. 5 and 6). Nematodes were the main meiofaunal grazers. In the spatial comparisons, bacteria removed by grazers in station 2 reached 332 mgC m^{-2} d^{-1}, representing 18.9% of bacterial stock and

28.6% of bacterial production (Table 5). In the deeper layer of sediment, the fraction of bacteria removed by nematodes represented 0.003, 0.002 and 0.001 % of bacterial stock at station 1, 2 and 3 respectively.

Table 4. Correlation coefficients (r_S, Spearman rank) between grazing rates of meiofauna and abotic and biotic parameters of the environment (n = 13) (* $p < 0.05$; ** $p < 0.01$; *** ; $p < 0.001$).

	Grazers of bacteria		
	Nematoda	Copepoda	*A. tepida*
pH	0.099	-0.391	-0.175
Salinity	-0.119	0.140	-0.119
Temperature	0.320	-0.122	0.450
Sediment water content	-0.599 *	-0.390	-0.475
Median grain size	0.566 *	-0.104	0.265
Organic Matter	-0.456	-0.209	-0.431
C/N	0.275	0.022	0.425
Carbohydrate	0.016	0.253	0.182
Protein	0.390	0.203	-0.055
Algal biomass	-0.731 **	-0.143	-0.829 ***
Flagellate	-0.560 *	-0.214	-0.525
Bacterial biomass	0.247	0.050	0.470
Bacterial production	0.209	-0.170	0.569 *
Bacterial grazing by *A. tepida*	0.547	0.083	
Bacterial grazing by copepoda	0.143	-0.313	0.083
Bacterial grazing by nematoda		0.143	0.547

Table 5. Bacteria taken up (mgC m^{-2} d^{-1}) by grazers in superficial and deep layers of sediment along the transect. * data estimated with grazing rates measured at station 3.

	Sampling point		
Bacterial biomass grazed	**1**	**2**	**3**
Nematode in surficial sediment (mgC m^{-2} d^{-1})	**2.89**	**0.79**	**0.66**
Nematode in deeper sediment (mgC m^{-2} d^{-1})	**0.18**	**0.11**	**0.08**
Copepod (mgC m^{-2} d^{-1})	**0.43**	**0.11**	**0.14**
***A. tepida* * (mgC m^{-2} d^{-1})**	**0.003**	**0.060**	**0.003**
***H. ulvae* * (mgC m^{-2} d^{-1})**	**59.9**	**331.7**	**2.7**

Discussion

Methodology

During each sampling session, grazers were collected and rapidly put in contact with ^{15}N labeled bacteria in microcosms. In a previous paper, we confirmed that labelled bacteria presented characteristics of size, activity and diversity roughly similar to natural bacteria (Chapter I). Grazing experiments are based on the assumption that grazing rates remain unchanged between the natural environment and the microcosms. This assumption implies that (i) environmental factors influencing feeding behaviour of grazers in the natural environment still have an influence in microcosms and (ii) grazing rates measured permit an evaluation of the fraction of bacterial stock removed by the studied grazers in the natural environment.

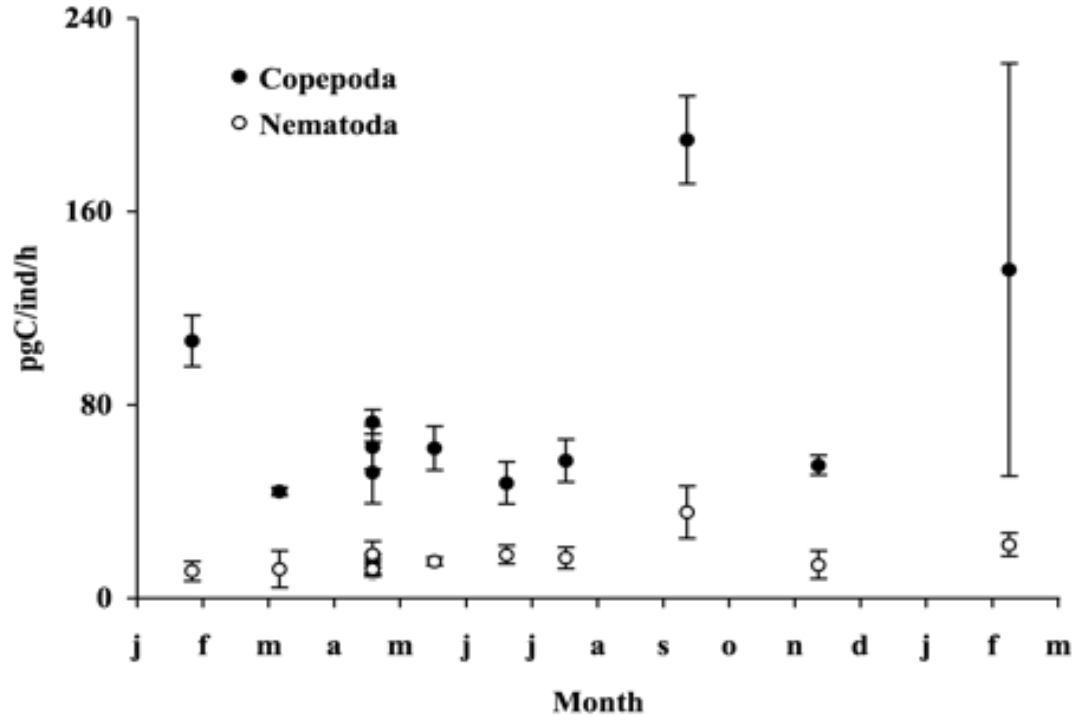

Figure 3. Individual grazing rates (pgC ind^{-1} h^{-1}) of copepods and nematodes through the year at station 3 (means ± SD).

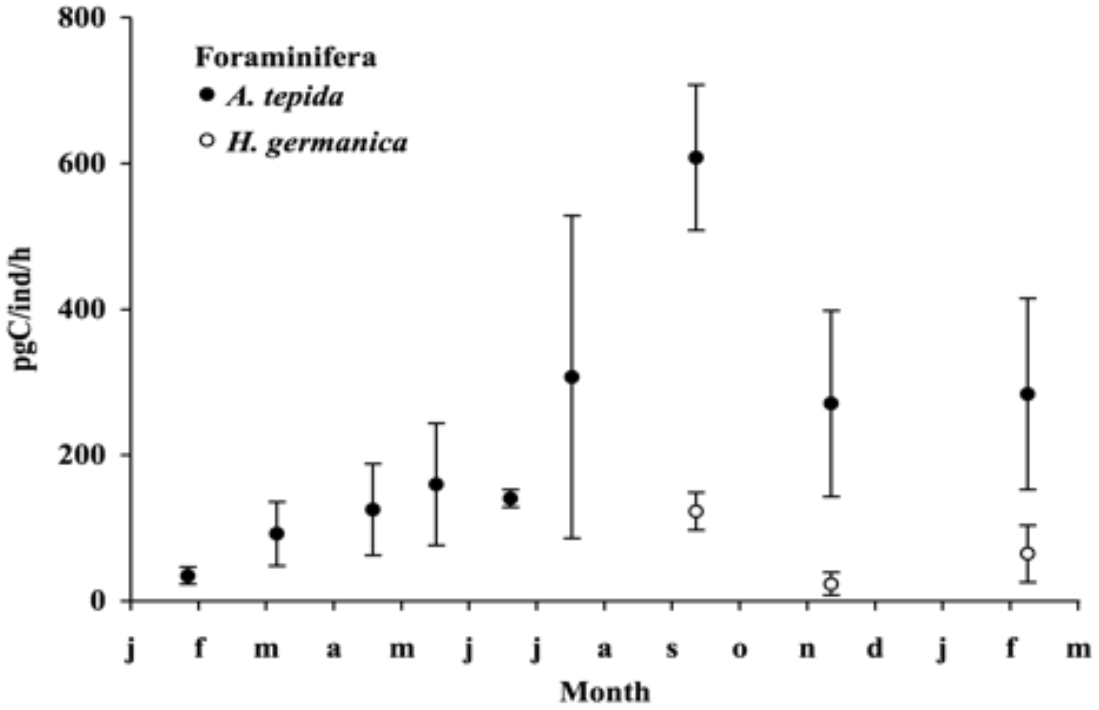

Figure 4. Individual grazing rates (pgC ind^{-1} h^{-1}) of foraminifera (*A. tepida* and *H. germanica*) through the year at station 3 (means ± SD).

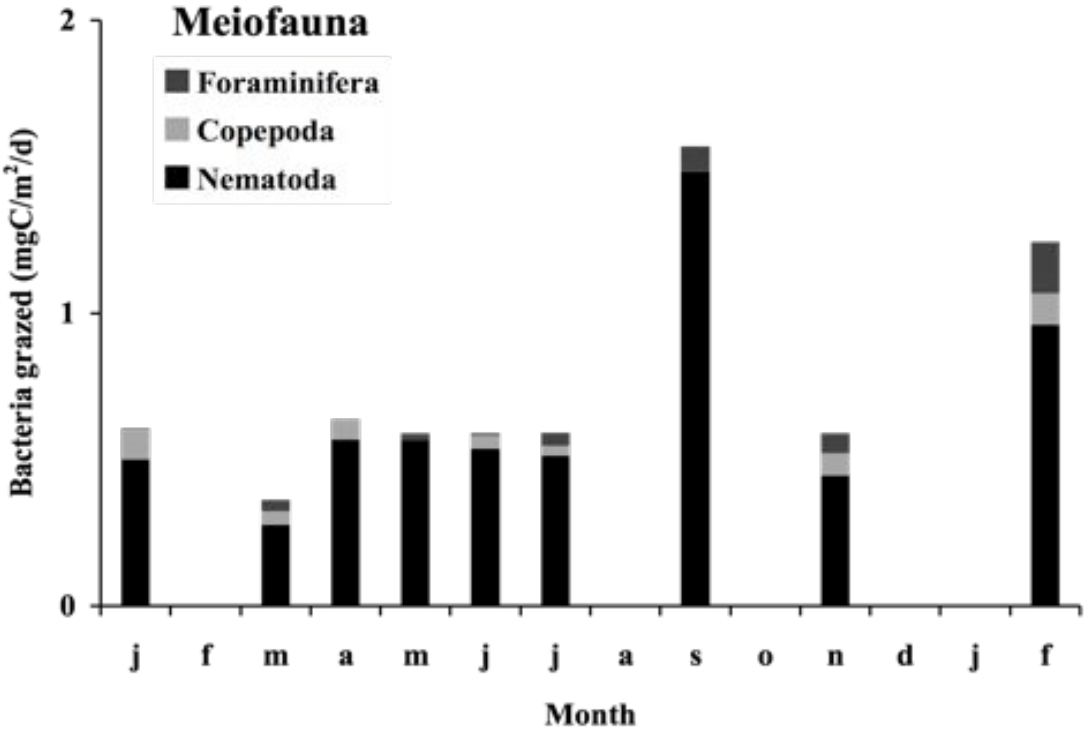

Figure 5. Bacteria grazed (mgC m^{-2} d^{-1}) by meiofauna in surficial sediment at station 3 through the year.

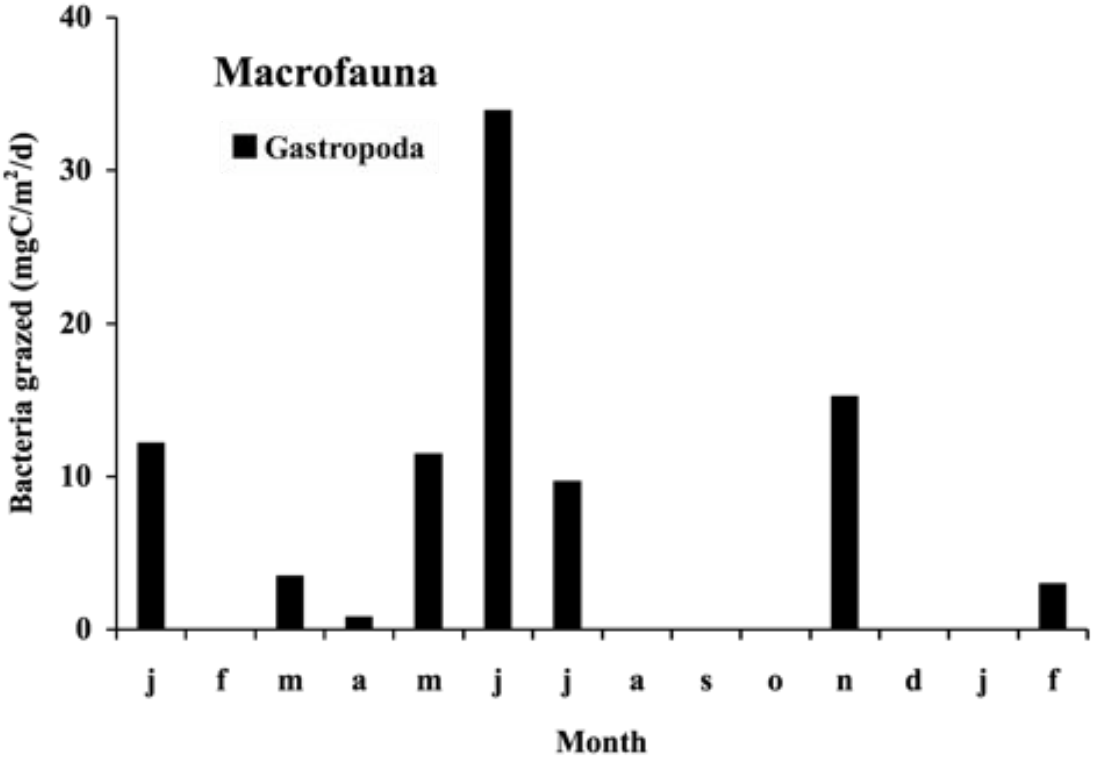

Figure 6. Bacteria grazed (mgC m^{-2} d^{-1}) by *H. ulvae* in surficial sediment at station 3 through the year.

Measured bacterial abundances and productions are within the range of values reported for other intertidal sediments (Cammen 1991; Epstein and Shiaris 1992b; Hamels et al. 2001). Grazing rates of bacteria established with ^{15}N prelabeled bacteria method in experimental conditions were previously found in the range from literature (Chapter I). Moreover, grazing rates found in this previous experimental study (Chapter I) and in the present work were 27 versus 8-36 pgC ind^{-1} h^{-1} for nematodes, 67 versus 35-308 pgC ind^{-1} h^{-1} for *A. tepida* and 1149 versus 883-2339 pgC $ind^{-1}h^{-1}$ for *H. ulvae*. Indeed, our results are in a similar range of values from the literature despite slight differences in methodology: in the laboratory experimental study, grazers were allowed to graze on a slurry of natural and enriched bacteria whereas in the present study, they only grazed on enriched bacteria.

One goal of the present study was to determine the effect of environmental factors on grazing rates. We assumed that environmental conditions impacting grazers might principally vary temporally. The seasonal aspect was consequently favoured over the spatial in the sampling strategy. This assumption was confirmed as PCA showed a higher separation of sampling sessions over seasons than over spatial locations or day-to-day time scales. Sampling strategy used in the present study should therefore cover a large range of environmental conditions supporting identification of the factors that most influence grazing rates. Indeed, similarly to environmental conditions, meiofaunal grazing rate significantly changed along the year but remained constant at short temporal and spatial scales, similar to environmental conditions. Copepod grazing did not follow this trend but showed spatial differences in grazing rates. This suggests that feeding behaviour of copepods may be driven by factors not taken into consideration in the present study.

Effects of biotic and abiotic factors on bacterivory

The mudsnail *H. ulvae* can realise large-scale movements in the studied mudflat (Haubois et al. 2002). This mobility implies that snails are not necessarily adapted to the local conditions where they are collected. Consequently, we did not study the effects of environmental factors on grazing rates of *H. ulvae*. During experiments, incubations were performed with temperatures ranging from 7.2 to 33.7°C. Despite this large range of fluctuation, meiofaunal grazing was not significantly affected by temperature (Table 4). In experimental conditions, temperature was found to affect grazing rates of *A. tepida* (Chapter IIb), nematodes (Moens and Vincx 2000) and copepods (Lonsdale and Levinton 1989). In those experiments, grazers were not acclimatised at each tested temperature.

