Réponse du zooplancton à la restauration de l'estuaire de l'Escaut et test d'un modèle de sélectivité trophique

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THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par *l'Université Toulouse III - Paul Sabatier*
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Avant propos

Dans la majeure partie de ce mémoire, la zone d'étude concerne la partie belge de l'estuaire de l'Escaut. Les noms des villes et des lieux-dits correspondant aux stations échantillonnées peuvent varier selon la langue employée (français, néerlandais ou anglais). Dans un souci de clarté et pour éviter toute confusion, ces stations seront désignées par leur distance par rapport à Vlissingen (point de repère pour l'embouchure) en kilomètres. Dans le second chapitre, du fait du changement du point de référence correspondant à la fin de l'estuaire (l'embouchure même et non Vlissingen), les distances en km diffèrent légèrement de celles employées dans le reste du mémoire.

Si dans la littérature la salinité est exprimée en ‰, en $g.kg^{-1}$ ou en PSU (practical salinity units), elle n'est désormais plus présentée comme un rapport de masse. Elle s'exprime donc sans unité, ce qui sera respecté dans ce manuscrit.

Les listes des références bibliographiques citées sont détaillées à la fin de chaque chapitre. Une liste reprenant toutes les références bibliographiques citées est présentée à la fin du manuscrit.

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Introduction

1. Généralités sur le milieu estuarien

1.1. Définitions

Selon Pritchard (1967), « un estuaire est un système constitué par une masse d'eau côtière semi-close, ayant une connexion libre avec la mer ouverte et à l'intérieur duquel l'eau marine est diluée d'une façon mesurable avec l'eau douce issue du drainage continental ». A cette définition, limitée par un critère de salinité, s'est ajoutée celle de Perillo (1995), qui intègre l'influence tidale et la présence d'organismes aquatiques. Selon l'auteur, « un estuaire est une masse d'eau semi close qui s'étend jusqu'à la limite d'influence des marées, dans laquelle l'eau de mer entrant par une ou plusieurs connexions libres avec la mer ouverte ou avec quelconque autre masse d'eau salée côtière, est significativement diluée avec de l'eau douce dérivant par drainage continental et qui peut contenir des espèces euryhalines pendant une partie ou l'ensemble de leur cycle de vie ».

Les estuaires sont qualifiés de microtidaux, mesotidaux, ou macrotidaux, selon si l'amplitude de la marée est respectivement faible, moyenne ou forte. Celle-ci est en général stoppée par des structures d'origine anthropique, comme les écluses ou les barrages hydrauliques. C'est à cet endroit précis que débute un estuaire. On ne parle pas d'estuaire pour les fleuves se déversant dans les mers closes non soumises à la marée (Pritchard, 1967).

La marée saline, correspondant à l'extension maximale du gradient de salinité, se distingue de la marée dynamique, correspondant à la propagation de l'onde de marée dans la vallée fluviale jusqu'au point où le courant du fleuve vers l'aval n'est plus inversé par les marées (Reid, 1961).

1.2. Influences continentale et marine

L'antagonisme des forces hydrauliques provenant du débit fluvial et de la marée conditionnent majoritairement la physicochimie du milieu estuarien et la distribution spatiale des organismes vivants. Parmi les facteurs environnementaux les plus caractéristiques, le

gradient de salinité est directement lié à ce mélange et décroît d'aval en amont. Trois zones estuariennes sont définies en fonction de la salinité (Reid, 1961) : la zone oligohaline, de 0,5 à 5 ; la zone mesohaline, de 5 à 18 ; la zone polyhaline, de 18 à 30.

Le mélange des eaux marines et continentales dépend des caractéristiques géomorphologiques des estuaires. Ainsi, en plus d'un gradient de salinité longitudinal, la stratification verticale de la salinité peut être plus ou moins importante, l'eau salée étant plus dense que l'eau douce. Une classification des estuaires, du plus stratifié au plus homogène, est proposée par Pritchard (1955) (Figure 1).

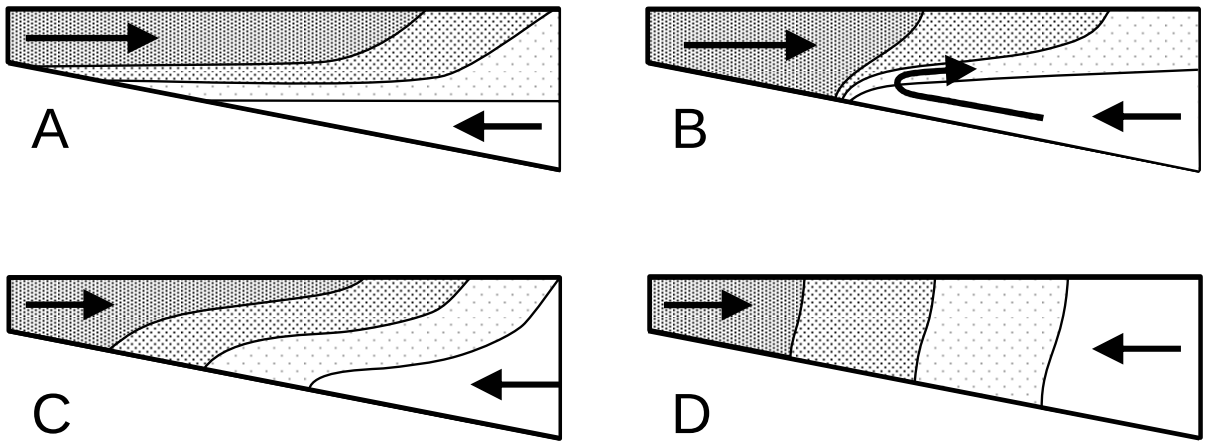

Figure 1. Représentation schématique des différents types d'estuaires selon Pritchard (1955). L'eau douce est représentée en blanc, l'eau de mer en gris foncé. La gamme de gris correspond au gradient de salinité.

Type A : Estuaires complètement stratifiés. L'eau salée pénètre sous l'eau douce et s'y mélange très peu.

Type B : Estuaires à coin salé. La stratification verticale reste forte malgré un léger mélange, dû à des courants de marées plus importants et/ou des débits fluviaux plus faibles que pour le type A.

Type C : Estuaires partiellement mélangés. Sous l'influence de forts courants de marées, la stratification verticale est limitée au profit d'une stratification longitudinale.

Type D : Estuaires bien mélangés et verticalement homogènes. Les courants de marées étant les plus importants, la stratification verticale est inexistante et le gradient de salinité est uniquement longitudinal.

De plus, les courants résiduels résultant de l'opposition de ces eaux convergent vers un point nodal. La matière en suspension, constituée de particules organiques et minérales, s'y accumule pour former une zone de turbidité importante appelée « bouchon vaseux » (maximum turbidity zone) (Glangeaud, 1938). Celui-ci migre d'amont en aval en fonction de l'importance du débit fluvial et des marées. La forte concentration en matière organique au niveau du bouchon vaseux induit une hausse de la consommation en oxygène par les organismes décomposeurs hétérotrophes (Klap and Heip, 1993).

1.3. Caractéristiques biotiques

Les estuaires sont, de par l'influence marine et continentale, des milieux variables, en perpétuel changement. Les espèces typiquement estuariennes sont celles qui y effectuent un cycle de vie complet. Mais les variations quotidiennes de salinité et de température font que peu d'organismes y sont réellement inféodés. La plupart des espèces trouvées dans la zone estuarienne sont soit originaires de la mer (zones poly et mesohalines) soit originaires de l'eau douce (zones meso et oligohalines) et leur présence n'est que transitoire. D'amont en aval, les espèces d'eau douce disparaissent progressivement au profit des espèces plus tolérantes à la salinité. Inversement, d'aval en amont, les espèces marines se raréfient. Dans la zone mésohaline, la plus éloignée des deux milieux limitrophes, la diversité biologique est réduite (figure 2).

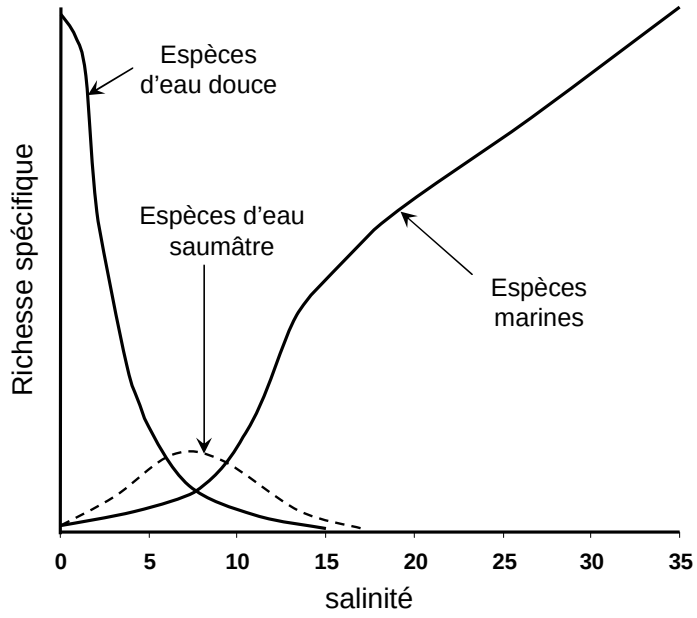

Figure 2. Répartition de la richesse spécifique sur un gradient de salinité selon Remane (1934)

La richesse en éléments nutritifs font des estuaires des systèmes hautement productifs (Cabecadas et al., 1999). Si leur diversité est relativement faible dans les estuaires, la production en invertébrés planctoniques (zooplancton) est importante. Ils représentent une ressource nutritive abondante pour les organismes des niveaux trophiques supérieurs : les mysidacés (Fockedey and Mees, 1999) ou les alevins de poissons (Maes et al., 2005). La productivité importante et le nombre limité de poissons prédateurs font des estuaires également des zones de fraies et de nurseries attractives pour les espèces de vertébrés vivant ordinairement hors de l'estuaire. De plus, les zones humides et vasières périodiquement émergées permettent d'accueillir une avifaune diversifiée et abondante. De nombreuses aires sont désormais protégées par des directives européennes (79/409/EEC).

1.4. Anthropisation

Environ 80 % de la population mondiale est concentrée le long des côtes (Costil et al., 2002). La majorité des villes de plus de 2,5 millions d'habitants est située à proximité des estuaires. Véritables carrefours des lignes commerciales maritimes, les estuaires concentrent les activités anthropiques et les impacts écologiques qu'elles peuvent avoir sur eux (Meire et al., 2005). Malgré la sérieuse dégradation des habitats estuariens, la prise de conscience sur le

besoin de conserver et de restaurer les fonctionnalités écologiques des estuaires est croissante. Ce n'est que récemment qu'il a été admis qu'une telle démarche ne pouvait se faire sans une connaissance approfondie et intégrée du fonctionnement de tous les systèmes qui composent un estuaire (Cloern, 2001; Van Damme et al., 2005; Schindler, 2006; UNEP, 2006; Ducrotoy and Dauvin, 2008).

2. L'estuaire de l'Escaut

2.1 Caractéristiques générales

Le bassin versant de l'Escaut (dit « Schelde » en néerlandais, « Scheldt » en anglais) s'étale sur trois pays de l'Union Européenne : la France, la Belgique et les Pays-Bas. L'Escaut prend sa source à Gouy-le-Câtelet, en France (Aisne) et se déverse dans la mer du Nord, 355 km plus loin, au niveau de Vlissingen, dans les Pays-Bas (Fig. 3). L'influence des marées se termine à 160 km de l'embouchure, près de la ville de Gand (Belgique), suite à la construction de barrages-écluses. La hauteur moyenne de la marée est de 5 m à Anvers (point kilométrique 78,5 ou « km 78,5 ») et de 2 m à Gand (Baeyens et al., 1998). Les profondeurs moyennes sont de 14 m à l'embouchure et de 7 m à Gand.

Sa source étant située à seulement 97 m d'altitude, la région traversée par l'Escaut présente un dénivelé très faible. Les faibles débits qui en découlent ainsi que la grande portée de l'influence des marées induisent un temps de résidence variable mais élevé, estimé à 2 ou 3 mois (Soetaert and Herman, 1995). L'estuaire est fortement influencé par le débit originaire des marées, supérieur au débit fluvial. De ce fait, excepté dans la région d'Anvers, où une stratification verticale survient occasionnellement, l'estuaire de l'Escaut est de type bien mélangé (Type D sur la classification de Pritchard, 1955) (Baeyens et al., 1998).

Des appellations particulières sont données aux différentes parties de l'Escaut. Le « Bovenschelde » (Escaut supérieur ou rivière Escaut) correspond au tronçon d'eau douce allant de la source jusqu'au début de la zone estuarienne. Ensuite, le « Zeeschelde » (Escaut maritime) s'étend de Gand jusqu'à Anvers (km 78,5) et correspond à la zone d'eau douce

estuarienne (salinité < 2). Enfin, le « Westerschelde » (Escaut occidental) correspond au tronçon estuarien situé au delà d'Anvers et comprend une zone d'eau saumâtre (du km 78,5 au km 36 ; salinité entre 2 et 10) et une zone d'eau salée (en aval du km 36 ; salinité supérieure à 10). La partie estuarienne d'eau douce constitue un écosystème unique en Europe (Meire et al., 2005; Van Damme et al., 2005).

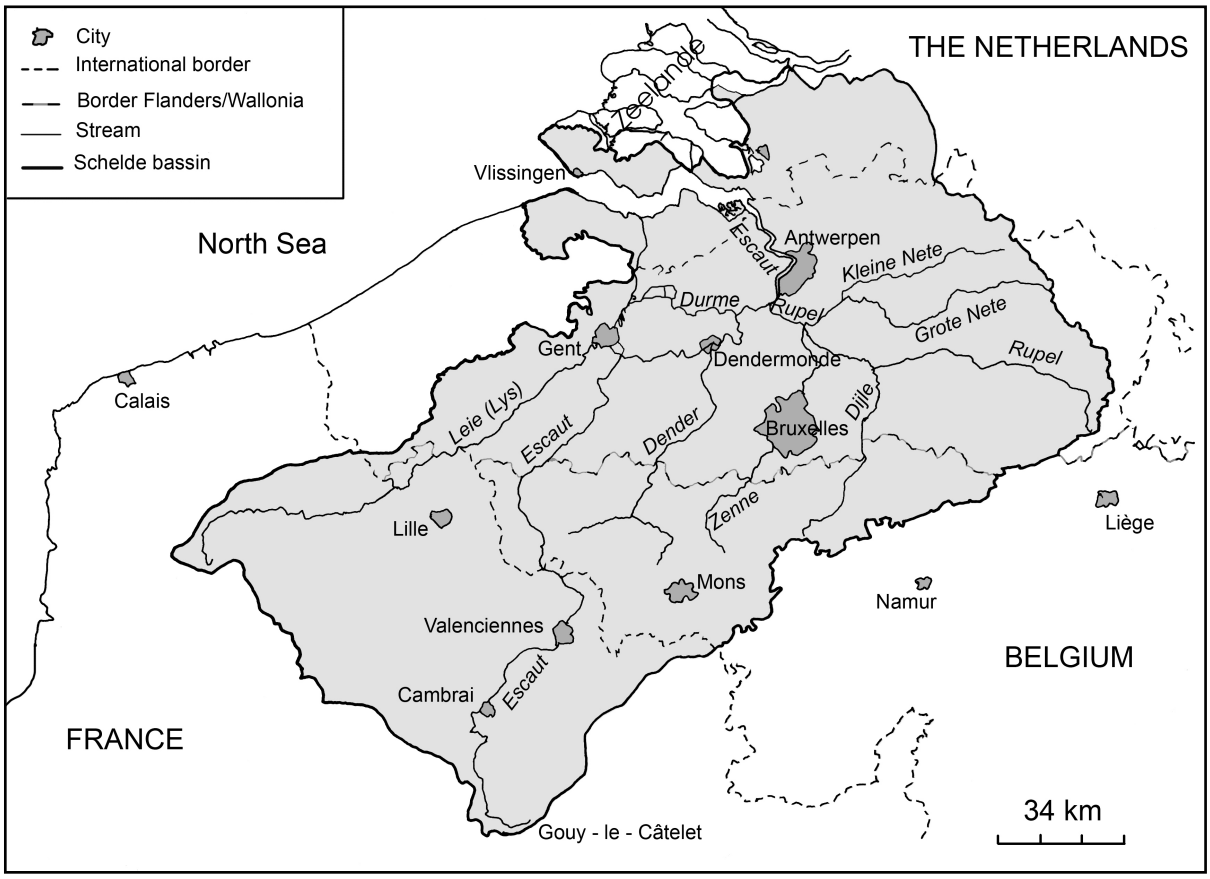

Figure 3. Carte du bassin versant de l'Escaut.

Les tributaires majeurs de l'Escaut sont la Durme, la Dendre et le Rupel. Le Rupel et la Durme sont soumis à l'influence des marées. L'Escaut supérieur ne contribue qu'à 25 % de l'apport hydrique de l'estuaire. Le reste est apporté par les tributaires et canaux, plus particulièrement par le Rupel (35 %) (Soetaert et al., 2006). Après la confluence avec le Rupel (km 90), le débit moyen de l'Escaut atteint 125 $m^3.s^{-1}$.

Le bassin versant de l'Escaut est soumis à un climat marin tempéré. La température de l'eau varie peu, d'une moyenne de 6 °C en hiver à une moyenne de 20 °C en été. Les variations

saisonnières de débit et de température sont plus marquées dans la zone d'eau douce que dans les zones saumâtres et marines.

2.2 Matière en suspension

Les matières en suspension (MES) sont des particules solides, d'origine minérale ou organique, insolubles dans l'eau. Les estuaires étant soumis à un mélange perpétuel de la colonne d'eau sous l'influence des marées, les concentrations en matières en suspension y sont importantes. Comme le montre le tableau 1, les concentrations en MES mesurées dans l'Escaut sont comparables à celles mesurées dans l'Elbe (Allemagne) mais très inférieures à celles trouvées dans la Gironde (Tableau 1). En revanche, la fraction organique et la proportion de matière organique labile au niveau du bouchon vaseux sont supérieures dans l'Escaut en comparaison à celles des deux autres estuaires Européens. Par conséquent, cette matière organique entraine un déficit de la concentration en oxygène dissous au niveau d'Anvers (km 78,5) sur une distance de 30 km. Jusque dans les années 90, cette charge organique a provoqué des conditions anoxiques dans la colonne d'eau douce en période estivale. En effet, dans l'estuaire de l'Escaut, plus de 30 % de la consommation en oxygène sont dus à la nitrification (Ouboter et al., 1998). Dans la zone d'eau douce, cette proportion peut atteindre 50 à 75 % (Cox et al., 2009). La charge en matière organique a donc un fort impact sur la concentration en oxygène du milieu et représente un enjeu important pour la gestion de la qualité de l'eau.

Tableau 1. Caractéristiques de la matière en suspension dans différents estuaires européens. Les données sont tirées de Burdloff (1993), Gasparini (1997), Chen et al. (2005) et Van Damme et al. (2005).

	Concentration en MES	Fraction organique	Matière organique labile (bouchon vaseux)
	$mg.l^{-1}$	%	%
Escaut	50-500	10	30
Elbe	80-300	6	20-30
Gironde	500-1000	3	10-15

Dans l'Escaut, le bouchon vaseux est difficile à localiser. S'il a été dans un premier temps situé aux environs d'Anvers (entre le km 55 et le km 78) par Baeyens et al. (1998), Van Damme et al.(Van Damme et al., 1999) l'ont situé plus en amont (entre le km 90 et le km 110). D'autres études considèrent qu'il y a deux (Hannouti, 2003) ou même trois (Chen et al., 2005) zones de turbidité maximale, réparties dans différentes parties de l'estuaire. La notion même de bouchon vaseux est parfois difficilement applicable dans l'Escaut.

2.3 Production Primaire et secondaire

Le temps de résidence élevé, dû aux caractéristiques géomorphologiques du fleuve Escaut, favorise une production primaire importante (Muylaert et al., 2000). Dans le lit majeur du cours d'eau, la turbidité élevée induite par la quantité importante de matière en suspension, empêche la production phototrophe benthique. De même, les macro-algues sont absentes de ce système et ne jouent pas un rôle important dans les estuaires macrotidaux turbides (Heip et al., 1995). Les phanérogames peuplant le bassin versant de l'Escaut sont diversifiés. Plus que le phytoplancton, ces macrophytes sont des consommateurs majeurs des nutriments des eaux de surfaces. Cependant, leur effet sur les flux en nutriments en aval sont minimes (Van Belleghem, 2007) en comparaison aux apports anthropiques encore importants

Créant un lien entre les producteurs primaires et les consommateurs secondaires, le zooplancton occupe une position clé dans les réseaux trophiques pélagiques des estuaires (Tackx et al., 2003; Maes et al., 2005). De plus, des études ont attribué à certains taxons un rôle de potentiel bioindicateur (Wilson, 1994; Appeltans et al., 2003). L'intérêt écologique qu'il suscite fait que le zooplancton de l'estuaire de l'Escaut est étudié depuis 1996 dans le contexte du projet OMES (Azémar, 2007).

Parmi les espèces les plus étudiées, *Eurytemora affinis* (Fig. 4) est un copépode calanoïde planctonique ou épibenthique dominant la communauté zooplanctonique de nombreuses zones saumâtres de la zone tempérée de l'hémisphère nord. Cependant, de nombreuses invasions des habitats d'eau douce ont été constatées en Amérique du Nord, en Europe et en Asie au cours de la seconde moitié du 20ème siècle (Lee, 1999).

Ce copépode étant fréquemment trouvé dans des zones à forte turbidité (Sautour and Castel, 1995), son association spatiale avec les zones à milieu détritique suggère que les détritus représentent une source de nourriture importante pour le copépode (Heinle et al., 1977). Parallèlement, des études ont suggéré un comportement trophique sélectif d'*Eurytemora affinis* en faveur des algues (Billones et al., 1999; Gasparini et al., 1999).

Figure 4. Spécimen adulte d'*Eurytemora affinis*, prélevé dans l'estuaire de l'Escaut (Photo : Frédéric Azémar)

2.4 Anthropisation

Durant la seconde moitié du 20ème siècle, l'estuaire de l'Escaut était considéré comme étant l'un des plus pollués d'Europe (Heip, 1989). En effet, son bassin versant subit une pression anthropique très dense (Fig. 5, Fig. 6), en commençant par une forte densité de population (10,4 millions d'habitants). L'agriculture intensive, apporte une importante quantité d'engrais et de pesticides jusqu'au cours d'eau (Billen et al., 1985; Voorspoels et al., 2004). Les complexes industriels concentrés autour de l'estuaire, principalement vers Gand, Anvers et Vlissingen, ont contribué à un apport direct et important en polluants (métaux lourds) dans les sédiments (Valenta et al., 1986; Bouezmarni and Wollast, 2005; Du Laing et al., 2007).

La partie supérieure de l'estuaire (Zeeschelde) reçoit d'importants apports en matière organique. La majeure partie des eaux usées, en provenance de l'agglomération de Bruxelles, arrive dans l'Escaut par le Rupel, à environ 90 km de l'embouchure Cette pression anthropique a causé une hyper eutrophisation du milieu et une dégradation des habitats (Abril et al., 2002; Meire et al., 2005; Soetaert et al., 2006).

En raison de l'enjeu économique que représente la zone portuaire d'Anvers, l'estuaire est en grande partie canalisé (Hoffmann and Meire, 1997). De nombreux canaux forment un réseau navigable, connectant les principaux ports de la région. Le plus important, reliant Gand à Terneuzen (Pays-Bas), détourne une grande partie du débit fluvial vers le Westerschelde pour supporter le trafic fluvial.

Figure 5. Porte conteneur circulant sur l'Escaut

2.5 Le plan SIGMA

Les plaines littorales de la Belgique et des Pays-Bas ayant de basses altitudes, elles sont fortement exposées au risque d'inondation. En février 1953, de fortes marées associées a de violentes tempêtes ont provoqué une montée des eaux et une rupture de digues. Des inondations particulièrement meurtrières et destructrices sont survenues dans la région

(Slinger et al., 2007). Les Pays-Bas ont ensuite réalisé, dans le cadre du plan Delta, une série de barrages afin de protéger les polders. Lors des tempêtes de janvier 1976, la Belgique, et notamment les rives de l'Escaut ont été touchées par de graves inondations. Par analogie au plan Delta, le plan Sigma a été lancé par le gouvernement Flamand. Le plan original décrit trois solutions :

- L'élévation des digues le long de l'Escaut et de certains tributaires de la zone d'eau douce (La Durme, la Nete, la Zenne et le Rupel).
- L'aménagement de zones d'inondations contrôlées, pour contenir les crues.
- La construction d'un barrage contre les tempêtes dans la région d'Anvers (annulée).

L'endiguement important de la zone d'eau douce réalisé au début du plan Sigma a supprimé une grande partie de l'espace attribué aux zones de marées d'eau douce, en dépit de leur rôle écologique important (Struyf et al., 2005).

Figure 6. Centrale nucléaire de Doel (à proximité d'Anvers)

2.6 La restauration

Depuis le début des années 90, d'importants efforts de restauration ont été menés par différents pays sur l'ensemble du bassin versant de l'Escaut. Ces mesures variées, comme la réduction d'apports en polluants émis dans le bassin versant (agriculture, industries et collectivités) ou la restauration de routes migratoires, furent précoces à l'échelle de l'Europe (Soetaert et al., 2006). En revanche, l'usine des traitements d'eaux usées à la sortie de Bruxelles n'a été mise en route qu'au cours de l'année 2007 (Posel, 2007).

En 2005, le plan d'aménagement de l'Escaut (SIGMA) a été actualisé, en tenant compte de l'impact important qu'il a eu sur les écosystèmes. Une attention particulière à la conservation des milieux a été intégrée à ce projet qui fut auparavant uniquement basé sur un aspect sécuritaire. Ainsi, le gouvernement flamand prévoit de restituer de l'espace aux zones humides. Avant d'entreprendre ces travaux à grande échelle, des sites-pilotes comme celui du Lippenbroek (Fig. 7), où l'entrée et la sortie d'eau sont contrôlées par des écluses, servent de support pour étudier l'effet écologique de tels aménagements (Cox et al., 2006; Jacobs et al., 2008; Jacobs et al., 2009).

Figure 7. Site expérimental du Lippenbroek (km 115), à marée haute (gauche) et à marée basse (droite).

2.7 Le programme OMES

Jusqu'en 1996, la plupart des études écologiques portaient sur la partie centrale et aval de l'Escaut (Westerschelde). Le manque de connaissances sur la partie supérieure (Zeeschelde) était flagrant, tant pour les conditions physicochimiques que pour les communautés biologiques, d'autant que cette partie représente plus du tiers de la longueur totale de l'estuaire (Van Damme et al., 2005). De manière générale, la connaissance des milieux d'eau douce estuariens est peu documentée.

Un suivi conséquent de l'écologie de la partie d'eau douce estuarienne de l'Escaut a été initié par le programme OMES (« Onderzoek Milieu-Effecten Sigmaplan », ou « Recherches sur les conséquences du plan SIGMA »). Cette étude multidisciplinaire, commandée par le gouvernement Flamand, a trois objectifs : (1) développer une base de données en ligne disponible pour la communauté scientifique, (2) créer un modèle général de fonctionnement de l'estuaire total de l'Escaut (MOSES), (3) fournir un support scientifique pour améliorer la politique de gestion de l'estuaire de l'Escaut. Le zooplancton fait partie du compartiment pélagique de ce programme. Depuis novembre 1995, des échantillonnages mensuels sont effectués par bateau, sur la partie flamande de l'estuaire de l'Escaut (eau douce et eau saumâtre) (Van Damme et al., 2005).

3. Objectifs de l'étude et organisation du mémoire

3.1 Réponse de la communauté zooplanctonique à la restauration de l'estuaire de l'Escaut

La première partie des travaux présentés dans ce manuscrit s'inscrit dans le cadre du programme OMES. Le suivi régulier depuis 1996 et la restauration progressive de son estuaire font de l'Escaut un système expérimental grandeur nature permettant d'étudier l'impact de l'augmentation de la qualité de l'eau sur les communautés aquatiques. Ceci constituera le premier objectif de l'étude, en considérant plus particulièrement le zooplancton.

Le **chapitre 1** traite de la réponse des crustacés zooplanctoniques à la restauration de l'estuaire de l'Escaut. L'évolution de leur répartition spatio-temporelle au cours de la période étudiée sera décrite. Les facteurs environnementaux potentiellement responsables des changements observés sont identifiés au moyen d'analyses multivariées.

Le **chapitre 2** s'inscrit dans le même contexte que le chapitre 1, mais se focalise sur le copépode dominant *Eurytemora affinis*. A partir des résultats obtenus, un schéma synthétique est élaboré pour tenter d'expliquer les conditions contrôlant l'expansion du copépode dans l'estuaire.

Le **chapitre 3** se consacre à l'aspect trophique de la réponse du zooplancton à la restauration de l'Escaut.

Il a été montré que la concentration en chlorophylle *a* mesurée dans le tube digestif d'*Eurytemora affinis* était indépendante de celle mesurée dans le milieu (Billones et al., 1999; Gasparini et al., 1999). *E. affinis* peut donc sélectionner les algues du milieu malgré une faible proportion de phytoplancton par rapport à la matière en suspension totale. Partant de ce fait, Tackx et al. (2003) ont mis au point un modèle montrant une relation de type Monod entre la concentration en chlorophylle *a* mesurée dans les contenus digestifs d'*E. affinis* et la proportion d'algues présentes dans la matière en suspension du milieu. Plus généralement, la proportion de proies préférentielles capturées par le copépode est fonction du rapport « proies préférentielles / proies potentielles totales » présentes dans le milieu estuarien. Pour étudier une possible réponse trophique d'*E. affinis* à l'amélioration de la qualité de l'eau, une comparaison des contenus digestifs d'*E. affinis* est faite entre deux périodes : le début des années 1990 et les années 2007-2008. Elles correspondent respectivement à des périodes antérieures et postérieures aux premières constatations d'une amélioration de la qualité de l'eau.

