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Facteurs de contrôle de l'activité et de la diversité du bactérioplancton dans des réservoirs de différents niveaux trophiques

Sébastien Delgoulet

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Soutenue le 21 Février 2007

Sébastien DELGOULET

**FACTEURS DE CONTROLE DE L'ACTIVITE ET DE LA
DIVERSITE DU BACTERIOPLANCTON DANS DES RESERVOIRS
DE DIFFERENTS NIVEAUX TROPHIQUES**

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INTRODUCTION

REVUE BIBLIOGRAPHIQUE

I. Introduction

Dans un écosystème, un des liens les plus importants qui unit les espèces est d'ordre alimentaire. Une vision classique et très simplifiée distingue trois catégories d'organismes :

- Les producteurs primaires comprenant essentiellement les végétaux chlorophylliens, capables, grâce à la photosynthèse, de fabriquer de la matière organique à partir de dioxyde de carbone en utilisant l'énergie lumineuse. Mais aussi des organismes chemoautotrophes capables d'utiliser l'énergie obtenue par oxydation de nutriments inorganiques.

- Les consommateurs ou producteurs secondaires (les animaux); il existe trois types de consommateurs :

- les herbivores qui se nourrissent des producteurs primaires, appelés aussi consommateurs primaires
- les carnivores primaires ou encore consommateurs secondaires qui se nourrissent des herbivores
- les carnivores secondaires appelés également consommateurs tertiaires qui se nourrissent des carnivores primaires

- Les décomposeurs (bactéries et champignons) qui dégradent les matières organiques de toutes les catégories et restituent au milieu les éléments minéraux.

Ces relations forment des séquences où chaque individu mange le précédent et est mangé par celui qui le suit, on parle alors de **chaîne alimentaire**. Chaque maillon forme un **niveau trophique**. La caractéristique principale des chaînes alimentaires est un transfert de matière et d'énergie avec recyclage continu.

A partir de cela, un **réseau trophique** peut être défini comme l'ensemble des interactions alimentaires entre les différentes chaînes alimentaires d'un écosystème.

Le réseau trophique pélagique concerne le réseau prenant place dans "la colonne d'eau". L'étude réalisée à la suite de cette revue s'intéresse plus particulièrement au milieu d'eau douce. Dans cet écosystème, le producteur primaire principal est le phytoplancton supportant l'ensemble du réseau trophique pélagique. Les consommateurs sont constitués de zooplancton herbivores consommant le

phytoplancton, ces herbivores étant consommés à leur tour par des planctonivores et des prédateurs. Ces derniers pouvant être piscivores ou consommateurs d'autres types d'animaux. Le régime omnivore est très commun dans le réseau trophique pélagique avec des organismes capables d'obtenir de la nourriture à partir de plusieurs niveaux trophiques (consommation de phytoplancton et de zooplancton).

Mais cette notion de réseau trophique pélagique a fortement évolué.

En effet, jusqu'à quelques décennies auparavant, seul le réseau décrit ci-dessus été considéré. Ce réseau est désormais appelé **chaîne trophique classique**.

Cette vision du réseau trophique pélagique a été remise en question durant les années 70 et 80 par des scientifiques comme Pomeroy (1974), Azam (1983) et Sherr (1984) qui ont montré une voix alternative au réseau classique : un flux de carbone passant par les bactéries vers des organismes eucaryotes unicellulaires (les protozoaires) puis des métazoaires. La matière organique dissoute (MOD) étant consommée comme substrat par les bactéries. Ce modèle de chaîne trophique est appelé **boucle microbienne**. Cette boucle vient se connecter au réseau trophique classique contribuant ainsi aux flux d'énergie et de carbone vers les niveaux trophiques supérieurs (figure 1).

Dans cette boucle microbienne, les bactéries planctoniques ou **bactérioplancton** sont les organismes les plus abondants (particulièrement dans les lacs d'eau douce) (Fenchel et al. 1998, Simon et al. 1998)

Ce bactérioplancton joue **un rôle essentiel** dans les systèmes lacustres (Gurung et Urabe 1999), d'une part par sa biomasse (Cho et Azam 1988, Simon et al. 1992) et d'autre part, par son action comme agent principal de recyclage des nutriments et de décomposition de la matière organique (Fischer et al. 2000, Sorokin 1999). Ces bactéries régulent donc une grande partie des flux de matières organiques et inorganiques par leur action de dégradation et d'assimilation. Elles constituent un réservoir majeur de carbone dans la zone euphotique. Cependant, ce nouveau réseau trophique apparaît avoir une structure différente entre un système oligotrophe et un milieu eutrophe (Dortch et Packard 1989, Gasol et al. 1997).

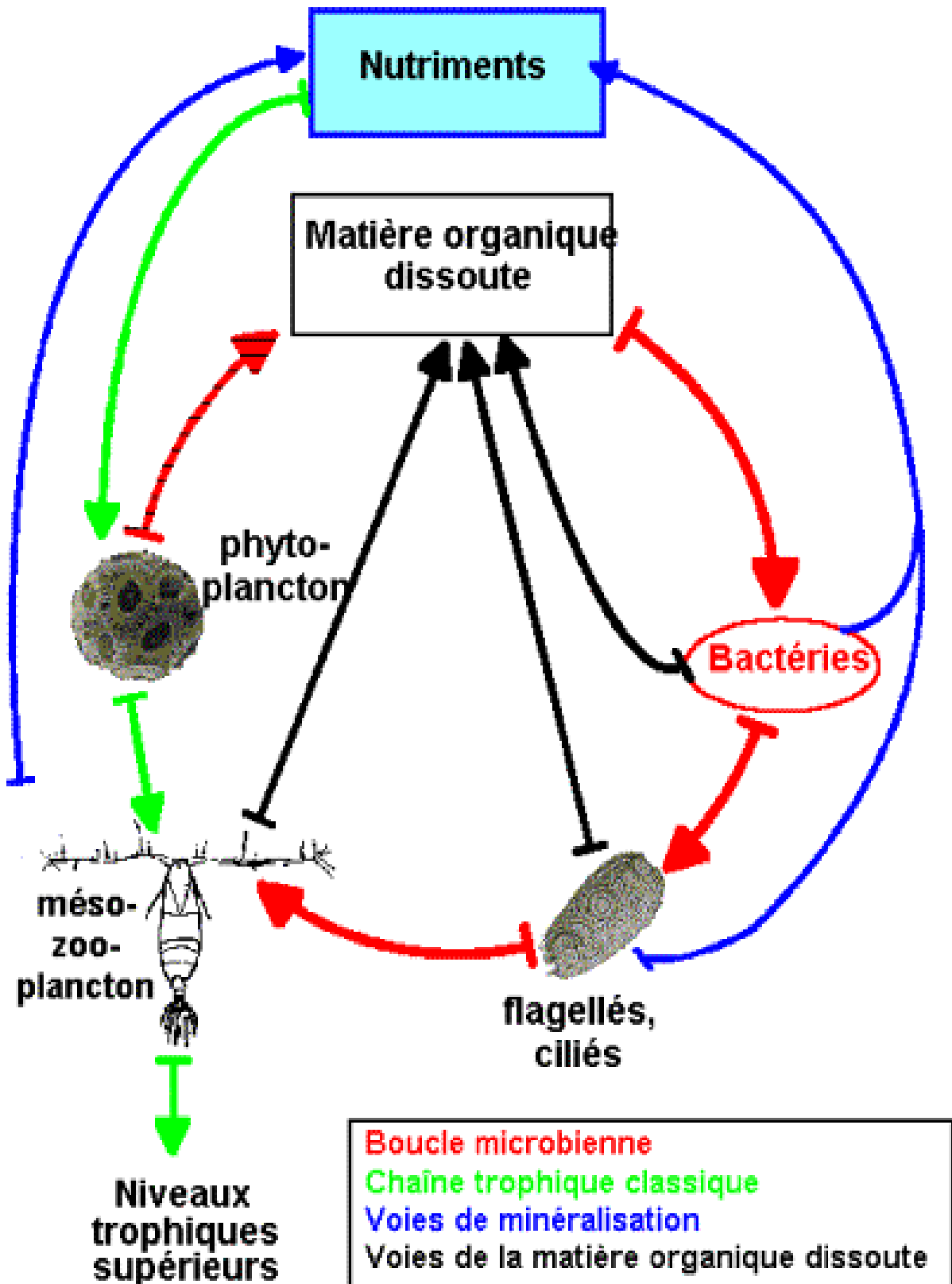


Figure1: Réseau trophique pélagique simplifié représentant la chaîne trophique classique et la boucle microbienne. (source : <http://www.com.univ-mrs.fr/IRD>)

Deux grandes régulations interviennent sur la structure et le fonctionnement des peuplements. Une régulation des populations est due aux **prédateurs** qui réduisent les effectifs de leurs proies (facteurs descendants ou effet *top-down*). Une autre correspond aux **ressources** disponibles (facteurs ascendants ou effet *bottom-up*) (Dajoz 2000). Les facteurs ascendants auront un impact au niveau de la croissance et de la reproduction, tandis que les facteurs descendants auront essentiellement des répercussions sur la mortalité des individus (Gliwicz 2002). Toutefois lorsque les éléments nutritifs sont en dessous du seuil compensant les besoins métaboliques il y a mortalité. Les facteurs ascendants ont donc aussi un impact sur la mortalité des organismes. **Ces régulations** ne sont pas incompatibles et leurs effets **peuvent être simultanés** : c'est le cas en milieu aquatique (Gasol et al. 2002b, Matz et Jürgens 2003, Selph et al. 2003). Par ailleurs, **l'importance relative de ces différents facteurs est susceptible de dépendre du niveau trophique de l'écosystème** : l'impact des ressources serait plus important en milieu oligotrophe et celui de la prédation dominant en milieu eutrophe (Sanders et al. 1992).

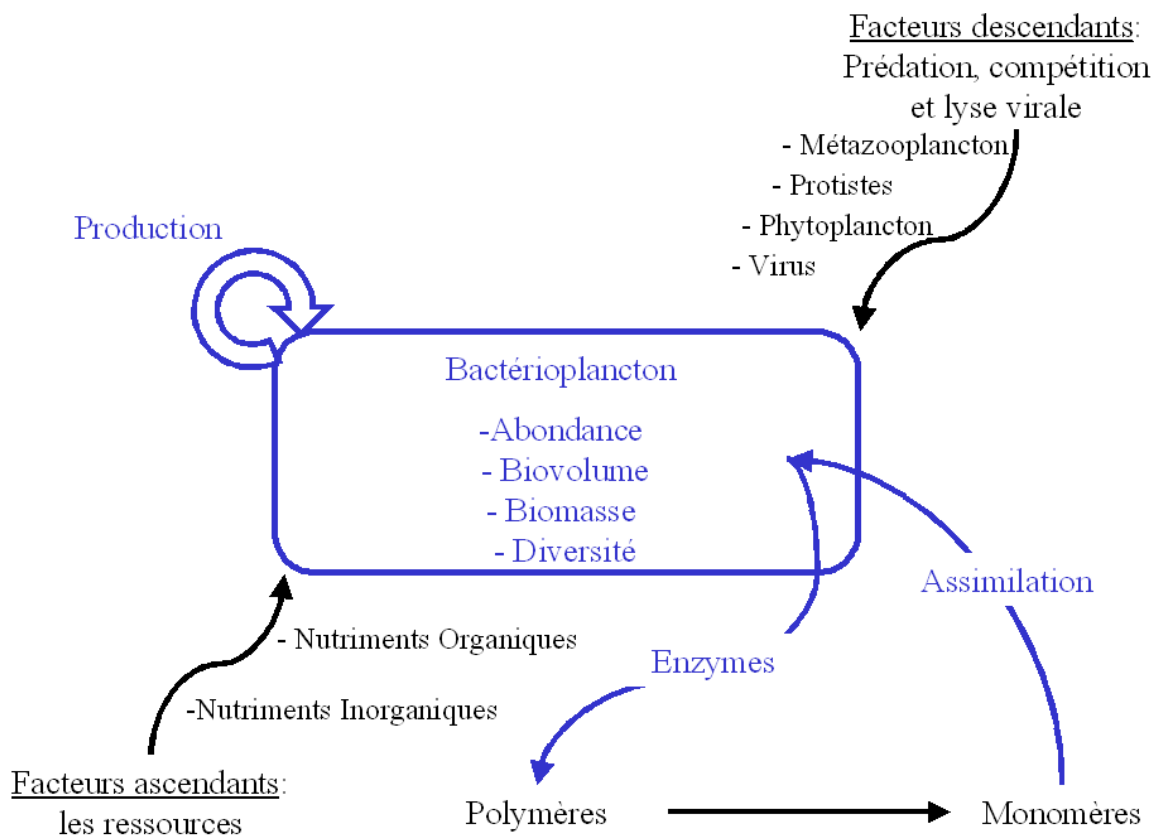
Naturellement, différents facteurs abiotiques comme la température, la teneur en oxygène, le pH, l'hydrologie, entre autres. (Pomeroy et Wiebe 2001, Martins et al. 2001, Muylaert et al. 2002) interviennent comme facteurs de régulation.

L'étude de ces facteurs et de leur importance relative encore trop peu explorées à ce jour, est donc de première importance pour la compréhension du fonctionnement des écosystèmes. Pour comprendre les mécanismes régulant le flux de matière dans les environnements aquatiques il donc est essentiel de clarifier les facteurs déterminants la communauté bactérienne en termes de biomasse et de structure ainsi que leur impact et leur importance relative.

Nous allons décrire dans les parties suivantes les principales variables associées au bactérioplancton et présenter l'état de l'art concernant les facteurs écologiques connus pour influencer les variables bactérioplanctoniques.

II. Dynamiques du bactérioplancton

- **Abondance**
 - **Taille cellulaire**
 - **Biomasse**
 - **Diversité**
 - **Activité enzymatique**
 - **Production**



II.1. Abondance

La première étape dans le processus d'étude des relations entre les bactéries et leur environnement est généralement la détermination des densités bactériennes. La méthode la plus commune pour compter les bactéries planctoniques est aujourd'hui **la microscopie à épifluorescence**, qui utilise des colorants fluorescents appelés fluorochromes (Hobbie et al. 1977, Porter et Feig 1980). Cette évolution technologique survenue dans les années 70 a permis une véritable révolution de l'écologie microbienne puisque seul un faible pourcentage des communautés naturelles peut être cultivé (Amann et al. 1995).

L'ensemble des études qui ont été possibles grâce au développement de cette nouvelle technique a permis de visualiser les communautés microbiennes naturelles et de révéler leurs dynamiques. Ces études ont donc montré que les populations bactériennes ont une distribution saisonnière hautement variable de lac à lac et d'année en année dans un même lac. Cependant, quelques généralisations peuvent être avancées grâce aux données de population déterminées avec une fréquence d'échantillonnage adéquate :

Ainsi, les bactéries apparaissent comme les organismes **les plus abondants dans les milieux aquatiques** (Fenchel et al. 1998, Simon et al. 1998). Le nombre et la biomasse des bactéries sont généralement élevés dans l'épilimnion, atteignent un minimum dans le métalimnion et l'hypolimnion supérieur, et augmentent dans l'hypolimnion inférieur, spécialement s'il y a anoxie. Dans certains systèmes oligotrophes, le nombre et la biomasse bactérienne peuvent être maximales dans le métalimnion (Coveney et Wetzel 1995, Simon et al. 1998).

Les biomasses bactériennes sont généralement plus basses durant l'hiver que durant l'été dans les réservoirs et les lacs tempérés. **Cette dynamique est corrélée avec les basses températures d'hiver** (Shiah et Ducklow 1994, Ochs et al. 1995a, Ochs et al. 1995b), la réduction de la charge particulaire et des substrats organiques dissous venant des sources autochtones (phytoplancton et plantes du littoral) et allochtones lorsque les sols sont gelés.

Statut trophique des eaux	Type de bassin	Densité		
		Nombre total	Biomasse	Production
Oligotrophe	Eaux océaniques profondes (> 800m)	0,01-0,02	1-2	0,02-0,06
	Eaux de l'Antarctique de l'Océan Pacifique	0,1-0,2	10-20	3-5
	Eaux océaniques tropicales	0,1-0,4	10-30	10-40
	Lacs	0,5-0,8	40-70	15-50
Mésotrophe	Eaux de l'Antarctique (été-automne)	1,0		
	Régions océaniques tempérées	1,0-2,0	100-300	50-200
	Mers internes, lagons, lacs d'eau douce	1,5-3,0	200-400	100-300
Eutrophe	Upwelling océanique côtier	2-5	400-2000	200-500
	Lacs	3-8	600-2000	300-800
	Côtes lagunaires, baies maritimes	5-10	2000-3000	500-1500
Hypereutrophe	Lagunes côtières polluées	10-40	2000-10000	2000-10000

Tableau 1 : Densité, biomasse et production des populations bactériennes dans différents systèmes aquatiques. Le nombre total est exprimé en 10^6 cellules.mL⁻¹ ; la biomasse et la production sont exprimées en mgC.m⁻³ (d'après Sorokin, 1999)

Enfin, **la densité dépend du niveau trophique** étudié. Plusieurs auteurs (Azam et al. 1983, Fuhrman et al. 1980, Bird et Kalff 1984, Cho et Azam 1990, Gasol et Vaquer 1993, Pace et Coel 1994) ont montré une croissance de la concentration en bactéries avec l'augmentation du niveau trophique. Le tableau 1 montre les densités moyennes des bactéries planctoniques dans différents systèmes aquatiques.

II.2. Taille cellulaire/Biomasse

L'épifluorescence a non seulement permis d'étudier les densités d'organismes trop petits pour être visibles à l'œil nu mais aussi de **déterminer leur forme** (bacille ou coque) et leurs **dimensions**. Bratbak (1985) a ainsi démontré que par un simple calcul géométrique, considérant les coques telles une sphère et les bacilles tels un cylindre avec des demi-sphères à leurs extrémités, il était possible d'obtenir la taille cellulaire et donc suivre l'évolution de ce volume cellulaire au cours du temps ainsi que la biomasse. En effet, si certains auteurs ont proposé des conversions directes entre la cellule et son contenu en carbone, il est apparu plus intéressant d'avoir une conversion qui prendrait en compte le volume cellulaire. Ainsi Posh et al. (2001) ont comparé différentes formules (relativement proches) dépendantes du fluorochrome employé, l'une des formules apparaissant la plus adéquate est la formule de Simon et Azam (1989), recalculée par Norland (1993) qui donne : contenu en C = **120 x Volume cellulaire^{0.86}**. Le volume est exprimé en μm^3 et le contenu en C est exprimé en fg C.

L'étude de la modification de la taille cellulaire bactérienne apporte d'importantes informations sur les dynamiques bactériennes et leurs réactions à l'environnement. Ainsi une **réduction de taille permet d'acquérir des nutriments plus efficacement** (Cotner et Biddanda 2002) et **une augmentation du volume permet d'échapper aux petits prédateurs** (Jürgens et al. 1994). De plus, la réduction de taille peut aussi permettre d'échapper à la prédation par les bactérivores de plus grande taille tels que des ciliés ou des organismes zooplanctoniques du genre *Daphnia* (Degans et al. 2002, Zollner et al. 2003) qui sont capables de couvrir une large gamme de taille de proies. Enfin une réduction de taille a aussi été observée en présence de nanoflagellés hétérotrophes (Langenheder et Jurgens 2001).

II.3. *Activité*

Rapidement ces paramètres seuls sont apparus insuffisants pour comprendre et étudier le rôle du bactérioplancton dans les systèmes aquatiques. En effet, une difficulté inhérente à l'énumération par microscope est **la discrimination des cellules viables et des cellules mortes**. L'utilisation de l'iodure de propidium, un fluorochrome ne pénétrant que les cellules ayant perdu leur intégrité membranaire, permet de discriminer les cellules mortes. De plus, dans cette fraction viable seule **une part des bactéries aquatiques est métaboliquement active**, le reste de la fraction étant en dormance (Jugnia et al. 2000, Amann et al. 1995, del Gioglio et al. 1997, Gasol et al. 1995, Lebaron et al. 2001). La densité totale et la biomasse reflètent donc une partie de la dynamique picoplanctonique (organismes planctoniques inférieurs à 2 μm) mais ne représentent en rien l'activité du bactérioplancton et donc l'impact du bactérioplancton sur l'écosystème. Pour mesurer cette activité, il s'avère ainsi nécessaire d'utiliser d'autres méthodes.

Ainsi, le 5-cyano-2,3-di-4-tolyl-tetrazolium chloride (**CTC**) est utilisé pour détecter les cellules bactériennes ayant une activité métabolique. Il agit tel un accepteur d'électron dans le système de chaînes de transfert d'électrons des membranes bactériennes (Smith et Mc Feters 1997). En effet, il se trouve réduit par ces derniers, précipite et devient fluorescent (Créach et al. 2003). Il peut donc être utilisé pour faire des mesures directes de l'abondance des cellules bactériennes engagées dans la respiration (Smith 1998). Par filtration puis observations sous lumière bleue, les échantillons présentent des précipités fluoresçant en rouge (Gasol et al. 1995). Les précipités comptés sont alors comparés à la concentration totale du picoplancton.

Cette **fraction "active" du picoplancton reste assez faible**, elle est généralement inférieure à **20% de la densité totale** (Jugnia et al. 2000, Berman et al. 2001, Haglund et al. 2002). Les pourcentages maxima obtenus montrent parfois une corrélation entre la température et le pourcentage de bactéries actives. De plus, ces courbes de cellules "actives" sont parallèles aux densités totales (Haglund et al. 2002) mais il ne semble pas y avoir de modèle saisonnier clair (Berman et al. 2001). Ceci peut s'expliquer par le fait que toutes les souches bactériennes ne sont pas capables de réduire le CTC (Smith 1997) et que celui-ci peut être toxique pour les bactéries (Ullrich et al. 1996).

L'utilisation du CTC est donc très controversée : certains auteurs promeuvent l'utilisation de ce réactif (del Giorgio et al. 1997) alors que d'autres mettent en avant les différents inconvénients de la méthode (Servais et al. 2001). En effet, la méthode n'est que peu standardisée. De même, les différentes procédures d'utilisation peuvent influencer le comptage des cellules réagissant au CTC. En contre partie l'utilisation du CTC reste relativement simple. Cette fraction active du picoplancton est donc relativement faible, mais serait responsable des activités microbiennes aquatiques.

Il existe une autre méthode développée par Lebaron et al. (2001) qui consiste à déterminer le contenu cellulaire en acides nucléiques. Ainsi, par l'utilisation de fluorochrome il est possible de discriminer des cellules en haute teneur en acides nucléiques (cellules fortement actives) de cellules à faible teneur en acides nucléiques (cellules non actives). Bien que cette méthode ne permette pas de différencier les intensités d'activités, elle permet de mettre en évidence la quantité de cellules responsable de la production bactérienne. A l'inverse du CTC, cette méthode montre que 50 à 80% des cellules totales sont impliqués dans la production bactérienne.

II.4. Activités enzymatiques

Entre 80 et 90 % de la matière organique des environnements aquatiques est composée de polymères de haut poids moléculaire (Münster et Chrost, 1990). Seules les molécules de faible poids moléculaire (molécules simples < 600 Da) peuvent être transférées de l'environnement vers l'intérieur de la cellule bactérienne (Rogers 1961) et ceci aussi vite qu'elles sont produites (Sondergraad et Middleboe 1995). Ainsi seule une faible fraction de la totalité de la matière organique dissoute est assimilable par les bactéries (Azam et Cho 1987). Malgré cela, les bactéries aquatiques présentent une biomasse et une production élevées. D'où la **capacité des bactéries hétérotrophiques à utiliser des polymères de haut poids moléculaire** (Hoppe 1983, 1991).

Pour cela **elles produisent des enzymes capables de dépolymériser et d'hydrolyser ces molécules** (Ammerman 1991, Billen 1991, Chròst 1991).

Ce processus a deux conséquences importantes :

- 1) les bactéries décomposent la biomasse organique et recyclent les éléments clefs (carbone, azote, phosphore, soufre...).
- 2) à travers la boucle microbienne, le pool de carbone organique dissous et particulaire mort devient disponible pour les organismes de niveaux supérieurs de la chaîne trophique (Azam et al. 1983).

Le bactérioplancton peut donc être non seulement considéré comme **l'organisme reminéralisateur** des composés organiques mais aussi un **intermédiaire indispensable** pour transmettre la matière organique vers les niveaux supérieurs de la chaîne trophique.

Du fait de leur intervention en amont dans les processus de dégradation, **l'activité des enzymes est**, dans la plupart des cas, **le facteur limitant** la décomposition du matériel organique et la croissance bactérienne (Chròst 1991). Il est donc important quand on étudie le devenir des productions biologiques, notamment à travers la dynamique du réseau microbien, de bien définir ce paramètre d'hydrolyse bactérienne (Riemann et al. 2000). Un certain nombre d'approches ont été utilisées pour mesurer les activités des enzymes extracellulaires dans les systèmes aquatiques. Les méthodes appliquées les plus communes impliquent l'apport d'un substrat traceur, typiquement constitué de monosaccharides ou d'un acide aminé, lié à un fluorophore. Sous l'hydrolyse de la liaison entre le monomère et le fluorophore, ce dernier devient fluorescent, et l'hydrolyse est mesurée par le signal fluorescent rapporté au temps (Hoppe 1983, Somville and Billen 1983). Les substrats fluorophores fréquemment utilisés incluent, entre autres, la methylumbelliferyl (**MUF**). Cette méthode est rapide et suffisamment sensible pour des milieux naturels (Somville et Billen 1983).

Les enzymes impliquées dans la transformation et la dégradation des substrats polymériques sont situés à l'extérieur de la membrane cellulaire et sont qualifiés **d'enzymes "extracellulaires"** ou ectoenzymes/exoenzymes (Priest 1984), en référence aux enzymes produites qui traversent la membrane cytoplasmique mais restent associées aux producteurs. Ces enzymes sont soit proches de la surface de la cellule, soit situés dans l'espace périplasmique des cellules bactériennes et réagissent avec les polymères du milieu environnant. La plupart des enzymes extracellulaires sont des hydrolases. Des enzymes peuvent être aussi présentes sous forme libre, dissoutes dans l'eau (Somville et Bilen 1983) ou absorbées sur d'autres surfaces (Wetzel 1991) que celles de leurs

producteurs (particules détritiques...). D'autres sources d'enzymes non bactériennes existent, venant de champignons (Unanue et al. 1993) ou du plancton eucaryote (Chròst 1990).

L'ensemble des **ces enzymes peut être activement sécrété** par des cellules viables mais aussi libéré dans l'environnement après un dommage cellulaire ou une lyse, ou bien encore le résultat du sloppy feeding de prédateurs sur des organismes unicellulaires.

D'autre part, comme suggéré par Boschker et Cappenberg (1998), le suivi de l'activité enzymatique permet de suivre l'évolution des différents apports en matière organique dans le milieu aquatique. En effet, les lacs reçoivent du carbone organique de sources très diverses qui n'apporte pas la même contribution à la chaîne trophique microbienne. Les réactions enzymatiques sont très diverses par rapport à cet apport. De plus, le bactérioplancton est capable de produire rapidement des enzymes (dans les 12h) pour réagir à l'apport de matière organique.

II.5. Production

Les milieux dystrophiques mis à part, le bactérioplancton vit dans des milieux environnementaux relativement peu favorables à une croissance rapide. **La croissance naturelle des bactéries est à des taux plus bas que leur capacité** car elle est limitée par la disponibilité des nutriments aussi bien que par les facteurs physico-chimiques.

La production bactérienne exprime la vitesse de formation de la biomasse bactérienne. En effet, le simple suivi des densités picoplanctoniques ne permet pas d'estimer les pertes causées par le grazing et la lyse. Pour observer une diminution de la densité, une très forte prédation sur le picoplancton est nécessaire. Il est alors possible de voir des dynamiques de croissances et de mortalités au cours du temps.

En pratique, la production bactérienne est normalement déterminée par la prise de précurseur radioactif, tels que la thymidine ou la leucine (Fuhrman et Azam 1980, Kirchman et al. 1985) marqués au tritium ou au carbone 14. Mais l'approche la plus prometteuse est **l'incorporation de leucine dans les protéines bactériennes** (Kirchman et al. 1985, Simon et Azam 1989). En effet, cette technique permet une

mesure directe de la production de carbone bactérien (Kaplan et al. 1992, Fuhrman et Azam 1980) en suivant la production de protéines. De plus, elle a un ordre de grandeur plus sensible car les cellules bactériennes absorbent 10 fois plus de leucine que de thymidine (Simon et Azam 1989). L'incorporation de leucine dans les bactéries a été intensivement testée dans les systèmes pélagiques (Jorgensen 1992, Kirchman et al. 1985, Riemann et Azam 1992, Simon et Azam 1989) et est désormais communément utilisée pour la mesure de production bactérienne dans ces milieux (Kirchman 1993).

L'estimation de la production bactérioplanctonique fournit une opportunité **d'évaluer le taux de croissance hypothétique** de la population indépendamment des facteurs de contrôle de la croissance tels que le grazing, le parasitisme et la sédimentation. **La production et la croissance de population sont donc deux concepts fortement différents.**

Généralement **la production diminue avec l'éloignement du bord et la profondeur**. Dans certains cas, elle peut être plus élevée durant la nuit (Scavia et Laird 1987). D'autre part, la moyenne annuelle de production bactérienne augmente avec le statut trophique des lacs et montre une corrélation directe avec la production du phytoplancton (Cole et al. 1988). Cependant, elle **montre des variations saisonnières différentes suivant le statut trophique**. Ainsi dans les milieux oligotrophes, la production bactérienne montre des variations saisonnières qui sont très proches de celles de la production phytoplanctonique, c'est-à-dire que les plus fortes valeurs sont trouvées durant l'été et l'automne et les plus basses durant l'hiver et le début du printemps (Simon et al. 1998). La production bactérienne augmente plus rapidement que la production primaire phytoplanctonique avec l'augmentation de la température (Coveney et Wetzel 1992). A l'inverse dans les milieux eutrophes, la production bactérioplanctonique décroît durant l'automne, diminution qui peut être accentuée par un bloom de cyanobactéries (Kisand et Tammert 2000).

II.6. La Diversité

Les communautés bactériennes ont été considérées pendant très longtemps comme une véritable « boîte noire » d'un point de vue taxonomique. Grâce à l'avènement des techniques de biologie moléculaire, la diversité bactérienne a pu être étudiée.

En **ciblant l'ARN ribosomal** soit directement, soit indirectement (gène codant pour cette molécule) il est possible de réaliser une étude phylogénétique (Woese 1987) de la communauté procaryotique. Sa structure est en grande partie conservée, tout en présentant des zones variables entre différents taxons.

L'ensemble des études rendues possibles grâce au développement de ces nouvelles techniques a permis d'explorer les communautés microbiennes naturelles et leur diversité. Ainsi, en milieu lacustre, le groupe phylogénétique dominant semble être celui des **bêta-protéobactéries**, puis celui **des alpha-protéobactéries** ou du groupe des *Cytophaga-Flavobacterium* (Tab. 2). D'autres études utilisant des techniques de clonage et séquençage, tendraient aussi à montrer que les *Actinobacteria* représenteraient une forte proportion du bactérioplancton. D'autre part, il apparaît que de nombreuses espèces actuellement non cultivées ont été détectées (Giovanni et Rappé, 2000) et que, si la diversité est élevée, seulement certains taxons dominent le bactérioplancton (Konopka et al. 1999 ; Casamayor et al. 2000, Pinhassi et Hagström, 2000, Schauer et al. 2000). Les résultats de Lindström et Leskinen (2002) indiquent que **quelques taxa de procaryotes sont présents dans les lacs, indépendamment de la période de l'année, de la profondeur et du niveau trophique du lac**. Par ailleurs, l'étude de Yannarell et al. (2003) dans des lacs de niveaux trophiques différents suggère l'existence **d'assemblages d'organismes indigènes à des lacs particuliers ou à des lacs de niveau trophique similaire**. Cependant, il est probable que ces organismes dont l'existence a été étudiée dans des travaux précédents (Zwart et al. 1998, Glöckner et al. 2000, Lindström et Leskinen 2002), présentent une distribution étendue et soient communs aux systèmes d'eau douce. A ces différences ou similitudes entre écosystèmes, viennent s'ajouter, pour un site donné, de fortes variations saisonnières (Zwisler et al. 2003) et/ou spatiales (Pernthaler et al. 1998, Höfle et al. 1999) dont les causes restent encore à déterminer.

Niveau trophique de référence	Auteurs	% EUB	%ARCH	%ALF	%BET	%GAM	%CF	%HGC
Oligotrophe	Pernthaler et al., 1998	55	1.4	21	24	<2	4	ND
Mésotrophe	Glöckner et al., 1999	46 (±9)	ND	0 (±3)	21 (±4)	0 (±3)	3 (±5)	ND
Mésotrophe	Glöckner et al., 2000	69	ND	ND	6	ND	1	25
Mésotrophe	Zwisler et al., 2003	40.3 (±11.4)	ND	4.3 (±4.6)	12.8 (±2)	2.6 (±1.9)	5.7 (±3.3)	ND

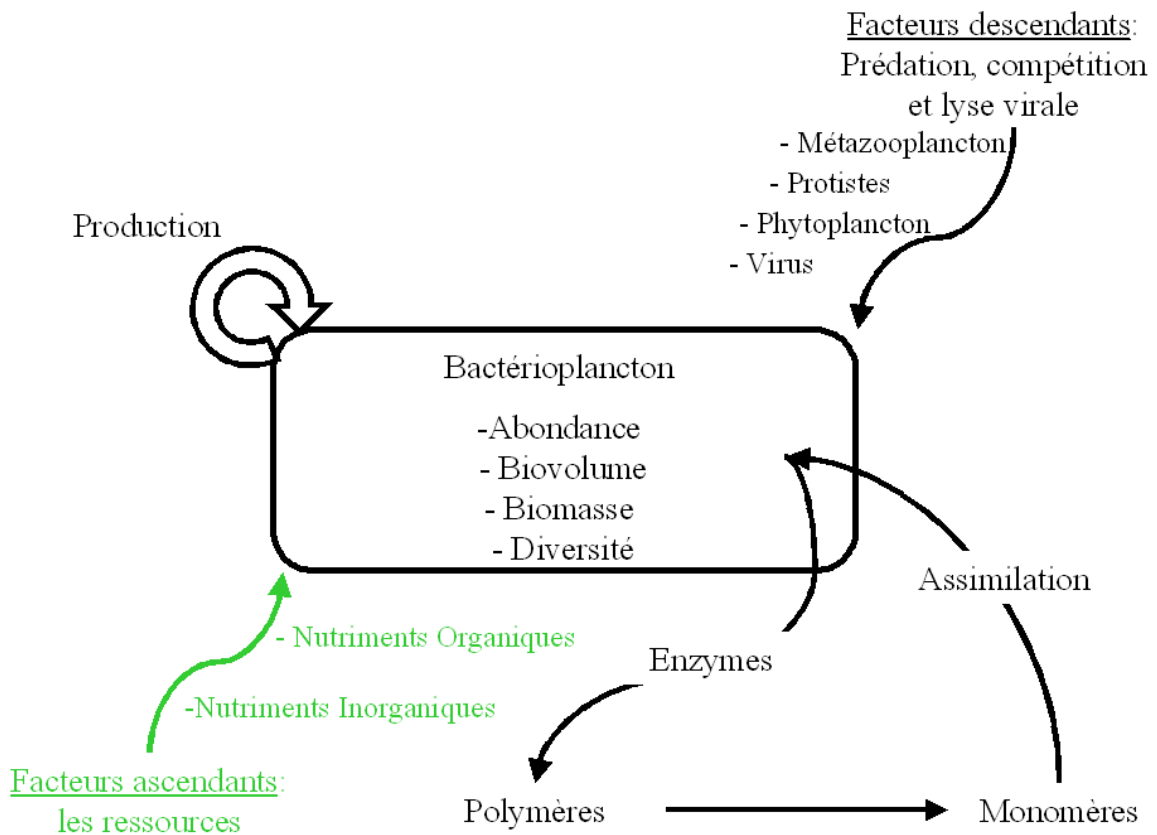
Tableau 2 : Importance relative des eubactéries, des Archaea et de différents groupes et sous-classes bactériens (BET : bêta-protéobactéries, ALF : alfa-protéobactéries, GAM : gamma-protéobactéries, CF : *Cytophaga Flavobacterium*, HGC : Actinobacteria), exprimée par rapport à l'abondance totale dans différents systèmes aquatiques (ND : non déterminée). D'après Jardillier, 2004.

III. Régulations des dynamiques bactériennes

III.1. Facteurs ascendants ou Contrôle “Bottom-up”

Les ressources organiques : Matière Organique Dissoute

Les ressources Minérales : Phosphore et Azote



La limitation de la croissance du bactérioplancton par les nutriments inorganiques et le carbone organique a été observée tant dans les milieux lacustres que marins (Morris et Lewis 1992, Elser et al. 1995, Vrede 1996, Caron et al. 2000). **La croissance bactérienne a été montrée comme limitée par les nutriments inorganiques et le C organique, seuls ou combinés**, dans les lacs oligotrophes (Chrzanowski et al 1995 ; Vrede 1996), mésotrophes (Schweitzer et Simon 1995, Vrede et al. 1999) et eutrophes (Wang et al. 1992, Felip et al. 1996). Le contrôle de l'abondance par les éléments minéraux est un phénomène courant dans les écosystèmes limniques. Ces ressources minérales peuvent, par ailleurs, faire **l'objet d'une compétition entre le picophytoplancton et les bactéries hétérotrophes**, le bactérioplancton semblant plus compétitif dans ce domaine (Drakare et al. 2002).

Durant les décennies passées, **l'importance du phosphore comme facteur de régulation a été montrée** (Jansson et al. 1996, Toolan et al. 1991). Sur ce sujet ont d'abord été proposés deux modèles décrivant la relation entre l'abondance du phytoplancton et du bactérioplancton : soit le phosphore total détermine l'abondance algale qui en retour détermine l'abondance bactérienne, soit algues et bactéries entrent en compétition pour le phosphore (Currie 1990). Les différentes études (Currie et Kalff, 1984, Vadstein et al. 1988, Toolan et al. 1991, Coveney et Wetzel 1992) qui ont examiné les cinétiques d'absorption du phosphore et les taux de croissances spécifiques du bactérioplancton en comparaison avec ceux du phytoplancton indiquent ces points primordiaux :

1. Le bactérioplancton a des besoins en phosphore plus élevés que le phytoplancton ;
2. Sa consommation en phosphore est plus large (4 à 10 fois plus) ;
3. Le contenu bactérien en quantité de phosphore est supérieur (10x)
4. Le phosphore peut limiter le bactérioplancton ;
5. Le bactérioplancton peut entrer en compétition avec le phytoplancton et inversement.

Les substrats organiques pour les bactéries pélagiques sont dérivés du carbone organique dissous (COD) de la colonne d'eau. Le COD est une mixture hétérogène de

molécules dont certaines sont importées du bassin versant (COD allochtone) et d'autres sont produites par les autotrophes du système (COD autochtone). **Les exudats produits par le phytoplancton apparaissent importants pour le bactérioplancton** (Cole et al. 1982, 1988 ; Baines et Pace 1991, White et al. 1991). Des études en laboratoires ont montrées que **les bactéries pouvaient rapidement utiliser le carbone organique d'origine algale** (Chen et Wangersky 1996). Kritzberg et al. (2004), grâce à des expériences sur des lacs entiers (dans le Wisconsin), trouvent que le COD autochtone reste préférentiellement utilisé par rapport à l'allochtone. Cependant l'autochtone ne semble pas capable de supporter seul la croissance bactérienne. En effet, les biomasses bactériennes sont constituées de 35 à 70% de carbone allochtone.

Toutefois, les **écosystèmes lacustres peuvent différer en COD**, puisqu'il peut être en grande partie d'origine terrestre, provenant du bassin versant. Aussi, dans de tels systèmes, **la production bactérienne peut être plus ou moins indépendante de la production phytoplanctonique de COD** (Jansson et al., 1999, Richardot et al. 1999), **augmentant l'importance de compétition pour les éléments inorganiques**. Par ailleurs, d'autres processus peuvent générer du carbone utilisable par les bactéries, comme le recyclage et la production de carbone lors de l'excrétion ou de l'égestion par les prédateurs (Pace et Cole 1994). Ainsi, Olsen et al. (1986) ont montré qu'en eau douce, **30% du carbone excrété par les cladocères du genre *Daphnia* était directement disponible sous forme de COD, qui était rapidement incorporé par les bactéries hétérotrophes** (Richardot et al. 2000). Plus récemment Vadstein et al. (2003) ont confirmé que **les organismes brouteurs étaient la principale source de COD**, source plus importante que les excréments algales. De la même façon, Drakare et al. (2002) ont montré que le carbone excrété par le picophytoplancton était d'importance mineure pour le développement des bactéries hétérotrophes.

En outre, différentes stratégies permettent la survie des cellules dans des conditions de faible disponibilité en nutriments. L'une est de réduire les besoins de la cellule pour un nutriment donné par une croissance plus lente ; une autre est **d'acquérir plus efficacement les nutriments** que les autres organismes en **limitant la taille cellulaire** ou en réduisant la quantité relative d'un nutriment donné dans la cellule (Cotner et Biddanda 2002).

Cependant **l'incorporation de nutriments à l'intérieur de la cellule bactérienne** ne peut être exécutée que si ces éléments sont sous formes de monomères, d'où le **besoin bactérien de produire des enzymes**.

Le contrôle moléculaire des synthèses enzymatique bactériennes est assuré par la matière organique dissoute (MOD) et la matière organique dissoute utilisable (MODU) (Chróst 1991a). Toutefois, l'induction des enzymes est assez complexe. Pour que les enzymes soient sécrétées aux taux appropriés, les microorganismes doivent être capables de suivre l'activité enzymatique se déroulant hors de la cellule. En effet, la plupart des substrats sont des composés polymères trop larges pour pénétrer dans la cellule et induire une synthèse. Ce problème peut être évité par une faible sécrétion d'enzymes. Si le substrat est présent et que les composés de faibles poids moléculaires produits atteignent un certain niveau, ils pénètrent dans la cellule, et servent d'inducteur (Priest 1984). Une répression s'effectue lorsqu'une certaine concentration de monomères est atteinte. Chróst (1991b) propose un modèle conceptuel pour les interactions entre enzymes microbiens et la MOD, représenté en figure 2.

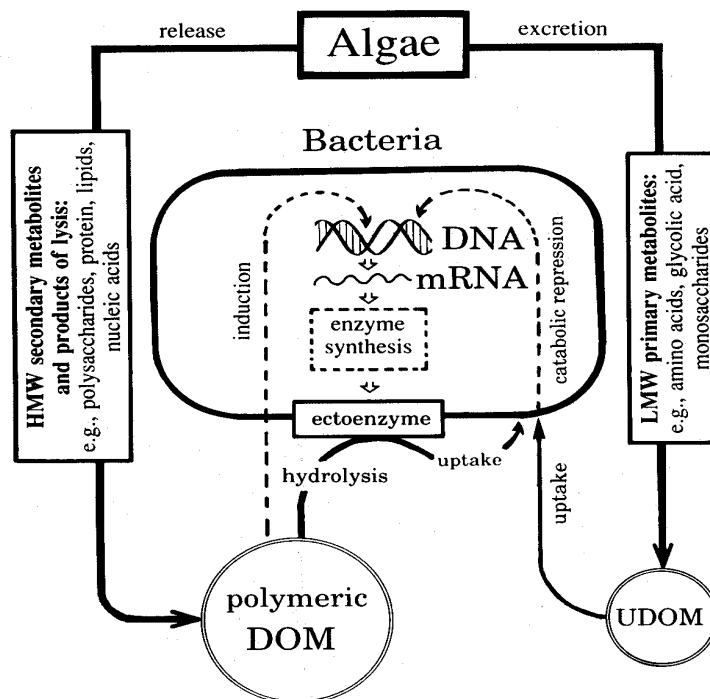


Figure 2 : Modèle conceptuel des mécanismes de régulation des ectoenzymes pour les interactions entre les bactéries et les substrats utilisables et polymériques. LMW=low-molecular-weight ; HMW=high-molecular-weight (modifié de Chróst, 1989, 1990).

Durant la croissance active du phytoplancton, les populations algales excrètent dans l'eau une variété de **métabolites primaires de faible poids moléculaire** et des produits issus de la photosynthèse, incluant des substances rapidement assimilables (Chrøst et Faust 1983, Fogg 1983), qui **supportent la croissance** et le métabolisme bactériens (Chrøst 1986). Ces composés de MODU (monosaccharides, acides aminés, acides organiques, esters simples, etc. ; Münster et Chrøst 1990) **inhibent l'activité** et répréssent **la synthèse d'exo-ectoenzymes** dans les bactéries hétérotrophiques. Cependant, les algues en sénescence relarguent des métabolites secondaires de haut poids moléculaires (Fogg 1966) et/ou libèrent, à travers l'autolyse ou à cause de l'activité de lyse virale (Suttle et al. 1990), une grande quantité de MOD polymérique, telle que les polysaccharides, les protéines, les lipides, des acides nucléiques. **La MOD et une grande quantité de polymères** (substrats pour exo-ectoenzymes) dans l'eau en même temps, **enlèvent la répression et induit la synthèse** d'exoenzymes par les bactéries aquatiques. Ainsi des enzymes impliquées dans la dégradation de polymères de glucose telles que l'alpha-glucosidase et la bêta-glucosidase réagissent aux changements à court terme de l'activité photosynthétique et du relarguage cellulaire de COD (Kamer et Rassoulzadegan 1995). Et les activations des carbohydrolases telles l'alpha-glucosidase, la bêta-glucosidase et la N-acétyl-glucosaminidase sont stimulées par les débris de macrophytes. Cependant la MOD d'origine terrestre peut supprimer l'activité des alpha-glucosidases et bêta-glucosidases (Wehr 1999).