Consequently, the rapid transfer of grazers to different temperature demonstrates the effects of short-time scale variations of temperature. In the present study, grazers were first incubated at a temperature matching with the environmental conditions. Thus, our results are not conflicting with those from other experimental studies: meiofauna may be affected by short-term variation of temperature but may acclimatize and adjust to local ambient circumstances along the year rendering it unaffected by seasonal scale temperature variations. Grazing of nematodes decreased when sediment presented high water content and low median grain size, whereas grazing of copepods and *A. tepida* was not affected. Caution must be taken in the interpretation of those results, as p-values of correlations were low. The decrease of median grain size of sediment was previously found to affect grazing of harpaticoid copepods (De Troch et al. 2006) and foraging of predacious nematodes (Gallucci et al. 2005). Sediments with low grain size have reduced pore space, which may impact the mobility of grazers (De Troch et al. 2006). None of the grazers was affected by changes in salinity, pH, organic matter, C/N or protein and carbohydrates of sediments. Consequently, the feeding behaviour of meiofauna appeared to be poorly influenced by abiotic factors in the study area.

Grazing activity can also be limited by biotic factors such as resource limitation or competition between grazers. The grazing rates of the nematode community and the foraminifera *A. tepida* on bacteria decreased with increasing algal abundance. This result suggests a change in food preference in response to changes in algal abundance. The natural isotopic composition of meiofauna suggests a similar conclusion, since low algal abundances imply a shift in meiofaunal diets and ingestion of other food sources (Carman and Fry 2002; Goldfinch and Carman 2000). Results of Epstein (1997b) also suggest that there are alternative states in microbial food web dynamics, and that ingestion rates of algae and bacteria are inversely correlated. In this study, there is no relationship between grazing rates on bacteria and bacterial abundance. If meiofauna was food limited with bacteria then it should respond with increasing feeding rates when bacterial biomass increases. Moreover, the absence of negative interactions between grazing activities of each grazer implies an absence of competition for the bacterial resource. For both reasons, bacteria may not constitute a limiting resource in this study area. Nematode and *A. tepida* appeared to graze preferentially on algae suggesting that the bacterial resource constitutes an alternative food source when algal abundances are low. This result is in accordance with (i) stable isotope analysis suggesting dominance of microphytobenthos in the diet of the Brouage nematode community (Riera et al. 1996) and (ii) the dominance of this community by epigrowth feeders, i.e. diatom consumers

(Rzeznik-Orignac et al. 2003). Moreover, grazing experiments performed with dual labelled prey establish dominance of algae over bacteria in the nematode diet (Chapter IIb; Montagna 1984b; Sundbäck et al. 1996). The foraminifera *Ammonia* was also found taking up algae with high efficiency (Moodley et al. 2000) and at a higher rate than bacteria (Chapter IIb). There are several possible explanations for why bacteria are less attractive than algae to grazers. Bacteria are generally less abundant than algae in temperate intertidal sediment (Rublee 1982). Bacteria may lack essential components such as fatty acids present in diatoms (Zhukova and Kharlamenko 1999). Moreover bacteria and algae differ in their spatial distribution. Benthic microalgae migrate in sediment and concentrate at the air-sediment interface during diurnal low tide. For grazers, feeding in this algal biofilm limits the waste of energy in (i) selection of food particles and/or (ii) rejection of non-digestible material. More homogenous vertical distribution of bacteria (Joint et al. 1982) would make bacteria less profitable than algae for grazers.

Trophic fate of bacteria

The present study suggests that bacterial abundance and production are not negatively influenced by grazer activity. Consequently, we must consider whether grazing constitutes a significant fate of bacterial production.

In general, pelagic bacterial production is grazed principally by protozoa (e. g. Sherr et al. 1986). Benthic systems differ from pelagic systems in that protozoa are proportionally less abundant relative to bacteria while ingestion rates may be comparable (Kemp 1990). Ciliates favour interstitial volumes (Fenchel 1967; Hamels et al. 2004) and low organic matter content (Bak and Nieuwland 1989; Sundbäck et al. 1996). As a result, ciliates present the highest abundance in fine sands (Fenchel 1969) and lowest abundance in muddy sediments (Alongi 1986). We did not measure ciliate abundance in the present study, as it has been shown that they have limited abundance and grazing activity in intertidal mudflat sediment (Epstein and Shiaris 1992a). In his review, Kemp (1990) attests that flagellates should ingest a large fraction of bacterial production only when their relative abundance is in the order of 1 flagellate to 1000 bacteria or more. Indeed, more recent studies confirmed that in silty sediment this ratio was lower than $1{:}10^4$ and flagellates would never graze more than 1% of bacterial stock per day (Hamels et al. 2001). In muddy sediments, with a ratio of $6.8{:}10^4$, flagellates removed 0.2% of bacterial stock per day (Epstein and Shiaris 1992a). In the present

study, this ratio never exceeded $1.3{:}10^5$. We can consequently assume that flagellate grazing may have a limited impact on bacterial stock.

In the present study, meiofauna grazed 0.04-0.34% of bacterial standing stock per day in the surficial centimeter of sediment. A majority of previous studies on the fraction of bacterial biomass grazed by meiofauna are based on the radioactive tracer technique developed by Daro (1978) and modified by Montagna (1984b; 1993). A radioactive tracer is introduced to the sediment and bacteria are labeled while they are being grazed. Using this in situ labeling technique, the fraction of bacterial biomass grazed daily by meiofauna has been found to reach 7% in beaches (Montagna and Bauer 1988), 7-12% in sandy sediments in mesocosms (Nilsson et al. 1991), 0.3-37% in shallow sandy sediments (Sundbäck et al. 1996) and 14% in sandy sediments from an hydrocarbon seep (Montagna et al. 1995). In muddy sediments, this fraction was 24% in a shallow estuary (Montagna and Yoon 1991) and reached 81% in a salt marsh (principally due to polychaetes) (Montagna 1984b). Bacterial grazing rates found in the present study lie within the lower range of those measured with in situ labeling. The use of fluorescent pre-labeled bacteria (FLB) also permits measurement of meiofaunal grazing rates. Using FLB, meiofauna was found to remove 1.5% of bacterial standing stock per day in intertidal sandflats (Epstein 1997a) whereas in intertidal mudflats, meiofaunal removal was limited to 0.03% (Epstein et al. 1992). Results of integrated modeling and in situ isotope tracer experiments suggested that 3% of bacterial biomass was grazed daily by meiofauna in a muddy intertidal flat (Van Oevelen et al. 2006). Our results are more in accordance with those last findings. It has been suggested that bacteria are principally affected by grazing in comparatively organic-poor habitats (Alongi 1989; Alongi and Hanson 1985; Alongi and Tenore 1985). As intertidal mudflats are typically organic rich habitats, differences observed between studies may be attributed to different locations and the low grazing effect may be characteristic of intertidal mudflats.

Differences between studies may also be attributed to the use of different techniques. Direct addition of label for estimating grazing rates presents several limitations, including direct ingestion of free label and absorption into epicuticular bacteria associated with surface tissues of grazers (Carman 1990; Montagna 1983; Montagna 1984a; Montagna and Bauer 1988). Other potential bias rarely taken into account is the direct ingestion of substances released by labeled prey. Sediment bacteria produce copious amounts of extracellular polymeric secretions (EPS) (Decho 1990), which are assimilated with high efficiency by grazers (Baird and Thistle 1986; Couch et al. 1996; Decho and Moriarty 1990) and may represent a more important carbon source

than bacterial cells in grazers diet (Hall and Meyer 1998). Direct injection of label in sediment induces labelling of active bacteria and of exopolymers secreted by those cells. High grazing rates observed in this type of experiment may be due to high consumption of labeled EPS by grazers. In the present study excretion of polymers may be reduced since only 10 % of labelled bacterial cells were active (Chapter I). Two other methods avoid uncertainties inherent with polymer ingestion by grazers: ingested FLB are individually identified in grazers (Epstein et al. 1992) and dissolved organic carbon and bacterial uptake are dissociated in the van Oevelen et al. (2006) experiment. Analogous discrepancies observed with studies dealing with algal grazing make more convincing this hypothesis concerning the effect of EPS on measured grazing rates. Diatoms also secrete a considerable amount of exopolymers and authors using in situ labelling conclude that meiofaunal grazing has a significant impact on algal stock (Blanchard 1991; Montagna 1995; Montagna and Yoon 1991). Lyophilised algae do not secrete exopolymers and grazing impacts found with this type of prey are low (Franco 2007; Moens et al. 2002; Urban-Malinga and Moens 2006). Moens et al. (2002) suggest that nematodes preferentially utilise labile organic matter derived from microphytobenthos and settled phytoplankton. Determination of the importance of EPS in meiofaunal diet appears to be a promising avenue for further explorations.

The grazing rate of nematodes on bacteria was higher in deeper than in surficial sediment in the lower part of the mudflat (station 3). Conversely, at the two other stations, there were no vertical differences in feeding rates. It is generally accepted that the proportion of epigrowth feeding decreases with sediment-depth (Heip et al. 1985). Consequently, grazing of algae decrease with depth. Indeed, the existence of two distinct food webs segregated by depth has been suggested (Franco 2007; Rudnick 1989). The present study suggests that this segregation is not systematic and may be dependent of tidal elevation. Alongi (1989) and Kemp (1990) consider that the fraction of bacteria grazed declines roughly in proportion to the decrease of predator abundance with increasing depth. Our results are not conflicting with this assumption and in the three locations studied, the fraction of bacterial stock grazed was more than 200 times higher in surficial than in deeper sediment. This result confirms that meiofaunal grazing constitutes a limited fate of bacteria both in surficial and in deeper sediment.

In the lower part of the Brouage mudflat, bacteria were on average grazed by macrofauna at a rate 12 times higher than by meiofauna. Similarly van Oevelen et al. (2006) conclude that macrofauna grazed bacteria at a rate 8 times higher than meiofauna. Extensive literature exists about the role of bacteria as food for macrofauna

(Review in Lopez and Levinton 1987). The question on the control of bacteria by macrofauna has received less attention. In his review, Kemp (1990) concludes that macrofauna have not been shown to reduce bacterial abundance, except when exceptionally high densities of macrofauna are present. In the studied mudflat, *H. ulvae* are mainly distributed in the upper half of the mudflat (Bocher et al. 2006; Haubois et al. 2002). Patterns of distribution found in the present study were similar, with the highest biomass in stations 1 and 2 and the lowest in station 3. Snails grazed daily 6, 19 and 0.3 % of bacterial stock respectively in stations 1, 2 and 3. Results of the present study are consequently in accordance with Kemp's conclusions (1990) and *H. ulvae* presents highest impact in the upper part of the mudflat where snail abundances are high. Bacterial stock grazed by *H. ulvae* fluctuated greatly during the year (Fig. 6). Those variations were principally due to large snail density fluctuations also observed by Haubois et al. (2002).

Conclusions concerning the general impact of macrofauna must be made with caution since all potential grazers were not taken into consideration. Bivalves represent the dominant fraction of macrofaunal biomass in the mudflat (Degré et al. 2006). *Scrobicularia plana* dominates the bivalve community followed by *Abra tenuis* and *Macoma balthica* (Bocher et al. 2006). Natural isotopic compositions indicate that *S. plana* and *M. balthica* feed preferentially on a mixture of benthic diatoms and marine phytoplankton (Riera et al. 1999). However, by their deposit feeding activity, bivalves may also influence bacterial stock. Moreover, the impact of *H. ulvae* on bacterial stock may have been overestimated as five hour long grazing experiments give access to ingestion rather than assimilation rates (Chapter IIc). Bacteria can resist digestion by consumers and stay intact and viable after passage through consumer gut. This bias was limited with nematode grazing because label accumulation reflects assimilation more than ingestion during a five hour incubation (Moens et al. 1999; Schiemer 1987).

Others biases to be taken into consideration are the determinations of bacterial biomass and production which present uncertainties common to all benthic experiments. Principal uncertainties in determination of bacterial biomass are (i) inefficient separation of bacteria from sediment before counting under the microscope and (ii) conversion factor from cell volume to carbon content varying by a factor of 5 in literature (Review in Kemp 1990). Conversion of thymidine uptake to bacterial biomass production also necessitates conversion factors highly varying according to authors (Moriarty 1986; Moriarty 1988). One of the aims of the present study was to compare our results with other studies dealing with the trophic fate of bacteria and therefore we tried to employ

conversion factors as similar as possible to those employed in other studies (e. g. Sundbäck et al. 1996).

In the upper and middle part of the mudflat, grazing estimates represented respectively 7 and 28% of bacterial production and throughout the year never represented more than 3% of bacterial production in the lower part of the mudflat. Even with the potential biases discussed above, grazing can be considered as a moderate and minor fate of bacterial production respectively in the higher and lower Brouage mudflat. This general conclusion is in accordance with other studies conducted in intertidal muflats (Epstein and Shiaris 1992a; Van Oevelen et al. 2006). If grazing fate is minor, fate of unconsumed bacterial production remains unexplained and poorly documented (Meyer-Reil 1984). Benthic bacteria can enhance planktonic biomass through resuspension of the top few centimetres of sediments (Blanchard et al. 1997) but this flux is also poorly documented. Bacterial mortality can be induced by programmed cell death (Yarmolinsky 1995) and viral lysis (e. g. Glud et al. 2003). After cell death, bacterial carbon joins the sediment organic carbon pool and has two potential fates: (i) recycling by degradation and (ii) burial. Veuger et al. (2006) concluded that burial is not a major fate of bacterial carbon in intertidal mudflats and Novitsky (1986) came to a similar conclusion in tropical marine beach sediments. According to Alongi (1989; 1994) the major part of bacterial production remains ungrazed but instead lyses, the lysate is recycled back to the bacterial community and participates in biogeochemical cycles maintaining the cycling of essential compounds.

In pelagic systems, bacteria are principally consumed by small grazers (<20 µm) and grazing is generally the principal fate of bacterial production (Azam et al. 1983; Ducklow and Carlson 1992; Sherr et al. 1987). In the sediment of intertidal mudflats, bacteria appear to be grazed principally by macrofauna (>500 µm) and grazing is a minor to moderate fate of bacterial production. This result does not necessarily imply that bacteria are insignificant in grazer diet, particularly when the algal resource is in low abundance. However, the present study also suggests that algal resources have more influence than bacteria in feeding behaviour of at least two of the studied grazers.

DISCUSSION GENERALE ET PERSPECTIVES

DISCUSSION GENERALE ET PERSPECTIVES

En milieu pélagique, les bactéries sont largement consommées par les protozoaires hétérotrophes. Elles présentent un rôle important au sein du réseau trophique, en rendant accessible une plus grande partie de la production primaire aux consommateurs supérieurs. Par analogie, il a souvent été considéré qu'un fonctionnement comparable existait en milieu benthique. Dans le sédiment, les bactéries sont généralement 1000 fois plus abondantes que dans la colonne d'eau et leur productivité y est très élevée. Les bactéries benthiques peuvent être consommées par la microfaune, la méiofaune et la macrofaune. Cependant, en raison des difficultés techniques, peu d'études se sont employées à décrire le niveau de consommation de ces divers compartiments. En outre, les résultats consignés dans la littérature à ce sujet sont très variables, les bactérivores consommant selon les études la majorité ou une part négligeable de la production bactérienne. Le devenir des bactéries au sein des réseaux trophiques des sédiments marins demeure donc incertain.

Principaux résultats de la thèse

Les études se sont focalisées sur la meiofaune et la macrofaune car des résultats préliminaires ont suggéré que la microfaune, très peu abondante, aurait un impact limité à Brouage. Le premier objectif de cette thèse a été la mise au point d'une méthode permettant la quantification de la bactérivorie de la méiofaune et de la macrofaune.