3.2 Etude de la sélectivité trophique des organismes benthiques dans un milieu à gradient de proies potentielles

Le second objectif de ce travail de thèse, faisant l'objet du **chapitre 4**, vise à tester la validité du modèle (Tackx et al., 2003) dans un milieu aquatique différent, comprenant un gradient du rapport « proies préférentielles / proies potentielles totales ». Cette étude s'inscrit dans un objectif à long terme, envisageant de tester la potentielle application de ce modèle à une large gamme d'écosystèmes, afin de mettre en évidence d'éventuels patterns communs concernant les relations trophiques entre producteurs microphytes et consommateurs primaires.

Pour pouvoir tester ce modèle, plusieurs conditions sont à vérifier au préalable :

- La présence d'une gamme variée des valeurs du ratio « proies préférentielles / proies potentielles totales », formant un gradient sur une échelle spatiale ou temporelle.
- La présence de taxons de consommateurs primaires dominants présents sur la totalité de ce gradient.
- Le comportement sélectif de ces organismes envers un type de proies.

Dans la première partie de ce chapitre, la validité des conditions précédemment décrites est vérifiée à partir de données issues d'une étude portant sur la dynamique temporelle des communautés benthiques du biofilm épilithique phototrophe de la Garonne. Ce chapitre forme donc une base de départ pour tester la faisabilité de l'étude dans cet écosystème aquatique. Une deuxième partie présente les résultats préliminaires de l'étude de la sélectivité d'un groupe meiobenthique dominant, les rotifères, envers la communauté algale. Dans cette optique, une méthode précédemment utilisée pour les copépodes benthiques marins (Buffan-Dubau and Carman, 2000b), basée sur analyses pigmentaires de leurs contenus digestifs par HPLC, est adaptée pour la première fois à l'étude de la sélectivité trophique des rotifères benthiques.

Chapitre 1

Réponse du zooplancton à la restauration de l'estuaire de l'Escaut (Belgique)

Article soumis à la revue **Estuarine, Coastal and Shelf Science** (juillet 2010)

Résumé de l'article

Introduction et objectifs

Bien que l'estuaire de l'Escaut ait été considéré comme l'un des plus pollués d'Europe durant la seconde moitié du 20ème siècle (Heip, 1988; Baeyens et al., 1998), sa qualité de l'eau s'est cependant améliorée de façon importante depuis les années 1990 en résultat des décisions politiques en faveur d'un effort de restauration (Van Damme et al., 2005; Cox et al., 2009). Considérant sa position importante dans les réseaux trophiques (Tackx et al., 2003; Maes et al., 2005b) et son rôle potentiel de bioindicateur (Wilson, 1994; Appeltans et al., 2003), le zooplancton de l'estuaire de l'Escaut est étudié depuis 1996 dans le cadre du programme OMES.

Cette étude a deux objectifs. Dans un premier temps, elle décrit la répartition spatio-temporelle des crustacés zooplanctoniques de l'estuaire de l'Escaut au niveau spécifique durant un cycle annuel. Dans un deuxième temps, elle a pour but d'observer les variations de la répartition spatio-temporelle du zooplancton au cours de la période 1996–2009 et de les mettre en relation avec les changements environnementaux observés.

Principaux résultats et discussion

Depuis 1996, d'importants changements de la qualité de l'eau sont observés, avec une diminution forte et régulière de la DBO_5, du $N\text{-}NH_4^+$, du $N\text{-}NO_2^-$ et du $P\text{-}PO_4^+$; de même qu'une forte augmentation en O_2 dissous. Ces changements sont plus importants dans le tronçon d'eau douce que dans le tronçon d'eau saumâtre de l'estuaire. Les tendances observées sont les mêmes que celles observées dans les études précédentes, entre la fin des années 1990 et le début des années 2000 (Van Damme et al., 2005; Cox et al., 2009).

L'identification des crustacés zooplanctoniques (cladocères et copépodes) au niveau spécifique pour l'année 2002 a permis de recenser 16 nouveaux taxons dans l'estuaire de

l'Escaut, en comparaison aux études précédentes (De Pauw, 1975; Soetaert and Van Rijswijk, 1993; Tackx et al., 2004).

La distribution spatio-temporelle du zooplancton est similaire à celle observée en 1996 (Tackx et al., 2004), avec notamment, une dominance des copépodes *Eurytemora affinis* (printemps, automne) et *Acartia tonsa* (été) en eau saumâtre, ainsi qu'une dominance des copépodes *Cyclops vicinus vicinus* (hiver) et *Acanthocylcops trajani* (été, automne) en eau douce. Les cladocères sont aussi abondants que les copépodes en eau douce.

L'analyse RDA montre que la distribution des calanoïdes est plus influencée par les facteurs associés à la qualité de l'eau (O_2, DBO_5, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$ et $P\text{-}PO_4^+$) que par la saisonnalité (température). En revanche, les études précédentes, menées avant les changements physicochimiques survenus dans l'Escaut, ont noté une influence prépondérante de la saisonnalité (Soetaert and Van Rijswijk, 1993; Tackx et al., 2004). Ces résultats suggèrent un rôle important de l'amélioration de la qualité de l'eau dans la détermination des conditions de vie de certains organismes aquatiques. Les copépodes calanoïdes semblent notamment réagir à cette amélioration de la qualité de l'eau par un déplacement de leurs populations vers le tronçon d'eau douce. En contrepartie, les copépodes cyclopides et les cladocères semblent beaucoup plus affectés par la disponibilité en phytoplancton que par la qualité de l'eau.

Depuis 2007, les calanoïdes et particulièrement *Eurytemora affinis*, ont peuplé en l'espace de quelques mois toute la zone d'eau douce estuarienne, atteignant des abondances supérieures à celles précédemment observées en eau saumâtre, alors qu'auparavant ils étaient principalement trouvés dans la zone saumâtre de l'estuaire de l'Escaut (Soetaert and Van Rijswijk, 1993). Simultanément, les populations de cyclopides ont fortement diminué en eau douce. Plusieurs hypothèses peuvent expliquer les changements importants survenus au sein des populations de copépodes :

(1) Au cours des printemps 2007 et 2008, les diatomées de grande taille ont dominé la communauté algale (Van Burm, Comm. Pers.) tandis qu'au printemps 1993, elle était dominée par les diatomées de petite taille (Muylaert and Sabbe, 1999). Ce changement peut avoir influencé l'aptitude du mesozooplancton à sélectionner les algues.

(2) Les calanoïdes, et particulièrement *E. affinis*, pourraient chasser plus efficacement les rotifères que les cyclopides. Ces derniers, privés de leur ressource alimentaire principale, pourraient en être défavorisés (Brandl, 2005).

(3) Depuis l'amélioration des conditions de vie du milieu, les populations de vertébrés (hareng, spratt) ou d'invertébrés hyperbenthiques (mysidacés) pourraient avoir investi la zone estuarienne (Verslycke et al., 2004) et effectué une prédation sélective sur les cyclopides. Cependant, des études montrent que cette prédation sélective agirait plutôt en faveur d'*E. affinis* (Fockedey and Mees, 1999; Maes et al., 2005a).

Les cladocères n'ont pas montré de tendance claire concernant leur distribution, si ce n'est des abondances supérieures en eau douce en comparaison à celles de l'eau saumâtre durant les années à fortes concentrations en chlorophylle *a*. Leur caractère filtreur différent du mode de nutrition des copépodes leur permet probablement d'éviter une compétition avec ces derniers (De Mott, 1988).

Cette étude révèle une implication importante de la qualité de l'eau dans la détermination des conditions de vie de certains organismes aquatiques tout en ayant soulevé de nombreuses questions sur les interactions biotiques au sein de ces communautés.

Response of zooplankton to improving water quality in the Scheldt estuary (Belgium)

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Abstract

Data obtained from 14 years of monthly samplings (1996–2009) were used to investigate the response of the crustacean zooplankton community to improving water quality in the Scheldt estuary. A strong reduction of poor water quality indicators, such as NH_4^+ and BOD_5, as well as an increase in oxygen and in chlorophyll *a* concentrations were observed during the study period. The inventory of the taxonomic composition of crustacean zooplankton at species or genus level revealed 27 taxa of copepods and 32 of cladocerans, with respectively 6 and 10 new reports for this estuary. During the study period, important changes were observed in the zooplankton community composition and spatial distribution. From 2007 onwards, calanoids, previously mainly found in the brackish water reach of the estuary, moved the bulk of their population to the freshwater, where they reached higher abundances than previously observed. Simultaneously, cyclopoids populations strongly decreased in freshwater while cladocerans did not change their abundance, except during years with high chlorophyll *a* concentrations. Redundancy analyses (RDA) showed that the variability within the calanoid population responds positively to an improvement in water quality. Variability within the

cyclopoids and cladoceran community is mainly explained by chlorinity and chlorophyll *a* concentrations. Their presence in the most polluted upstream area up till 2007 suggests they are more tolerant to poor water quality than calanoids. Several hypotheses to explain the disappearance of cyclopoids after the move of calanoids to the freshwater are discussed.

Keywords: estuaries, restoration, Scheldt, spatio-temporal distribution, water pollution, zooplankton.

1. Introduction

Understanding how taxa respond to environmental conditions provides an essential knowledge for ecosystem and biodiversity management. This paper focuses on the planktonic crustaceans of the Scheldt estuary (Belgium/The Netherlands). The Scheldt estuary is one of the few European estuaries that still have an extensive freshwater tidal zone (salinity < 0.5) in its upper reaches, which represents a rare habitat in Europe (Meire et al., 2005). Having a drainage basin which is heavily impacted by anthropogenic activity, the Scheldt was considered as one of the most polluted systems in Europe during the second half of the 20th century especially in its freshwater part (Heip, 1988; Baeyens et al., 1998). As a result of substantial emission reduction efforts throughout the watershed and the construction of water purification plants in the Brussels area, the water quality of the Scheldt estuary has substantially improved since the nineteen nineties. Oxygen concentration improved considerably in the freshwater stretch from 1996 to 2006, associated with a decrease in N concentrations, mainly of ammonium and 5-day biochemical oxygen demand (Van Damme et al., 2005; Cox et al., 2009). Associated with the rise in oxygen concentration, there has been an increase in nitrate concentration as a result of more intensive nitrification (Cox et al., 2009). To follow-up the restoration process and to provide managers of this estuary with appropriate advice, the Government of Flanders sponsored a multi-disciplinary monitoring program, “OMES”. The general aim of the OMES study, which began in 1996, is to describe the changes in the estuarine community during its restoration, and to understand which environmental conditions have led to this pattern.

Because of its key position as a link between primary producers and higher trophic levels (Tackx et al., 2003; Maes et al., 2005b), as well as its potential role as bioindicator (Wilson,

1994; Appeltans et al., 2003; Mialet et al., 2010) zooplankton of the Scheldt estuary is studied since 1996 in the context of the OMES project. In a first inventory, (Tackx et al., 2003; Tackx et al., 2004) reported a strong dominance in abundance of rotifers in freshwater. A detailed report of the taxonomic composition and yearly cycle of the rotifer community as observed in 2002 is given by (Azémar et al., 2010). Also, A detailed report of the crustacean taxonomic composition as observed in 1996 is given by Tackx et al.(2004)

This paper completes the taxonomic list given by Tackx et al. (2004) with a taxonomic list of crustaceans, in which most taxa are determined at species level. The seasonal evolution of this community, as observed in 2002, is also shown. We further report the data at phylum level for copepods (cyclopoids, calanoids and harpacticoids) and at genera level for cladocerans over the entire period 1996–2009 and relate the spatio-temporal evolution of the crustacean community to the changes in the environmental conditions occurring during this period in the Scheldt estuary. The dominant calanoid copepod, *Eurytemora affinis*, is considered separately.

2. Material & Methods

2.1. Study site

The Scheldt estuary has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands (Fig. 1). Contrarily to most of the other temperate estuaries, the Scheldt estuary is characterized by a vertically well mixed water column (Baeyens et al., 1998), showing little salinity or current stratification (Heip, 1988). Within the framework of the OMES project, samples are taken monthly at 16 stations (Fig. 1) since 1996 until present, with an interruption between 2000 and 2002. All stations are situated in the brackish (salinity > 0.5) and freshwater part (salinity < 0.5) of the estuary (Fig. 1).

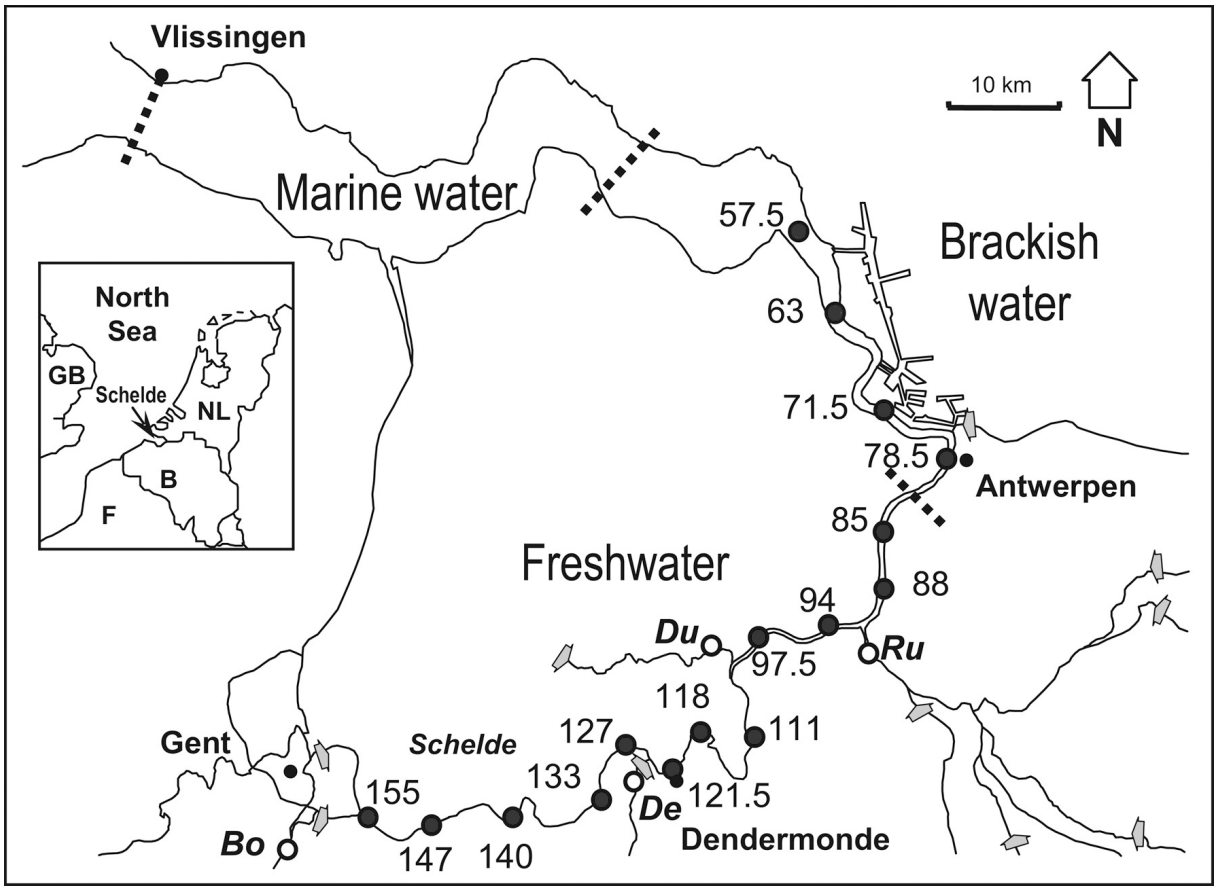


Fig. 1. Map of the Scheldt estuary with OMES sampling stations, designated by their distance, in km, upstream from Vlissingen (mouth). Dotted lines indicate limits between marine water, brackish water and freshwater reaches. Grey arrows indicate the end of tidal influence in the Scheldt and tributaries (Bo: Bovenschelde, De: Dender, Du: Durme, Ru: Rupel).

2.2. Sampling and analysis

At each station, surface water samples were collected in the middle of the river throughout the entire studied period, by means of bucket hauls from the ship. A set of environmental variables were measured. 5-day biochemical oxygen demand (BOD_5) was estimated using a WTW OXI 96 oxymeter. pH and temperature were measured using a CONSORT C832 electrode and dissolved oxygen concentration (O_2) a WTW OXI 325, equipped with Clark electrode. Samples were taken for the determination of the concentrations of chlorine (Cl^-), ammonium (NH_4^+-N), nitrates (NO_3^--N), nitrites (NO_2^--N), and orthophosphates (PO_4-P) within 24 h after sampling. They were stored at 4 °C, and analyzed colorimetrically using a SKALAR SA 5100 segmented flow analyzer. Suspended particular matter (SPM) samples

were filtered on pre-combusted Whatman GF/C filters. From 1996 to 2001, Chlorophyll *a* (Chl *a*) samples were filtered on 45 µm Sartorius filters, extracted in 90 % acetone and analysed using reversed phase HPLC. The reader is referred to Van Damme et al. (1997; 2005), for more details on the methodologies used. From 2002 to present, Chlorophyll *a* (Chl *a*) samples were filtered over a 25 mm diameter Whatman GF/F glass fibre filter. Pigments were then extracted and analyzed by HPLC according to the method of Wright et al. (1997). More details on the methodologies used are presented in Lionard et al. (2008).

The Flemisch Administration for Waterways and Maritime Affairs provides daily discharge measures (Q) of the Bovenschelde, the Dender and the Rupel. The upstream discharge data at these stations were used to estimate downstream discharge, taking into account all the physical features of the Scheldt estuary. Daily average discharges for km 68 are used in our data set. This station is located downstream in the study area and integrates discharge values of upstream stations.

For zooplankton sampling, a volume of 50 liter of surface water was collected at each station by means of bucket hauls and filtered through a 50 µm net. The collected zooplankton was anaesthetised with carbohydrated water and subsequently fixed in a formaldehyde solution (4 % final concentration). Samples were analyzed with stereomicroscope (90 x magnification) and microscope (400 x) for zooplankton taxa composition and abundance. For some years (1996, 1997, 1998, 2002) data on the abundance are available for all 16 stations. For the other years, zooplankton sampling was limited to 6 stations (km 57.5, 78.5, 98, 121.5, 140 and 155) and hence abundance data are only available for these stations.

2.3. Data analysis

Spatio-temporal trends in the zooplankton community, and their relationships to some environmental factors (BOD_5, Chl *a*, Cl^-, NH_4^+-N, NO_3^--N, NO_2^--N, PO_4-P, O_2, pH, SPM, T, Q) were analyzed using multivariate statistics. The environmental factors used are known to be important in structuring the Scheldt zooplankton community (Tackx et al., 2004), and most of them have changed in recent years. Taxa abundance data were log (x + 1) transformed prior to multivariate analysis to obtain a normal distribution. The data contained 1184 samples, 24 taxa and 12 environmental factors.

The CANOCO software package, version 4.5 (ter Braak, 1987, 1994) was used. The modality of the environmental factors was first analyzed by a Detrended Correspondence Analysis (DCA), using detrending by segments and downweighting for rare species. As the total inertia observed was less than 2.6, a predominance of linear species response curves could be expected. So a Principal Components Analysis (PCA) was used to investigate the relationships among environmental factors (ter Braak and Smilauer, 2002). Then the same DCA for the taxa distribution was performed. As for the environmental factors, the total inertia observed was less than 2.6, and so a redundancy analysis (RDA) was used to investigate the relationships between environmental factors and taxa composition. Forward selection of variables was used to select those most closely associated with the spatio-temporal structure of the zooplankton taxa, and to quantify their relative importance. The statistical significance was tested with Monte Carlo permutation tests (499 unrestricted permutations) ($P < 0.05$) and a Bonferroni correction for multiple test was applied. The minimum model so obtained explains the distribution without co-linear extra fitting. For each analysis, the variance explanation of each environmental variable (marginal effect), and their additional variance explained when included in the model (conditional effect) (ter Braak and Smilauer, 2002) were also shown.

Non parametrical tests and boxplot graphs were performed with Statistica 6 (version 6.0; Statsoft Inc., Tulsa, USA).

3. Results

3.1. Changes in water quality during the studied period

As can be seen from Fig. 2, axe 1, explaining the majority of the variance, was strongly correlated to factors associated with water quality (BOD_5, NH_4^+-N, NO_2^--N, PO_4-P, O_2). Along the entire studied period and area, changes in the water quality were dominated by a clear tendency towards a decrease in organic matter load and nutriments (BOD_5, NH_4^+-N, NO_2^--N, PO_4-P, O_2) (Fig. 2a) associated with an increase in oxygen concentration and in NO_3^--N (Fig. 2b).

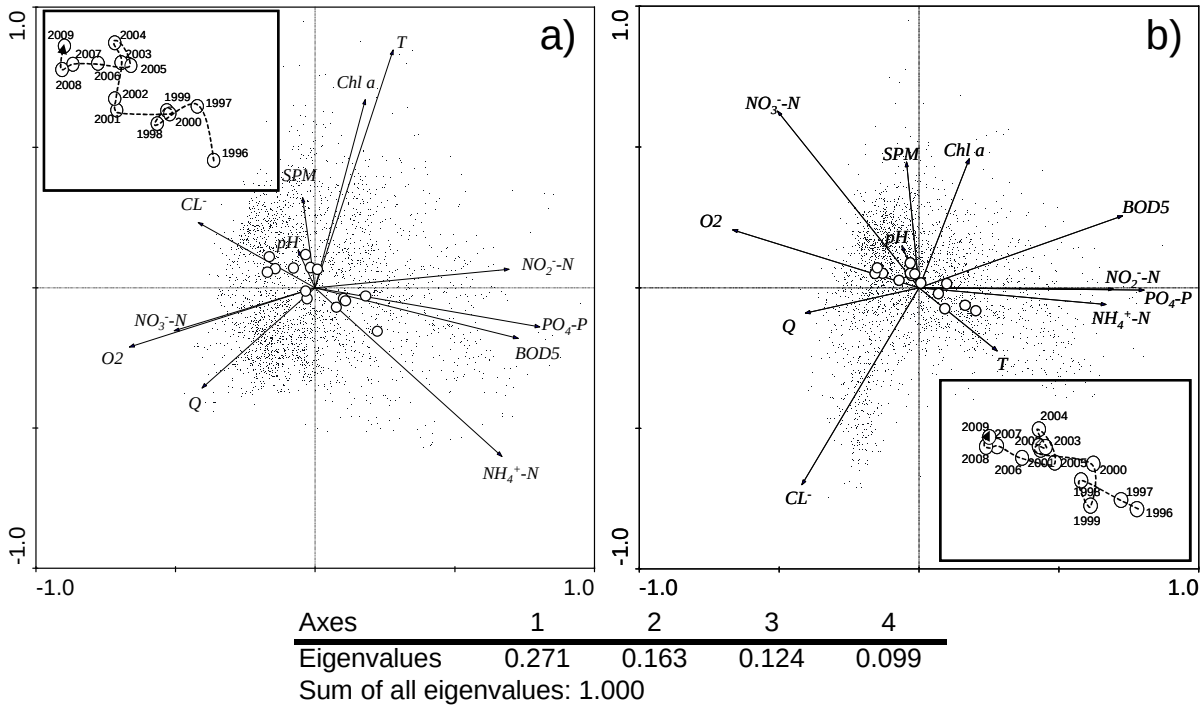
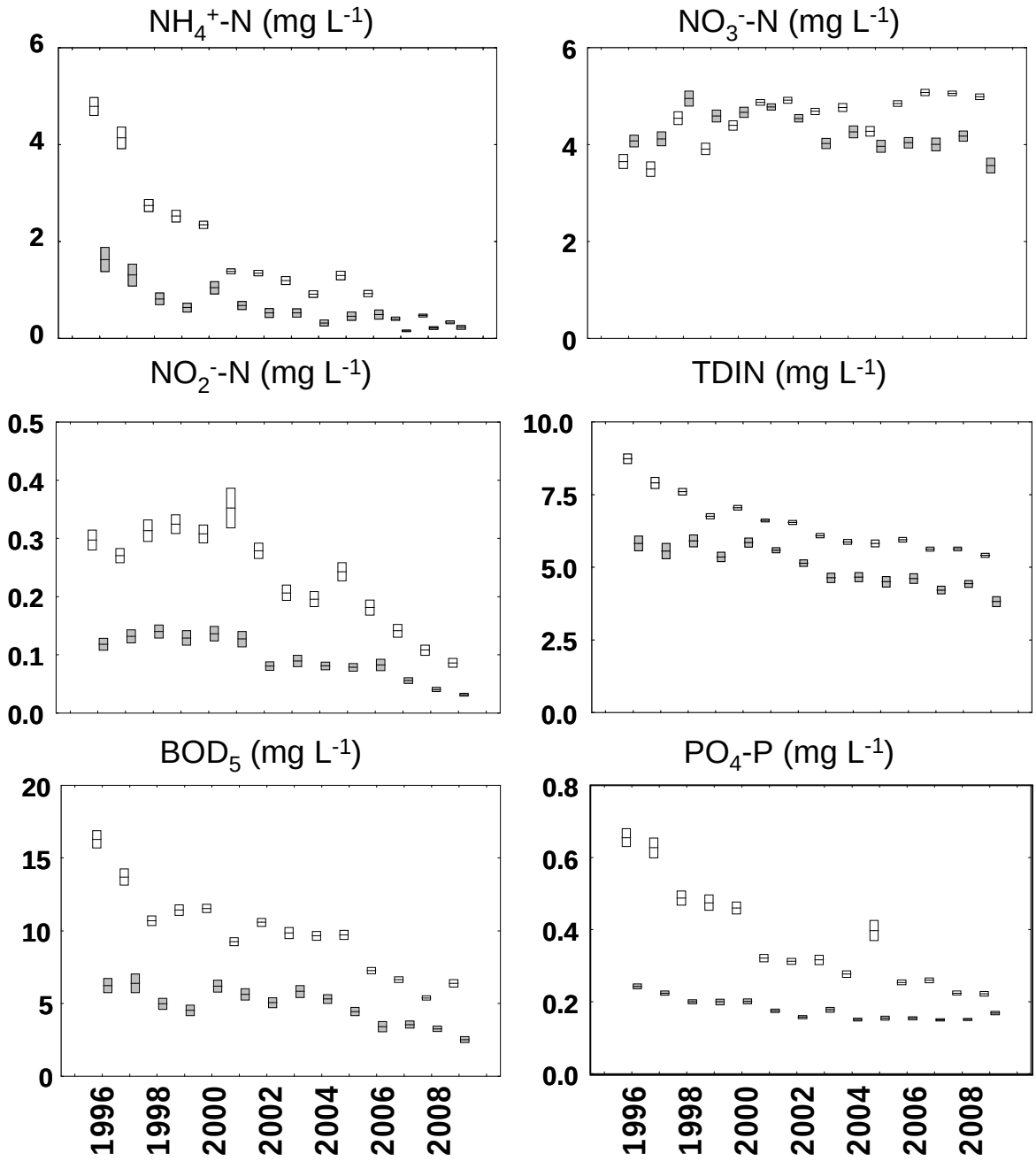


Fig. 2. PCA biplot axis 1 & 2 (a); axis 1 & 3 (b) showing the ordination of environmental factors (arrows), samples (dots) and annual means (circles) in the Scheldt estuary. See text for the abbreviations of the environmental factors.

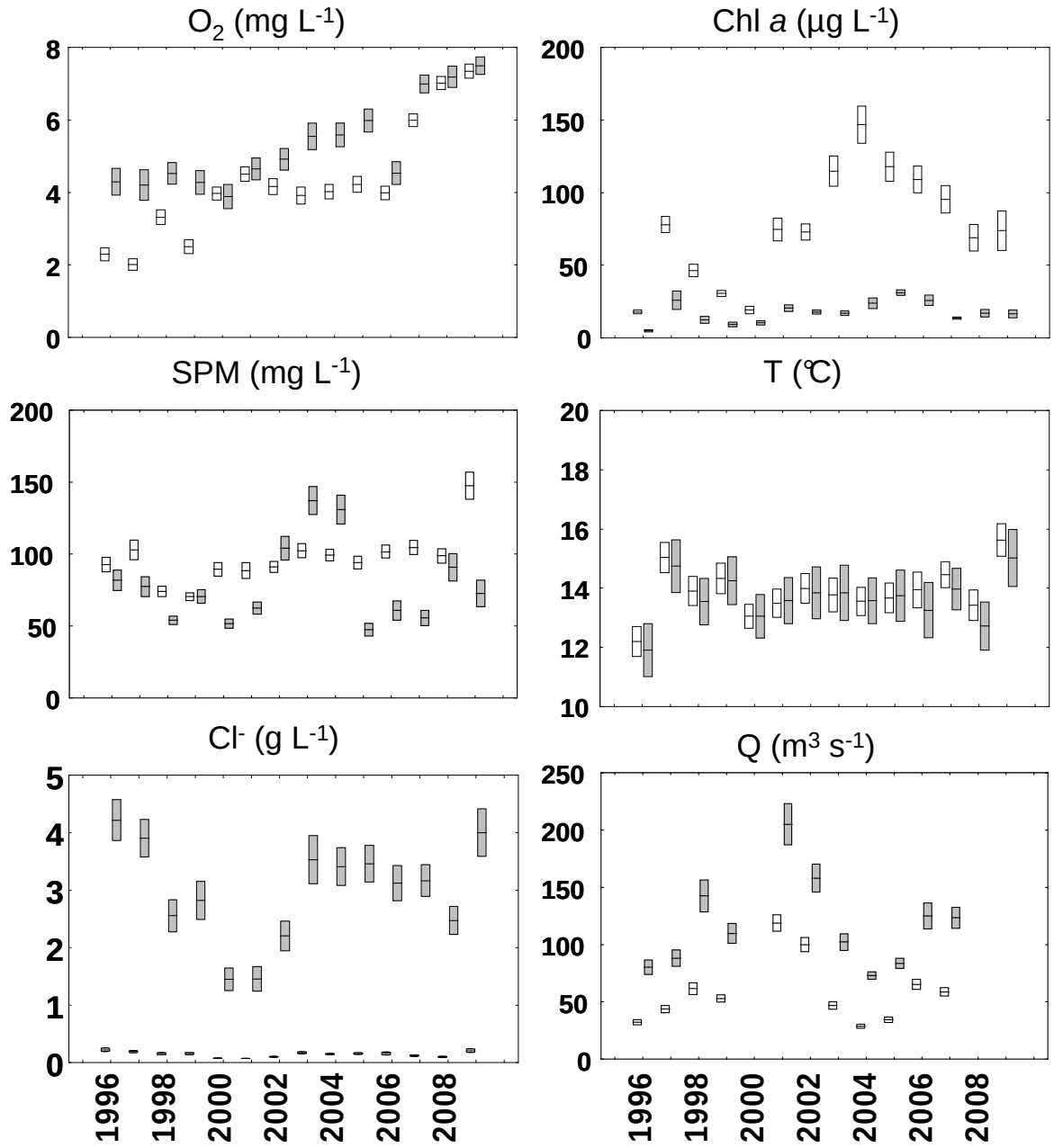
The evolution of the environmental variables is shown for the brackish and the freshwater stretch separately, using the salinity of 0.5 to distinguish between both zones (Fig. 3). Annual mean BOD_5, NH_4^+-N and PO_4-P (proxy for organic pollution), as well as NO_2^--N, showed a substantial decrease, especially in the freshwater part. NO_3^--N tended to decrease in brackish water while it increased in freshwater. Chl *a* concentration showed no clear tendency with time but increased in freshwater from 2000 to 2004, reaching a maximum mean value of 147 µg l^{-1}, then decreased regularly to 74 µg l^{-1} in 2009. O_2 concentration increased in both reaches; SPM significantly increased in the freshwater reach but showed strong variation between years. Along the studied period, for most factors associated with water quality (BOD_5, NH_4^--N, NO_2^--N, PO_4-P, O_2), the values observed in the freshwater reach became closer to the range observed in the brackish reach. In both zones, considered over the entire 1996–2009 period, Q and T showed no tendency with time, but Q increased strongly during the first half of the study period and decreased afterwards. Cl^-, as correlated with Q (a spearman rank test between mean annual values was significant with $p < 0.001$) showed the opposite trend with time.