Le taux d'activité de la bêta-glucosidase montre une réponse positive à l'apport de glucose et de COD dérivant du phytoplancton (Chrøst 1989, 1991b). La dégradation partielle des feuilles peut fournir suffisamment de COD et de nutriments inorganiques pour l'utilisation microbienne durant l'automne et l'hiver lorsque les sources d'origine algale et macrophytes se réduisent (Wehr 1999).

De plus **l'absorption et l'activité enzymatique ont des dynamiques réciproques qui peuvent être une indication de régulations simultanées**. Ainsi les enzymes bactériennes peuvent avoir une forte activité mais avec une faible affinité pour le substrat ou une faible activité mais avec une forte affinité. D'autre part, si l'hydrolyse et l'absorption sont fortement couplées les composés de faible poids moléculaire vont être absorbés. Si l'absorption est supérieur à l'hydrolyse alors la concentration en

MODU pourra limiter la croissance. Enfin si l'hydrolyse est plus rapide que l'absorption, les composés de faible poids moléculaire vont s'accumuler et éventuellement augmenter le flux de COD perdu par sédimentation.

Bien que les bactéries utilisent préférentiellement le carbone disponible (comme le carbone dissous) plutôt que celui produit par l'hydrolyse des polymères (Kisand et Tammert 2000), **elles répondent aux changements de nutriments par la régulation de la synthèse et l'activité de leurs enzymes** (Gotschalk 1985). D'où une **modification d'activité avec le statut trophique**.

Une étude conduite sur 13 lacs a révélé une **similarité de la composition des communautés bactériennes pour les systèmes présentant une productivité analogue** mais également **une relation positive entre la diversité bactérienne et la productivité** des lacs (Yannarell et Triplett 2004). De plus, Casamayor et al. (2000) ont observé que les communautés microbiennes étaient dépendantes de gradients verticaux et temporels des conditions physico-chimiques du milieu. Plus généralement, des études conduites en milieux marin et d'eau douce ont montré que **la disponibilité des ressources affectait la composition de la communauté bactérienne** (Höfle 1992, Donner et al. 1996, Pinhassi et al. 1999), et ce à une échelle de temps relativement courte, de l'ordre de la journée (Höfle 1999). De la même manière, les ressources pourraient aussi constituer un facteur de contrôle de la composition de la communauté des *Archaea*. En outre, les paramètres environnementaux tels que la profondeur, la salinité, l'oxygène et les macronutriments tendent à expliquer les différences de répartition entre les bactéries et les *Archaea* qui occuperaient ainsi des biotopes différents (Wells et Deming 2003).

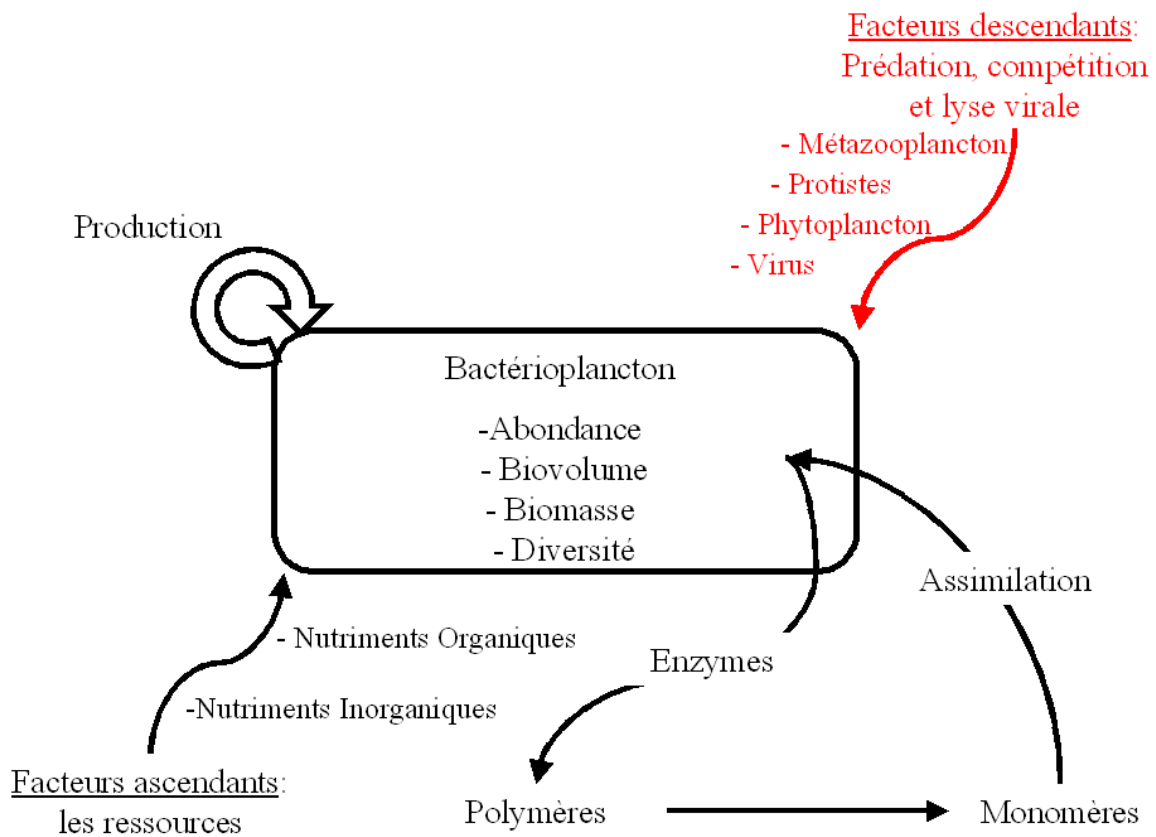
III. Régulations des dynamiques bactériennes

III.2. Facteurs descendants ou Contrôle “Top-down”

➤ **La prédation**

- Les protozoaires
- Le métazooplancton

➤ **La lyse virale**



a) Les protozoaires

Les facteurs de mortalité des bactéries par prédation jouent naturellement un rôle important dans le contrôle de la communauté bactérienne. Les organismes prédateurs de bactéries, comme **les nanoflagellés hétérotrophes et les ciliés, sont généralement considérés comme les organismes bactérovores majeurs** (Sanders et al. 1989, Simek et al. 1990, Berninger 1991, Kisand et Zingel, 2000). L'impact des protistes flagellés sur la communauté bactérienne a pu être mise en évidence en laboratoire (Simek et al. 1997) et *in situ* (Jürgens et al. 1999, Simek et al. 1999, Suzuki 1999, Langenheder et Jürgens 2001, Gasol et al. 2002a).

La plupart des protozoaires sélectionnent activement leur nourriture, selon la taille, la morphologie ou la nature biochimique de leurs proies (Gaul et Antia 2001). Outre la concentration des proies dans le milieu, **leur taille est un des facteurs déterminants de l'ingestion par les prédateurs**. Des travaux ont montré que les nanoflagellés ingèrent préférentiellement les bactéries de taille comprise entre 1,1 et 2,4 μm (Tuomi et Kuupo 1999, Jürgens et al. 1999, Hahn et Höfle 2001). Parmi les ciliés, certains sont capables de consommer une large gamme de taille de nourriture, allant de moins 2 μm à 4 μm , incluant ainsi le bactérioplancton, les nanoflagellés hétérotrophes et les algues (Simek et al. 2000). D'autre part, **la pression de prédation des nanoflagellés hétérotrophes peut amener des changements morphologiques**. Ainsi, une augmentation de la taille cellulaire bactérienne permet de lutter contre cette prédation (Wetzel 2001). De plus, en réponse à la pression de prédation de protistes flagellés, certaines bactéries peuvent présenter des formes filamenteuses (Jürgens et al. 1994).

Dans les eaux eutrophes où les populations de nanoflagellés hétérotrophes peuvent atteindre 10^5 cellules.mL⁻¹, le taux maximal de grazing pourrait supprimer 10^7 bactéries.mL⁻¹.h⁻¹. Ceci est le taux potentiel pour une suppression massive de bactéries par les nanoflagellés hétérotrophes dans la colonne d'eau de ces systèmes aquatiques (Sigeo 2005). Suivant la saison et la profondeur, **les ciliés peuvent être responsables de 30% de l'action bactérovore** (Sanders et al. 1989). Mais la plupart des études sur la production bactérienne et le grazing (Riemann 1985, Sanders et al. 1989, Nagata 1988, Servais et al. 1985) montrent que dans différents systèmes environnementaux, le taux de grazing est normalement inférieur à celui-ci. Les valeurs s'échelonnent de 10^5 dans les

lacs eutrophes à 10^4 bactéries.mL⁻¹.h⁻¹ dans les masses d'eau mésotrophes. Ces taux de grazing sont de même amplitude que la production bactérienne de ces milieux, suggérant que **le grazing des nanoflagellés hétérotrophes peut contrebalancer en totalité n'importe quelle augmentation de biomasse bactérienne**. Les pertes par grazing sous forme de pourcentage de production bactérienne sont fortement variables : de 0 à 200% suivant le système étudié (Sanders et al. 1989, Simek et al. 1990, Berninger 1991). Cela suggère que **l'impact des nanoflagellés hétérotrophes peut varier suivant la dynamique de l'écosystème** (Sigeo 2005).

Les changements morphologiques au sein des communautés bactériennes peuvent aussi correspondre à des changements taxonomiques (Hahn et Höfle 1999). Ainsi lorsque la prédation des protozoaires constitue la pression de sélection majeure, **des taxa sous-représentés peuvent alors devenir dominants** *via* la formation de filaments résistants à la prédation (Jürgens et al. 1999).

b) Le métazooplancton

Dans les écosystèmes lacustres, les métazoaires du **genre *Daphnia* sont également consommateurs de bactéries** (Jürgens 1994, Thouvenot et al. 1999). Certains copépodes (cyclopoïdes et calanoïdes) se nourrissent de manière sélective, préférant les algues d'une certaine taille par rapport à d'autres et aux bactéries (Burns et Schallenberg 1998). Bien que le zooplancton puisse consommer d'apparentes grandes quantités de bactéries, les taux de grazing sont relativement bas.

Outre les protistes phagotrophes, le zooplancton métazoaire, essentiellement **les cladocères, peut entraîner des changements de composition taxonomiques** picoplanctonique. Ainsi, Langenheder et Jürgens (2001) ont observé dans des réservoirs eutrophes allemands que **les daphnies**, lorsqu'elles sont présentes à de fortes densités, **peuvent constituer un facteur de régulation** des communautés picoplanctoniques plus important que les nanoflagellés hétérotrophes. Höfle et al. (1999) ont également observé dans le lac eutrophe PluBsee (Allemagne) que, lors d'évènements saisonniers comme la phase d'eau claire, caractérisée par une forte prédation par les cladocères, ceux-ci pouvaient influencer notablement la composition de la communauté et réduire la

diversité du bactérioplancton en milieu pélagique. **Cet impact des cladocères sur la composition de la communauté picoplanctonique est difficile à expliquer pour des organismes filtreurs.** Aussi, l'une des principales hypothèses est que **toutes les bactéries ne sont pas digérées dans le tractus des organismes zooplanctoniques** (King et al. 1991). La viabilité des proies au niveau du tractus de *Daphnia* sp. constituerait ainsi un mécanisme permettant, notamment, leur survie face à la prédation du zooplancton métazoaire (Langenheder et Jürgens 2001) entraînant une **sélectivité passive**. La composition de la communauté picoplanctonique peut être également modifiée par la MOD relâchée lors de l'activité de prédation (Vadstein et al. 2003), qui peut alors favoriser certaines bactéries.

c) La lyse virale

Différentes études ont pu mettre en évidence que **la lyse virale pouvait avoir un rôle important dans la mortalité bactérienne suivant les écosystèmes** (Weinbauer 2004). En effet, les virus sont très abondants, de l'ordre de 10^8 virus.mL⁻¹, dans les systèmes aquatiques (Wommack et Colwel 2000).

Dans des milieux aquatiques non-extrêmes, la mortalité induite par les virus peut être **égale à celle provoquée par le grazing des nanoflagellés hétérotrophes**, et représenter en moyenne 25% de la mortalité bactérienne. A l'inverse dans des milieux extrêmes, peu favorables à la croissance des flagellés, la lyse virale **peut représenter la quasi totalité de la mortalité bactérienne** (Paul et Kellogg 2000).

Cependant la moitié des virus peut ne pas être infectieuse (Wilhelm et al. 1998). D'autre part certains auteurs (Hara et al. 1996, Tuomi et al. 1995, Weinbauer et Peduzzi 1995) ont trouvé que **l'infection virale pouvait être dépendante de la morphologie bactérienne** (coque, bâtonnet, spirale...): Lors d'expériences d'enrichissements en virus, seules les bactéries en bâtonnets étaient infectées (Weinbauer et Peduzzi 1995) alors que les formes filamenteuses ne présentaient aucune infection (Simek et al. 2003). De plus, par la réduction du nombre de récepteurs viraux à la surface de la cellule ou un changement de structure (Wommack et Colwel 2000), **une large partie des bactéries pourraient résister à l'infection virale** (Winter et al. 2004). Enfin, il existe des virus

“lysogéniques et pseudolysogéniques” (Ackermann et DuBow 1987) dont l’infection n’entraîne pas de lyse cellulaire mais pourrait avoir un impact sur le génotype bactérien. Ainsi, **le virioplancton peut limiter sélectivement la densité de population de souches bactériennes sensibles.**

Les modèles proposés par Thingstad et Lignell (1997), Wommack et Colwell (2000) et Middleboe et al. (2001) montrent que **la communauté virale pourrait aussi contrôler la composition de la communauté bactérienne.** Ainsi, en présence de virus, la densité de certains phylotypes augmentent ou diminuent tandis que d’autres ne montrent aucune modification (Schwalbach et al. 2004). D’autre part les virus réguleraient la diversité bactérienne par “**killing the winner**” (Fuhrman et Suttle 1993), **infectant préférentiellement les bactéries les plus abondantes** (pour une synthèse voir Wommack et Colwell 2000, Weinbauer 2004).

IV. Organisation du mémoire et objectifs des études

Comme montré dans cette revue bibliographique, la compréhension du fonctionnement des écosystèmes est l’une des préoccupations majeures de l’écologie.

Parmi les écosystèmes aquatiques, les lacs-réservoirs subissent une dégradation accélérée de la qualité de l’eau. Or, ces systèmes sont conçus pour répondre aux besoins économiques et culturels des populations, fournissant de l’eau pour la consommation et offrant des activités récréatives. De part ces fonctions, il est nécessaire de connaître la dynamique, l’activité et la composition des communautés bactériennes présentes ainsi que de déterminer les principaux facteurs de régulation. Etant à ce jour trop peu explorés, ils sont donc de première importance pour la compréhension du fonctionnement des écosystèmes.

L’objectif de cette thèse a donc été, dans un premier temps, d’étudier la dynamique saisonnière du bactérioplancton pour déterminer des périodes clefs dans l’année et émettre des hypothèses quant aux régulations possibles. Puis, dans un second temps, de valider ces hypothèses par des expériences *in situ*, testant ainsi les contrôles *bottom-up* et *top-down* durant ces périodes.

Ce mémoire se divisera donc en trois chapitres.

Le premier chapitre porte sur le suivi saisonnier effectué sur une année dans trois réservoirs présentant un gradient d'eutrophisation s'échelonnant de l'oligomésotrophe à l'eutrophe. Les communautés bactériennes ont été suivies en prenant en considération la densité, le volume cellulaire, l'activité et la diversité. En parallèle des paramètres biologiques (métazooplancton, protozoaires, Chl *a* et virus) et abiotiques (température, oxygène...et nutriments) ont été mesurés.

Le deuxième chapitre présente les expériences réalisées en mésocosmes et en sacs de dialyse sur les réservoirs pour tester les effets des ressources par enrichissements ou diminution de nutriments sur les paramètres bactériens (densité, activité, diversité).

Enfin, pour tester l'impact des facteurs *top down* sur nos communautés bactériennes, une expérience de filtrations différentielles a été réalisée pour déterminer les différents degrés d'influence de chaque niveau du réseau trophique. Cette partie forme le troisième chapitre.

CHAPITRE I

LE SUIVI SAISONNIER: Les dynamiques bactériennes

Avant d'envisager des expérimentations *in situ* ayant pour but de déterminer l'importance relative des contrôles ascendants et descendants sur les communautés bactériennes, il était nécessaire de connaître les dynamiques bactériennes des réservoirs étudiés et de déterminer des périodes « clefs » dans l'année en terme d'activité biologique.

Un suivi saisonnier a donc été effectué durant une année sur trois réservoirs présentant des niveaux trophiques différents s'échelonnant de l'oligomésotrophe à l'eutrophe. Les communautés bactériennes ont été suivies en prenant en considération la densité, le volume cellulaire, l'activité et la diversité. En parallèle, des paramètres biologiques (abondance du protozooplancton hétérotrophe, du phytoplancton, du mézozooplancton et des virus) et abiotiques (température, oxygène, nutriments, ...) ont été mesurés. Ces valeurs ont été mises en relation par des analyses multivariées.

Dans ce premier chapitre, sont présentés les analyses multivariées (analyses de redondance et analyses canoniques de correspondances) et de partition de variance réalisées à partir des dynamiques saisonnières observées. Ces analyses synthétisent les variations saisonnières bactériennes mais mettent aussi en évidence les facteurs de régulation potentiel du bactérioplancton.

L'accumulation de données (biotiques et abiotiques) nécessaires à ce chapitre n'est pas présentée ici mais est disponible en annexes (cf dynamiques saisonnière p 165).

Relationship between bacterial dynamics and bottom-up versus top-down variables along a trophic gradient.

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Abstract

The dynamics of bacterial activity and diversity were intensively monitored during one year in three freshwater reservoirs with different trophic statuses (oligomesotrophy, mesoeutrophy and eutrophy). Seasonal changes in dynamics were related to the bottom-up (resources), top-down (grazers) and physical parameters using multidimensional statistical analyses. On one hand, the seasonal patterns of bacterial activity presented high similarities between reservoirs. On the other hand, the ordination patterns of eubacterial diversity showed that changes across seasons became more abrupt as the trophic status increased.

However, bacterial activity had same significant bottom-up factors in all reservoirs. Temperature and nutrients appeared to play an important role in the regulation of the bacteria by limiting density and activity of picoplankton during winter and the stratification period respectively. Whereas the significant top-down factors depended on the reservoir status. Crustacean for the oligomesotrophic reservoir and heterotrophic nanoflagellates in the mesoeutrophic reservoir, alone viruses were a significant factor in all reservoirs. For eubacterial diversity, the significant variables varied according to the reservoir. Temperature and viruses were significant for the oligomesotrophic system, for mesoeutrophic freshwater factors significant were heterotrophic nanoflagellates and chlorophyll *a* and in the eutrophic reservoir, heterotrophic nanoflagellates and ammonia appeared as significant factors. Nitrates were significant for all reservoirs.

We used variation partitioning to evaluate top-down regulation of bacterial community after bottom-up regulation has been accounted for. Using this approach, we found the major significant environmental factors were water temperature and bottom-up factors (nutrients) in all reservoirs.

Introduction

For a long time, lake bacterial communities have been considered as “black boxes” in ecological studies due to their unculturability and their small size. These very abundant organisms are however known to be responsible for key processes in aquatic ecosystems such as matter recycling and energy transfer towards upper trophic levels (2). The understanding of their dynamics are therefore of prime importance for grasping the significance of the bacterial processes in the functions and productivity of aquatic ecosystems. Since the development of staining procedures and more recently molecular methods (21), bacterioplankton can be studied without cultivation. Despite the development of such methods, relevant data are still too scattered to infer a general model of seasonal dynamics in lakes and reservoirs (53).

Indeed few papers describe a complete seasonal dynamics of bacterioplankton. Most studies dealt with specific periods such as planktonic bloom (14, 58). Some studies encompassed the entire year (29, 41), but dealt with only one type of freshwater ecosystem or included only a few variables . Most papers describing the evolution of the community structure in time (11, 22) and space (24) did not include data on the physiological and activity of bacterioplankton. Nevertheless, on an ecological point of view, bacterial metabolism is fundamental for the understanding of biological processes. All anthropogenic perturbations (i.e. pollution, eutrophication...) affecting the bacterioplankton's dynamics can have major effects on other constituents of the planktonic food web, and thus on the structure of the entire ecosystem. Studying bacteria will allow a better understanding of changes in the ecosystems and the prediction of the evolution of freshwaters. Timescale is an important parameter too. Although bacteria are much less variable in abundance than other components of the pelagic food web (17), bacteria undergo strong fluctuations (10, 53). A monthly sampling strategy could missed some planktonic peaks (36). Freshwater environments are more variable than the oceanic ones, both in terms of water chemistry and in the range of biological productivities they support (3). Series of sampling sufficiently extensive are required to establish a seasonal pattern.

Moreover substantial research efforts have been invested in evaluating the factors regulating bacteria in aquatic ecosystems. Bottom-up (resources) as well as top-down

(predation) factors have been shown to regulate bacterial population. Currently, substrate supply, grazing, temperature and viruses are known to be significant factors controlling both bacterial abundance and production (52). The relative importance of these factors appears however to vary according to the water body characteristics and to the season (52).

Many authors have shown that bacterial abundance changes with the trophic state in freshwater and marine systems (for a review see 56). Sanders et al. (50) proposed that planktonic biomass and production in oligotrophic waters are more likely substrate-limited, whereas predation more likely controls them in eutrophic environments. In a more complicated scenario, (13) proposed that substrate-limiting control of bacterioplankton growth in spring shifts to top-down control in summer. We can suppose that variation of the bacterial parameters is under regulating mechanisms that differ with the seasons and the trophic status.

Multivariate analyses techniques provide the appropriate statistical tools for describing variation in ecological communities and describing ways to relate this variation to changes in environment. These techniques yield measured of correlation between explanatory variables and bacterial community variables and provide statistical tests for this correlation. Interpretative problems may arise when many different explanatory variables are intercorrelated. Multi-collinearity between several explanatory variables makes it difficult to distinguish the separate effects of these different variables on the bacterial community. Variation partition analysis, used to separate these effects in a way analogous to partial correlation analysis, provides a solution for this problem (5).

Therefore, in addition to understanding principles of bacterioplankton growth control in general, it is also important to identify which controlling factors are relevant in a given ecosystem. In the present study we examine the seasonal dynamics of activity and the diversity of bacterioplankton in relation to selected biotic and abiotic factors across a gradient of trophic levels. For this study, the bacterial community activity and composition in each reservoir were monitored during one year. Seasonal changes in bacterial variables were related to bottom-up and top-down variables by using multivariate analysis methods (redundancy analysis and canonical correspondence

analysis). The effects of bottom-up and top-down variations were subsequently separated using variation partitioning.

Materials and methods

Three lakes with contrasting morphometric characteristics were selected for this study. The main features of the reservoirs are given in table 1. The oligomesotrophic is located in Germany. Mesoeutrophic and eutrophic reservoirs, built on the Sûre-river in Luxembourg, are interconnected forming a dam and a pre-dam. The sampling of water was made using a 2.2-L Ruttner bottle during the year 2003. For the oligomesotrophic reservoir, samples were collected twice a month from April to November. The samples were taken at a depth interval of 1 meter across the epilimnion when thermal stratification occurred and from 0 to 10 m during the mixing period. Discrete samples were pooled in a sterile 20-L polypropylene carboy. Identical sampling was carried out for the mesoeutrophic reservoir, but the samples were collected monthly in January, February and November; twice a month for March and October; and weekly since April until September. In the eutrophic reservoir, the waters samples were collected every 0.5 m from the surface to the bottom (maximum depth: 3 m) at a frequency that is similar to that of the mesoeutrophic reservoir. Water temperature, dissolved oxygen, pH and conductivity were determined with a multiparameter probe (Hydrolab DS4). Orthophosphate ($\text{PO}_4\text{-P}$), ammonium ($\text{NH}_4\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), nitrate ($\text{NO}_3\text{-N}$), chlorine (Cl), sulphate (SO_4), dissolved organic carbon (DOC) and silica (SiO_2) concentrations were analysed in laboratory according to standard methods of APHA (1).

For bacterial enumeration, ten mL of the pooled sample were preserved with formalin (2% final concentration) at 4°C in the dark until counting. Cells in suspension were stained with the fluorescent dye Sybr Green I (Molecular Probes) (0.06% final concentration) for 15 min at room temperature in the dark. They were then filtered onto black 0.2- μm polycarbonate membrane filters (Millipore). Cells were counted by epifluorescent microscopy at 1,000x magnification under blue excitation. At least 500 cells were counted for each prepared filter. The mean cell biovolume (V , in μm^3) was calculated using digital pictures of the microscope fields with a formula: $V = (\pi/4) a^2 (A - a/3)$ where a and A are respectively the small axis and the large axis of

the cells measured in μm (7). Autotrophic picoplankton was enumerated, using its autofluorescence capacity. Ten mL of the pooled sample were preserved with formalin (2% final concentration) at 4°C in the dark during maximum 24 hours. Whole sample was filtered onto black $0.2\text{-}\mu\text{m}$ polycarbonate membrane filters (Millipore). Cells were counted by epifluorescent microscopy at 1,000x magnification under green excitation. At least 300 cells were counted on each filter. Bacterial ectoenzymatic activities were monitored as described by Hoppe (23). Alpha-glucosidase and beta-glucosidase substrates were added to a final concentration that proved to be saturating concentrate (50 and $25\ \mu\text{mol L}^{-1}$ respectively). Samples were incubated at *in situ* temperature in the dark during 4 hours. At times 0, 2 and 4 hours, fluorescence was measured by a fluorometer (Turner Design 700). The slope of the regression gives the enzymatic degradation rate. Bacterial production (BP) was assessed using ^{14}C -leucine incorporation (33). Ten ml of pooled water were incubated in duplicate during two hours at *in situ* temperature in the dark. Two formalin-killed blanks were incubated in the same way. Incubations were stopped with the addition of formalin (2% final concentration). Protein precipitation was achieved with hot (80°C) 5% trichloroacetic acid (TCA) for 120 min. Samples were filtered onto cellulose acetate filters, and washed by cold TCA 5%. Filters were transferred in a 7-mL scintillation vials (PackardBioscience) before being dissolved in ethyl acetate and scintillation (PerkinElmer) cocktail. Disintegration rate of the radioactive molecules was measured using a scintillation liquid analyzer. Assimilation of ^{14}C -glucose follows the same procedure as leucine incorporation without TCA extraction step. Samples were directly filtered onto cellulose acetate filters.

For analysis of bacterial diversity using DGGE, a 200-mL subsample was filtered over a membrane filter (pore size, $0.22\ \mu\text{m}$; filter diameter, 45 mm; Supor200; Pall Corporation). The filter was put in 2 mL of lysis buffer (40 mM EDTA, 400 mM NaCl, 0.75 M sucrose, 50 mM Tris-HCl pH : 8.3) and stored at -20°C until extraction. DNA was extracted by phenol-chloroform 50/50 (vol/vol) and ethanol precipitation. Fragments suitable for subsequent DGGE analysis were obtained as described by Muyzer and Smalla (43) with the primer combination of 341f with a GC clamp (40-nucleotide GC-rich sequence, 5'-CCT ACG GGA GGC AGC AG-3') and 907r (5'-CCG TCA ATT CCT TTG AGT TT-3') PCR amplification procedures were performed with a

Biometra temperature cycler. Each mixture, containing 2 ng of DNA, each primer at 0.5 μM , each desoxynucleoside triphosphate at 200 μM , 1.5 mM MgCl_2 , 5 μl of 10x PCR buffer and 1 U of *Taq* DNA polymerase (Amersham), was adjusted to a final volume of 50 μl with sterile water (Sigma). Following incubation for 5 min at 94°C, a touchdown PCR was performed using 37 cycles consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and primer extension at 72°C for 1 min. The tubes were then incubated for 7 min at 72°C. DGGE for bacteria was run as described before with the D-Code System from Bio-Rad Laboratories. PCR samples were loaded onto 1-mm-thick 8% (wt/vol) polyacrylamide gels in 1x TAE (20 mM Tris-acetate [pH 7.4], 10 mM acetate, 0.5 mM disodium EDTA). The denaturing gradient contained 20 to 70% denaturant (100% denaturant corresponded to 7 M urea and 40% [vol/vol] deionised formamide). Equal amounts of PCR product were applied to the DGGE gel. Electrophoresis was performed for 15 h at 100 V. The temperature was set at 60°C. DGGE gels were stained with Sybr Gold (Molecular Probes) and photographed on a UV transillumination table.

Phytoplankton biomass was assessed through pigment analysis of the particulate fraction retained on 47 mm GF/C filters by filtration of at least 1000 ml under a moderate vacuum. The extraction of the algal material and analysis by HPLC followed the procedure detailed in (12). Samples for nanoflagellates analysis were fixed in glutaraldehyde (1% final concentration). The samples for ciliated protozoa were preserved with mercuric chloride (2.5% final concentration). Nanoflagellates were stained with primulin and collected onto black 0.8 μm filters. Nanoflagellates (counting of at least 300 cells) were enumerated at a magnification of 1000x by epifluorescence microscopy (Leica, DMRB) equipped with blue light (excitation: 450-490 nm, emission > 515 nm) and UV light (excitation: 340-380 nm, emission > 430 nm), respectively. Ciliates were allowed to settle for 12-24 h in 25- to 100-mL plankton chambers. Their densities were estimated with an inverted Leitz microscope by scanning the whole chamber area (at a magnification of 200), and they were identified to the genus level. Zooplankton was preserved in a formalin solution added with sucrose 12 g L^{-1} (4% final concentration). In the laboratory, rotifers and crustaceans were determined at the genus level under an inverted microscope and at least 200 individuals for each sample were counted. Biovolumes of ciliates and auto- and heterotrophic nanoflagellates were

calculated from measurements of dimensions and approximations to simple geometric forms. Biovolumes were converted to carbon using $220 \text{ fgC } \mu\text{m}^{-3}$ for ciliates and $190 \text{ fgC } \mu\text{m}^{-3}$ for nanoflagellates. Zooplankton biomass was estimated from length-weight relationships found in the literature (6). Chlorophyll *a* concentrations were converted to carbon using a carbon/chlorophyll *a* ratio of 42.3, which is the average value for the phytoplankton in the mesoeutrophic reservoir (unpublished data). Virus-like particles (VLP) were recovered from a 250- μl formaldehyde-fixed subsample by filtration on a $0.02 \mu\text{m}$ pore size Al_2O_3 filter (Whatman). VLP were stained with the fluorochrome YoPro (50 μM final concentration) in solution in NaCN (2 mM final concentration) using microwaves (59).

We used multivariate statistics to investigate the relation between bacterial dynamics and explanatory variables (including the chemical, biological and physical parameters listed in Table 1). The software package CANOCO 4.0 for Windows (54) was used for all analyses. Explanatory variables were $\log(x + 1)$ -transformed where necessary to approximate normal distribution. The significance of the relation between explanatory variables and bacterial dynamics was tested using Monte Carlo permutation tests (999 unrestricted permutations, $P < 0.05$). Variation in bacterial dynamics and the relationship of this variation to explanatory variables were analyzed for each lake separately. Preliminary exploratory DCA (detrended canonical analyses) analyses of the datasets of the three reservoirs showed that redundancy analysis (RDA) was the most adequate analysis for exploring the patterns of the bacterial “activity” variables. The obtained ordination axes are linear combinations of environmental variables, assuming a linear species-environmental relationship (54). For the bacterial community composition, analyses were done on presence-absence of band data. To link a presence-absence matrix to environmental variables we used a canonical correspondence analysis (CCA).

Variation partitioning analysis (VPA) was used to evaluate top-down and bottom-up effects on the bacterial dynamics as described by Muylaert et al (2002). All explanatory variables were divided into two groups: variables related to bottom-up regulation (concentration of $\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, Cl, SO_4 , DOC and SiO_2 as well as temperature, transparency, pH, O_2 and conductivity) and variables related to top-

down regulation (biomass of heterotrophic nanoflagellates, ciliates, rotifers, crustaceans and viruses abundance). For each reservoir, we selected only variables that independently explained a significant amount of variation by the forward selection. Then, for the set of bottom-up and top-down variables separately, we generated a minimal set of explanatory variables for distinguishing top-down and bottom-up effects as well as their synergistic effects.

Gels DGGE were analysed using a software program (Genetools for Syngene) and transformed in a matrix with presence/absence of bands. Non metric multidimensional scaling (NMDS) was used to distinguish clusters of samples on the basis of their DGGE profiles. NMDS analyses were performed with the Primer version 5 software using the Sorenson similarity index.

Results

Main physico-chemical characteristics of the study site

Table 1 shows the morphometric and physico-chemical characteristics of the three reservoirs. Concentration in PO₄-P was often under the limit detection (0.05 mg.L⁻¹) in oligomesotrophic and mesoeutrophic reservoirs. An increase in the median values for chlorophyll *a* (Chl *a*) and phosphorus (PO₄) concentrations, with a decrease of water transparency were observed with the increase trophic status of the reservoirs. This trophic status range from oligomesotrophic for the Olefalsperre Reservoir to mesoeutrophic and eutrophic for the Esch-sur-Sûre and Pont-Misère reservoirs respectively according to the OECD index (45). Several other abiotic variables followed the same pattern and seemed related to lake trophic status, such as nitrogen element, dissolved organic carbon (DOC) and conductivity (Table 1). Except biomass of rotifers, all the measured biomasses increased with the trophic status. All reservoirs presented relatively similar pH values. pH was circum neutral or alkaline during summer. The water temperature ranged between 3 and 24°C over the year in all reservoirs. No differences in the median values of temperature have been observed between the reservoirs (Table 1). The eutrophic reservoir was a shallow, unstratified

Table 1. Surface area, maximum and mean depths, and range of determined values of chemical, physical, and biological parameters in the three studied reservoirs (biomasses of the different planktonic group in $\mu\text{g C L}^{-1}$) from January to December 2003.

	Oligomesotrophic reservoir	Mesoeutrophic reservoir	Eutrophic reservoir
Surface area (km^2)	1.0	3.2	0.2
Maximum depth (m)	52	46	~4
Mean depth (m)	20	17	~2
	Median (minimum-maximum)	Median (minimum-maximum)	Median (minimum-maximum)
PO4-P ($\mu\text{g P L}^{-1}$)	< 5	6 (<5-31)	16 (8-55)
NH4-N ($\mu\text{g N L}^{-1}$)	21 (3-48)	32 (11-116)	115 (30-288)
NO3-N (mg N L^{-1})	1.03 (0.72-1.28)	3.26 (1.32-4.57)	1.79 (0.29-4.26)
DOC (mg.L^{-1})	1.92 (1.7-6.7)	2.35 (1.3-11.4)	6.60 (2.1-11.0)
Transparency (m)	6.4 (2.3-7)	3.2 (1-5.4)	0.9 (0.35-1.95)
Temperature ($^{\circ}\text{C}$)	16.1 (6.2-21.6)	18.4 (3.5-23.7)	15.2 (5.0-22.2)
Conductivity ($\mu\text{S cm}^{-1}$)	68 (62-71)	141 (114-146)	159 (130-188)
pH	7.4 (6.6-8.4)	8.2 (6.9-9.5)	7.4 (6.9-8.4)
Viruses (10^6 VLP mL^{-1})	26 (6-52)	62 (24-116)	104 (45-275)
Chl a ($\mu\text{g C L}^{-1}$)	41 (0-257)	84 (0-535)	570 (38-1605)
Bacterioplankton ($\mu\text{g C L}^{-1}$)	30 (13-67)	50 (19-187)	95 (30-340)
Flagellates ($\mu\text{g C L}^{-1}$)	5 (1.9-8.3)	5 (0.05-22)	17 (0.3-107)
Ciliates ($\mu\text{g C L}^{-1}$)	15 (4.65-231)	29 (4.3-274)	111 (1.3-2359)
Rotifers ($\mu\text{g C L}^{-1}$)	1.31 (0.19-5.23)	0.82 (0.18-14)	0.94 (0.18-23.51)
Crustaceans ($\mu\text{g C L}^{-1}$)	19 (2.63-37)	36 (4-611)	53 (3-854)

lake whereas other reservoirs were deeper and presented stratification from May to September.

Multivariate analysis of seasonal patterns

Seasonal succession of bacterial activity variables and community composition were studied using RDA and CCA analyses respectively. Results are presented in figures 1 and 2. The three RDA showed clear seasonal trends with the highest values of bacterial abundance and activities in spring and summer. It was possible to find biotic and abiotic variables that were significantly correlated to the seasonal pattern of bacterial variables. Oligomesotrophic reservoir showed a seasonal dynamics with 5 distinct periods. Large cells with a low activity were observed just after the ice cover disappeared. During a second phase, water temperature increased, bacterial cell volume decreased and the extracellular enzymatic activities rose. The maximal temperature was observed during the third period together with high bacterial density and heterotrophic production. Then the fourth period was characterized by an important development of picoautotrophs and by smaller bacterial cell volume. The cell volume then increased progressively during the last period when the highest water conductivity was observed. Six different phases can be distinguished in the seasonal dynamics of bacterial variables in the mesoeutrophic reservoir. The specificity of the first period is the presence of large cells and a relatively high production at the beginning of the year. With water warming up, an important development of picoautotrophs occurred during the second period. A sample of the second period corresponded to the clear water phase; all picoplankton variables are in their median values. Maximal picoplankton densities and activities characterized the third period. The fourth period corresponded to the collapse of thermal stratification in autumn. At this time of the year, conductivity increased and the mean cell volume was the smallest of the year. During the last period, inorganic nutrient concentrations were high. The eutrophic reservoir showed a seasonal dynamics with 5 distinct periods. Large picoplankton cells and low activities characterized the first period. Then, during water warming up, the cell volume decreased and the enzymatic activities increased. The third period corresponded to summer with the maximal values for water temperature and bacterial variables (density, production and activities) and a

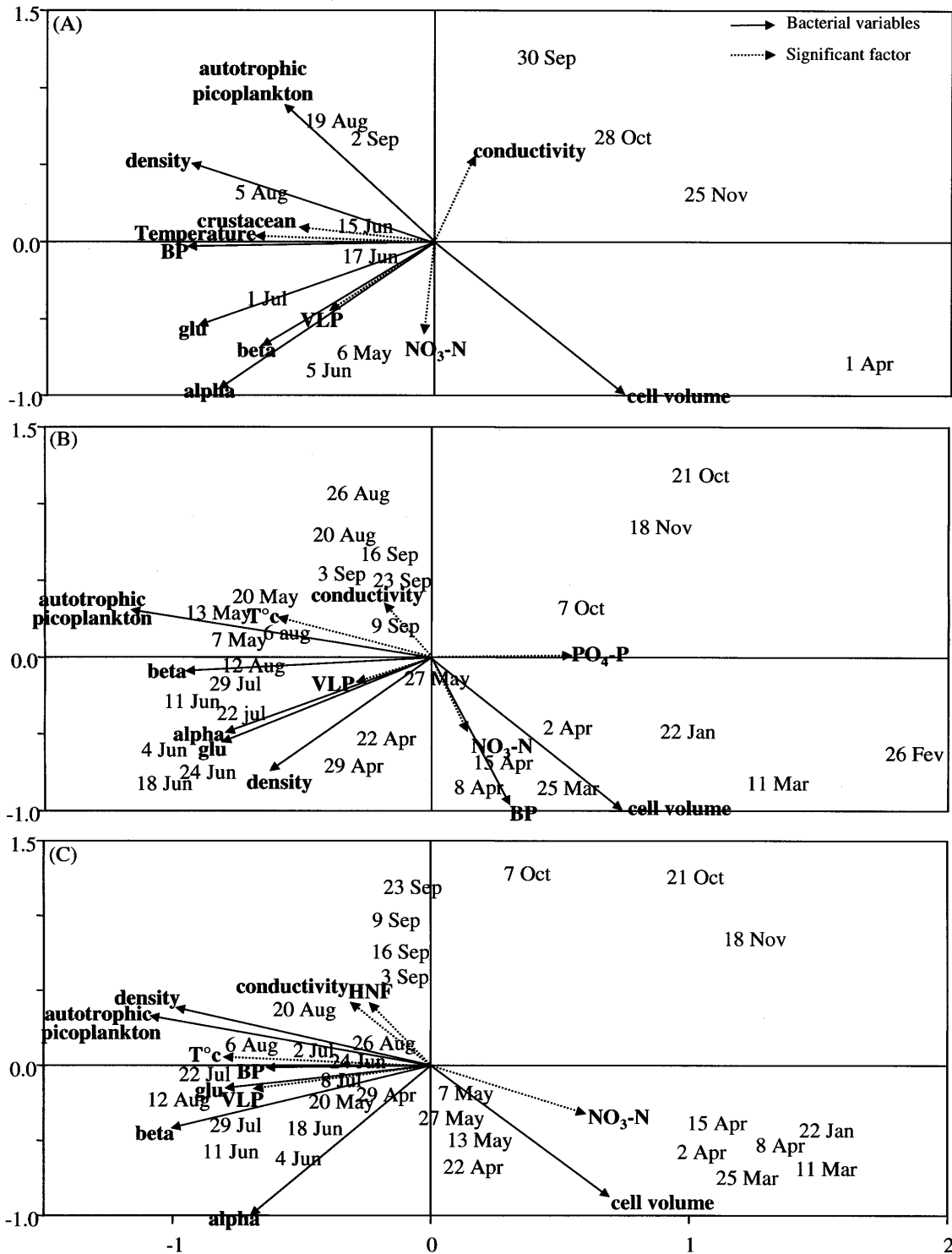


Figure 1. Results from Redundancy Analyses (RDA) using picoplankton variables and environmental factors (glu = glucose assimilation; alpha = alpha-glucosidase; beta = beta-glucosidase, BP = bacterial production, VLP = virus like particles). (A) Triplot of the oligomesotrophic reservoir. The percentage of variance explained by axes 1 and 2 were 40% and 25% respectively. (B) Triplot of the mesoeutrophic reservoir. The percentage of variance explained by axes 1 and 2 were 51% and 23% respectively. (C) Triplot of the eutrophic reservoir. The percentage of variance explained by axes 1 and 2 were 65% and 15% respectively. Only the significant factors (Monte-Carlo test, $p < 0.05$) were represented.