Des bactéries ont été pré-enrichies en ^{15}N et utilisées comme traceurs durant des expériences de broutage.

Toutes les méthodes existant actuellement pour mesurer la bactérivorie en milieu benthique présentent des inconvénients et des biais potentiels. La méthode décrite dans le premier chapitre de la thèse ne fait pas exception à cette règle. Le principal biais serait lié au tamisage du sédiment servant à éliminer une partie des bactéries naturelles avant les expériences de broutage. Cette étape est nécessaire car les bactéries enrichies doivent être suffisamment abondantes pour que leur ingestion soit détectable. Ce tamisage est susceptible de perturber les organismes, notamment en modifiant leur comportement alimentaire. Cependant, cette méthode a été utilisée dans des conditions différentes au cours de la thèse : lors d'expériences *ex situ* (Chapitre II A, Chapitre II B, Chapitre II C) et lors d'expériences *in situ* (Chapitre III). Les résultats obtenus avec les deux approches sont relativement proches, indiquant une bonne répétabilité de la méthode. Par ailleurs, cette méthode présente un certain nombre d'avantages par

rapport à celles de la littérature. Les bactéries enrichies présentent une taille, une activité respiratoire et une diversité relativement proches de celles du milieu naturel. Ainsi cette méthode limiterait les biais liés à la sélectivité de l'ingestion de bactéries par les bactérivores. En outre, fondée sur l'usage d'isotopes stables, cette méthode ne connaît pas de restriction légale que se soit en laboratoire ou sur le terrain.

Les vasières intertidales constituent des milieux aux conditions environnementales très changeantes. Le deuxième objectif de cette thèse était donc de déterminer l'influence de facteurs biotiques et abiotiques sur les taux d'ingestion de bactéries.

Des expériences de broutage ont donc été réalisées en conditions expérimentales contrôlées pour mesurer l'influence de facteurs abiotiques (température, salinité, et lumière) et biotiques (abondances d'algues et de bactéries) sur les taux d'ingestion du foraminifère *Ammonia tepida*, du peuplement de nématodes et du gastéropode *Hydrobia ulvae*.

Les variations de température et de salinité ont influencé la consommation de bactéries du foraminifère *A. tepida* mais ont été sans incidence sur le niveau de consommation du peuplement de nématodes et du gastéropode *H. ulvae*. La lumière n'a pas eu d'effet négatif sur le comportement alimentaire des trois bactérivores. Ces résultats suggèrent donc que les organismes étudiés ont un comportement alimentaire bien adapté à l'environnement hypervariable que constitue l'interface air/sédiment à marée basse. C'est au niveau de cette interface que se forme le biofilm algal durant les exondations diurnes. Les trois organismes étudiés ont ingéré les algues à un taux systématiquement plus élevé que celui des bactéries. Les algues semblent donc avoir une importance plus grande que les bactéries dans la nutrition des organismes étudiés.

Le troisième objectif de la thèse était de déterminer le devenir trophique de la production bactérienne au cours de l'année.

Les conclusions des expériences de broutage réalisées sur la vasière de Brouage au cours d'un cycle annuel sont comparables à celles des études menées en conditions expérimentales : les organismes bactérivores sont peu influencés par les variations des facteurs abiotiques. Contrairement à ce qui a été observé en conditions expérimentales, la température et la salinité n'ont pas eu d'impact sur le niveau de bactérivorie manifesté par *A. tepida*. En conditions expérimentales, les nématodes et le foraminifère *A. tepida* ont présenté une bonne capacité de sélection des algues par rapport aux

bactéries. Les expériences de terrain confirment que les bactéries ne sont pas ingérées préférentiellement car l'ingestion de bactéries par ces organismes est négativement corrélée aux abondances d'algues mais n'est pas corrélée aux abondances bactériennes. Ces résultats suggèrent que pour ces organismes, les bactéries constituent une **ressource alternative**, consommée préférentiellement quand les abondances d'algues sont faibles. Sur la vasière de Brouage, la consommation de bactéries est largement dominée par le gastéropode *H. ulvae* si bien que la fraction de bactéries consommées est principalement dépendante de l'abondance du gastéropode. Le broutage des bactérivores étudiés ne représente jamais plus de 6% de la production bactérienne. **En conséquence, le broutage apparaît comme un devenir limité de la production bactérienne dans la vasière de Brouage (Fig. 1).**

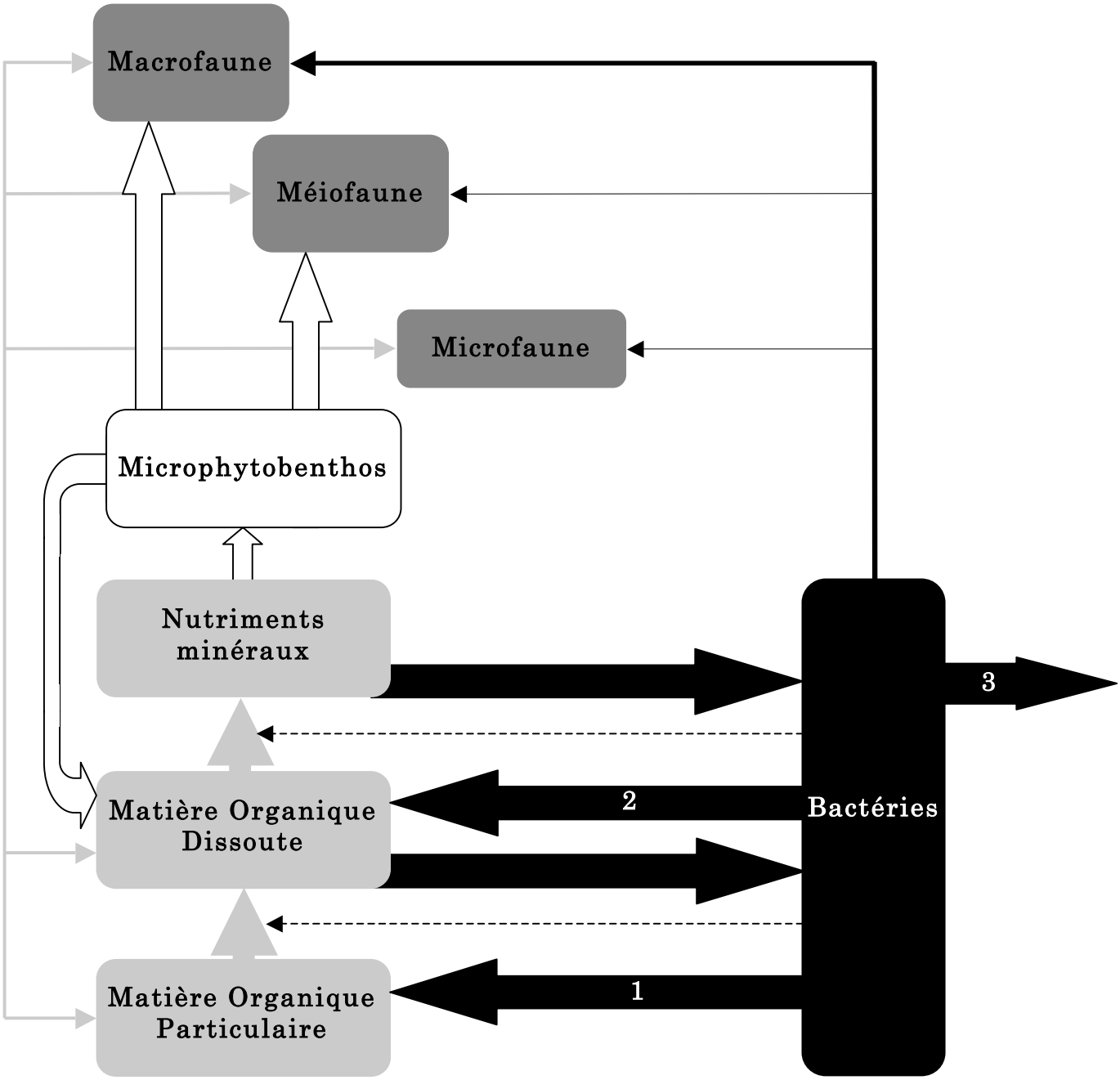

Figure 1. Schéma simplifié présentant le devenir des bactéries dans un réseau trophique benthique. La taille des flèches n'est pas proportionnelle aux flux. Les flèches pointillées correspondent à des processus de minéralisation réalisés par les bactéries. La flèche 1 représente la mortalité bactérienne induite par lyse des bactéries. La flèche 2 représente le DOM exsudé par les bactéries et la mortalité bactérienne induite par lyse des bactéries. La flèche 3 représente la remise en suspension et sortie du système.

Discussion et perspectives

Biais potentiels

La conclusion majeure de cette thèse est donc que la part de production bactérienne consommée par les taxons étudiés est faible à Brouage. Avant de discuter de cette conclusion, il convient de discuter des biais potentiels susceptibles d'influencer cette conclusion. *A priori*, le biais le plus important pourrait venir de la détermination des biomasses et productions bactériennes nécessitant des facteurs de conversion qui selon les études varient respectivement d'un facteur de 2 et 5 (Kemp 1990). Dans la présente étude, des facteurs « moyens » ont été utilisés pour minimiser ces biais. Ces biais constituent un problème récurrent dans toutes les études portant sur les communautés de bactéries. Une meilleure connaissance des facteurs de conversion spécifiques aux différents types de communautés bactériennes benthiques pourrait sensiblement améliorer la connaissance du compartiment bactérien benthique et rendre plus fiable les conclusions des études de flux trophiques.

Dans la présente étude, les bactérivores potentiels n'ont pas tous été pris en compte. En effet, les expériences se sont focalisées sur les espèces dominantes de la méiofaune et de la macrofaune. La bactérivorie des flagellés n'a pas été étudiée. Cependant, leur faible abondance permet de supposer que leur impact sur les bactéries est limité (Kemp 1990). Parmi les différents compartiments de la macrofaune, seul le gastéropode déposivore *H. ulvae* a été étudié. D'autres sont cependant susceptibles de jouer un rôle non négligeable. Localisé dans la partie haute de la vasière, *Scrobicularia plana* est le bivalve déposivore dominant. *Macoma balthica* et *Abra tenuis* présentent des abondances plus faibles (Bocher et al. 2006). La mesure de la consommation de bactéries par ces bivalves permettrait d'affiner la connaissance du devenir trophique des bactéries. Comme les abondances des bivalves sont faibles dans la partie basse de la vasière (point 3), il est cependant peu probable que ces études modifient les conclusions sur la faible consommation des bactéries benthiques. En revanche, ces conclusions pourraient être modifiées localement dans la partie haute.

Influence des bactérivores sur les bactéries

L'ingestion de bactéries par les bactérivores présenterait donc un impact quantitatif réduit sur le compartiment bactérien. Ce résultat n'implique pas nécessairement l'absence d'effet qualitatif. En effet, en ingérant sélectivement les

bactéries, les bactérivores pourrait modifier la composition de la communauté bactérienne (De Mesel et al. 2004), et ceci en dépit de faibles taux d'ingestion. La sélection de souches bactériennes a été observée chez les nématodes (Tietjen et al. 1970, Tietjen & Lee 1973, 1977, Moens et al. 1999) et les foraminifères (Lee et al. 1966, Bernhard & Bowser 1992). En revanche, en raison de sa taille et de son mode d'alimentation, il est peu vraisemblable que *H. ulvae* soit capable d'ingérer sélectivement les bactéries.

Les bactérivores peuvent également influencer l'abondance des bactéries par d'autres phénomènes indirects. Les bactérivores peuvent stimuler la production bactérienne par leur bioturbation qui permet une diffusion importante de l'oxygène et des nutriments (Abrams & Mitchell 1980, Nehring et al. 1990, Alkemade et al. 1992, Aller & Aller 1992) ou par des sécrétions de composés riches en nutriments comme le mucus (Jensen 1996, Riemann & Helmke 2002). Enfin, par son activité bioturbatrice, *H. ulvae* augmente la remise en suspension du sédiment (Orvain et al. 2004) et donc l'exportation des bactéries benthiques vers le milieu pélagique.

Influence des bactéries sur les bactérivores

Pour deux des bactérivores étudiés, la communauté de nématodes et *A. tepida*, les bactéries semblent constituer une ressource alternative, consommée quand les algues sont peu abondantes. Cette préférence peut s'expliquer par une abondance des algues plus élevée que celle des bactéries dans les sédiments intertidaux (Zhukova & Kharlamenko 1999). En effet, certain auteurs considèrent que les bactéries ne seraient pas assez abondantes pour remplir les besoins alimentaires de la plupart des espèces détritivores (Lopez & Levinton 1987, Alongi 1989, Kemp 1990). Cette préférence pour la ressource algale peut aussi s'expliquer par le comportement de migration des algues. Les diatomées se concentrent à la surface du sédiment durant la marée basse sous forme d'un biofilm pouvant dépasser 50 µm d'épaisseur (Herlory et al. 2004). S'alimenter au sein de ce biofilm algal limite la perte d'énergie liée à la sélection des particules alimentaires et/ou à l'élimination des particules non digestes ingérées. Contrairement aux algues, les bactéries présentent une distribution beaucoup plus homogène les rendant ainsi moins intéressantes pour les consommateurs. La préférence des algues par rapport aux bactéries pourrait aussi s'expliquer par une meilleure qualité nutritive des algues liée à leur composition en acide gras et en lipides plus complexes (Alongi 1998, Zhukova & Kharlamenko 1999). Ainsi, les bactéries ne constitueraient pas la ressource alimentaire la plus convoitée sur la vasière. Cependant, elles pourraient présenter un

rôle trophique important lors de pénuries de la ressource algale. Il a aussi été suggéré que les bactéries pouvaient comporter des éléments indispensables pour certains organismes comme les foraminifères qui ne peuvent pas se reproduire sans bactéries (Muller & Lee 1969).

Par l'excrétion d'enzymes extracellulaires, les bactéries dégradent la Matière Organique Particulaire (MOP) en Matière Organique Dissoute (MOD) (Fig. 1). Les organismes vivants au sein de la vasière assimilent la MOD : la microfaune (Tranvik et al. 1993), la méiofaune (Jensen 1984, Montagna 1984, Riemann & Helmke 2002) et la macrofaune (Wright & Manahan 1989, Alber & Valiela 1995). Bien que cette assimilation ait été mise en évidence qualitativement, ce flux trophique a rarement été quantifié. Diverses études se sont intéressées au rapport entre MOP et matière vivante dans l'alimentation des déposivores (Synthèse dans Lopez & Levinton 1987). En revanche, le rôle de la MOD demeure peu documenté. Les bactéries pourraient donc jouer un rôle important en augmentant la quantité de MOD disponible pour les organismes de la vasière. Connaître l'importance trophique de la MOD constitue donc une perspective de recherche intéressante.

Que peut-on en déduire sur le déterminisme de la production bactérienne ?

La biomasse bactérienne peut être contrôlée (i) par l'apparition de nouvelles bactéries (« bottom-up ») et (ii) par la disparition de bactéries existantes (« top-down »). L'observation de la relation entre les biomasses et les productions des bactéries donne une information sur la nature du contrôle qui s'exerce sur les communautés bactériennes (Billen et al. 1990, Ducklow 1992, Thingstad 2000). Plus ces paramètres sont corrélés, plus les bactéries sont contrôlées par un phénomène « bottom-up ». En revanche, plus le coefficient directeur de la droite de corrélation est proche de 0, plus les bactéries sont contrôlées par des processus de disparition (« top-down »). Dans le cas de Brouage, la corrélation est faible (Fig. 2), ce qui indiquerait donc que les bactéries ne sont pas limitées par les ressources nécessaires à la synthèse de nouvelles bactéries. Cette hypothèse est appuyée par le fait qu'à Brouage, l'ammonium n'est jamais limitant pour les bactéries (Vouvé 2000). De plus, l'absence de couplage entre biomasses et productions bactériennes a déjà été observée dans les vasières intertidales (van Duyl et al. 1999).