Spearman rank R (variable – year):

Variable	Freshwater	Brackish water
NH_4^+-N	-0.97 ***	-0.94 ***
NO_3^--N	0.79 ***	-0.58 *
NO_2^--N	-0.83 ***	-0.87 ***
TDIN	-0.98 ***	-0.91 ***
PO_4-P	-0.95 ***	-0.84 ***
BOD5	-0.93 ***	-0.82 ***

Fig. 3. Evolution of environmental factors in the Scheldt estuary from 1996 to 2009, with annual mean values (lines) and standard errors (whiskers), in the brackish water part (salinity > 0.5, grey boxes) and in the freshwater part (salinity < 0.5, blank boxes). The resulting R values of a Spearman rank test for tendencies between mean annual values and time for both zones (*: $p < 0.05$; ***: $p < 0.001$; ns: non significant) are given below the figure.



Spearman rank R (variable – year):

Variable	Freshwater	Brackish water
Chl *a*	ns	ns
O_2	0.87 ***	0.87 ***
SPM	0.57 *	ns
Cl^-	ns	ns
Q	ns	ns
T	ns	ns

Fig. 3 (continued). Evolution of environmental factors in the Scheldt estuary from 1996 to 2009, with annual mean values (lines) and standard errors (whiskers), in the brackish water part (salinity > 0.5, grey boxes) and in the freshwater part (salinity < 0.5, blank boxes). The resulting R values of a Spearman rank test for tendencies between mean annual values and time for both zones (*: $p < 0.05$; ***: $p < 0.001$; ns: non significant) are given below the figure.

59 crustacean species, belonging to 37 genera, were identified (Table 1). About 27 % (16 species) were new reports for the Scheldt estuary. 29 species were only found in freshwater, whereas 5 were only found in brackish water.

Table 1

Taxonomic composition of the crustaceans of the Scheldt estuary. Symbols: *****, new taxa for the Scheldt; **b**, taxa present in the brackish water zone; **f**, taxa present in the freshwater zone. The taxonomic list is based on observations from all 2002 samples and on some other punctual samples.

CRUSTACEA					
Copepoda					
Cyclopoida					
* *Acanthocyclops trajani* Mirabdullayev, 2002	bf	*	*Oithona brevicornis* Giesbrecht, 1891	b
Cyclops vicinus vicinus Ulianine, 1875	bf	*	*Paracyclops imminutus* (Kiefer, 1929)	f
Cyclops strenuus Fischer, 1851	bf		*Paracyclops fimbriatus* (Fischer, 1853)	f
Diacyclops bicuspidatus (Claus, 1857)	bf		*Paracyclops poppei* (Rehberg, 1880)	f
Eucyclops serrulatus (Fischer, 1851)	f		*Thermocyclops crassus* (Fisher, 1853)	f
Eucyclops speratus (Lilljeborg, 1901)	f		*Thermocyclops oithonoides* (G. O. Sars, 1863)	bf
Metacyclops gracilis (Lilljeborg, 1853)	b		*Tropocyclops prasinus* (Fisher, 1860)	f
Mesocyclops leukarti (Clauxs, 1857)	f			
Harpacticoida				
Canthocamptus staphylinus	f	*	*Ectinosoma barroisi* (Richard, 1893)	b
* *Bryocamptus (Br.) minutus* (Claus, 1863)	bf		*Microarthridion littorale* (Poppe, 1881)	bf
* *Halectinosoma curticorne (* Boeck, 1872)	b		*Nitocra lacustris* (Schmankevitch, 1875)	bf
Euterpina acutifrons (Dana, 1848)	b		*Pseudobradya* sp.	bf
Calanoida				
Acartia tonsa Dana, 1848 1849?	bf		*Eudiaptomus gracilis* (G.O. Sars, 1863)	bf
Eurytemora affinis (Poppe, 1880)	bf		*Temora longicornis*	bf
Branchiopoda				
* *Acroperus harpae* (Baird, 1835)	f		*Daphnia obtusa* Kurz, 1874	f
* *Alona affinis* (Leydig, 1860)	bf		*Daphnia pulex* Leydig, 1860	bf
* *Alona rectangula* Sars, 1862	f		*Disparalona leei* (Chien, 1970)	f
Anchistropus emarginatus	bf		*Disparalona rostrata* (Koch, 1841)	f
Bosmina coregoni Baird, 1857	bf		*Ilyocriptus agilis* Kurz, 1878	f
Bosmina longirostris (O. F. Müller, 1785)	bf		*Ilyocryptus sordidus* (Liévin, 1848)	f
Ceriodaphnia quadrangula (O. F. Müller, 1785)	bf		*Leydigia acanthocercoides* (Fischer, 1854)	bf
* *Ceriodaphnia laticaudata* P.E. Müller, 1867	f		*Leydigia leydigi* (Schoedler, 1858)	f
* *Ceriodaphnia pulchella* Sars, 1862	f		*Macrothrix laticornis* (Jurine, 1820)	bf
* *Ceriodaphnia reticulata* (Jurine, 1820)	f		*Moina brachiata* (Jurine, 1820)	bf
Chydorus sphaericus (O. F. Müller, 1785)	bf		*Moina micrura* Kurz, 1874	f
Daphnia cucullata Sars, 1862	f	*	*Pleuroxus aduncus* (Jurine 1820)	f
Daphnia galeata Sars, 1864	f	*	*Pleuroxus uncinatus* Baird, 1850	f
Daphnia hyalina Leydig, 1860	f	*	*Scapholeberis mucronata* (O. F. Müller 1785)	f
Daphnia longispina O. F. Müller, 1785	bf	*	*Simocephalus exspinosus* (Koch, 1841)	f
Daphnia magna Straus, 1820	bf		*Simocephalus vetulus* (O. F. Müller 1776)	f

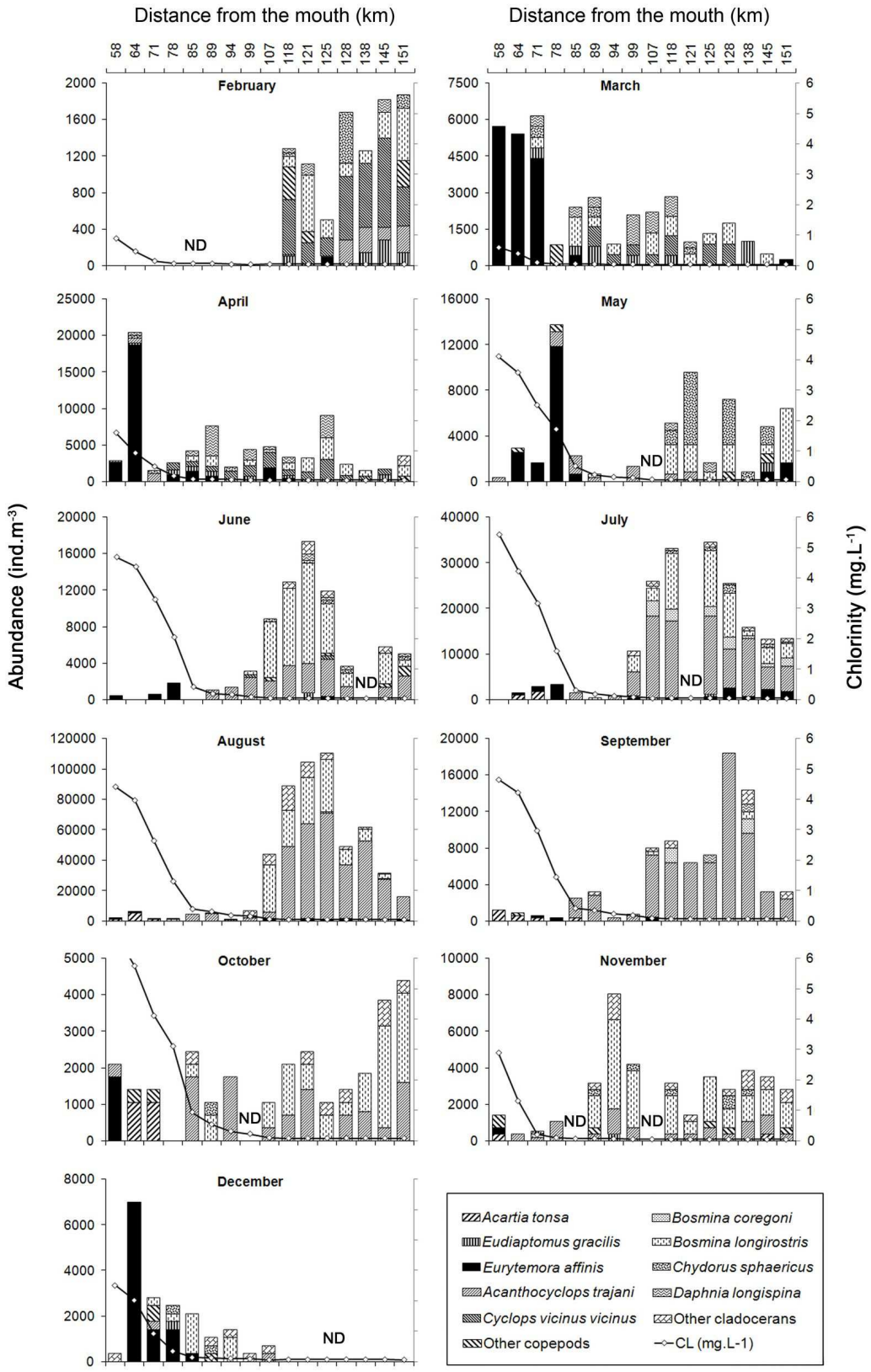


Fig. 4. Spatio-temporal distribution of crustaceans as observed in 2002 in the Scheldt estuary. The X axis shows the distance from the mouth of the estuary (in km). The left Y axis indicates the crustacean abundance (bars); the right Y axis indicates the chlorinity (line). ND: no data available.

As shown in Fig. 4, crustacean 2002 abundance was low during autumn and winter, and then increased up to maximal abundance during summer (reaching 110 000 ind m^{-3} in August). During spring, the abundances were higher in brackish water (km 57.5–78.5) than in freshwater (km 85–155), varying from 3 000 to 20 000 ind m^{-3}; whereas during summer, the abundances were much higher in freshwater, varying from 3 000 to 110 000 ind m^{-3}.

Concerning the most abundant species, the calanoid copepod *Eurytemora affinis* was found in high abundance during spring and late autumn in the brackish water part. In the freshwater part of the estuary, the calanoid copepod *Eudiaptomus gracilis* occured in winter and spring in low abundance. The cyclopoid copepod *Cyclops vicinus vicinus* was present in high numbers during winter (February) in the freshwater part. *Acanthocyclops trajani* dominated during summer and autumn in the freshwater while *Acartia tonsa* dominated the brackish water (notice that the abundance scale varies because of strong cyclopoid abundance in freshwater). The cladoceran *Bosmina longirostris* was the only taxa present the whole year in various abundance, in both brackish and freshwater reaches. Cladocerans and copepods were present in similar abundance in freshwater whereas *E. affinis* dominated the brackish water part except in the late summer.

3.3. Changes in zooplankton distribution during the 1996–2009 study period

The evolution of the abundance of planktonic taxa considered is shown in Fig. 5 and in Fig. 6. Few crustaceans were observed in the brackish water zone, except calanoids. Between 1996 and 2006, *E. affinis* was observed mainly downstream of km 98.5 and during spring (Fig. 5), in varying abundance, with a mean of 610 ind m^{-3} over the year. *E. affinis* was occasionally found in freshwater, in relative low abundances. Its freshwater abundance increased significantly from 1996 to 2009, especially from 2007 onwards (Fig. 6). This increase was associated with an upstream spreading of populations up to km 155 (Fig. 5). Moreover, *E. affinis* is now present also during summer.

Other calanoids essentially followed the same evolution as *E. affinis* (Fig. 5). Between 1996 and 2006, they were found mainly in freshwater water during winter (*Eudiaptomus gracilis*) and in brackish water during summer (*Acartia tonsa*) in relatively low abundance (Fig. 5), with a mean of 130 ind m^{-3} (Fig. 6). From 2007 to 2008, an important increase in abundance

(Fig. 6) and a spreading toward the upstream reach (Fig. 5) were observed. However, they were found in very low abundance in the study area during 2009 (Fig. 6).

Cyclopoids were typically observed in the freshwater zone upstream from km 98.5 (Fig. 5, notice the spatial scale is inversed for clarity reasons), mainly during late spring and summer. They occured in varying abundance with a mean of 3390 ind m^{-3} between 1996 and 2006 (Fig. 6). Their freshwater abundance decreased significantly from 1996 to 2009 (Fig. 6), especially from 2007 onwards. No spatial shift was observed for these taxa (Fig. 5).

As the cyclopoids, cladocerans also essentially inhabited the freshwater reach, upstream of km 98.5, mainly during late spring and summer (Fig. 5). Along the study period, their freshwater mean abundance was around 5000 ind m^{-3}. A marked period of high abundance occured in the freshwater zone between 2003 and 2005, with a mean abundance of 28 600 ind m^{-3} (Fig. 6), reaching maximum abundance in 2004.

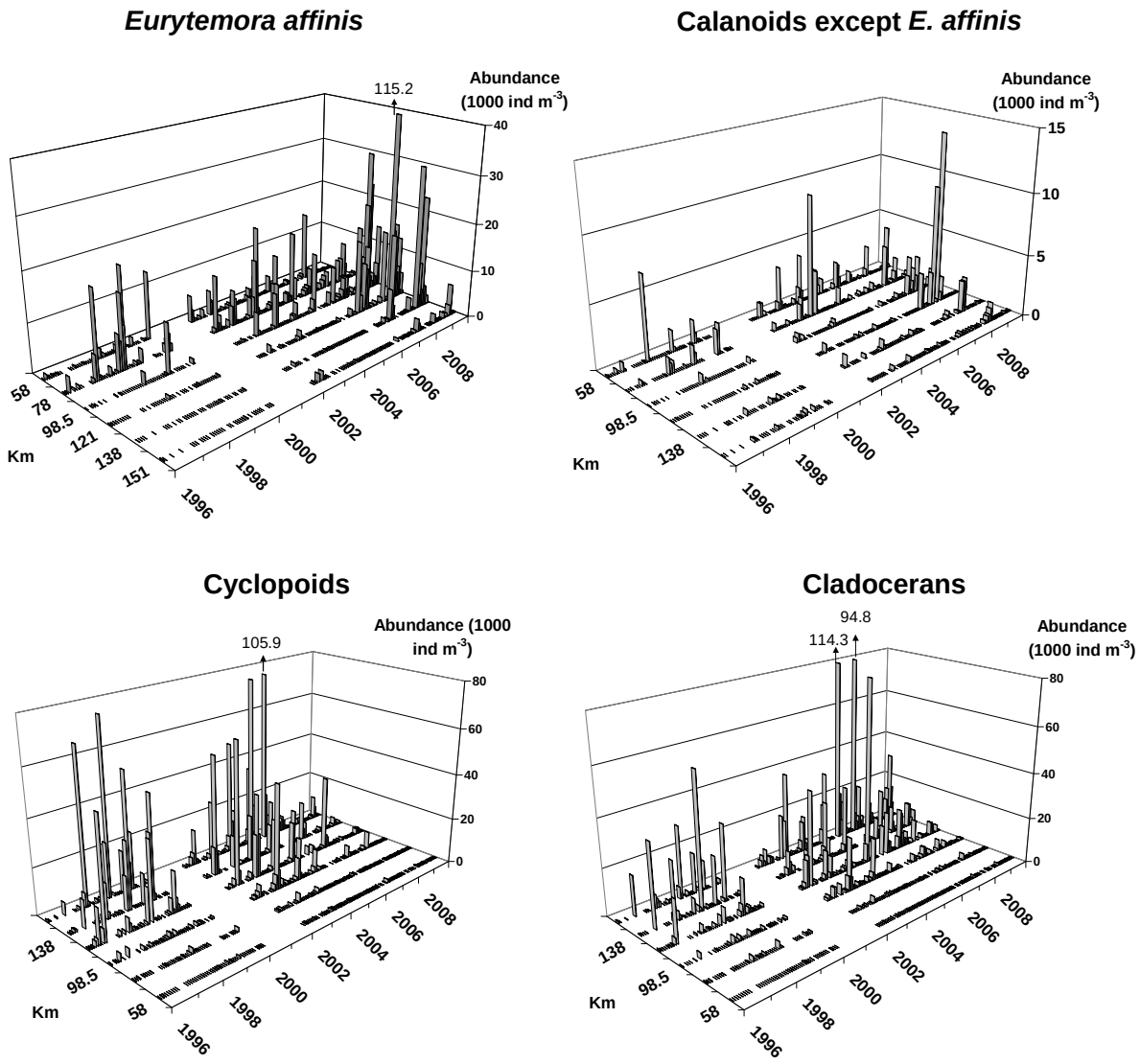
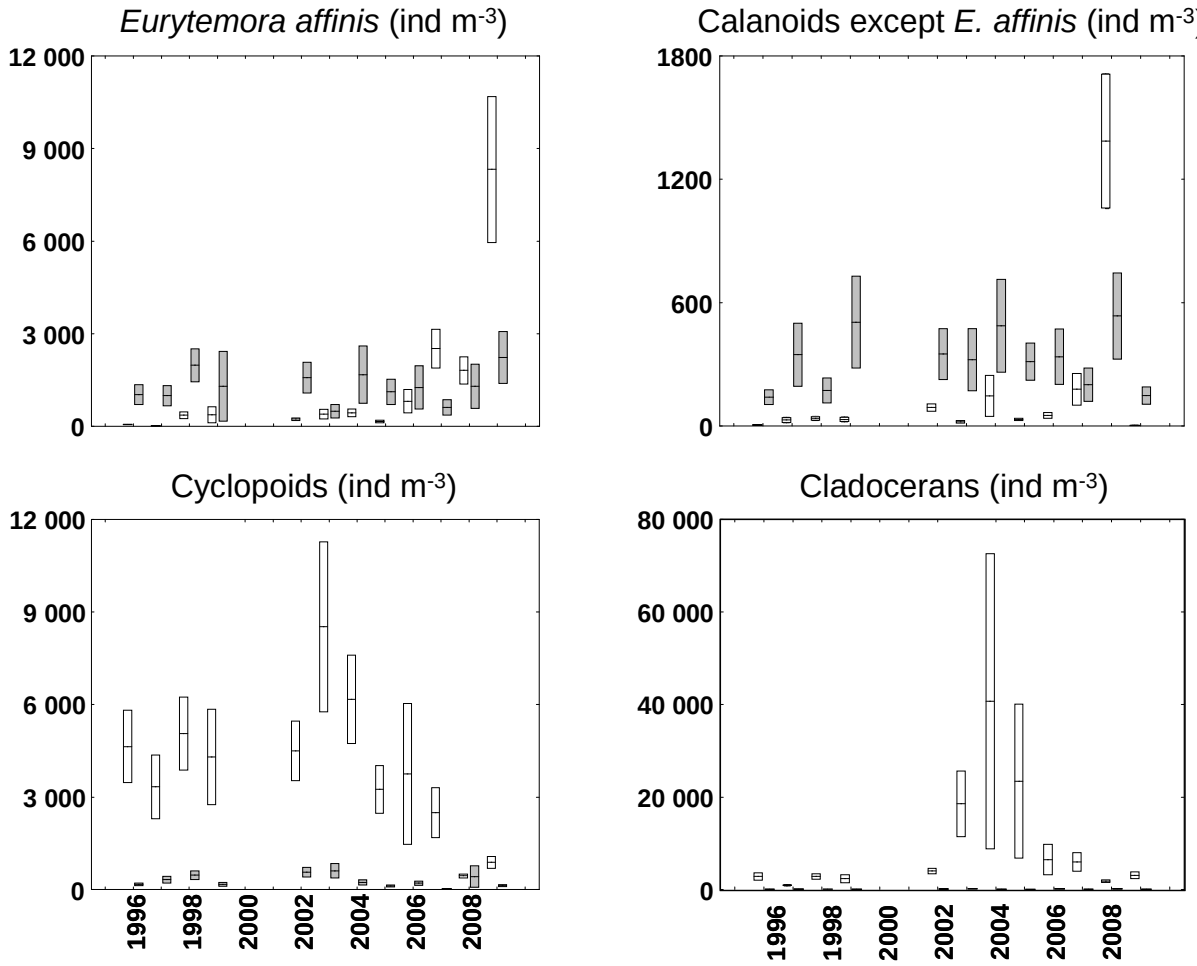


Fig. 5. Evolution of spatial and temporal distribution of abundance (monthly values for each station) of planktonic crustaceans in the Scheldt estuary, from 1996 to 2009: *Eurytemora affinis* adults (a), other calanoids adults (b), cyclopoids adults (c) and cladocerans adults (d). Spatial scale is inversed for freshwater species (cyclopoids and cladocerans).



Spearman rank R (variable – year):

Variable	Freshwater	Brackish water
E. affinis	0.86 ***	ns
Calanoids except *E. affinis*	ns	ns
Cyclopoids	-0.63 *	ns
Cladocerans	ns	ns

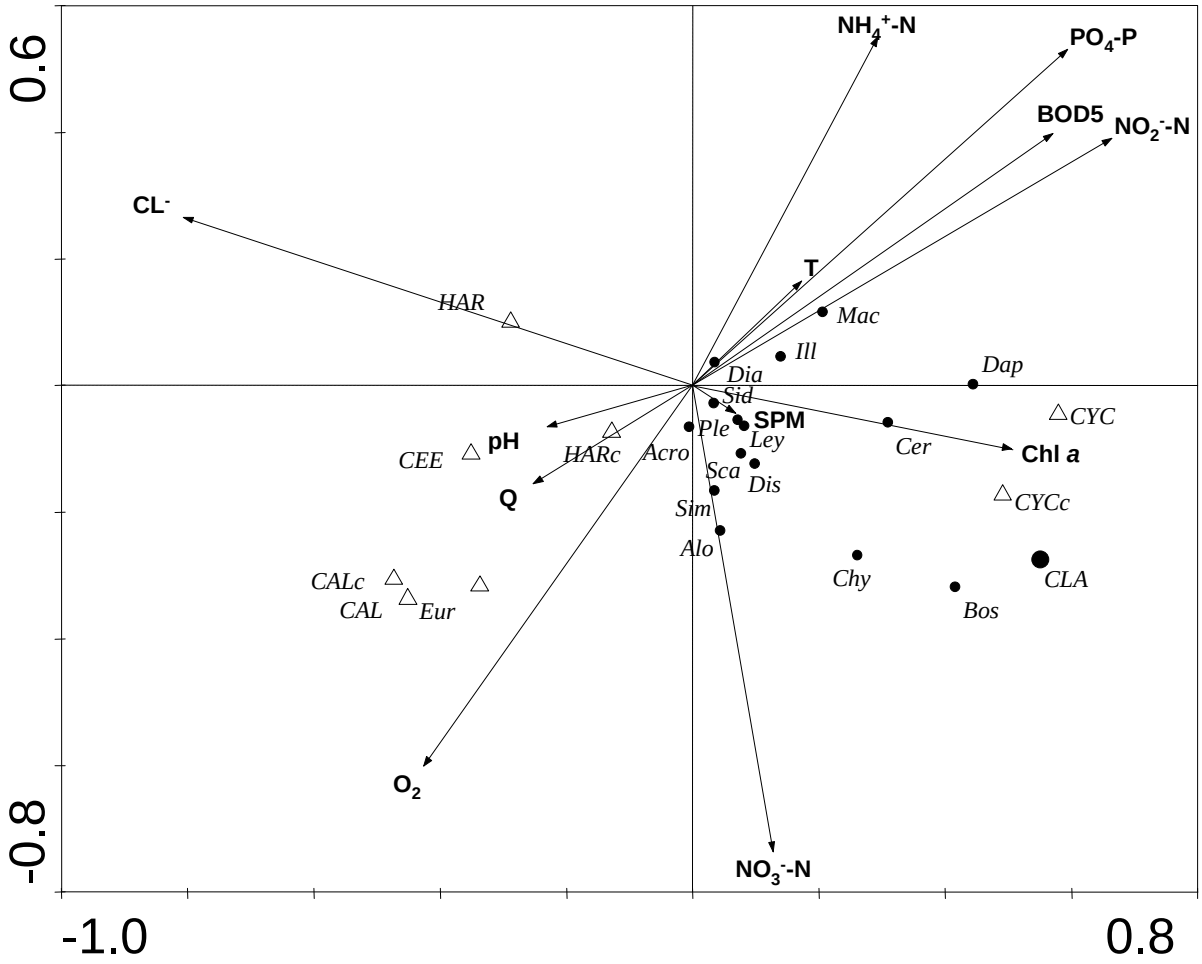
Fig. 6. Evolution of the abundance of planktonic crustaceans in the Scheldt estuary, with annual mean values (lines) and standard errors (whiskers), in the brackish water part (salinity > 0.5, grey boxes) and in the freshwater part (salinity < 0.5, blank boxes). The result of a Spearman rank test for tendencies with time for both zones (*: $p < 0.05$; ***: $p < 0.001$; ns: non significant) are given below the figure.

3.4. Interactions between zooplankton community and water quality

The first RDA analysis, using all environmental factors, explained 27.5 % of the total variance in the taxa distribution (Fig. 6). The taxa ordination was first explained by Cl^- (Tab 1a) then by factors representing organic pollution (NO_2^--N, PO_4-P, NH_4^--N, BOD_5). Other factors seemed to play a minor role.

The biplot shows a typical estuarine setting, with *E. affinis* and other calanoids placed in the left hand side, negatively correlated with factors representing organic pollution (see above) and positively correlated with Cl^- and O_2 concentrations (Fig. 7). Cyclopoids are placed on the right-hand zone, negatively correlated with Cl^- and positively correlated with Chl *a* concentration. Total cladocerans showed the same correlations than cyclopoids, but genera showed various affinities, with some taxa more correlated with T (*Illyocryptus, Macrothrix*), than others. NO_2^--N, PO_4-P, NH_4^--N, BOD_5 are typical for the freshwater area, where water quality is worse than in the brackish part of the estuary and Chl *a* concentrations are higher (Fig. 2). They were also associated with temperature, typically at maximum values during summer months. Harpacticoids adults seem correlated with Cl^-. As they were observed in few numbers, essentially in the brackish water part, we chose to not show results of a RDA based on this taxa (11.4 % of the variance explained). Rare cladocerans genera and harpacticoids copepodits are located in the centre of the diagram, with no particular affinities with environmental variables.

RDA analysis performed with freshwater and backish water data separately, gave essentially the same associations between taxa and environmental factors.



Cladocerans	
Alo	*Alona sp*
Acro	*Acroperus sp*
Bos	*Bosmina sp*
Cer	*Ceriodaphnia sp*
Chy	*Chydorus sp*
Dap	*Daphnia sp*
Dia	*Diaphonosoma sp*
Dis	*Disparalona sp*
Ill	*Illyocryptus sp*
Ley	*Leydigia sp*
Mac	*Macrothrix sp*
Ple	*Pleuroxus sp*
Sca	*Scapholeberis sp*
Sid	*Sida sp*
Sim	*Simocephalus sp*
CLA	Total cladocerans adults

Copepods	
Eur	*Eurytemora affinis*
CEE	Calanoids except *E. affinis*
CAL	Total calanoids adults
CYC	Total cyclopoids adults
HAR	Total harpacticids
CALc	Calanoids copepodits
CYCc	Cyclopoids copepodits
HARc	Harpacticids copepodits

Axes	1	2	3	4
Eigenvalues	0.189	0.059	0.012	0.005

Sum of all canonical eigenvalues: 0.275

Fig. 7. RDA biplots axis 1 & 2, showing the distribution of planktonic crustaceans (blank triangles: copepods; black circles: cladocerans) in the Scheldt estuary, as a function of the environmental factors. See text for the abbreviations of the environmental factors. Taxa CAL and CLA, as they already include several taxa, were added as supplementary data after the analysis.