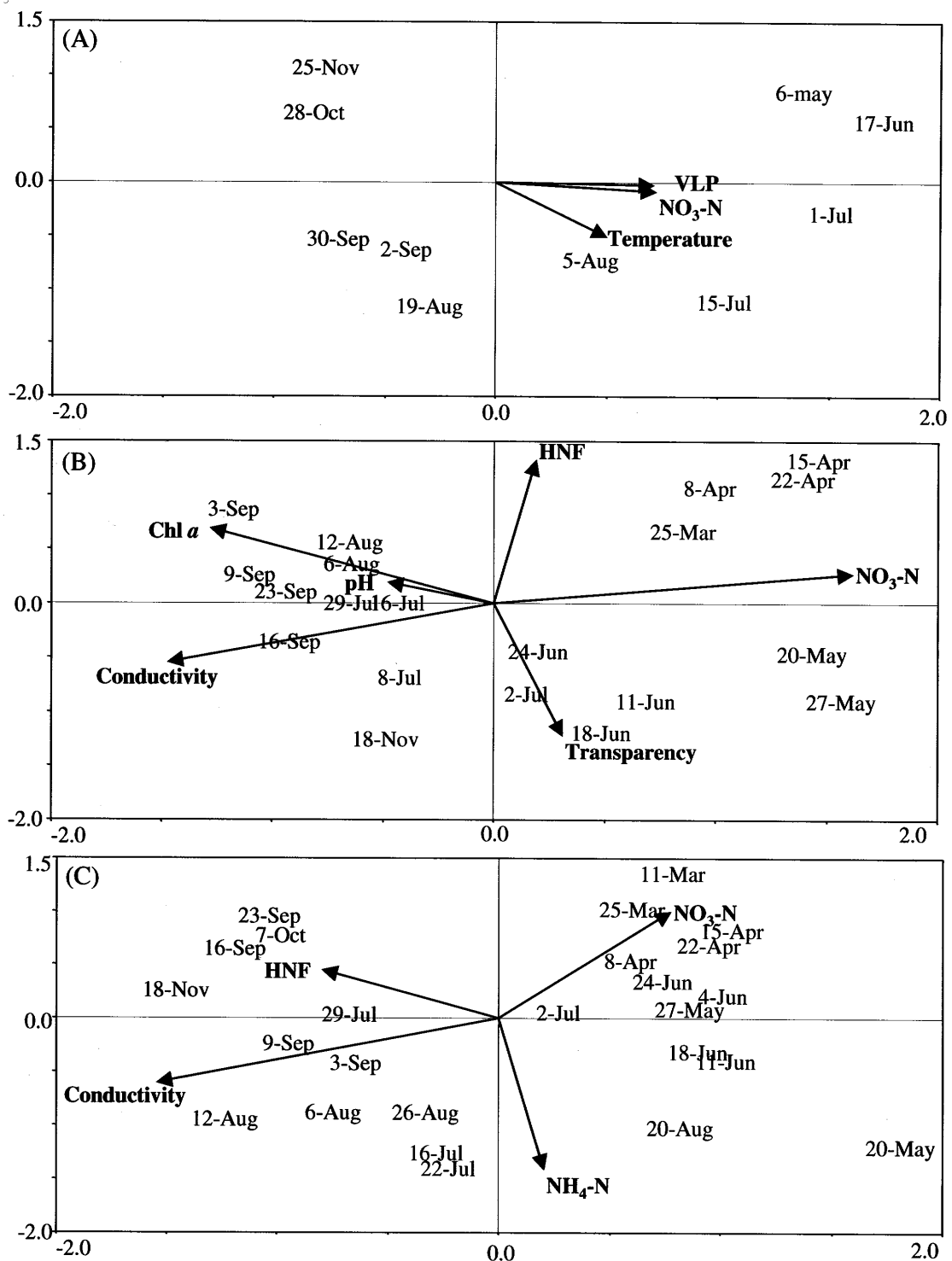


Figure 2. Results from Canonical Correspondence Analyses (CCA) of the bacterial diversity and environmental factors (VLP = virus like particles; HNF = hetrotrophic nanoflagellates; Chl *a* = chlorophyll *a*). (A) Biplot of the oligomesotrophic reservoir. The percentage of variance explained by axes 1 and 2 were 38 % and 34 % respectively. (B) Biplot of the mesoeutrophic reservoir. The percentage of variance explained by axes 1 and 2 were 40 % and 21 % respectively. (C) Biplot of the eutrophic reservoir. The percentage of variance explained by axes 1 and 2 were 35 % and 31 % respectively. Only the significant factors (Monte-Carlo test, $p < 0.05$) were represented.

development of the picoautotrophs. During the fourth period, conductivity increased and picoplanktonic activities decreased. During the last period, the mean cell volume increased and activities were low. In all reservoirs, there was a seasonal separation between the maximum values for the mean cell volume and for the metabolic activities. We found large cells with a low density and a weak activity in winter and abundant cells with a small cell size and high activities in summer.

We obtained usable band patterns for a large majority (80%) of the samples. In total 41 different bands were detected with the DGGE gels (Table 2). The average number of band detected per sampling date decreased with the increase of the trophic status. In oligomesotrophic and mesoeutrophic reservoirs between 16 and 20% of the bands detected were restricted to single samples, whereas 2 and 4 bands were found on every sampling occasions. In contrast with these two reservoirs, the eutrophic reservoir presented only one sampling date with a band that was not present during the rest of the year. Moreover there was no taxon that was present all the year long. 20% of total bands (8 bands) were found to be similar in the three reservoirs. 30% were common to two reservoirs. 4 bands were specific to oligomesotrophic and mesoeutrophic reservoirs, 5 bands were found similar in both mesoeutrophic and eutrophic reservoirs, and 3 bands in oligomesotrophic and eutrophic reservoirs. 51% are specific to one reservoir: respectively 5, 8 and 8 specific bands for oligomesotrophic, mesoeutrophic and eutrophic reservoirs. A test of similarity (nMDS) (Fig. 3) showed that each our freshwater system had specific bacterial community. Seasonal succession in bacterial community composition was studied using a CCA with presence/absence of band. The three CCA (Fig. 2) of the bacterial community composition showed patterns with rising complexity with increasing trophic status. In oligomesotrophic reservoir, a clear seasonal appeared, indicating a gradual succession of bacterial communities. In mesoeutrophic reservoir, bacterial community composition appeared to shift from a stable state to another. In eutrophic reservoir, bacterial communities have strong fluctuations in their composition.

In oligomesotrophic reservoir, temperature ($p = 0.010$), $\text{NO}_3\text{-N}$ ($p = 0.040$), crustaceans, ($p = 0.025$) and VLP ($p = 0.025$) appeared to be significantly correlated with the bacterioplankton pattern. $\text{PO}_4\text{-P}$ was under the limit detection most of the year. In mesoeutrophic reservoir, temperature ($p = 0.005$), $\text{PO}_4\text{-P}$ ($p = 0.045$), $\text{NO}_3\text{-N}$ ($p =$

0.005) and VLP ($p = 0.05$) appeared significant by the ordination. In eutrophic reservoir, temperature ($p = 0.005$), $\text{NO}_3\text{-N}$ ($p = 0.04$), HNF ($p = 0.005$) and VLP ($p = 0.005$) significantly explained the variation in bacterial variables.

In oligomesotrophic reservoir, temperature ($p = 0.010$), $\text{NO}_3\text{-N}$ ($p = 0.020$) and VLP ($p = 0.030$) significantly explained the variance in bacterial community composition. In mesoeutrophic reservoir, $\text{NO}_3\text{-N}$ ($p = 0.015$), transparency ($p = 0.05$), pH ($p = 0.035$), conductivity ($p = 0.013$), Chl *a* ($p = 0.005$) and biomass of HNF ($p = 0.05$) significantly explained the variance in the dataset. In eutrophic reservoir, all the significant factors were abiotic: conductivity ($p = 0.001$), $\text{NH}_4\text{-N}$ ($p = 0.002$), $\text{NO}_3\text{-N}$ ($p = 0.034$) and biomass of HNF ($p = 0.038$) were significantly related to bacterial community composition.

The results of the variation partitioning analysis are summarized in Fig. 4. In all cases, top-down factors explained only a small part of the variation of the bacterioplankton activity and composition (from 1.4 to 15.6 %). The variation in bacterioplankton activity was mainly explained by a shared of the two factors. But in the mesoeutrophic reservoir, bottom-up factors explained the highest part of variance. For the bacterial community composition, the unexplained part of variance increased with the trophic status (from 20.2 to 72.1%). But in the three reservoirs bottom-up factors were the main explanatory factors, explaining from 23.2 to 46.6% of the total variance.

Discussion

The present study is characterised by a high frequency of sampling, the simultaneous monitoring of three reservoirs with contrasting trophic status and the concomitant examination of bacterial diversity and activity. The high sampling frequency allowed to better ascertain the general plan of seasonal dynamics despite the inevitable presence of outlier values. Use of multivariate analyses also allow us to determine a set of significant explanatory variables including both biotic and abiotic variables. Lindström (38) and Muylaert et al. (42) used similar approaches to relate bacterial diversity and the ecological factors. But no paper compared activity and diversity in time and along a trophic gradient.

Table 2. Results from the DGGE analyses in the three reservoirs during the year 2003: number of different electrophoretic bands detected throughout the year, average number of bands per date, number of bands that were only detected on one occasion during the study period, number of bands that were observed on every sampling dates

	Total number of different bands	Average number of bands per date (min-max)	Number of bands only present on one sampling date (percentage of the total number of bands)	Number of band common to all the samplings dates
Oligomesotrophic reservoir	20	10.9 (10-12)	4 (20 %)	4
Mesoeutrophic reservoir	25	9.2 (6-12)	4 (16 %)	2
Eutrophic reservoir	25	6.8 (3-12)	1 (4 %)	0

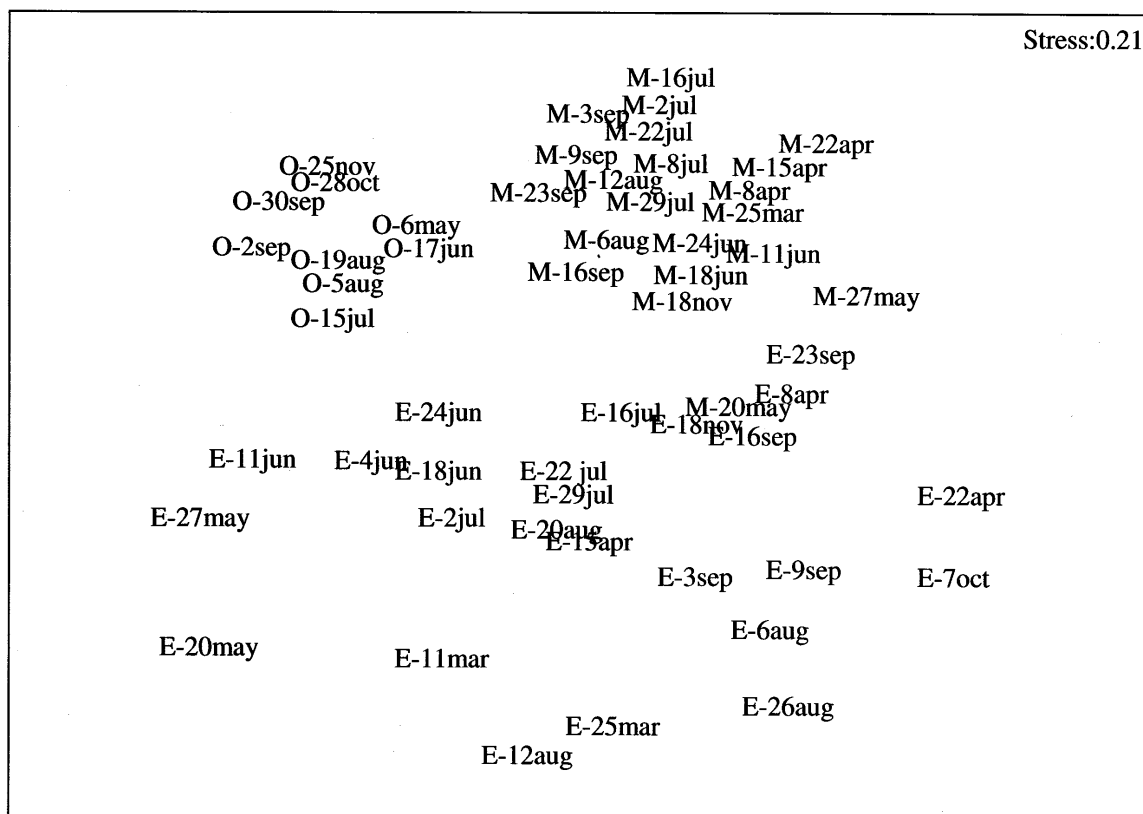


Figure 3. Results from NMDS analysis of data concerning the community composition of bacterioplankton in the three studied reservoirs (O = oligomesotrophic, M = mesoeutrophic and E = eutrophic reservoirs).

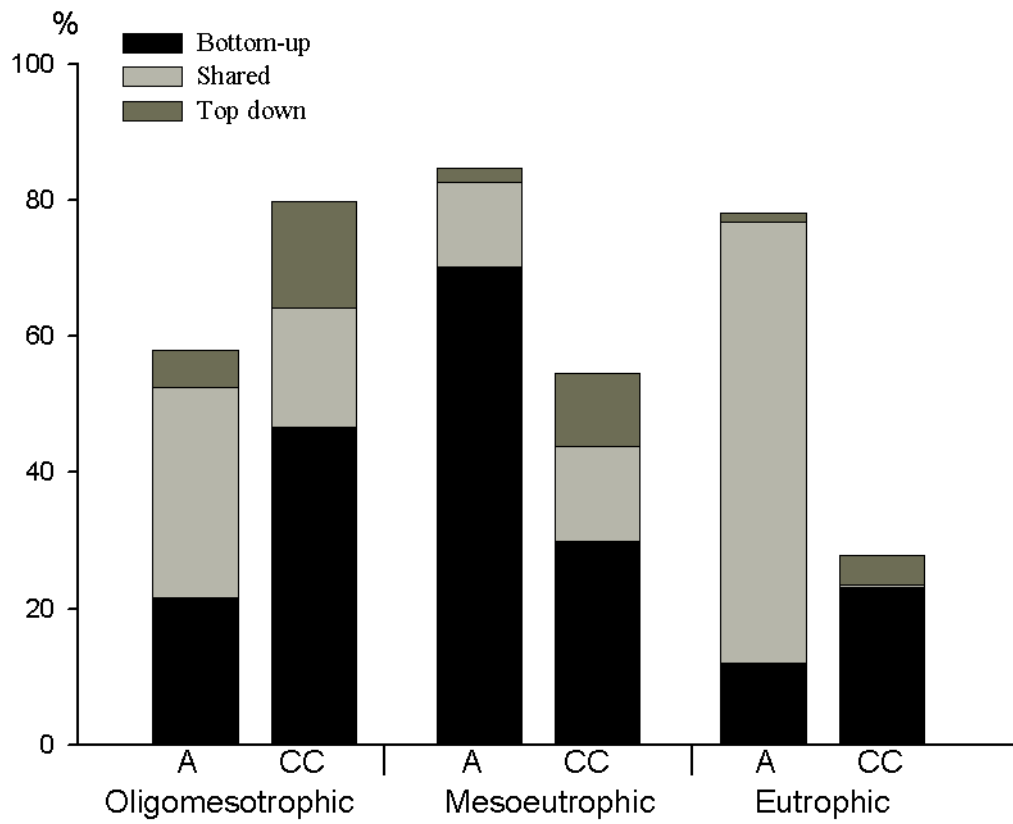


Figure 4. Results of variation partitioning for the three reservoirs. For each reservoir, the total variation explained in picoplankton activity (A) and bacterial composition community (CC) is partitioned between bottom-up variation and top-down variation.

Comparison of the RDA patterns obtained for each reservoir highlights similarity in the seasonal patterns of picoplankton dynamics. A clear seasonal trend was observed, beginning from large inactive cells in winter to high values of abundance and activities of small bacterial cells in spring and summer. In winter, picoplanktonic cells were able to reach a large size allowing stocking of carbon reserves (48). Then, by the reduction of their cell size bacteria facilitated the incorporation of nutrients (9, 16), providing an adaptive advantage compared to planktonic algae. The summer was characterized by intense biological interactions with fluctuating populations, leading to rapid changes in picoplankton variables (28). These seasonal patterns are close to those observed in mesotrophic lake Constance in Germany (53) and the general annual cycle summarized by Wetzel (56). Even so, the timing of the different events was not exactly the same among the reservoir. The dissimilarities in timing were essentially due to differences in climatic conditions. The oligomesotrophic reservoir was located in an area where winters are generally colder than in the area of the two other interconnected reservoirs. In 2003 ice cover was observed on the oligomesotrophic reservoir until March while there was no ice cover on the two other reservoirs. On the other hand, functioning as a pre-dam, eutrophic reservoir is strongly influenced by the river flow regime. The spring water warming and biological development are therefore delayed compared to the other reservoirs.

Concerning the diversity of the bacterial community, the average number of band detected per sampling date is in accordance with Lindström (38). In oligomesotrophic and mesoeutrophic reservoirs between 16 and 20% of the bands detected were restricted to single samples, whereas 2 and 4 bands were found on every sampling occasion. These results are in agreement with those from previous studies in freshwater environments (34, 38). This indicates that few taxa have an ubiquitous nature and the appearance of most taxa is restricted to peculiar period of the year. In contrast with these two reservoirs, the eutrophic reservoir presented only one sampling date with a band that was not present during the rest of the year. Moreover there was no taxon that was present all the year long. The three CCA showed that a seasonal succession occurred in all reservoirs. However changes across seasons became more abrupt as the trophic status increased. For the oligomesotrophic reservoir the pattern appeared to be more gradual probably due to the lower number of samples. Data from DGGE and other

methods have also demonstrated gradual changes in bacterial community, some of which may be linked to seasonal trends (47, 60).

The multivariate analysis moreover highlighted that a mix of biotic and abiotic variables drives the seasonal cycles of the bacterial ecophysiology, density and diversity in the studied temperate lakes. Despite the difference in the trophic status, bacterial activity variables showed close seasonal patterns with same significant bottom-up factors (temperature, conductivity and nitrogen concentration). Each RDA displayed water temperature as the major abiotic discriminant variables along the axis 1. Bacteria in temperate lakes were known to be temperature-dependent up to a certain threshold above which other factors regulate their growth (44). Water conductivity and nitrate concentration were the major abiotic discriminant variables along the axis 2, presenting a high negative inter-correlation. Indeed, nutrient concentration may directly influence bacterial biomass (8). In oligomesotrophic and mesoeutrophic reservoirs during summer, accentuated by water stratification, nitrate and phosphorus remained below a threshold for growth for planktonic bacteria (16). The eutrophic pre-dam is a shallow reservoir and this weak depth allowed a release of nutrients from sediment to the whole water column (49). Although organic nutrients estimated on the basis of DOC presented high concentrations and some fluctuations, it was not a significant variable, contrary to phosphates or nitrates. But the global assessment of DOC is not representative of the bioavailable carbon fraction, which could be a limiting factor for bacteria. Viruses are potentially ubiquitous mortality factors that were found to be significant factors in all reservoirs. In laboratory experiments, Guixa-Boixereu et al.(19) showed an increase in viral production after the increase of bacterial heterotrophic production. However, in our reservoirs no breakdown in bacterial production occurred during highest viral abundance. Viral lysis cannot therefore be considered as a dramatic mortality factor. VLP may even have a positive influence on bacteria rather than a negative one as viral lyses lead to organic matter release (55). The influence of selective grazing by cladocerans on bacterial size distribution leads to small single bacteria (18) or large colonial bacteria (31). This could describe the cell volume dynamic that occur in our reservoirs. Moreover, the flow of C, N and high quality-substrates to the dissolved pool due to the zooplankton grazing and excretion can indeed be comparable in magnitude to phytoplankton exudation (25). This nutrient input by zooplankton may be more

important in oligomesotrophic reservoir, since crustaceans appeared as a significant factor in this reservoir. This production of nutrient is a case of interaction between a top-down factor and a bottom-up factor corresponding probably to a shared part in VPA. VPA allowed us to separated top-down from bottom-up effects and quantify factors effect on the seasonality of bacterioplankton. In the three reservoirs, VPA showed that bottom-up factors or shared part explained more variation than top-down factors. But the explained part by bottom-up decreased in the eutrophic reservoir, supporting the hypothesis of Sander et al. (50) that inversion in major factors occurred with the trophic status.

Contrary to what has been observed in the analyses of activity patterns, the significant variables for eubacterial diversity were different according to the reservoirs.

On the whole temperature, HNF, NH_4 , NO_3 , Chl *a*, VLP, transparency, and conductivity appeared as significant variables. Our results were in accordance with the results of some authors who designate water flow, temperature, nutrients and viral infection as variables affecting the diversity of bacterial community (37, 47). We found relationships between HNF and bacterial community structure, HNF have long been viewed to be mainly responsible for bacterial biomass control (3, 20). During prey capture, HNF select certain bacteria over others (4, 30), and a strong influence of HNF grazing on bacterial community composition has also been demonstrated in experiments (32, 35) and in various lakes (38). In mesoeutrophic and eutrophic reservoirs, nitrogen was a significant factor. These observations and the VPA analysis highlight the important role of nutrients in the variation of the bacterial community composition in both ecosystems. Jardillier et al. (27) found that nitrate and ammonium accounted for a significant part of bacterial community composition variation. Likewise, Fisher et al. (15) and Lindström (38) showed that nitrogen and phosphorus were responsible for the variation in the bacterial community composition. Chlorophyll *a* concentration was a significant factor in the mesoeutrophic reservoir. Primary producers are directly connected by microbial food web with bacterioplankton. It suggests that the bacterial community depends strongly on the organic matter excreted by specific phytoplankton groups (26) while the bacterioplankton in other reservoirs depended mainly on other carbon sources, possibly allochthonous sources. Covariation between bacterioplankton community composition and phytoplankton biomass has been observed in some lakes

(22, 46). In oligomesotrophic VLP present an impact on the BCC dynamics. As described by Middleboe et al. (40) and Schwalbach et al. (51) viruses modified composition of the bacterial community by selective lytic activities (57), or by nutrient recycling promoting certain types of competing bacteria (39). Like for bacterial activity, bottom-up factors were the main factor explained variation in the dataset of bacterial composition. Decrease of the shared part occurred with the increase of the trophic status. But in eutrophic reservoir the significant factors explained only 30% of the bacterial community composition variation. Some other factors, which were no studied, could have an impact on the bacterial composition. For example, water flow could be an important parameter (37)

The present study gives an overview of the picoplanktonic dynamics occurring in three freshwater reservoirs with contrasting trophic levels during a complete year cycle. The dynamics of microorganisms in aquatic ecosystems results in a large part from complex interactions between abiotic and biotic factors (20). In our studies, seasonal patterns of the picoplankton dynamics were similar even if the reservoirs displayed some specificity. Although bacterial diversity is different among reservoirs, their physiologic variables showed clearly similar seasonal trends with differences in the timing due to climatic conditions and differences in the intensity of the activities depending on the trophic status of the reservoir. Contrary to activity patterns, diversity patterns were different among reservoirs. They indeed appeared to be linked to different influencing variables. In summary, the major significant environmental factors were water temperature and nutrients. A second degree of regulation includes predation intensity, which depended on the reservoir status. This regulation by grazers and viruses can influence picoplankton either in a positive (e.g. sloppy feeding/cascading effect of crustaceans in oligomesotrophic reservoir) or a negative way (e.g. grazing by NFH in the eutrophic reservoir).

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CHAPITRE II

EXPERIMENTATIONS: Les facteurs bottom-up

Pour étudier l'impact des nutriments sur les communautés bactériennes, nous avons choisi 2 approches expérimentales:

- des microcosmes
- des sacs de dialyse

Bien que ces expériences introduisent inévitablement des biais dans l'évolution des dynamiques bactériennes dus aux effets de confinement et de maniement, ces outils expérimentaux sont très utiles. Ils permettent de comprendre comment des processus environnementaux (telle que l'addition ou la diminution de nutriments) induisent des variations temporelles dans la structure et l'activité des communautés bactériennes (Simek et al. 2001, Lebaron et al. 2001, Jardillier et al. 2004).

Peu d'études se déroulent sur une aussi longue période d'incubation que la nôtre (une semaine) et avec un pas de temps d'échantillonnage aussi étroit (1 prélèvement toutes les 24 heures). Seuls quelques auteurs (Simek et al., 2001 ; Gasol et al., 2002) ont utilisé les mêmes types de suivis pour des réservoirs.

Ce chapitre se divise en deux parties :

1. La première partie présente des expériences d'enrichissement dans les réservoirs oligomésotrophe et mésoeutrophe durant deux périodes de l'année contrastées :

- l'une présentant de fortes concentrations en nutriments et de faibles densités de phytoplancton.

- l'autre une diminution en nutriments inorganiques et des populations algales abondantes.

Ces expériences permettent de suivre l'évolution d'une communauté picoplanctonique lors d'un fort apport de nutriments bien particuliers, telle une pollution arrivant par un affluent dans le réservoir.

2. La deuxième partie du chapitre présente des expériences de translocation qui modifient les concentrations de l'ensemble des nutriments y compris les micro-éléments dans le sens d'un enrichissement ou d'un appauvrissement lors de trois périodes bien distinctes de l'année (assimilables à trois saisons : printemps , été et automne).

La démarche adoptée ici permet de confronter les résultats qui seront obtenus par deux méthodes conceptuellement différentes mais ayant pour but la démonstration d'un même phénomène.

Response of bacterial communities to nutrient availability: mesocosm enrichments in reservoirs with different trophic statuses

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Abstract

Mesocosm experiments were performed to study the influence of nutrients on activity and diversity of bacterial assemblages from two reservoirs with different trophic statuses. Changes in natural bacterial assemblages were monitored in mesocosms with either inorganic nutrient (ammonia + phosphate) or organic nutrient (glucose) addition as well as with concurrent of inorganic and organic nutrient additions. Variations in the bacterial density and production showed that in both reservoirs bacteria were nutrient-limited during the summer period, this nutrient limitation was weaker during the clear water phase. On the contrary the nutrient C:N:P ratio appeared to be an influencing factor for the bacterial activity whatever the period considered. As inferred from the gel band patterns obtained by DGGE, only moderate changes took place within the bacterial community when nutrients were added alone. Supplementing the mineral nutrients with glucose led to major changes in band patterns. We suggested that bacterial assemblages in our reservoirs were dominated by a majority of non-specialized bacteria that are efficient in using a large array of food resource varying in both quality and quantity.

Introduction

The bacterioplankton is now recognized as an important regulator of the energy and matter fluxes in the aquatic ecosystems via the decomposition of the organic matter, the nutrient recycling, and the carbon and energy transfer to the higher trophic levels [1, 5, 30]. The current challenge is to understand the mechanisms controlling this bacterioplankton dynamics. Temperature, nutrients, predation by zooplankton and viral lysis are thought to be the main control factors of the spatio-temporal dynamics of aquatic bacteria [17, 34, 48].

Few authors [37, 39] have however studied the dynamics of bacteria in freshwater reservoirs. The evaluation of the strength of the top-down control has received most of the attention in reservoirs [2, 38, 39]. These studies showed that protists, especially heterotrophic nanoflagellates, consume most of bacterial production during an annual cycle, except for the clear-water phase. Moreover, a significant shift in bacterial community composition occurs under nanoflagellate bacterivory. However, with increasing trophic ciliates become as important bacterivores. Concerning bottom-up factors, changes in limiting nutrients are known to yield shifts in bulk properties of bacterioplankton communities, in terms of abundance, production and diversity [6, 35, 38]. During the last decade, the importance of the limitation in nutrient phosphorus (P) was more particularly examined [9, 40, 43]. It was found that P limited bacterioplankton growth in oceanic and freshwater systems. Likewise, the labile organic substrates produced by phytoplankton through exudation are also a regulating factor of bacterial growth [8, 47]. As a result, limitations of the bacterioplanktonic growth by a concomitant depletion of inorganic nutrients and organic carbon were observed in lakes as well as in marine ecosystems [3, 6, 14, 15, 25, 35, 42, 46]. The seasonal variations of the bottom-up control of bacterioplankton remain largely unknown.

Our purpose was to ascertain the intensity of nutrient limitation in lentic ecosystems characterized by contrasted nutrient conditions. To determine the impact of nutrients on the bacterial communities, we chose an oligomesotrophic reservoir and a mesoeutrophic reservoir. These water bodies were studied at two main periods of the year: the clear water phase and the summer season. These periods were chosen for their contrasted characteristics. Clear water phase is a nutrient-rich period with presumably weak competition between algae and bacteria. On the contrary, summer is a period with

inorganic nutrient depletion and potentially a high availability of organic exudates from algae. Microcosms enriched with organic and inorganic nutrients alone or in combination were used. These experimental tools are known to be very useful to understand the influence of environmental factors on the structure and the activity of the bacterial communities [22, 24, 39]. Bacterioplankton communities were monitored from a metabolic as well as a diversity point of view.

Materials and methods

Study Sites and Experimental design. The Esch-sur-Sûre reservoir is located in the upper part of the Sûre river in Luxembourg (latitude 49°54'; longitude 5°53'). This reservoir is a stratified mesoeutrophic water body with a surface of 3.2 km² and a mean depth of 17 m. The Oleftalsperre reservoir is a stratified oligomesotrophic reservoir located in Germany (latitude 50°29; longitude 6°25'). It is used for drinking water production. It has a surface of 1.0 km² and a maximum depth of 20 m. Enrichment experiments were conducted in these reservoirs (oligomesotrophic and mesoeutrophic) at two different periods of the year (Table 1): during the clear water phase and during the summer algal development. The sampling of water was made using a 2-L Ruttner bottle. For the oligomesotrophic reservoir, samples were collected every meter, on 24th May 2004 (clear water phase) and on 25th July 2005, across the epilimnion. Discrete samples were pooled in a sterile 50-L polypropylene carboy. Identical sampling was carried out in the mesoeutrophic reservoir, but the samples were collected on 9th May 2005 (clear water phase) and on 1st August 2005.

A fraction (1.5 L) of the pooled sample were poured into 2-L autoclaved bottles (Schott). Inorganic phosphorus, inorganic nitrogen and organic carbon were added to the bottles, alone and in combinations, as Na₂HPO₄, NH₄ and C₆H₁₂O₆ respectively. Each treatment was carried out in triplicate. Three additional bottles received no nutrient additions and served as controls. Na₂HPO₄, NH₄ and C₆H₁₂O₆ were added to reach final concentrations of 40 µg P L⁻¹, 600 µg N L⁻¹, and 4000 µg C L⁻¹, respectively. This represents a molar C:N:P ratio of 100:15:1. This ratio was chosen to be similar to an average C:N:P ratio of eutrophic water [27] and to the Redfield ratio [31]. The bottles were incubated [11] *in situ* at a depth where only 15% of incident light penetrated. Bacterioplankton variables were measured every day during five days.

Bacterial variables. For bacterial enumeration, ten mL of the pooled sample were preserved with formalin (2% final concentration) at 4°C in the dark until counting. Cells in suspension were stained with the fluorescent dye Sybr Green I (Molecular Probes) (0.06% final concentration) for 15 min at room temperature in the dark. They were then filtered onto black 0.2 µm polycarbonate membrane filters (Millipore). Cells were counted by epifluorescent microscopy at 1,000x magnification under blue excitation. At least 500 cells were counted for each prepared filter.

Bacterial production (BP) was assessed using ¹⁴C-leucine incorporation. Ten ml of pooled water were incubated in duplicate during two hours at *in situ* temperature in the dark. Two formalin-killed blanks were incubated in the same way. Incubations were stopped with the addition of formalin (2% final concentration). Protein precipitation was achieved with hot (80°C) 5% trichloroacetic acid (TCA) for 120 min. Samples were filtered onto cellulose acetate filters, and washed by cold TCA 5%. Filters were transferred in a 7-mL scintillation vials (PackardBioscience) before being dissolved in ethyl acetate and scintillation (PerkinElmer) cocktail. Disintegration rate of the radioactive molecules was measured using a scintillation liquid analyser (Tricarb 2900TR, Packard). Assimilation of ¹⁴C-glucose follows the same procedure as leucine incorporation without TCA extraction step. Samples were directly filtered onto cellulose acetate filters.

For analysis of bacterial community composition (BCC) using DGGE, a 200-mL subsample was filtered over a membrane filter (pore size, 0.22 µm; filter diameter, 45 mm; Supor200; Pall Corporation). The filter was put in 2 mL of lysis buffer (40 mM EDTA, 400 mM NaCl, 0.75 M sucrose, 50 mM Tris-HCl pH : 8.3) and stored at -20°C until extraction. DNA was extracted by phenol-chloroform 50/50 (vol/vol) and ethanol precipitation. Fragments suitable for subsequent DGGE analysis were obtained as described by Muyzer and Smalla [26] with the primer combination of 341f with a GC clamp (40-nucleotide GC-rich sequence, 5'-CCT ACG GGA GGC AGC AG-3') and 907r (5'-CCG TCA ATT CCT TTG AGT TT-3'). PCR amplification procedures were performed with a Biometra temperature cycler. Each mixture, containing 2 ng of DNA, each primer at 0.5 µM, each desoxynucleoside triphosphate at 200 µM, 1.5 mM MgCl₂, 5 µl of 10x PCR buffer and 1 U of *Taq* DNA polymerase (Amersham), was adjusted to a

final volume of 50 µl with sterile water. Following incubation for 5 min at 94°C, a touchdown PCR was performed using 37 cycles consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and primer extension at 72°C for 1 min. The tubes were then incubated for 7 min at 72°C. DGGE for bacteria was run with the D-Code System from Bio-Rad Laboratories. PCR samples were loaded onto 1-mm-thick 8% (wt/vol) polyacrylamide gels in 1x TAE (20 mM Tris-acetate [pH 7.4], 10 mM acetate, 0.5 mM disodium EDTA). The denaturing gradient contained 20 to 70% denaturant (100% denaturant corresponded to 7 M urea and 40% [vol/vol] deionised formamide). Equal amounts of PCR product were applied to the DGGE gel. Electrophoresis was performed for 15 h at 100 V. The temperature was set at 60°C. DGGE gels were stained with Sybr Gold (Molecular Probes) and photographed on a UV transillumination table.

Statistical analyses. For comparing abiotic variables of the different reservoirs, we used a Student's t-test. Analyses of covariance (ANCOVA) and analyses of variance (ANOVA) were used to test changes potentially induced in the bacterial variables by the different bottom-up treatments. ANCOVA was used for testing full experiment during time and ANOVA was used for comparing treatment at a giving time. Tukey's Honest Significant Difference (HSD) post hoc comparisons were used to test individual treatment effects. Gels DGGE were analysed using a software program (Genetools for Syngene) and transformed in a matrix with presence/absence of bands. Clustering is based on the simple matching algorithm, while the dendrogram is drawn applying the group average method. Analyses of similarities (ANOSIM) were used to compare changes in the bacterial community composition [7].

Results

In the oligomesotrophic reservoir: the main characteristics of the experimental periods were summarized in Table 1. The enrichment experiment performed during the clear water phase (Fig.1) showed that the concurrent addition of inorganic and organic nutrients (CNP treatment) was significantly different (ANCOVA $p < 0.05$) from the other treatments after 24 h (heterotrophic production), 48 h (glucose assimilation) or 72 h (abundance) of incubation. After 96 h of incubation, bacterial abundance was 3 times

higher in the CNP treatment than in the control. At this time, abundances in the treatments with organic C addition (C treatment) and with inorganic nutrients (NP treatment) were not different from each other and 1.5 times higher than in the control treatment. After 96 h, the glucose assimilation and the heterotrophic production were respectively 175 and 100 times higher in the CNP treatment than in the control treatment.

During the summer period (Fig. 2) the CNP treatment was significantly different than other treatments as far as the abundance, the glucose assimilation or the heterotrophic production were concerned (ANCOVA $p < 0.05$). The bacterial abundance and the glucose assimilation were significantly higher in the CNP treatment compared to the other treatments (ANOVA $p < 0.05$). The heterotrophic production increased regularly and steeply in NP treatment and CNP treatment. After 96 h, these two treatments were not significantly different from each other and their heterotrophic production were significantly higher than that observed in the control and the treatment with C addition only (C treatment).

Table 1: Main characteristics of the experimental periods (clear water phase and

Study site	Oligomesotrophic		Mesoeutrophic	
	Clear Water Phase	Summer period	Clear Water Phase	Summer period
PO ₄ -P (mg P L ⁻¹)	<0.005	<0.005	0.005	<0.005
NH ₄ -N (mg N L ⁻¹)	0.014	0.016	0.068	0.025
NO ₃ -N (mg N L ⁻¹)	1.38	1.17	4.79	3.73
DOC (mg.L ⁻¹)	1.90	2.67	2.30	2.38
Temperature (°C)	14.5	20.9	13.1	21.0
Oxygen (% of saturation)	102	72	79	75
Conductivity (µS cm ⁻¹)	752	820	1862	1851
pH	7.3	7.9	7.1	8.8
Rotifers (ind. L ⁻¹)	136	567	12	23
Crustaceans (ind. L ⁻¹)	12	245	489	551

summer period) in the oligomesotrophic and the mesoeutrophic reservoirs. The values are means of two measures.

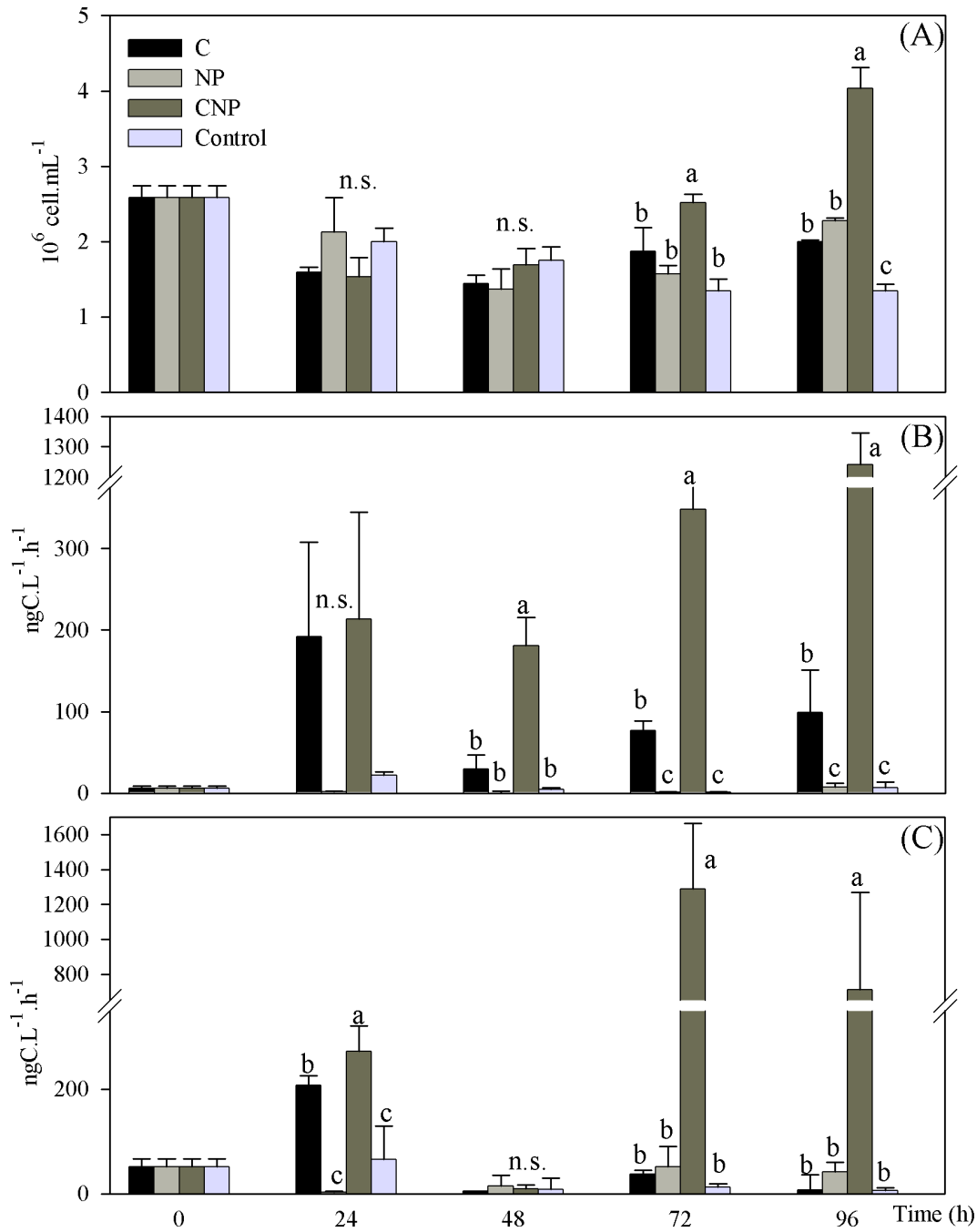


Figure 1: Changes in bacterial parameters in different treatments (C, NP, CNP and Control) in mesocosms during the clear water phase experiment in the oligomesotrophic reservoir. (A) Bacterial abundance. (B) Bacterial glucose assimilation. (C) Bacterial production. Letters represent significant differences between treatments (ANCOVA). (n.s. is no significant)

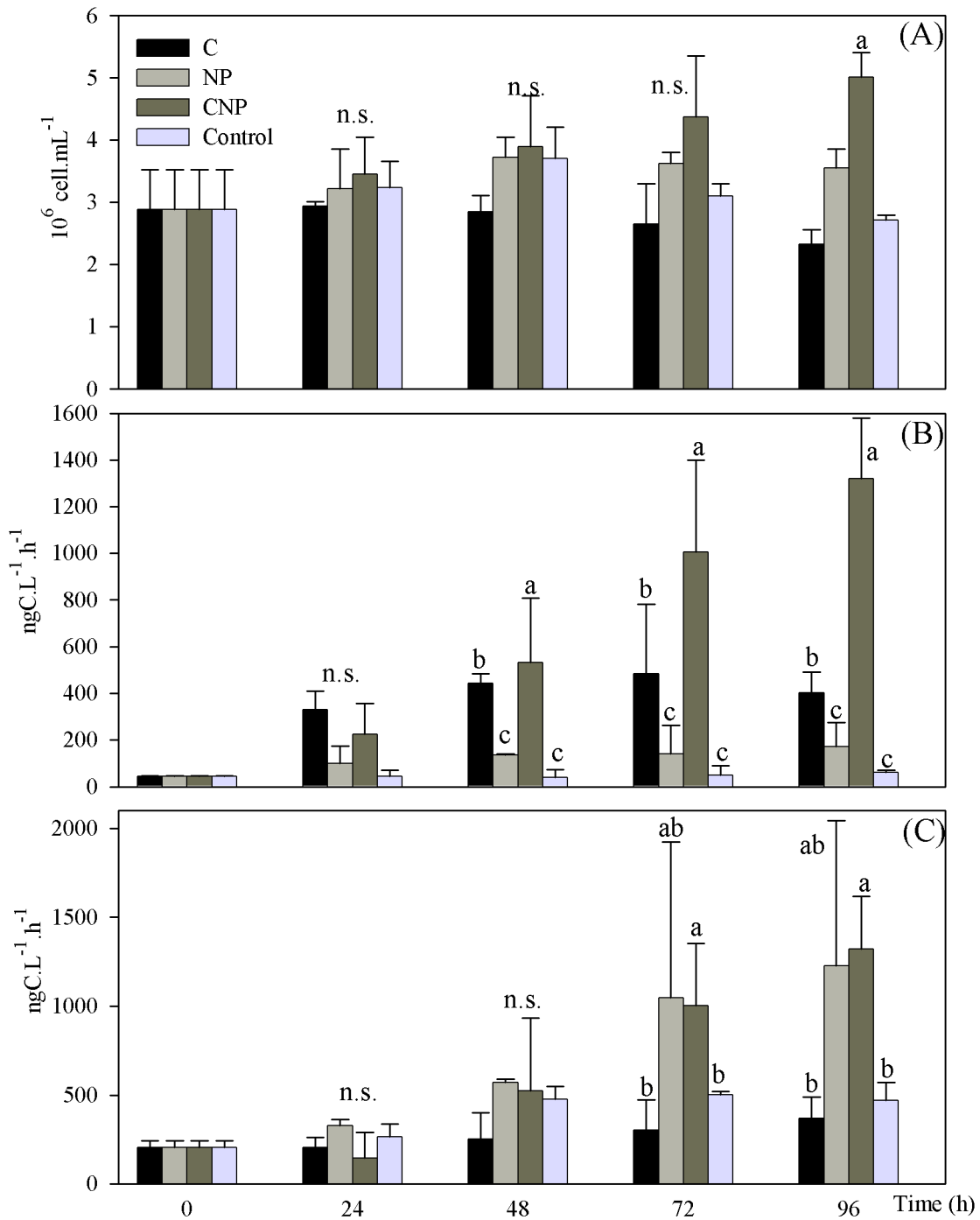


Figure 2: Changes in bacterial parameters in different treatments (C, NP, CNP and Control) in mesocosms during the summer period experiment in the oligomesotrophic reservoir. (A) Bacterial abundance. (B) Bacterial glucose assimilation. (C) Bacterial production. Letters represent significant differences between treatments (ANCOVA). (n.s. is no significant)

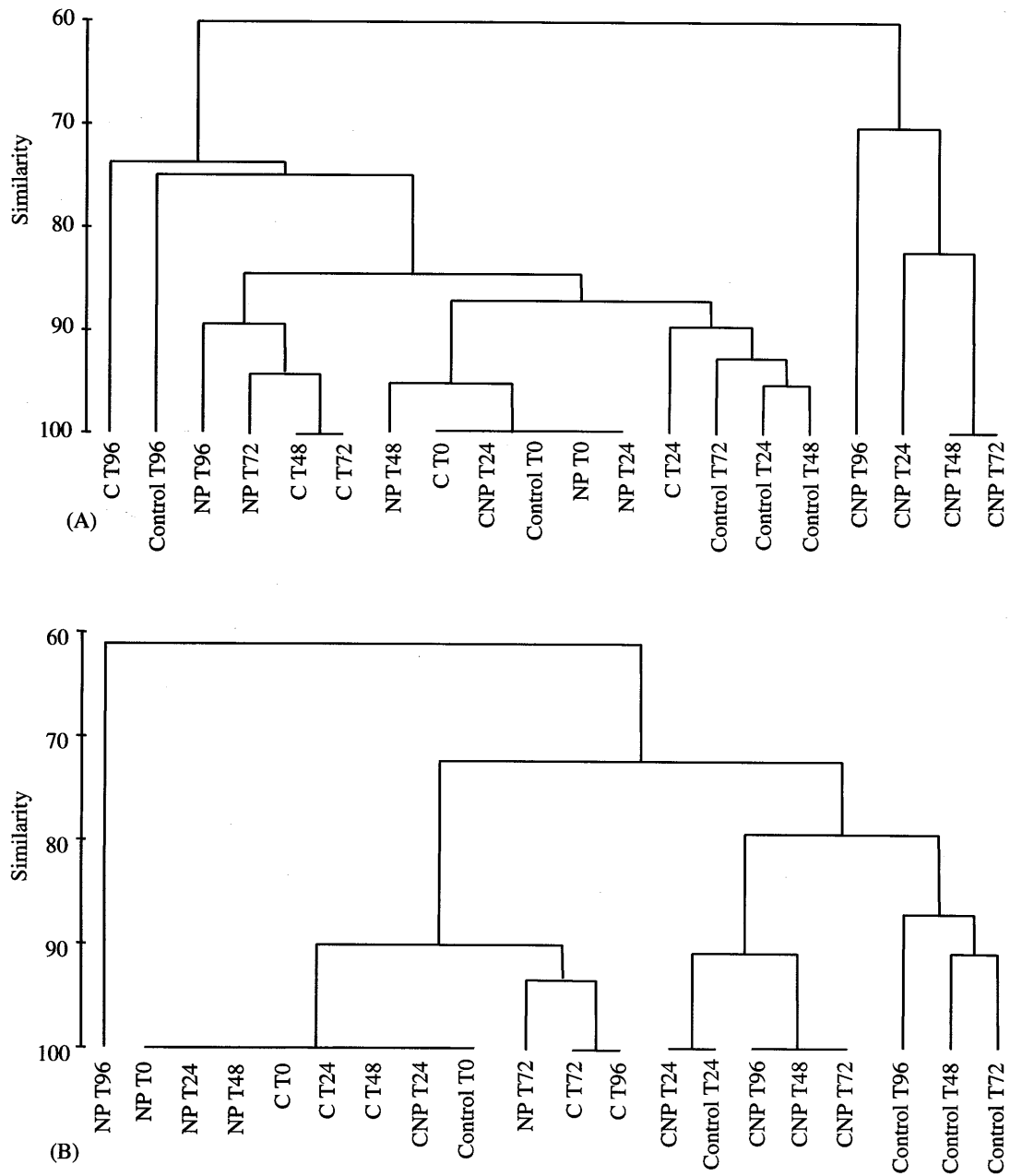


Figure 3: Dendrogram showing the similarity of DGGE patterns from mesocosms and sampling time. Clustering is based on the simple matching algorithm, while the dendrogram is drawn applying the group average method. (A corresponding to the clear water phase period and B to the summer period)

Reproducible DGGE fingerprints were obtained using each microbial assemblage. During the clear water phase (Fig.3A) the bacterial community composition (BCC) consisted in 11 Operational Taxonomic Units (OTUs). As inferred from the DGGE band pattern, the control mesocosms had a stable bacterioplankton community during the experiment except after 96h, where 3 OTUs disappeared compared to the community at 72 h of incubation. The addition of inorganic (NP) and organic nutrients (C) alone induced a moderate change in the band pattern compared to the control before 72 h of incubation, having 2 or 3 bands less than in the control experiment. The most marked changes in band pattern were found in the CNP treatment. The number of bands decreased markedly during the experimental period. CNP treatment displayed about 40% of dissimilarity compared to the Control treatment. During the summer period (Fig. 3B), the bacterial community diversity was strongly reduced including only 6 OTUs. Contrary to what has been observed during the clear water phase, 1 or 2 bands OTUs appeared during the time course of the experiments. The total number of OTUs was constant in the Control treatment but some OTUs were replaced by new ones during the time course of the experiment. Differences in the diversity of the bacterial communities among treatments were weak and not significant (ANOSIM $p < 0.05$).