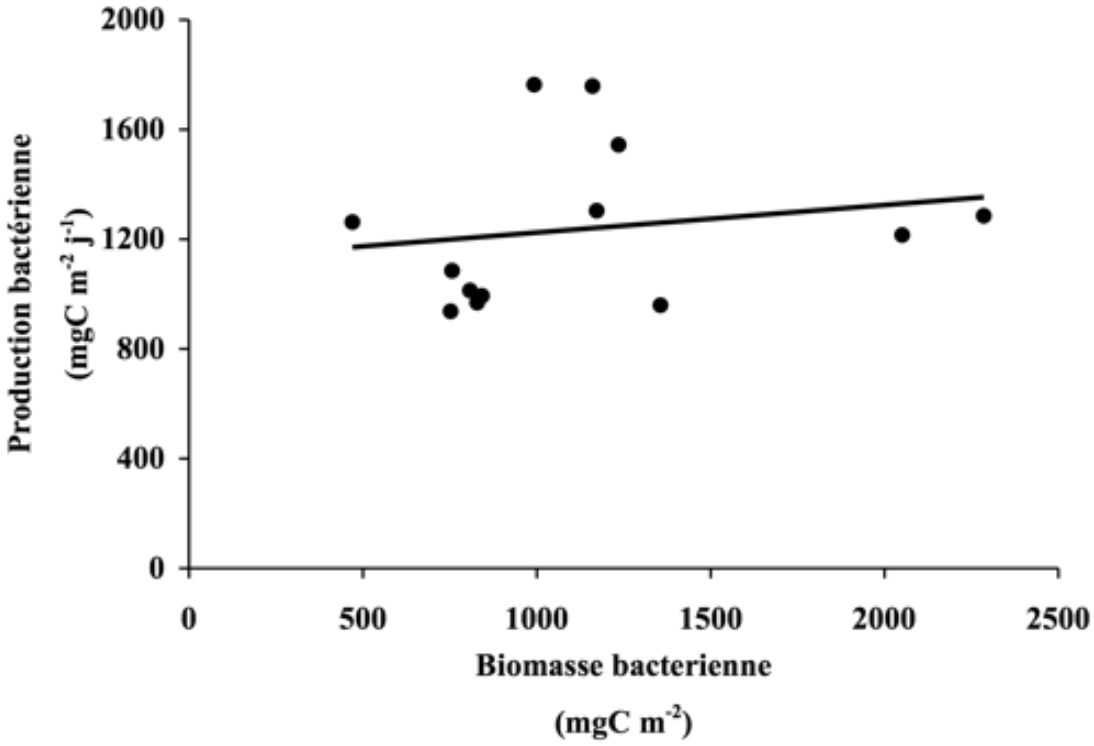

Figure 2. Relation entre production (mgC m^{-2} j^{-1}) et biomasse (mgC m^{-2}) bactériennes dans le sédiment de la vasières de Brouage (Chapitre III).

Ainsi la biomasse bactérienne serait principalement contrôlée par la disparition de bactéries existante (« top down »). Cependant, la principale conclusion de cette thèse est que la consommation de bactéries est faible et ne dépasse pas 6% de la production bactérienne. La disparition des bactéries doit donc être induite par d'autres phénomènes qui représenteraient plus de 94% de la production bactérienne.

La disparition des bactéries pourrait être liée à une remise en suspension du sédiment à marée haute (Blanchard et al. 1997). Une fois dans la colonne d'eau, les bactéries seraient exportées vers les eaux côtières (Teal 1962). Ces bactéries, isolées ou en agrégats, pourraient aussi être consommées par les organismes filtreurs et les bactérivores pélagiques (Wainright 1987). La remise en suspension du sédiment peut être engendrée par l'action du courant de marée ou par le clapot lié au vent (Blanchard et al. 2002). Cette remise en suspension est accentuée par la bioturbation effectuée par les organismes de la vasière (Andersen et al. 2002, Orvain et al. 2004). Cette remise en suspension concerne essentiellement les couches très superficielles du sédiment (Le Hir et al. 2000) alors que les couches profondes sont peu affectées. La très faible production bactérienne consommée dans cette zone profonde (Chapitre III), suggère donc l'existence d'autres processus pour réguler les communautés bactériennes.

L'apoptose (ou mort cellulaire programmée) est un processus par lequel les cellules déclenchent leur auto destruction. L'apoptose a été observée chez des bactéries de culture (Yarmolinsky 1995), mais n'a pas été quantifiée chez des bactéries en milieu naturel. Les bactéries pourraient donc s'autodétruire, mais pourraient aussi détruire d'autres bactéries. En effet, les bactéries ont la capacité d'émettre des antibiotiques ou des bactériocines qui détruisent les autres bactéries vivant dans le même environnement. Cependant l'impact de ces processus sur les communautés naturelles de bactéries reste très peu documenté.

La lyse des bactéries par des virus pourrait représenter une importante cause de mortalité bactérienne. Les virus sont abondants dans tous les écosystèmes aquatiques et leur impact sur les dynamiques bactériennes a fait l'objet de nombreux travaux (Fuhrman 1999). En milieu pélagique, les virus peuvent induire 40% de la mortalité bactérienne (Fuhrman & Noble 1995). En revanche, les données concernant les virus benthiques sont très limitées. Le domaine benthique pourrait constituer un environnement idéal pour les virus car les abondances d'algues et de bactéries sont beaucoup plus importantes que dans la colonne d'eau, favorisant ainsi la probabilité de rencontre phage/hôte (Danovaro & Serresi 2000). Il a donc été suggéré que les virus pourraient avoir un impact plus grand en milieu benthique qu'en milieu pélagique

(Corinaldesi & Danovaro 2003). Selon le peu d'études réalisées, la mortalité virale représenterait 20% (Glud & Middelboe 2004) ou pourrait dépasser 40% de la production bactérienne benthique (Mei & Danovaro 2004). Le nombre limité d'études des benthovirus est lié aux difficultés techniques (i) de séparation des virus de la matrice sédimentaire et (ii) de leur identification visuelle (Danovaro & Serresi 2000). En effet, les propriétés de surface des virus provoquent leur agrégation ou leur fixation sur les différents matériaux de leur environnement. Ainsi les protocoles d'extraction actuels sont imparfaits et nécessitent des améliorations (Glud et al. 2003). Il a été suggéré qu'en milieu pélagique, le rapport entre la mortalité bactérienne liée au virus et celle liée à la prédation est plus forte en milieu eutrophe qu'en milieu oligotrophe (Furham 2000). En milieu benthique, plusieurs auteurs suggèrent que l'impact de la prédation serait plus important dans les milieux pauvres que dans les milieux riches en matière organique (Alongi & Hanson 1985, Alongi & Tenore 1985, Alongi 1989). Comme il existe une relation inverse entre la teneur en matière organique et la granulométrie des sédiments (e.g. Alongi 1998), les sédiments sableux peuvent être apparentés à des milieux oligotrophes s'ils sont comparés aux sédiments vaseux qui seraient eutrophes (Fig. 3). Une perspective de recherche intéressante serait donc de comparer le rapport de la mortalité liée aux virus à celle liée à la prédation dans des sédiments de granulométrie différente (Fig. 3).

La boucle microbienne en milieu benthique

Dans la boucle microbienne planctonique, la MOD produite par le phytoplancton est assimilée par les bactéries qui sont elles-mêmes consommées par les flagellés (Azam et al. 1983). Ainsi, les bactéries jouent un rôle central en rendant accessible une partie de la production primaire, sous forme de MOD, aux flagellés puis à l'ensemble du réseau trophique. L'importance de la boucle microbienne étant proportionnelle à la consommation des bactéries par les bactérivores, son rôle devrait être limité dans la vasière de Brouage. En milieu pélagique, les systèmes eutrophes sont dominés par les herbivores et leurs prédateurs alors que les systèmes oligotrophes sont dominés par les bactéries et leurs prédateurs (Cotner & Biddanda 2002). La contribution des bactéries hétérotrophes aux flux de matière et d'énergie est donc maximale dans les écosystèmes oligotrophes et diminue dans les systèmes mésotrophes et eutrophes (Legendre & Rassoulzadegan 1995). Le fonctionnement du milieu benthique pourrait être comparable avec une importance maximale de la boucle microbienne dans les milieux pauvres en matières organiques tels que les sables (Fig. 3).

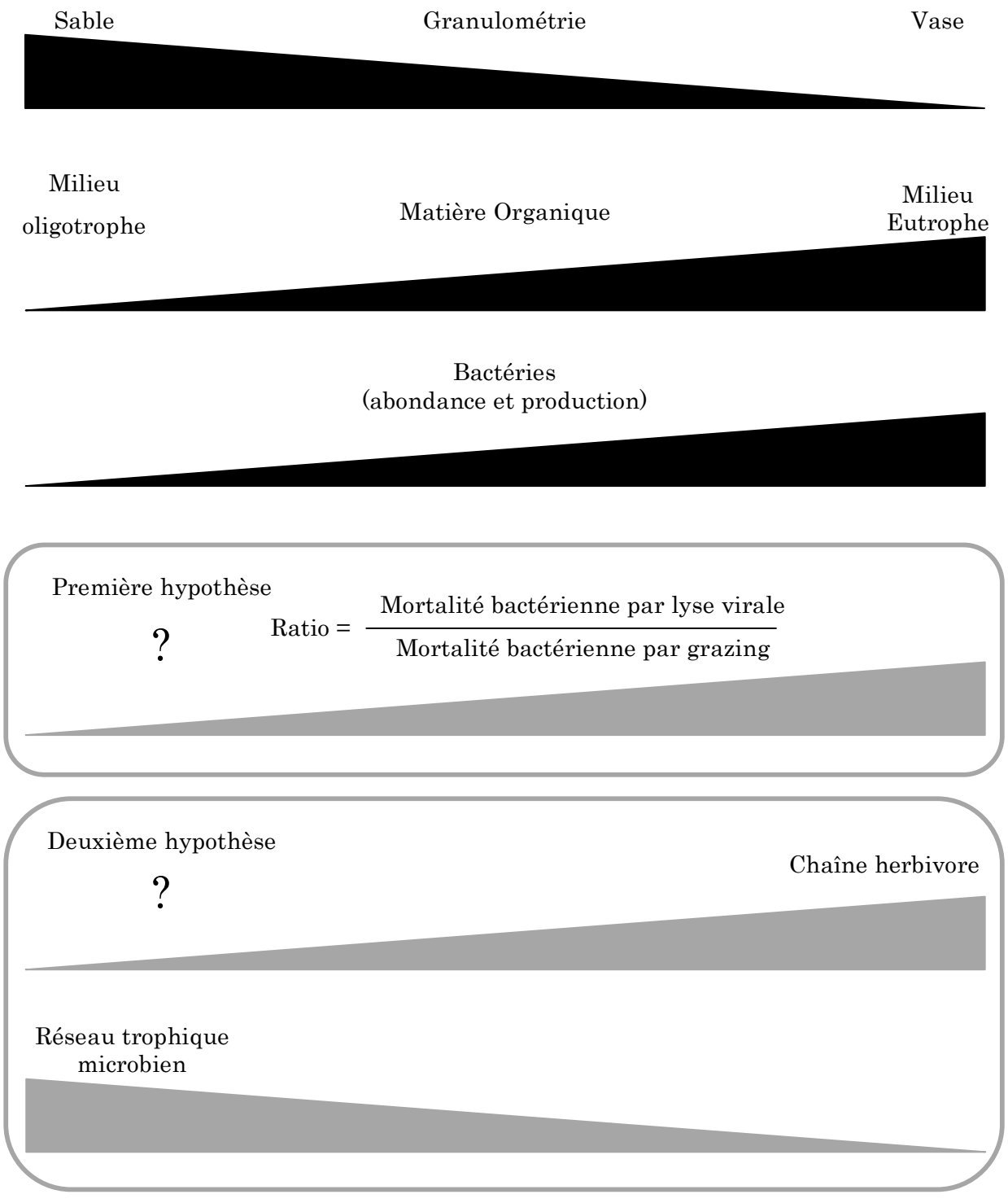

Figure 3. Schémas théoriques du fonctionnement des systèmes benthiques selon la richesse en matière organique. Les modèles présentés dans les encadrés gris clairs ont été observés en milieux pélagiques et restent à valider en milieu benthique.

La lyse des bactéries conduit à la création de MOP et de MOD qui peuvent être dégradées et utilisées à nouveau par les bactéries (Furham 1992) (Fig. 1). Le matériel résistant à la dégradation s'accumule dans le sédiment. Cette accumulation serait cependant limitée dans les vasières intertidales (Veuger et al. 2006). Alongi (1989) suggère que dans les environnements marins benthiques, la majorité des bactéries n'est pas consommée mais détruite par lyse et que le matériel issu de la lyse est recyclé par le compartiment bactérien. Ce schéma de fonctionnement semble confirmé par la présente étude. En effet, la consommation de bactéries est faible dans la vasière de Brouage et le devenir principal des bactéries serait la lyse. Des études dans des sédiments de nature différente permettraient d'étendre ou non ce schéma à l'ensemble des milieux benthiques.

BIBLIOGRAPHIE

Abrams BI, Mitchell MJ (1980) Role of nematode-bacterial interactions in heterotrophic systems with emphasis on sewage suldge decomposition. Oikos 35:404-410

Alber M, Valiela I (1995) Organic aggregates in detrital food webs: incorporation by bay scallops *Argopecten irradians.* Mar Ecol Prog Ser 121:117-124

Alkemade R, Wielemaker A, de Jong SA, Sandee AJJ (1992) Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella bruciei* in stimulating the mineralization of *Spartina anglica* detritus. Mar Ecol Prog Ser 90:149-155

Aller RC, Aller JY (1992) Meiofauna and solute transport in marine muds. Limnol Oceanogr 37:1018-1033

Almogi-Labin A, Perelis-Grossovicz L, Raab M (1992) Living *Ammonia* from a hypersaline inland pool, Dead Sea area, Israël. J Foraminifer Res 22:257-266

Alongi DM (1986) Quantitative estimates of benthic protozoa in tropical marine systems using silica gel: a comparison of methods. Est Coast Shelf Sci 23:443-450

Alongi DM (1988) Microbial-meiofaunal interrelationships in some tropical intertidal sediments. J Mar Res 45:349-365

Alongi DM (1989) The fate of bacterial biomass and production in marine benthic food chains. In: Press JSS (ed) 5th International Symposium on Microbial Ecology, Tokyo, p 355-359

Alongi DM (1991) The role of intertidal mudbanks in the diagenesis and export of dissolved and particulate material from the Fly Delta, Papua New Guinea. J Exp Mar Biol Ecol 149:81-107

Alongi DM (1992) Benthic infauna and organism-sediment relations in a shallow, tropical coastal area: influence of outwelled mangrove detritus and physical disturbance. Mar Ecol Prog Ser 81:229-245

Alongi DM (1994) The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems. Hydrobiologia 285:19-32

Alongi DM (1998) Coastal ecosystem processes, CRC Press448 pp

Alongi DM, Hanson RB (1985) Effect of detritus supply on trophic relationships within experimental benthic food webs. II. Microbial responses, fate and composition and decomposing detritus. J Exp Mar Biol Ecol 88:167-182

Alongi DM, Tenore KR (1985) Effect of detritus supply on trophic relationships within experimental benthic food webs. I. Meiofauna-polychaete (*Capitella capitata*) interactions. J Exp Mar Biol Ecol 88:153-166

Altenbach AV (1992) Short term processes and patterns in the foraminiferal response to organic flux rates. Mar Micropaleontol 19:119-129

Altenbach AV, Pflaumann N, Schiebel U, Thies A, Timm S, Trauth M (1999) Scaling percentages and distribution patterns of benthic foraminifera with flux rates of organic carbon. J Foraminifer Res 29:173-185