Table 2

Variance in the zooplankton abundance explained by the environmental factors in different RDA analysis.

a) RDA with all taxa

Marginal effects		Conditionnal effects	
Variable	Lambda1	Variable	LambdaA
Cl^-	0.13	Cl^-	0.13
NO_2^--N	0.09	PO_4-P	0.05
PO_4-P	0.08	NO_2^--N	0.03
BOD5	0.07	NH_4^+-N	0.02
O_2	0.06	O_2	0.01
Chl *a*	0.05	Chl *a*	0.01
NH_4^+-N	0.04	pH	0.01
NO_3^--N	0.04	NO_3^--N	0.01
Q	0.02	SPM	0.01
T	0.02	T	0
pH	0.01	BOD5	0
SPM	0	Q	0

Sum of all canonical eigenvalues : 0.275

b) RDA with only taxa EUR, CEE, CALc

Marginal effects		Conditionnal effects	
Variable	Lambda1	Variable	LambdaA
PO_4-P	0.15	PO_4-P	0.15
NO_2^--N	0.14	O_2	0.04
BOD5	0.12	Cl^-	0.03
O_2	0.11	NO_2^--N	0.02
NH_4^+-N	0.09	T	0.01
Cl^-	0.09	Chl *a*	0.01
Chl *a*	0.03	Q	0.01
pH	0.02	BOD5	0
NO_3^--N	0.02	pH	0
Q	0.02	NO_3^--N	0
T	0.01	NH_4^+-N	0
SPM	0	SPM	0

Sum of all canonical eigenvalues : 0.271

c) RDA with only taxa CLA, CYC, CYCc

Marginal effects		Conditionnal effects	
Variable	Lambda1	Variable	LambdaA
Cl^-	0.22	Cl^-	0.22
Chl *a*	0.1	Chl *a*	0.05
NO_2^--N	0.09	NH_4^+-N	0.02
BOD5	0.06	PO_4-P	0.02
PO_4-P	0.05	NO_2^--N	0.01
NO_3^--N	0.04	O_2	0.01
O_2	0.03	NO_3^--N	0.01
Q	0.02	T	0.01
T	0.02	Q	0
NH_4^+-N	0.01	SPM	0
SPM	0.01	pH	0
pH	0	BOD5	0

Sum of all canonical eigenvalues : 0.355

Two additional RDA analyses have been specifically performed for brackish water and freshwater groups (Table 2b, c). The first one (Table 2b) explained 27.1 percent of the total variance of all calanoids adults and copepodits. Their distribution is almost exclusively negatively correlated to factors associated with water quality (Table 2b and Fig. 7). Other factors, including Cl^-, seem not to be very explicative (see conditional effects). The second analysis (Table 2c) explained 35.5 % of the variance of cladocerans, cyclopoids adults and copepodits. The distribution of these freshwater species was mainly explained by Cl^- then by Chl *a* (Table 2c) with a positive correlation with Chl *a* (Fig. 7).

As can be seen from Fig. 3, during the study period, the abundance of cladocerans in freshwater followed the same pattern as chlorophyll *a* concentration. Fig. 8 shows an increase in mean annual cladoceran abundance with mean annual chlorophyll *a* concentration (spearman rank test $p < 0.001$). This increase was particularly strong from 100 µg l^{-1} onwards.

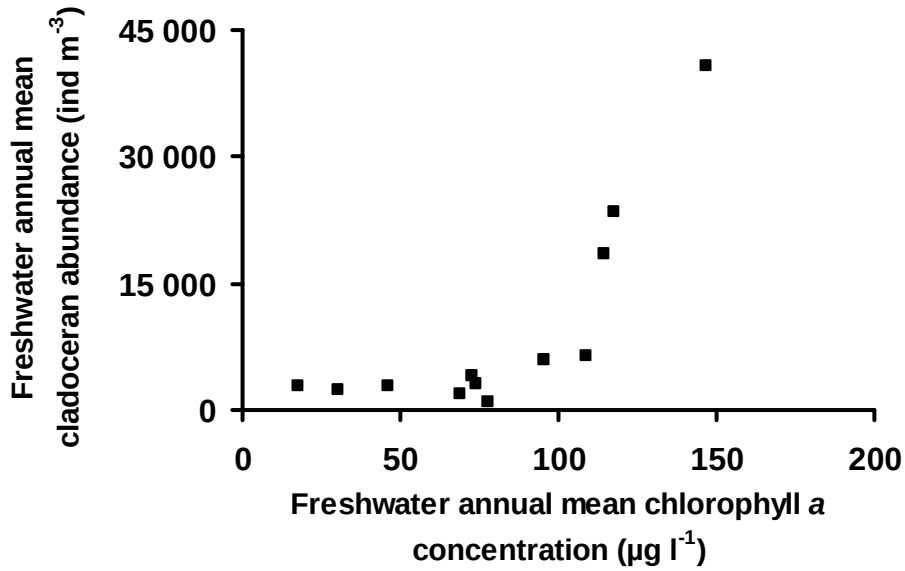


Fig. 8. Relation between annual mean cladoceran abundance and chlorophyll *a* concentration in the freshwater part of the Scheldt estuary.

4. Discussion

4.1. Environmental changes in the Scheldt estuary

Historically, water quality has been worse in the freshwater zone than in the brackish part (Van Damme et al., 1995). Also during this study, high BOD_5, PO_4-P, NH_4^+-N, NO_2^--N values and low O_2 concentrations were associated with the freshwater area (Fig. 2). Logically, O_2 was negatively related to temperature, as minimum O_2 concentrations values occur during the warm season.

Since the middle of the Nineties, the water quality of the Scheldt estuary had shown very important changes. Major tendencies observed in this study confirm those previously observed (Van Damme et al., 1995; Cox et al., 2009). BOD_5, NH_4^+-N, NO_2^--N and PO_4-P concentrations continue to decrease particularly in the freshwater reach (Fig. 3). O_2 still increases regularly in both the brackish and in the freshwater reach. Slight increasing of NO_3^--N in the freshwater part can be explained by the fact that since the second half of the Seventies, the nitrification front moved upstream due to the increase of oxygen concentrations and caused a substantial impact on the N-load (Soetaert, K. and Herman, P. J. M., 1995).

Our data, considering the entire km 57.5–155 transect, showed the same trend than described by Cox et al. (2009) in both the fresh and brackish water area (Fig. 3). Cox et al. (2009) explain this evolution as a switch between two steady states: from a high nutrient — hypereutrophic state — which inhibited phytoplankton primary production, the Scheldt has recently evolved to a lower nutrient — high phytoplankton production — eutrophic equilibrium.

It should be mentioned that, apart from runoff, Cl^-, SPM and Chl *a* concentrations, all environmental variables considered follow a gradual, unidirectional change either during the entire study period, either from a certain year onwards in both in the fresh and brackish water stretch. The pattern observed for Cl^- can probably be explained by variations in runoff, as it is significantly correlated with Q.

4.2. Taxonomic composition and abundance of zooplankton

With 16 new taxa reported in the Scheldt estuary, the present mesozooplankton taxonomic list shows a considerable extension since previous studies (De Pauw, 1975; Soetaert and Van Rijswijk, 1993; Tackx et al., 2004). This is probably due to the taxonomic effort provided in this study, although the heavy shipping activity is known to introduce exotic species in estuaries (Carlton, 1996; Johnson and Padilla, 1996; Ruiz et al., 2000; Wasson et al., 2001). The pattern of taxa spatio-temporal distribution observed in 2002 (Table 1, Fig. 2) is quite similar to the one found in 1996 (Tackx et al., 2004).

4.3. Effect of environmental changes on the zooplankton community

The use of a 14 year monitoring database allows us to analyse the association of the earlier mentioned improvement of water quality to the evolution of the zooplankton community. Important changes in the abundance and spatial distribution of the dominant mesozooplankton groups have clearly occurred in freshwater during the study period. For *E. affinis* and other calanoids, most strong changes have occurred from around 2007 onwards. For cyclopoids a gradual decrease in abundance occurred from 2003 onwards, while cladocerans reached very high abundances between 2003 and 2005.

Even with a Bonferroni correction, all the environmental factors considered contributed significantly to explain the distribution of crustaceans in the Scheldt. The dataset shows the colinearity of environmental factors, typical for an estuarine gradient situation. This is well shown by differences between marginal and conditional effects (Table 2): after having taken into account the variance explained by the first factors (Cl^- for Table 2a and 2c; PO_4-P for Table 2b), the variance explained by following factors is considerably reduced in the conditional effect. In the present study, after the salinity, environmental factors associated with water quality (NO_2^--N, PO_4-P, NH_4^+-N, BOD_5 and O_2) are the dominant factors which explain the ordination of crustacean taxa (Table 2a). Contrarily to the strong seasonal influence reported by Soetaert and Van Rijswijk (1993) and Tackx et al. (2004), in this long term study, factors such as T and Chl *a*, as seasonal and food availability proxies, seem to be less important than water quality (Table 2a). No obvious strong influence of the water quality

on the taxa distribution was mentioned in above cited studies. However, the fact that these studies were run before the major physicochemical changes which occurred in the estuary, noticed by Van Damme et al. (2005), has to be considered. This suggests that improving water quality played an important role in determining living conditions of zooplankton taxa in the Scheldt estuary.

Considering the RDA analyses on calanoids, cyclopoids and cladocerans separately (Table 2b, c), the marginal effect of the environmental factors showed that the distribution of calanoids is strongly affected by water quality. The distribution of cyclopoids and cladocerans is mainly explained by Chl *a* in addition to Cl^- and not by other factors associated with water quality. Their strongest abundances were found the same years (2003–2005) as the strongest phytoplankton blooms (Fig. 3, Fig. 6). The fact that cladoceran mean annual abundance increases with Chl *a* concentration, especially from 100 $\mu g\ l^{-1}$ onwards (Fig. 8), suggests a potential threshold effect of the phytoplankton availability on the cladoceran abundance. In Chilean lakes, De los Rios and Soto (2007) have also shown a strong response of daphnid abundance to eutrophication.

The strong reduction of cyclopoid abundance in the brackish water is probably due to their decrease in freshwater reaches, which limited their expansion in downstream direction. Indeed, for the 1996 (Tackx et al., 2004) and 2002 samples, cyclopoids copepods that were present in the brackish zone almost always belonged to the dominant freshwater species *Cyclops vicinus vicinus* and *Acanthocyclops trajani*.

Among the copepods, *Eurytemora affinis* shows the most spectacular displacement. This calanoid is known to be typical of the brackish–freshwater transition zone in European estuaries (Sautour and Castel, 1995). In the Scheldt, previous studies have noticed the absence of *E. affinis* from the freshwater–brackish water fringe and upstream reaches during the Sixties (De Pauw, 1973) and the late Eighties (Soetaert and Van Rijswijk, 1993). This was explained by the very bad water quality in this salinity range, situated around the highly polluted zone of Antwerp (Fig. 1). First observations of the copepod there occurred in the middle of the Nineties and this population shift was attributed to the improving oxygen concentrations (Appeltans et al., 2003). Since 1996 (fig.4a) *E. affinis* is regularly abundant around Antwerp (km 78.5) and as such well settled in the same salinity range as in other European temperate estuaries (Sautour and Castel, 1995). Between 2001 and 2006, *E. affinis*

was sporadically observed in the upstream freshwater part of the estuary (Fig. 5). From 2007 onwards, these occasional occurrences have switched to a consistent high abundance of *E. affinis* in the freshwater area. *E. affinis* reaches now very high abundance with maxima (155 000 ind m^{-3}) comparable with those of freshwater stretch of the Seine estuary (Mouny and Dauvin, 2002). In the Gironde and in the Ems, maximal freshwater abundances where inferior to 20 000 ind m^{-3} (Sautour and Castel, 1995). Other calanoids, although much less abundant, have also increased substantially in the freshwater reach in 2007–2009. Thus, since its water quality has improved, the freshwater part of the Scheldt estuary seems to have become a suitable area for calanoids. However, in the last 3 years, the increase in calanoid abundance in the freshwater area is spectacular. Is this a sporadic boom or a real trend? Mialet et al. (2010), using Scheldt spring data on *E. affinis* between 1996 and 2007, have shown that *E. affinis* can occur in the upstream freshwater stretch (salinity > 0.5)when mean upstream O_2 concentration is > 4 mg l^{-1} and the minimum O_2 concentration, which is always observed around the middle of the estuary, is > 1.3 mg l^{-1}. A possible explanation to the abrupt invasion of the freshwater reach by *E. affinis* (and other calanoids) since 2007 could be that environmental conditions are since then steadily above limit level. Indeed, verifying the 2007–2009 spring data shows that conditions are permissive over the entire study zone for *E. affinis* upstream expansion during 91 % of the monthly sampling campaigns, while during 1996–2006, this was only the case in 56 % of the campaigns. It remains to be investigated if this recent apparent acceleration in the water quality in the Scheldt estuary is related to the start-up of the purification plant built in Brussels, aimed at reducing organic loads reaching the Scheldt trough the Rupel tributary (Fig. 1) (Van Damme et al., 2005).

Tackx et al. (2003) have shown that *E. affinis* is capable of selecting phytoplankton among the detritus-dominated suspended matter in the Scheldt estuary, and can satisfy its nutritional demands by its phytoplankton ingestion solely. It is thus unlikely, that the higher phytoplankton concentration in the freshwater stretch than in the brackish water (Fig. 3), have acted as an incentive for *E. affinis*'s move to the freshwater, or that *E. affinis* really profited from better feeding conditions in this area. Moreover, chlorophyll *a* concentration in the freshwater zone is decreasing since 2004 (Fig. 3). Considering the above, the improving water quality (strong reduction of NH_4^+-N, and BOD_5, increase in O_2 concentration) seems the most likely cause of the shift of *E. affinis* from the brackish water to the upstream freshwater Scheldt.

Previous studies suggested that some *E. affinis* populations which originally live in brackish water environments could adapt to freshwater in only few generations (Lee et al., 2003). Lee (1999) also showed that in the past century, *E. affinis* brackish populations invaded freshwater reaches, in European systems as well as in North American and in Asian ones, in a short time range. But factors responsible for these invasions were not clearly identified. It would be interesting to make an inventory of water quality changes in these *Eurytemora* invaded systems.

4.4. Potential effect of biotic interactions on zooplankton community

The shift of calanoids toward upstream is concomitant with a quasi-complete disappearance of cyclopoids from this area. This suggest that cyclopoids, which appear to be less sensitive to poor water quality (Table 2c), were able to live there as long as calanoids were not. Once water quality in the freshwater stretch became permissive for calanoids, they rapidly became dominant. To our knowledge, there is no literature comparison of the salinity, pollutant or oxygen concentration tolerance for calanoids, cyclopoids or cladocerans.

As it is difficult to imagine that improving water quality has a negative effect on cyclopoids, a likely explanation is that calanoids reduced cyclopoid abundance by either competition or predation. Using long term (8 months) enclosure experiments with (1) lake calanoids only, (2) cyclopoids only and (3) a mix of both, Soto and Hurlbert (1991) showed that interactions between these two groups are very complex, and both direct and indirect. In an early stage of the experiment, cyclopoids predation on calanoid nauplii and copepodits caused a reduction in calanoid abundance. After a few months however, calanoid abundance was found to be superior in the mixed mesocosms than in the ones with only calanoids. This is explained by the fact that when only calanoids are present, they overexploit the edible phytoplankton, favouring development of non edible species. In the mixed mesocosms, the original reduction of calanoid abundance by cyclopoids reduces calanoid grazing pressure in phytoplankton and as such assures a more sustainable environment for calanoids. In the same experiments, calanoids strongly depressed cyclopoid populations in the mixed mesocosm as compared to the ones with only cyclopoids. Here also, mainly nauplii and cyclopoids were impacted. Soto and Hurlbert (1991) explain this effect of calanoids on cyclopoids by a reduction of

phytoplankton or rotifer food for cyclopoid nauplii or by calanoid predation on cyclopoid nauplii.

Using stable isotope signatures to study the lower trophic web in the York river, (Virginia, USA) Hoffman et al. (2008) have shown that *E. affinis*, the dominant cladoceran *Bosmina freyi* and cyclopoids have similar signatures, and can switch from autochthonous produced phytoplankton to allochthonous matter. In the Scheldt estuary however, *E. affinis* occurs in spring, while *B. freyi* and cyclopoids occur later in the year. Considering the above mentioned capacity for phytoplankton selection of *E. affinis*, it is possible that this dominant calanoid performs better in selecting high quality food than cyclopoids. Moreover, algae species composition during spring and summer in the Scheldt estuary seems to have changed between 1996 and nowadays (Van Burm, unpublished data). During spring 2007 and 2008, large diatoms (*Actinocyclus normanii*) dominated the community whereas during spring 1993, small species (*Cyclotella meneghiniana, Skeletonema costatum*) dominated (Muylaert and Sabbe, 1999). This shift in phytoplankton size composition could also influence the feeding efficiency and development of the various mesozooplankton species, but also of rotifers, which are numerically dominant in the entire estuary (Tackx et al., 2004) and form another potential high quality prey to the mesozooplankton.

The capacity of both freshwater and marine copepods to feed on rotifers is well known (Williamson, 1987; Conde-Porcuna and Declerck, 1998; Ciros-Perez et al., 2004). Lapesa et al (2004) report that the marine calanoid *Arctodiaptomus salinus* feeds more efficiently on rotifers that the co-occurring cyclopoid *Diacyclops bicuspidatus*. As for algae, it is possible that *E. affinis* feed better on rotifers than cyclopoids., Azémar et al. (2010) showed that during 2002, rotifers reached their peak abundance in spring and autumn (up to 2 500 000 ind m^{-3} in the freshwater zone), thus alternating with cladocerans and cyclopoids population peaks. This alternation could reflect a predation of cyclopoids and/or cladocerans on rotifers. Indeed, the two dominant cyclopoids species in the Scheldt estuary, *Cyclops vicinus vicinus* and *Acanthocyclops trajani*, are able to develop to the adult stage on a pure algal diet (Hansen and Santer, 1995; Hopp and Maier, 2005), but feed essentially on rotifers as adults (Brandl and Fernando, 1978; Brandl, 2005). Rotifers were shown to increase by two orders of magnitude (from 10 000 ind m^{-3} to 2 000 000 ind m^{-3}) in abundance in the freshwater reach between 1967–1969 and 2002 (Tackx et al. 2005). Rotifer abundance data for the Scheldt are not yet available for the complete period studied. While awaiting more recent abundance data, it is

difficult to evaluate their potential role in the recent evolution of the zooplankton community in the Scheldt estuary.

We have no knowledge of carnivorous feeding by *E. affinis*, but in view of numerous demonstrations of flexibility in feeding behaviour of calanoids (De Mott, 1988), this possibility cannot be excluded. Dodson (1975) has shown that in freshwater ponds, calanoids and cyclopoids can feed on each other's nauplii.

Also the top down control on the mesozooplankton could influence the dominance among taxa, for example by selective predation (De Mott, 1995; Lu and Xie, 2001). During the early Nineties, when *E. affinis* was still very abundant in the brackish water part of the Scheldt, the diet of the mysid *Neomysis integer* in this zone consisted practically solely of *E. affinis* (Fockedey and Mees, 1999). In addition, in the low salinity zone of the Scheldt (km 78.5 and downstream), *E. affinis* and various hyper benthic species form an important food resource for the diet of juveniles of dominant fish species such as sprat and herring (Maes et al., 2005a). While water quality improvement has probably resulted in higher abundance of zooplankton predators in the freshwater area (Verslycke et al., 2004), there is no obvious reason that these predators would feed selectively on cyclopoids rather than on other copepods.

Contrarily to cyclopoids, cladocerans showed no decrease since 2007. So they are apparently less hampered by biological interactions occurring in the freshwater zone than cyclopoids. While capable of selective feeding (Wong et al., 2006), cladocerans are generally considered as less selective and more filter feeding than copepods (De Mott, 1988). Because of this feeding mode, they perhaps enter little in competition with calanoids and cyclopoids. In the higher mentioned experiments of Sotto et al (1991), addition of cladocerans and of a mosquitofish to the mixed cyclopoid–calanoid tanks showed that the presence of cladocerans had no effect on cyclopoids and increased the abundance of some species of calanoids, while decreasing that of others.

The fact that changes in some other parameters, not measured in OMES, could have lead to the observed changes in the zooplankton community cannot be excluded: besides pollutant loads, also potential changes in the benthos could interact with the pelagic community. In a long term study over zooplankton community of a German lagoon (Feike et al., 2007) observed important and rapid changes in calanoids (*Eurytemora affinis* and *Acartia tonsa*) and

rotifers abondance just after the introduction of an invasive polychaete species. These authors suggest that this benthic species caused the depletion of rotifer resting eggs by grazing or bioturbation. Also in the upper Scheldt estuary, improving water quality could have affected the benthic community and indirectly changed living conditions for the plankton.

In conclusion, what is clear is that the improving water quality in the Scheldt estuary, and mainly in the freshwater stretch, is paralleled with major shifts in the spatial distribution of the zooplankton taxa: an increase in calanoid copepod and cladoceran abundance, while cyclopoids are strongly diminishing. These changes in the zooplankton community structure raise a number of fascinating questions on tolerance for environmental conditions and trophic competition between the phyla calanoids, cyclopoids and cladocera. Unfortunately, little information on water quality tolerance or biotic interactions among freshwater estuarine crustacean plankton taxa is available. Answering these questions will require mesocosm experiments using freshwater estuarine taxa, combine with *in situ* studies on trophic interactions (stable isotopes, fatty acids, observation of gut contents).

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Chapitre 2

Distribution spatio-temporelle du copépode *Eurytemora affinis* (Copepoda, Calanoida) dans un estuaire en restauration, l'Escaut (Belgique)

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Résumé de l'article

Introduction et objectifs

Comme indiqué dans le chapitre précédent, d'importants changements de la qualité de l'eau sont survenus dans l'estuaire de l'Escaut à partir des années 1990 (Van Damme et al., 2005; Cox et al., 2009). Cependant, une zone de déficit en oxygène résultant des rejets d'eaux usées en provenance de Bruxelles, persiste aux alentours de la ville d'Anvers. Parmi les taxons du zooplancton de l'estuaire de l'Escaut étudiés dans le cadre du programme OMES, un intérêt particulier est porté sur le copépode *Eurytemora affinis*. Ce copépode printanier domine une grande partie des zones d'eau saumâtre de l'hémisphère Nord (Lee, 1999). Néamoins, des observations dans la Gironde et dans l'Ems montrent que son optimum de salinité se trouve à des salinités aux alentours de 0-2, donc en amont des estuaires (Sautour and Castel, 1995). Les travaux d'Appeltans et al. (2003) suggèrent un effet seuil de la concentration en oxygène dissous sur la présence de ce copépode. Les auteurs émettent l'hypothèse que, dans l'Escaut, les conditions hypoxiques aux alentours d'Anvers agiraient comme une barrière écologique, empêchant les populations de ce copépode de migrer d'aval en amont. Depuis 1996, on observe l'espèce de façon ponctuelle dans le tronçon d'eau douce en amont de l'Escaut.

L'analyse d'un jeu de données issu de 11 années d'échantillonnages a été utilisée afin de répondre à deux objectifs. Le premier consiste à comprendre quels facteurs environnementaux influencent la distribution spatiale d'*E. affinis* dans l'estuaire de l'Escaut, en particulier sa présence sporadique en eau douce entre 1996 et 2007. Le second est de vérifier l'hypothèse d'Appeltans et al. (2003).

Principaux résultats et discussion

Une régression multiple de type PLS (Partial Least Square) a montré que les facteurs influençant la distribution d'*E. affinis* dans l'estuaire de l'Escaut éliés à la qualité de l'eau (concentrations en oxygène dissous, en azote Kjeldahl, en matière en suspension), ainsi qu'à la saisonnalité (le débit et la température). La température et la charge en matière organique

(azote Kjeldahl) semblent influencer la concentration en oxygène du milieu, chacune de façon indépendante. Plusieurs résultats pèsent en faveur de l'hypothèse de l'hypoxie localisée agissant comme barrière écologique à l'expansion du copépode vers l'amont de l'estuaire :

(1) L'influence des concentrations minimales en oxygène, observées en permanence à proximité d'Anvers (notées « O_2 min »), sur l'abondance d'*E. affinis* en amont de l'estuaire est statistiquement plus importante que celle de la concentration moyenne en oxygène dissous en amont.

(2) Le copépode est quasiment absent de l'amont de l'estuaire lorsque ces concentrations minimales en oxygène sont inférieures à 1,3 mg l^{-1}. Ce seuil est en accord avec ceux estimés dans les précédentes études (Stalder and Marcus, 1997; Appeltans et al., 2003).

(3) Globalement, lorsque les conditions d'oxygénation sont bonnes en amont de l'estuaire (> 4 mg l^{-1}), le copépode y est présent si les concentrations minimales en oxygène sont élevées (> 3 mg l^{-1}), alors qu'il est absent ou peu abondant si ces concentrations minimales sont faibles (< 3 mg l^{-1}). Dans le premier cas, son abondance en amont est corrélée à celle mesurée en aval de l'estuaire.

Les résultats suggèrent qu'il y a toujours une seule origine des populations printanières d'*E. affinis*, située en eau saumâtre. Dans de rares cas, ces populations semblent provenir d'une rivière tributaire, la Durme, dont la confluence avec l'Escaut est située dans la zone d'eau douce, à 115 km en amont de l'embouchure. L'hypothèse la plus probable est que des populations originaires d'eau saumâtre se seraient auparavant préétablies dans cette zone avant de recoloniser l'estuaire.

Un schéma conceptuel synthétisant les principaux résultats de cette étude est établi en fin de chapitre, pour tenter d'expliquer la distribution d'*E. affinis* dans l'estuaire de l'Escaut.

Spatial spring distribution of the copepod Eurytemora affinis (Copepoda, Calanoida) in a restoring estuary, the Scheldt (Belgium).

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Abstract

The spatial spring distribution of *Eurytemora affinis* (adults and C5) in the Scheldt estuary (Belgium) brackish and freshwater reaches was studied in between 1996 and 2007. The bulk of the *E. affinis* population being generally situated in the brackish water reach (salinity > 0.5), we specifically studied which environmental factors are responsible for its recent sporadic occurrence in the freshwater estuarine reach. Using PLS analysis, it is shown that its presence upstream is conditioned by a sufficient oxygen concentration (> 4 mg l^{-1}) that is associated with temperature. However, not only the environmental conditions in the upstream

zone are important: the frequent presence of an O_2 minimum zone in the mid estuary (O_2 min < 1.3 mg l^{-1}) seems to block the transition of the downstream *E. affinis* population in upstream direction. Occasionally, the bulk of the population is however situated upstream. During these periods, high *E. affinis* abundance is also observed in the Durme tributary. Our findings suggest the possibility to use *E. affinis* as an "indicator" species of water quality, but also lead us to stress the necessity to consider conditions over the entire estuary when studying restoration effects, not exclusively in the zone of interest.

Keywords: distribution, estuaries, *Eurytemora affinis*, oxygen, restoration, zooplankton

1. Introduction

The Scheldt is one of the few remaining extensive salt – brackish – freshwater tidal river systems in Europe. In particular its freshwater (< 0.5 salinity) tidal upstream reach is a rare habitat in Europe (Meire et al., 2005). Having a drainage basin which is heavily impacted by anthropogenic activity, the Scheldt was considered as one of the most polluted systems in Europe during the second half of the 20th century (Baeyens et al., 1998; Heip, 1988). The most polluted zone of the estuary, the downstream freshwater area, situated between Rupelmonde (km 103 from the mouth) and Antwerpen (km 90), was heavily impacted by several sources of disturbance and pollution, because of port infrastructure and industrial activities surrounding Antwerpen, but also by organic pollution coming from untreated wastewater of the Brussels agglomeration arriving in the Scheldt through the Rupel tributary (km 103). This area also coincides with the downstream part of the maximum turbidity zone (MTZ) of the estuary, and hence concentration of organic matter in the region is very high. In the Seventies, this situation led to – among other pollution characteristics – very low oxygen concentrations in this part of the estuary (Van Damme et al., 1995).

However, as a result of substantial emission reduction efforts throughout the watershed and the construction of water purification plants in the Brussels area, an improvement of the water quality is observed since the Nineties. Oxygen concentration improved considerably in the freshwater stretch from 1996 to 2006, associated with a decrease in N concentrations, mainly of NH_4 (Van Damme et al., 2005). As such, oxygen concentration can be considered as a

proxy for the global water quality in the Scheldt estuary. At present, some stretches of the estuary still present indications of poor water quality. In the zone between 82 and 110 km from the mouth, oxygen concentrations below 0.5 mg l^{-1} are still regularly encountered.

Because of its key position as a link between primary producers and higher trophic levels, the zooplankton community has been studied since 1996 within the context of a multi disciplinary follow-up of the evolution of this restoring estuary (OMES project) (Tackx et al., 2003; Tackx et al., 2004). As in most temperate estuaries, the Scheldt zooplankton spring community in the brackish – freshwater fringe is dominated by the calanoid copepod *Eurytemora affinis* (Castel et al., 1986; Soetaert et al., 1993; Peitsch et al., 2000; Devreker et al., 2008). This paper considers the spatial distribution of this species in the context of the improving water quality of the Scheldt estuary, from 1996 till 2007.

The calanoid euryhaline copepod species complex *Eurytemora affinis* (Poppe, 1880) is generally known to inhabit brackish systems such as estuaries and salt marches in the Northern hemisphere. It is also able to invade freshwater reservoirs and lakes (Lee, 1999). Lee (2003) demonstrates true installations of freshwater populations in various systems. The spatial distribution of *E. affinis* in the Scheldt estuary at first received attention in the frame of a comparative study of the spring zooplankton communities in European estuaries carried out during spring 1992. This study showed that, in the Ems (The Netherlands) and the Gironde (France) estuary, *E. affinis* had its peak abundance at salinity around 2 (Sautour et al., 1995). In the Scheldt however, peak abundance of *E. affinis* during the same period was observed further downstream, at salinities between 10 and 12. This difference in spatial distribution was explained by the very low water quality around the brackish water – freshwater fringe in the Scheldt estuary, which – as explained earlier – at that time characterised the highly polluted maximum turbidity zone around the harbour of Antwerpen (Soetaert et al., 1993; Sautour et al., 1995) (Fig. 1). Apparently, *E. affinis* in the Scheldt could not survive at its supposed salinity optimum of a few units (as deduced from the positioning of the bulk of its populations in the other estuaries). Following the improvement of water quality, Appeltans et al. (2003) demonstrated a tenfold increase in *E. affinis* abundance at Antwerpen between the periods 1989 – 1991 and 1996 – 1998. This shift in positioning was correlated to an increase in oxygen concentration around Antwerpen. Appeltans et al. (2003) suggest that below a threshold oxygen concentration between 0.6 and 1.6 mg l^{-1}, *E. affinis* could not remain in the Antwerp region and oxygen deficiency could act as a "barrier effect" to the upstream or

downstream expansion of the copepod. Since the observations of (Appeltans et al., 2003), we sporadically observe *E. affinis* upstream of Antwerpen, sometimes in considerable abundance (cf. results).