In the mesoeutrophic: no statistical difference was found between densities in the different treatments (ANCOVA $p > 0.05$) during the clear water phase experiment reservoir (Fig.4). On the contrary, the assimilation of glucose in the CNP treatment presented a strong increase during the experiment, rising from 35 $\text{ngC.L}^{-1}.\text{h}^{-1}$ at the beginning of the study to 2700 $\text{ngC.L}^{-1}.\text{h}^{-1}$ after the 96-h incubation. C treatment presented the same pattern. Moreover the production in CNP treatment was statistically higher than that in the other treatments (ANCOVA $p < 0.001$). The CNP treatment had the highest production increase, reaching 500 $\text{ngC.L}^{-1}.\text{h}^{-1}$ after 96 h. It was significantly different from other treatments (ANCOVA $p < 0.05$).

During summer period (Fig.5), the bacterial abundance had a significantly increase in NP and CNP treatments (ANCOVA $p < 0.001$ and $p < 0.05$, respectively). For the latter treatment, the density was the highest after 48 h and declined to a value that

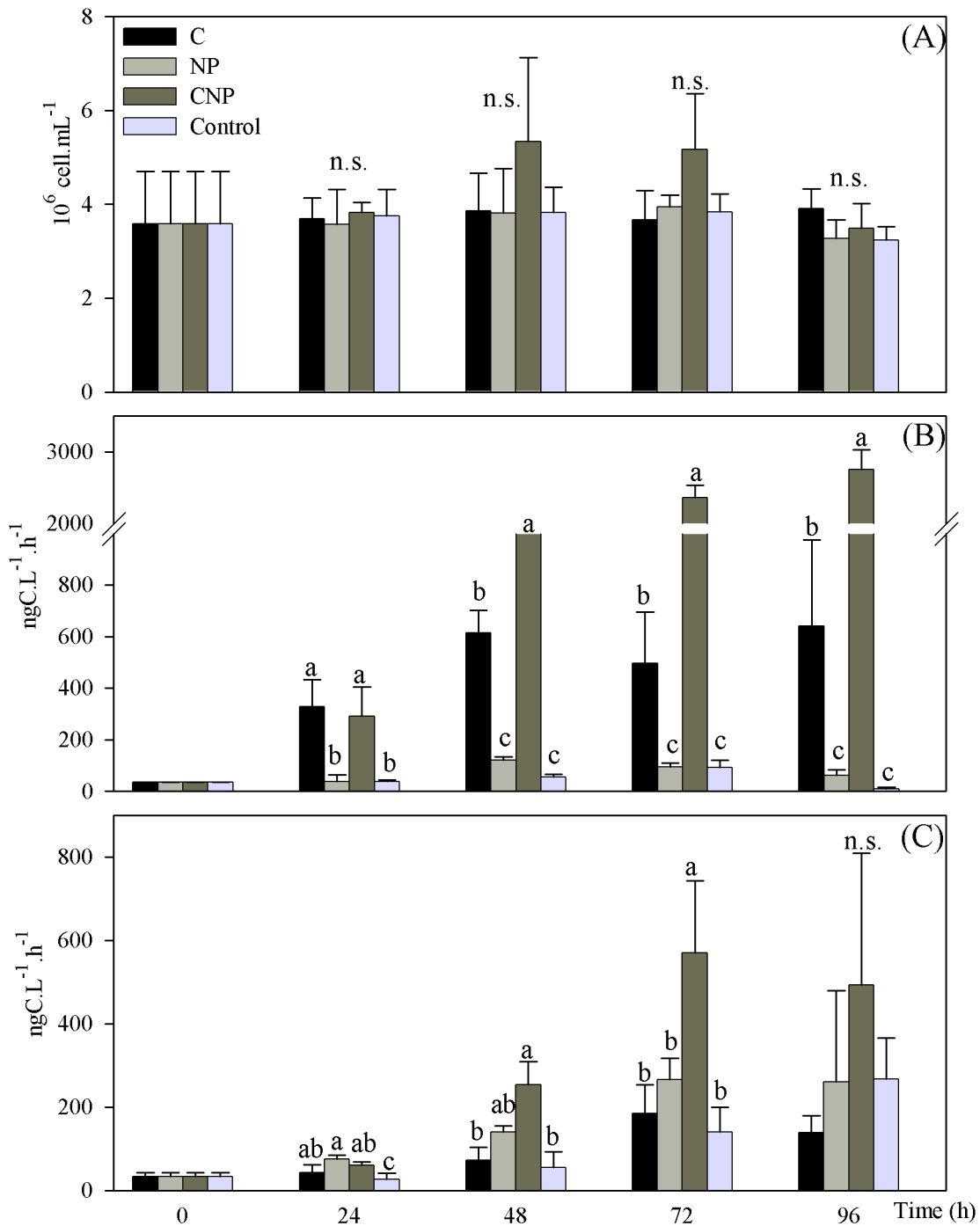


Figure 4: Changes in bacterial parameters in different treatments (C, NP, CNP and Control) in mesocosms during the clear water phase experiment in the mesoeutrophic reservoir. (A) Bacterial abundance. (B) Bacterial glucose assimilation. (C) Bacterial production. Letters represent significant differences between treatments (ANCOVA). (n.s. is no significant)

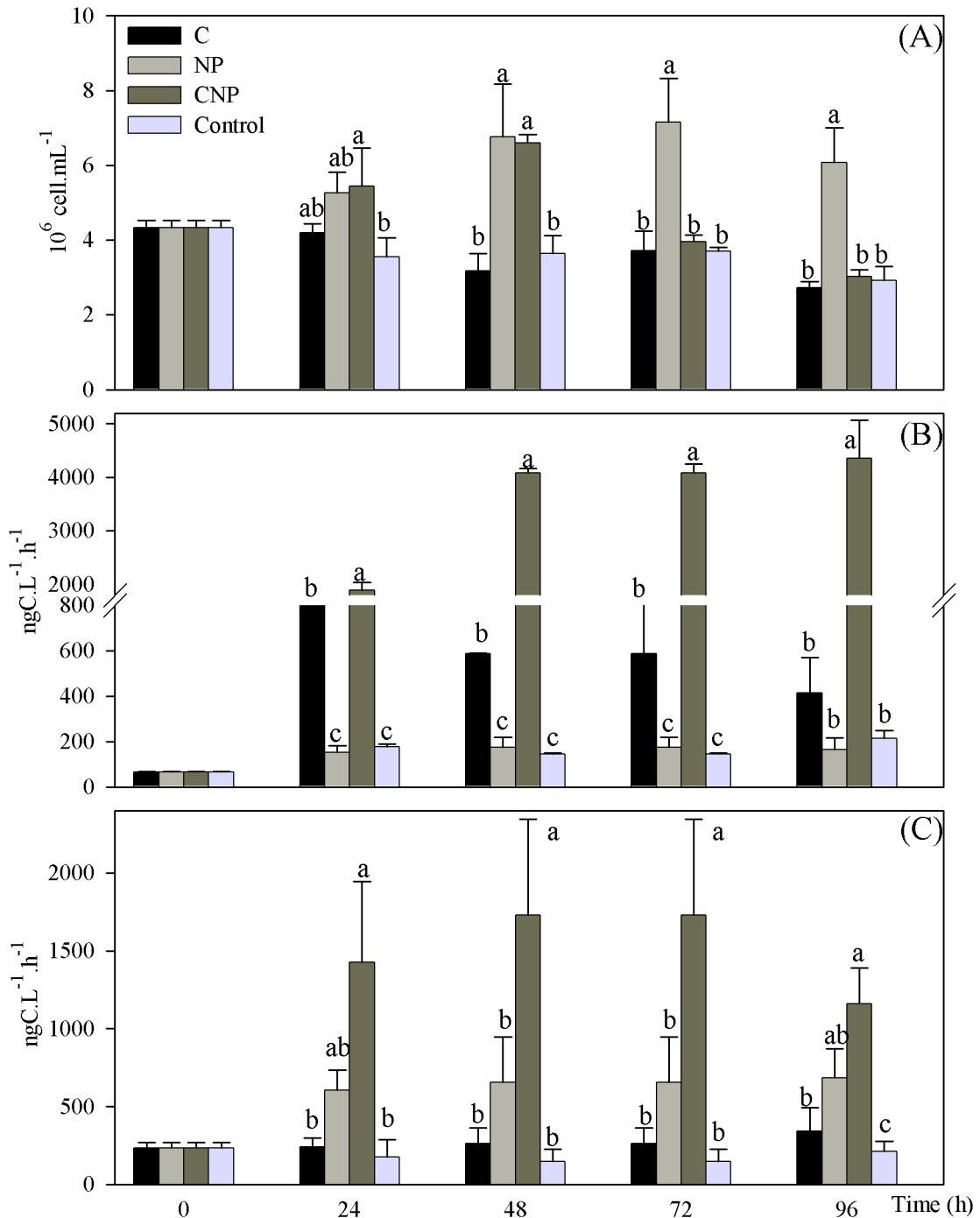


Figure. 5: Changes in bacterial parameters in different treatments (C, NP, CNP and Control) in mesocosms during the summer period experiment in the mesoeutrophic reservoir. (A) Bacterial abundance. (B) Bacterial glucose assimilation. (C) Bacterial production. Letters represent significant differences between treatments (ANCOVA). (n.s. is no significant)

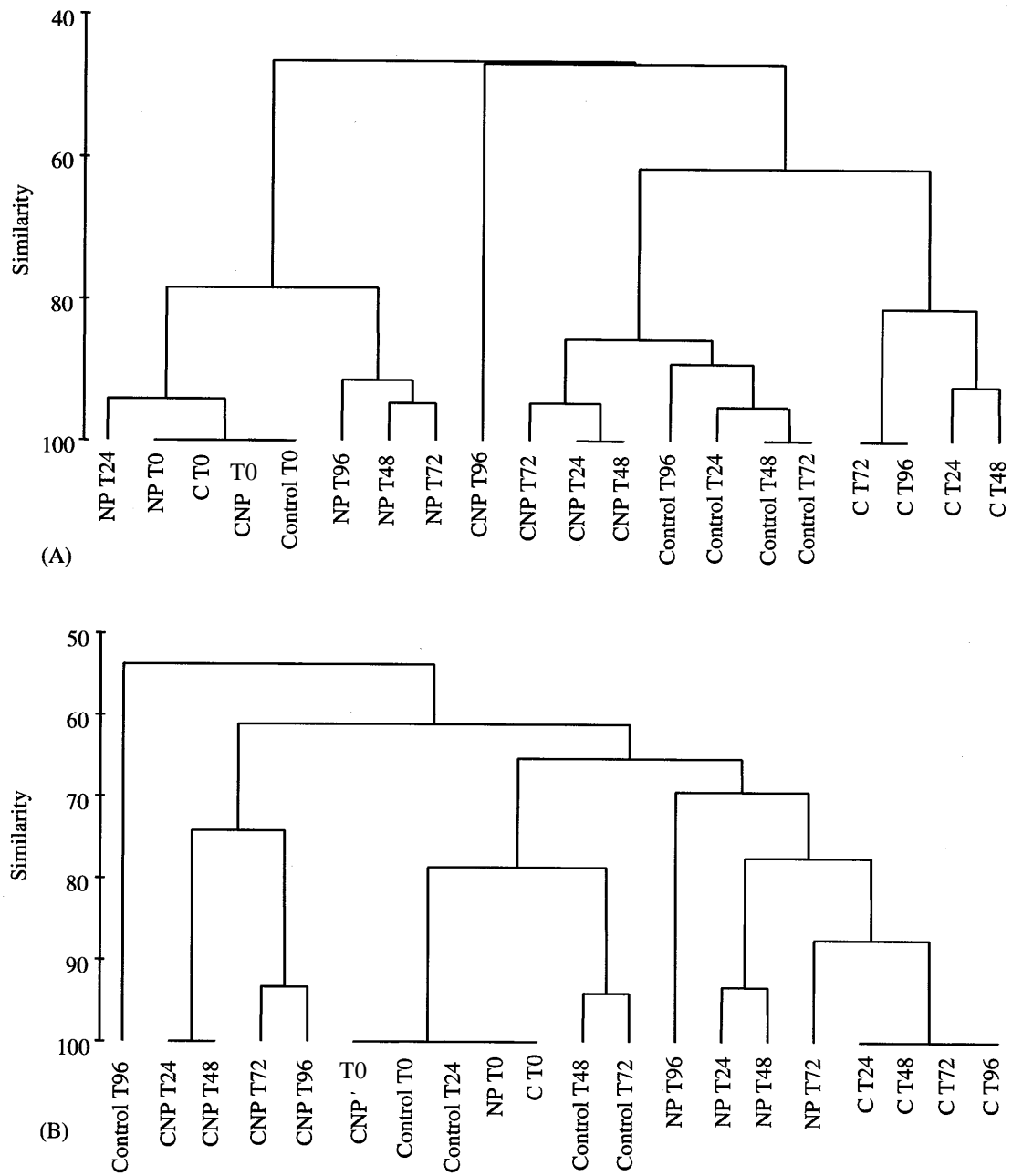


Figure. 6 : Dendrogram showing the similarity of DGGE patterns from mesocosms and sampling time. Clustering is based on the simple matching algorithm, while the dendrogram is drawn applying the group average method. (A corresponding to the clear water phase period and B to the summer period)

Glucose assimilation of CNP treatment (ANCOVA $p < 0.001$) had a strong increase from 0 to 48 hours passing from $65 \text{ ngC.L}^{-1}.\text{h}^{-1}$ to $4300 \text{ ngC.L}^{-1}.\text{h}^{-1}$ and remained at this value for the end of the experiment. Highest value of glucose assimilation in C treatment was at 24 h and declined after. The production increased in all treatments compared to Control. The NP treatment had a strongly production increase with $200 \text{ ngC.L}^{-1}.\text{h}^{-1}$ the first day until $700 \text{ ngC.L}^{-1}.\text{h}^{-1}$ the last day (ANCOVA $p < 0.001$) and the CNP treatment had the strongest increase with $1700 \text{ ngC.L}^{-1}.\text{h}^{-1}$ at 76 h and then fell to $1100 \text{ ngC.L}^{-1}.\text{h}^{-1}$ after 96h (ANCOVA $p < 0.05$).

During the clear water phase (Fig. 6A), the bacterial community was constituted of 9 OTUs. All the mesocosms, including the Control treatment, showed a modification of composition at 24 h. Then, the Control treatment presented a stable bacterial community. The CNP treatment remained close to the Control except after 96 h, displaying at this moment weak similarities (45%) with the other samples. The addition of inorganic (NP) or organic (C) nutrients induced changes during the experimental period. The number of OTUs had strong fluctuation in NP treatment and the C treatment lost 2 OTUs. After 96h, the different treatments had less than 70% of similarities with the Control. During summer period (Fig. 6B), the bacterial community presented 8 OTUs. Control mesocosms differed from the original communities. The CNP treatment had the strongest fluctuation in OTUs. The C treatments had a stable community (>75% of similarities). The NP treatment evolved during experimental period for reaching 60% of similarities with the original communities. At the end of the experiment similarities between treatments and Control were only 50%. But no statistical differences were found by ANOSIM test in the both periods ($p > 0.05$).

Discussion

Experimental Design. We chose an experimental approach using microcosms to be able to control the action of various resources. The enrichment allowed following the evolution of the bacterial communities during a strong increase of particular nutrients, such as during a pollution arriving by a tributary in a reservoir.

Few studies used so long incubation periods (96 h) with a short time sampling interval (24 h). Only Simek and al [39] and Lebaron and al [24] carried out similar studies. However most of the studies were about a specific period of the year and did

not compared the seasons [16, 18, 33, 37]. Likewise, when experiments occurred in different periods, they were on only one freshwater system [20, 45].

The bacterial variables. Bacterial variables remained constant in Controls mesocosms. The confinement of communities in bottles did not affect the microbial dynamics. There was no nutrient depletion and therefore no experiment-driven limitations during the control experiment. On the contrary, in enrichment mesocosms, various reactions were observed. Treatments enriched with organic substrate (C treatment) do not present significant stimulation of the bacterial production and density. Donachie and al. [13] and Shiah and al. [36] also observed this phenomenon. Thus, the dissolved organic carbon (DOC) naturally present in the water was sufficient in both quantitative and qualitative terms to support bacterial growth. Coveney and Wetzel [10] came to similar conclusions after the addition of a mixture of organic substrates in an oligotrophic reservoir (lake Laurence, Michigan). On the contrary, Verde et al. [45] observed an effect of C addition during autumn. However, they observed nutrient limitation in their enclosures; a phenomenon that was not observed in our experiments whatever the period or the reservoir considered. Glucose assimilation was on the contrary stimulated in many cases in the C treatment. This indicates that bacteria shifted from an unknown source of carbon, energy for respiration to glucose.

The enrichments with nitrogen and phosphorus did not induce increase in bacterial production, contrary to the works of Fisher et al. [16]. The clear water phases in both reservoirs presented lower limitations by these nutrients compared to the summer period. Experiments by Coveney and Wetzel [10] showed an increase of bacterial production and density due to enrichments with inorganic nutrients and more particularly phosphorus. The physico-chemical parameters in our reservoirs showed a strong decrease in phosphorus during the stratification period, under the detection limit. We can conclude that our reservoirs presented no limitations of phosphorus and/or nitrogen for bacteria during clear water phase and summer period.

However, CNP treatments showed production and density stimulations whatever the period or the reservoir considered. This occurred when C treatments showed that organic nutrients did not limit bacteria and NP treatments showed that inorganic nutrients did not limit bacteria. Moreover, an increase in glucose assimilation

occurred in all reservoirs when glucose was added, labile C may have accumulated during P limitation in aquatic systems [44]. Bacteria would therefore be limited at first by inorganic nutrients and would become in a second time limited by the carbon after addition of inorganic nutrients [4, 16]. Furthermore, bacteria are better competitors for P [12] when they are not limited by C [21]. Synergistic effect may therefore occur between nutrients. Indeed, the N-species allow a better absorption of the glucose [13] conferring to bacterial cells a better capacity to development in the treatment with double contributions. Furthermore, the absorption of nutrients depends on internal molecular ratios in bacteria [13] but also ratio in the environment. Thus, the nutrients recycling by bacteria may increase according to the molecular ratio. When the ratio C:N is higher than 6:1, the recycling of the NH_4^+ increased, on the contrary a ratio close to 1 bring a weaker recycling and thus a lower growth. Moreover, more C:P ratio is high and more the release of P is possible [19]. But, it was difficult to know the exactly molecular ratio in our reservoirs.

During the summer periods, the monitored bacterial variables reacted quickly to the nutrients contributions. Lebaron and al. [24] found the same velocity of growth with, in parallel, a strong consumption of nutrients added during the first 3 days during the experiment. During the clear water phase experiments, the oligomesotrophic reservoir, and to a lesser extent the mesoeutrophic reservoir, showed a later bacterial development (after 48 hours). Fisher et al. [16] observed the same development in a dilution culture experiment. In the present study, bacterioplankton production appeared to be a more sensitive measure of the bacterial community response compared with bacterial abundance [29]. Vrede et al. [45] found that bacterial abundance needs 3-4 d to response to the nutrient manipulations. But glucose assimilation appeared as a sensitive measure too with a fast response to glucose loading and intensity of assimilation seemed to be an indicator of inorganic limitation.

Bacterial community composition. Few changes appeared in the community composition in single contribution treatment (NP or C). A modification of 1 or 2 OTUs was observed in time. The community presented in the double contributions treatment showed more important changes, confirming the works of Ovreas et al. [28] who observed major changes in DGGE patterns led to a *Vibrio*-dominated bacterial

community. The strongest modification was observed during the clear water phase in the oligomesotrophic reservoir with a strong decrease of the number of OTUs. So, a large part of the community would be competent to take advantage of double enrichments. Vadstein [41] observed that some groups of bacteria reacted to one pulse of nutrients while the others were competent when the concentrations of nutrients remained constant. Thus, some bacteria have a better adaptation of competition. The weak modifications obtained in the community could also be due to some bacteria with fast growth, indeed Riemann and Winding [32] found that some bacteria (*Beta-Proteobacteria* and *Cytophagales*) were bacteria with fast growth that can be preferentially selected during particular events (decomposition of biomass algae). These bacteria were probably capable of fitting quickly their growth rate to new conditions quickly but weakly changes of composition of the bacterial communities.

Conclusion

It is always delicate to extrapolate the processes observed in mesocosm experiments to the processes taking place *in situ* at a larger scale. However our results suggest that nutrients play synergistically a key role in the regulation of abundance and the bacterial activity. On the other hand, nutrients seem to have only a weak effect on the eubacterial composition at a short time scale, producing only a modification of one in two OTUs during the experiments.

The weak changes in the bacterial community composition compared to the large variations in the bacterial activity and density indicates that this community was predominantly composed of generalist bacteria that are efficient in using a large array of food resource varying in both quality and quantity. The existence of generalists and specialists in bacterial communities has already been hypothesised by Langehender et al. [23].

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**Expériences de translocation dans un complexe de réservoirs formant un gradient
d'eutrophisation : effets sur les communautés bactérioplanctoniques.**

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Résumé

Nous avons effectué des expériences de translocations dans le complexe de réservoirs (barrage et pré-barrage) de la Sûre (Luxembourg) pour estimer les facteurs qui contrôlent l'abondance, l'assimilation du glucose et la production bactérienne ainsi que la composition de la communauté bactérienne. Les échantillons du barrage et du pré-barrage sont incubés dans des sacs de dialyse placés *in situ* dans leur milieu d'origine et dans l'autre milieu durant trois périodes au cours de l'année. Les sacs sont échantillonnés toutes les 24h pour estimer l'abondance, l'assimilation, la production et la composition de communauté bactérienne (par DGGE). Nos résultats montrent que les réservoirs sont rarement limités en nutriments, que les intensités des activités mesurées dépendent plus du milieu que de la communauté bactérienne. Cette dernière est capable de s'adapter phénotypiquement à de nouveaux milieux sans fortes modifications génotypiques.

1. INTRODUCTION

Les réservoirs présentent un grand intérêt socio-économique. Ces masses d'eau artificielles ont été créées pour des raisons spécifiques de gestion de l'eau potable et des eaux de crues. Stockage d'eau, contrôles de flux, création d'électricité et loisirs font partis de leurs diverses fonctions. Ces pratiques peuvent être mises en péril par une eutrophisation rapide de l'eau (VOLLENWEIDER, 1990). L'eutrophisation des systèmes aquatiques est généralement causée par l'apport régulier en nutriments à partir du bassin versant et leur longue rétention au sein du système, notamment au niveau du sédiment. Ainsi, par leur fonction de rétention d'eau, les réservoirs peuvent subir une accentuation de l'eutrophisation. Malgré l'importance de ces systèmes, ils restent peu étudiés (GASOL *et al.*, 2002 ; SIMEK *et al.*, 2003).

Lors d'apports en matière organique, une partie va être recyclée par le bactérioplancton, qui est le principal décomposeur de la matière organique. Ce bactérioplancton regroupe l'ensemble des organismes hétérotrophes les plus abondants à l'intérieur du réseau trophique et se situe à la base de la boucle microbienne (AZAM *et al.*, 1983). Il a un rôle clef dans le fonctionnement des systèmes aquatiques à travers les processus de recyclage et les flux d'énergie (CHO et AZAM, 1988). De plus, ils constituent une source de nourriture alternative aux algues pour le zooplancton de certains lacs et réservoirs (JÜRGENS, 1994 ; THOUVENOT *et al.*, 1999 ; BURNS et SCHALLENBERG, 1998) L'étude de ces organismes dans les réservoirs permettrait de mieux comprendre la réponse de ces systèmes aquatiques au phénomène d'eutrophisation.

Un défi actuel est de comprendre les mécanismes contrôlant ce bactérioplancton. De manière générale la température et les ressources, la prédation et la lyse virale ont été identifiées comme étant les processus majeurs de contrôle des dynamiques spatiales et/ou temporelles des bactéries (WRIGHT, 1988 ; SANDERS *et al.*, 1992 ; FUHRMAN, 1999). Les changements dans les nutriments limitants sont connus pour modifier l'ensemble des propriétés des communautés bactérioplanctoniques, en terme d'abondance, de production et de diversité (CHRZANOWSKI *et al.*, 1995 ; SCHWEITZER et SIMON, 1995 ; SIMEK *et al.*, 1999). Cependant, ces facteurs limitant peuvent montrer des variations saisonnières à travers différents systèmes aquatiques (MORRIS et LEWIS, 1992 ; SCHWEITZER et SIMON, 1995 ; SKOOG *et al.*, 1999). L'estimation et la compréhension des facteurs qui contrôlent les bactéries facilitera la connaissance de la circulation des nutriments dans la chaîne trophique planctonique. Notre objectif est d'élargir la compréhension concernant la limitation des bactéries par les nutriments dans différents réservoirs par des expériences de translocation de communautés bactériennes entre réservoirs de statuts trophiques différents.

Nous reportons ici ces expériences qui consistent à incuber l'entièreté des communautés planctoniques *in situ* dans des sacs à dialyse. Cette méthode permet de modifier l'ensemble

des conditions physico-chimiques du milieu sans modifier la communauté planctonique (HERNDL *et al.*, 1993).

2. MATERIELS ET METHODES

2.1. Sites d'étude

Le complexe de réservoirs interconnectés construits sur la rivière Sûre au Luxembourg formé d'un réservoir pré-barrage eutrophe (Pont-misère) et d'un lac de barrage mésoeutrophe (Réservoir d'esch-sur Sûre). Ce dernier (latitude 49°54'; longitude 5°53') est un réservoir stratifié d'une surface de 3,2 km² et d'une profondeur moyenne de 17 m. Le prébarrage est un réservoir peu profond (profondeur maximale = 4 m) d'une surface de 0,2 km².

2.2. Echantillonnage

Des échantillons ont été effectués grâce à une bouteille de Ruttner (2 litres) tous les mètres dans l'épilimnion durant la stratification ou de la surface jusqu'à 10 m de profondeur durant la période de brassage des eaux dans le barrage mésoeutrophe. Ce même échantillonnage a été effectué tous les ½ mètres sur la totalité de la colonne d'eau pour le prébarrage eutrophe. Pour chaque réservoir, les échantillons sont réunis dans une bouteille stérile de 50 L. Le volume d'eau prélevé ne subit aucune filtration. Les communautés planctoniques sont ainsi non modifiées dans leurs proportions relatives par rapport à la situation d'origine.

2.3. Dessin expérimental

Le complexe de réservoirs de la rivière Sûre présentant une différence de niveau d'eutrophisation de l'amont vers l'aval, il se prêtait parfaitement à des expériences de translocation afin d'évaluer l'effet d'une variation de la charge nutritive (enrichie ou appauvrie) sur les communautés bactériennes. La translocation consiste à transférer des communautés enfermées dans des sacs à dialyse d'un milieu dans un autre. Ainsi les communautés du réservoir eutrophe ont été placées dans le réservoir mésoeutrophe et inversement. Ces communautés planctoniques sont retenues dans des sacs de dialyse (spectra/por) d'une porosité de 25 Angströms ne laissant passer que les molécules inférieures à 12 kDa dont les nutriments. Les sacs de dialyse d'une longueur de 30 cm et d'un volume de 1,5 L sont maintenus à une profondeur où seul 15% de la lumière incidente pénètre. Les intensités lumineuses ont été déterminées à l'aide d'un luminomètre submersible (Li-Cor 1000).

L'évolution des communautés transférées est comparée à celle des communautés du milieu récepteur, également contenues dans des sacs à dialyse et servant de contrôle.

Ces expériences de translocation ont été exécutées dans les deux réservoirs à trois reprises au cours de l'année : aux mois d'avril, de juillet et d'octobre. Ces périodes ont été choisies car elles se sont révélées être des périodes clefs dans les dynamiques saisonnières picoplanctoniques de ces réservoirs (Chapitre I). Ainsi, les échantillonnages ont été effectués le 12 et le 19 juillet 2004, le 4 et le 11 octobre 2004 et enfin le 11 et le 18 avril 2005. Ces expériences ont duré 4 jours avec une fréquence d'échantillonnage journalière.

2.4. Variables suivies

La température de l'eau, la concentration en oxygène dissous, le pH et la conductivité ont été déterminés par une sonde multiparamétrique (Hydrolab DS4). Les concentrations en orthophosphates ($\text{PO}_4\text{-P}$), en ammonium ($\text{NH}_4\text{-N}$), en nitrite ($\text{NO}_2\text{-N}$), en nitrate ($\text{NO}_3\text{-N}$), en chlorure (Cl), en sulfate (SO_4) et en silice (SiO_2) ont été analysés en laboratoire selon des méthodes standards (APHA 1995). Les échantillons pour le carbone organique dissous (COD) ont été filtrés sur des membranes GF/F grillé (taille de pore 0,8 μm , Whatman) puis congelés dans de l'azote liquide. Le COD a été estimé par un analyseur élémentaire (TOC-5000 Shimadzu)

Pour l'énumération bactérienne, dix mL d'échantillon ont été conservés avec du formol (concentration finale 2%) à 4°C et dans l'obscurité jusqu'au comptage. Les cellules ont été colorées au Sybr Green I (Molecular probes) (concentration finale de 0,06%) pendant 15 minutes à température ambiante dans l'obscurité (NOBLE et FUHRMAN, 1998), puis filtrés sur membrane noire de polycarbonate 0,2 μm (Millipore). Les cellules ont alors été comptées par microscopie epifluorescente (Leica, DMRB) (HOBBIE *et al.*, 1977) avec un grossissement de 1.000x sous excitation bleue (excitation: 450-490 nm, émission > 515 nm). Un minimum de 500 cellules a été compté sur chaque filtre préparé.

La production bactérienne (BP) a été évaluée en mesurant l'incorporation de ^{14}C -leucine (KIRCHMAN *et al.*, 1985). Dix mL d'eau échantillonnée ont été incubés en duplicat pendant deux heures à température *in situ* et dans l'obscurité. Deux blancs immédiatement fixés au formaldéhyde (concentration finale 2%) ont été incubés de la même manière que les échantillons. Les incubations des échantillons ont été arrêtées par ajout de formaldéhyde (concentration finale 2%). L'extraction des protéines a été réalisée avec de l'acide trichloroacétique (TCA) 5% à chaud (80°C) pendant 120 min. Les échantillons ont ensuite été filtrés sur filtres en acétate de cellulose et rincés avec du TCA 5% froid. Les filtres ont alors été transférés dans des fioles à scintillation (PackardBioscience) de 7 mL avant dissolution par l'acétate d'éthyle et le liquide de scintillation. Le taux de désintégration des molécules radioactives a été mesuré par un analyseur de liquide scintillant (PerkinElmer). L'assimilation de

¹⁴C-glucose suit la même procédure que l'incorporation de leucine mais sans extraction au TCA. Les échantillons ont été directement filtrés sur filtres en acétate de cellulose.

Pour l'analyse de la composition de la communauté bactérienne par DGGE (électrophorèse sur gel à gradient dénaturant), un échantillon de 200 mL est filtré sur membrane (diamètre des pores, 0,22 µm ; diamètre de la membrane 47mm ; Supor200 ; Pall corporation). La membrane est déposée dans 2 mL de tampon de lyse (40 mM EDTA, 400 mM NaCl, 0.75 M sucrose, 50 mM Tris-HCl pH : 8.3) et conservé à -20°C jusqu'à extraction. L'ADN est extrait par phénol-chloroforme 50/50 (vol/vol) et précipité par éthanol. Les fragments utilisables pour les analyses DGGE ont été obtenus comme décrits par MUYZER et SMALLA (1998) avec la combinaison de l'amorce 341f ayant une attache polyGC (séquence riche 40 nucléotides GC, 5'-CCT ACG GGA GGC AGC AG-3') et 907r (5'-CCG TCA ATT CMT TTG AGT TT-3'). L'amplification PCR est effectuée avec un thermocycleur Biometra. Chaque mélange contient 2 ng d'ADN, 0,5 µM des 2 amorces, 200 µM de triphosphate désoxynucléosides, 1,5 mM de MgCl₂, 5 µl de tampon PCR 10x et 1 U de *Taq* polymérase (Amersham), avec ajustement à un volume final de 50 µl avec de l'eau stérile. La PCR consiste en une incubation à 94°C durant 5 min, suivie de 37 cycles constitués d'une dénaturation à 94°C pendant 1 min, hybridation à 55°C pendant 1 min et extension à 72°C pendant 1 min. Ensuite, les tubes sont incubés pendant 7 min à 72°C. La DGGE pour les bactéries est réalisée avec un System D-Code (Bio-Rad). Les échantillons PCR sont déposés dans un gel à 8% (masse/vol) d'acrylamide d'épaisseur 1mm dans un tampon TAE 1x (20 mM Tris-acétate [pH 7.4], 10 mM acétate, 0.5 mM disodium EDTA). Le gradient dénaturant s'échelonne de 20 à 70% (le 100% correspond à 7 M d'urée et 40% [vol/vol] de formamide déionisé). La migration s'effectue à 100 V pendant 15 h. La température est de 60°C. Puis les gels DGGE sont révélés au Sybr Gold (Molecular Probes) et photographiés sur une table à transillumination UV.

2.5. Outils statistiques

Un test-t de Student (logiciel Sigma Stat) est utilisé pour comparer les variables abiotiques des différents réservoirs, mais aussi pour tester les changements potentiels induits dans les variables bactériennes par les translocations. Les gels DGGE sont analysés en utilisant le logiciel « Genetools for Syngene » et transformés en matrice de présence/absence de bandes. La création de clusters est basée sur un algorithme simple (« simple matching algorithm ») et les dendogrammes sont conduits en appliquant la méthode de moyenne de groupe grâce au logiciel Primer. Les analyses de similarités (ANOSIM) sont utilisées pour comparer la composition de la communauté bactérienne (CLARKE, 1993).

3. RESULTATS

3.1. Printemps

Durant l'expérience de printemps (avril 2005), le pré-barrage est significativement ($p < 0,05$) plus riche en nutriments inorganiques que le barrage (Tab.1). La quantité de COD est statistiquement identique dans les deux réservoirs. Il en va de même pour la température. Cependant le barrage mésoeutrophe présente une meilleure oxygénation ainsi qu'un pH à tendance basique.

Au début de l'expérience (0h), les densités bactériennes et les productions bactériennes entre les deux réservoirs sont très proches (Fig.1), ne présentant pas de différences statistiques. Seule l'assimilation de glucose est statistiquement différente ($p < 0,001$) ; les communautés du barrage mésoeutrophe présentant des taux d'assimilation de 43% plus élevés que la communauté du pré-barrage eutrophe. Au cours des expériences, les communautés transloquées présentent des dynamiques très semblables à celles des contrôles situés dans le même réservoir. Ainsi une augmentation tout à fait parallèle des assimilations de glucose est observée pour les deux traitements dans le pré-barrage. La seule tendance qui ressort clairement des observations faites en avril 2005 est une augmentation significative de la production bactérienne dans le contrôle situé dans le pré-barrage par rapport au traitement transloqué.

La comparaison des caractéristiques des traitements transloqués par rapport aux caractéristiques des contrôles restés dans le milieu d'origine est présentée dans la Table 2. De nombreuses différences significatives sont observées en ce qui concerne le milieu mésotrophe dont la densité, l'assimilation de glucose et la production de ses communautés bactériennes augmentent à la faveur de la translocation. A l'inverse la comparaison de la translocation vers le barrage (Trans-) et le contrôle du pré-barrage (Ctr+) montre simplement une légère diminution significative de la production par l'échantillon Trans- à partir de 48h.

D'un point de vue diversité, les communautés bactériennes des deux réservoirs sont distinctes et le resteront tout au long de l'expérience (Fig. 2). Au printemps, la communauté transloquée du pré-barrage eutrophe vers le barrage mésoeutrophe (Trans-) ne se modifie pas au cours de l'expérience. Le contrôle dans le barrage (Ctr-) se différencie légèrement (90% de similarité) à partir de 48h par rapport à la communauté d'origine. La communauté transloquée du barrage vers le pré-barrage (Trans+) et le contrôle dans le pré-barrage (Ctr+) se modifient faiblement à partir de 48h. Cependant les tests ANOSIM ne montrent aucune différences significatives ($p > 0,05$).

Table 1: Caractéristiques physico-chimiques entre le barrage et le pré-barrage lors des trois périodes d'expérimentation : avril 2005, juillet 2004 et octobre 2004. Comparaison effectuée par test-t de Student, le seuil de signification utilisé est 0,05.

Périodes	Avril			Juillet			Octobre		
	Site	Pré	Barrage	Pré	Barrage	Pré	Barrage	Pré	Barrage
PO ₄ -P (mg P L ⁻¹)	0.018	<0.005	***	0.043	<0.005	***	0.021	<0.005	***
NH ₄ -N (mg N L ⁻¹)	0.046	0.015	***	0.113	0.044	**	0.078	0.020	***
NO ₃ -N (mg N L ⁻¹)	3.7	5.2	***	1.7	3.8	***	2.4	2.7	***
COD (mg.L ⁻¹)	2.5	2.3	n.s.	5.6	3.7	***	3.8	3.8	n.s.
Température (°C)	11.3	10.2	n.s.	17.8	18.9	n.s.	11.5	15.2	***
Oxygène (% de saturation)	96	108	**	79	97	**	87	99	**
Conductivité (µS cm ⁻¹)	1479	1262	*	1657	1557	***	1600	1615	n.s.
pH	7.6	8.4	*	7.8	8.9	***	7.8	10.1	***

Table 2: Résultats des comparaisons par test-t de Student entre échantillons restés dans leur milieu d'origine et échantillons transloqués (comparaison : Ctr-/Trans+ et Ctr+/Trans-) au cours du temps et suivant les différentes périodes d'expérimentation. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

		Avril			Juillet			Octobre		
		T24	T48	T72	T24	T48	T72	T24	T48	T72
Barrage	Densité		*	*				***	*	
	Assimilation	*	**	***				**	***	
	Production	**	***	**						***
Prébarrage	Densité									
	Assimilation							***	***	
	Production		*	*						

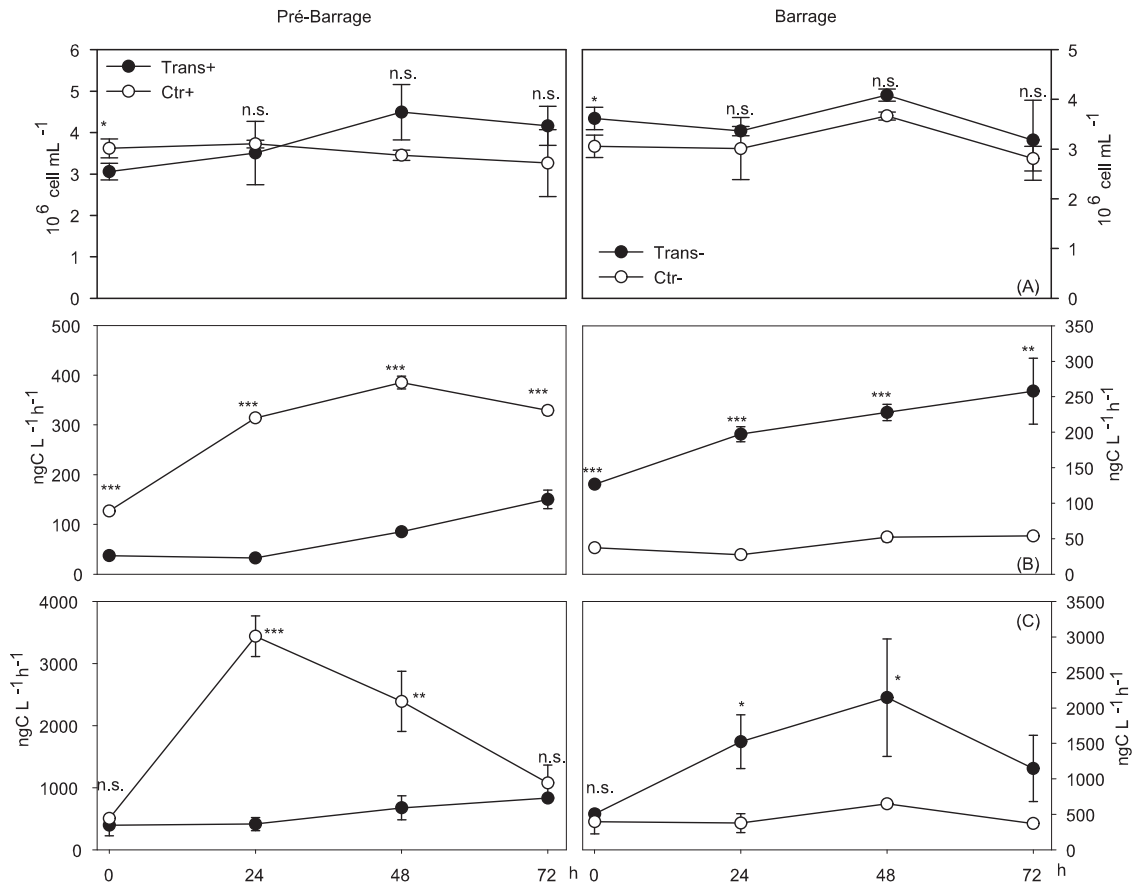


Figure 1 : Suivi de la densité (A), de l'assimilation de glucose (B) et de la production (C) des échantillons transloqués (Trans) et des Contrôles (Ctr) dans le pré-barrage (+) et le barrage (-) de la Sûre au cours de l'expérience d'avril 2005. (Moyenne et écart-type sont représentés). Les astérisques représentent les résultats des tests t entre les différents traitements (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s.= non significatif).