Andersen TJ, Jensen KT, Lund-Hansen L, Mouritsen KN, Pejrup M (2002) Enhanced erodability of fine-grained marine sediments by *Hydrobia ulvae.* J Sea Res 48:51-58

Antia NJ, Cheng JY (1970) The survival of axenic cultures of marine phytoplankton algae from prolonged exposure to darkness at 20°C. Phycologia 9:179-183

Armynot du Chatelet E, Debenay J-P, Soulard R (2004) Foraminiferal proxies for pollution monitoring in moderatly polluted harbors. Environ Pollut 127:27-40

Avery L, Shtonda BB (2003) Food transport in the *C. elegans* pharynx. J Exp Biol 206:2441-2457

Azam F, Fenchel T, Field JG, Gray J, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257-263

Bachelet G, Yacine-Kassab M (1987) Post-recruitement phase population dynamics of the intertidal gastropod *Hydrobia ulvae* (Pennant). J Exp Mar Biol Ecol 111:37-60

Bacher C, Duarte P, Ferreira JG, Raillac O (1998) Assessment and comparison of the Marennes-Oléron Bay (France) and Carlingford Lough (Ireland) carrying capacity with ecosystem model. Aquat Ecol 31:379-394

Bacher C, Sauriau P-G (1995) Structure spatiale et cartographie des peuplements benthiques du bassin de Marennes-Oléron : première approche par la méthode de krigeage. ICES Mar Sci Symp 192:41-47

Baird BH, Thistle D (1986) Uptake of bacterial extracellular polysaccharides by a deposit-feeding holothurian (*Isostichopus badionotus*). Mar Biol 92:183-187

Bak RPM, Nieuwland G (1989) Seasonal fluctuations in benthic protozoa populations at different depths in marine sediments. Neth J Sea Res 24:37-44

Bak RPM, van Duyl FC, Nieuwland G, Kop AP (1991) Benthic heterotrophic nanoflagellates in the north sea field/mesocosm bottoms and their response to algal sedimentation. Ophelia 33:187-196

Barnes RSK (1986) Daily activity rythms in the intertidal Gastropod *Hydrobia ulvae* (Pennant). Est Coast Mar Sci 22:325-334

Barnes RSK (1990) Reproductive strategies in contrasting populations of the coastal gastropod *Hydrobia ulvae*: longevity and life-time egg production. J Exp Mar Biol Ecol 138:183-200

Barnes RSK (2006) Variation in feeding rate of the intertidal mudsnail *Hydrobia ulvae* in relation to the tidal cycle. Mar Ecol 27:154-159

Beck MW, Heck KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51:633-641

Bernhard JM (2003) Potential symbionts in bathyal foraminifera. Science 299:861

Bernhard JM, Bowser SS (1992) Bacterial biofilms as a trophic resource for certain benthic foraminifera. Mar Ecol Prog Ser 83:263-272

Bianchi M, Marty D, Bertrand JC, Caumette P, Gauthier M (1989) Micro-organismes dans les écosystèmes océaniques, Masson, Paris, 445 pp

Bianchi TS, Levinton JS (1981) Nutrition and food limitation of deposit-feeders. II. Differential effects of *Hydrobia totteni* and *Ilyanassa obsoleta* on the microbial community. J Mar Res 39:547-556

Bianchi TS, Levinton JS (1984) The importance of the microalgae, bacteria and particulate organic matter in the somatic growth of *Hydrobia totteni*. J Mar Res 42:431-433

Billen G, Servais P, Becquevort S (1990) Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environment: bottom up or top down control? Hydrobiologia 207:37-42

Blackburn TH (1988) Benthic mineralization and bacterial production. In: Blackburn TH, Sørensen J (eds) Nitrogen cycling in Coastal Marine Environment. John Wiley & Sons, pp 175-190

Blanc C, Sy M, Djigal D, Brauman A, Normand P, Villenave C (2006) Nutrition on bacteria by bacterial-feeding nematods and consequences on the structure of soil bacterial community. Eur J Soil Biol 42:S70-S78

Blanchard GF (1991) Measurement of meiofauna grazing rates on microphytobenthos: is primary production a limiting factor? J Exp Mar Biol Ecol 147:37-46

Blanchard GF, Guarini JM (1996) Studying the role of mud temperature on the hourly variation of the photosynthetic capacity of microphytobenthos in intertidal areas. CR Acad Sci Paris 319:1153-1158

Blanchard GF, Guarini JM, Gros P, Richard P (1997a) Seasonal effect on the relationship between the photosynthetic capacity of intertidal microphytobenthos and temperature. J Phycol 33:723-728

Blanchard GF, Guarini JM, Orvain F, Sauriau PG (2001) Dynamic behaviour of benthic microalgal in intertidal mudflats. J Exp Mar Biol Ecol 264:85-100

Blanchard GF, Guarini JM, Provot L, Richard P, Sauriau P-G (2000) Measurement of ingestion rate of *Hydrobia ulvae* (Pennant) on intertidal epipelic microalgae: the effect of mud snail density. J Exp Mar Biol Ecol 255:247-260

Blanchard GF, Guarini JM, Richard P, Gros P, Mornet F (1996) Quantifying the short-term temperature effect on light-satured photosynthesis of intertidal microphytobenthos. Mar Ecol Prog Ser 134:309-313

Blanchard GF, J.-M. G, Bacher C., Huet V (1998) Contrôle de la dynamique à court terme du microphytobenthos intertidal par le cycle exondation-submersion. C R Acad Sci Paris 321:501-508

Blanchard GF, Sauriau P-G, Cariou-Le Gall V, Garet MJ, Olivier F (1997b) Kinetics of tidal resuspension of microbiota: testing the effects of sediment cohesiveness and bioturbation using flume experiments. Mar Ecol Prog Ser 151:17-25

Blanchard GF, Simon-Bouhet B, Guarini JM (2002) Properties of the dynamics of intertidal microphytobenthos biomass. J Mar Biol Ass U K 82:1027-1028

Bocher P, Piersma T, Dedinka A, Kraan C, Yates MG, Guyot T, Folmer EO, Radenac G (2006) Site- and species-specific distribution patterns of molluscs at five intertidal soft-sediment areas in northwest Europe during a single winter. Mar Biol 151:577-594

Boucher G (1974) Premières données écologiques sur les nématodes libres marins d'une station de vase côtière de Banyuls. Vie Milieu 23:69-100

Bouchet VMP, Debenay J-P, Sauriau P-G, Radford-Knoery J, Soletchnik P (2007) Effects of short-term environmental disturbances on living benthic foraminifera during the Pacific oyster summre mortality in the Marennes-Oléron Bay (France). Mar Environ Res 64:358-383

Bouvy M (1988) Contribution of the bacterial and microphytobenthic microflora in the energetic demand of the meiobenthos in an intertidal muddy sediment (Kerguelen Archipelago). P S N Z I: Mar Ecol 9:109-122

Bradshaw JS (1957) Laboratory studies of the rate of growth of the foraminifera *Streblus beccarii* (Linné), var. *tepida* Cushman. J Paleontol 31:1138-1147

Bradshaw JS (1961) Laboratory experiment on the ecology of foraminifera. Contr Cushman Found Res 12:87-106

Bratbak G, Dundas I (1984) Bacterial dry matter content and biomass estimations. Appl Environ Microbiol 48:755-757

Buffan-Dubau E, Carman KR (2000) Diel feeding behavior of meiofauna and their relationships with microalgal resources. Limnol Oceanogr 45:381-395

Calow P (1975) The feeding strategies of two freshwater gastropods. *Ancylus fluviatilis* Mull. and *Planorbis contortus* Linn. (Pulmonata) in terms of ingestions rates and absorption efficiencies. Oecologia 20:33-49

Cammen LM (1980a) Ingestion rate: an empirical model for aquatic deposit feeders and detritivores. Oecologia 44:303-310

Cammen LM (1980b) The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea.* Mar Biol 61:9-20

Cammen LM (1991) Annual bacterial production in relation to benthic microalgal production and sediment oxygen uptake in an intertidal sandflat and an intertidal mudflat. Mar Ecol Prog Ser 71:13-25

Carman KR (1990a) Mechanisms of uptake of radioactive labels by meiobenthic copepods during grazing experiments. Mar Ecol Prog Ser 68:71-83

Carman KR (1990b) Radioactive labeling of a natural assemblages of marine sedimentary bacteria and microalgae for trophic studies: an autoradiographic study. Microb Ecol 19:279-290

Carman KR, Dobbs FC, Guckert JB (1989) Comparison of three techniques for administering radiolabeled substrates for trophic studies: uptakes of label by harpaticoid copepods. Mar Biol 102:119-125

Carman KR, Fry B (2002) Small-sample methods for $\delta^{13}C$ and $\delta^{15}N$ analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. Mar Ecol Prog Ser 240:85-92

Carman KR, Thistle D (1985) Microbial food partitioning by three species of benthic copepods. Mar Biol 88:143-148

Carriou-Le Gall V, Blanchard GF (1995) Monthly measurement of pigment concentration from an intertidal muddy sediment of Marennes-Oléron Bay, France. Mar Ecol Prog Ser 121:171-179

Chandler GT (1989) Foraminifera may structure meiobenthic communities. Oecologia 81:353-360

Chatterji A, Ansari ZA, Mishra JK, Parulekar AH (1995) Seasonality in meiofaunal distribution on a tropical beach at Balramgari, northwest coast of India. Indian J Mar Sci 24:49-55

Clough LM, Lopez GR (1993) Potential carbon sources for the head down deposit-feeding polychaete *Heteromastus filiformis*. J Mar Res 51:595-616

Colijn F (1982) Light absorption in the waters of the Ems-Dollard Estuary and its consequences for the growth of phytoplankton and microphytobenthos. Neth J Sea Res 15:196-216

Colijn F (1987) Primary production of microphytobenthos in the Ems-Dollard Estuary. Mar Ecol Prog Ser 14:185-196

Corinaldesi C, Danovaro R (2003) Ecology of virus in aquatic sediments. Rec Res Dev Microbiol 7:119-134

Cotner JB, Biddanda BA (2002) Small player, larger role: microbial influence on biogeochemical process in pelagic aquatic ecosystems. Ecosystems 5:105-121

Couch CA, Meyer JL, Hall RO (1996) Incorporation of bacterial extracellular polysaccharide by black fly larvae (Simuliidae). J North Am Benthol Soc 15:289-299

Croll NA (1970) The behaviour of nematodes: their activity, senses and responses, Edward Arnold, London, 117 pp

Danovaro R, Serresi M (2000) Viral density and virus-to-bacterium ratio in deep-sea sediments of the Eastern Mediterranean. Appl Environ Microbiol 66:1857-1861

Daro MH (1978) A simplified ^{14}C method for grazing measurements on natural planktonic populations. Helgol wiss Meeresunters 13:241-248

De Mesel I, Derycke S, Moens T, Van der Gucht K, Vincx M, Swings J (2004) Top-down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. Environ Microbiol 6:733-744

De Troch M, Houthoofd L, Chepurnov V, Vanreusel A (2006) Does sediment grain size affect diatom grazing by harpaticoid copepods? Mar Environ Res 61:265-277

Debenay J-P, Guillou J-J (2002) Ecological transitions indicated by foraminiferal assemblages in paralic environments. Estuaries 25:1107-1120

Decho AW (1988) How do harpaticoides grazing rates differ over a tidal cycle? Field verification using chlorophyll-pigment analyses. Mar Ecol Prog Ser 45:263-270

Decho AW (1990) Microbial exopolymer secretions in ocean environments : their role(s) in food webs and marine processes. Ocenogr Mar Biol Annu Rev 28:73-153

Decho AW, Moriarty CM (1990) Investigation of bacterial mucus-exopolymer utilization by marine animals: methodology and results using harpaticoid copepods. Limnol Oceanogr 35:1039-1049

Degré D, Leguerrier D, Armynot du Chatelet E, Rzeznik-Orignac J, Auguet J-C, Dupuy C, Marquis E, Fichet D, Struski C, Joyeux E, Sauriau P-G, Niquil N (2006) Comparative analysis of the food webs of two intertidal mudflats during two seasons using inverse modelling: Aiguillon Cove and Brouage Mudflat, France. Est Coast Shelf Sci 69:107-124

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350-356

Ducklow HW (1992) Factors regulating bottom-up control of bacterial biomass in open ocean plankton communities. Arch Hydrobiol Beih 37:207-217

Ducklow HW (2000) Bacterial production and biomass in the oceans. In: Kirchman DL (ed) Microbial ecology of the ocean. Wiley-Liss, New York, pp 85-120

Ducklow HW, Carlson CA (1992) Oceanic bacterial production. Adv Microb Ecol 12:113-181

Duncan A, Schiemer F, Klekowski RZ (1974) A preliminary study of feeding rates on bacterial food by adult femals of a benthic nematode, *Plectus palustris* de Man 1880. Pol Arch Hydrobiol 21:249-258

Epstein SS (1997a) Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. Microb Ecol 34:188-198

Epstein SS (1997b) Microbial food webs in marine sediments. II. Seasonal changes in trophic interactions in a sandy tidal flat community. Microb Ecol 34:199-209

Epstein SS, Burkovsky IV, Shiaris MP (1992) Ciliate grazing on bacteria, flagellates, and microalgae in a temperate sandy-flat : ingestion rates ans food niche partitioning. J Exp Mar Biol Ecol 165:103-123

Epstein SS, Shiaris MP (1992a) Rates of microbenthic and meiobenthic bacterivory in a temperate muddy tidal flat community. Appl Environ Microbiol 58:2426-2431

Epstein SS, Shiaris MP (1992b) Size-selective grazing of coastal bacterioplancton by natural assemblages of pigmented flagellates, colorless flagellates, and ciliates. Microb Ecol 23:211-225

Fenchel T (1967) The ecology of marine microbenthos I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. Ophelia 4:121-137

Fenchel T (1969) The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. Ophelia 5:1-182

Fenchel T (1972) Aspects of decomposer food chains in marine benthos. Verh Dtsch Zool Ges 65:14-22

Fenchel T (1975) Character displacement and coexistance in mud snails (Hydrobiidae). Oecologia 20:19-32

Fenchel T, Kofoed LH, Lappalainen A (1975) Particle size-selection of two deposit feeders: the Amphipod *Corophium volutator* and the Prosobranch *Hydrobia ulvae*. Mar Biol 30:119-128

Fontanier C, Jorissen FJ, Licari L, Alexandre A, Anschutz P, Carbonel P (2002) Live benthic foraminiferal faunas from the Bay of Biscay: faunal density, composition and microhabitats. Deep-Sea Res 49:751-785

Forbes VE, Lopez GR (1986) Changes in feeding and crawling rates of *Hydrobia truncata* (Prosobranchia: Hydrobiidae) in response to sedimentary chlorophyll a and recently egested sediment. Mar Ecol Prog Ser 33:287-294

Forbes VE, Lopez GR (1989) The role of sediment particle size in nutritional energetics of a surface deposit-feeder. I. Ingestion and absorption of sedimentary microalgae by *Hydrobia truncata* (Vanatta). J Exp Mar Biol Ecol 126:181-192

Forster SJ (1998) Osmotic stress tolerance and osmoregulation of intertidal and subtidal nematodes. J Exp Mar Biol Ecol 224:109-125

Franco MA (2007) Nematode response to changing food conditions in shallow marine and estuarine sediments. PhD Thesis, University of Gent, 191 pp

Fuhrman JA (1981) Influence of method on the apparent size distribution of bacterioplankton cells: epifluorescence microscopy compared to scanning electron microscopy. Mar Ecol Prog Ser 5:103-106

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 69:541-548

Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar Biol 66:109-120

Fuhrman JA, Noble RT (1995) Viruses and protist cause similar bacterial mortality in coastal water. Limnol Oceanogr 40:1236-1242

Furham JA (1992) Bacterioplankton roles in cycling of organic matter: the microbial food web. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum press, New York, pp 361-383