So, the first aim of the present paper is to understand which environmental factors influence the spatial distribution of *E. affinis* in the Scheldt, and particularly its presence and abundance upstream in the freshwater reach. Following the hypothesis of Appeltans et al. (2003), we also test if the persistent presence of a low oxygen concentration zone in the Scheldt acts as a barrier for upstream or downstream expansion of *E. affinis*.

2. Material and Methods

2.1. Study site

The Scheldt estuary has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands (Fig. 1). Contrarily to most of the other temperate estuaries, the Scheldt estuary is characterised by vertically well mixed water flows (Baeyens et al., 1998), inducing most of the time no salinity or current stratification (Heip, 1988). Within the framework of the OMES project, samples are taken monthly at 16 stations (Fig. 1) since 1996 until present, with an interruption between 2000 and 2002. All stations are situated in the brackish and freshwater part of the estuary (Fig. 3). This paper considers only the months of February – May of this dataset, because of the occurrence of *E. affinis* in the Scheldt mainly in this period of the year.

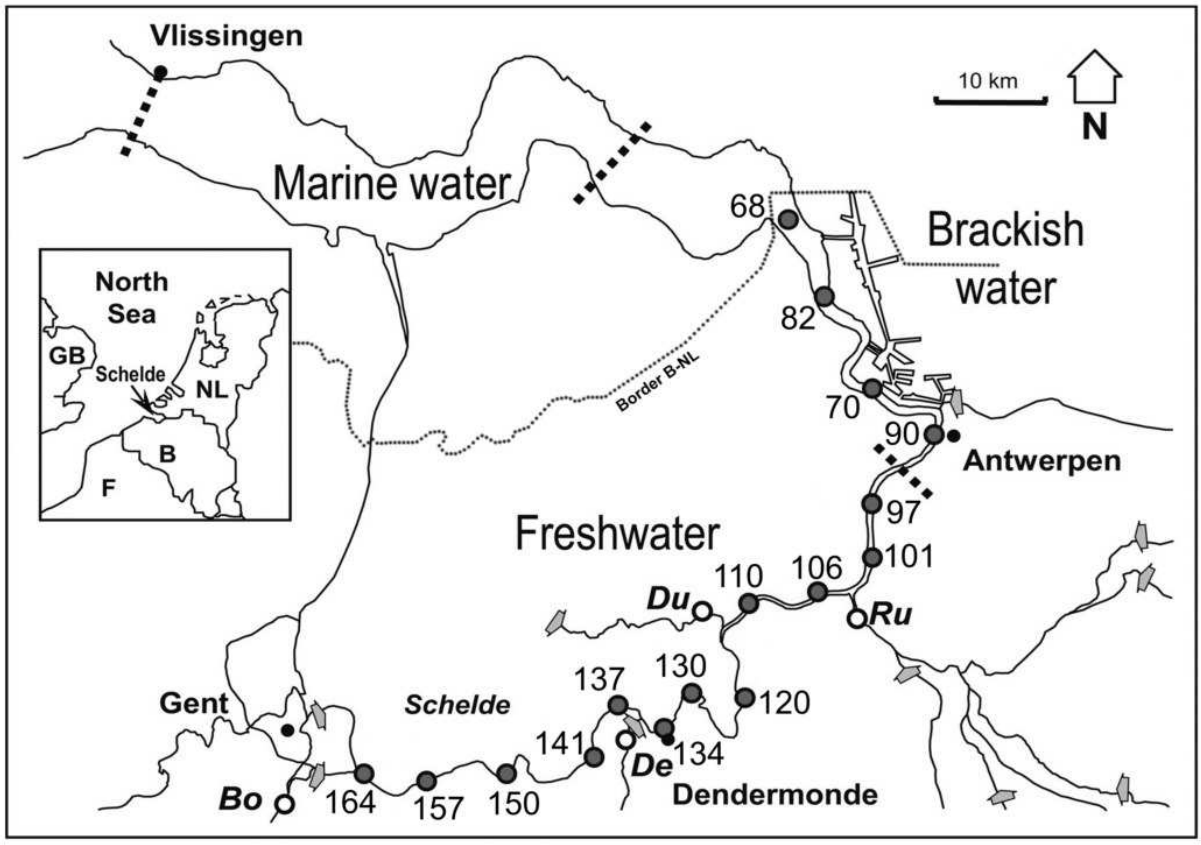

Fig. 1. Map of the Scheldt estuary with OMES sampling stations, designated by their distance in km upstream from Vlissingen. Arrows indicate the end of tidal influence on the tributaries (Bo: Bovenschelde, De: Dender, Du: Durme, Ru: Rupel).

2.2. Sampling and analysis

2643 water samples where collected in the middle of the river throughout the entire studied period, by means of bucket hauls from the ship. At each station, a set of environmental variables were measured. Temperature (T) and dissolved oxygen (O_2) were measured *in situ* with a 'WTW OXI 91' oxygen meter, salinity was measured with a 'WTW LF 91' conductivity-meter using the Practical Salinity Scale, Kjeldahl nitrogen (Kj-N) and total phosphorus (P) were measured by colorimetry using a SKALAR SA 5100 segmented flow analyser. Dissolved organic carbon samples where filtered on Whatman GF/C glass fibre filters, then treated with H_2SO_4 acidification and flushing with nitrogen, then set free by UV-irradiation. Suspended particular matter (SPM) samples were filtered on pre-combusted Whatman GF/C filters. From 1995 to 2001, Chlorophyll *a* (Chl *a*) samples were filtered on pre-combusted 45µm Sartorius filters, extracted in 90% acetone and analysed using reversed

phase HPLC. The reader is referred to Van Damme et al. (1997; 2005), for more details on the methodologies used. From 2002 to present, Chlorophyll *a* samples were filtered over a 25-mm diameter Whatman GF/F glass fibre filter. Pigments were then extracted and analysed by HPLC according to the method of Wright et al. (1997). More details on the methodologies used are presented in Lionard et al. (2008).

Since 1995, at each station, a volume of 50 litter of surface water was collected by means of bucket hauls and filtered through a 50 µm net. The collected zooplankton was anaesthetised with carbohydrated water and subsequently fixed in a formaldehyde solution (4 % final concentration). Samples were analysed by binocular microscope (90 x magnification) for zooplankton species composition and abundance. For some years (1996, 1997, 1998, 2002) data on the abundance of *E. affinis* are available for all 16 stations. For the other years, zooplankton sampling was limited to 6 stations (km 68, 90, 110, 134, 150 and 164) and hence *E. affinis* abundance data are only available for these stations. In the tributaries, environmental variables and zooplankton samples were taken from the shore, 10 km upstream of the mouth, within 24 hours of the estuarine sampling. The same methods were used as in the estuary.

The Administration of Waterways and Sea (AWZ) provides daily discharge measures of the Bovenschelde, the Dender and the Rupel. The upstream discharge data at these stations were used to estimate downstream discharge, taking into account all the physical features of the Scheldt estuary. Daily average discharges for km 68 are used in our dataset. This station is located at the end of the study area and integrates discharge values of upstream stations.

2.3. DATA analysis

A strong linkage exists between the abundance distribution of *E. affinis* and the brackish water – freshwater gradient observed for almost all environmental variables in the estuary (Soetaert et al., 1993; Tackx et al., 2003; Van Damme et al., 2005). Thus, the relatively small changes in the upstream abundance, compared to the abundance of the bulk of the population, cannot be studied using general linear models (GLM) or principal component analysis (PCA) on data over the entire zone studied. Moreover, there are some missing values in the dataset. We therefore choose to carry out partial least square (PLS) regressions to identify the

environmental variables that best explain the upstream distribution of *E. affinis*, based on predictors importance and regressions coefficients (Höskuldsson, 1988). Indeed, this method provides a mean to solve the problem of colinearity between tested variables, thanks to the variables importance index. It shows which predictors are significantly more influent on the dependant variables than others. The R^2Y index is the proportion of the total variability of the dependant variables explained by the regression. A PLS regression is significant when its Q^2 index is equal or superior to 0.05. All variables, except temperature, were log-transformed to improve normality.

Simple regressions, equality of variances, k-mean clustering, parametrical and non parametrical tests were performed with Statistica 6 (version 6.0; Statsoft Inc., Tulsa, USA). SIMCA-P (version 9.0; Umetrics AB, Umeå, Sweden) was used to perform PLS regression. All graphs and statistical tests, including PLS analysis, were based on the same dataset, including 38 month of samplings.

Spring abundance data of adult and C5 *E. affinis* from 1996 till 2007 were used in this study. In the following, the term *"E. affinis"* refers *to Eurytemora affinis* adults and C5. We considered two ways to characterise the distribution of *E. affinis* in the estuary. Firstly, we quantified it simply by its upstream (cf. below) abundance. Secondly, we characterised its relative abundance in the upstream part using an « Upstream/Downstream Homogeneity index » for every sampling date, using the following formula:

$$UDH = 1 - \frac{|D - U|}{D + U}$$

D: Downstream mean *E. affinis* abundance
U: Upstream mean *E. affinis* abundance

In order to test a potential barrier effect of the low oxygen zone, we considered O_2 min, the lowest dissolved oxygen concentration measured in the estuary, as a spatial fringe between upstream and downstream abundances. The distance to the mouth of O_2 min is strongly correlated to the distance to the mouth of 0.5 salinity (Spearman rank test $p = 0.000005$), so the station corresponding to the O_2 minimum can effectively be considered as a spatial fringe between upstream and downstream reaches. Mean *E. affinis* abundance downstream to the O_2

min station was calculated considering the stations which distance to mouth is inferior to the distance where the O_2 min was measured. Upstream mean abundance was calculated considering the stations which distance to mouth is superior or equal to it.

This UDH varies from 0 (total heterogeneity) to 1 (total homogeneity), whether maximal abundance is located upstream or downstream.

3. Results

3.1 Spring distribution of salinity, dissolved oxygen and E. affinis abundances in the studied area

As shown in Fig. 2, the mean spring oxygen concentration as measured during 1996-2007 decreases from km 68 in upstream direction to reach a minimum in between km 82 and 110 and increases further upstream. In the low concentration zone, values can be as low as 0.1 mg l^{-1} at some stations. So, oxygen concentrations are still generally low in the middle zone covering some 40 km of the Scheldt estuary.

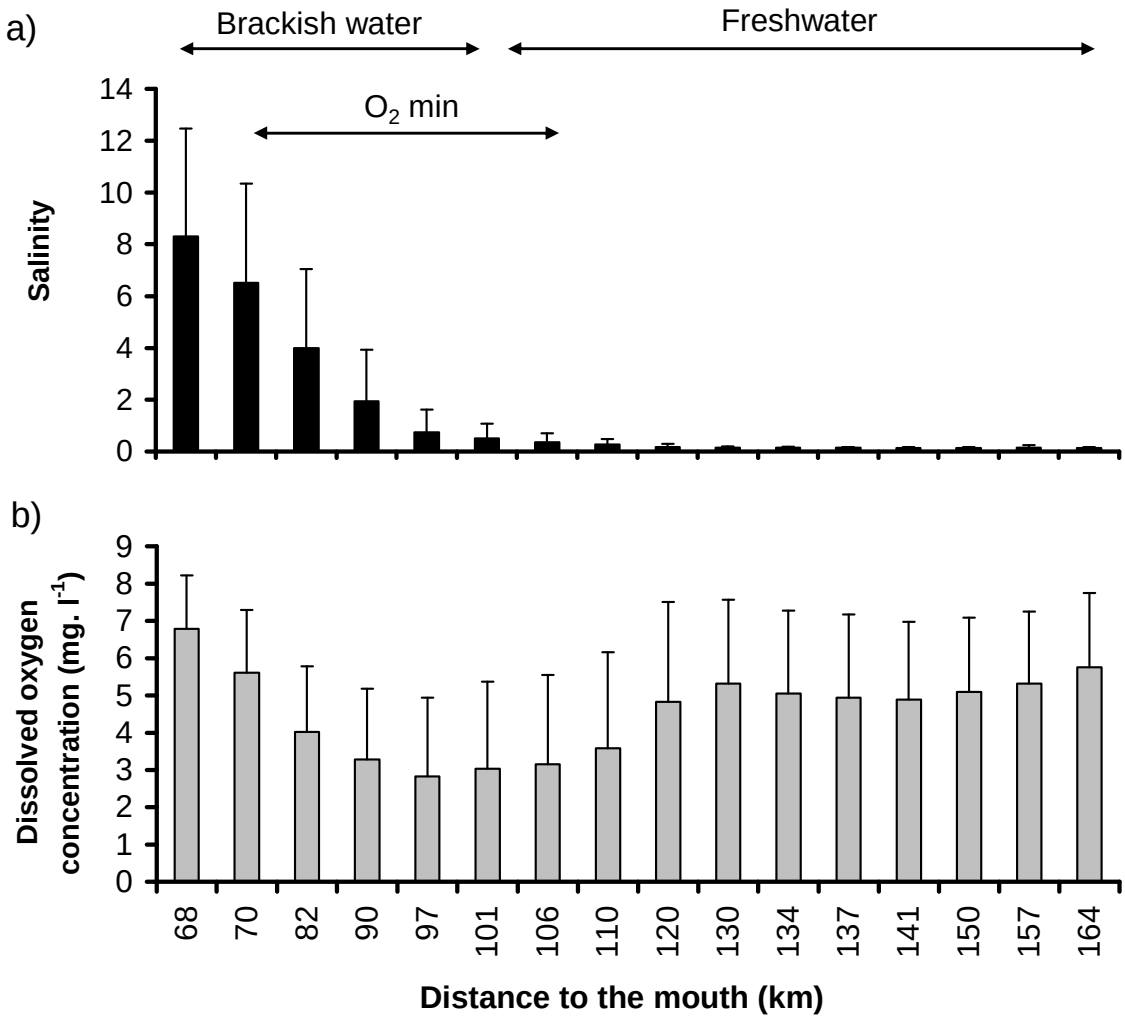

Fig. 2. Spring spatial distribution of mean salinity (a) and mean oxygen concentration (b), with their standard deviation, in the Scheldt estuary over the period 1996-2007. The locations of the minimal oxygen concentrations observed for all samplings are situated in the "O_2 min" range.

The spatial distribution of *Eurytemora affinis* as observed in between 1996 and 2007 practically always peaks in the zone between km 70 and Antwerpen (km 90), at salinities between 4 and 8 (Fig. 3a). At the same time, *E. affinis* adults are also occasionally observed upstream of Antwerpen in the freshwater reach of the Scheldt, even as far upstream as Melle (km 164) (Fig. 1, Fig. 3a, Fig. 4). The *E. affinis* population in the Scheldt now seems to have its peak abundance at similar salinity reaches as earlier observed in the Ems and the Gironde (Sautour et al., 1995). It even penetrates in the freshwater (< 0.5 salinity) reach. Its presence in the upstream part of the estuary seems however very variable.

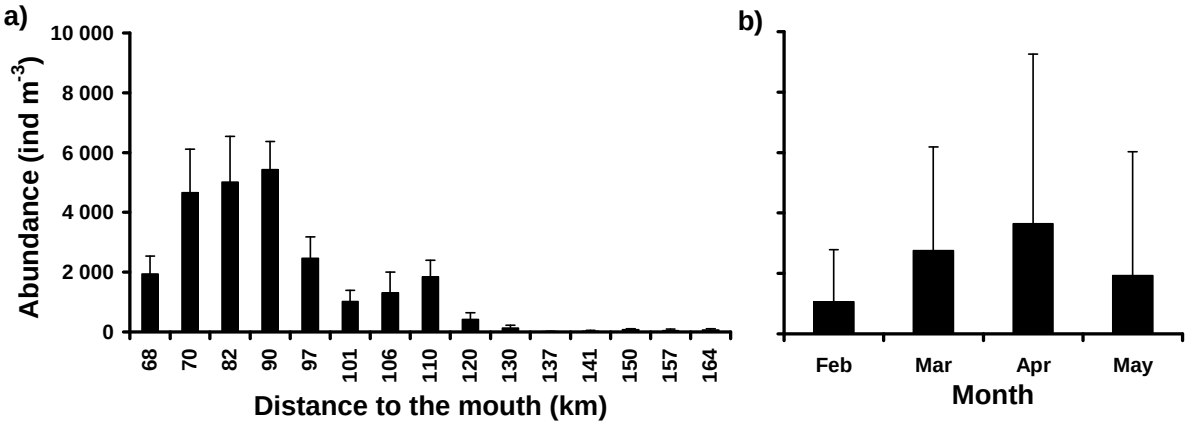

Fig. 3. Mean spring (February - May) abundance of *Eurytemora affinis* adults and C5 measured in the Scheldt estuary from 1996 to 2007, sorted by stations (a) or by months (b). Black lines show standard deviation.

An example of this variability is given in Fig. 4. During some months, such as March 1998, *E. affinis* is present and abundant as far upstream as km 134 (Fig. 4a) whereas during other months, such as April 1997, *E. affinis* remains downstream km 106 and is quasi absent upstream from this station (Fig. 4b). Inversely, it sometimes happens that the bulk of the population is located in the freshwater reaches, upstream km 103, such as for example during February and March 2004 (Fig. 4c).

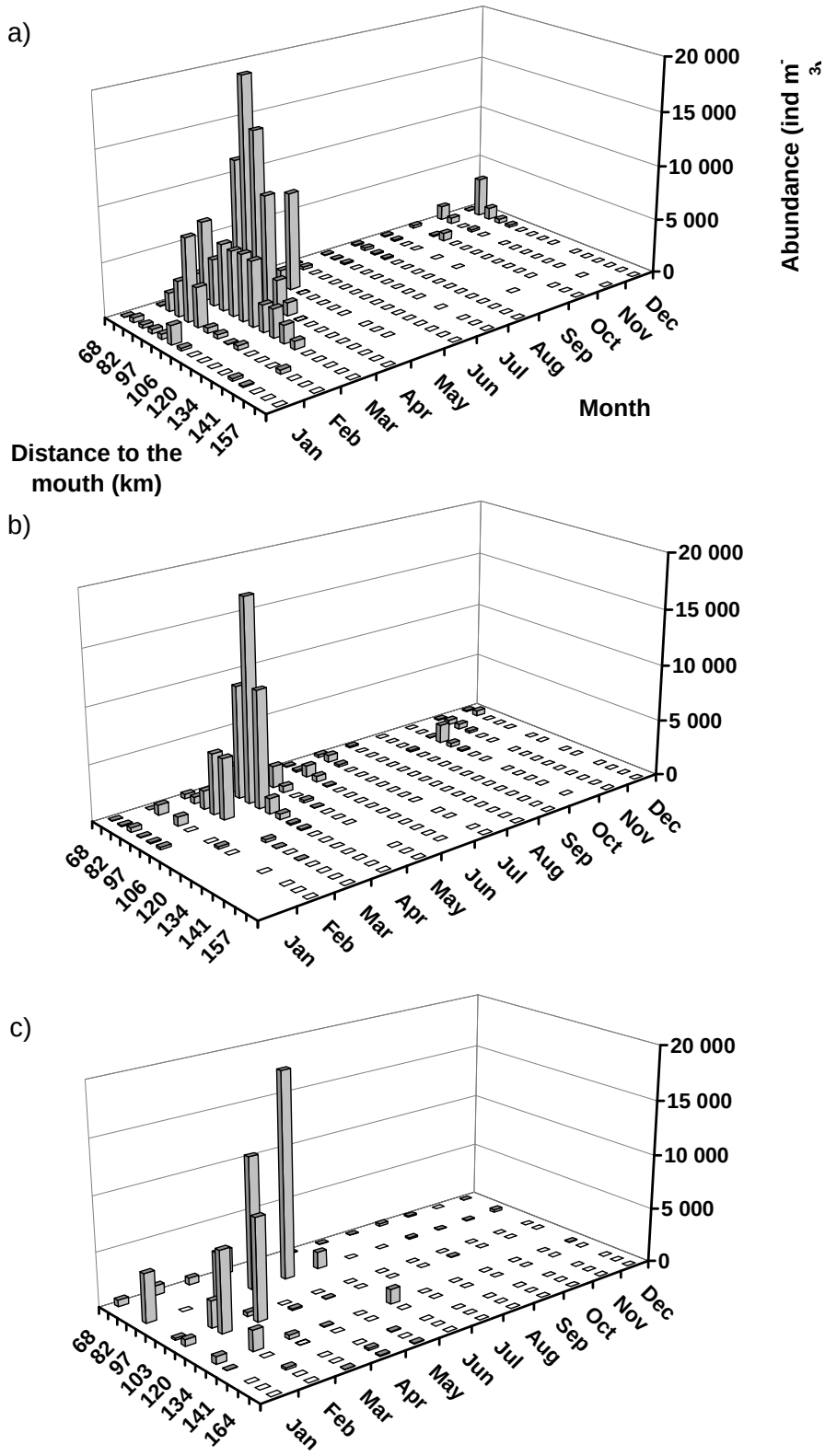

Fig 4. Examples of various spatial distributions of *Eurytemora affinis* adults in the Scheldt estuary, during 1998 (a), 1997 (b) and 2004 (c). White squares mean null values.

3.2. Influence of environmental factors on the distribution of Eurytemora affinis

A PLS analysis was carried out to determine which environmental variables influence the upstream mean abundance of *E. affinis* and its UDH. In addition, we tested relations between the significant predictors to surface any possible correlations between these. The results are shown in table 1.

Table 1

Partial least squares regression results, using the station where the lowest dissolved oxygen concentration was measured as upstream/downstream fringe. Significant results are figured in bold and marked with an asterisk. O_2 min: lowest dissolved oxygen concentration measured in the estuary, O_2: upstream mean dissolved oxygen concentration, Kj-N: upstream Kj-N mean concentration, tot P: upstream tot P mean concentration, CHL *a*: upstream mean Chl *a* concentration, SPM: upstream mean SPM concentration, T: upstream mean temperature, Q: Mean run off at km 68 (from day -7 to sampling day). See text for explanation.

Dependant variables		***E. affinis* Upstr.**	**UDH**	**O_2**	**Kj-N**
Predictors importances	**O_2 min**	**1.37 ***	**1.41 ***	-	1.12
	O_2	**1.49 ***	**1.37 ***	-	1.06
	Kj-N	**0.97 ***	0.48	**1.08 ***	-
	tot P	0.94	0.42	**1.36 ***	**2.09 ***
	CHL *a*	0.08	0.01	0.12	0.37
	SPM	1.28	**1.30 ***	0.83	0.05
	T	0.62	**1.38 ***	**1.38 ***	0.27
	Q	0.14	0.37	0.64	0.20
Predictors coefficients	**O_2 min**	**+ 0.18 ***	**+ 0.18 ***	-	- 0.19
	O_2	**+ 0.19 ***	**+ 0.18 ***	-	- 0.18
	Kj-N	**- 0.13 ***	- 0.06	**- 0.23 ***	**- 0.36 ***
	tot P	- 0.12	- 0.06	**- 0.30 ***	
	CHL *a*	- 0.01	0	- 0.03	- 0.06
	SPM	+ 0.17	**+ 0.17 ***	+ 0.18	- 0.01
	T	- 0.08	**- 0.18 ***	**- 0.30 ***	- 0.05
	Q	- 0.02	- 0.05	+ 0.14	- 0.03
n		38	38	38	38
R^2Y		0.38	0.39	0.41	0.47
Q^2		0.33	0.32	0.24	0.33

R^2Y indexes are rather good in all analyses. O_2 min and O_2 are the most important and significant factors explaining upstream abundance and UDH, with a positive influence. SPM is significant in explaining UDH and upstream mean abundances, with a positive influence. Kj-N negatively influences upstream mean abundance. T and Q respectively negatively and positively influence UDH. Contrarily to upstream mean abundance, UDH is better explained by O_2 min than by O_2. O_2 is negatively influenced by Kj-N, tot P and T, but is strongly and positively influenced by Q (Table 1). Kj-N is strongly related to tot P but not to T, which therefore has an impact on O_2 which is independent from Kj-N. Given that the Kj-N importance coefficient is superior to that for the tot P in explaining upstream abundance of *E. affinis*, we can consider Kj-N concentration to represent both the Kj-N and tot P effect on *E. affinis* upstream – downstream distribution. O_2 or O_2 min importance are higher than that of Kj-N, tot P and T in explaining UDH and upstream abundance. Therefore, oxygen concentration has its own independent effect on the distribution of *E. affinis*.

To summarise, O_2 min, O_2, Kj-N, SPM, T and Q seem to be the most likely factors governing the upstream-downstream distribution of *E. affinis* in the Scheldt estuary. To visualise the combined effect of upstream O_2 concentration and the potential O_2 min barrier, we plotted UDH values in a O_2 min and O_2 biplot (Fig. 5). We divided UDH values in 2 groups with a k-mean analysis to separate higher and lower values.

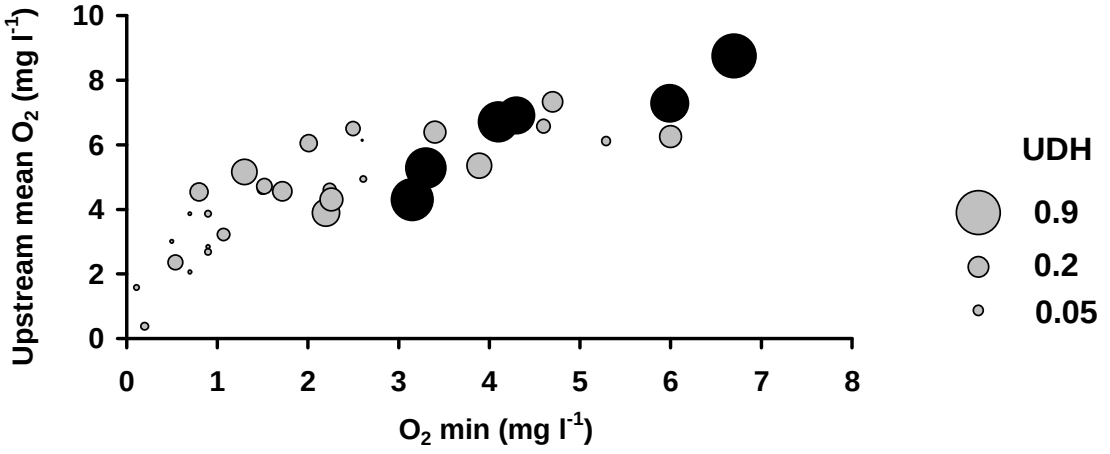

Fig. 5. Relation between UDH, O_2 min and O_2. Two UDH groups (high values in black, lower values in grey) were divided by a k-mean analysis.

A set of very high UDH values (Fig. 5) are observed when O_2 min is superior to 3 mg l^{-1}. If we consider upstream O_2, we find these high UDH values above 4 mg l^{-1}. Nevertheless, at these oxygen concentrations, several low homogeneity values are observed as well, mainly (8 cases out of 14) in the area corresponding to O_2 min < 3 mg l^{-1}. In the zone corresponding to upstream O_2 < 4 mg l^{-1} and O_2 min <1.3 mg l^{-1}, only very low UDH values are observed. This figure also illustrates that there is a very clear relation ($p < 10^{-13}$) between upstream mean O_2 and O_2 min.

In addition, considering the influence of environmental variables, we also considered the possibility that the abundance of the *E. affinis* population itself influences its spatial distribution. In other words, the population spreads out (in upstream or downstream direction, depending on where the population maximum abundance is situated), when its abundance becomes too high. Considering the previous results, we have therefore tested the relation between the maximal *E. affinis* abundance and the mean upstream abundances under several conditions (Fig. 6).

When O_2 min is superior to 3 mg l^{-1}, a clear relation exists between maximal *E. affinis* abundance observed and mean upstream or downstream abundance (Fig. 6 a, c), depending on whether maximal abundance is found downstream or upstream. At lower O_2 min values, no correlation exists (Fig. 6 b, d).

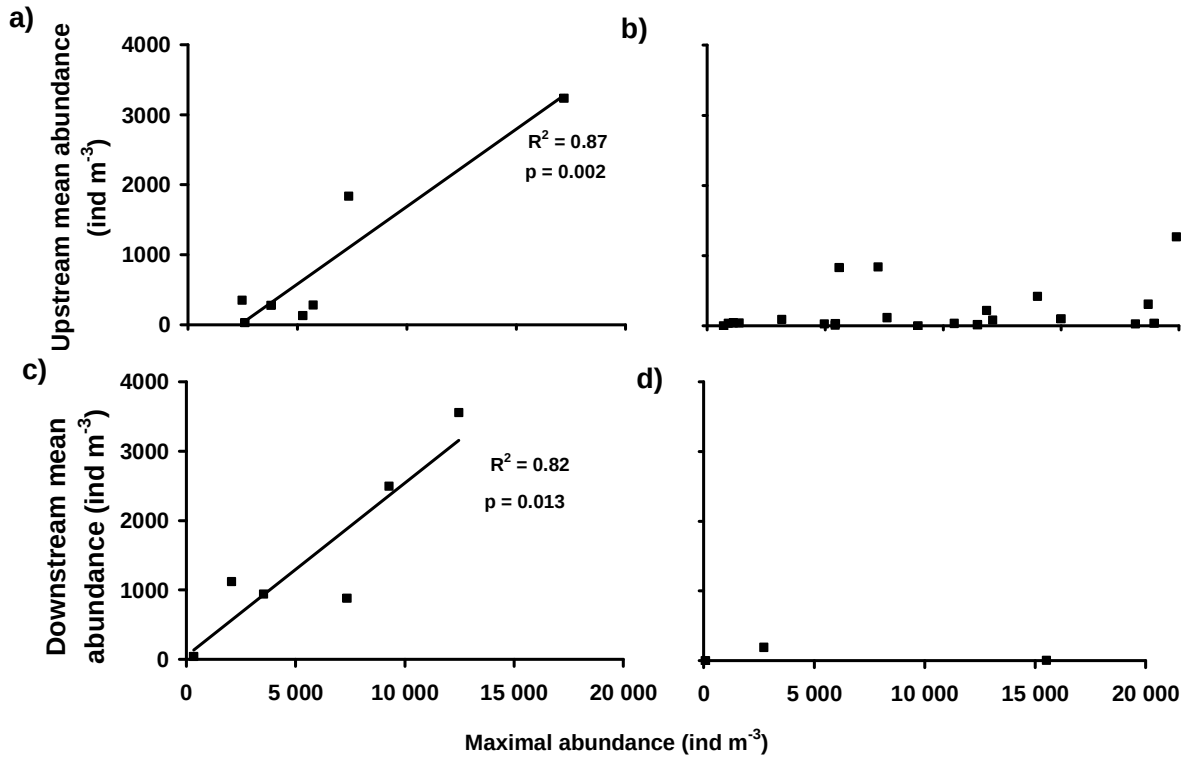

Fig. 6. Relation between maximal *E. affinis* abundance and upstream or downstream mean abundance of the copepod *Eurytemora affinis* in the study area, when O_2 min > 3 mg l^{-1} (a, c) and when O_2 min < 3 mg l^{-1} (b, d). O_2 min was used as upstream/downstream fringe value. When maximal abundances are located downstream the O_2 min location, the mean upstream abundance is considered (a, b). When maximum abundance is situated upstream the O_2 min location, the mean downstream abundance is considered (c, d).