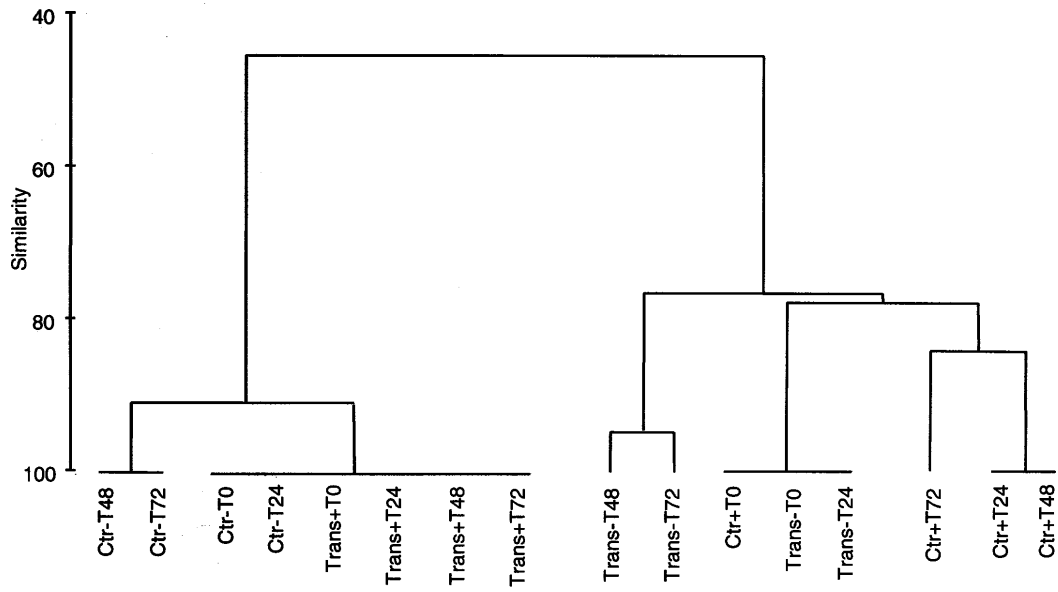


Figure 2 :Dendrogramme de similarités des schémas obtenus par DGGE suivant l'échantillon et le temps. Expérience de printemps. (Trans = translocation, Ctr = contrôle, + = eutrophe, - = mésoeutrophe)

3.2. *Eté*

La température est identique dans les deux réservoirs (Tab.1). Comme au printemps le pré-barrage eutrophe est plus riche en nutriments que le barrage mésoeutrophe. Cependant contrairement à la période printanière le COD a une concentration plus élevée dans le pré-barrage que dans le barrage. Les autres conditions sont les mêmes qu'au printemps avec toujours un pH basique et une bonne oxygénation du milieu mésoeutrophe.

L'expérience se déroulant en été (Fig.3) montre de fortes différences entre les deux réservoirs en début d'expérience (0h) pour toutes les variables bactériennes suivies. Au cours du temps, les variables des communautés transloquées et des contrôles vont tendre à devenir similaire. Ainsi les densités statistiquement distinctes en début d'expérience, se décalent l'une vers l'autre pour ne plus présenter de différence significative en fin d'expérience (72 h). Dans le pré-barrage eutrophe, l'échantillon transloqué (Trans+)venant du barrage mésoeutrophe présente une augmentation d'assimilation se rapprochant du contrôle de ce milieu(Ctr+). Cependant il y a toujours différence significative en fin d'expérience (72 h). A l'inverse une légère diminution de l'assimilation de glucose du Trans- et une forte augmentation du Ctr- ont lieu dans le barrage, amenant à une différence non statistiquement significative. De même, les productions distinctes à 0h, présentent une forte augmentation dans l'échantillon Trans+, ne montrant plus de différence avec le Ctr+ dès 24h. De même la production dans l'échantillon Trans- augmente mais reste statistiquement distincte du contrôle (Ctr-).

Les comparaisons des échantillons transloqués et des échantillons restés dans le milieu d'origine ne présentent aucune différences significatives (Tab.2).

Les communautés bactériennes montrent une diversité relativement stable tout au long de l'expérience (Fig. 4) avec une faible modification par rapport à la communauté de départ vers 24 h. Cependant l'ensemble des traitements montre une seconde modification vers 72 h, seul le Ctr- a une modification de communauté vers 48 h. Toutefois, les tests ANOSIM ne montrent pas de différences significatives par rapport à la communauté de départ. De plus aucun rapprochement de composition de communauté n'apparaît entre les communautés Trans-/Ctr- et Trans+/Ctr+.

3.3. *Automne*

Durant cette période (octobre 2004), la température est distincte entre les deux réservoirs, le barrage mésoeutrophe ayant une température de l'eau supérieure au pré-barrage. Le pré-barrage eutrophe reste plus riche en nutriments que le barrage mésoeutrophe. Cependant le COD est identique dans les deux milieux. Le barrage présente toujours un pH basique et une bonne oxygénation.

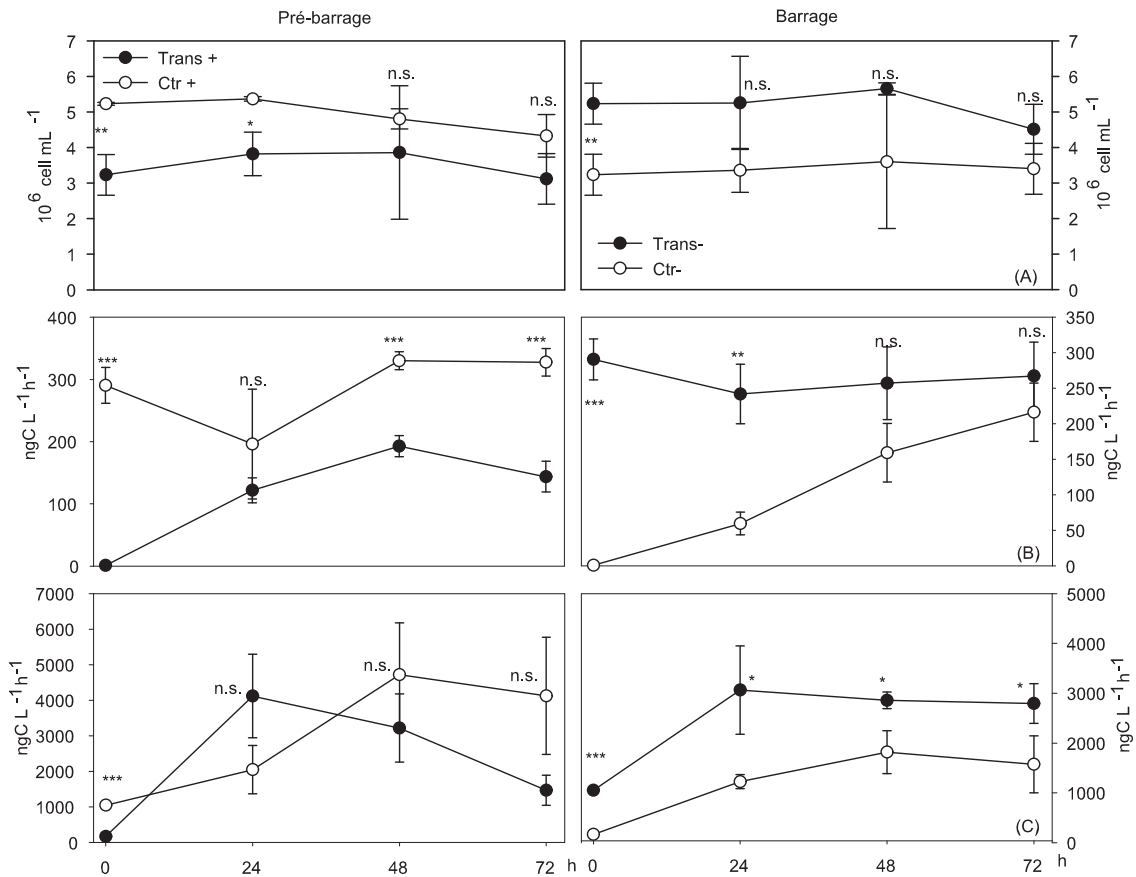


Figure 3 : Suivi de la densité (A), de l'assimilation de glucose (B) et de la production (C) des échantillons transloqués (Trans) et des Contrôles (Ctr) dans le pré-barrage (+) et le barrage (-) de la Sûre au cours de l'expérience de juillet 2004. (Moyenne et écart-type sont représentés). Les astérisques représentent les résultats des tests t entre les différents traitements (* <0.05 ; ** <0.01 ; *** <0.001 ; n.s.= non significatif).

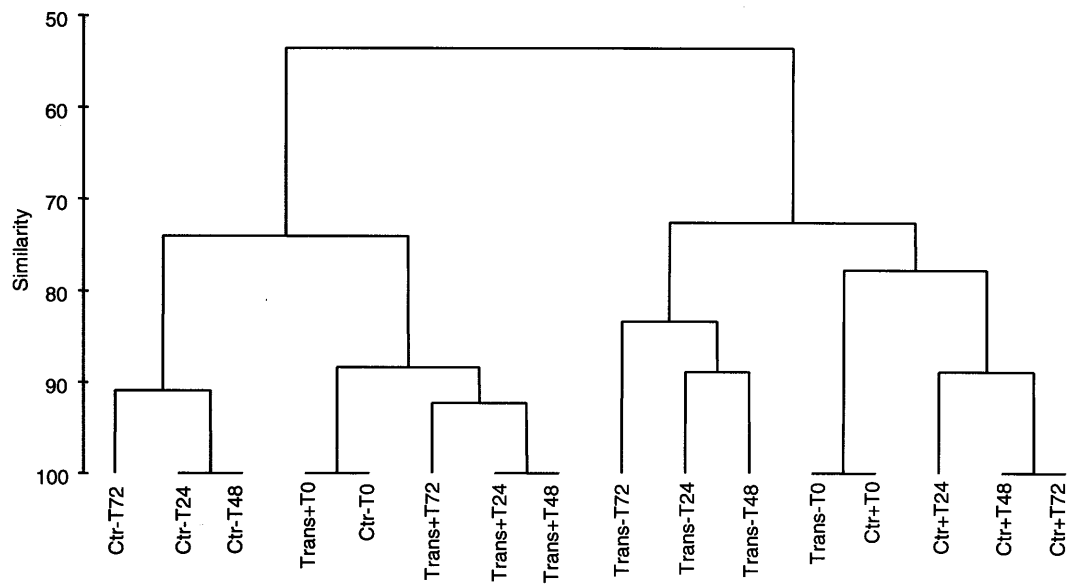


Figure 4 :Dendrogramme de similarités des schémas obtenus par DGGE suivant l'échantillon et le temps. Expérience d'été. (Trans = translocation, Ctr = contrôle, + = eutrophe, - = mésoeutrophe)

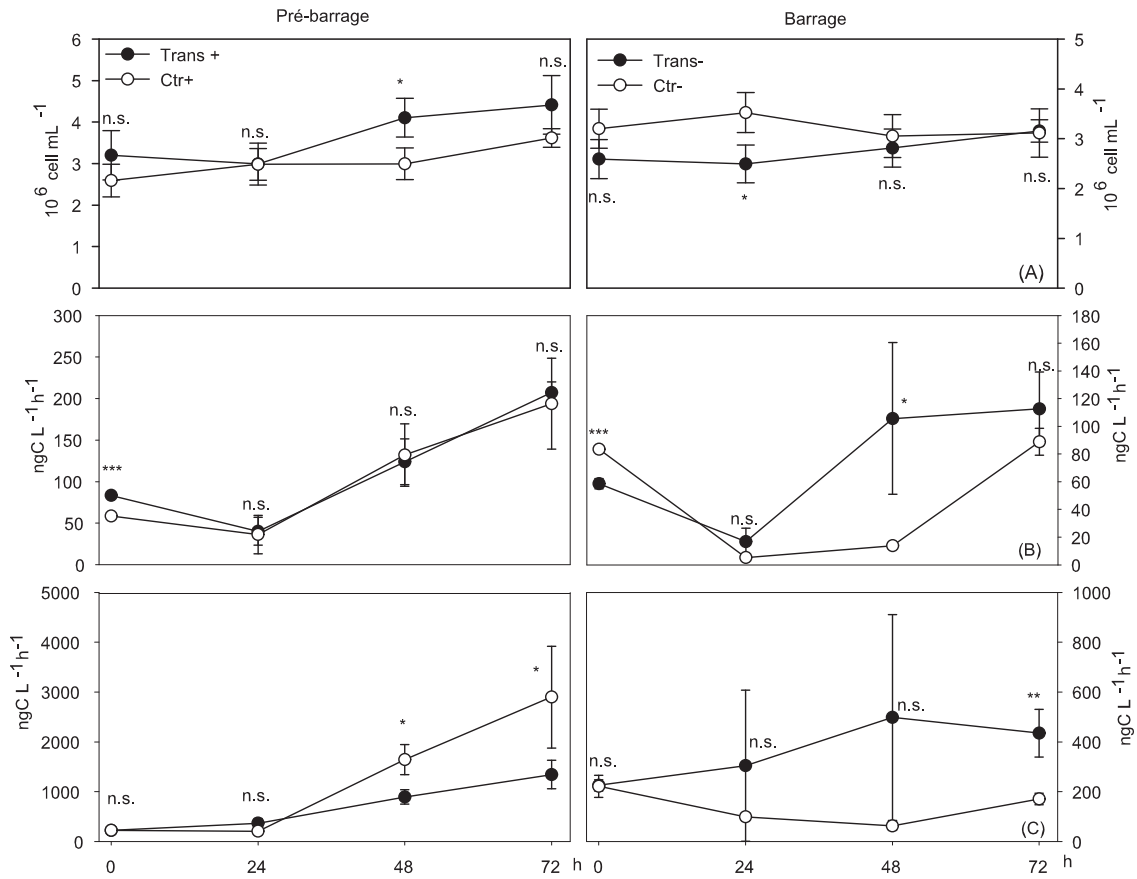


Figure 5: Suivi de la densité (A), de l'assimilation de glucose (B) et de la production (C) des échantillons transloqués (Trans) et des Contrôles (Ctr) dans le pré-barrage (+) et le barrage (-) de la Sûre au cours de l'expérience de juillet 2004. (Moyenne et écart-type sont représentés). Les astérisques représentent les résultats des tests t entre les différents traitements (* <0.05 ; ** <0.01 ; *** <0.001 ; n.s.= non significatif).

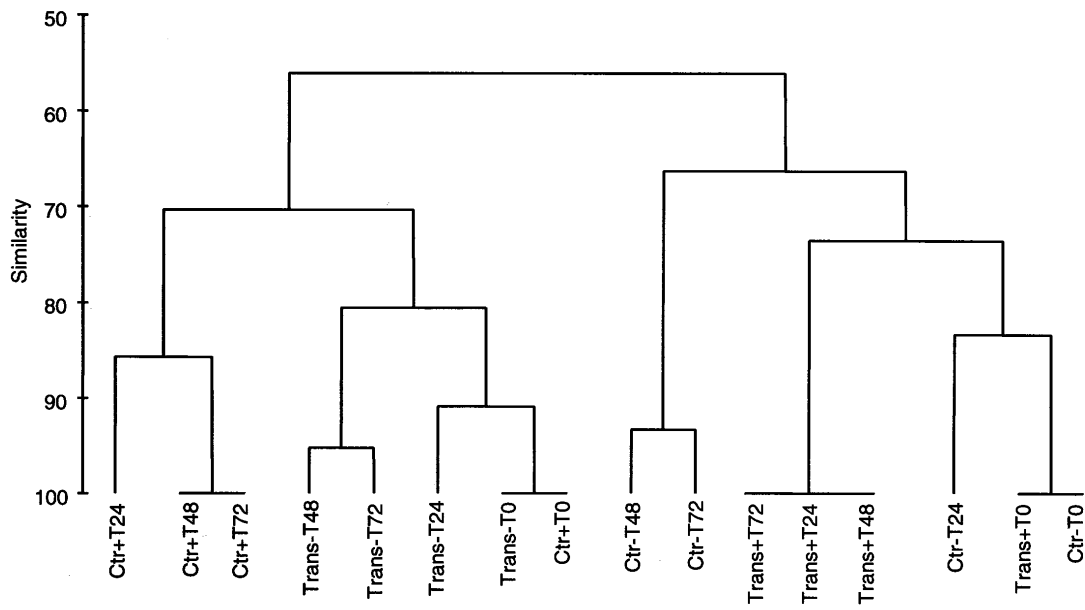


Figure 6 :Dendrogramme de similarités des schémas obtenus par DGGE suivant l'échantillon et le temps. Expérience d'automne. (Trans = translocation, Ctr = contrôle, + = eutrophe, - = mésoeutrophe)

Les communautés automnales se développant dans les deux réservoirs ont des densités et des assimilations différentes mais des productions identiques (Fig. 5). Très rapidement, après 24 h, les densités ne présentent plus de différence statistique entre transloqués et contrôles. Les communautés du pré-barrage présentent des vitesses d'assimilation plus élevées quel que soit le milieu dans lequel elles se trouvent (Ctr+ ou Trans-) et croissent par rapport au début de l'expérience. Alors que les productions sont identiques à 0h, les communautés originaires du pré-barrage (Ctr+ et Trans-) montrent un accroissement de leur production à 24 et 48 h se distinguant statistiquement des communautés du barrage. Toutefois une forte diminution de cette production a lieu à 72 h et ne présente plus de différences significatives.

Cependant la comparaison (Fig. 5 et Tab. 2) entre les activités des communautés transloqués du barrage vers le prébarrage (Trans+) avec celles des communautés du barrage (Ctr-) restées dans leur milieu d'origine montre une augmentation d'activité significative pour l'ensemble des variables suivies. A l'inverse la comparaison Trans- et Ctr+ montre simplement une légère diminution significative de l'assimilation de glucose par l'échantillon Trans- à partir de 48h.

Au cours de cette expérience, les communautés présentent dans le milieu eutrophe (Trans+ et Ctr+) ont une légère modification de composition de communauté à partir de 48 h. A l'inverse les communautés immergés dans le milieu mésoeutrophe (Trans- et Ctr-) ont une modification de composition de communauté dès 24 h (Fig. 6).

4. DISCUSSION

4.1. Aspect méthodologique :

La méthode utilisée permet aux organismes de se développer dans un environnement différent du point de vue physico-chimique et physique de leur milieu d'origine, sans modification du compartiment biotique. Les sacs de dialyse fonctionnent, en effet, comme des cages fournissant aux bactéries un accès à un environnement modifié. Ce type d'expérience présente le grand intérêt de pouvoir faire varier en totalité l'ensemble des nutriments y compris ceux présents sous forme de micro-éléments. Cependant les sacs de dialyse ne sont pas des cages parfaites tout en étant beaucoup plus proche des conditions *in situ* que des expériences en bouteilles (GASOL *et al.*, 2002). En effet, le degré de limitation par les nutriments peut être exagéré par des enclos car l'apport de nutriments par l'hypolimnion ou les sédiments est stoppé (VREDE *et al.*, 1999).

Le complexe de la haute Sûre formé d'un barrage et d'un pré-barrage se prêtait parfaitement à ces expériences, non seulement d'un point de vue logistique (rapidité de mise en place) mais aussi pour des contraintes physiques relativement proches. Ainsi, la température

qui est reconnue comme un facteur de régulation (SHIAH et DUCKLOW, 1994), est la même entre les deux réservoirs au cours de l'année permettant de tester seulement l'impact des nutriments. Toutefois, au cours de l'automne, la température du pré-barrage eutrophe est inférieure à celle du barrage mésoeutrophe sans atteindre le seuil en dessous duquel la température devient limitant pour les bactéries (OCHS *et al.*, 1995). Enfin, dans une étude exploratrice précédente faisant appel à des analyses de redondance et de correspondances, les nutriments sont apparus comme des facteurs significatifs pour les dynamiques bactériennes (Chapitre I). D'où l'intérêt de tester ces nutriments suivant la période et le milieu.

4.2. Activités bactériennes

Suivant la période, la production bactérienne réagit rapidement au changement de milieu, en effet, GASOL *et al* (2002) ont montré qu'il existait un temps de latence de 12 h lors de translocation. Mais passé ce délai, une modification de production est visible dès 24 h.

Nos expériences présentent régulièrement une augmentation de production sans augmentation des densités. La production bactérienne apparaît comme une mesure plus sensible aux changements que l'abondance (PACE, 1993). Cependant cette stabilité de densité peut se traduire par une mortalité bactérienne due à la prédation. De même, l'assimilation de glucose apparaît telle une variable prompte à la réaction lors de changement de milieu, des variations apparaissant dès 24 h.

Malgré les différences en nutriments et en composition de communauté entre le barrage et le pré-barrage, les communautés de printemps (avril 2005) présentent les mêmes densités et les mêmes productions. Ainsi seule l'assimilation de glucose semble une variable dépendante du niveau trophique. La température et le COD ne présentent pas de différence significative entre les deux réservoirs. Ainsi, la température pourrait être le facteur prépondérant au printemps (SHIAH et DUCKLOW, 1994) de même que le carbone qui est relié à la production et à l'abondance bactérienne (COLE *et al.*, 1988). Les bactéries ne pourraient pas se développer en dessous d'un certain seuil de température (OCHS *et al.*, 1995) ou d'une certaine concentration en COD (EILER *et al.*, 2003) malgré la présence de nutriments inorganiques en suffisante quantité. Toutefois, les communautés transloquées du barrage vers le pré-barrage (Trans+) présentent un développement significatif des activités suivies par rapport aux communautés restées dans le barrage (Ctr-). Ainsi cette croissance limitée des bactéries dans le barrage pourrait être due aux faibles concentrations en nutriments. Cet état a aussi été constaté par SIMEK *et al.* (2003) dans le réservoir Rimov (Rép. Tchèque). Ce résultat est aussi confirmé par l'échantillon Trans- (pré-barrage vers barrage) qui présente une diminution de production par rapport au Ctr+ à partir de 48 heures, sans provoquer de diminution de densité. La translocation dans un milieu plus riche pourrait entraîner l'activation de cellules viables mais

métaboliquement inactives décrites par STEVENSON (1978). La translocation dans un milieu plus pauvre diminuerait l'activité pour aboutir à cet état d'inactivation.

A l'inverse durant l'été (juillet 2004), lorsque la température dépasse le seuil permettant un développement des bactéries (OCHS *et al.*, 1995), les communautés ont des activités distinctes d'un milieu à l'autre. Les communautés transloquées du barrage vers le pré-barrage (Trans+) profitent des conditions favorables de ce nouveau milieu à la levée d'inhibition (augmentation d'assimilation et de production). Cependant les communautés restées dans le barrage présentent aussi une augmentation d'assimilation et de production, ceci pouvant s'expliquer par des modifications des conditions du milieu. Cette élévation d'activité explique les différences non statistiquement significatives entre les communautés transloquées du barrage vers le pré-barrage et les communautés restées dans le barrage. Toutefois, le barrage apparaît limité en nutriments et l'échantillon Trans+ présente de fortes valeurs de production et d'assimilation après la translocation. Cependant, les densités ne bougeant pas et présentant même une légère diminution, nous pouvons supposer que cette production bactérienne est tout aussi profitable aux bactériovores présents. De plus, les milieux plus riches tel notre pré-barrage sont plus propices au développement d'organismes hétérotrophes comme les nanoflagellés hétérotrophes ou les ciliés (SAMUELSSON *et al.*, 2002) connus pour être des régulateurs de la communauté bactérienne (BERNINGER *et al.*, 1991). Enfin, si les échantillons transloqués du pré-barrage eutrophe vers le barrage mésoeutrophe ne présentent pas de diminution d'activité malgré la translocation, ce peut être due aux changements environnementaux qui ont pu se dérouler dans le barrage.

En automne (octobre 2004) les réservoirs n'apparaissent pas limités en nutriments, les communautés semblent trouver des conditions favorables à leur développement dans un milieu ou dans l'autre. Toutefois la comparaison entre les échantillons transloqués et les communautés restées dans leur milieu d'origine montrent des différences significatives (augmentation légère de production et d'assimilation par rapport au temps 0) tendant à prouver que le barrage présenterait une limitation en nutriments. Cependant un très fort développement de production apparaît dans le milieu eutrophe sans entraîner une augmentation de la densité. Au cours de suivis saisonniers effectués précédemment (Chapitre I), ce milieu a régulièrement présenté de forts développements de flagellés hétérotrophes au cours de cette saison. Les flagellés hétérotrophes étant des organismes bactériovores, il est probable que ceci empêchent un accroissement des densités bactériennes. D'autre part, les bactéries actives subissent une prédation quatre fois supérieure aux bactéries inactives (DEL GIORGIO *et al.*, 1996), ce fort taux de production les rendrait plus attractives.

4.3. Diversité

A l'inverse de certains auteurs (GASOL *et al.*, 2002 ; SIMEK *et al.*, 2003), les communautés ne montrent pas de modifications drastiques de leur composition par les nutriments sur une échelle de temps de 72 h. De plus, l'ensemble des communautés transloquées tendent à rapprocher leurs activités de celles des communautés du milieu récepteur. Ceci ne s'accompagne pas d'un rapprochement de composition de communauté. Ainsi les bactéries s'adaptent aux concentrations en nutriments présentes. D'où une production qui apparaît plus dépendante du milieu que de la composition de la communauté bactérienne. Les organismes généralistes ont un avantage de compétition par rapport aux organismes spécialisés (NYGAARD et TOBIESEN, 1993) lors de changements des conditions du milieu. Nous pouvons donc considérer que la majorité des bactéries présente dans ce complexe de réservoirs sont des bactéries généralistes, la prédominance de ce type de bactéries ayant déjà été observé par LANGENHEDER *et al.* (2005).

Cependant, une légère modification de composition à partir de 48 h apparaît régulièrement et qui pourrait être dû à l'activation de cellules dormantes (STEVENSON, 1978) ou à l'inhibition d'autres cellules. Il faut tout de même prendre en compte que l'analyse DGGE des fragments d'ADNr 16S amplifiés par PCR ne peut détecter les populations les moins abondantes dans une communauté (EILER *et al.*, 2003). Ainsi, les organismes dont les bandes disparaissent au cours de l'expérience peuvent être toujours présents mais non détectables.

5. conclusion

Les translocations montrent une stabilité de la composition malgré des modifications de concentrations en nutriments. Nos réservoirs étant interconnectés, les organismes présents peuvent être régulièrement amenés à subir ce type de variations, d'où leur adaptation phénotypique. Une modification de composition de la communauté aurait pu être probablement visible avec un changement de qualité ou de biodisponibilité des nutriments plutôt qu'une variation quantitative. D'autre part, LINDSTRÖM et BERGSTRÖM (2005) ont montré que le bassin versant pouvait être un important facteur dans la formation de la composition de la communauté bactérienne par des apports en bactéries. Permettraient ainsi un certain maintien de la composition bactérienne.

Les modifications d'activités ne s'accompagnant pas de modifications de composition taxonomique, l'intensité des activités bactériennes est plus dépendante du milieu que de la composition de la communauté. Cette communauté possède donc de large capacité d'adaptation. Ces résultats doivent être cependant modérés car une forte limitation en nutriments d'une ou plusieurs espèces peut ne pas être détectée dans une communauté « moyenne », si une grande partie ou toutes les espèces ne sont pas limitées par le même éléments (SOMMER, 1988).

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CHAPITRE III

EXPERIMENTATIONS: Les facteurs top-down

Les analyses exploratrices effectuées au chapitre I ont montré que les facteurs descendants (*top-down*) étaient spécifiques d'un réservoir. Pour étudier l'impact de ces facteurs sur les communautés bactériennes, nous avons utilisé comme approche expérimentale des filtrations différentielles, pour séparer les différents organismes suivant leur taille. Ceci sont incubés *in situ* durant 5 jours avec un suivi journalier.

Contrairement aux nutriments qui peuvent être consommés directement par les bactéries, les prédateurs présentent un grand nombre d'interconnexions. Ainsi, par le fractionnement de taille, tous les niveaux trophiques ont été manipulés. Au contraire des expériences de dilution, il n'y a pas d'altération du pool de matière dissoute consommé par les bactéries.

Les réservoirs ont été étudiés à différentes périodes correspondantes au développement d'organismes prédateurs ou à leur absence (période estivale et phase d'eau claire).

Plusieurs variables bactériennes ont été suivies, la densité seule ne pouvant suffire, car la production bactérienne peut contrebalancer la mortalité

Sont présentés dans ce chapitre les relations trophiques qui prennent part au réseau planctonique ainsi qu'une évaluation de la méthode de fractionnement par filtrations.

Critical evaluation of size fractionation method to evaluate top-down control on freshwater bacterioplankton

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Introduction

Bacteria are at the basis of the heterotrophic aquatic food chain and therefore they are linked by several direct and indirect interactions to other planktonic organisms. Bacteria mediate biogeochemical cycles in water ecosystems through recycling processes and energy fluxes. Their key role in the functioning of aquatic ecosystems justifies the important research efforts which are developed to evaluate the importance of factors controlling them. There is evidence that grazing is one of the major forces shaping the bacterial community structure (Jürgens and Jeppessen, 2000; Simon et al, 1998; Güde, 1989). Heterotrophic flagellates are known as the main predator of bacteria in aquatic ecosystems (Šimek et al., 1999, 2001, 2005; Jürgens et al., 1999). Nevertheless, it has been shown that cladocerans (Jardillier et al., 2004; Degans et al, 2002; Kamjunke et al, 1999) or ciliates (Kisand and Zingel, 2000) can also in several cases be the dominant bacterivores. Some rotifer taxa such as brachionids are also able to exert a strong impact on bacteria in lakes (Arndt, 1993). Furthermore, cascading effects of metazoans on intermediate consumers finally also affect bacteria. The multiple connexions within microbial and classical food web have numerous direct and indirect impacts on bacteria but these interactions have been relatively few examined. This gap arises somewhat from the difficulty to choose an appropriate experimental design allowing assessing the magnitude of these trophic linkages on bacterioplankton at *in situ* concentrations and in natural conditions. Manipulation of planktonic communities by sequential filtrations on membrane filters of different pore sizes allows separating planktonic communities according to their size and seems to be adapted for the investigation of trophic relationships within planktonic food webs. Size fractionation is commonly used to study lower trophic level (bacteria-flagellates relationships)

(Šimek et al., 2005, 2001, 1999; Jürgens et al., 1999) but received less success for investigating the effects of the whole planktonic food web on bacteria.

The dilution technique (Landry and Hassett, 1982) is also commonly used to quantify the impact of protozooplankton grazing on phytoplankton or bacteria but Landry (1994) cautioned that the method is not appropriate for bacteria because of the possible alteration of feedback relationships between pools of dissolved matter and bacteria.

Moreover, generalization about the trophic interactions with bacterioplankton is made difficult because the composition of microbial loop components may fluctuate according to lake productivity or within the annual cycle. Such fluctuations may distort the results of manipulations based on the size of planktonic organisms. Indeed, size spectra could markedly differ between lakes of different trophic status (Auer et al., 2004; Kalinowska, 2004; Taylor & Carter, 1997) or along the seasonal cycle (Tamigneaux et al., 1997). The natural variation of size occurring in the aquatic environment could necessitate adapting the mesh size of membranes filters in the case of fractionation experiments carried-out in periods with different planktonic assemblages. At our knowledge, few authors, except Carrias et al. (2000), paid a critical attention on this potential source of variation.

Usually, manipulation of planktonic communities had a weak effect on bacterial density (Adrian and Schneider-Olt, 1999; Wickham, 1998; Jürgens et al., 1994). Increasing predation triggered generally higher bacterial production rates that counterbalance bacterial mortality rates (Auer et al., 2004). It is therefore necessary to investigate several types of bacterial responses to display top-down effects. Nowadays, planktonic bacteria cannot still be considered as a single functional unit but they are recognized as a heterogeneous community able to respond to the regulating environmental factors by changing their taxonomic composition, their phenotypic characters and/ or their metabolism. Conflicting findings between studies could result from the different bacterial responses considered. Moreover, by integrating the aspects of diversity and function in microbial studies, limnological research gains in ecological significance and pertinence.

The present study aimed to describe the trophic relationships taking place within the planktonic web and more specifically, the responses of heterotrophic

bacterioplankton to manipulated planktonic food web in reservoirs of increasing trophic statuses, during key periods of the seasonal succession. The investigated bacterial responses were numeric, morphologic, metabolic and genomic. Furthermore, we examined the relevance of fractionation method for studying trophic interactions. Advantages and disadvantages of the method and comparability of results between reservoirs and seasons are discussed.

Methods

Experimental design and study sites

Four fractionation experiments were conducted in three reservoirs differing in trophic statuses: the oligomesotrophic Olef reservoir (Germany), the mesoeutrophic Esch-sur-Sûre reservoir and its eutrophic pre-reservoir (Grand-duchy of Luxembourg). Key periods of the seasonal succession of planktonic communities were chosen in order to achieve different situations of dominance by one planktonic group offering contrasting predation conditions: the clear-water phase, characterised by a dominance of cladocerans and low densities of protozoans and phytoplankton, the early-spring and autumn periods dominated by protozoans. For details on the abiotic and biotic characterizations of the reservoirs, see table 1.

The vertical profile of water temperature was firstly measured to determine stratification layers and water was taken within the warm and homogeneous upper strata: the epilimnion for deep reservoirs (0-10 m layer during non-stratification period) and the whole column for shallow reservoirs. A pool (50-l) was constituted from water samples taken every meter with a 2.2-l Ruttner bottle (HYDROBIOS). Then, water was sequentially filtered through 250, 50, 5 μm nylon mesh and then through 0.8 μm A/D glass fibre filters (Gelman) to obtain different size fractions containing theoretically: bacteria, nanoflagellates, ciliates, rotifers and nauplii in the “< 250 μm ” fraction; bacteria, nanoflagellates, ciliates in the “< 50 μm ” fraction; bacteria and nanoflagellates in the “< 5 μm ” fraction; bacteria in the “< 0.8 μm ” fraction. Each fraction was duplicated and filled in 25-l polypropylene tanks. Two tanks were also filled with unfiltered samples (control) and served as controls. Two liters were immediately taken from each tank for the determination of initial conditions and the tanks were immersed

in the reservoirs at a depth where $\pm 20\%$ of incident light was attenuated. Gas exchange with the atmosphere was allowed through a silicone tube. Incubation proceeded for 5 days and daily sampling of 2-l was achieved after homogenisation of the tanks for the monitoring of bacterial, abiotic and biotic parameters. *In situ* conditions were surveyed at the same frequency during the course of the experiments.

Abiotic variables measurements

Water temperature, dissolved oxygen concentration, pH and conductivity were determined with a multi-parameters probe (HYDROLAB-DS 4). Water transparency was estimated as the disappearance depth of a Secchi disk (\varnothing 20 cm). Light attenuation in the water column was measured using a light probe (Li-Cor-1000). Hundred millilitres of water were filtered on pre-ignited GF/F filters and immediately frozen in liquid nitrogen for the determination of the dissolved nutrients. Determination of the ammonium, soluble reactive phosphorus and silicates concentrations were carried out following standard colorimetric methods (APHA et al, 1995). Other ions concentrations (NO_3 , NO_2) were analysed by ionic chromatography (DIONEX DX 500, equipped with IonPac AG 12A or CG 12A ion exchange column). Dissolved organic carbon was measured with an elemental analyser (TC-5000 SHIMADZU).

Bacterial variables measurements

Bacterial abundance and biomass. Samples were fixed with formaldehyde (2% final concentration), stained with Sybr Green I (Molecular probes) (0,1% final concentration) (Noble and Fuhrman, 1998) and filtered onto black 0.2 μm polycarbonate membrane filters (Millipore). Cells were enumerated at a magnification of 1000x by epifluorescence microscopy (Leica, DMRB) under blue excitation (excitation: 450-490 nm, emission > 515 nm). The mean cell volume (V , in μm^3) was calculated using digital pictures of microscopical fields with a formula: $V = (\pi/4) a^2 (A - a/3)$ where a and A are respectively the small axis and the large axis of the cells (Bratbak, 1985).

Bacterial production (BP) was measured via leucine incorporation (Kirchman et al., 1985). In brief, bacteria were allowed to incorporate ^{14}C -labelled leucine (final concentration 25 nM) during two hours in dark conditions and at *in situ* temperature.

After stopping incubation (formaldehyde 2% f.c.), proteins were extracted by the hot TCA method (Fitch *et al.*, 1955) and precipitates were collected on 0.2 μm cellulose acetate filters. Their radioactivity content was measured by liquid scintillation in disintegration per minute. For the detailed protocol, see chapter 1.

The procedure for the measurement of **glucose assimilation and respiration** was similar to bacterial production assessment with ^{14}C -labelled glucose as substrate (final concentration 25nM) (Skoog *et al.*, 1999). The respired amount of glucose was considered as the difference between the total incorporated radioactivity in the cell and the radioactivity incorporated in cell constituents (after extraction in TCA 50%) and was expressed in % of the total glucose assimilated.

Biotic variables measurements

We included in biotic variables the major components of the planktonic food web interacting directly or indirectly with bacteria. For **Virus-like particles (VLP)**, we used the same preservation and enumeration procedure as for bacteria using YO-PRO in place of Syber Green as dye (Xénopoulos and Bird, 1999) and filtration on 0.02 μm Al_2O_3 filter (Whatman).

Chlorophyll-*a* concentration was used as an estimation of algal biomass and thus considered as a biotic variable. Chlorophyll-*a* was assessed through pigment analysis of the particulate fraction retained on 47 mm GF/C filters by filtration under a moderate vacuum. Pigments were extracted in 90% acetone according to Descy *et al.* (1999), separated and quantified by HPLC (column C18, Zorbax Bonus-RP 4.6 x 250 mm, Agilent Technologies, USA) using a modified ternary gradient of Wright *et al.* . HPLC analysis was performed using a Dionex SummitTM system (Sunnyvale, CA, USA) equipped with a P580 gradient pump, a GINA 50 autosampler and a UVD 340S diode array detector (DAD). A 15 μl aliquot was injected onto a 4.6 x 250 mm 5 μm Zorbax Bonus-RP C18 column (Agilent Tech., USA).

Samples for **protozoans** enumeration were fixed with a final concentration of 2.5% mercuric chloride for ciliates and 1% glutaraldehyde for flagellates. After specific staining with primulin (Bloem *et al.*, 1986; Caron, 1983), nanoflagellates were collected onto black 0.8 μm filters and enumerated at a magnification of x1000 by epifluorescence microscopy (Leica, DMRB) equipped with UV light (excitation: 340-

380 nm, emission > 430 nm). Ciliates were allowed to settle for 12-24 h in 25 to 100 ml plankton chambers and enumerated using an inverted Leica microscope.

Rotifers and crustaceans were concentrated onto 50 µm nylon mesh, narcotised in carbonated water and preserved in 12/4% sucrose/formaldehyde. Their densities were estimated with an inverted Leica microscope.

Denaturing gel gradient electrophoresis

Bacterial 550-bp fragments suitable for subsequent DGGE analysis were obtained with the primer combination of 341 f with a GC clamp (40-nucleotide GC-rich sequence, 5'-CCT ACG GGA GGC AGC AG-3') and 907 (5'-CCG TCA ATT CCT TTG AGT-3') (Muyzer and Smalle, 1998; Muyzer et al., 1993). DGGE for bacteria was run as described before (Casamayor et al., 2000) for 15 h at a constant voltage of 100 V and at 60°C in a 20 to 70% vertical denaturant gradient (the 100% denaturant agent is 7 M urea and 40% deionised formamide).

Statistical analysis

The differences in planktonic communities composition between fractions were tested using the multivariate two-way crossed analysis of similarity (PRIMER). ANCOVA (STATISTICA) tested for differences of bacterial and abiotic variables between fractions with time as covariable. Post hoc comparisons were performed by Scheffé tests. The temporal pattern of bacterial diversity was analysed by Multidimensional Scaling analysis (MDS) (PRIMER) from a similarity matrix of presence-absence bands.

Results

Initial abiotic and biotic conditions

Fractionation experiments were carried out between May 2003 and June 2004 at 4 periods characterized by contrasted abiotic and biotic conditions (Table 1).

Table 1: Abiotic and biotic characterisations of the reservoirs at the start of fractionation experiments (mean \pm distance to the mean). The major components of planktonic biomass are noted in bold

<i>Reservoir</i>	Oligomesopic		Mesoeutrophic 1		Mesoeutrophic 2		Eutrophic	
Experiment period	24/05/04 28/05/04		19/05/03 23/05/03		30/03/04 02/04/04		23/08/03 29/08/03	
Mean depth (m)	18		15		15		2	
Incubation depth (m)	4		2		4		0.75	
Sampled layer (m)	0-6		0-6		0-10		0-3.5	
Secchi depth (m)	6.0		3.7		2.4		0.8	
Temperature (°C)	13.4		14.7		6.1		18.2	
Oxygen (%)	83.3		100.9		103.1		44.2	
pH	7.4		8.0		8.1		6.8	
Conductivity ($\mu\text{S cm}^{-1}$)	70.5		133.8		167.9		174.0	
Orthophosphates (mgL^{-1})	<0.005		<0.005		0.007		0.020	
Nitrates (mgL^{-1})	1.38		3.74		5.35		1.62	
DOC (mgL^{-1})	1.25		4.13		2.59		7.16	
Taxa	Density (cf column 1)	Biomass ($\mu\text{gC L}^{-1}$)	Density (cf column 1)	Biomass ($\mu\text{gC L}^{-1}$)	Density (cf column 1)	Biomass ($\mu\text{gC L}^{-1}$)	Density (cf column 1)	Biomass ($\mu\text{gC L}^{-1}$)
Virus (10^6cell mL^{-1})	n.d.		54.3 \pm 1.8		n.d.		97.8 \pm 3.9	
ANF (cell mL^{-1})	87.6 \pm 9.8	3.0 \pm 0.0	1195.1 \pm 25	28.2 \pm 0.8	442.5 \pm 7.5	5.9 \pm 2.5	558.4 \pm 8.3	34.1 \pm 5.2
HNF (cell mL^{-1})	2480.6 \pm 85.9	8.6 \pm 0.8	920.1 \pm 10.0	5.1 \pm 0.7	1425.1 \pm 295.0	6.4 \pm 1.4	3995.4 \pm 1605.1	18.1 \pm 5.3
Total ciliates (cell mL^{-1})	15.3 \pm 1.0	15.3 \pm 0.1	5.7 \pm 1.4	20.3 \pm 4.4	39.9 \pm 0.9	112.0 \pm 39.8	52.5 \pm 3.0	245.1 \pm 31.6
Prostomatids (cell mL^{-1})	1.7 \pm 0.8	1.4 \pm 0.7	0.9 \pm 0.2	0.3 \pm 0.1	29.7 \pm 2.0	9.2 \pm 0.6	10.4 \pm 0.4	97.0 \pm 22.6
Oligotrichs (cell mL^{-1})	12.0 \pm 0.1	12.2 \pm 0.7	3.7 \pm 1.0	15.1 \pm 5.1	1.5 \pm 0.1	3.7 \pm 0.4	16.1 \pm 1.3	96.1 \pm 37.1
Tintinnids (cell mL^{-1})	0.1 \pm 0.1	0.0 \pm 0.0	0.2 \pm 0.1	0.2 \pm 0.1	2.8 \pm 0.5	2.9 \pm 0.5	15.9 \pm 0.4	0.0 \pm 0.0
Scuticociliates (cell mL^{-1})	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.4 \pm 0.1	0.5 \pm 0.0	2.2 \pm 0.4	3.0 \pm 0.5
Peritrichs (cell mL^{-1})	0.0 \pm 0.0	0.0 \pm 0.0	0.1 \pm 0.0	2.0 \pm 1.5	0.1 \pm 0.1	0.3 \pm 0.3	5.0 \pm 1.7	49.0 \pm 16.6
Unidentified cil. (cell mL^{-1})	1.5 \pm 0.4	1.7 \pm 0.1	0.7 \pm 0.3	2.70.8	5.4 \pm 0.6	95.3 \pm 39.6	2.9 \pm 0.3	0.0 \pm 0.0
Zooplankton (ind. L^{-1})	147.9 \pm 30.0	49.7 \pm 1.0	1685.7 \pm 1044.3	397.2 \pm 213.4	500.8 \pm 101.0	24.0 \pm 3.2	1882.6 \pm 52.5	179.3 \pm 13.0
Rotifers (ind. L^{-1})	136.0 \pm 30.0	4.2 \pm 0.9	901.7 \pm 598.3	51.6 \pm 36.8	485.0 \pm 99.0	14.6 \pm 3.0	1291.5 \pm 92.6	38.7 \pm 2.8
Crustaceans (ind. L^{-1})	11.9 \pm 0.0	45.5 \pm 0.1	784.5 \pm 446.0	345.6 \pm 176.6	15.8 \pm 2.0	9.4 \pm 0.3	591.1 \pm 40.1	140.7 \pm 15.7
Bosminidae (ind. L^{-1})	0.2 \pm 0.0	0.2 \pm 0.0	147.0 \pm 93.0	91.6 \pm 57.6	1.3 \pm 0.0	1.4 \pm 0.0	23.8 \pm 6.2	15.3 \pm 3.8
Daphniidae (ind. L^{-1})	0.2 \pm 0.0	0.3 \pm 0.0	53.0 \pm 27.0	100.5 \pm 51.4	0.2 \pm 0.0	0.0 \pm 0.0	0.4 \pm 0.4	0.0 \pm 0.0
Holopedidae (ind. L^{-1})	8.1 \pm 0.0	27.5 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
Sididae (ind. L^{-1})	0.0 \pm 0.0	0.0 \pm 0.0	1.0 \pm 1.0	0.9 \pm 0.9	0.0 \pm 0.0	0.0 \pm 0.0	0.4 \pm 0.4	0.4 \pm 0.4
Nauplii (ind. L^{-1})	0.0 \pm 0.0	4.2 \pm 0.1	466.0 \pm 284.0	65.0 \pm 39.7	8.0 \pm 2.0	1.0 \pm 0.3	489.3 \pm 22.0	68.2 \pm 3.1
Calanoid cop. (ind. L^{-1})	2.0 \pm 0.0	9.1 \pm 0.0	1.0 \pm 1.0	3.2 \pm 2.9	0.6 \pm 0.0	2.0 \pm 0.0	0.0 \pm 0.0	0.3 \pm 0.0
Cyclopoid cop. (ind. L^{-1})	1.4 \pm 0.0	4.1 \pm 0.0	116.0 \pm 44.0	84.4 \pm 31.7	5.6 \pm 0.0	4.9 \pm 0.0	77.1 \pm 12.8	56.4 \pm 9.2
Chl- <i>a</i> ($\mu\text{g L}^{-1}$)		65.0 \pm 9.4		194.1 \pm 140.8		1139.5 \pm 153.8		968.3 \pm 438.3
Bacteria (10^6 cell mL^{-1})	2.3 \pm 0.1	65.6 \pm 9.1	3.8 \pm 0.3	41.0 \pm 8.8	3.1 \pm 0.1	115.9 \pm 7.6	7.0 \pm 0.9	92.5 \pm 6.4

In the oligomesotrophic reservoir, the experiment was performed during the clear water phase. Planktonic biomass was dominated by the large sized cladoceran *Holopedium gibberum* ($27.5 \mu\text{gC L}^{-1}$). Rotifers, ciliates, flagellates and algae (mainly cryptophytes) displayed low biomasses. Epilimnion reached a 6 meter depth. Water temperature, oxygen saturation and conductivity averaged respectively $13.4 \text{ }^\circ\text{C}$, 83.3% and $70.5 \mu\text{S cm}^{-1}$. Circumneutral pH was observed.