Furham JA (2000) Impact of viruses on bacterial processes. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, pp 327-350

Gallucci V, Steyaert M, Moens T (2005) Can field distributions of marine predacious nematodes be explained by sediment constraints on their foraging success? Mar Ecol Prog Ser 304:167-178

Garet MJ (1996) Transformation bactérienne de la matière organique dans les sédiments côtiers. Relation entre les métabolismes respiratoires et les activités exoprotéolytiques bactériennes. PhD Thesis, University of Bordeaux II,

Garet MJ, Moriarty DJW (1996) Acid extraction of tritium label from bacterial DNA in clay sediment. J Microbiol Methods 25:1-4

Gee JM (1985) Seasonal aspects of the relationship between temperature and respiration rate in four species of intertidal harpaticoid copepod. J Exp Mar Biol Ecol 93:147-156

Gelsomino A, Keijzer-Wolters AC, Cacco G, Van Elsas JD (1999) Assessment of bacterial community diversity in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. J Microbiol Methods 38:1-15

Gerlach SA (1971) On the importance of marine meiofauna for benthos communities. Oecologia 6:176-190

Gerlach SA (1978) Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. Oecologia 33:55-69

Glud RN, Gundersen JK, Røy H, Jørgense BB (2003) Seasonal dynamics of benthic O_2 uptake in a semi enclosed bay: importance of diffusion and fauna activity. Limnol Oceanogr 48:1265-1276

Glud RN, Middelboe M (2004) Virus and bacteria dynamics of a coastal sediment: Implications for benthic carbon cyling. Limnol Oceanogr 49:2073-2081

Goldfinch AC, Carman KR (2000) Chironomid grazing on benthic microalgae in a Louisana salt marsh. Estuaries 23:536-547

Goldstein ST, Corliss JO (1994) Deposit feeding in selected deep-sea and shallow-water benthic foraminifera. Deep-Sea Res 41:229-441

Gooday AJ (1988) A response by benthic foraminifera to the deposition of phytodetritus in the deep-sea. Nature 332:70-73

Gooday AJ, Levin LA, Linke P, Heeger T (1992) The role of benthic foraminifera in deep-sea food webs and carbon cycling. In: Rowe GT, Parient V (eds) Deep-sea Food Chains and the global Carbon Cycle. Kluwer, Dordrecht, pp 63-91

Grewal PS, Wright RT (1992) Migration of *Caenorhabditis elegans* (Nematod: Rhaabditidae) larvae towards bacteria and the nature of bacterial stimulus. Fund Appl Nemat 15:159-166

Groß O (2002) Sediment interactions of foraminifera: implications for food degradation and bioturbation processes. J Foraminifer Res 32:414

Grudemo J, André C (2001) Salinity dependence in the marine mud snails *Hydrobia ulvae* and *Hydrobia ventrosa.* J Mar Biol Ass U K 81:651-654

Guarini JM, Blanchard GF, Gros P (2000a) Quantification of the microphytobenthic primary production in European intertidal mudflats - a modelling approach. Cont Shelf Res 20:1771-1788

Guarini JM, Blanchard GF, Gros P, Gouleau D, Bacher C (2000b) Dynamic model of the short-term variability of microphytobenthos biomass on temperate intertidal mudflats. Mar Ecol Prog Ser 195:291-303

Guarini JM, Blanchard GF, Gros P, Harrison SJ (1997) Modeling the mud surface temperature on intertidal flats to investigate the spatio-temporal dynamics of the benthic microalgal photosynthetic capacity. Mar Ecol Prog Ser 153:25-36

Haglund AL, Törnblom E, Boström B, Tranvik L (2002) Large differences in the fraction of active bacteria in plankton, sediments, and biofilm. Microb Ecol 43:232-241

Hagström A, Larsson U, Horstedt P, Normark S (1979) Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. Appl Environ Microbiol 37:805-812

Hahn MW, Höfle MG (1999) Flagellate predation on a bacterial model community: interplay of size-selective grazing specific bacterial cell size, and bacterial community composition. Appl Environ Microbiol 65:4863-4872

Hahn MW, Höfle MG (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol Ecol 35:113-121

Hall RO, Meyer JL (1998) The trophic significance of bacteria in a detritus-based stream food web. Ecology 79:1995-2012

Hamels I, Koenraad M, Casteleyn G, Vyverman W (2001) Uncoupling of bacterial production and flagellate grazing in aquatic sediments: a case study from intertidal flat. Aquat Microb Ecol 25:31-42

Hamels I, Sabbe K, Muylaert K, Vyverman W (2004) Quantitative importance, composition, and seasonal dynamics of protozoan communities in polyhaline versus freshwater intertidal sediments Microb Ecol 47:18-29

Hargrave BT (1970) The effect of a deposit-feeding amphipod on the metabolism of benthic microflora. Limnol Oceanogr 15:21-30

Haubois AG, Guarini JM, Richard P, Blanchard GF, Sauriau PG (2002) Spatio-temporal differentiation in the population structure of *Hydrobia ulvae* on an intertidal mudflat (Marennes-Oléron Bay, France). J Mar Biol Ass U K 82:605-614

Haubois AG, Guarini JM, Richard P, Fichet D, Radenac G, Blanchard GF (2005a) Ingestion rate of the deposit-feeder *Hydrobia ulvae* (Gastropoda) on epipelic diatoms: effect of cell size and algal biomass. J Exp Mar Biol Ecol 317:1-12

Haubois AG, Sylvestre F, Guarini JM, Richard P, Blanchard GF (2005b) Spatio-temporal structure of the epipelic diatom assemblage from an intertidal mudflat in Marennes-Oléron Bay, France. Est Coast Shelf Sci 64:385-394

Heip CHR, Herman P, Coomans A (1982) The productivity of marine meiobenthos. Academia Analecta 44:1-20

Heip CHR, Vincx M, Vranken G (1985) The ecology of marine nematodes. Ocenogr Mar Biol Annu Rev 23:399-489

Héral M, Bacher C, Deslous-Paoli JM (1989) La Capacité trophique des bassins ostréicoles. In: Troadec JP (ed) L'homme et les ressources halieutiques : essai sur l'usage d'une ressource renouvelable Ifremer, Brest, pp 225-259

Héral M, Razet D, Deslous-Paoli JM, Bertome JP, Garnier J (1982) Caractéristiques saisonnières de l'hydrologies du complexe estuarien de Marennes-Oléron (France). Revue des travaux de l'institut des pêches maritimes 46:97-119

Herlory O, Guarini JM, Richard P, Blanchard GF (2004a) Microstructure of microphytobenthic biofilm and its saptio-temporal dynamics in an intertidal mudflat (Aiguillon Bay, France). Mar Ecol Prog Ser 282:33-44

Herlory O, Guarini JM, Richard P, Blanchard GF (2004b) Microstructure of microphytobenthos biofilm and its spatio-temporal dynamics in an intertidal mudflat (Aiguillon Bay, France). Mar Ecol Prog Ser 282:33-44

Herman PMJ, Vranken G (1988) Studies of the life history and energetics of marine and brackish water nematodes. II. Production, respiration and food-uptake by *Monhystera disjuncta*. Oecologia 77:457-463

Holling CS (1959) Some characteristics of simple types of predation and parasistism. Can Entomol 91:385-398

Hondeveld BJM, Nieuwland G, van Duyl FC, Bak RPM (1994) Temporal and spacial variations in heterotrophic nanoflagellate abundance in North Sea Sediments. Mar Ecol Prog Ser 109:235-243

Hondeveld BMJ, Bak RPM, van Duyl FC (1992) Bacterivory by heterotrophic nanoflagellates in marine sediments measured by uptake of fluorescently labeled bacteria. Mar Ecol Prog Ser 89:63-71

Hylleberg J (1975) The effect of salinity and temperature on egestion in mud snails (Gastropoda: Hydrobiidae). A study in niche overlap. Oecologia 21:279-289

Jacob U, Mintenberg K, Brey T, Knust R, Beyer K (2005) Stable isotope food web studies: a case for standardized sample treatment. Mar Ecol Prog Ser 287:251-253

Jensen KT (1984) Food ingestion and growth of the diatom-feeding nematode *Chromadorita tenuis*. Mar Biol 81:307-310

Jensen KT (1987) Feeding ecology of free-living aquatic nematodes. Mar Ecol Prog Ser 35:187-196

Jensen KT, Siegismund HR (1980) The importance of diatoms and bacteria in the diet of *Hydrobia* species. Ophelia 1:193-199

Jensen P (1996) Burrows of marine nematodes as centres for microbial growth. Nematologica 42:320-329

Joint IR, Gee JM, Warwick RM (1982) Determination of fine-scale vertical distribution of microbes and meiofauna in an intertidal sediment. Mar Biol 72:157-164

Jonge VND (1980) Fluctuations in the organic carbon to chlorophyll-*a* ratios in estuarine benthic diatom populations. Mar Ecol Prog Ser 2:345-353

Juniper SK (1987) Deposit-feeding ecology of *Amphibola crenata*. I. Long-term effcts of deposit feeding on sediment micro-organisms. N Z J Mar Freshwater Res 21:235

Kang CK (1999) Structures trophiques et production secondaire dans les réseaux benthiques intertidaux du bassin de Marennes-Oléron: utilisation du tracage isotopique naturel. PhD Thesis, University of Nantes, 272 pp

Kawasaki N, Benner R (2006) Bacterial release of dissolved organic matter during cell growth and decline: molecular origin and composition. Limnol Oceanogr 51:2170-2180

Kelly JA, Honeywill C, Paterson DM (2001) Microscale analysis of chlorophyll-a in cohesive intertidal sediments: the implications of microphytobenthos distribution. J Mar Biol Ass U K 81:151-162

Kemp PF (1986) Direct uptake of detrital carbon by the deposit-feeding polychaete *Euzonus mucronata* (Treadwell). J Exp Mar Biol Ecol 99:49-61

Kemp PF (1987) Potential impact on bacteria of grazing by a macrofaunal deposit-feeder, and the fate of bacterial production. Mar Ecol Prog Ser 36:151-161

Kemp PF (1988) Bacterivory by benthic ciliates: significance as a carbon source and impact on sediment bacteria. Mar Ecol Prog Ser 49:163-169

Kemp PF (1990) The fate of benthic bacterial production. Rev Aquat Sci 2:109-124

Kirchman DL (2000) Microbial ecology of the oceans, Wiley-Liss, New York, 512 pp

Kirchman DL, K'nees E, Hodson RE (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural waters. Appl Environ Microbiol 49:599-607

Klekowski RZ, Schiemer F, Duncan A (1979) A biogenic study of a benthic nematode, *Plectrus palustris* de Man 1880, throughout its life cycle. Oecologia 44:119-124

Kofoed LH (1975) The feeding biology of *Hydrobia ventrosa* (Montague). I. The assimilation of different components of food. J Exp Mar Biol Ecol 19:233-241

Kofoed LH, Forbes VE, Lopez G (1989) Time-dependent absorption in deposit feeders In: Lopez G, Taghon GL, Levinton JS (eds) Ecology of marine deposit feeders. Springer, New-York, pp 128-148

Koury ST, Bowser SS, MacGee-Russel SM (1985) Ultrastructure changes during reticulopod withdrawal in the foraminiferan protozoan *Allogromia sp.*, Strain NF. Protoplasma 129:149-156

Kuipers BR, de Wilde PAWJ, Creutzberg F (1981) Energy flow in a tidal flat ecosystem. Mar Ecol Prog Ser 5:215-221

Langer MR, Gehring CA (1993) Bacteria farming: a possible feeding strategy of some smaller, motile foraminifera. J Foraminifer Res 23:40-46

Langezaal AM, Jannink NT, Pierson ES, van der Zwaan GH (2005) Foraminiferal selectivity towards bacteria: an experimental approach using cell-permeant stain. J Sea Res 54:256-275

Lassen HH, Kristensen JH (1978) Tolerance to abiotic factors in mudsnails (Hydrobiidae). Nat Jutl 20:243-250

Le Hir P, Roberts W, Cazaillet O, Christie M, Bassoullet P, C. B (2000) Characterization of intertidal flat hydrodynamics. Cont Shelf Res 20:1433-1459

Lee JJ, Anderson OR (1991) Symbiosis in foraminifera. In: Lee JJ, Anderson OR (eds) Biology of foraminifera. Academic Press, New-York, pp 157-220

Lee JJ, Faber WW, Lee RE (1991) Granular reticulopodial digestion - a possible preadaptation to benthic foraminifera symbiosis ? Symbiosis 10:47-61

Lee JJ, Mc Enery M, Pierce S, Freudenthal HD, Muller WA (1966) Tracer experiments in feeding littoral foraminifera. J Protozool 16:659-670

Lee JJ, Muller WA (1973) Trophic dynamics and niches of salt marsh foraminifera. Am Zool 13:215-223

Legendre L, Rassoulzadegan F (1995) Plankton and nutrient dynamics in marine waters. Ophelia 41:153-172

Legendre L, Rassoulzadegan F (1996) Food-web mediated export of biogenic carbon in oceans: hydrodynamic control. Mar Ecol Prog Ser 145:179-193

Leguerrier D (2005) Contribution et étude d'un modèle de réseau trophique de la vasière de Brouage (Bassin de Marennes-Oléron, France). Prise en compte de la saisonnalité et des échanges physiques pour la synthèse constructive des connaissances sur une zone intertidale d'une région tempérée. PhD Thesis, University of La Rochelle,

Leguerrier D, Niquil N, Boileau N, Rzeznik J, Sauriau PG, Le Moine O, Bacher C (2003) Numerical analysis of the food web of an intertidal mudflat ecosystem on the Atlantic coast of France. Mar Ecol Prog Ser 246:17-37

Leguerrier D, Niquil N, Petiau A, Bodoy A (2004) Modeling the impact of oyster culture on a mudflat food web in Marennes-Oléron Bay (France). Mar Ecol Prog Ser 273:147-162

Levinton JS (1979) Deposit feeding and coastal oceanography. In: Lopez G, Taghon GL, Levinton JS (eds) Ecology of marine deposit feeders. Springer-Verlag, New-York, pp 1-23

Levinton JS (1987) The body size-prey-size hypothesis and *Hydrobia*. Ecology 68:229-231

Levinton JS, Bianchi M, Stewart S (1984) What is the role of particulate organic mater in benthic invertebrate nutrition? Bull Mar Sci 35:270-282

Levinton JS, Bianchi TS (1981) Nutrition and food limitation of deposit-feeders. I. The role of microbes in the growth of mud snails (Hydrobiidae). J Mar Res 39:531-545

Linke P, Altenbach AV, Graf G, Heeger T (1995) Responses of deep-sea benthic foraminifera to a simulated sedimentation event. J Foraminifer Res 25:75-82

Lipps JH (1983) Biotic interactions in benthic foraminifera. In: Tevesz MJS, Mc Call PL (eds) Biotic interactions in recent and fossil benthic communities. Plenum Press, New-York, pp 331-373

Lonsdale DJ, Levinton JS (1989) Energy budgets of latitudinally separated *Scottolana canadensis* (Copepoda: Harpaticoida). Limnol Oceanogr 34:324-331

Lopez G, Riemann F, Schrage M (1979) Feeding biology of the brackish-water Oncholaimid nematod *Adoncholaimus thalassophygas.* Mar Biol 54:311-318

Lopez GR, Cheng IG (1983a) Epipsammic browsing and deposit-feeding in mud snails (Hydrobiidae). J Mar Res 38:585-599

Lopez GR, Cheng IJ (1983b) Synoptic measurements of ingestion rate, ingestion selectivity, and absorption efficiency of natural foods in the deposit-feeding molluscs *Nucula annulata* (Bivalvia) and *Hydrobia ulvae* (Gastropoda). Mar Ecol Prog Ser 11:55-62

Lopez GR, Kofoed LH (1980) Epipsammic browsing and deposit-feeding in mud snails (Hydrobiidae). J Mar Res 38:585-599