4. Discussion

This study aims to get a better understanding of the factors which control the spatial distribution and more specifically the recent expansion of *Eurytemora affinis* upstream the Scheldt estuary.

Tidal phase at sampling cannot be controlled for logistic reasons, but a recent study (Toumi, unpublished data) has shown that, in the Scheldt estuary, *E. affinis* surface abundance is representative of the entire water column abundance when considering mean values over 15 sampling occasions.

4.1. Influence of environmental factors on the distribution of Eurytemora affinis

A possible explanation for the sporadic occurrence of *E. affinis* upstream could be the importance of runoff. The upstream migration of *E. affinis* could be possible only during low runoff periods, and hampered by high runoff. However, runoff shows a significant but positive influence on the UDH values. So this hypothesis can be ruled out. This is in contradiction to major changes in the positioning of the *E. hirundoides* (synonym of *E. affinis*; Busch et al., 1992) population at high and low runoff periods, observed in the Gironde estuary (Castel et al., 1986). However, these authors considered the March to October period, while our study considers only the spring bloom of *E. affinis*, during which runoff variations are smaller (200-600 m^3 s^{-1}) that considered in the Gironde study over an entire year (200-2000 m^3 s^{-1}; Gasparini, 1997).

Feeding conditions such as phytoplankton abundance could also influence the spatial distribution of *E. affinis*. Chlorophyll *a* concentrations in the study area are higher upstream than downstream and increasing with time over the 1996-2007 period (unpublished results). Chl *a* concentration did not appear as significantly influencing *E. affinis* upstream abundance or UDH values in the PLS analysis. This can be explained by the fact that, already during 1997, grazing experiment using natural Scheldt water showed that the ratio phytoplankton/suspended matter was sufficiently high for *E. affinis* to select phytoplankton at maximum rate (Gasparini et al., 1999; Tackx et al., 2003). So the subsequent increase in phytoplankton concentration probably did not improve feeding conditions for *E. affinis*. However, the fact that SPM has a significant and positive effect on UDH and upstream mean abundance can be explained by the fact that SPM concentration is higher in the freshwater region than in the downstream, brackish water zone. As, most of the time, the bulk of the *E. affinis* population is situated downstream, high UDH values correspond to a spreading upstream, towards these higher SPM concentrations. As explained above an effect of SPM concentration on the feeding conditions for *E. affinis* is unlikely (Gasparini et al., 1999; Tackx et al., 2003).

The PLS regression performed demonstrated that, O_2 min, O_2, Kj-N, SPM, T and Q significantly influenced the upstream-downstream distribution of *E. affinis* in the Scheldt estuary. Moreover, an independent impact of the oxygen concentration was distinguished from the seasonal influence. O_2 min, O_2 and Kj-N can be considered as representing "water

quality". The fact that O_2 is more influent than O_2 min in explaining upstream abundances is quite logic considering that O_2 and upstream abundance are both based on an upstream mean. The fact that O_2 min is slightly more influent than O_2 on UDH suggests an independent effect of O_2 min and so a potential barrier effect on *E. affinis* expansion. So our results confirm the earlier suggestion by Appeltans et al. (2003) that oxygen concentration has an important impact on the distribution of *E. affinis* in the Scheldt estuary. According to our results, there is always a strong heterogeneity between upstream and downstream abundance when upstream mean oxygen concentration is inferior to 4 mg l^{-1} or when the O_2 min threshold value is less than 1.3 mg l^{-1} (Fig. 5). The oxygen threshold values found in this study are in the range of the 0.6 - 1.6 mg l^{-1} range reported by Appeltans (2003). In semi-enclosed coastal waters (Turkey Point, Florida, USA), Stalder et al. (1997) also reported shifts in populations of three calanoids species (*Labidocera aestiva*, *Acartia tonsa* and *Centropages hamatus*) below 2 mg l^{-1} and experimentally observed declines in survival at oxygen concentrations below 0.9 mg l^{-1}. We demonstrated that a good relationship exists between the O_2 min and the upstream mean oxygen concentration, indicating that the water quality of these two zones is linked (Fig. 5) and that O_2 min can be used as an indicator of water quality upstream of the O_2 minimum. O_2 min can as such represent conditions for *E. affinis* presence upstream. Some relative high values of upstream oxygen concentrations (> 4 mg l^{-1}) are associated with low UDH values (Fig. 5), representing situations when *E. affinis* is scarce or absent upstream, even when O_2 concentration seems to be sufficiently high in the area. Verification showed that these values correspond to situations where the bulk of the abundance is located downstream and O_2 min values are below 3 mg l^{-1} (Fig. 5). So these are situations in which upstream oxygen conditions are permissive to the expansion of *E. affinis*, but the O_2 min value is not. So occasionally, *E. affinis* seems to be effectively blocked by a low oxygen barrier in its expansion upstream. This also explains the superior importance of O_2 min to the upstream mean oxygen concentration in influencing UDH in PLS regression (Table 1).

As to the influence of Kj-N on the *E. affinis* distribution, it should be reminded that we have considered Kjeldahl nitrogen as representing the associated phosphorous concentrations as well. As shown from table 1, high Kj-N concentrations generally co-occur with low O_2 min, and hence low upstream O_2 concentrations. High concentrations of nitrogen and phosphorous manifest a high eutrophication level, which induces a strong consumption of oxygen in the water column. Indeed, in the Scheldt estuary, more than the third of the oxygen consumption is due to nitrification, inducing a strong impact on the N-load (Ouboter et al., 1998).

The respectively negative and positive effect of T and Q on UDH is obviously explained by seasonality. UDH values are indeed higher in early spring (February and March), when temperatures are colder and discharge values stronger, than later in the study period (not shown). Moreover, oxygen concentration, which itself greatly explains UDH, is also linked to these two factors.

The absence of relation between T and *E. affinis* upstream abundances is not surprising in our analysis, because this relationship is probabely not linear. Indeed, as in the Seine estuary (Mouny et al., 2002), maximal abundances are found when temperatures varies between 10 and 15° C in the Scheldt estuary, during April, and not when they are warmer. Devreker et al. (2004; 2009) also found optimal temperature for naupliar survival and hatching time around 15°C.

It should be born in mind that oxygen concentration is related to Kj-N but that Kj-N is not related to T or Q. As such, low oxygen concentrations upstream or in the O_2 min area can be explained by a blended effect of seasonality and water quality.
In conclusion, the UDH of the distribution of *E. affinis* seems to be first limited by low oxygen concentration, itself limited independently by a high eutrophication level and/or by the natural seasonal influence.

Water quality seems adequate to explain most of the *E. affinis* upstream expansions. However, the potential influence of biotic interactions, which are not taken into account in our study, could also influence the abundance and the distribution of *E. affinis*. Predation pressure, for example, could block the upstream expansion of the copepod, or reduce its abundance. It has been shown for the spring 1993 period that, in the brackish part of the Scheldt, the diet of the mysid *Neomysis integer* consisted practically solely of *E. affinis* (Fockedey et al., 1999). As suggested by Verslycke et al. (2004), it is possible that *N. integer* populations shifted upstream the estuary since the improvement of the water quality in the maximum turbidity zone, but the distribution of this species in the upstream part of the Scheldt estuary since this period has not been studied yet. In addition, in the low salinity zone of the Scheldt, *E. affinis* and various hyperbenthic species form an important food resource for the diet of juveniles of dominant fish species such as sprat and herring (Maes et al., 2005). In our study, the upstream expansion of *E. affinis* decreased in late spring (during higher

temperatures and lower discharge values). This period also corresponds to the bloom of cyclopids in the freshwater part of the estuary (Tackx et al., 2004). Thus, competition could also hamper *E. affinis* upstream expansion in late spring.

4.2. Influence of the bulk of the population size on the distribution of Eurytemora affinis

The relation between observed maximal abundance and mean upstream or downstream abundance (Fig. 5) is significant when O_2 min is superior to 3 mg l^{-1}, and totally absent when O_2 min is inferior to 3 mg l^{-1}. This result suggests that there is indeed a spreading out of the *E. affinis* population with increasing size of its population, but this spreading out is hampered when oxygen concentrations are low. This enforces the concept of the oxygen as an ecological barrier for zooplankton. When this barrier is absent (O_2 min > 3 mg l^{-1}), expansion of populations towards upstream or downstream is possible, otherwise it becomes limited.

Further verification of the data revealed that very high UDH values (> 0.7) are only found when upstream mean abundances are slightly superior to downstream ones, and/or when maximal abundances are located upstream to the O_2 min value. When these maximal abundances were located downstream, UDH values remain lower. This suggests that the expansion of the copepod is easier in downstream than in upstream direction. Nevertheless, it is also possible that the upstream population receives individuals from another source than the downstream population and/or develops independently from the downstream population, at least during conditions which result in high UDH values.

4.3. Origin of the populations

The most evident sources of the upstream population – apart from the downstream one – are potential populations harboured in the tributaries Dender, Durme and Rupel (Fig. 1). Only the Durme regularly shows a considerable abundance of *E. affinis* (up to 3800 ind m^{-3}). In order to test if this tributary could play a reservoir role, we considered all cases when bulk of the *E. affinis* abundance is located upstream the Sheldt estuary, and compared it to *E. affinis*

abundance in the Durme. In these specific cases, there is a significant correlation between Durme *E. affinis* abundance and mean upstream abundances (Fig. 7).

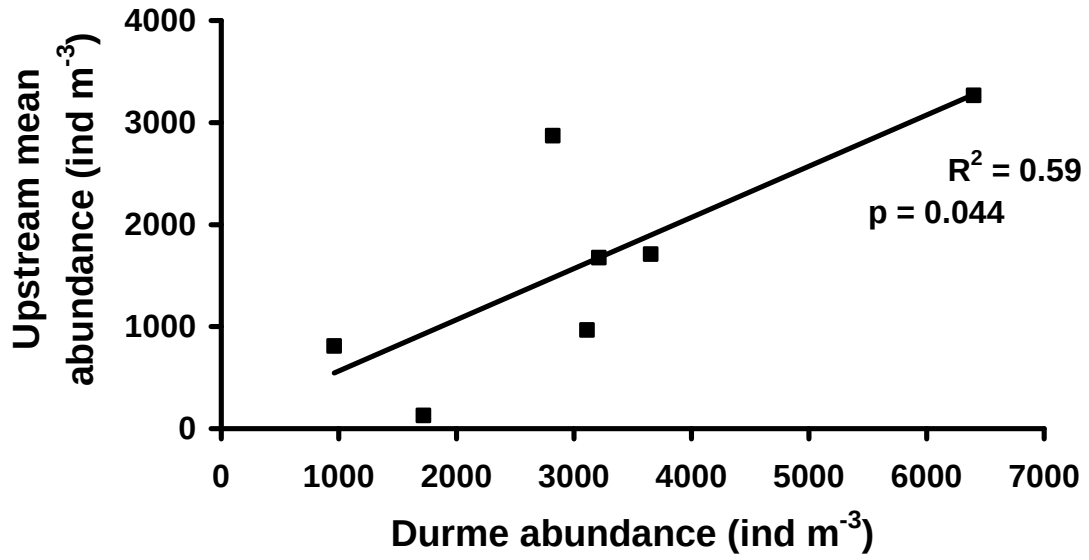

Fig. 7. Comparison between *Eurytemora affinis* abundance in the Durme tributary and its mean abundance in the upstream part of the Scheldt estuary, under conditions when the bulk of the population is located in the upstream reach.

The fact that, in these cases, *E. affinis* abundance in the Durme is higher than in the upstream Scheldt (Fig. 7), suggests that the Durme population is either fed by an inland source or that it has previously been imported from the downstream Scheldt population and developed well in this tidal inlet, which has environmental conditions similar to the upstream Scheldt area (unpublished data). The first possibility seems unlikely, as the Durme drainage is reduced to a few local polders.

Considering the cases when the bulk of the population is located downstream (Fig. 2), which represents the majority of our samplings, it seems logic to suppose this downstream population is the source of the upstream individuals. We support this statement by several arguments. When there is a rather good upstream/downstream homogeneity (UDH > 0.2), the abundance of *E. affinis* at the station where the O_2 min was observed is never null and always varies between 1000 and 17 000 ind.m^{-3}. So there seems to be a persistent linkage between

upstream and downstream reaches under permissive water quality conditions, which is also represented by the correlations between maximum abundance and upstream or downstream mean abundance (Fig. 6 a, c). We checked *E. affinis* abundance in the Durme in all cases when bulk of the abundance is located downstream the Scheldt estuary. In these cases, Durme abundances are never superior to 700 ind m^{-3}. So it seems unlikely that, when the bulk of the population is located downstream, the origin of upstream individuals lies in the Durme.

As we saw previously, when upstream oxygen conditions are permissive and O_2 min values are not, a real ecological barrier exists. In our database, this situation corresponds to 11 cases. If there were two independent populations, we would sometimes record considerable abundances simultaneously in the upstream and downstream reaches under permissive conditions. There would indeed be no reason for the upstream population to be hampered in its development because of a downstream low O_2 min value. Actually, this situation never happens. The bulk of the population is always situated upstream or downstream and associated with low UDH values (< 0.2). Moreover, *E. affinis* abundance in the Dender tributary, which is closed to estuarine input by locks, always remains below 80 ind m^{-3}. There is no obvious reason why local populations should develop in the Durme and not in the Dender. In the Rupel tributary, which does receive freshwater input from the Zenne, Dijle and the Nete rivers, *E. affinis* abundance is generally low (< 200 ind m^{-3}) except at occasions when abundance in the upstream Scheldt is also high.

Another argument in favour of a “one population” hypothesis is the fact that Lee (1999), in a study on *E. affinis* of North America, Europe and Asia, shows that genetic variance among *E. affinis* within drainages is only 5 %. We have little historic information on the presence of *E. affinis* in the Scheldt drainage. De Pauw (1973), reports the *E. affinis* population to be present from the mouth to Schijn (km 78) and to consistently peak around Zandvliet (km 65), as was the case during 1989-1991 (Soetaert et al., 1993; Sautour et al., 1995). It should be mentioned that during the period studied by De Pauw (1967-1969), the Scheldt was already heavily polluted (Van Damme et al., 1995; Heip, 1988), which explains the absence of *E. affinis* upstream of km 78 (Schijn). Verraes (1968) at the time also reports a paucity of copepods in the Scheldt estuary, and its absence from the Rupel during the Nineteen Sixties. During our study period, the mean upstream abundance of *E. affinis* shows no pattern with time by simple regression ($p = 0.38$). This also suggest that *E. affinis* is not stably installed in the upstream area. So while at present, we cannot definitively exclude the existence of an upstream

population which would be totally independent of the downstream one, this seems very unlikely.

We conclude that the recent upstream occurrence of *E. affinis* in the Scheldt is clearly linked to water quality, as represented by oxygen, Kjeldahl nitrogen (and associated total phosphorous). These factors have a direct influence, but are themselves seasonally influenced by temperature. Our results also show the importance that environmental conditions in one zone can have on living conditions for a species in another area of the system. Hence the necessity to take into account the water quality of the entire estuary to understand expansion of living populations. Fig 8 represents a synthesis of the various environmental situations occurring in the Scheldt with regard to the spatial distribution of *E. affinis.*

UDH analysis could also be used to study the influence of hypoxia (or other limiting conditions) on the distribution of other pelagic organisms than *E. affinis,* in estuaries or river systems under two conditions. First, the system must include a local and periodic hypoxic area, which is located somewhere in the organism's expansion area. Secondly, the system must transit by this particularly area: the organisms should not be able to expand towards upstream or downstream reaches by any other way. More generally, the UDH analysis presented here can be applied to any ecosystem which spatial scale can be studied in one dimension (e.g. rivers or estuaries) and which includes a potential abiotic or biotic barrier.

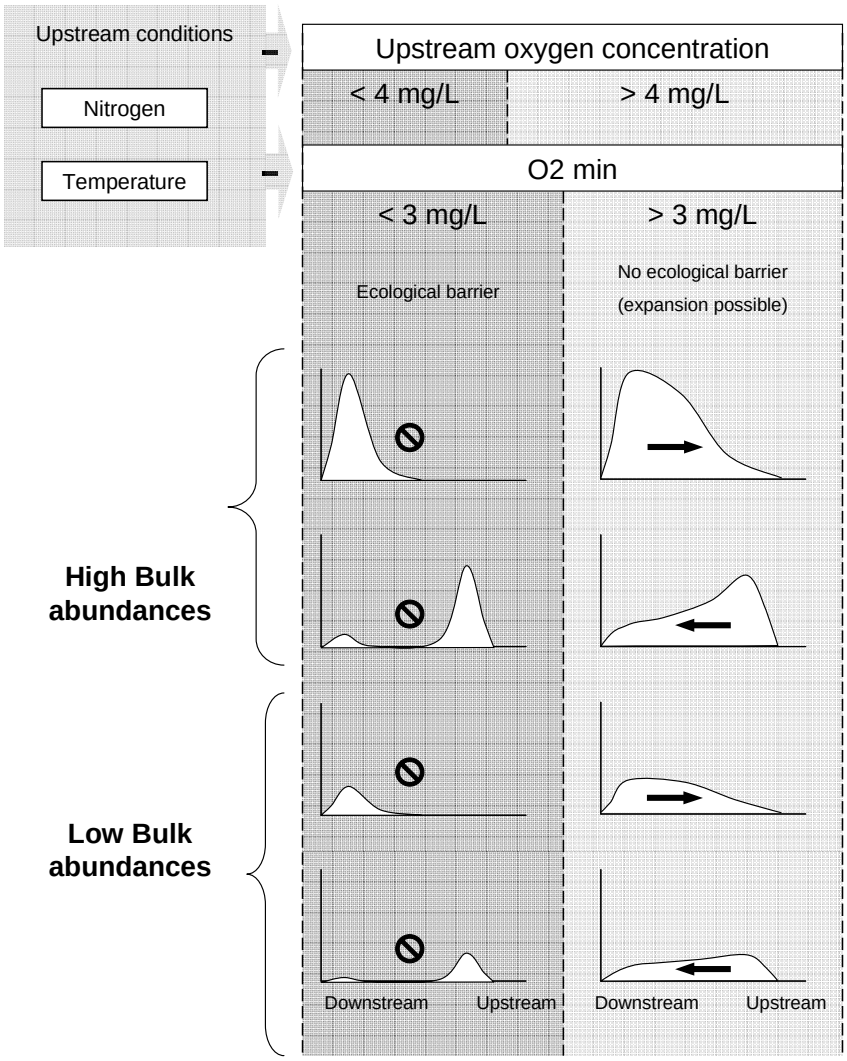

Fig. 8. Conceptual scheme representing the environmental conditions for the spatial distribution of *Eurytemora affinis* adults in the Scheldt estuary. Distance to the mouth is represented on the X-axis, *E. affinis* abundance on the Y-axis.

The occurrence of *E. affinis* in the upstream Scheldt area can to some extent be considered as a tracer of the success of the restoration process in the Scheldt. As the conditions for its existence seem to be clearly related to threshold values of environmental variables, this opens perspectives for modelling its occurrence. *E. affinis* being an easily recognisable species, and considering its importance as prey for mysids and fish in the Scheldt (Fockedey et al., 1999; Maes et al., 2005) and temperate estuaries in general (Knutson et al., 1983; Mouny et al., 2002; Winkler et al., 2003; Winkler et al., 2004) it seems to be a good "indicator" candidate.

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Chapitre 3

Changements dans le comportement trophique d'*Eurytemora affinis* (Copepoda, Calanoida) suite à l'amélioration de la qualité de l'eau en milieu estuarien

Article en cours de rédaction pour être soumis à la revue **Aquatic Ecology**

Résumé de l'article

Introduction et objectifs

Comme démontré dans les chapitres 1 et 2, depuis l'amélioration de la qualité de l'eau constatée dans l'estuaire de l'Escaut au cours des années 1990 (Van Damme et al., 2005; Cox et al., 2009), un déplacement plus ou moins sporadique des populations d'*E. affinis*, de la zone d'eau saumâtre vers la zone plutôt d'eau douce, aux alentours d'Anvers, a été observé (Appeltans et al., 2003; Mialet et al., 2010). Comme la qualité de l'eau est à présent toujours meilleure en aval d'Anvers qu'en amont, la question de l'avantage pour *E. affinis* d'un tel déplacement vers l'amont se pose.

Si en milieu estuarien *Eurytemora affinis* a longtemps été considéré comme un détritivore (Heinle and Flemer, 1975; Heinle et al., 1977), des études plus récentes ont mis en évidence son comportement sélectif vis à vis du phytoplancton (Gasparini et al., 1999; Tackx et al., 2003). Un modèle de type Monod, mettant en évidence la relation entre la disponibilité en phytoplancton et le contenu digestif en phytopigments (G) du copépode a été établi par Tackx et al. (2003).

L'objectif de cette étude est de comprendre si, d'un point de vue nutritif, les conditions environnementales actuelles dans l'estuaire de l'Escaut sont meilleures pour *E. affinis*, que celles décrites par Tackx et al. (2003) pour la période 1993-1994. Cette analyse se base sur la comparaison de données anciennes et récentes sur le sujet.

Principaux résultats et discussion

La calibration du modèle des contenus digestifs en phytopigments (G) en fonction du rapport phytoplancton / matière en suspension présenté dans Tackx et al. (2003), n'a pas été possible avec des données obtenues en 2008 et en 2009 sur des populations d'*E. affinis* dans l'Escaut. Les différentes causes pouvant expliquer ce résultat seront discutées ci-dessous.

Les valeurs des contenus digestifs spécifiques en phytopigments (Gs) d'*Eurytemora affinis* mesurées en 2007 et en 2008 étaient en moyenne plus de deux fois inférieures à celles mesurées en 1992. Ce résultat suggère qu'actuellement le copépode se nourrit moins de phytoplancton qu'auparavant. Pourtant la condition des organismes, mesurée à partir du poids sec individuel et de la longueur du prosome, montrent des animaux en aussi bonne voire meilleure condition que pendant les années 1990.Ceci montre que même si *E. affinis* se nourrit moins de phytoplancton qu'au par avant, il n'est pas en pénurie de nourriture dans le tronçon de l'estuaire qu'il occupe actuellement. Deux hypothèses peuvent expliquer ce phénomène :

1. Un changement dans la composition phytoplanctonique peut entraîner une réduction de la disponibilité des algues pour le copépode, résultant d'une augmentation de la taille cellulaire globale ou d'une distribution taxonomique différente.

2. D'autres ressources alimentaires peuvent être désormais accessibles et préférées par le copépode, comme les organismes microzooplanctoniques (Hétérotrophes nanoflagellés, rotifères, ciliés).

L'observation microscopique des contenus digestifs d'*Eurytemora affinis* a montré une préférence du copépode pour les algues de type diatomées discoïdes ayant un diamètre d'environ 30 à 40 µm, parmi le phytoplancton. L'analyse des spectres de tailles des algues de la colonne d'eau montre que cette catégorie d'algues n'était pas moins abondante en 2007 et en 2008 qu'en 1992. La variabilité interannuelle (1992, 2007, 2008) de la composition spécifique des diatomées dans l'estuaire de l'Escaut est importante. Toutefois, ce phénomène n'est pas nouveau et a été constaté depuis de nombreuses années (Rijstenbil et al., 1993; Muylaert and Sabbe, 1999). C'est pourquoi l'hypothèse d'un basculement du régime alimentaire causé par un changement de taille ou de la composition taxonomique de la communauté algale semble donc peu probable.

Plusieurs études ont montré qu'*E. affinis* adopte un régime alimentaire composé d'organismes hétérotrophes dans la Gironde (Gasparini et al., 1999; David, Valérie et al., 2006) et la Charente (Modéran, 2010). De plus, Gasparini et al. (1997) ont montré, en milieu expérimental, qu'à une concentration en matières en suspension (MES) supérieure à 150 mg l^{-1}, le copépode consommait majoritairement des proies hétérotrophes. Or, les MES mesurées

dans l'Escaut augmentant sensiblement depuis 1996 et ayant dépassé une valeur annuelle moyenne de 150 mg l^{-1} depuis 2006 (cf. chapitre 2), cette tendance vers la carnivorie serait donc envisageable.

Changes in feeding behaviour of *Eurytemora affinis* (Copepoda, Calanoida) with water quality improvement in estuarine environments

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Abstract

Previous studies have shown that in the Scheldt estuary, zooplankton spatial and temporal distributions have changed with improving water quality since 1996. *Eurytemora affinis*, an estuarine spring dominant copepod, is now spreading toward the freshwater reach of the estuary. The purpose of the present paper is to investigate the potential changes in *E. affinis*' feeding conditions co-occurring with its recent upstream expansion, and possibly being an incentive to this new positioning. Because of the increasing primary production in the upstream part of the estuary, we focus on the grazing activity of *E. affinis* and its potential role in controlling phytoplankton blooms in the upstream Scheldt estuary. To this purpose, past and recent data were compared. *E. affinis'* condition, as quantified by female individual dry weight and prosome length was significantly better in 2007–2008 than in 1996. Clutch size was not different between the two periods. Present chlorophyll *a*-eq specific gut content (Gs) values decreased and were around 1/3 of the past ones. This means that in the present Scheldt situation, *E. affinis* feeds less on phytoplankton than before. Observation of *in situ* gut content showed that the copepod essentially ingested centric diatoms (25–45 µm). This result, associated with a comparison of past and recent algal composition, suggested that the availability of phytoplankton for *E. affinis* is probably not lower at present in the Scheldt than before. Although no rotifer mastax have been found in *E. affinis'* guts, the more likely explanation for these lower Gs values is a regime shift to microzooplankton organisms. Recent *E. affinis*' clearance rates values on total phytoplankton are lower than past ones, but they have to be calculated specifically for diatoms to assess if the copepod could regulate their blooms.

Keywords: *Eurytemora affinis*, gut content, restoration, Scheldt estuary.

1. Introduction

Since the middle of the nineteen nineties, water quality in the Scheldt estuary (Belgium/The Netherlands) has substantially improved, especially around the region of Antwerpen and upstream. Among the main changes are an increase in chlorophyll *a* (Chl *a*) concentration and oxygen concentration as well as a reduction in nutrient concentrations (Van Damme et al.,

2005; Cox et al., 2009). In recent years, chlorophyll *a* concentrations in the upstream part varied between 50 and 700 µg l^{-1} during summer. These blooms of phytoplankton are expected to boost zooplankton development, and consequently also higher trophic levels. It has been also observed that phytoplankton taxonomic composition in the Scheldt has changed over time (Muylaert and Sabbe, 1999; Van Burm, unpublished data).

In the frame of a follow-up of the response of the zooplankton community to the improving water quality in the Scheldt, *E. affinis* is one of the key species which is monitored. This calanoid copepod species complex is a typical inhabitant of temperate estuaries (Soetaert and Van Rijswijk, 1993; Sautour and Castel, 1995; Peitsch et al., 2000). Previous studies suggested that some *E. affinis* populations which originally live in brackish water environments can adapt to freshwater in only few generations (Lee et al., 2003). Lee (1999) also showed that in the past century, *E. affinis* brackish populations invaded freshwater reaches, in European systems as well as in North American and in Asian ones, in a short time range. But factors responsible for these invasions were not yet clearly identified.

In the Scheldt estuary, *E. affinis* occurs mainly in spring (Tackx et al., 2004). Some recent studies have shown a response of this calanoid copepod to water quality improvement both in terms of an increase in abundance (Tackx et al., 2005) and of spatial distribution (Mialet et al., submitted; Appeltans et al., 2003; Mialet et al., 2010). From the nineteen nineties to around 2006, *E. affinis* has first displaced its peak abundance from the 10–12 salinity zone (Sautour and Castel, 1995) towards 0–2 salinity range (Appeltans et al., 2003; Mialet et al., 2010). In a second step, from 2007 onwards, the bulk of its population has switched to the freshwater zone, in which it occurs in very high abundance (up to 115 ind l^{-1}) (Mialet et al., submitted). As such, the question rises to what extent zooplankton will in the future control phytoplankton biomass in the upstream reaches of the Scheldt (Cox et al., 2009).

Multivariate analyses have shown the main factors associated with *E. affinis* positioning to be representative of water quality: NO_2^--N, PO_4-P, NH_4^--N, BOD_5 and O_2 concentrations (Appeltans et al., 2003; Mialet et al., 2010; Mialet et al., submitted). Thus, this change has been explained as a reaction to improving water quality: populations of the copepod could have shifted towards upstream as water quality became more appropriate for their development. However, as water quality has always been better in the brackish part of the

estuary, and remains to be so, the question arises which is the benefit for *E. affinis* to move towards the freshwater reach around Antwerp and further upstream.