Two experiments were carried out in the mesoeutrophic reservoir. The first experiment (mesoeutrophic 1) was carried out at the beginning of the clear water phase, a period characterised by low algal (mainly cryptophytes) and protozoan biomasses and by a dominance of cladocerans (*Daphnia* and *Bosmina*). Bacterial density and biomass were low. The epilimnion (depth = 6 meters) was well oxygenated (100.9 % saturation), displayed a slightly basic pH of 8, a temperature of $14.7 \text{ }^\circ\text{C}$ and a conductivity of $133.8 \mu\text{S cm}^{-1}$ respectively. The second experiment (mesoeutrophic 2) took place during the spring algal bloom (composed mainly of diatoms) in the presence of a high ciliate biomass. The water column being not thermally stratified, we considered the 0-10 m water layer characterized by a low temperature ($6.1 \text{ }^\circ\text{C}$), a good oxygenation (103.1 %), a somewhat basic pH (8.1) and a conductivity of $167.9 \mu\text{S cm}^{-1}$.

The trophic interactions in the shallow eutrophic reservoir were investigated in summer during the development of algae, ciliates and zooplankton. In this shallow reservoir, the whole water column was sampled (0-3.5 m). At this period, water temperature was maximal (18.2°C), oxygenation was low (44.2%), pH was circumneutral and the conductivity was similar to that of the mesoeutrophic reservoir.

The efficiency of sequential filtration to separate planktonic communities in distinguishable fractions was tested for each experiment (ANOSIM). The “< $0.8 \mu\text{m}$ ”, “< $5 \mu\text{m}$ ”, “< $50 \mu\text{m}$ ” and “< $250 \mu\text{m}$ ” fractions consisted in significantly different planktonic communities in all reservoirs and experiments ($p < 0.05$). The biotic communities in fractions differed significantly from the control in oligomesotrophic and mesoeutrophic 1 reservoirs. However, in mesoeutrophic 2 and eutrophic reservoirs, biotic compositions were similar in the “< $250 \mu\text{m}$ ” fraction and in the control since these reservoirs contained mainly small sized zooplankton (rotifers and nauplii) while the 2 other experiments (oligomesotrophic and mesoeutrophic 1 reservoirs) were performed in cladocerans-dominated conditions. Planktonic composition in the control

and *in situ* conditions were significantly comparable for all experiments. Physico-chemical and chemical conditions as well as bacterial parameters in the control were statistically similar to *in situ* environment (ANOSIM).

Initial densities or concentrations (chlorophyll-*a*) of planktonic groups are illustrated in figure 1. In oligomesotrophic and mesoeutrophic 2 reservoirs, heterotrophic nanoflagellates were removed from the “< 0.8 μm ” fraction but some cells subsisted in mesoeutrophic 1 and eutrophic reservoirs (respectively 0.9% and 2.8% of the heterotrophic nanoflagellates density in the control). Ciliates and zooplankton were absent and undetectable chlorophyll-*a* concentrations were measured in the “< 0.8 μm ” fraction for all reservoirs. The “< 5 μm ” fraction contained mainly heterotrophic nanoflagellates and small algae excepted in the oligomesotrophic reservoir where ciliates remained abundant. In all reservoirs, ciliates density in the “< 50 μm ” fraction was similar or higher to the density in the control. Zooplankton density was low (maximum 13 % of the density in control) or null. In all reservoirs, the density of zooplankton in the “< 250 μm ” fraction was reduced except in mesoeutrophic 2 reservoir when the control and the “< 250 μm ” fraction displayed similar zooplanktonic abundances.

Effects of fractionation on bacteria

The effects of fractionation on bacterial density, mean cell volume and diversity were analysed at time 0 by comparison with the control (Fig. 2). In most cases, the proportion of bacteria removed by filtration was lower than 15% (Fig. 2A). The more obvious effect was observed in the “< 0.8 μm ” fraction in the eutrophic reservoir when 32% of bacteria were removed compared to the control. Inversely, a slight but significant increase of bacterial density occurred in the oligomesotrophic reservoir (3 to 10 % more bacteria in “< 5 μm ”, “< 50 μm ” and “< 250 μm ” fractions compared to the control).

Sequential filtration affected the bacterial cell volume in variable ways (Figure 2B). Large bacterial cells (present in oligomesotrophic and mesoeutrophic 2 reservoirs) were more affected by filtration than small cells (present in mesoeutrophic 1 and eutrophic reservoirs). In the oligomesotrophic reservoir, bacterial cell volumes were highly variable making difficult to evaluate the effects of fractionation. Mean cell

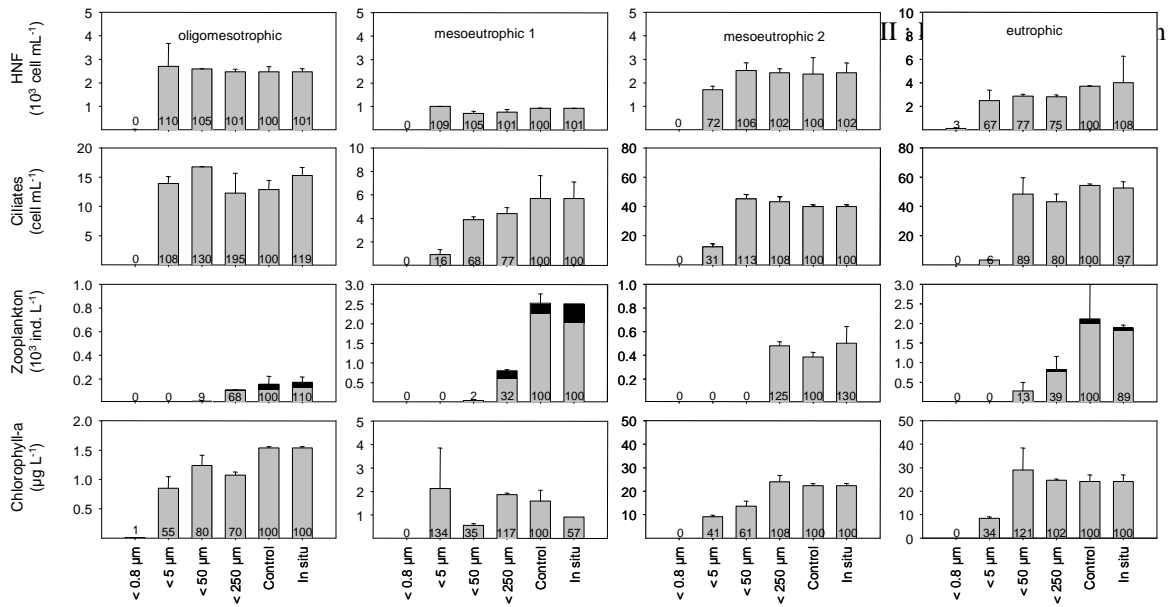


Figure 1: Initial density of heterotrophic nanoflagellates, ciliates, total zooplankton, zooplankton > 250 µm (black) and chlorophyll-*a* concentrations in the different treatments and reservoirs. Values inside the vertical bars give, in percent, the ratio between the density (or concentration) in the fraction and in the control (percentage of organisms passed through the filter membrane).

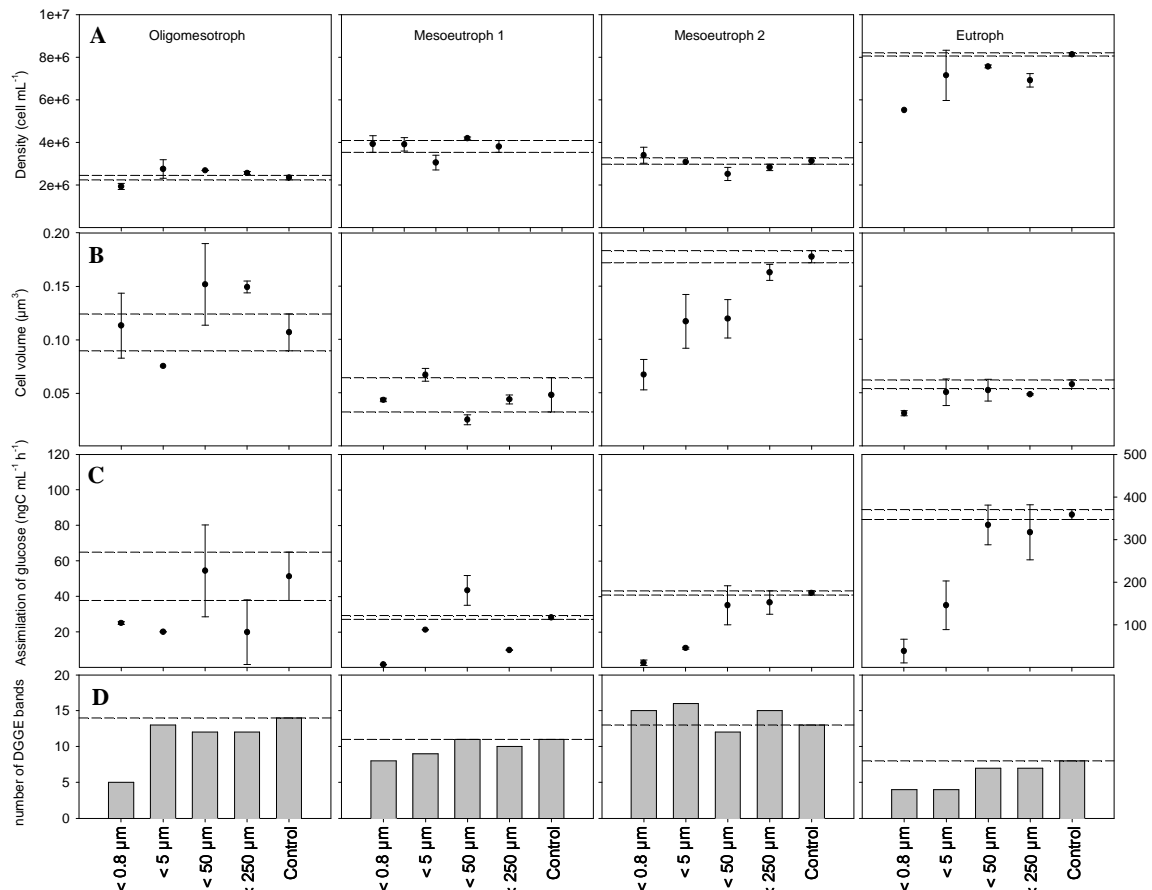


Figure 2: Effects of sequential filtration on bacterial density (A), cell volume (B), glucose assimilation (C) and diversity (expressed as the number of DGGE bands) (D) at time 0. Vertical bars represent standard error. Unfiltered treatment is considered as control and represented by the dotted line.

volume was unaffected in the “< 0.8 μm ” fraction, reduced in the “< 5 μm ” fraction and increased in “< 50 μm ” and “< 250 μm ” fractions compared to the control. In the mesoeutrophic 1 reservoir, cell volume did not differ significantly from the control excepted in the “< 50 μm ” fraction when the cell volume was slightly reduced. In the mesoeutrophic 2 reservoir, all fractionations affected significantly the bacterial cell volume. Smaller was the mesh size of the membrane filter; greater was the effect on cell volume. In the eutrophic reservoir, the bacterial cell volume decreased significantly in the “< 0.8 μm ” fraction. A small effect was also observed in the “< 250 μm ” fraction. Whatever the experiment, fractionation induced a strong weakening decrease of bacterial activity (expressed as glucose assimilation). (Fig. 2C). The effect was emphasized by low mesh sizes. Bacterial diversity was affected by fractionation whatever the mesh size of the membrane filter (Fig. 2D). In many cases, fractionation resulted in a limited variation of DGGE band numbers (1 or 2 bands) compared to the control. A considerable reduction of the bands number occurred in the “< 0.8 μm ” fraction in the oligomesotrophic reservoir (decrease from 14 to 5 bands) and in “< 0.8 μm ” and “< 5 μm ” fractions in the eutrophic reservoir (decrease from 8 to 4 bands). In the mesoeutrophic reservoir, the number of bands increased from 1 or 2 bands in “< 0.8 μm ”, “< 5 μm ”, “< 250 μm ” fractions compared to the control. As shown on the clustering analysis of the bacterial diversity (Fig. 7), sequential fractionation affected bacterial community composition in a variable way according to the mesh size of the filter. However, these divergences in bacterial community composition were not statistically significant according to the ANOSIM analysis.

Temporal evolution of bacterial and biotic parameters throughout experiments

Physico-chemical conditions in the experimental tanks (temperature, dissolved oxygen concentration, pH and conductivity) remained stable during the course of the experiment. No significant difference was observed between the treatments and with *in situ* conditions. Nutrients concentrations in the different treatments were comparable except for ammonium and nitrates. Significantly higher ammonium and nitrates concentrations were measured in “< 0.8 μm ” and “< 5 μm ” fractions in 3 experiments out of 4 (ANCOVA, $p < 0.05$).

The temporal evolutions of biotic and bacterial variables throughout the experiments are shown in figures 3 to 6. The analysis of table 2 allows identifying the bacterial variables significantly influenced by the treatment.

In the oligomesotrophic reservoir, bacteria assimilated significantly more glucose in the “< 250 μm ” fraction (Table 2, Fig. 3C). Assimilations of glucose were low and stable in “< 0.8 μm ”, “< 5 μm ” and “< 50 μm ” fractions while higher but more variable assimilations were measured in the control. Other bacterial variables (density, cell volume and production) displayed similar and quite stable temporal evolutions in all treatments. Similar chlorophyll-*a* concentrations ($\pm 1,1 \mu\text{g L}^{-1}$) and patterns were observed in fractions larger than 5 μm (Fig. 3E). Heterotrophic nanoflagellates declined promptly during the first 48 h to reach low and stable abundance (Fig. 3F). The strongest decrease occurred in “< 5 μm ” and “< 250 μm ” fractions. Ciliates density increased progressively in small fractions (“< 5 μm ” and “< 50 μm ”) while they decreased gradually in presence of metazooplankton (“< 250 μm ” fraction and control) (Fig. 3G). Zooplankton density was low and mainly composed of rotifers in “< 5 μm ” and “< 250 μm ” fractions. A small peak of rotifers occurred at time 24h in the “< 250 μm ” fraction, followed by a decrease in abundance and a succeeding relative steady population (Fig. 3H). In the control, zooplankton remained stable until time 48h and then decreased rapidly at time 72h.

Some data are missing for experiment 1 in the mesoeutrophic reservoir because water losses occurred in some tanks. Bacterial densities displayed variable and inconsistent patterns in all treatments (Fig. 4A). Bacterial mean cell volume was significantly higher in the “< 5 μm ” fraction (Table 2, Fig. 4B). The assimilation of glucose augmented in all fractions after 48h and was significantly higher in the “< 250 μm ” fraction (Table 2, Fig. 4C). Bacterial production increased progressively, in the same way, in all treatments. A decrease in chlorophyll-*a* occurred in all fractions except in the “< 50 μm ” fraction that displayed low and stable concentrations (Fig. 4E). Heterotrophic nanoflagellates abundance declined after 48h in all treatments and especially fast in the “< 50 μm ” fraction and the control (Fig. 4F). Ciliates were largely removed in the “< 5 μm ” fraction but the population started to grow again from time 48h. Inversely, a marked drop occurred in fractions containing higher trophic levels (“< 50 μm ”, “< 250 μm ” fractions and control) (Fig. 4G).

Table 2: Statistical differences between treatments measured by ANCOVA for bacterial parameters

	Oligomesotroph	Mesoeutroph 1	Mesoeutroph 2	Eutroph
Density (cell mL ⁻¹)	n.s.	n.s.	< 0.001	0.004
Cell volume (µm ³)	n.s.	0.002	0.015	< 0.001
Bacterial assimilation of glucose (ngC L ⁻¹ h ⁻¹)	0.017	0.011	< 0.001	n.s.
Cell assimilation of glucose (pgC 10 ⁶ cell ⁻¹ h ⁻¹)	0.017	n.s.	< 0.001	0.040
Respiration (%)	n.s.	n.s.	n.s.	n.s.
Bacterial production (ngC L ⁻¹ h ⁻¹)	n.s.	n.s.	0.009	n.s.
Cell production (pgC 10 ⁶ cell ⁻¹ h ⁻¹)	n.s.	n.s.	n.s.	n.s.

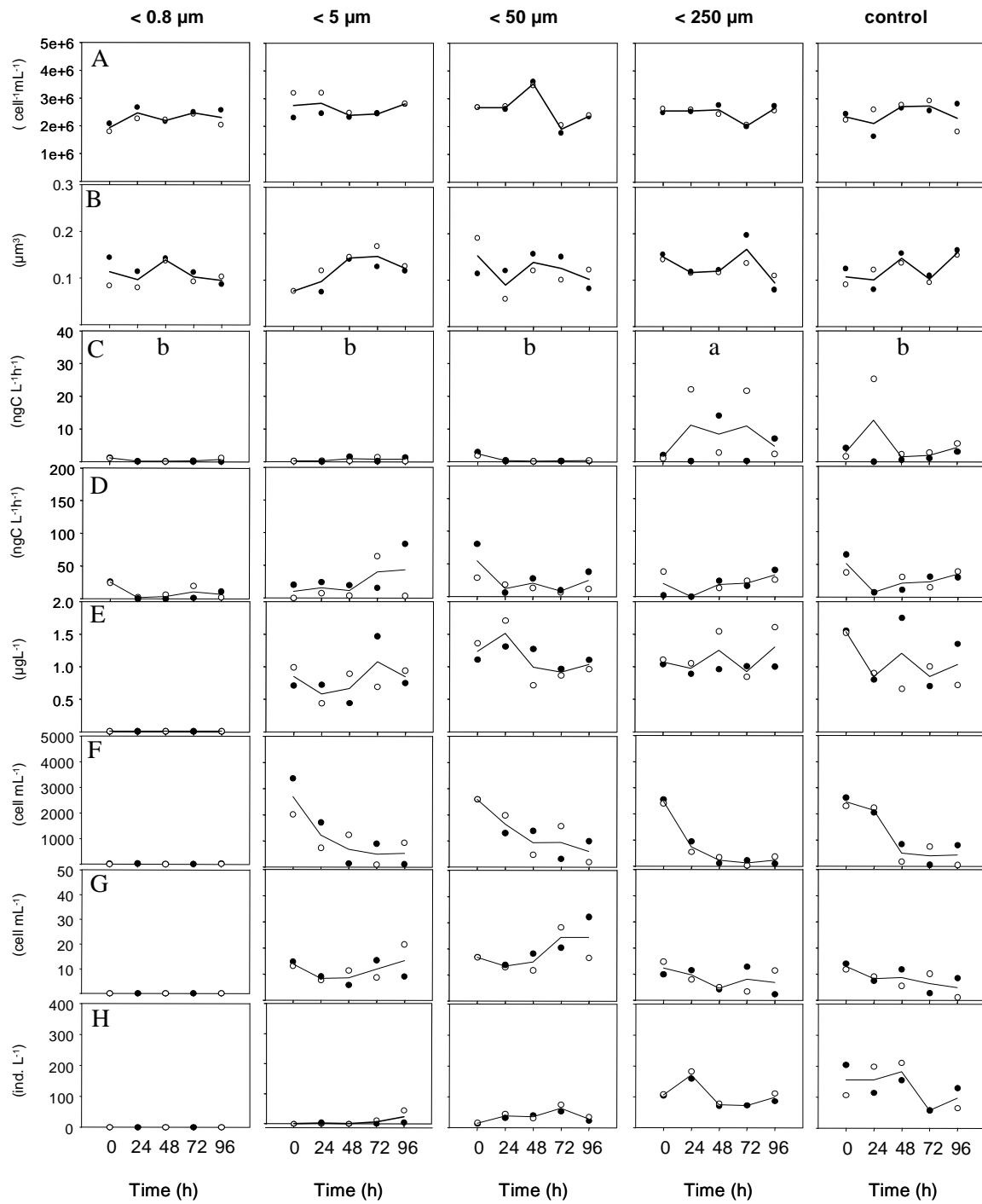


Fig. 3

Figure 3: Temporal pattern of bacterial density (A), bacterial mean cell volume (B), bacterial assimilation of glucose (C), bacterial production (D), chlorophyll-*a* concentration (E), heterotrophic nanoflagellates density (F), ciliates density (G), zooplankton density (H) within fractions in the oligomesotrophic reservoir. Line is the mean for two replicate treatments respectively represented by black and white circles. Letters represent significant differences between treatments (ANCOVA).

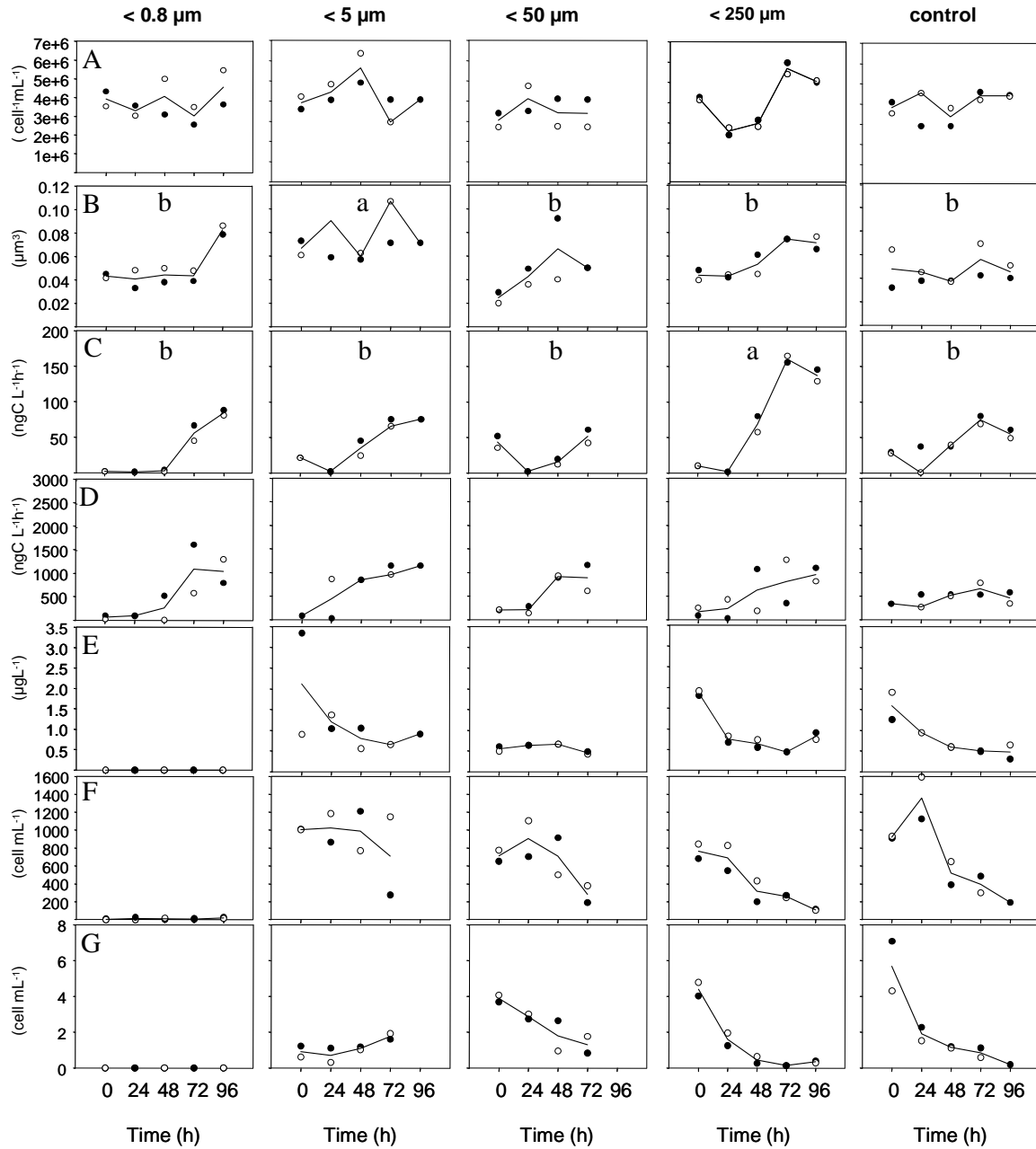


Figure 4: Temporal pattern of temporal pattern of bacterial density (A), bacterial mean cell volume (B), bacterial assimilation of glucose (C), bacterial production (D), chlorophyll-*a* concentration (E), heterotrophic nanoflagellates density (F), ciliates density (G), within fractions in mesoeutrophic reservoir (exp. 1). Line is the mean for two replicate treatments respectively represented by black and white circles. Letters represent significant differences between treatments (ANCOVA).

In the mesoeutrophic 2 reservoir, the density of bacteria augmented progressively in all treatments except in the “< 0.8 μm ” fraction where a significant decrease was observed (Table 2, Fig. 5A). The mean cell volume increased in smaller fractions (“< 0.8 μm ” and “< 5 μm ” fractions) and was stable in larger fractions (“< 50 μm ”, “< 250 μm ” fractions and control) (Fig. 5B). The difference was only significant between “< 0.8 μm ” and “< 5 μm ” fractions. Activities (bacterial assimilation of glucose and production) were significantly reduced in the “< 0.8 μm ” fraction compared to other fractions (Fig. 5C, D). Maximal activities were measured in the “< 5 μm ” fraction. Chlorophyll-*a*, heterotrophic nanoflagellates and ciliates increased in all treatments throughout the experiment (Fig. 5E, F, G). The increase of heterotrophic nanoflagellates was more rapid in the “< 50 μm ” fraction and in the control while the heterotrophic nanoflagellates development was slowed down in “< 5 μm ” and “< 250 μm ” fractions (Fig. 5F). Ciliates rose more rapidly and reached higher densities in “< 50 μm ” and “< 250 μm ” fractions while they maintained more stable densities in the control. Zooplankton abundance stayed low in the “< 50 μm ” fraction (Fig. 5H). In larger fractions, zooplankton kept stable densities until 72h and then increased suddenly at 96h.

In the eutrophic reservoir, the “< 0.8 μm ” fraction displayed significantly higher bacterial density but lower cell volume and cell assimilation of glucose (Table 2). Bacteria developed in the “< 0.8 μm ” fraction while their abundance declined over time in the presence of predators (“< 5 μm ”, “< 50 μm ”, “< 250 μm ” fractions and control) (Fig. 6A). Bacterial cell volume increased slightly in “< 5 μm ” and “< 50 μm ” fractions and remained unchanged in other treatments (Fig. 6B). Bacterial cell assimilation of glucose and production remained low in the “< 0.8 μm ” fraction (Fig. 6C, D) while a marked increase occurred in fractions with predators (“< 5 μm ”, “< 50 μm ”, “< 250 μm ” fractions and control). Chlorophyll-*a* concentration increased in all treatments except in the “< 0.8 μm ” fraction where algae were eliminated (Fig. 6E). Although they were completely removed by filtration on 0.8 μm , heterotrophic nanoflagellates developed again after 60h (Fig. 6F). In larger fractions (“< 5 μm ”, “< 50 μm ”, “< 250 μm ” fractions and control), heterotrophic nanoflagellates increased progressively. A marked peak occurred at time 36h in the “< 5 μm ” fraction. Ciliates were eliminated from “< 0.8 μm ” and “< 5 μm ” fractions but they started to grow again after 12h in the

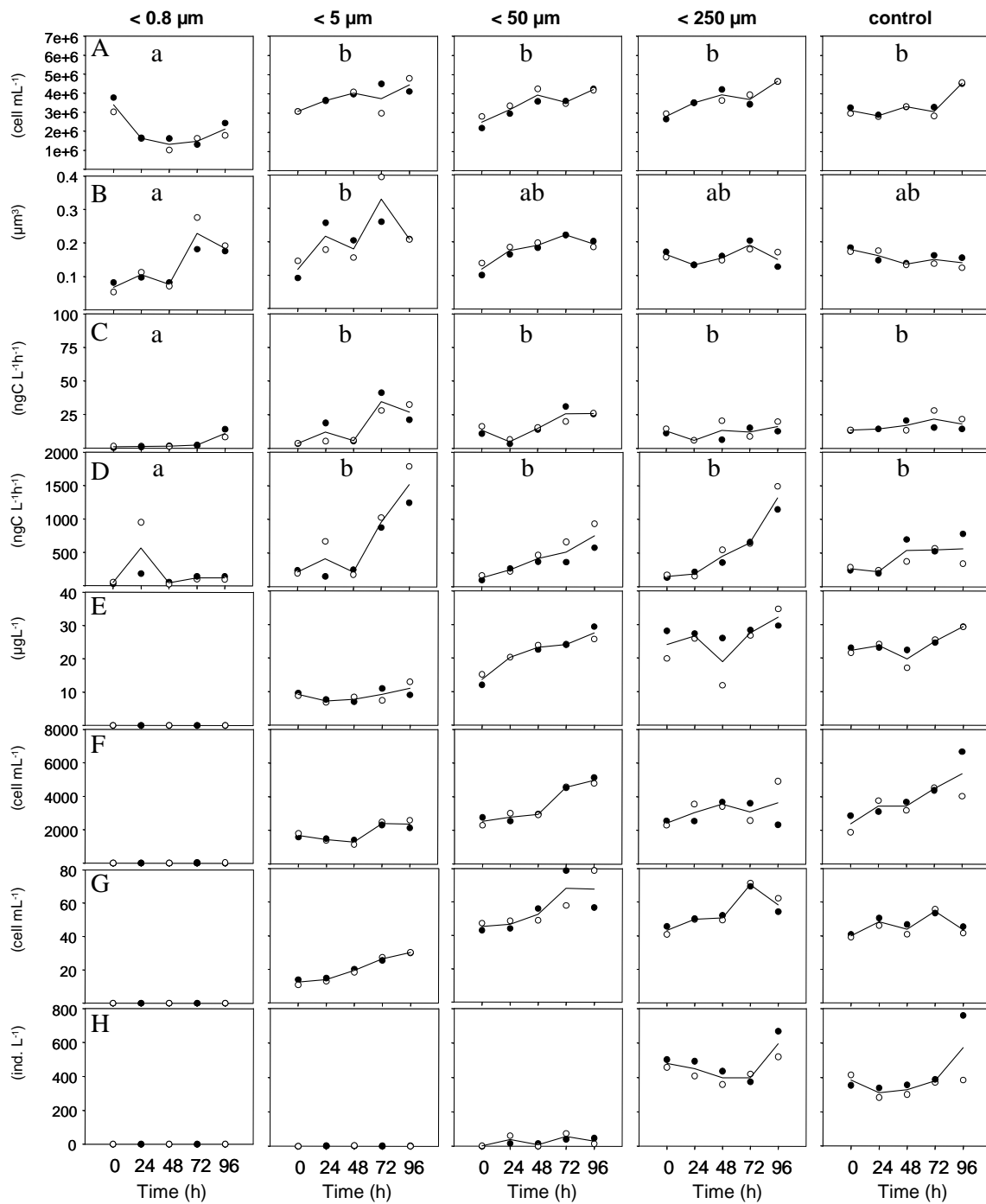


Figure 5 : Temporal pattern of temporal pattern of bacterial density (A), bacterial mean cell volume (B), bacterial assimilation of glucose (C), bacterial production (D), chlorophyll-*a* concentration (E), heterotrophic nanoflagellates density (F), ciliates density (G) within fractions in mesoeutrophic reservoir (exp. 2). Line is the mean for two replicate treatments respectively represented by black and white circles. Letters represent significant differences between treatments (ANCOVA).

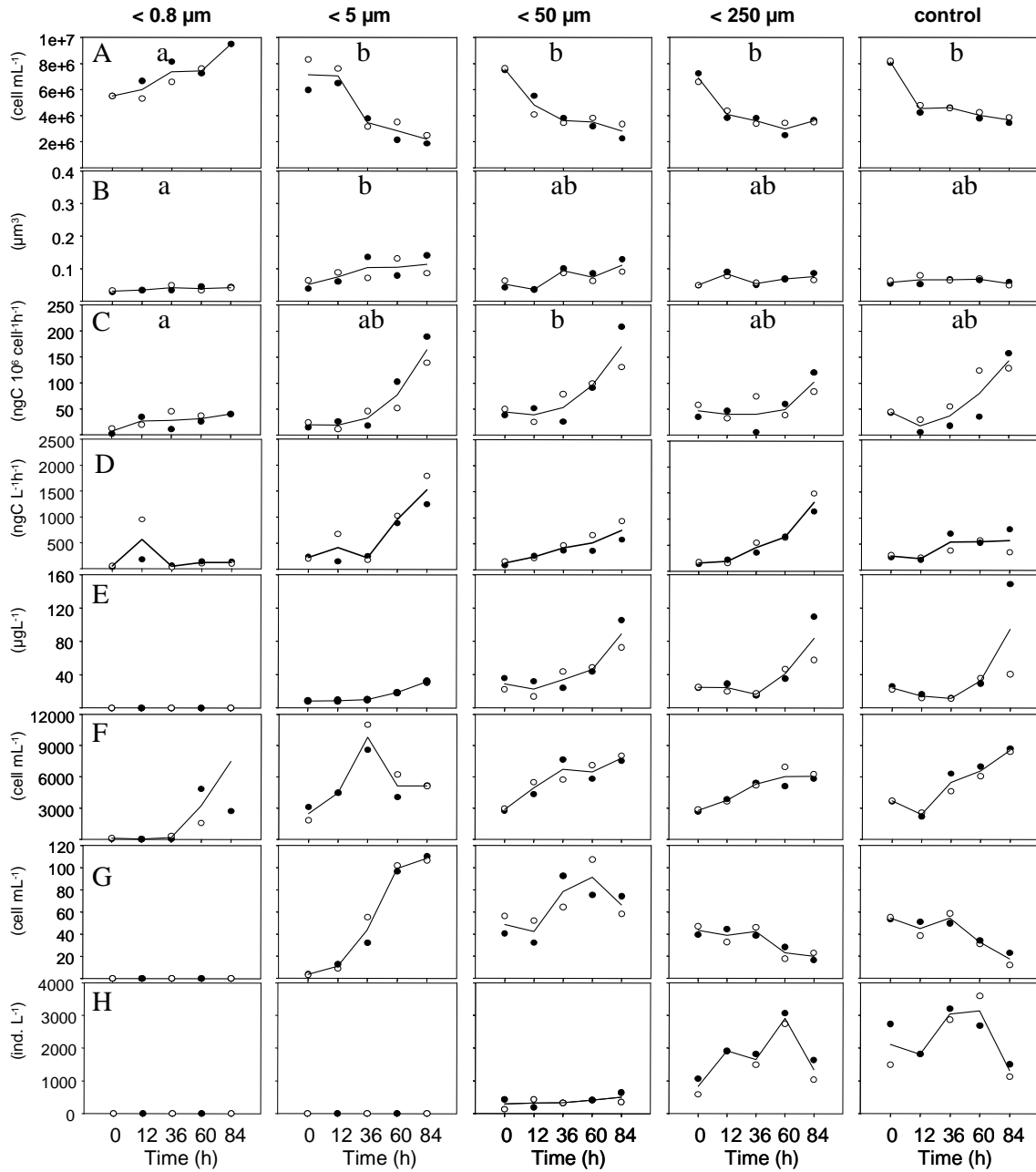


Figure 6: Temporal pattern of bacterial density (A), bacterial mean cell volume (B), cell assimilation of glucose (C), bacterial production (D), chlorophyll-*a* concentration (E), heterotrophic nanoflagellates density (F), ciliates density (G) within fractions in eutrophic reservoir. Line is the mean for two replicate treatments respectively represented by black and white circles. Letters represent significant differences between treatments (ANCOVA).

“< 5 μm ” fraction and reached the highest densities at the end of the experiment (Fig. 6G). The “< 50 μm ” fraction allowed also a good development of ciliates while the presence of zooplankton (“< 250 μm ” fraction and control) limited their growth. Low zooplankton densities were observed in the “< 50 μm ” fraction (Fig. 6H). In larger fractions (“< 250 μm ” fraction and control), a peak occurred at time 60h, followed by a prompt decline.

Taxonomic response

According to the reservoir, bacterial community composition (BCC) in the control presented 92% to 74% of similarity with *in situ* BCC. Similarities between the different fractions varied between 52 and 100%. In the oligomesotrophic reservoir, BCC in the different fractions presented more than 74% of similarity excepted for BCC in the “< 0.8 μm ” fraction that displayed only 60% of similarity and underwent a strong change between 0 and 24h. BCC within the others fractions experienced limited changes. In the mesoeutrophic 1 reservoir at the beginning of the experiment, BCC displayed some dissimilarities (less than 70% of similarity) between small (“< 0.8 μm ” and “< 5 μm ”) and large fractions (“< 50 μm ”, “< 250 μm ”, control and *in situ*). After 24h, BCC in the “< 0.8 μm ” and “< 5 μm ” fractions differentiated. BCC in the “< 50 μm ” fraction diverged slightly from the control and from the “< 250 μm ” fraction that followed a parallel temporal evolution. At the beginning of the mesoeutrophic 2 experiment, the fractions can be sorted in 3 groups according to their similarities in BCC. The first group gathered the large fractions (“< 50 μm ”, “< 250 μm ” and control) and the second assembled the small fractions (“< 0.8 μm ” and “< 5 μm ” fractions) (88% similarity). *In situ* BCC was intermediary between these two groups. Rapidly, BCC in each fraction evolved rapidly except *in situ* and in the “< 0.8 μm ” fraction that underwent minor changes. In the eutrophic reservoir at the start of the experiment, highly similar bacterial communities were observed between the “< 250 μm ” fraction, the control and *in situ* (85% of similarity). BCC in the “< 50 μm ” fraction presented 73% of similarity with the control while BCC in smaller fractions (“< 0.8 μm ” and “< 5 μm ” fractions) differentiated more clearly from the control (56% of similarity). *In situ* BCC and in the control progressed in the same way but at different speeds. BCC in “< 250 μm ” and “< 0.8 μm ” fractions underwent reduced changes. Bacterial community in the “< 50 μm ”

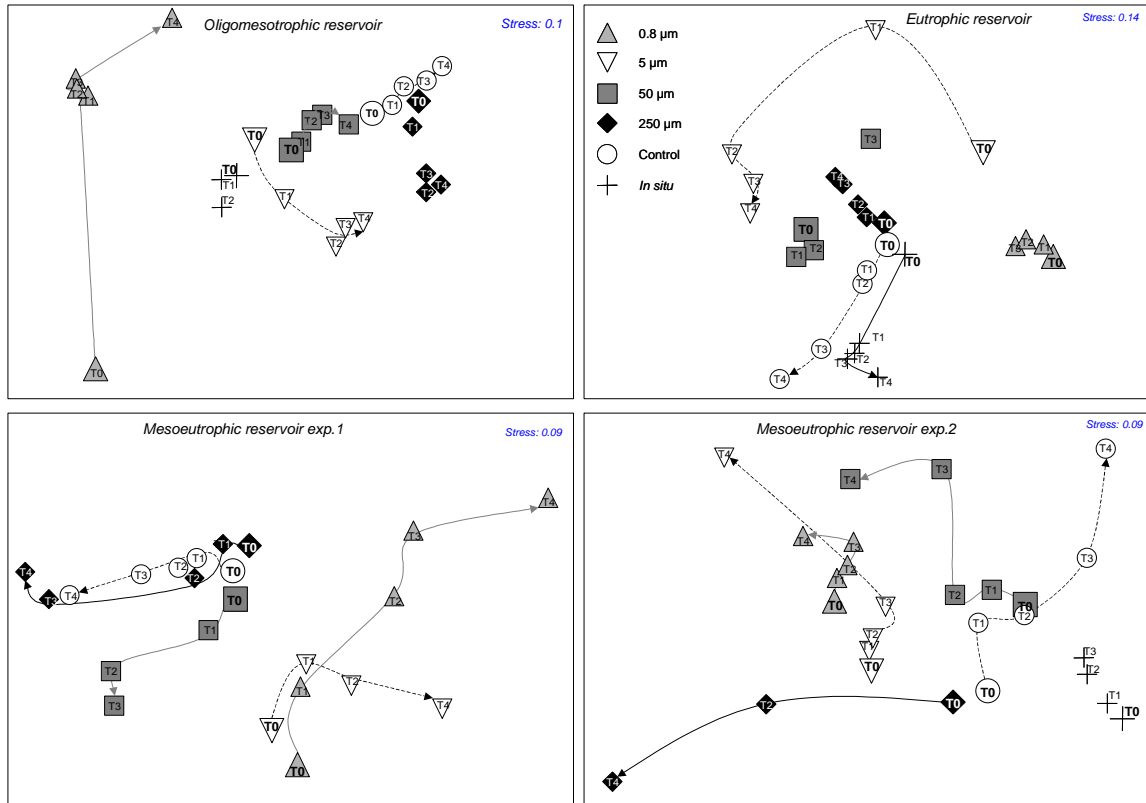


Figure 7 : Temporal pattern of bacterial diversity (MDS) in the different treatments and reservoirs

fraction remained stable during the first 48h and afterwards changed suddenly. After 24h, BCC in the “< 5 μm ” fraction modified strongly and became similar to BCC in the “< 50 μm ” fraction (85% of similarity).

Discussion

By their position at the base of the planktonic food web, bacteria experience several trophic influences: predation (grazing), growth stimulation (nutrients recycling), competition for substrates (algae, protozoans), parasitism (viruses). In multi-levels predatory chains, these mechanisms are complicated by the cascading influences. A major concern of microbial ecologists is to identify the top-down factors controlling bacterioplankton. A large research effort was already achieved to explore this issue but, most often, only limited parts of the planktonic food web were considered. Actually, little is known about the cascading trophic interactions from metazooplankton to bacteria. Different ways exist for investigating such processes. Between purely descriptive field observations and laboratory studies conducted under very reduced conditions, the manipulation of natural communities in micro- or mesocosms represents a relevant compromise (Srivastova et al., 2004). Manipulation experiments of the planktonic food web are generally achieved by adding increasing densities of predators (one species or an entire trophic level) or by removing one or several trophic levels. By truncating the grazer chain at different trophic levels, size fractionation method seems adapted to study trophic cascades effects on bacteria while respecting natural planktonic abundance and composition. However, size-fractionation was rarely used to manipulate all the trophic levels from bacteria to metazooplankton. The method was largely employed to investigate the bacteria-flagellates chain (Jardillier et al., 2005; 2004; Šimek et al., 2005; 2001) or in a less extend the bacteria-flagellates-ciliates chain (Šimek et al., 1999) and the bacteria-flagellates-cladoceran chain (Degans et al., 2002; Langenheder and Jürgens, 2001). Some marine studies took into account the planktonic web from bacteria to metazooplankton (Calbet et al., 2001; Calbet and Landry, 1999). In this study, we assessed the relevance of size fractionation for investigating the cascading trophic interactions proceeding, via direct and indirect effects, from metazoan to bacteria. Indeed, besides modifying predation conditions, fractionation induces also

changes in available substrates and nutrients. Therefore, the resulting bacteria responses stem from the combined top-down and bottom-up influences. The numeric, physiologic and genotypic responses of bacterial communities to the various predator chains were considered in order to determine both the structural and the functional responses of bacterial communities. Cascading influences on bacteria are susceptible to vary according to the water system considered and the season. Therefore, we studied reservoirs with contrasted trophic status at different periods in order to obtain distinct planktonic compositions and size structures.