Lopez GR, Levinton JS (1978) The availability of microorganisms attached to sediment particles as food for *Hydrobia ventrosa* (Montague) Gastropoda, Prosobranchia. Oecologia 32:263-375

Lopez GR, Levinton JS (1987) Ecology of deposit-feeding animals in marine sediments. Q Rev Biol 62:235-260

Lorenzen S (1966) A method for the continuous measurement of *in vivo* chlorophyll concentration. Deep-Sea Res 13:223-227

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275

Luna GM, Manini E, Danavoro R (2002) Large fraction of dead and inactive bacteria in coastal marine sediments : comparison of protocols for determination and ecological significance. Appl Environ Microbiol 68:3509-3513

MacIntyre HL, Geider RJ, Miller DC (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

MacLachlan A (1985) The biomass of macro- and interstitial fauna on clean and wrack-covered beaches in Western Australia. Est Coast Shelf Sci 21:587-599

Mariotti A (1982) Apports de la géochimie isotopique à la connaissance du cycle de l'azote. PhD Thesis, Université Pierre et Marie Curie Paris VI, 476 pp

McLusky DS (1989) The estuarine ecosystem, Chapman & Hall, New York, 215 pp

Mei ML, Danovaro R (2004) Virus production and lifes strategies in aquatic sediments. Limnol Oceanogr 49:459-470

Meyer-Reil LA (1984) Bacterial biomass and heterotrophic activity in sediments and overlying waters. In: Hobbie JE, Williams PJ (eds) Heterotrophic activity in the sea. Plenum Press, New York, pp 523-546

Meyer-Reil LA, Faubel A (1980) Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. Mar Ecol Prog Ser 3:251-256

Meyer-Reil LA, Köster M (1991) Fine-scale distribution of hydrolytic activity associated with foraminiferans and bacteria in deep-sea sediments of the Norwegian-Greenland sea. Kieler Meeresforsch, Sonderhelf 8:121-126

Middelburg JJ, Barranguet C, Boschker HTS, Herman PMJ, Moens T, Heip CHR (2000) The fate of intertidal microphytobenthos carbon. An *in situ* ^{13}C labelling study. Limnol Oceanogr 45:1224-1234

Moal JJF, Samain JF, Le Coz JF, Daniel JY (1985) Protéines, glucides et lipides particulaires: aspects methodologiques. Oceanis 11:487-502

Moens T (1999) Feeding ecology of free-living estuarine nematodes. An experimental approach. PhD Thesis, University of Gent, 302 pp

Moens T, Bergtold M, Traunspurger W (2006) Feeding ecology of free-living benthic nematodes. In: Eyulaem A, Andrassy I, Traunspurger W (eds) Freshwater nematodes: ecology and taxonomy. CAB International publishing, Cambridge, pp 105-131

Moens T, Herman P, Verbeeck L, M. S, Vincx M (2000) Predation rates and prey selectivity in two predacious estuarine nematode species. Mar Ecol Prog Ser 205:185-193

Moens T, Luyten C, Middelburg JJ, Herman PMJ, Vincx M (2002) Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. Mar Ecol Prog Ser 234:127-137

Moens T, Verbeeck L, de Maeyer A, Swings J, Vincx M (1999a) Selective attraction of marine bacterivorous nematodes to their bacterial food. Mar Ecol Prog Ser 176:165-178

Moens T, Verbeeck L, Vincx M (1999b) Feeding biology of a predatory and a facultatively predatory nematode (*Enoploides longispiculosus* and *Adoncholaimus fuscus*). Mar Biol 134:585-593

Moens T, Verbeeck L, Vincx M (1999c) Preservation and incubation time-induced bias in tracer-aided grazing studies on meiofauna. Mar Biol 133:69-77

Moens T, Vierstraete A, Vincx M (1996) Life strategies in two bacterivorous marine nematods: preliminary results. P S N Z I: Mar Ecol 17:509-518

Moens T, Vincx M (1997) Observations on the feeding ecology of estuarine nematodes. J Mar Biol Ass U K 77:211-227

Moens T, Vincx M (2000) Temperature, salinity and food thresholds in two brackishwater bacterivorous nematode species: assessing niches from food absorption and respiration experiments. J Exp Mar Biol Ecol 243 137–154

Moloney CL, Field JG (1989) General allometric equations for rates of nutrient uptake, ingestion, and respiration in plankton organisms. Limnol Oceanogr 34:1290-1299

Montagna PA (1983) Live control for radioisotope tracer food chain experiment using meiofauna. Mar Ecol Prog Ser 12:43-46

Montagna PA (1984a) Competition for dissolved glucose between meiobenthos and sediment microbes. J Exp Mar Biol Ecol 76:177-190

Montagna PA (1984b) *In situ* measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. Mar Ecol Prog Ser 18:119-130

Montagna PA (1993) Radioisotope technique to quantify *in situ* microbivory by meiofauna in sediments. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Pubishers, Boca Raton, pp 745-753

Montagna PA (1995) Rates of metazoan meiofaunal microbivory: a review. Vie Milieu 45:1-9

Montagna PA, Bauer JE (1988) Partitioning radiolabeled thymidine uptake by bacteria and meiofauna using metabolic blocks and poisons in benthic feeding studies. Mar Biol 98:101-110

Montagna PA, Bauer JE, Hardin D, Spies RB (1995a) Meiofaunal and microbial trophic interactions in a natural submarine hydrocarbon seep. Vie Milieu 45:17-26

Montagna PA, Bauer JE, Toal J, Hardin D, Spies RB (1987) Temporal variability and the relationship between benthic meiofaunal and microbial populations of a natural coastal petroleum seep. J Mar Res 45:761-789

Montagna PA, Blanchard GF, Dinet A (1995b) Effect of production and biomass of intertidal microphytobenthos on meiofaunal grazing rates. J Exp Mar Biol Ecol 185:149-165

Montagna PA, Yoon WB (1991) The effect of freshwater inflow on meiofaunal consumption of sediment bacteria and microphytobenthos consumption of sediment bacteria and microphytobenthos in San Antonio Bay, Texas, U. S. A. Est Coast Shelf Sci 33:529-547

Moodley L, Boshker HTS, Middelburg JJ, Pel R, Herman P, de Deckere E, Heip CHR (2000) Ecological significance of benthic foraminifera: ^{13}C labelling experiments. Mar Ecol Prog Ser 202:289-295

Moodley L, Heip HR, Middelburg JJ (1998) Benthic activity in sediments of the northwestern Adriatic Sea: sediment oxygen consumption, macro- and meiofauna dynamics. J Sea Res 40:263-280

Moodley L, Middelburg JJ, Boschker HTS, Duineveld R, Pel R, Herman PMJ, Heip CHR (2002) Bacteria and foraminifera: key players in a short-term deep sea benthic response to phytodetritus. Mar Ecol Prog Ser 236:23-29

Moodley L, Middelburg JJ, Soetaert K, Boschker HTS, Herman PMJ, Heip CHR (2005) Similar rapid response to phytodetritus deposition in shallow and deep-sea sediments. J Mar Res 63:457-469

Moriarty DJW (1986) Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Adv Microb Ecol 9:245-292

Moriarty DJW (1988) Accurate conversion factors for calculating bacteria growth rates from thymidine incorporation into DNA: elusive or illusive? Arch Hydrobiol Beih 31:211-217

Moriarty DJW, Pollard PC, Hunt HW, Moriarty CM, Wassenberg TJ (1985) Productivity of bacteria and microalgae and the effect of grazing by holothurians in sediments on a coral reef flat. Mar Biol 85:293-300

Motoda S (1959) Devices of simple plankton apparatus. Memoirs of the Faculty of Fisheries Hokkaido University 7:73-94

Muller WA (1975) Competition for food and other niche-related studies of three species of salt-marsh Foraminifera. Mar Biol 31:339-351

Muller WA, Lee JJ (1969) Apparent indispensability of bacteria in foraminiferan nutrition. J Protozool 16:471-478

Murray JW (1991) Ecology and paleoecology of benthic foraminifera, New-York, 397 pp
Murray JW, Bowser SS (2000) Mortality, protoplasm decay rate and reliability of staining techniques to recognise "living" foraminifera: a review. J Foraminifer Res 30:66-70
Nehring S, Jensen P, Lorenzen S (1990) Tube-dwelling nematodes: tube construction and possible ecological effect on sediment-water interfaces. Mar Ecol Prog Ser 64:123-128
Newell RC (1965) The role of detritus in the nutrition of two marine deposit-feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macoma balthica*. Proc Zool Soc Lond 138:49-75
Nicholas WL, Grassia A, Viswanathan S (1973) The efficiency with which *Caenorhabditis briggsae* (Rhabditinae) feeds on the bacterium *Escherichia coli*. Nematologica 19:411-420
Nilsson P, Jönsson B, Lindström-Swanberg I, Sundbäk K (1991) Response of a shallow-water sediment system to an increased load of inorganic nutrients. Mar Ecol Prog Ser 71:275-290
Noble RT, Furham JA (1998) Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat Microb Ecol 14:113-118
Nomaki H, Heinz P, Hemleben C, Kitatzao H (2005a) Behavior and response of deep-sea benthic foraminifera to freshly supplied organic matter: a laboratory feeding experiment in microcosm environments. J Foraminifer Res 35:103-113
Nomaki H, Heinz P, Nakatsuka T, Shimanaga M, Kitazato H (2005b) Species-specific ingestion of organic carbon by deep-sea benthic foraminifera and meiobenthos: *In situ* tracer experiments. Limnol Oceanogr 50:134-146
Norland S, Fagerbakka KM, Heldal M (1995) Light element analysis of individual bacteria by X-ray microanalysis. Appl Environ Microbiol 61:1357-1362
Novitsky JA (1986) Degradation of dead microbial biomass in a marine sediment. Appl Environ Microbiol 52:504-509
Novitsky JA (1990) Protozoan abundance, growth, and bacterivory in the water column, on sedimenting particles, and in the sediment of Halifax Harbor. Can J Microbiol 36:859-863
Nozais C, Desrosiers G, Gosselin M, Belzile C, Demers S (1999) Effect of ambient UVB radiation in a meiobenthic community of a tidal. Mar Ecol Prog Ser 189:149-158
Ogawa H, Amagai Y, Koike I, Kaiser K, Benner R (2001) Production of refractory dissolved organic matter by bacteria. Science 292:917-920
Orvain F, Sauriau P-G (2002) Environmental and behavioural factors affecting activity in the intertidal gastropod *Hydrobia ulvae*. J Exp Mar Biol Ecol 272:191-217
Orvain F, Sauriau P-G, Sygut A, Joassard L, Le Hir P (2004) Interacting effect of *Hydrobia ulvae* bioturbation and microphytobenthos on the erodibility of mudflat sediments. Mar Ecol Prog Ser 278:205-223
Pérez-Uz (1996) Bacterial preferences and growth kinetic variation in *Uronema marinum* and *Uronema nigricans* (Ciliophora : Scuticociliatida). Microb Ecol 31:189-198
Platt HM (1977) Vertical and horizontal distribution of free-living marine nematodes from Strangford Lough, Northern Ireland. Cah Biol Mar 18:261-273
Platt HM, Subba-Rao DV, Irwin B (1983) Photosynthesis of picoplankton in the oligotrophic ocean. Nature 301:702-704
Platt HM, Warwick RM (1980) The significance of free-living nematodes to the littoral ecosystem. In: Price JH, Irvine DEG, Farnham WF (eds) The shore environment, Vol 2. Academis Press, London and New-York, pp 729-759

Pollard PC, Moriarty DJW (1984) Validity of the tritiated thymidine method for estimating bacterial growth rates: the measurement of isotope dilution during DNA synthesis. Appl Environ Microbiol 48:1076-1083

Pomeroy LR (1974) The ocean's food web, a changing paradigm. Bioscience 24:499-504

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943-948

Price CA, Reardon EM, Guillard RRL (1978) Collection of dinoflagellates and other marine microalgae by centrifugation in density gradients of a modified silica gel. Limnol Oceanogr 23:548-553

Proctor LM, Souza AC (2001) Method for enumeration of 5-cyano-2,3-ditolyl tetrazolium chloride (CTC)-active cells and cell-specific CTC activity of benthic bacteria in riverine, estuarine and coastal sediments J Microbiol Methods 43:213-222

Rassoulzadegan F (1993) Protozoan patterns in the Azam-Ammerman's bacteria-phytoplankton mutualism. In: Guerro R, Pedros-Alio C (eds) Trends in Microbial Ecology. Spanish society for microbiology, Barcelona, pp 435-440

Rice DL, Rhoads DC (1989) Early diagenesis of organic matter and the nutritional value of sediment. In: Lopez G, Taghon GL, Levinton JS (eds) Ecology of marine deposit feeders. Springer, New-York, pp 59-97

Riemann B, Schrage M (1978) The mucus-trap hypothesis on feeding of aquatic nematods and implication for biodegradation and sediment texture. Oecologia 34:75-88

Riemann F, Helmke E (2002) Symbiotic relations of sediment-agglutinating nematodes and bacteria in detrital habitats: the enzyme-sharing concept. Mar Ecol 23:93-113

Rieper M (1978) Bacteria as food for marine harpaticoid copepods. Mar Biol 45:337-345

Riera P, Richard P (1996) Isotopic determination of food sources of *Crassostrea gigas* along a trophic gradient in the estuarine bay of Marennes-Oléron. Est Coast Shelf Sci 42:347-360

Riera P, Richard P (1997) Temporal variations of $\delta^{13}C$ in particulate organic matter and oyster Crassostrea gigas in Marennes-Oléron Bay (France): effect of freshwater inflow. Mar Ecol Prog Ser 147:105-115

Riera P, Richard P, Grémare A, Blanchard GF (1996) Food source of intertidal nematods in the Bay of Marennes-Oléron (France), as determined by dual stable isotope analysis. Mar Ecol Prog Ser 142:303-309

Riera P, Stal LJ, Nieuwenhuize J, Richard P, Blanchard GF, Gentil F (1999) Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen stable isotopes: importance of locally produced sources. Mar Ecol Prog Ser 187:301-307

Rivkin RB, De Laca TE (1990) Trophic dynamics in Antartic benthic communities. I. *In situ* ingestion of microalgae by foraminifera and metazoan meiofauna. Mar Ecol Prog Ser 64:129-136

Rivkin RB, Legendre L, Deibel D, Tremblay JE, Klein B, Crocker K, Roy S, Silverberg N, Lovejoy C, Mesplé F, Romero N, Anderson MR, Matthews P, Savenkoff C, Vézina AF, Therriault JC, Wesson J, Bérubé C, Ingram RG (1996) Vertical flux of biogenic carbon in the ocean: Is there food web control? Science 272:1163-1166

Romeyn K, Bouwman L (1983) Food selection and consumption by estuarine nematodes. Hydrobiol Bull 17:103-109

Rublee PA (1982) Bacteria and microbial distribution in estuarine sediments. In: Kennedy VS (ed) Estuarine comparisons. Academic Press, New-York, pp 159-182

Rudnick DT (1989) Time lags between the deposition and meiobenthic assimilation of phytodetritus. Mar Ecol Prog Ser 50:231-240

Rzeznik-Orignac J (2004) Contribution de la méiofaune dans le transfert des métaux dans le réseau trophique de la baie de Gdansk (Baltique) et de la baie de Marennes-Oléron (Atlantique). PhD Thesis, University of La Rochelle & University of Gdansk, 243 pp

Rzeznik-Orignac J, Fichet D, Boucher G (2003) Spatio-temporal structure of the nematode assemblages of the Brouage mudflat (Marennes Oléron, France). Est Coast Shelf Sci 58:77-88

Samir AM (2000) The response of benthic foraminifera and ostracods to various pollution sources: a study from two lagoons in Egypt. J Foraminifer Res 30:83-98

Sander BC, Kalff J (1993) Factors controlling bacterial production in marine and freshwater sediments. Microb Ecol 26:79-99