In order to understand *E. affinis* recent upstream expansion, this paper explores if the present position occupied by *E. affinis* in the Scheldt estuary represents better feeding conditions than those reported by Tackx et al. (2003), for the 1993–1994 period. Emphasis will be put on phytoplankton ingestion by *E. affinis*, because, as explained above, its impact on phytoplankton is relevant for ecosystem management. Being associated with detritus rich environments, *E. affinis* has long been considered as a detritivore (Heinle and Flemer, 1975; Heinle et al., 1977). Gasparini et al. (1999) studied the feeding of *E. affinis* on natural estuarine water in three European estuaries during 1993 and 1994: the Elbe (Germany, 1993), the Gironde (France, 1993) and the Scheldt (Belgium / the Netherlands, 1993). They showed that *E. affinis'* gut pigment content, quantified as chlorophyll *a* equivalent (Chl *a*-eq) pigment concentration per unit of body weight (Gs), is independent of the Chl *a* concentration in the water, but decreases with suspended particular matter (SPM) concentration. This suggested that *E. affinis* to be a selective feeder, which does not gather food particles in proportion to their abundance in the environment, but can select phytoplankton when the ratio of phytoplankton to SPM is not too low. Tackx et al. (1995; 2003) showed that *E. affinis* in the Scheldt and the Gironde estuaries indeed selects phytoplankton over SPM. Tackx et al. (2003) also fitted a model to Gasparini's data which shows that gut pigment content, quantified as Chl *a*-eq pigment concentration per individual (G) of *E. affinis*, follows a Monod-type function as a function of the proportion of phytoplankton–carbon to particulate organic carbon. The efficiency of phytoplankton selection by *E. affinis* reaches 80 % of maximum gut pigment content when phytoplankton POC contributes 5 % of the total POC. Considering the 1996–2007 dataset, Chl *a* concentration did not show up as a significant factor in relation to *E. affinis'* spatio-temporal distribution (Mialet et al., 2010). This is not surprising, as *E. affinis* was already 80 % satiated with phytoplankton ingestion in the mid nineteen nineties (Tackx et al., 2003), when Chl *a* concentration in freshwater was much lower (on average 18 µg l^{-1}) than at present (on average 74 µ l^{-1} during 2008) (Mialet et al., submitted).

The present study will be divided in 4 parts. Firstly, past and present condition of *E. affinis* females is compared in term of individual dry weight (DW), prosome length and clutch size measurements in samples from the periods 1996–1998 and 2007–2009. Secondly, the validity of the phytoplankton selectivity pattern detected in the mid nineteen nineties is verified in the

present situation. Phytopigments gut contents were measured in the Gironde and in the Scheldt to revisit the model under recent conditions. In a third part, past and recent feeding conditions for *E. affinis* are compared. For that, in addition to *in situ* SPM and Chl *a* measurements, supplementary parameters were measured in the Scheldt, including algal composition and size distribution in the medium and in *E. affinis'* gut. Considering indications of carnivorous feeding by *E. affinis* (Gasparini et al., 1999; David, V. et al., 2006; Modéran, 2010), the presence of rotifer mastax in the gut of *E. affinis* was also checked. Finally, in the last part, clearance rates are calculated for past and recent conditions in order to compare the impact of the *E. affinis* population on phytoplankton in the Scheldt estuary.

2. Materials and methods

2.1. Field samplings

In order to validate the model with recent samplings (2007–2009 period), and to efficiently compare various parameters with the 1993–1994 period, spring measurements in the Gironde estuary were performed at a similar salinity range as in the previous studies, during 14 and 15 April 2009 (Gasparini et al., 1999). The recent data were compared with those of Gasparini et al. obtained in 1994 (Table 1, Fig. 1). In the Scheldt estuary, as *E. affinis* is now observed more upstream than before (Appeltans et al., 2003; Mialet et al., 2010), samplings were carried out around the region of Antwerpen, on two occasions (12 April 2007, 13 to 15 March 2008). In 2008, 3 to 4 samplings were performed at 3 stations during different times of the day, which cover a large salinity gradient. In total, 23 samplings spread over the 0.2–6 salinity range were carried out for temperature, salinity, SPM concentration, Chl *a* concentration, zooplankton gut pigment content and abundance.

Temperature and salinity were measured at each sampling in the water column using portable instruments (Lf 95Temp, conductimeter, WTW, Weilheim, Germany). Water samples of 50 to 500 ml, depending on the suspended matter concentration, were filtered onto glassfiber filters (GF/C) in sixfold. 3 replicates were stored in a coolbox for SPM measurements, and 3 others were stored in liquid nitrogen for phytopigments quantification. Filters for SPM were weighted before sampling.

Zooplankton was collected with a 200 µm mesh net. A part of the catch, to be used for dry weight, prosome and clutch size measurements, was stored in 4 % formaldehyde. A second part of the zooplankton sample, to be used for gut phytopigments measurements, was filtered trough a 5 x 5 cm 200 µm gauze, then immediately immersed in liquid nitrogen. Samples were then stored in a -80 °C freezer prior to treatment which was carried out between 3 to 12 months after sampling. During the 2008 campaigns, water was also sampled in 120 ml bottles and fixed with lugol (10 ml) for algae observation.

2.2. Laboratory analysis of samples

For copepod weighing, 3 replicates of 10 female adults of *Eurytemora affinis* were isolated from 2007 and 2008 formalin-preserved samples (total of 13 samples) into a small aluminium pre-weighted dish. Samples were dried 24 h at 40 °C before weighing.

Measurements of prosome length and clutch size were performed on 4 % formaldehyde preserved samples from two periods: 1996–1998 (the oldest samples available for these measurements) and 2008–2009. All samples were obtained from the OMES monitoring program (Van Damme et al., 2005). The reader is referred to Tackx et al. (2004) for more details about sampling methods. For each period, 26 to 45 adult females were isolated. Prosome length was measured under inverted microscope and the number of eggs per clutch was counted under binocular..

SPM was measured by weighting filters after drying them at 60 °C for 24 h. Filters for algal pigments were then extracted three times (15 min at -20 °C) with a total of 25 ml (10, 10, and 5 ml) 98 % cold-buffered methanol (with 2 % of 1 M ammonium acetate), following (Buffan-Dubau and Carman, 2000a; Caramujo et al., 2008). Algal pigment release was favoured at each extraction step by an ultrasonication probe (Sonifier 250A, Branson Ultrasonics corp., 200 Danbury, CT, U.S.A.). One ml of the pigment solution so obtained was then filtered on 0.2 µm PTFE syringe filter and analyzed using a Hewlett Packard 1100 liquid chromatograph consisting of a 100 µl loop auto-sampler and a quaternary solvent delivery system coupled to a diode array spectrophotometer. The diode array detector was set at 665 nm for detection of chlorophyll *a*, pheophytin *a* and pheophorbid *a* (Wright et al., 1991). The Separation of

pigments was performed by reverse-phase liquid chromatography using a C8, 5 µm column coupled to a guard column. The mobile phase was prepared and programmed according to the analytical gradient protocol described by Barlow et al. (1997). All organic solvents and water were HPLC grade. Data analysis was performed using Hewlett Packard HP ChemStation software. Pigments were identified by comparing their retention time and absorption spectra with those of authentic standards (DHI). Each pigment concentration was calculated in relating its chromatogram's peak area with the corresponding area of calibrated pigment standards (DHI). Chlorophyll a, pheophytin *a* and pheophorbid *a* concentrations were summed and expressed in µg Chl *a*–eq l^{-1}.

For 2007 and 2008 gut phytopigments measurements, frozen samples were defrosted, corresponding to the same stations and dates as for copepod weighing. Three replicates of 20 to 40 female adults of *Eurytemora affinis*, depending on their abundance, were isolated under binocular microscope into 500 µl of 98 % cold-buffered methanol (2 % ammonium acetate). Extraction and HPLC analysis followed the same procedure as described above.

To observe and measure algae and to check the presence of rotifer mastax in *E. affinis*' gut, frozen zooplankton samples were defrosted. About 75 adult females, from 3 samples taken in 2008 at different salinities, were observed with 60 x magnification, and then dissected to isolate the gut. Gut content observations and measurements were performed at a 400 x magnification. Green algae and diatoms of the medium, obtained from 2008 samples, were observed and measured with 400 x magnification. Other minor taxa were not taken into account.

2.2. Data analysis

The fitting of the present gut content data to the model of Tackx et al. (2003) and non parametrical tests were performed with Statistica 6 software (version 6.0; Statsoft Inc., Tulsa, USA).

For both past and recent periods, ingestion rates (I) of *E. affinis* on phytoplankton were calculated by multiplying G (ng Chl *a*-eq ind^{-1}) values shown in Fig. 1 by Gut Clearance Rate (GCG), using the regression: GCR (h^{-1}) = 0.0117 + 0.001794 T (°C) (Dam and Peterson,

1988). Clearance rates (F) on phytoplankton were calculated by dividing I by the concentration of Chl *a* and pheopigments (µg Chl *a*-eq l^{-1}) measured in the water at the time of sampling. *E. affinis*' community clearance rates on phytoplankton per day were calculated by multiplying F by the abundance of *E. affinis* (ind l^{-1}) and by 24 hours. Temperature and *E. affinis* abundance values for the recent period were obtained from OMES monitoring data which samplings were the closest to those performed for gut content measurements in the present study.

In the results and discussion section, measurements and data from the past period will be compared with those of the present period. The grouping of data into these two periods is given in Table 1.

Table 1. Detailed information about different data involved in the present study.

	Past period			Recent period (this study)	
Data / measurement	Source	Salinity range	Sampling year	Salinity range	Sampling year
E. affinis female G and Gs, Chl *a* , SPM	Gasparini et al. (1999)	Gironde: 0.5–18	1993–1994	Gironde: 0.5–10	2009
	"	Scheldt: 5–12	"	Scheldt: 0.2–6	2007–2008
E. affinis female filtration rate, individual DW	"	"	"	"	"
Pheopigment / Chl a ratio in medium and gut	Billiones, unpublished	Scheldt: 4–8	1997	"	"
E. affinis promosome length and clutch size	OMES monitoring	Scheldt: 0.5–2	1996–1998	"	2008–2009
Central diatoms abundance and biovolume	Tackx et al. (1995)	Scheldt: 2–8	1992	"	2008

3. Results and discussion

3.1. Eurytemora affinis' past and recent condition in the Scheldt estuary

Mean female individual dry weights (DW) in the Scheldt were 12.5 µg ind^{-1} in 2007–2008, which is significantly higher (Mann–Whitney, $p = 0.006$) than the average of 10.2 µg ind^{-1} measured in 1994 (Fig. 1). Female prosomes were significantly longer (Mann–Whitney, $p = 0.002$) in 2008–2009 (mean of 1008.8 µm), than in 1996–1998 (mean of 930.2 µm) (Fig. 1). The number of eggs per clutch is not significantly different (Mann–Whitney, $p = 0.93$) between the two periods (Fig. 1).

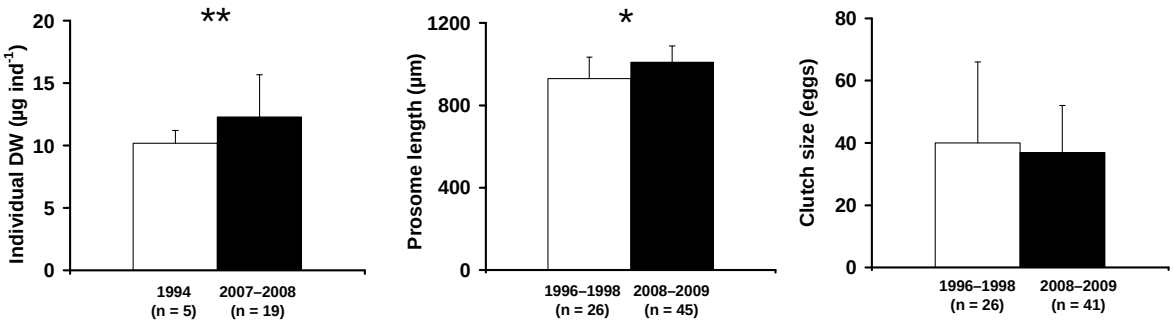

Fig. 1. Comparison of *Eurytemora affinis*' female individual dry weight (DW), prosome length and clutch size between two periods in the brackish water reach of the Scheldt estuary (Antwerpen). Mean values are given with their standard deviations. n for DW correspond to the number of treated samples, n for prosome length and clutch size correspond to the number of copepods analysed. Mann–Whitney tests significance levels are indicated (*: $p < 0.05$; **: $p < 0.01$).

So, *E. affinis* females' condition in the Scheldt estuary seems to have improved over time (dry weight and prosome length respectively + 20.8 % and + 8.4 %). These changes in condition seem moreover not to affect the mean clutch size, which did not change significantly. However the variability between females (see error bars) seems to be lower in the recent period.

3.2. E. affinis' pigments gut contents: revisiting the selective feeding model

The model presented in Tackx et al. (2003), is based on the dependence of G on body weight, SPM and Chl *a* concentrations in the water:

$$G = aW^{b} \frac{\text{Chl a}}{k\text{SPM} + \text{Chl a}}$$

G is the gut pigment content and W is the dry weight measured on *E. affinis* adult females (mg ind^{-1}). Chl *a* (µg l^{-1}) and SPM (mg l^{-1}) are chlorophyll *a* and suspended particular matter concentrations in the water column. Using values of the 1993–1994 period, it followed a Monod-type curve which shows that when the ratio of Chl *a* to SPM was above 0.05 µg mg^{-1}, *E. affinis*'s G reached a limit of about 1.7 ng Chl *a*-eq ind^{-1} (Fig. 2, white symbols). As can be seen from Fig. 2, the range of the ratio of Chl *a* to SPM values in the Scheldt estuary was much wider during 2007–2008 (from 0.04 to 0.25 µg mg^{-1}) than during 1994 (from 0.02 to

0.08 µg mg^{-1}). Feeding conditions in the Scheldt at present are within the range giving rise to maximum G values, as observed mainly in the Elbe estuary in the 1994 (Fig. 1).

In the Scheldt, combining a significant increase in individual body weight (Fig. 1) and a significant decrease in Gs (Fig. 3a), the present data showed G values to be significantly lower (around 0.7 ng Chl *a*-eq ind^{-1}; Fig. 2, black symbols; Mann–Whitney, $p < 0.01$) than the 1994 values. While showing a plateau from around 0.05 Chl *a* / SPM (Spearman rank, $p = 0.92$), present maximum G values in the Scheldt were around 1/3 of the plateau values observed by Gasparini et al. (1999) and were rather variable. *E. affinis* either sometimes can not obtain satiating amount of phytoplankton, or that sometimes it eats other food. Such a switch in diet could be either imposed by an insufficient availability of phytoplankton, or be the result of a more advantageous feeding on other preys. No satisfactory calibration of the feeding model has been possible with the present measurements, indicating that the model proposed by Tackx et al. (2003) is not valid under recent conditions.

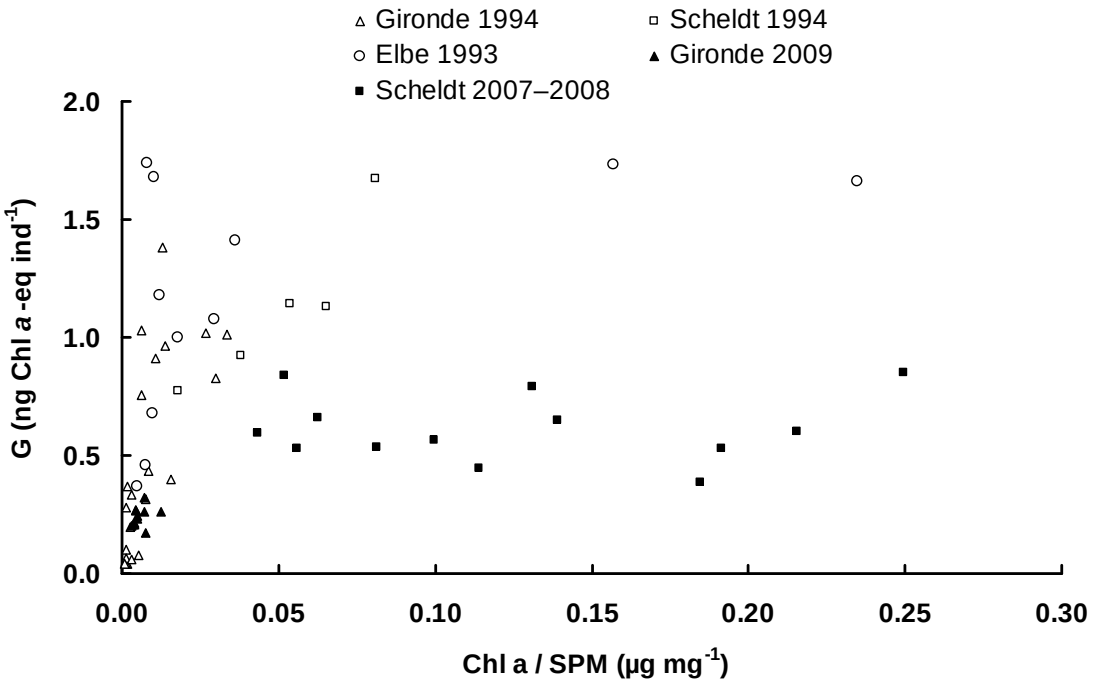

Fig. 2. *Eurytemora affinis* individual gut pigments contents (G) as a function of the ratio of Chl *a* to SPM, in the Gironde (triangles), the Elbe (circles) and the Scheldt estuaries (squares), during two periods: 1993–1994 (white symbols) and 2007–2009 (black symbols).

The ratio of pheopigments to chlorophyll *a* (Fig. 3b) was much more elevated in 2007–2008 than in 1994 (Mann–Whitney, $p < 0.0001$), with a mean of 4.3 versus 0.6. This increase

indicates that gut phytopigments were more degraded in the second period than in the first one. This results suggest that during 1994 the copepod tended to directly ingest phytoplankton organisms whereas during the 2007–2008 period, it tended to ingest heterotrophic organisms which fed on phytoplankton organisms is their guts.

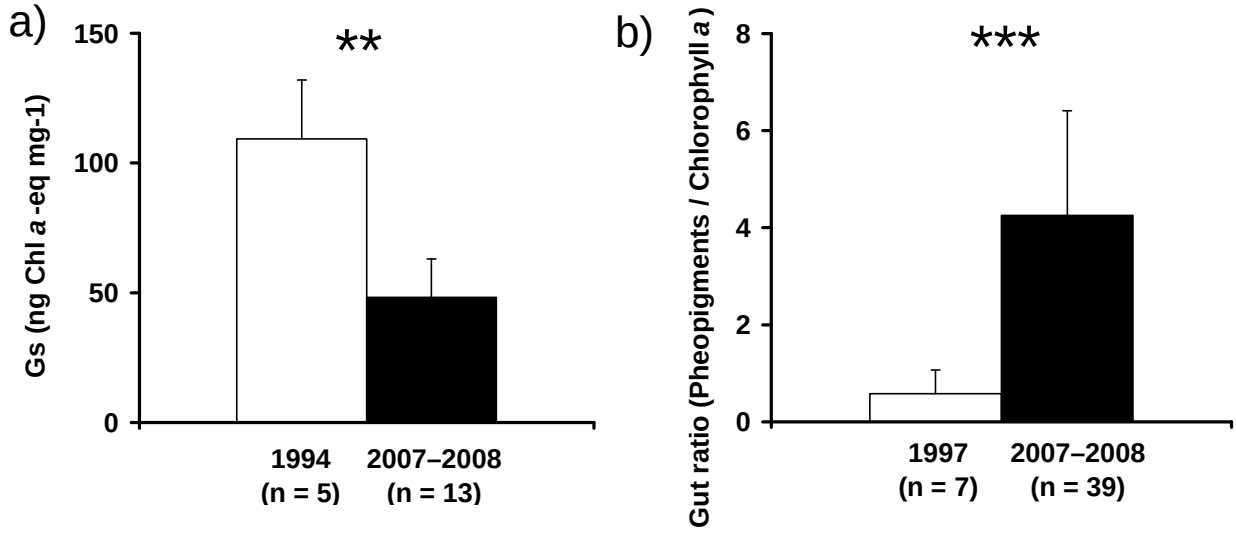

Fig. 3. Comparison of *Eurytemora affinis*' (a) specific phytopigments gut contents and (b) gut ratio of pheopigments to chlorophyll *a* (means and standard deviations) between several periods, in the brackish water reach of the Scheldt estuary. n correspond to the number of measurements. Mann–Whitney tests significance levels are indicated (**: $p < 0.01$; ***: $p < 0.001$).

The model proposed by Tackx et al. (2003) was not valid under recent conditions. This suggests that *Eurytemora affinis* did very likely change its feeding behaviour since major water quality improvements occurred in the Scheldt estuary. Considering the 80 % satiation of gut pigment content under Chl *a* / SPM conditions during 1993–1994 (Tackx et al., 2003) and considering the increase in Chl *a* / SPM ratio concentration which has taken place in the Scheldt (fig. 2), *E. affinis* G values were expected to be the same or possibly slightly higher under recent conditions. As *E. affinis* individual body weight has increased, somewhat higher G values seemed likely. So in the following, we focus on understanding why, contrary to the expectation, *E. affinis* G and Gs values measured in the present study are lower than the ones observed in 1994.

3.3. Comparison of methods

To verify that the difference in G(s) values measured is not a consequence of methodological differences, a comparison of methods employed to measure Chl *a* concentrations between Gasparini et al. (1999) and the present study is necessary. Gasparini et al. (1999) used a fluorimeter and Lorenzen's equations (Lorenzen, 1967) to quantify Chl *a*, and consequently G and Gs values, for the period 1993–1994. In the present study, Chl *a* was measured by HPLC, following Barlow et al. (1997). In the literature, comparisons between Lorenzen's method (1967), employed by Gasparini et al. (1999), and HPLC method are numerous but the results are inconsistent (Jeffrey et al., 1997). To verify if methodological differences could be responsible for the lower recent *E. affinis* Gs values measured in the Scheldt in comparison with 1994 measurements in the Gironde during both periods were compared. Feeding under past and recent Chl *a* / SPM ratios which were in the same range, *E. affinis*' Gs values were not significantly different (Table 2; Fig. 1).

Table 2. Comparison of various parameters measured in the Gironde estuary. n correspond to the number of measurements. Mann–Whitney tests significance levels are indicated.

		March–May 1994	**April 2009**	**Mann–Whitney p**
Chl *a*	µg l^{-1}	2.1 ± 1.2	3.4 ± 1.0	0.0037
SPM	mg l^{-1}	236.6 ± 133.3	645.7 ± 279.5	0.0004
Chl *a* / SPM	µg mg^{-1}	0.012 ± 0.010	0.006 ± 0.003	ns
Gs	ng Chl *a*-eq ind^{-1}	78.7 ± 43.6	64.3 ± 13.1	ns
n		10	12	

Considering the general trend in Chl *a* and SPM with time in the Gironde estuary, in the salinity range, Chl *a* has been observed to slightly and regularly decreasing between the middle of the nineteen nineties and 2008 of our 2009 sampling, (Chaalali, unpublished data). Thus, as Chl *a* and Gs measurements carried out in the Gironde for the present study were not lower than in 1994, present Gs measurements carried out in the Scheldt estuary were obviously not underestimated compared to Gasparini et al. (1999). This means that in the present Scheldt situation, *E. affinis* feeds less on phytoplankton in the Scheldt estuary than before. Two hypotheses can be considered to explain this:

1. The switch in phytoplankton composition could make the phytoplankton less available for *E. affinis*, by a change in size of dominant species or by a different species composition that could be induced by changes in water quality (Tas et al., 2009).
2. Other food items could now be available and preferred by *E. affinis*. Obvious candidates are microzooplankton organisms.

3.4. Analysis of phytoplankton availability for E. affinis in the Scheldt estuary

As phytoplankton taxonomic composition in the Scheldt has changed over time, we verified if the present phytoplankton community is as available to *E. affinis* as the past one. In the guts of the 75 *E. affinis* adult females which were dissected, numerous diatoms frustules intact or partially broken and some green algae have been observed (Fig. 4). However, no rotifer mastax have been observed.

Comparison of gut and water column algae composition, from water samples that have been taken at the same time and stations in the Scheldt, are given in Fig. 5. Diatoms, and especially centric diatoms, were largely dominant (on average 87 % of the total algae) in the gut, while they were proportionally less abundant (on average 41 % of the total algae) in the medium (Fig. 5a).

Measurements of centric diatom diameters found in the guts of copepods are shown in Fig. 5b. Centric diatoms in copepod guts were significantly larger than the ones found in the medium (Mann–Whitney, $p < 0.001$). The mean diameter ingested by *E. affinis* was around 35–45 µm. As can be shown by Fig. 5c, algae diameters which were most frequently ingested by *E. affinis* were less frequently found in the water column, showing a feeding selectivity of *E. affinis* for this size range of centric diatoms.

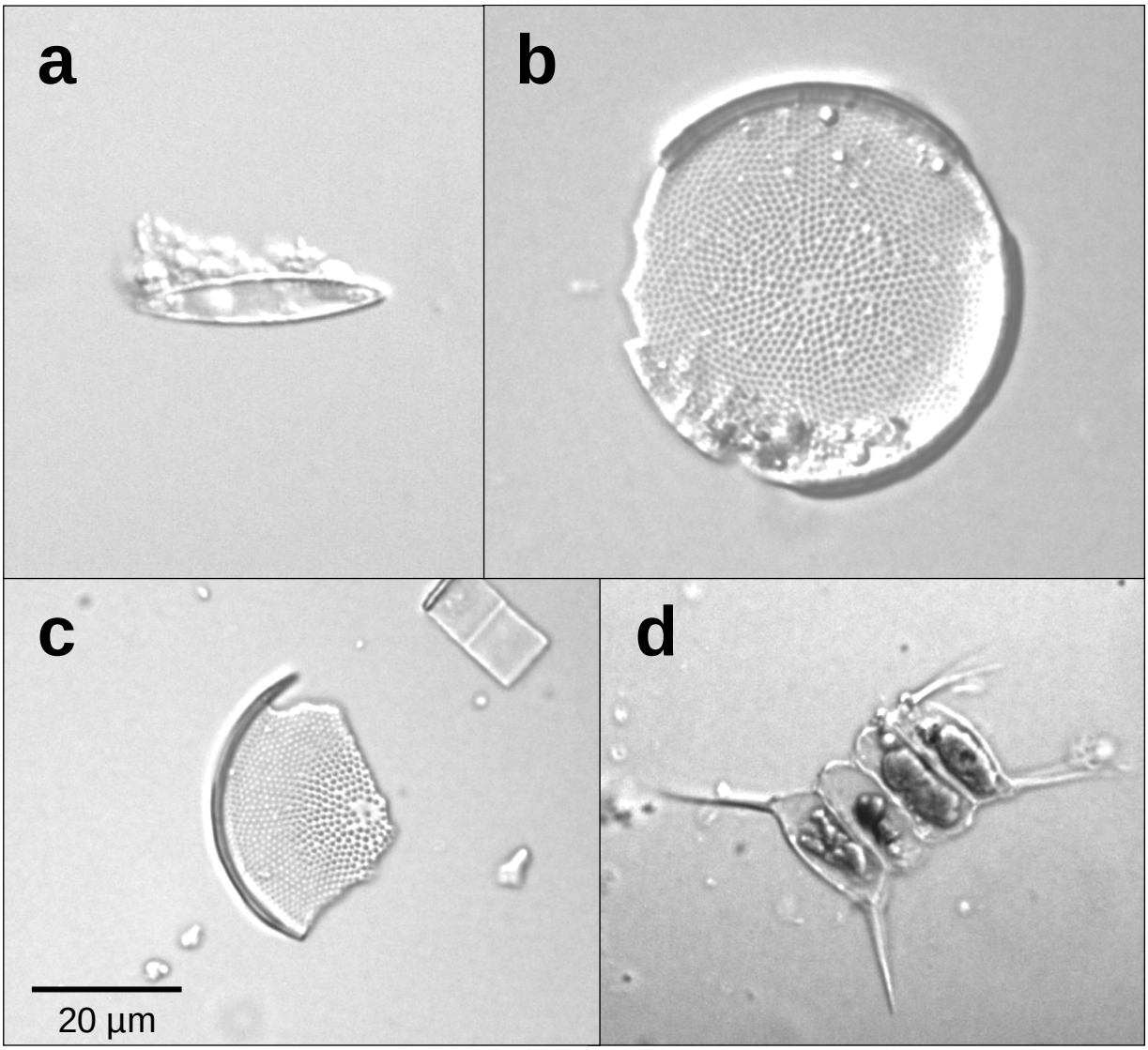

Fig. 4. Examples of algae observed in *E. affinis*' gut: pennate diatom (a), centric diatoms (b, c), green alga (d).