Methodological limits of size fractionation method

First, the efficiency of the sequential filtration to separate plankton in different trophic levels was assessed by comparing the abundance and composition of planktonic assemblages in the fractions at the beginning of the experiments. The fractionation process provided significantly different planktonic chains. The “< 0.8 μm ” fraction can be described as free of predator (in oligomesotrophic and mesoeutrophic reservoirs) or as a low predator treatment (in eutrophic reservoir). In the “< 5 μm ” fraction, bacteria experienced the impact of flagellates as main predator (limited presence of small ciliates) except in the oligomesotrophic reservoir when the small size ciliates community passed through the 5 μm filter membrane. The “< 50 μm ” fraction consisted in a simple microbial chain (bacteria, algae and protists) where metazooplankton was quasi absent. The “< 250 μm ” fraction consisted in a planktonic web without large crustaceans. Large cladocerans (daphniidae, holopedidae) and adults copepods were efficiently removed by the 250 μm membrane filter but small crustaceans (bosminidae, copepodites and nauplii) stayed on. In mesoeutrophic 2 and eutrophic reservoirs, the “< 250 μm ” fraction did not differ significantly from the unfiltered treatment since large crustaceans were naturally absent *in situ* at these dates. In conclusion, the size-fractionation method allows separating efficiently the main trophic levels by sequential filtration. Although the plankton of the three reservoirs differed in size structure and composition, the chosen mesh sizes seem adequate and applicable to most lakes communities.

Secondly, the consequences of sequential filtrations on bacterial communities were checked. In most cases, the 0.8 μm membrane allowed separating bacteria from

predators without affecting (or affecting in a limited extend) the bacterial abundance except for the eutrophic reservoir when 30% of bacteria were removed by filtration. While the filtration process affected moderately the bacterial number, it reduced significantly the mean cell volume. This methodological bias occurred for low mesh size in eutrophic, mesoeutrophic 2 and in oligomesotrophic reservoirs. Fractionation on membranes with low porosity (0.8 and 5 μm) inhibited bacterial activity. The filtration process can stress bacteria. However, the inverse relationship between bacterial activity and membrane pore size suggested that the elimination of large bacterial cells affect severely the physiology of the bacterial assemblage. Indeed, large bacterial cells are also the most metabolically active (Lebaron et al., 2002; Servais et al., 1999; Gasol et al., 1995; Letarte and Pinel-Alloul, 1991). As showed by the DGGE analysis, the composition of the bacterial assemblage was altered by the filtration on 0.8 μm as well as on 5 μm in all experiments but, in most cases, only few taxonomic units were removed (1 or 2 bands). Indeed, large bacterial cells are generally not many numerous and composed of few genotypes (Bernard et al., 2000). In 3 cases, the filtration effect was more pronounced causing the elimination of half or more of the taxonomic units (" $< 0.8 \mu\text{m}$ " fraction in the oligomesotrophic reservoir and in " $< 0.8 \mu\text{m}$ " and " $< 5 \mu\text{m}$ " fractions in the eutrophic reservoir). Inversely, new taxonomic units appeared in mesoeutrophic 2 reservoir, resulting probably from the release of attached or associated bacteria during the filtration process. It has been shown that fractionation provoke the destruction of organisms and the release of particulate matter (Gasol and Moran, 1999). Such process probably occurred during our experiments leading to bacterial release.

In summary, the use of membrane of low porosity to separate bacteria from predators do not affect markedly bacterial density but may modify severely the composition, the morphology and mainly the metabolism of the bacterial assemblage altering the initial experimental conditions. Although size fractionation on 0.8 μm was commonly used to separate bacteria from predators (Šimek et al., 2005, 2003, 2001, 1999), few authors take into account this potential source of error. The use of 1 μm or 1.2 μm membranes could limit the elimination of large bacteria but allows the passage of numerous protists (Degans et al., 2005; Jardillier et al., 2004; 2005; Šimek et al., 1999).

The effect of the experimental device was evaluated by comparing biotic and bacterial assemblages as well as abiotic characteristics in the control treatment and *in situ* conditions. According to ANOSIM analysis, abiotic conditions, planktonic composition and bacterial characteristics did not differ significantly between the control and *in situ*. Therefore, we can assume that confinement and recycling effects do not significantly affect bacterial responses. The large volume used in our experiments (25 L) probably limits the containment effects typically found in bottle experiments (Simek et al., 2005; Srivastava et al., 2004; Roman and Rubble, 1980).

Trophic relationships

The trophic relationships going on during the clear water phase in oligomesotrophic and mesoeutrophic 1 reservoirs presented several similarities. The microbial loop (algae, HNF and ciliates) experienced a severe decline in all treatments suggesting a strong resource limitation accentuated by the top-down control of the abundant crustaceans population (copepods, *Daphnia* and/or *Holopedium*). At this period, planktonic organisms initiated the decreasing phase of the spring peak. This classical population decay has been well described in the PEG-model (Sommer et al., 1986). In both experiments, a clear top-down control of ciliates by metazooplankton larger than 50 μm occurred. This control stems likely from the grazing activity of cladocerans and copepodites that constituted the major part of the zooplankton population. Both planktonic groups are known to reduce significantly ciliates populations (Brett et al., 1994; Wiackowski et al., 1994). HNF population drop off whatever the predator considered (ciliates or crustaceans) and the predation intensity (variables ciliates and crustaceans abundances), suggesting the primary role of resources exhaustion in the HNF decline prior to the potential predation by ciliates (Ichinotsuka et al., 2006; Simek et al., 1997) and/or cladocerans (Degans et al., 2002; Langenheder and Jürgens, 2001; Jürgens et al. 1996).

The mesoeutrophic 2 experiment was performed during the ascending phase of the spring algal peak. The planktonic assemblage, mainly composed of ciliates and rotifers, was typical of this period. The rising temperature and the good nutrients conditions were favourable to all microbial components that displayed a clear developing trend. No

obvious cascading interactions were observed amongst the studied planktonic components.

In eutrophic reservoir, the abundant zooplankton population was dominated by rotifers and nauplii. A clear top-down control on ciliates is observed in the presence of zooplankton (" $< 250 \mu\text{m}$ " fraction and control) while ciliates developed freely when zooplankton was absent or reduced (" $< 5 \mu\text{m}$ " and " $< 50 \mu\text{m}$ " fractions). The ciliates and zooplankton populations were insufficient to control the rapid HNF development. The high initial bacterial density suffered a severe decline in all treatments suggesting an intense bacterivorous activity by the abundant HNF population.

Bacterial responses

In all reservoirs, bacterial abundance remained in the average range observed for the year 2003 (chapter 1). In oligomesotrophic and mesoeutrophic 1 experiments, bacterial density remained unchanged whatever the predation conditions. The grazing activity of the dominant cladocerans population present in both experiments provided likely large amounts of dissolved organic carbon (Richardot et al., 2001; Kamjunke and Zeher, 1999; Hygum et al, 1997) that stimulated bacterial growth and equilibrated the predation impact. The weak numeric response of bacteria to changes in predation pressure is common in biomanipulation studies because predation mortality is counterbalanced by nutrients recycling resulting in stable bacterial abundances (Adrian and Schneider-Olt, 1999; Wickham, 1998; Jürgens et al., 1994). Inversely, in mesoeutrophic 2 and eutrophic reservoirs, the complete removal of predators (protozoans and small zooplankton) resulted in a significant but opposite numeric responses of bacteria. The bacterial abundance decreased in the mesoeutrophic 2 experiment whereas it increased in the eutrophic experiment. The bacterial decline in the mesoeutrophic 2 experiment cannot be related to any change in the bacterial community structure that remained stable during the course of the experiment. The presence of predators (protozoans and small zooplankton) likely stimulated bacterial growth by excreting nutrients compared to the free-predator treatment where growth factors were probably limiting. In the eutrophic reservoir, the favourable growth conditions (high temperature and nutrients concentrations) allowed bacterial growth in the absence of predators. Whereas the high HNF abundances present in all predation

conditions induced a significant bacterial mortality. The temporal patterns of bacterial density in our experiments suggested that a threshold in predation pressure should be reached to bring the balance between growth and mortality down. The required predation level was only reached in the eutrophic reservoir in summer. In all cases, bacteria seemed able to respond quickly to predation conditions by adapting their growth rates.

The presence of predators induced a significant increase of the mean bacterial volume in all experiments excepted in the oligomesotrophic reservoir. The cell volume response was particularly obvious in the flagellates-dominated treatment (“< 5 μm ” fraction). The cell volume increase was not related to taxonomic changes excepted in the eutrophic reservoir when the composition of the bacterial community displayed strong changes in the “< 5 μm ” fraction. Colonial or filamentous bacterial forms were absent in all experiments. The absence of cell volume response in the oligomesotrophic reservoir could mean that in low trophic water bodies, bacteria are less influenced by predation because they are more severely nutrients-limited or because the predation pressure is below a certain threshold. At our knowledge, no similar study has been performed in an oligotrophic system excepted in marine system (Calbet et al., 2001). In systems of higher trophy, increasing its cell volume could limit the risk to be consumed by predators. Grazing-induced morphological shifts towards larger or lower bacterial sizes (or both) have been reported in many predation experiments (see the mini-review of Hahn and Höffle, 2001).

In all experiments, the presence of predators stimulated the assimilation of glucose at the cell or community level as well as the bacterial production in the eutrophic reservoir. The stimulation stemmed likely from the recycling action of planktonic grazers recognized to produce high quality substrates for bacterial growth (Hygum et al., 1997). The increase in glucose assimilation occurred in various grazer treatments according to the reservoir, suggesting the non-specific action of grazing-mediated nutrients. The association between bacteria and heterotrophic plankton or the attachment of bacteria on particular matter is known to enhance bacterial activity (Kragh & Sondergaard, 2004 ; Strom et al., 1997). Such association could also explain the higher assimilations measured in treatments containing predators and/or particulate

matter compared with predator-free treatment since the used method does not distinguish between free and attached bacterial activity.

The bacterial community composition (expressed by DGGE analysis) displayed variable temporal patterns according to the fraction and the reservoir. In the main, BCC evolved strongly when predators were removed and remained quite stable in multilevel predatory chains (from “< 50 μm ” fraction until intact planktonic chain) excepted in the mesoeutrophic reservoir during spring algal bloom when all treatments induced BCC variations. The stronger modifications occurred in free-predators treatments or in the flagellates-dominated treatment. This suggested that strong changes in predation-resource balance are needed to induce a change in BCC.

In summary, the fractionation method allows separating efficiently the main planktonic components in most water systems but the use of low-porosity membrane may affect severely the integrity of the bacterial assemblage especially their metabolism. The method allows displaying significant bacterial responses but the respective influences of ciliates, rotifers and crustaceans on bacterial responses are difficult to distinguish since the truncated food webs remained quite complex. Such multilevel design allows displaying clear relationships between higher trophic levels. Bacteria display multiple and variable responses to predation. They increase their cell volume and their metabolic activity in presence of predators. In a multi-level trophic chain, bacterial community composition remained stable while a strong predator removal induces (in most cases) a strong BCC change. Up to a certain level, predation mortality is counterbalance by a growth rate enhancement resulting in stable or rising bacterial density.

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DISCUSSION GENERALE

CONCLUSION

ASPECTS METHODOLOGIQUES

Par l'engouement provoqué par le développement des outils moléculaires, beaucoup d'études se focalisent sur la diversité bactérienne au détriment de la connaissance de l'activité bactérienne. Or, l'approche écophysologique procure des informations capitales pour comprendre le fonctionnement des écosystèmes. Ainsi l'approche multiparamétrique choisie dans le chapitre I tend à répondre à la demande de descriptions des cycles bactériens. Les observations obtenues permettent de proposer des théories écologiques. Cependant une telle approche présente comme principal inconvénient de prendre en considération l'ensemble des composantes de l'écosystème, augmentant ainsi la complexité et limitant la possibilité et la pertinence de l'interprétation des résultats. Elle répond toutefois au besoin de connaître les dynamiques des communautés étudiées en conditions naturelles.

Nombre de techniques récentes ont été employées pour suivre les différents paramètres bactériens. La coloration au Sybr green I est apparue comme la coloration la plus adaptée à notre suivi des populations picoplanctoniques, ce fluorochrome étant le plus puissant et présentant peu de diminution d'intensité au cours du temps (Marie et al. 1997). Deux inconvénients surgissent cependant de cette utilisation : la difficulté à savoir clairement si l'ensemble des cellules colorées sont bien des bactéries et la discrimination entre des cellules viables et des cellules mortes. Cette seconde difficulté est inhérente à l'énumération par microscope (Jugnia et al. 2000, Del Giorgio et al. 1997). A cela s'ajoute la présence de cellule "ghost", présentant de l'ADN non fonctionnelle (Zweifel et Hagström 1995), correspondant à des résidus de lyses virales ou du grazing des protozoaires sur les bactéries.

D'autre part, une question se pose quant aux activités suivies (enzymatiques et production), les mesures effectuées correspondent-elles réellement aux bactéries ? En effet, Vrba et al. (2004) ont montré que des eucaryotes (champignons, diatomées, protozoaires...) pourraient être considérés comme des producteurs d'enzymes potentiellement très importants. La même problématique se pose pour l'assimilation de

molécules marquées radioactivement (sucres et leucine), certains organismes (plus particulièrement les protistes) seraient-ils capables d'ingérer directement ces substrats ?

L'utilisation du CTC aurait pu répondre partiellement à ces interrogations. Cependant la controverse exposée dans la Revue Bibliographique sur son utilisation, nous a plutôt amené à tester les limites de cette méthode. Ainsi les petites amplitudes de densité des cellules capables de réduire le CTC (CTC+) sont peu informatives. Mais la comparaison entre les densités de CTC+ et les densités totales montre un modèle saisonnier qui peut être relié à la disponibilité en nutriments, au « sloppy feeding » des cladocères ou à la disponibilité en oxygène. De plus, la proportion de CTC+ augmente le long du statut trophique (cf l'article présenté en annexe).

Dès lors, dans la suite de notre propos, nous utiliserons plutôt le terme « picoplancton » pour l'ensemble des paramètres suivis et resterons prudents quant aux activités mesurées.

L'électrophorèse sur gel à gradient dénaturant (DGGE) est couramment utilisée pour comparer la répartition des populations le long de différentes échelles (verticale, horizontale, concentration de nutriments) ou pour visualiser des changements de peuplement au cours de série saisonnière. C'est pourquoi, dans notre étude la composition de la communauté bactérienne a été déterminée par DGGE, qui a permis d'obtenir une image de la diversité des différentes communautés. Cette technique est plus rapide à mettre en œuvre que celle du clonage-séquençage. Les résultats obtenus ne dépendent pas du volume d'échantillon filtré puisque la PCR amplifie les organismes majoritairement présents dans les échantillons, de plus il est possible d'exciser les bandes obtenues et de les séquencer. Alors que la méthode n'apparaissait que qualitative, Muylaert et al. (2002) ont montré qu'il était possible de corrélérer l'intensité des bandes avec l'abondance relative d'espèce. Cependant la méthode présente quelques limites : la comparaison inter gels nécessite l'utilisation d'un standard interne (Peterson et Dahllöf, 2005) et d'autre part une dilution effectuée sur des échantillons entraîne une modification des résultats.

Nos approches expérimentales *in situ* (Chap. II et III) permettent par simplification du système ou par le contrôle de paramètres choisis (Fraser et Keddy 1997) de mettre clairement en évidence l'action de facteurs de régulation donnés sur le

modèle étudié, tout en limitant les biais liés aux co-variations (Srivastava et al. 2004). Nous avons utilisé des volumes adaptés aux différentes communautés étudiées avec des temps d'incubation adaptés pour obtenir des réponses des communautés picoplanctoniques aux différents traitements.

Cependant les filtrations différentielles peuvent apporter des biais potentiels par cette simplification de la boucle microbienne, c'est pourquoi nous avons utilisé de grands volumes afin de limiter l'effet de confinement qui peut entraîner un développement spécifique de certaines communautés. La comparaison des paramètres physico-chimiques entre les échantillons *in situ* et les échantillons total ne montrent pas de différences significatives (Chap. III).

L'ETUDE

L'ensemble des suivis saisonniers présenté dans le chapitre I, nous a permis d'appréhender les dynamiques picoplanctoniques, de discriminer certains facteurs significativement liés à ces dynamiques et ainsi, d'émettre des hypothèses quant aux mécanismes d'action des facteurs significatifs. Ceux-ci ont été par la suite testés par des expériences réalisées *in situ* (chapitre II et III) pour déterminer à quel niveau de régulation (biomasse, activité, diversité...) les facteurs agissaient.

Le suivi des communautés picoplanctoniques révèle une croissance picoplanctonique ainsi qu'une élévation de toutes les activités avec l'augmentation du niveau trophique des écosystèmes étudiés (Annexes-dynamiques bactériennes). Cette croissance était déjà connue grâce à d'autres études (Krustulovic 1997) et elle apparaît par ailleurs peu surprenante. Cependant, cette étude montre aussi que les cellules picoplanctoniques du réservoir oligomésotrophe présentent de meilleures performances d'activités enzymatiques et d'assimilation (Annexes-dynamiques bactériennes). Ces cellules présenteraient une plus grande affinité avec les substrats et donc une meilleure adaptation à un environnement oligotrophe entraînant un atout de compétition contre les algues (cf. Revue Bibliographique).

En se référant aux travaux de Yannarel et Triplett (2004), nous pouvions nous attendre à ce que des systèmes ayant des productions différentes présentent des

communautés différentes. En effet, les résultats obtenus montrent que les compositions des communautés eubactériennes sont spécifiques à chaque réservoir. Toutefois, 20% des bandes trouvées sur gels de DGGE sont communes aux trois réservoirs et 50% sont spécifiques d'un seul réservoir.

Activités picoplanctoniques

Malgré les fortes différences quantitatives et des communautés spécifiques, l'analyse exploratoire multivariée des suivis écophysologiques a mis à jour une dynamique picoplanctonique similaire parmi les réservoirs étudiés (Chap.I). Nous obtenons une séparation statistique entre de larges cellules peu actives en période « froide » et des cellules de petite taille fortement actives en période « estivale ». Un décalage temporel est toutefois observé s'expliquant par l'emplacement géographique des réservoirs, le réservoir oligomésotrophe se situant dans une région plus continentale. Cette dynamique est proche de celle observée dans le lac Constance, mésotrophe, en Allemagne (Simon et al. 1998) et correspond schématiquement au modèle de dynamique proposé par Wetzel (2001).

Les facteurs apparus significatifs par l'analyse de redondance exécutée sur nos trois réservoirs (Chap. I) montrent que les facteurs significatifs sont majoritairement des facteurs « *bottom-up* ». Plus particulièrement, ces facteurs *bottom-up* correspondent aux nutriments inorganiques tels que nitrates et phosphates auxquels nous pouvons rajouter la température. En effet, ce facteur physique peut être considéré (Vrede 2003) comme un des principaux facteurs de croissance. Bano et al (1997) ainsi que Pomeroy et Wiebe (2001) ont montré que la production bactérienne est principalement contrôlée par les ressources organiques ou minérales disponibles, directement dépendant des apports autochtones ou allochtones. Cependant les études exploratrices du Chapitre I ont mis en avant que le carbone organique dissous (COD) n'était pas un facteur significatif, alors que plusieurs études ont montré une production bactérienne plus ou moins dépendante de la production de COD par le phytoplancton (Jansson et al. 1999, Richardot et al. 1999) ou par les prédateurs (Pace et al. 1994, Olsen et al. 1986). Toutefois, ce résultat peut s'expliquer par la méthode exploratrice utilisée. Soit il existe une covariation avec

les paramètres suivis, soit le COD mesuré ne représente pas une bonne estimation de la fraction assimilable par les micro-organismes (Sondergaard et Middleboe 1995).

Il est à noter les facteurs bottom-up significatifs sont identiques d'un réservoir à l'autre. A l'inverse, les facteurs top-down significatifs divergent d'un réservoir à l'autre, apparaissant spécifique du réservoir.

Un seul facteur est significatif dans tous les réservoirs : les virus. En effet, la communauté virale constitue un facteur de mortalité pour la communauté picoplanctonique dont l'importance varie selon les écosystèmes et qui peut représenter de 0 à 50% de la mortalité bactérienne dans les écosystèmes lacustres (Weinbauer 2004). Toutefois, Fuhrman (1999) rapporte que la lyse virale pourrait ne pas avoir d'effet significatif sur la mortalité bactérienne. Elle pourrait même lui être bénéfique par le relargage de matière organique qui s'en suit, offrant des substrats profitables au picoplancton (Middleboe 2000). En accord avec ces résultats les pics de virus coïncident dans nos réservoirs avec les pics de production picoplanctonique. Cependant les corrélations entre virus et production peuvent simplement s'expliquer par le fait que les virus doivent être suffisamment abondants pour rencontrer leurs hôtes (Schwalbach et al. 2004) : dix à vingt fois supérieure à celle des bactéries (Wommack et Colwell 2000).

Par l'analyse à partition de variance, les facteurs *top-down* ont montré n'avoir qu'une faible intensité d'impact sur les dynamiques picoplanctoniques (Chap.I fig.4). Ce résultat a pu être confirmé par les expériences de filtrations différentielles (Chap. III). En effet, quelles que soient les conditions de prédatons l'abondance picoplanctonique reste stable.

La population de cladocères fournissant en quantité suffisante du carbone organique dissout (Richardot et al. 2001, Hygum et al. 1997). Toutefois certains travaux ont mis en avant que certains types de cladocères tel les *Daphnia* sp. pouvaient être responsables d'une mortalité importante du picoplancton (Jürgens 1994, Langenheder et Jürgens 2001, Vadstein et al. 2003). En réponse à cela King et al. (1991), posent la question de la viabilité bactérienne dans le tractus de ces organismes. Ce tractus offrirait un lieu propice de protection par rapport au zooplancton métazoaire.

Dans le réservoir eutrophe, le seul facteur biologique significatif (Chap. I) correspond aux nanoflagellés hétérotrophes. Ces organismes sont classiquement

considérés comme les principaux organismes régulant l'abondance bactérienne (Simek et al. 2000). Lors des expériences de prédatons réalisées dans ce réservoir la chute de l'abondance bactérienne la plus élevée est constatée dans le traitement $<5 \mu\text{m}$ contenant l'essentiel des nanoflagellés hétérotrophes (Chap.III fig.6).

Cette dissimilarité entre le comportement des facteurs *bottom-up* (nutriments + température) et des facteurs *top-down* en fonction du réservoir considéré est confirmée par les analyses à partitions de variances (Chap.I). **Les facteurs *bottom-up* seuls expliquent majoritairement les variations des dynamiques picoplanctoniques** (Chap.I, Fig.3). Peu de comparaison s'avère possible car les travaux portant sur la concomitance et l'estimation de l'importance relative des facteurs sur la dynamique d'activités picoplanctoniques sont, à notre connaissance, inexistant.

Cependant, nous observons une décroissance de la part expliquée par les ressources avec l'augmentation du niveau trophique au bénéfice de la part expliquée commune aux deux types de facteurs (Chap.I, Fig.3). Ainsi, l'impact strict des ressources sur l'activité picoplanctonique devient moins prépondérant dans des milieux riches en nutriments. Cela correspond à l'hypothèse de Sanders et al. (1992), selon laquelle les facteurs prépondérants s'inversent le long du gradient trophique.

Cette part commune correspond à une interaction entre les deux types de facteurs. En effet, une source importante de COD peut être libérée par la lyse virale et la prédation (Vadstein et al. 2003). De même, Olsen et al. (1986) ont montré que le picoplancton pouvait assimiler directement 30% du carbone excrété par les cladocères du genre *Daphnia*. Enfin, lors d'expériences réalisées en culture, Selph et al. (2003) ont observé que la croissance bactérienne pouvait être stimulée de 2 à 14 fois en présence de prédateurs flagellés, l'activité bactériovore étant probablement source des ressources nutritives.

Les expériences en microcosmes (Chap. II) ont montré que les réservoirs oligomésotrophe et mésoeutrophe étaient non limitées en N et P durant la phase d'eau claire et la période estivale. A l'inverse ces nutriments inorganiques constituent des facteurs de contrôle de production picoplanctonique durant la période estivale. Les enrichissements en COD (sous forme de glucose) n'ont montré que très peu d'impact sur les communautés présentes, corroborant par la même occasion les résultats obtenus

au Chapitre I et montrant que nos réservoirs n'étaient pas limités en COD durant la phase d'eau claire ou la période estivale. Cependant si le COD ne semble pas avoir un impact direct sur les communautés picoplanctoniques, nos travaux ont prouvé que la présence de nutriments carbonés en quantité suffisante entraînent une synergie d'activité. Celle-ci pouvant se traduire par une meilleure absorption des nutriments inorganiques, comme observée lors des expériences de Vrede et al (2002). En se basant sur les résultats de Donachie et al. 2001, **les quantités de nutriments apparaissent non seulement importantes mais leurs rapports moléculaires semblent primordiaux**. Ainsi, les espèces azotées (NH_4^+ et NO_3^-) semblent réguler l'absorption de glucose (Donachie et al. 2001).

La comparaison des réservoirs oligomésotrophe, mésoeutrophe et eutrophe ont permis de montrer que les activités sont liées aux concentrations en nutriments et plus particulièrement aux nutriments inorganiques. L'activité picoplanctonique apparaît donc très liée aux facteurs de contrôle « bottom-up ». A l'inverse les compositions taxonomiques de communautés ne se modifient que très faiblement lors de nos expériences (Chap. II, partie 1 fig 3 et 6, partie 2 fig 4).

Lors de ces expériences, la production picoplanctonique apparaît être une mesure plus sensible de la réponse de la communauté picoplanctonique, comparée à l'abondance (Pace 1993). Dans le même sens, Verde et al. (1999) trouvent que l'abondance bactérienne nécessite 3 à 4 jours pour répondre aux manipulations de nutriments. A l'inverse, l'assimilation de glucose apparaît tout aussi sensible aux changements de milieu, des variations apparaissant des 24h.

Diversité

Les analyses multivariées de la composition taxonomique de la communauté bactérienne (Chap.I) montrent des dynamiques distinctes entre les réservoirs (Yannarel et al. 2003), avec une complexité saisonnière croissante suivant le gradient trophique.

Les facteurs apparus significatifs par rapport aux données de diversité sont spécifiques du réservoir, toutefois les nitrates apparaissent comme un facteur significatif dans chaque réservoir et les flagellés hétérotrophes sont significatifs pour les réservoirs

mésoeutrophe et eutrophe. Des études conduites en milieux marins et d'eau douce ont montré que la disponibilité des ressources (dépendante du niveau trophique) affectait la composition de la communauté bactérienne (Höfle 1992, Donner et al. 1996, Pinhassi et al. 1999). Les analyse à partition de variance (Chap.I) ont montré qu'effectivement les facteurs « *bottom-up* » seuls expliquaient majoritairement les variations. Toutefois la "part totale" expliquée par les paramètres suivis chute fortement pour le milieu eutrophe (12 % et pour l'activité et 23 % la diversité). Cela sous-entendant que d'autres paramètres (non suivis dans notre étude) entreraient en jeu dans la dynamique de la composition bactérienne. Ainsi, les changements de niveau d'eau ou d'autres variables hydrauliques auraient pu être des paramètres intéressants à suivre (Boucher et al. 2006).

Cependant, lors des expériences d'enrichissements et de translocation réalisées sur les trois réservoirs (Chap II partie 2), peu de changements sont apparus dans la composition communautaire estimée par DGGE dans les traitements ne subissant qu'un seul type d'apport (traitements NP ou C). Une modification de 1 à 2 bandes a été observée parfois, sans pour autant être significatif. La communauté présente dans le traitement à double apports (traitement CNP) a montré des changements plus considérables, confirmant les travaux d'Ovreas et al. (2003) qui avaient montré des changements majeurs de la composition de la communauté bactérienne lorsque le glucose est ajouté en excès avec les nutriments inorganiques. La modification la plus forte correspond à la période de phase d'eau claire du réservoir oligomésotrophe montrant une forte diminution du nombre de bandes observées. Ainsi, une grande partie de la communauté serait apte à profiter du double enrichissement. Vadstein (1998) a observé que seules quelques groupes de bactéries réagissent à une arrivée brusque de nourritures tandis que d'autres sont favorisés lorsque les concentrations en nourritures restent constantes. Ainsi, quelques bactéries ont une meilleure adaptation de compétition. Les faibles modifications obtenues dans la communauté pourraient aussi être dues à quelques bactéries à croissance rapide. En effet Riemann et al. (2001) ont constaté que quelques groupes bactériens (Béta-Proteobacteria et Cytophagales) à croissance rapide, peuvent être favorisés pendant des événements particuliers (décomposition de biomasse algale). Ces bactéries sont probablement capables d'adapter rapidement leur taux de croissance à de nouvelles conditions, mais n'entraînent que de faibles changements de composition des communautés bactériennes.

Lors des expériences de filtrations différentielles (Chap. III), la stabilité de la composition de la communauté bactérienne à une période donnée apparaît dépendante de la chaîne de prédateurs. Ainsi, lors des filtrations, des changements de composition de communauté apparaissent dans les fractions $<50\mu\text{m}$. Les protistes apparaissent donc avoir un impact sur la diversité bactérienne, corroborant les analyses canoniques du Chapitre I. Ainsi les protistes exerceraient une mortalité différentielle au sein de la communauté bactérienne qui dépendrait des différentes caractéristiques des proies, tel la vitesse de nage (Matz et al. 2002), la motilité ou les propriétés membranaires (Matz et Jürgens 2003).

CONCLUSION

Il apparaît ainsi dans nos réservoirs que les différents facteurs qui exercent un contrôle sur l'abondance de la communauté ne sont pas les mêmes que ceux qui ont un effet sur la composition eubactérienne.

De plus les facteurs significatifs sont majoritairement représentés par des facteurs *bottom-up*. Ces résultats sont conformes à ceux de Muylaert et al. (2002) et de Jardillier et al. (2004).

Les enrichissements et les translocations effectués sur les réservoirs oligomésotrophe, mésoeutrophe et eutrophe ont permis de montrer qu'effectivement les activités sont fortement liées aux concentrations en nutriments et plus particulièrement aux nutriments inorganiques. L'enrichissement organique n'a montré que peu de réaction de la part du picoplancton. Cependant ces travaux ont aussi prouvé que la présence de nutriments organiques en suffisante quantité lors d'apports en nutriment inorganique (NP) entraînent une synergie d'activité et une meilleure absorption. Ainsi non seulement les quantités de nutriments apparaissent importantes mais surtout les rapports moléculaires entre eux apparaissent primordiaux. Ainsi même dans le milieu eutrophe nous observons des limitations dues aux nutriments.

Par contre les filtrations différentielles effectuées ont montré que les prédateurs n'avaient que peu d'effet sur l'activité picoplanctonique provoquant cependant une augmentation du volume cellulaire.

Par ailleurs, on remarque que la composition génotypique des communautés ne change pas de manière drastique lors des apports en nutriments. Ces communautés ont donc des capacités d'adaptations assez grandes vis-à-vis des variations en nutriments. Par contre l'intensité d'activité semble liée aux capacités des communautés présentent alors que l'activité proprement dite dépend des nutriments présents et non pas de la diversité bactérienne. A l'inverse, les nanoflagellés hétérotrophes apparaissent comme des organismes intervenant significativement dans la composition de la communauté bactérienne mais sur un laps de temps restreint. La prédation apparaissant comme un contrôle de maintien de la diversité en limitant l'abondance des bactéries les plus compétitives qui profitent au mieux des ressources.

De manière générale, il apparaît que les organismes picoplanctoniques sont fortement contrôlés par les ressources, et ce, quel que soit le niveau trophique. Nos résultats montrent que ce contrôle par les facteurs *bottom-up* semble s'appliquer également dans les milieux eutrophes alors que selon certains auteurs (Sanders et al. 1992, Gasol et al. 2002, Cotner et Biddanda 2002) la communauté picoplanctonique devrait être régulée principalement par la prédation et la lyse virale dans ce type de milieu.

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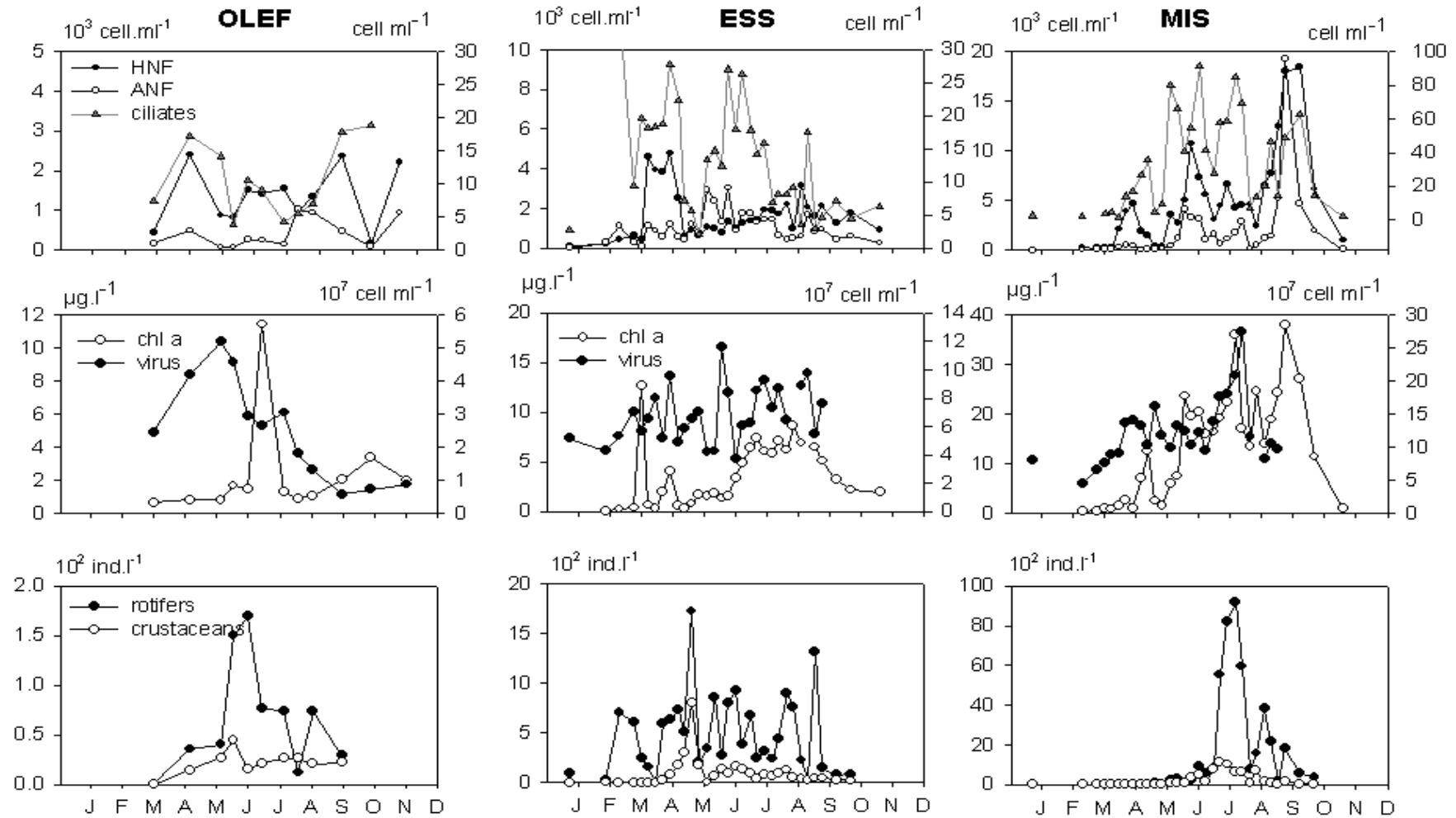
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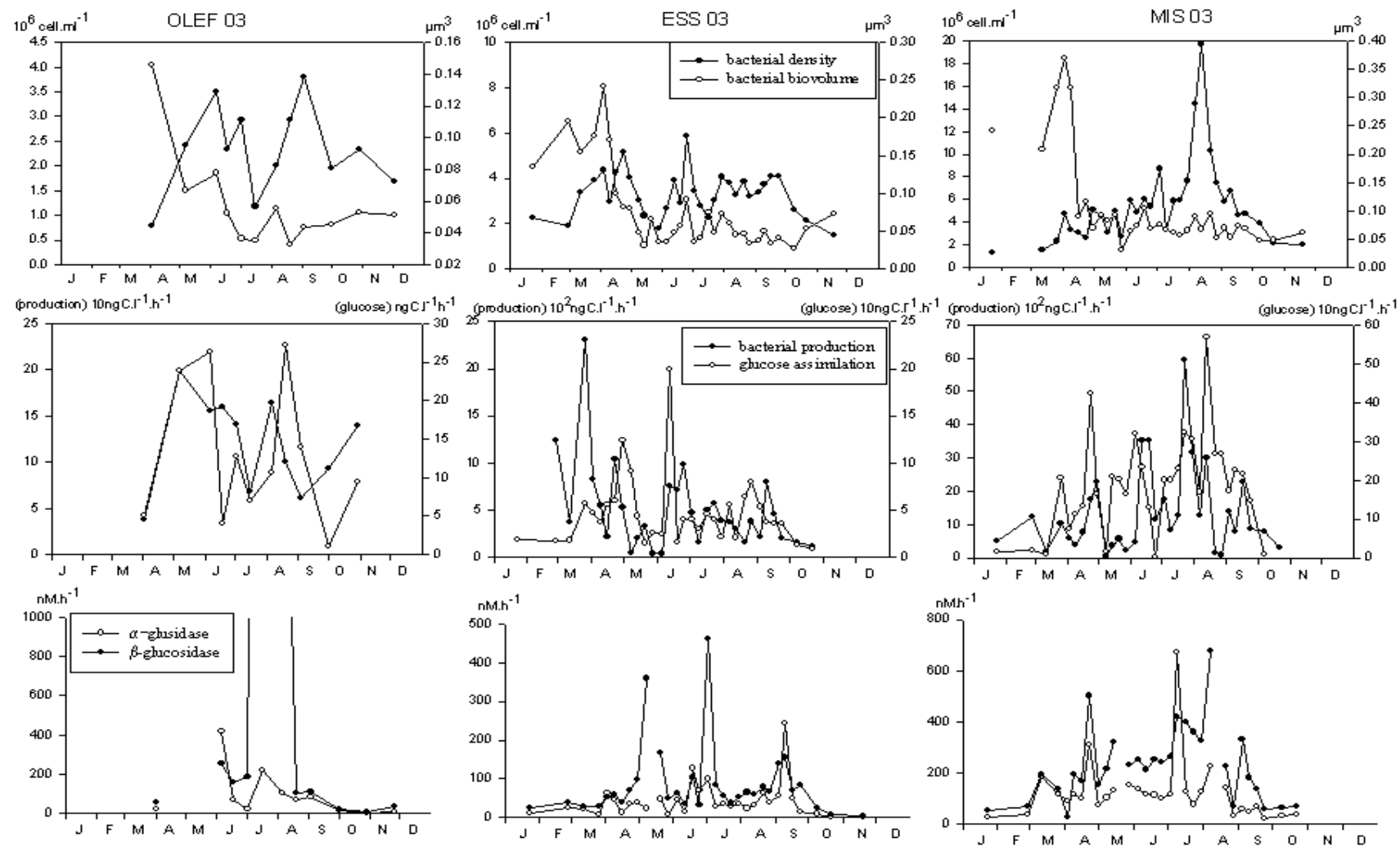
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ANNEXES

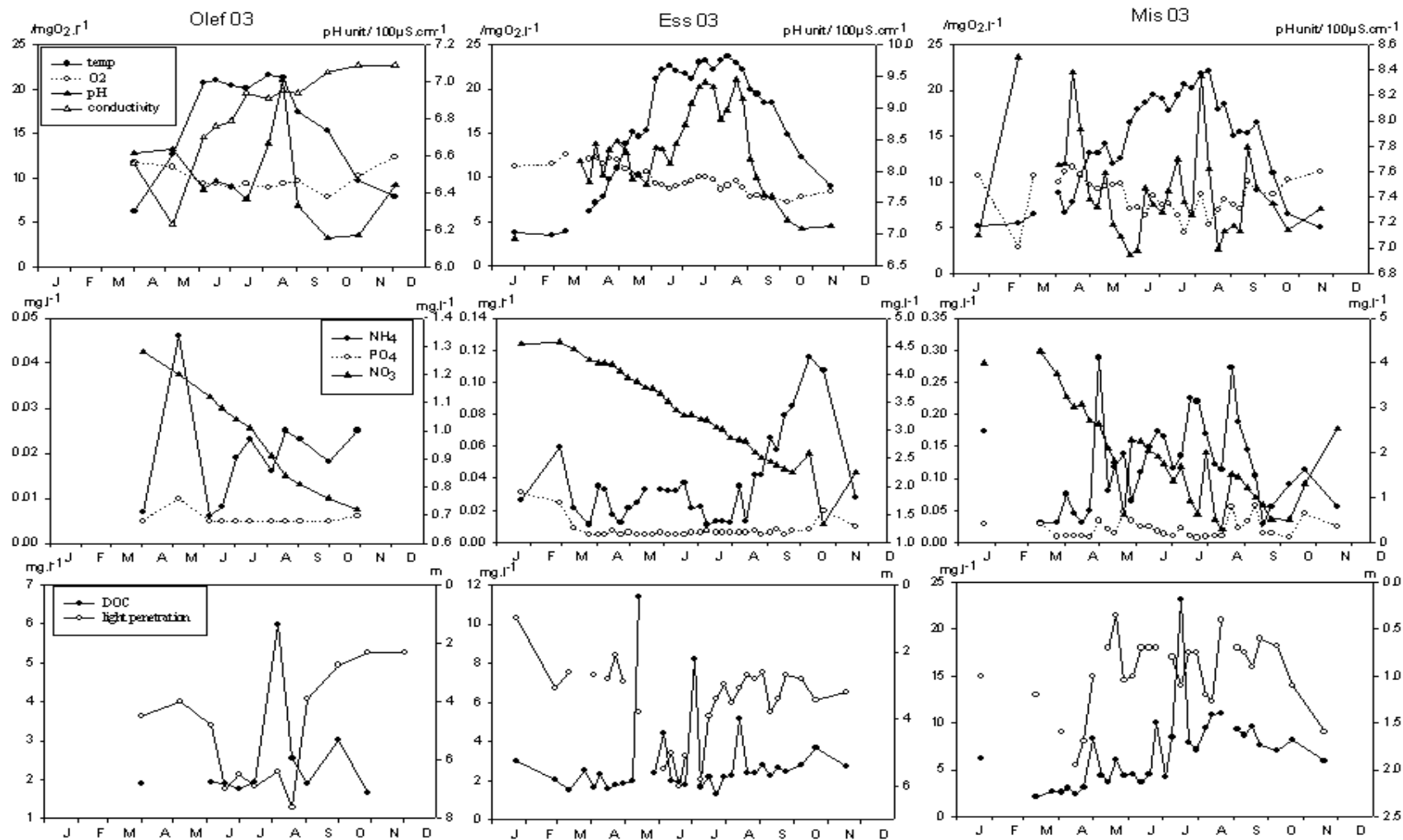
- Données des dynamiques saisonnières
 - Article sur l'utilisation du CTC



Dynamiques des variables biologiques : Nanoflagellés hétérotrophes (HNF), nanoflagellés autotrophes (ANF), ciliés, chlorophylle a (Chl a), virus, rotifères et crustacés, durant l'année 2003



Dynamiques des variables bactériennes : densité, volume cellulaire moyen, production, assimilation de glucose, activités enzymatiques (alpha et béta glucosidases) durant l'année 2003



Dynamiques des variables physico-chimiques : température (temp), oxygène (O₂), pH, Conductivité, Nutriments (NH₄-N, PO₄-P, NO₃-N), Carbone organique dissout (DOC), pénétration de la lumière durant l'année 2003

Spatio-temporal patterns of CTC+ bacteria in freshwater reservoirs

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Abstract

In a first methodological part, we examined the dynamics of cell staining using different concentrations of 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), a fluorochrome used to determine the “metabolically active” part of the bacterial communities. The density of CTC reducing bacteria (CTC+ cells) varied a lot according to incubation time and CTC concentration. The maximum CTC+ cell density was either observed after 2 hours at 5 mM or after 5 hours at 5 mM. This variability implies that using in every circumstance an incubation of 2 hours with a concentration of 5 mM (i.e. the conditions that are the most often encountered in the literature) can be misleading. We concluded that small amplitude variations in the CTC+ cell density are most probably non informative because of this significant methodological bias. However, in the second part of the paper, we examined spatial and temporal patterns of CTC+ cell densities and proportions in reservoirs with contrasted trophic statuses. The CTC+ cell proportion is the ratio between the CTC+ cell density and the total cell density as determined using the fluorochrome Sybr Green I. It appeared that the CTC+ cell density or proportion varied vertically in the water column, increasing in the most favourable, intermediate layer. On a seasonal scale, CTC+ cell proportion compared to the total cell density displayed a pattern that could be related to the availability of nutrient, sloppy feeding by cladocerans or oxygen availability. The CTC+ cell proportion also increased along the studied trophic status gradient. The CTC staining method seems therefore to be able to indicate relatively large-scale spatial and temporal patterns. However, the staining protocols or the conditions of use must be improved to give a reproducible result.