Sauriau P-G, Mouret V, Rince JP (1989) Organisation trophique de la malacofaune benthique non cultivée du bassin de Marennes-Oléron. Oceanol Acta 12:193-204

Schiemer F (1982a) Food dependence and energetics of freeliving nematodes. I. Respiration, growth and reproduction of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. Oecologia 54:108-121

Schiemer F (1982b) Food dependence and energetics of freeliving nematodes. II. life history parameters of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. Oecologia 54:122-128

Schiemer F (1983) Comparative aspects of food dependance and energetics of free living nematodes. Oikos 41:32-42

Schiemer F (1987) Nematoda. In: Pandian TJ, Vernberg FJ (eds) Animal energetic. Academic Press, New York, pp 185-211

Schiemer F, Duncan A, Klekowski RZ (1980) A bioenergetic study of a benthic nematod, *Plectus palustris* de Man 1880, throughout its life style. II Growth, fecundity and energy budget at different levels of bacterial food and general ecological considerations. Oecologia 44:205-212

Schmidt JL, Deming JW, Jumars PA, Keil RG (1998) Constancy of bacterial abundance in surficial marine sediments. Limnol Oceanogr 43:976-982

Schwab D, Hofer HW (1979) Metabolism in the protozoan *Allogromia laticollaris* Arnold. Z Mikrosk Anat Forsch 93:715-727

Serôdio J, da Silva JM, Catarino F (1997) Nondestructive tracing of migratory rhythms of intertidal benthic microalgae using in vivo chlorophyll a fluorescence. J Phycol 33:542-553

Severin K (1987) Laboratory observations of the rate of subsurface movement of a small miliolid foraminifer. J Foraminifer Res 17:110-116

Severin K, Erskian MG (1981) Laboratory experiments on the vertical movement of *Quinqueloculina impressa* Reuss through sand. J Foraminifer Res 11:133-136

Sherr EB, Sherr BF (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb Ecol 28:223-235

Sherr EB, Sherr BF (2000) Marine microbes: an overview. In: L. KD (ed) Microb Ecol. Wiley-Liss, New-York, pp 13-46

Sherr EB, Sherr BF, Albright LJ (1987) Bacteria: sink or link? Science 235:88-89

Sherr EB, Sherr BF, Fallon RD, Newell SY (1986) Small aloricate ciliate as a major component of the marine heterotrophic nanoplankton. Limnol Oceanogr 31:177

Snider LJ, Burnett BR, Hessler RR (1984) The composition and distribution of meiofauna and nanobiota in a central North Pacific deep-sea area. Deep-Sea Res 31:1225-1249

Soetaert K, Vincx M, Wittoeck J, Tulkens M (1995) Meiobenthic distribution and nematode community structure in five European estuaries. Hydrobiologia 311:185-206

Sokolowski A, Richard P, Fichet D, Radenac G (2005) Cd transfer in the deposit-feeder prosobranch *Hydrobia ulvae* (Pennant) from the benthic diatoms: the kinetics of rapid Cd assimilation and efflux. J Exp Mar Biol Ecol 317:159-174

Sola JC (1996) Population dynamics, reproduction, growth, and secondary production of the mud-snails *Hydrobia ulvae* (Pennant). J Exp Mar Biol Ecol 205:49-62

Somerfield PJ, Warwick RM, Moens T (2005) Methods for the study of marine benthos. In: Eleftheriou A, McIntyre AD (eds) Meiofauna techniques. Blackwell Science, Oxford, pp 229-272

Starink M, Krylova IN, Bär-Gilissen MJ, Bak RPM, Cappenberg TE (1994) Rates of benthic protozoan grazing on free and attached sediment bacteria measured with fluorescently stained sediment. Appl Environ Microbiol 60:2259-2264

Steele (1974) The structure of marine ecosystems, Harvard university press128 pp

Steyaert M, Herman PMJ, Moens T, Widdows J, Vincx M (2001) Tidal migration of nematodes of an estuarine tidal flat (the Molenplaat, Schelde Estuary, SW Netherlands). Mar Ecol Prog Ser 224:299-304

Steyaert M, Vanaverbeke J, Vanreusel A, Barranguet C, Lucas C, Vincx M (2003) The importance of fine-scale, vertical profiles in characterizing nematode community structure. Est Coast Shelf Sci 58:353-366

Struski C, Bacher C (2006) Preliminary etimate of primary production by phytoplancton in Marennes-Oléron Bay, France. Est Coast Shelf Sci 66:323-334

Sundbäck K, Nilsson C, Odmark S, Wulff A (1996a) Does ambient UV-B radiation influence marine diatom-dominated microbial mats? A case study. Aquat Microb Ecol 11:151-159

Sundbäck K, Nilsson P, Nilsson C, Jonsson B (1996b) Balance between autotrophic and heterotrophic components and processes in microbenthic communities of sandy sediments: a field study. Est Coast Shelf Sci 43:689-706

Taghon GL (1982) Optimal foraging by deposit-feeding invertebrates: roles of particle size and organic coating. Oecologia 52:295-304

Taghon GL, Jumars PA (1984) Variable ingestion rate and its role in optimal foraging behavior of marine deposit feeders. Ecology 65:549-558

Taghon GL, Self RLF, Jumars PA (1978) Predicting particle selection by deposit feeders: a model an its implications. Limnol Oceanogr 23:752-759

Teal JM (1962) Energy flow in the salt-marsh ecosystem of Georgia. Ecology 43:614-624

Theil-Nielsen J, Søndergaard M (1998) Bacterial carbon biomass calculated from biovolumes. Archiv Hydrobiol 141:195-207

Thingstad F (2000) Control of bacterial growth in idealized food webs. In: Kirchman DL (ed) Microbial ecology of the Oceans. Wiley-Liss, New York, pp 229-260

Thomas G, Héral M, Kantin R (1999) La Charente, un estuaire conchylicole. Recherches Marines 22:20-25

Thomas JH (1989) Genetic analysis of defecation in *Caenorhabditis elegans.* Genetics 124:855-872

Tietjen JH (1980) Microbial-meiofaunal interrelationships: a review. Microbiology 110:335-338

Tietjen JH, Lee JJ (1972) Life cycles of marine nematodes. Influence of temperature and salinity on the development of *Monhystera denticulata* Timm. Oecologia 10:167-176

Tietjen JH, Lee JJ (1973) Life history and feeding habits of the marine nematode *Chromadora macrolaimoides* Steiner. Oecologia 12:303-314

Tietjen JH, Lee JJ (1977a) Feeding behavior of marine nematodes. In: Coull BC (ed) Ecology of marine benthos. University of South Carolina press, Columbia, pp 21-35

Tietjen JH, Lee JJ (1977b) Life histories of marine nematodes. Influence of temperature and salinity on the reproductive potential of *Chromadorine germanica* Bütschli. Mikrofauna Meeresboden 61:263-270

Tietjen JH, Lee JJ, Rullman J, Greengart A, Trompeter J (1970) Gnotobiotic culture and culture physiological ecology of the marine nematode *Rhabditis marina* Bastian. Limnol Oceanogr 15:535-543

Tranvik L, Sherr EB, Sherr BF (1993) Uptake and utilization of "colloidal DOM" by heterotrophic flagellate in seawater. Mar Ecol Prog Ser 92:301-309

Travis JL, Bowser SS (1991) The mobility of foraminifera. In: Lee JJ, Anderson NH (eds) Biology of foraminifera. Academic Press, London, pp 91-155

Underwood AJ, Kromkamp JC (1999) Primary production by phytoplancton and microphytobenthos in estuaries. Adv Ecol Res 29:93-153

Underwood GJC, Kromkamp JC (2000) Primary production by phytoplancton and microphytoplancton in estuaries. In: Nedwell DB, Raffaelli DG (eds) Estuaries, Advances in Ecological Research. Academic Press, pp 93-153

Urban-Malinga B, Moens T (2006) Fate of organic matter in Artic sediments: Is utilisation by meiofauna important? J Sea Res 56:239-248

van de Kam J, Ens B, Piersma T, Zwarts L (2004) Shorebirds: an illustrated behavioural ecology, KNNV Publishers, Utrecht, 368 pp

van Duyl FC, de Winder B, Kop AJ, Wollenzien U (1999) Tidal coupling between carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats. Mar Ecol Prog Ser 191:19-32

van Duyl FC, Kop AJ (1990) Seasonal patterns of bacterial production and biomass in intertidal sediments of the western Dutch Wadden Sea. Mar Ecol Prog Ser 59:249-261

van Oevelen D, Middelburg JJ, Soetaert K, Moodley L (2006a) The fate of bacterial carbon in sediments: modeling an in situ isotope tracer experiment. Limnol Oceanogr 51:1302-1314

van Oevelen D, Moodley L, Soetaert K, Middelburg JJ (2006b) The trophic significance of bacterial carbon in a marine intertidal sediment: Results of an *in situ* stable isotope labeling study. Limnol Oceanogr 51:2349-2359

van Oevelen D, Soetaert K, Middelburg JJ, Herman PMJ, Moodley L, Hamels I, Moens T, Heip CHR (2006c) Carbon flows through a benthic food web: integrating biomass, isotope and tracer data. J Mar Res 64:453-482

Venette RC, Ferris H (1998) Influence of bacterial type and density on population growth of bacterial-feeding nematodes. Soil Biol Biochem 30:949-960

Veuger B, van Oevelen D, Boschker HTS, Middelburg JJ (2006) Fate of peptidoglycan in an intertidal sediment: An *in situ* ^{13}C-labeling study. Limnol Oceanogr 51:1572-1580

Vézina AF (1989) Construction of low networks using inverse methods. In: F. W, J.G. F, K.H. M (eds) Network ANALYSIS in marine ecology: Methods and applications. Springer-Verlag, Berlin, pp 62-81

Vézina AF, Platt T (1988) Food web dynamics in the ocean. I. Best-estimates of flow networks using inverse methods. Mar Ecol Prog Ser 42:269-287

Vézina AF, Savenkoff C (1999) Inverse modeling of carbon and nitrogen flows in the pelagic food web of the northest subarctic Pacific. Deep Sea Research II 46:2909-2939

Vouvé F (2000) Régénération benthique dans les vasières intertidales de Marennes-Oléron: nature de la matière organique , son impact sur la production et l'incorporation de l'ammonium. PhD Thesis, University of Perpignan, 263 pp

Vranken G, Herman PMJ, Heip CHR (1988) Studies of the life history and energetics of marine and brackish-water nematodes I. Demography of *Monhystera disjuncta* at different temperature and feeding conditions Oecologia 77:296-301

Wainright SC (1987) Stimulation of heterotrophic microplankton production by resuspended marine sediment. Science 238:1710-1712

Walton WR, Sloan BJ (1990) The genus *Ammonia* Brünnich, 1772: its geographic distribution and morphologic variability. J Foraminifer Res 20:128-156

Warwick RM, Price JH (1979) Ecological and metabolic studies on freeliving nematodes from an estuarine mudflat. Est Coast Mar Sci 9:257-271

Watson PG, Novitsky TJ, Quinby HL, Valois FW (1977) Determination of bacterial number and biomass in the marine environment. Appl Environ Microbiol 33:940-946

Wieser W (1953) Die Beziehungen zwischen Mundhölengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen nematoden. Ein ökologisch-morphhlogische studie. Arkiv fur Zoologi 4:439-484

Wieser W (1960) Benthic studies in Buzzards Bay. II. The meiofauna. Limnol Oceanogr 5:121-137

Wollast R (1991) The coastal organic carbon cycles: fluxes, sources and sinks. In: Martin JM, Wollast R (eds) Ocean margin processes in global change, Wiley

Wright SH, Manahan DT (1989) Integumental nutrient uptake by aquatic organisms. Annu Rev Physiol 51:585-600

Yarmolinsky MB (1995) Programmed cell death in bacterial populations. Science 267:836-837

Zhukova NV, Kharlamenko VI (1999) Sources of essential fatty acids in the marine microbial loop. Aquat Microb Ecol 17:153-157

RESUME

Les sédiments des vasières intertidales, très riches en matière organique, présentent des abondances et des productions bactériennes très élevées. Par analogie avec le fonctionnement des systèmes pélagiques, il a été suggéré que les bactéries joueraient un rôle important dans les réseaux trophiques benthiques. Cependant, en raison des difficultés techniques, les études traitant de bactérivorie benthique sont peu nombreuses et produisent des résultats contradictoires. La mise au point d'une méthode utilisant des communautés bactériennes de culture enrichies en ^{15}N comme traceur a permis de mesurer la bactérivorie d'organismes de la méiofaune et de la macrofaune. Les bactéries enrichies présentent des caractéristiques de taille, d'activité et de diversité relativement proches de celles des bactéries naturelles limitant ainsi les biais liés à une éventuelle sélectivité d'ingestion des bactérivores. Les vasières constituent des milieux dont les conditions environnementales varient très rapidement en fonction du cycle de marée. Ces variations sont susceptibles d'affecter les comportements d'ingestion de bactéries. L'influence de facteurs abiotiques (température, salinité et lumière) et biotiques (abondances algales et bactériennes) sur la bactérivorie du gastéropode *Hydrobia ulvae*, du foraminifère *Ammonia tepida* et d'un peuplement de nématodes de la vasière de Brouage (Baie de Marennes Oléron) a donc été estimé. À l'exception de *A. tepida*, les bactérivores sont peu affectés par les variations de facteurs abiotiques suggérant une aptitude à s'alimenter dans l'interface hypervariable air/sédiment où se forme le biofilm algal durant les exondations diurnes. Les algues ont été ingérées à un taux systématiquement plus élevé que les bactéries indiquant une importance moindre des bactéries dans la nutrition de ces organismes. Pour mesurer les variations de l'ingestion de bactéries au cours d'un cycle annuel, des expériences de broutage ont été conduites régulièrement *in situ* sur la vasière de Brouage. Pour les nématodes et *A. tepida*, la bactérivorie est négativement corrélée aux abondances d'algues du milieu ce qui indiquerait que les bactéries constituent une ressource alternative consommée lors de pénurie d'algues. Le broutage des bactérivores étudiés ne représente jamais plus de 6% de la production bactérienne. En conséquence, le broutage apparaît comme un devenir limité de la production bactérienne dans la vasière de Brouage.

ABSTRACT

Bacteria are highly abundant and productive in intertidal mudflat sediments. By analogy with pelagic system, it has been suggested that bacteria play an important role in benthic food web. Due to technical difficulties, numbers of studies dealing with benthic bacterivory are not numerous and give conflicting results. A method using community of ^{15}N enriched bacteria as tracer was developed in order to measure grazing of meiofauna and macrofauna. Since enriched bacteria present characteristics of size, activity and diversity close to natural ones, bias due to selective bacterial ingestion by grazers are limited. Due to tidal cycles, intertidal mudflat constitutes highly variable environment at short time scale. Since those variations may influence the feeding behavior of grazers, grazing experiments were performed in order to evaluate effects of abiotic (temperature, salinity and luminosity) and biotic (bacterial and algal abundances) factors on rates of bacterial uptake. Studied grazers were the mudsnail *Hydrobia ulvae*, the foraminifera *Ammonia tepida* and a nematode community from Brouage mudflat (Marennes-Oléron Bay). Except *A. tepida*, grazers were poorly affected by abiotic factors. This result suggests that grazers are adapted to feed in the highly variable environment constituted by the air/sediment interface where algal biofilm appears during diurnal ebb. Algae were ingested at a higher rate than bacteria suggesting a lower importance of bacteria in grazer diets. In order to measure annual variations of bacterial ingestion, regular grazing experiments were performed *in situ* in Brouage mudflat. Nematode community and *A. tepida* presented a bacterivory negatively correlated with bacterial abundance suggesting that bacteria represent an alternative resource that is grazed when algal abundances are low. The grazing of studied grazer never represented more than 6% of bacterial production. As a result, grazing constitutes a limited fate of benthic bacteria in the Brouage mudflat.