Introduction

Determining the density, biomass and production of bacteria is of prime interest in aquatic ecology because these microorganisms are responsible for a large part of major transformations such as aerobic and anaerobic respiration, and remineralisation. In the late 1970's, methods using fluorescent dyes that bind to nucleic acids were developed to detect and enumerate bacteria (Hobbie et al. 1977, Porter & Feig 1980). These methods however do not allow determining the physiological status of the bacteria. It is therefore unknown whether the cells are alive and metabolically active, alive but dormant, or moribund (Stevenson 1978). During the last decade, it has been highlighted that a substantial part of the bacterial communities were most probably metabolically inactive in many environments (Colwell & Grimes, 2000).

Different methods have been developed to determine the metabolically active state of bacteria. Microautoradiography allows identifying bacteria that actively assimilate radiolabelled substrates such as amino acids (Karner & Furhman 1997) or monosaccharides (Meyer-Reil 1978). On the other hand, the technique of Zweifel & Hagström (1995) allows the visualization of nucleoids in bacteria after washing out of the nonselectively bound DAPI by hot isopropanol. The cells with visible nucleoids are suggested to be the only active, or at least living bacteria. Universal 16S rRNA-targeted oligonucleotide probes can also be used to stain bacteria with high ribosome content that are supposed to be active. Jepras et al. (1995) tested the use of a fluorescent anionic membrane potential probe, DiBAC₄(3) as a viability marker. Finally, stains that change color (2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride – INT) or fluorescence (5-cyano-2,3-ditolyl tetrazolium chloride – CTC) upon reduction by energy intercepted along the electron transport system (ETS) can be used to detect cells with functional respiration (including not only oxygen consumption) (Maki & Remsen 1981, Rodriguez et al. 1992). More recently, Lebaron et al. (2001) proposed to distinguish between active and inactive cells on the basis of their nucleic acid content using flow cytometry.

Large discrepancies were often observed between the estimations of the active cell fractions using the methods cited above (see references in Créach et al. 2003). The

main reason for this is most probably that the methods cited above do not measure the same aspect of the bacterial metabolism. On the other hand, Servais et al. (2001) suggest that the active cell counts are underestimated because of methodological limitations such as the difficulty to detect the fluorescence signal visually at the microscope.

Despite criticisms again this technique, the staining of bacteria with CTC is still used. Reasons for this are the easiness of use and the increasing use of epifluorescence microscope in applied research. Much attention has been paid to the inadequacy of CTC as a global metabolic marker. The potential application of CTC as an easy rank index of a type of bacterial activity was not really studied until now.

The aim of the present paper is to determine whether the spatial and temporal patterns of the CTC-reducing bacterial cells (CTC+) appear to be related to the major environmental variables in reservoir and can help us to better understand the bacterial dynamics in freshwater reservoirs.

Material and methods

The study was conducted in four reservoirs exhibiting different trophic statuses. The Esch-sur-Sûre reservoir was studied in 1999 and 2003. It is located in the upper part of the Sûre river in Luxembourg (latitude 49°54'; longitude 5°53'). This reservoir is a stratified mesoeutrophic water body with a surface 3.2 km² of and a mean depth of 17 m. During 2003, three other reservoirs were included in the study. The Olefalsperre reservoir is a stratified oligomesotrophic reservoir located in Germany (latitude 50°29'; longitude 6°25'). It is used for drinking water production. It has a surface of 1.0 km² and a maximum depth of 20 m. The Pont-Misère reservoir is a shallow (maximum depth = 4 m) reservoir built upstream to the Esch-sur-Sûre reservoir. It is a eutrophic water body and serves as pre-dam to the Esch-sur-Sûre reservoir. The Differdange reservoir is a wastewater stabilisation pond. This is a small (surface = 0.1 km²), shallow (maximum depth = 3.0 m) and hypereutrophic water body.

In every reservoir, water samples were taken at an depth interval of 1 metre using a 2-l Ruttner bottle. Samples from different depths were pooled in a sterile carboy in order to obtain a bacterial community representative of the different parts of the water column. For the reservoirs that were thermally stratified (Oleftalsperre, Esch-sur-Sûre), the water samples were pooled according to the stratum they belong to during stratification (epi-, meta- or hypolimnion). When the water column was completely mixed, three pools were constituted: one with water from 0 to 10 metres deep, one with water from 11 to 15 metres deep, and one with water from under 15 metres down to the bottom. For the shallow reservoirs, only one pool was constituted on the basis of the samples taken every metre over the whole water column with the Ruttner bottle.

10 ml of each pool were immediately fixed with formaldehyde (final concentration = 2%) for the determination of the total bacterial density at the laboratory. The fluorochrome Sybr Green I (Molecular Probes) was used for staining bacterial DNA. On the other hand 10 ml of each pool were incubated during 2 hours at *in situ* temperature and in the dark in the presence of CTC (5 mM final concentration). At the end of the incubation, samples were immediately filtered on black polycarbonate membranes (porosity = 0.2 μm) and the cells stained with CTC (CTC+ cells) were enumerated under blue excitation using a Leica DMRB epifluorescence microscope. Samples for physico-chemical and other biological analyses were taken from the pool and were preserved and analysed as indicated later in the text.

On two occasions (15 January 1999 and 26 May 1999), the evolution with time of the density of CTC+ cells was monitored at four different CTC concentrations: 0.5, 1.0, 5.0 and 10.0 mM. For each concentration, triplicates of reservoir water (volume = 15 ml) were incubated in the laboratory at *in situ* temperature. One-ml to 2-ml subsamples were taken immediately at the beginning of the experiment and after 1, 2, 3, 4, 5 and 20 h of incubation. Subsamples were immediately fixed with formaldehyde (2% final concentration), filtered onto a black membrane (porosity: 0.2 μm) and CTC+ cells were enumerated under blue excitation using the epifluorescence microscope.

In parallel to the staining experiments, triplicates of ten times diluted reservoir water were incubated during one hour at in situ temperature and the rate of density increase was determined as the difference in bacteria density between the beginning and the end of the incubation. Membrane-filtered (0.2 μm) reservoir water was used for the dilution. Bacteria were stained with Sybr Green I, filtered onto a black polycarbonate membrane (0.2 μm) and enumerated using the epifluorescence microscope.

Water temperature, dissolved oxygen concentration, pH and conductivity were determined using a Hydrolab Multiprobe. Chlorophyll a concentration was determined spectrophotometrically according to Lorenzen (1967). The concentrations of Dissolved Inorganic Nitrogen (DIN), PO_4 , Total Phosphorus (TP), SiO_2 were determined using standard methods (APHA et al. 1995). Bacterial production was estimated using the incorporation of ^{14}C -leucine as tracer according to the method described by Kirchmann (1995). Nanoflagellates, ciliates and metazooplankton were fixed in glutaraldehyde (1% final concentration), mercuric chloride (2.5% final concentration) and formaldehyde, respectively. At the laboratory, nanoflagellates were stained with primulin and observed under the epifluorescence microscope. Ciliates and metazooplankton were counted using an inverted microscope. Rotifers and crustaceans were distinguished. The predation rate rather than the density of putative predators was used in the present study. The predation rate exerted by heterotrophic nanoflagellates (HNF), ciliates and metazoans (rotifers and crustaceans) was estimated as the density of predators multiplied by a mean *per capita* predation rate estimated on the basis of data from the literature. The mean (± 1 standard deviation) predation rate reached 23 , 406, 10, 800 and 2,288,000 cells $\text{ind}^{-1} \text{h}^{-1}$ for HNF, ciliates, rotifers and cladocerans, respectively (McManus & Fuhrman 1986, Borsheim & Andersen 1987, Sanders et al. 1989, Caron et al. 1991, Hondeveld et al. 1992, Ooms-Wilms et al. 1995, Sommaruga 1995, Carrias et al. 1996, Menon et al. 1996, Stabell 1996, Simek et al. 1997, Eisenmann et al. 1998, Nakano et al. 1998, Thouvenot et al. 1999, Wieltschnig et al. 1999). Copepodites were not taken into account because they do not consume significant amounts of bacteria (Thouvenot et al. 1999, Hwangm & Heath, 1999). In 2003 the density of viruses was moreover analysed using YO-PRO as fluorochrome (Xenopoulos & Bird 1997). The

relationships between the bacterial counts and the environmental variables were analyzed using Spearman correlation.

Results

The evolution of CTC staining with time at concentrations ranging from 0.5 to 10.0 mM was monitored on two occasions in 1999 (Fig. 1). In both experience, the density of the cells stained with CTC (CTC+) was the highest at 5.0 mM. In January 1999, the CTC+ density reached a maximum value of 105×10^3 cells ml⁻¹ after an incubation of two hours. In May, on the contrary, CTC+ density increased continuously during the five first hours of incubation. In January, the CTC+ density determined after an incubation of 24 h was not significantly different from the CTC+ density determined after 5 h. In May, a decrease in the CTC+ density was observed between the 5-h incubation and the 24-h incubation.

The rates of total density increase in the 10-times diluted samples during the first hour of incubation at different CTC concentration are presented in the Fig. 2. The rates observed at concentration higher than 0.5 mM were significantly lower than the rate in the control and the rate observed at a concentration of 0.5 mM. The rates at a concentration of 10 nM were virtually null.

The seasonal dynamics of the total bacterial density (Sybr Green stained bacteria) was similar in the three strata with the highest values observed from April to July (Fig. 3). The CTC-reducing cells (CTC+ cells) presented a significantly different seasonal dynamics. The density of CTC+ cells widely varied within each stratum. It ranged most of the time from 200 to 600×10^3 cells ml⁻¹ in the epilimnion and the metalimnion. It was globally lower in the hypolimnion, ranging most of the time from 100 to 300×10^3 cells ml⁻¹. The mean proportion of CTC+ cells compared to Sybr green counts was the highest in the metalimnion and the lowest in the hypolimnion (Table 1). However, the proportion varied a lot throughout the year, ranging from a minimum value between 1.5 and 2.0% to a maximum between 19.5 and 20.9% in the different strata.

The investigation was performed on one date at a higher space resolution in the Esch-sur-Sûre reservoir at the end of the water mixing period confirmed the higher CTC+ cell density in the upper layers of the water body (Fig. 4). The global pattern of bacterial production was similar to that of the CTC+ cell density; displaying the highest values in the upper layers and lower values in the deep, colder layer. However bacterial production presented a peak at a depth of 5 m when CTC+ cell density was very similar between the surface and the depth of 10 meters.

In the three reservoirs used for drinking water production the total bacterial densities followed a classical dynamics according to their trophic status (Fig.5). The cold period were characterised by stable, relatively low bacterial densities. Total densities increased during spring when temperature raised. The late spring and summer periods were characterised by high densities with frequent peaks. In the wastewater stabilisation pond of Differdange, total densities were very high ($6 \times 10^7 - 4 \times 10^8$ cells ml^{-1}) and globally increased from the winter to late summer. Contrary to the total density, the dynamics of CTC+ bacteria density was different among the reservoirs. When considering the proportion of CTC+ cells related to the total bacterial counts rather than the CTC+ bacterial density, similar temporal patterns appeared, especially when comparing the Esch-sur-Sûre and the Pont-Misère reservoirs. High CTC+ proportions were observed in spring, followed by a depression in May-June and a recovery during summer.

In 2003, the bacterial production was estimated in the four reservoirs concurrently to the CTC+ bacteria counts. The bacterial production has been plotted against the CTC+ cell densities and proportions as shown in Fig. 6. No clear relationship appeared between these variables. The significant linear relationships between the proportion of CTC+ cells and the ecological variables are presented in the Table 2. Significant ecological variables varied according to the stratum or the reservoir considered. In the upper stratum of the Esch-sur-Sûre reservoir, dissolved oxygen concentration appeared to be significantly correlated with CTC+ proportions in both studied years. On the contrary, dissolved inorganic nitrogen and cladoceran bacterivory were only significantly correlated in 1999.

Discussion

Our first task in the present study was to determine the conditions giving an optimal staining of the bacterial cells and compare it to existing protocols. Our results concerning the effects of the CTC concentration on the cell staining are consistent with those found by Rodriguez et al. (1992), Epstein & Rossel (1995), Servais et al. (2001) and Whiteley et al. (2003), among others, who observed that the concentrations resulting in an optimal staining was in the range of 2 to 6 mM. Higher CTC concentrations (10 mM) generally resulted in a lower CTC-formazan deposition within the cells (Rodriguez et al. 1992). However, this reduction in staining ability at concentration above 5 mM was not observed in every case (Griebe et al., 1997). The reduction kinetics presented in the literature showed a sharp increase in the density of CTC+ cells during the two or three first hours followed by a period with a slower increase, stability or decrease. We however observed an increase during up to five hours in May 1999. Using a 2-hour incubation in May 1999 resulted in a significant underestimation (32%) of the CTC+ count.

Beyond concentration and incubation duration, existing staining protocols also differ in the incubation temperature. Some authors incubated the water samples at 28 °C or at room temperature (Rodriguez et al., 1992; Epstein & Rossell, 1995; Cook & Garland, 1997; Griebe et al., 1997) according to the indications of the manufacturer. On the other hand, other authors incubate the samples with CTC at in situ temperature. To our knowledge, the effect of temperature on the staining with CTC is largely unknown. We observed a difference in the staining dynamics between the January and the May experiment. It is however impossible to ascertain whether this difference was due to temperature or to other factors. Given that the bacterial metabolism is known to be highly sensitive to temperature (Simon & Wünsch, 1998), it appears however more logical to use in situ temperature rather than room temperature.

The comparison of the rates of density increase indicates that an inhibition of the bacterial growth appears for CTC concentrations above 0.5 mM. The inhibitory effect was similar at 1.0 and 5.0 and increased severely at 10.0 mM. Inhibitory effect of CTC on bacterial growth, production and respiration has also been demonstrated by Ullrich et al. (1996). These authors estimated the EC_{50} (i.e. the concentration causing 50% inhibition in Microtox bioassay) of CTC to be 0.85 nM. According to our results, this EC_{50} is probably underestimated as we did not observed effects on growth between the control and the treatment with CTC 0.5 mM. The EC_{50} lays therefore more probably between 0.5 and 1.0 mM. In any case, a toxic effect in the range of efficient concentrations can severely weaken the usefulness of CTC as indicator of metabolically active bacteria as underlined by Créach et al. (2003).

Considering this first, methodological part of the study, we can conclude that a common protocol appears difficult to determine definitely. If one wants to use CTC, preliminary experiments have to be made to determine the kinetics of staining. As shown in the present study, the density of CTC + cells can reach a maximum value after 2 hours or after 5 hours according to the season. This variation in staining dynamics is therefore a first important drawback using CTC as a marker of “metabolically active” cells. Variations in the staining ability can lead to method-driven biases that can severely undermine the interpretation of the results. However in our study, like in the majority of other studies, a preliminary testing of the optimum CTC concentration appeared quite unpracticable routinely, notably due to the remote position of the study sites and to the limited time and resources available on site. We will therefore only discuss in the following part of the discussion the major variations in CTC+ cell densities considering that the smallest variations are too much affected by methodological uncertainties.

Given the drawbacks mentioned above, one can ask whether the results give anyway some large interpretable patterns. In the reservoir of Esch-sur-Sûre where both spatial and temporal data exists, it appeared that the dynamics of CTC+ cell density was not readily readable. On the contrary, patterns appeared when we considered the proportion of CTC+ cells compared to the total bacterial density. In all layers high

proportions of active bacteria were observed during the winter. In the upper and lower layers, CTC+ proportion decreased regularly from spring to the clear-water phase (May) and remained low until the beginning of the mixing period, at the end of summer. In the metalimnion, after a similar depression during the clear-water phase the CTC+ proportion increased again and remained higher than in the epilimnion during summer. In 2003, the seasonal dynamics of CTC+ cell proportion in the Esch-sur-Sûre reservoir was rather similar to that observed in 1999.

Several factors can explain a higher proportion of active bacteria in the metalimnion during thermal stratification. During the clear water phase, a higher UV penetration can induce a decrease in the bacterial activity in the epilimnion. For example, Wilhelm & Smith (2000) showed a significant photoinhibitory loss of bacterial production (14-30%) due to UV in the upper 5 m of the Lake Erie. In the reservoir of Esch-sur-Sûre, the maximum Secchi depth (5.6 m on May 26th, 1999) indeed coincided with one of the lowest CTC+ cell proportions during the year. After the clear water phase, a higher proportion of CTC+ cells appeared in the metalimnion compared to the epilimnion concurrently with a metalimnetic phytoplankton development (results shown in Jacquet et al. 2005). In this latter case, a higher availability of dissolved organic matter due to phytoplankton excretion and death probably led to an increased bacterial activity. Other controlling factors such as virus lysis or zooplankton grazing occurring in the epilimnion are also known to lead to a higher bacterial activity (mainly estimated on the basis of biomass production) in the metalimnion of certain lakes (e.g. Weinbauer & Höfle 1998) even if this is not the case in other lakes (Conevey & Wetzel 1995; Reichart & Simon 1996).

In our dataset, bacterivory and nutrients were in some situations correlated with CTC+ cell proportion (Table 2). It is noticeable that cladoceran bacterivory was positively rather than negatively correlated with CTC+ cell density. This reflects the positive effect of sloppy feeding by cladocerans on activity (Park et al. 1997). In *in vitro* experiments, sloppy feeding by the cladoceran *Daphnia galeata* has been proved to be sufficient to increase the bacterial cell production by more than 50% compared to a control without cladocerans (Riemann et al. 1986). Concerning nutrients, CTC+ cell

proportion was positively linked to total phosphorus concentration in the reservoir that was the most limited by nutrient scarcity, not in the other ones. On the contrary, it was linked to nitrate concentration in the hypertrophic reservoir. If we compare the annual mean values of CTC+ cell densities and proportions between the studied reservoirs, a significant increase of these values with the trophic status is observed. CTC staining seems therefore to be indicative of temporal changes of the nutrient level, the zooplankton-mediated nutrient recycling or the trophic status.

In a spatial dataset with a finer scale (Figure 4), we observed that the depth variation in the CTC+ cell density and the CTC+ cell proportion was not correlated with bacterial production. This absence of correlation questions the information that can be drawn from CTC+ counts or proportions in a bacterial ecophysiology perspective. The CTC staining indeed includes both growing and nongrowing bacteria (Créach et al. 2003). This is why CTC+ counts generally do not correlate with bacterial production.

The proportion of CTC+ cells with respect to the total bacterial density varies quite a lot in the literature. del Giorgio et al. (1997) found significantly higher proportions in Canadian lakes compared to our results. In their study, the mean and maximum proportions of CTC+ cells were around 20% and 58% in the epilimnion. On the contrary the densities of CTC+ cells were rather similar to those observed in our study. Berman et al. (2001) observed CTC+ cell proportions in Lake Kinneret that were similar (average values = 4.1 – 6.5%; maximum values = 12.8 – 27.3%) to our results. This draws our attention on the fact that different staining methods seems to yield significantly different estimations of total bacterial density. We observed such discrepancies according to the fluorochromes. Acridine Orange (AO) gave significantly lower counts than Sybr Green I which is a brighter dye. The Sybr Green:AO ratio reached on average 3.2 ± 1.2 for ten replicates of the same samples from the Esch-sur-Sûre reservoir (results not shown). Indeed the size distribution of stained bacteria appears to vary according to the fluorochromes. In a study concerning the bacteria from a mesotrophic lake, AO stained bacteria showed a wider range of cell lengths compared to DAPI stained bacteria, resulting in higher biovolumes with AO compared to DAPI

(Kato, 1996). Another potential problem is that some nucleic acid dyes are sensitive to physico-chemical conditions. Martens-Habbena & Sass (2006) showed that Sybr Green I and II, PicoGreen and Ethidium Bromide were sensitive to pH.8. Zipper et al. (2003) showed that humic acids binds to Sybr Green I, decreasing the fluorescence intensity of the Sybr green/DNA complex. The physico-chemical characteristics of the sample will therefore influence the fluorescence intensity of stained bacteria and finally their ability to be detected by eye using an epifluorescence microscope. Once again the lack of comparability between the CTC+ proportions among studies is certainly partly due to methodological biases. This claims once again for the establishment of common protocols.

Conclusions

Our study indicates, likewise other published studies, that CTC staining method seems to be able to indicate relatively large-scale spatial and temporal patterns. Severe methodological drawbacks prevent however this method to be functional for the detection of smaller variations in the abundance or proportion of “metabolically active” bacteria. CTC must therefore be used with care until it will be possible to agree on reproducible protocols.

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Legends of figures and tables

Fig. 1. Temporal dynamics of CTC reducing bacteria cell density according to four different CTC concentrations (final concentrations: 0.5, 1.0, 5.0 and 10 mM)

Fig. 2. Rate of bacterial density increase during the first hour after a 10-times dilution of the bacterial community. CTC was added in such a way to reach four different final concentrations: 0.5, 1.0, 5.0 and 10 mM.

Fig. 3. Temporal dynamics of total bacterial density (“Sybr Green” curves), CTC+ cell density (“CTC” curves) and CTC+ cell proportion (histograms) in the upper, intermediate and lower layers of the Esch-sur-Sûre reservoir (Luxembourg) during the year 1999.

Fig. 4. Temporal dynamics of total bacterial density (“Sybr Green” curves), CTC+ cell density (“CTC” curves) and CTC+ cell proportion (histograms) in the upper layers of four reservoirs during the year 2003 (OLEF = Oleftalsperre reservoir, Germany; ESCH = Esch-sur-Sûre reservoir, Luxembourg; PONT-MISERE = Pont-Misère reservoir, Luxembourg; DIFFERDANGE = wastewater stabilisation pond at Differdange, Luxembourg).

Fig. 5. Vertical profiles of bacterial production, CTC+ cell counts and proportions, total cell densities (Sybr Green counts) and three abiotic variables (water temperature, pH and dissolved oxygen concentration).

Fig. 6. Scatter plots showing the relationship between CTC+ cell density or proportion (expressed in 10^3 cells ml^{-1}) and the bacterial production (BP) (expressed in $\mu\text{gC l}^{-1} \text{h}^{-1}$).

Table 1. Mean values of CTC+ cell density (CTC+ counts) and CTC+ cell proportion in the different layers of the Esch-sur-Sûre reservoir in 1999 and in the upper layers of the Oleftalsperre and Esch-sur-Sûre reservoir and in the whole water column of the Pont-Misère and Differdange reservoirs in 2003.

Table 2. List of variables that were linearly correlated with the CTC+ cell proportions in the different layers of the Esch-sur-Sûre reservoir in 1999 and in the upper layers of the Oleftalsperre and Esch-sur-Sûre reservoir and in the whole water column of the Pont-Misère and Differdange reservoirs in 2003. (r = Spearman correlation coefficient, p = level of significance, HNF = heterotrophic nanoflagellates, TP = total phosphorus, DIN = dissolved inorganic nitrogen).

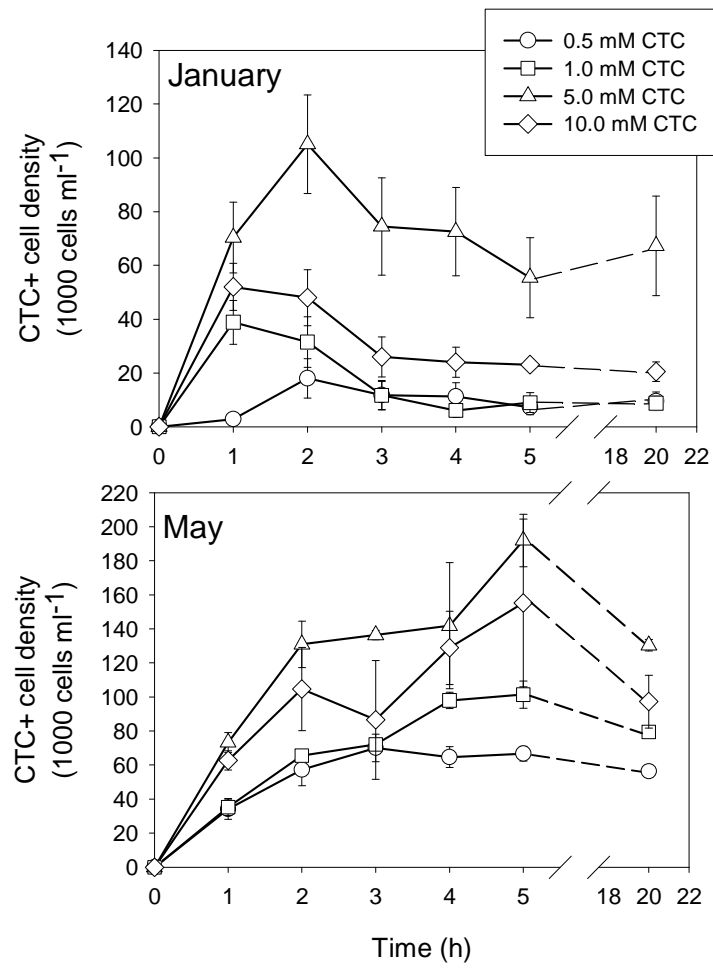


Fig.1.

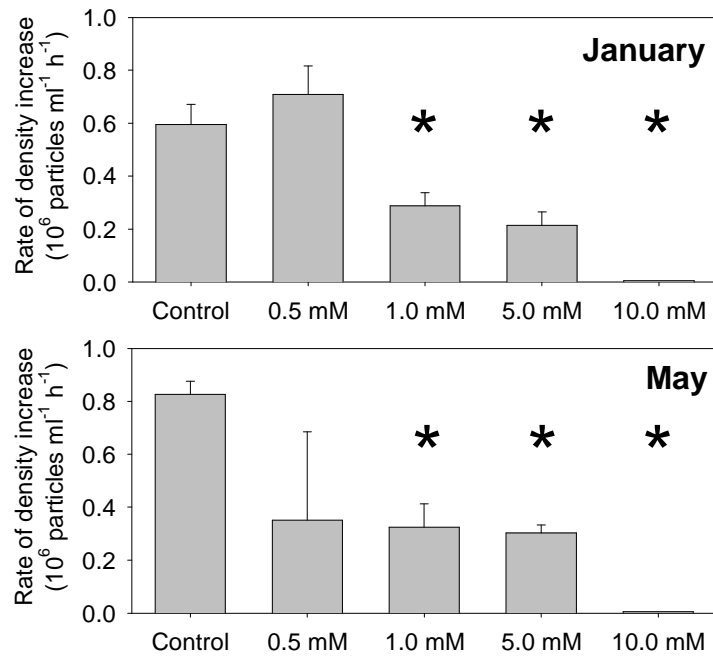


Fig.2.

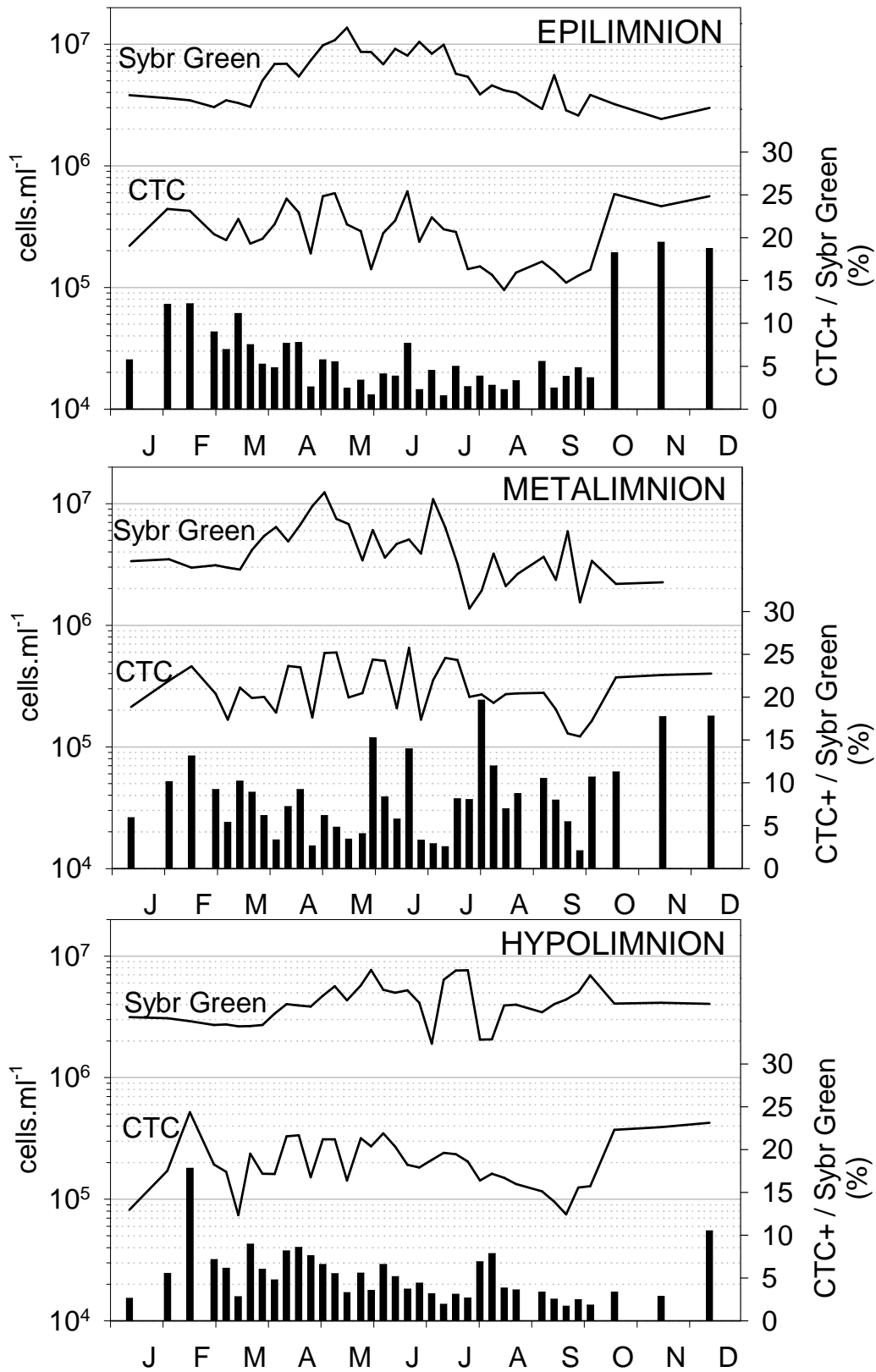


Fig.

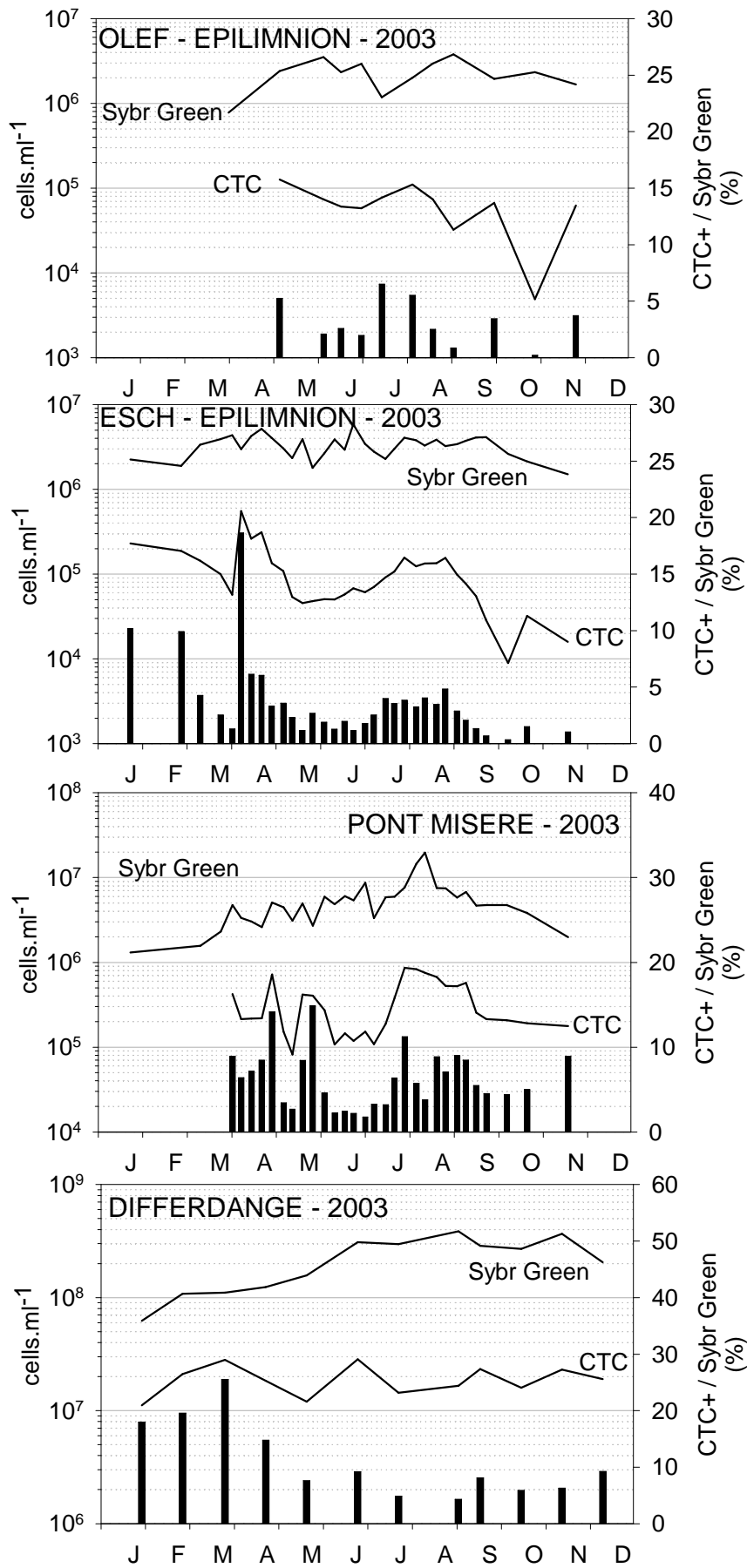


Fig. 4.

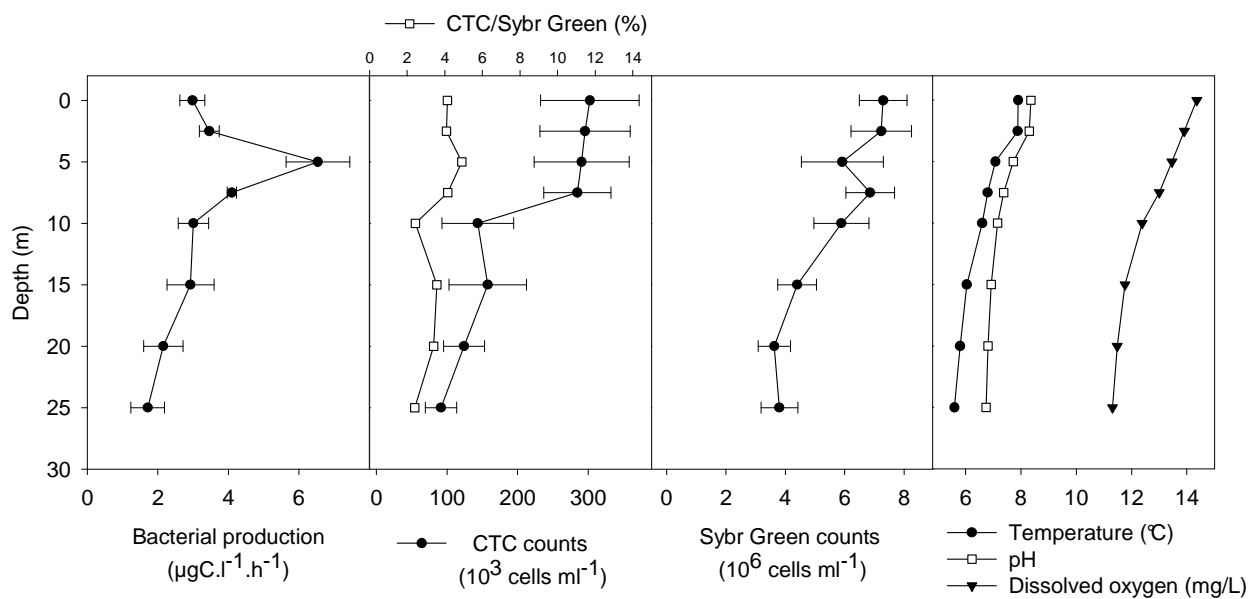


Fig.5.

Table 1.

	CTC+ cell counts ($10^5 \text{ cells.ml}^{-1}$)		CTC+ cell proportions (% of Sybr Green counts)			
	Mean	\pm SD	Mean	\pm SD	Min	Max
1999 – Esch Reservoir						
Epilimnion	3.0	1.6	6.3	4.7	1.5	19.5
Metalimnion	3.3	1.4	8.3	4.5	2.1	19.7
Hypolimnion	2.2	1.1	16.6	3.1	1.7	20.9
2003						
Oleftalsperre Reservoir	0.7	0.3	3.6	3.5	0.3	18.7
Esch-sur-Sûre Reservoir	1	0.5	3.2	1.9	0.2	6.5
Pont-Misère Reservoir	3.4	2.4	6.3	3.4	1.7	14.9
Differdange Reservoir	192.7	56.9	11.1	6.8	4.3	25.5

Table 2.

Variables significantly correlated with CTC+ cell proportions	
1999 – Esch Reservoir	
Epilimnion	Dissolved oxygen concentration ($r = 0.356$; $p = 0.029$) Dissolved inorganic nitrogen ($r = 0.430$; $p < 0.001$) Cladoceran bacterivory ($r = 0.333$; $p = 0.040$)
Metalimnion	no significant Spearman correlation
Hypolimnion	HNF bacterivory ($r = 0.348$; $p = 0.032$) Rotifer bacterivory ($r = 0.622$; $p < 0.001$) Cladoceran bacterivory ($r = 0.427$; $p < 0.001$)
2003	
Oleftalsperre Reservoir	TP concentration ($r = 0.728$; $p = 0.017$)
Esch-sur-Sûre Reservoir	Dissolved oxygen concentration ($r = 0.381$; $p = 0.035$)
Pont-Misère Reservoir	Virus density ($r = 0.564$; $p = 0.001$) Crustacean bacterivory ($r = 0.688$; $p < 0.001$)
Differdange Reservoir	DIN concentration ($r = -0.634$; $p = 0.036$)

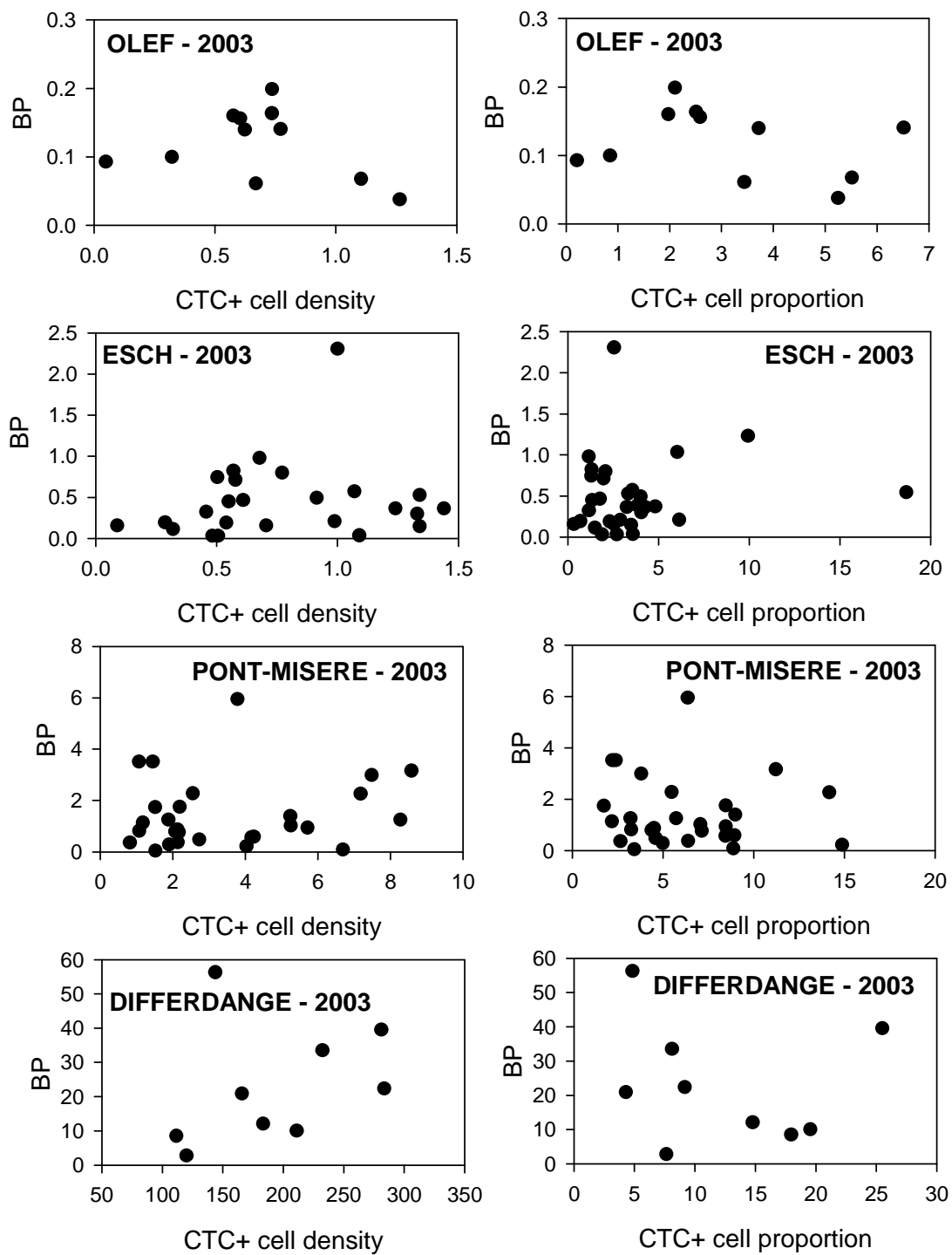


Fig. 6.

Résumé

Ce travail de thèse avait pour but de déterminer les facteurs de contrôle du bactérioplancton suivant un gradient d'eutrophisation.

La première étape a consisté en la collecte de données de dynamiques saisonnières tant bactériennes (densité, volume cellulaire, activité et diversité) que biologiques (abondance du protozooplancton hétérotrophe, du phytoplancton, du métazooplancton et des virus) ou physico-chimiques (température, oxygène, nutriments, ...). Puis, dans une seconde étape, déterminer l'impact des facteurs ascendants (les ressources) et descendants (prédation) ont été testés par des expériences *in situ*.

Le suivi saisonnier a permis de déterminer des périodes clés dans l'année pour effectuer les expérimentations mais aussi de mettre en avant une dynamique des activités bactériennes identique quel que soit le réservoir ainsi que des facteurs ascendants prépondérants.

Les différentes expérimentations *in situ*, ont montré que les bactéries apparaissaient fortement contrôlées par les ressources, y compris dans les milieux eutrophes. Par contre les prédateurs n'ont que peu d'impact sur l'activité bactérienne.

De plus, l'intensité d'activité semble dépendre des nutriments présents et non pas de la diversité bactérienne. Alors que l'intensité semble liée aux capacités des communautés présentes.

Les nanoflagellés hétérotrophes apparaissent significativement impliqués dans la composition de la communauté bactérienne mais sur un laps de temps restreint. La prédation apparaît comme un contrôle de maintien de la diversité en limitant l'abondance des bactéries les plus compétitives qui profitent au mieux des ressources.

The aim of this PhD study was to determine the factors controlling bacterioplankton along a trophic gradient

The first step consisted to monitor bacterial (density, biovolume, activities and diversity) and biological (heterotrophic protozooplanktonic abundance, phytoplankton, mesoplankton and viruses) and physico-chemical (temperature, oxygen, nutrients,...) seasonal dynamics. In a second step, the impacts of bottom-up factors (resources) and top-down factors (predation) were tested by *in situ* experiments.

The seasonal pattern allowed to determine some key-periods in the year and to realise experiments during these periods. It allowed to show an identical bacterial dynamics of the activities whatever the reservoir and the preponderance of ascending factors.

The *in situ* experiments showed that bacteria seemed strongly controlled by the resources, including eutrophic middle. On the other hand the predators have few impact on the bacterial activity.

Furthermore, the activity itself depends on the present nutrients and not on the bacterial diversity. While intensity of activity seems connected to the capacities of bacterial communities.

Heterotrophic nanoflagellates seem significantly involved in bacterial community composition but during a restricted time. The predation appears as a control for maintaining the diversity by limiting the abundance of the most competitive bacteria which take advantage of resources.