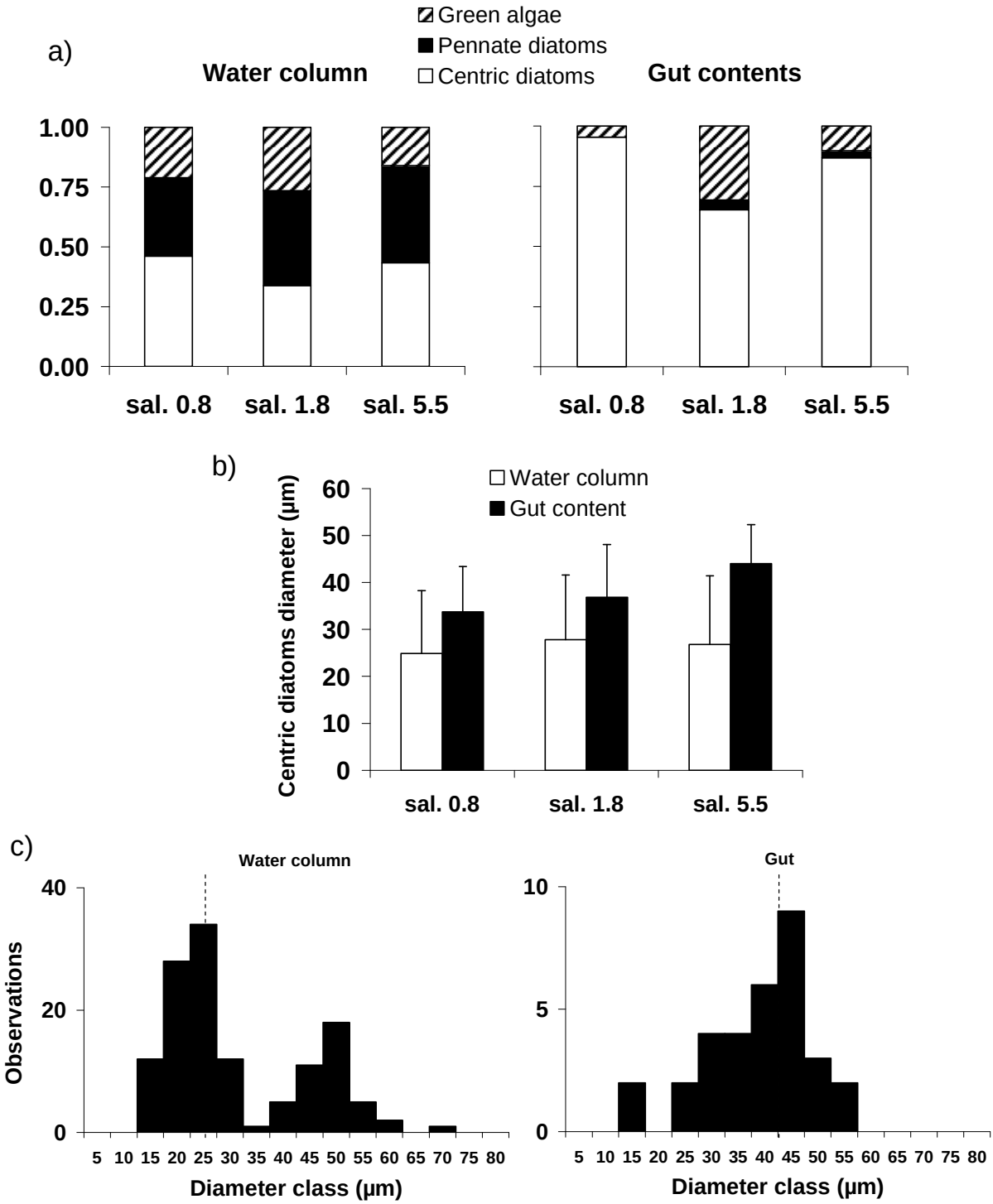

Fig. 5. Comparison of (a) relative abundance of algal groups and (b) centric diatoms diameters (mean and standard deviation), between the water column and *Eurytemora affinis*' gut contents during march 2008 in the Scheldt estuary, at 3 stations around Antwerpen. Stations are designed by their salinity. (c) Examples of centric diatoms diameter spectra in the medium and the gut. Median values are indicated with a dotted line.

Considering the dominance of centric diatoms in the gut contents, the comparison of column algal size distribution in the water column between both periods was focused on centric diatoms. The proportion of centric diatoms was significantly higher during spring 2007 and 2008 than during spring 1992 (Mann–Whitney, $p < 0.05$) in both term of abundance and biovolume (Fig. 6). As Chl *a* increased between the middle of the nineteen nineties and 2008 (Cox et al., 2009; Mialet et al., submitted; Fig. 7), the availability of centric diatoms can reasonably be supposed to be superior during 2007–2008 than during 1992.

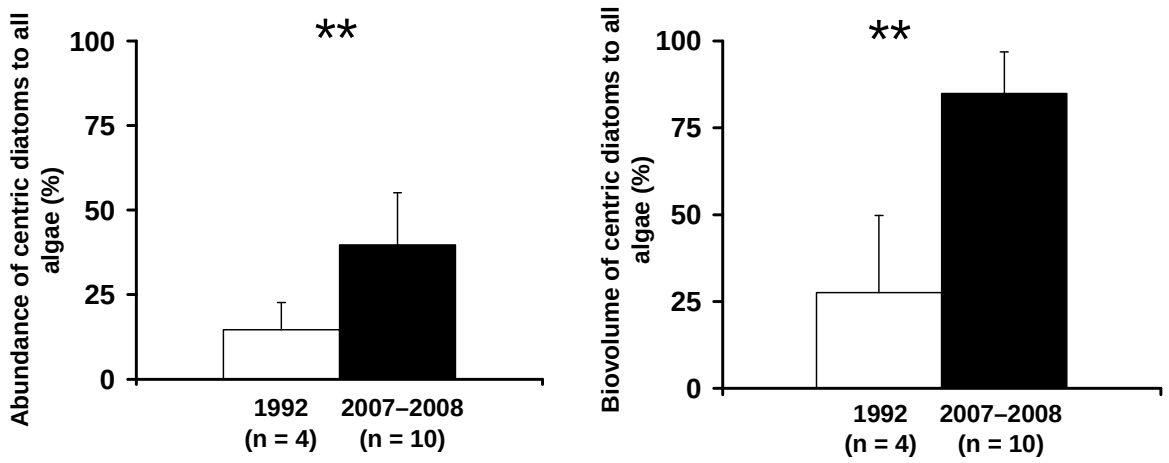

Fig. 6. Comparison of proportion of centric diatoms to all algae abundance and biovolume in the water column, (means and standard deviations) between the two periods. n correspond to the number of samples. Mann–Whitney tests significance levels are indicated (**: $p < 0.01$).

So these results suggest that, *E. affinis* selects rather big centric diatoms (35–45 µm) among the algae community. Ingestion of non siliceous algae was probably underestimated in the present microscopic gut analysis because they are more completely digested than diatoms**.** However, the selective consumption of diatoms by *E. affinis* in the Scheldt was also found by Tackx et al. (1995), using a method not biased by algae characteristics. Using microscopic counts of phytoplankton taxa in incubation experiments with *E. affinis* feeding on natural Scheldt water from the brackish zone, these authors showed that, when feeding on natural suspended matter in the Scheldt estuary, this copepod exerts clearance rates between 0.1 and 1.5 ml ind^{-1} h^{-1} on algae — most often diatoms — with a square equivalent diameter (SED) between 20 and 35 µm and of maximum 0.2 ml ind h^{-1} on total SPM Tackx et al. (1995). These preferred diatoms are as abundant at present (on average 0.18 ppm) as during the experiments of Tackx et al. (1995) during the years 1990 to 1992 (on average 0.20 ppm).

In conclusion, the availability of phytoplankton does not seem to be a likely cause for the variability in maximum *E. affinis* G values observed.

Above, only types and size of phytoplankton have been considered. After comparing spring dominant algae taxa (in biovolume and in density) from 1992 and from 2007 and 2008 OMES samples (not shown), inter-annual variability seemed to be important, except for *Nitzschia sp.* and *Desmodesmus sp.*, which were present during all years in various abundances (not shown). So basically, it could be probable that some species that belonged to *E. affinis*' diet during 1992 disappeared before the 2007–2008 period. However, Muylaert and Sabbe (1999) reported that only 52 % of the phytoplankton taxa they observed in the Scheldt estuary in 1993 (brackish and freshwater parts) were similar to those previously observed in 1984 in the brackish water part (Rijstenbil et al., 1993). In the same way, only 33 % of the diatom genera observed in the present study where common to those found by Muylaert and Sabbe (1999) in 1993. So it is unlikely that *E. affinis*, a typical estuarine species, would be adapted to feeding only on a few algae species, taking into account the general pattern of important variability encountered in estuaries (Cloern et al., 1983).

No rotifer mastax have been observed in the guts, but it could be possible that at present, as it has been already observed in the Gironde estuary (Gasparini et al., 1999; David, V. et al., 2006), the copepod feeds more on other heterotrophic organisms such as ciliates and bacteria, or detritus. Modéran (2010), from *in situ* measurements in the Charente estuary (France), found that the origin of *E. affinis*' $\delta^{13}C$ signature was associated with the $\delta^{13}C$ signature of estuarine particular organic matter. This signature corresponds to detritus or microzooplankton rather than to phytoplankton. Gasparini and Castel (1997) showed, with experimental incubations, that *E. affinis* gradually shifts its grazing pressure from autotrophic to heterotrophic nanoplankton with increasing SPM concentrations. Major shift seems to occur when SPM concentrations are above 150 mg l^{-1}, but it could be different under natural conditions. In the freshwater part of the Scheldt estuary, mean annual SPM concentrations significantly increased between 1996 and 2009 (Mialet et al., submitted). Mean annual values rose above 100 mg l^{-1} since 2006, to reach 150 mg l^{-1} in 2009. Thus, it is likely that recently, in the Scheldt estuary, *E. affinis* could have partially shifted its diet from autotrophic to heterotrophic organisms. While the organic fraction of SPM in the Scheldt has decreased from an average of 18 ± 5 % before 1996 to an average of 10 ± 2 % after 1996 till 2001 (Chen et al., 2005) it remains higher than in the Gironde estuary, thus rendering likely that

microzooplankton is sufficiently abundant in the Scheldt to represent an accessible food source to *E. affinis*. Spatial pattern and biodiversity of protozoan communities are related to environmental conditions (Mayer et al., 1997), especially BOD_5, NO_3-N and NH_4^+ (Tan et al., 2010), which are significantly changing since 1996 in the Scheldt estuary (Van Damme et al., 2005). A change in microzooplankton composition could have made them more available for *E. affinis*. Further studies based on stable isotopes and fatty acids analysis will be needed to obtain some decisive information about the recent feeding behaviour of *Eurytemora affinis* in the Scheldt estuary.

3.5. Analysis of E. affinis clearance rates on phytoplankton

As shown by previous studies (Van Damme et al., 2005; Cox et al., 2009), and as verified by Fig. 7, recent Chl *a* concentrations near Antwerpen were significantly higher than the past ones. *E. affinis*' clearance rates on phytoplankton were significantly lower (Mann Whitney, $p < 0.001$) in recent conditions (mean 0.12 versus 0.80 ml ind^{-1} h^{-1}), mainly because of recent lower G values. The mean population clearance rate of the whole *E. affinis* population on phytoplankton per day was significantly higher during the past conditions (on average 38.3 ml l^{-1} d^{-1}) than during present ones (on average 10.5 ml l^{-1} d^{-1}).

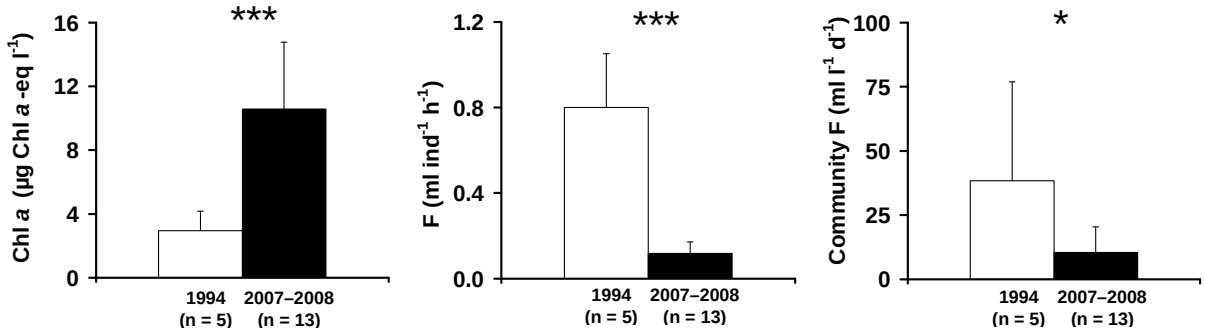

Fig. 7. Comparison of Chl *a* concentration, *Eurytemora affinis*' clearance rate on phytoplankton per hour and *E. affinis*' community clearance rate on phytoplankton per day, between two periods in the Scheldt estuary. Mean values are given with their standard deviations. n correspond to the number of measurements. Mann–Whitney tests significance levels are indicated (*: $p < 0.05$; ***: $p < 0.001$).

Due to a higher mean chlorophyll *a* concentration in the water and a lower mean clearance rate by *E. affinis*, the total *E. affinis* population F was substantially lower under recent conditions than under past ones. But the population F is strongly influenced by the high degree of variability in punctual *E. affinis* abundance values measured in the Scheldt. Considering this, it is difficult to assess if the grazing impact of *E. affinis* population on the whole phytoplankton community around Antwerpen did change under recent conditions.
F values on the entire algal community — as measured from Gut fluorescence values — are low but this copepod feeds essentially on diatoms. As shown by Tackx et al. (1995), clearance rate mean values of *E. affinis* on *Prorocentrum micans* (dinoflagellate) and *Coscinodiscus communtatus* (diatom) are superior to 1 ml ind^{-1} h^{-1} and these populations could almost be regulated by *E. affinis* only. Thus, by considering the fact that *E. affinis* feeds more on some types of phytoplankton than on others, it could be interesting to look into its grazing impact on different dominant algae species. *E. affinis*' clearance rates on diatoms have not been assessed in the present study. Dissolved silica is a major parameter in controlling diatoms populations (Wang and Evans, 1969). It has been observed that dissolved silica, controlling, significantly decreased in the Scheldt estuary during last years (Van Damme et al., 2005; Soetaert et al., 2006). Since a few years, summer dissolved silica deficiencies hamper diatom blooms in the upstream part (Carbonnel et al., 2009). If diatoms tend to be limited in the following years, other groups like green algae or cyanobacteria may increase their abundance. Considering the selectivity of *E. affinis* confirmed by our observations on recent samples, it seems rather unlikely that *E. affinis*, which is one of the dominant zooplanktonic grazers in the Scheldt, would be able to regulate these blooms. Nevertheless, to really assess its potential impact on phytoplankton, it will be first necessary to know if the *E. affinis* populations stabilize upstream Antwerpen, in the freshwater part of the Scheldt estuary.

4. Conclusion

To conclude, the fact that *Eurytemora affinis* now feeds less on phytoplankton and more on heterotrophic organisms is likely. Although no improvement of feeding conditions in comparison to the nineteen nineties could be shown, the present data show that feeding conditions for *E. affinis* at present are at least as good as before the restoration of the Scheldt estuary took place. Feeding conditions represent one possible cause to explain the recent expansion of the copepod upstream in the estuary, but remains to debate. Other causes, like

water quality influence or interactions with zooplankton organisms, are in progress (Mialet et al., submitted).

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Chapitre 4

Etude de la sélectivité trophique des organismes benthiques dans un milieu à gradient de proies potentielles

Dans le milieu estuarien, le comportement sélectif d'*Eurytemora affinis* envers le phytoplancton a été démontré, ce qui a permis d'établir une courbe d'efficacité de cette sélection en fonction du gradient de phytoplancton disponible dans la colonne d'eau (Tackx et al., 2003). Le second objectif de ce travail de thèse, est de tester la validité de ce modèle dans un milieu aquatique différent du milieu estuarien. Pour pouvoir tester ce modèle, plusieurs conditions sont à vérifier au préalable dans ce milieu :

- La présence d'une gamme variée des valeurs du ratio « proies préférentielles / proies potentielles totales », formant un gradient sur une échelle spatiale ou temporelle.
- La présence de taxons de consommateurs primaires dominants présents sur la totalité de ce gradient.
- Le comportement sélectif de ces organismes envers un type de proies.

De par les fréquentes perturbations naturelles auquel il est soumis et la diversité des communautés animales, végétales et microbiennes qui le composent (Majdi et al., soumis), le biofilm phototrophe de la Garonne a été choisi comme support d'étude pour tester ce modèle. L'étude de sa dynamique temporelle associée à celle de la méiofaune qui le compose (Majdi et al., soumis), présentée en annexe I, permettra de vérifier deux des trois conditions présentées ci-dessus.

La première condition est la présence d'un gradient du ratio « proies préférentielles / proies potentielles totales » pour des organismes phytophages, comprenant une gamme de valeurs relativement large sur une échelle temporelle ou spatiale. La deuxième est la présence de taxons méiobenthiques dominants présents sur la totalité de ce gradient.

La vérification de la troisième condition nécessaire au test du modèle, à savoir la mise en évidence du caractère sélectif de ces taxons dominants envers un type de proies, sera l'objet de l'article présenté à la fin de ce chapitre.

Contribution personnelle à cette étude dans le cadre du travail de thèse

Les différentes sorties de terrain et manipulations en laboratoire ayant contribué à l'élaboration de l'article présenté en Annexe I sont détaillées ci-dessous. Les détails propres aux matériels et méthodes employés sont explicités dans l'article de Majdi et al. (soumis).

Campagne 1 :

- Extractions et mesures des pigments photosynthétiques de l'épilithon par HPLC (96 échantillons sur 96)
- Tri et dénombrement de la méiofaune (56 échantillons sur 68)

Campagne 2 :

- Optimisation de la méthode (surfaçage des galets par photographie, mesures HPLC)
- Echantillonnages du biofilm épilithique de rivière (10 sorties terrain sur 57)
- Pesées de la biomasse du biofilm épilithique (matière sèche sans cendres) et surfaçage des galets (40 échantillons sur 228)
- Création et gestion d'une base de données ACCESS regroupant toutes les données de l'étude.

Analyses statistiques (30 %) et rédaction (10 %) de l'article.

Analyse du gradient de proies potentielles dans le biofilm épilithique de la Garonne

Dans les travaux de Tackx et al. (2003), la concentration en chlorophylle *a* du milieu est utilisée pour caractériser la concentration en proies préférentielles du copépode : les algues phytoplanctoniques. La concentration en matières en suspension, représentant l'ensemble des particules en suspension, organiques et inorganiques, est utilisée pour caractériser la concentration en proies potentielles totales. Pour l'étude du biofilm épilithique de la Garonne, les facteurs mesurés par Majdi et al. (soumis) qui se rapprochent le plus de ceux considérés par Tackx et al. (2003) sont la concentration en chlorophylle *a* du biofilm epilithique

(caractérisant la biomasse totale des microphytes du biofilm constituant des proies potentielles pour la méiofaune) et le poids sec des matières sèches du biofilm (MS) qui représente l'ensemble des éléments organiques et inorganiques de l'épilithon. Le rapport « Chl *a* / MS » représentera donc la disponibilité en proies potentielles dans ce milieu.

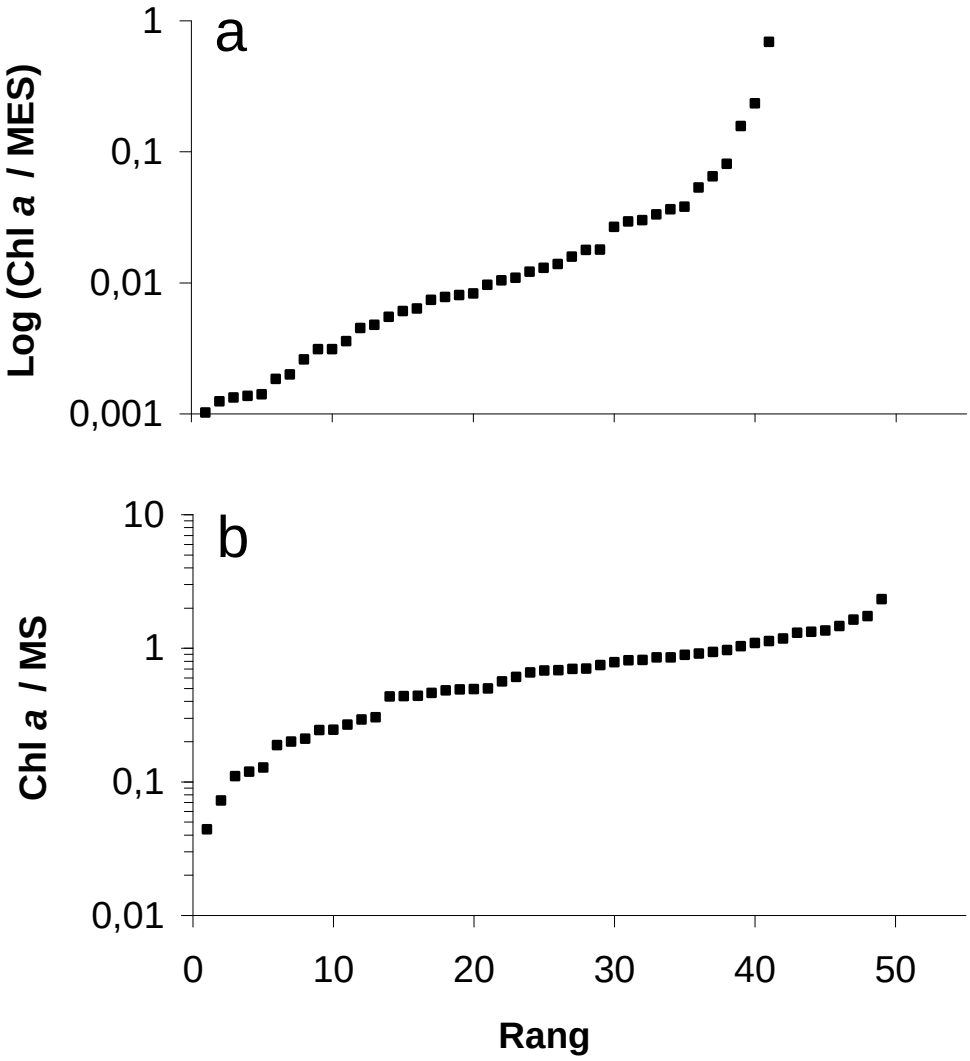

Fig. 1. Comparaison des valeurs totales représentant le rapport « proies préférées / proies potentielles totales » pour des organismes phytophages, dans deux milieux aquatiques : (a) la colonne d'eau de l'estuaire de l'Escaut (Tackx et al., 2003) et (b) le biofilm épilithique de la Garonne (Majdi et al., soumis).

Dans la colonne d'eau de l'Escaut, la plus grande valeur du rapport « Chl *a* / MS » est environ 600 fois plus importante que la plus petite (Fig. 1). Dans le biofilm de la Garonne, la plus grande valeur de ce rapport est environ 50 fois plus importante que la plus petite. Ceci présente la possibilité de tester le modèle dans des gammes différentes de disponibilité des proies préférées sur les proies potentielles totales. Une autre différence est que, dans le biofilm, les valeurs du gradient Chl *a* / MS sont distribuées de manière plus régulière que dans le milieu estuarien. La première condition nécessaire pour pouvoir tester le modèle est donc vérifiée.

Présence de taxons dominants à l'échelle du gradient de proies potentielles

La densité des principaux groupes méiobenthiques associés au biofilm épilithique de la Garonne a été comparée au gradient « Chl *a* / MS » pour vérifier la réalisation de cette condition (Fig. 2). Dans cet objectif, une limite de faisabilité a été fixée pour chaque groupe. En effet, si un organisme est présent mais en trop faible densité, il ne sera pas possible d'isoler suffisamment d'individus dans un délai raisonnable, pour pouvoir faire des mesures correctes de ses contenus digestifs. La limite concernant les chironomides est basée sur les travaux de Goldfinch et Carman (2000). Celle des rotifères est tirée des travaux présentés dans l'article en fin de chapitre. Enfin, celle des nématodes fais l'objet de travaux en cours (Majdi et al., in prep.).

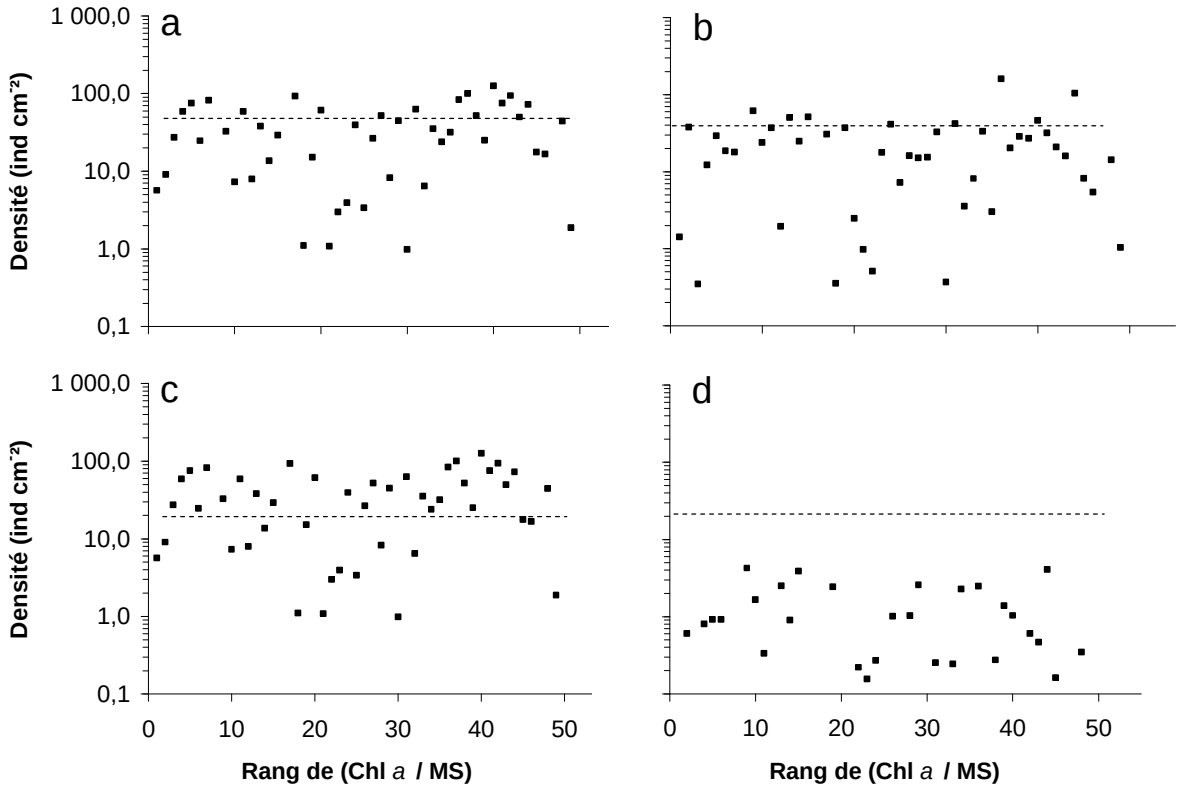

Fig. 2. Densité en organismes méiobenthiques en fonction des valeurs du rapport « Chl *a* / MS» du biofilm épilithique de la Garonne : (a) rotifères, (b) nématodes, (c) larves de diptères, (d) oligochètes (valeurs moyennes, n = 4). La ligne pointillée représente la limite pratique de faisabilité (voir texte).

En général, les rotifères, nématodes et oligochètes apparaissent en abondances suffisantes tout le long du gradient du rapport « Chl *a* / MS» pour pouvoir faire l'objet de mesures pigmentaires en laboratoire (Fig. 2), ce qui indique que la deuxième condition nécessaire pour pouvoir tester le modèle est vérifiée. Les chironomides ont déjà fait l'objet d'une étude basée sur des mesures de pigments dans leurs contenus digestifs par HPLC (Goldfinch and Carman, 2000). En revanche, aucune mesure de contenu digestif n'a été rapportée pour les nématodes et les rotifères (benthiques et planctoniques). Des études expérimentales effectuées en milieu pélagique ont déjà suggéré une sélectivité trophique des rotifères planctoniques sur la communauté algale (Chotiyaputta and Hirayama, 1978; Gilbert and Bogdan, 1984). C'est pourquoi ce groupe a été choisi comme sujet pour tester son comportement trophique sélectif dans les biofilms de la Garonne. Des travaux similaires sur la sélectivité des nématodes dans le biofilm de la Garonne sont aussi en cours (Majdi et al., in prep).

Mesure par HPLC des contenus digestifs des rotifères bdelloïdes dans un biofilm épilithique de rivière.

L'article suivant est en cours de rédaction.

Résumé de l'article

Introduction et objectifs

Le biofilm epilithique phototrophe de la Garonne (France) est composé en proportion importante de microalgues benthiques (Leflaive et al., 2008; Majdi et al, submitted). La contribution des cyanobactéries, des microalgues vertes et des diatomées varie en fonction des perturbations saisonnières auxquelles cet écosystème est soumis, même si les diatomées dominent la communauté (Eulin and Le Cohu, 1998; Majdi et al, submitted). De nombreuses espèces de rotifères bdelloïdes et de nématodes, sont supposées adopter un régime alimentaire phytophage.

L'objectif principal de cette étude est de réussir à mesurer le contenu digestif en pigments photosynthétiques des rotifères bdelloides issus de leur micro-habitat naturel (ici le periphyton) afin de mieux connaître leur régime alimentaire *in situ*. Il s'agira aussi de mettre en évidence un éventuel comportement trophique sélectif envers certains groupes d'algues, en comparant les rapports pigmentaires issus de leurs contenus digestifs avec ceux mesurés dans le biofilm épilithique.

Principaux résultats et discussion

L'analyse pigmentaire par HPLC a permis de quantifier la fucoxanthine, de la myxoxanthophylle et en moindre quantité de la lutéine, dans les rotifères prélevés *in situ*. Ces pigments biomarqueurs témoignent de l'ingestion de diatomées, de cyanobactéries et de

micro-algues vertes. Cette étude constitue une première preuve directe de l'ingestion *in situ* de cyanobactéries par ces organismes.

Une analyse de sélectivité a été faite en utilisant l'indice *D* de Jacobs (1974) et les rapports pigmentaires du biofilm et des rotifères. Elle suggère un comportement très sélectif des rotifères envers les cyanobactéries, et au contraire une tendance à l'évitement des microalgues vertes et des diatomées, ces dernières étant pourtant dominantes dans le milieu. Ce résultat se retrouve dans les trois échantillons prélevés à des années différentes. L'hypothèse de la sélection des algues selon un critère de taille est avancée, étant donné la taille réduite des cyanobactéries en comparaison aux diatomées et aux microalgues vertes (Leflaive et al., 2008).

Note concernant la poursuite des travaux

Dans l'optique de renforcer les résultats présentés dans cet article, plusieurs manipulations en laboratoire sont prévues. Premièrement, le jeu de données sera étendu à trois répliquas par échantillon, afin de confirmer la validité des résultats. La durée de cette opération est estimée à 4 semaines. Deuxièmement, une identification si possible à l'espèce, ou à défaut au genre, des rotifères issus des échantillons congelés sera faite, afin d'avoir une idée précise de la composition spécifique des communautés étudiées. Troisièmement, un échantillonnage automnal de biofilm épilithique est prévu, dans le but de répondre à deux objectifs. Le premier est de prélever des rotifères vivants en nombre suffisant et de les faire jeûner afin de constituer un échantillon témoin pour l'analyse des pigments des rotifères. Le second est d'obtenir un échantillonnage automnal supplémentaire pour équilibrer le jeu de données, avec un total de deux échantillons pour chaque saison (printemps et automne). La durée du traitement de l'échantillon supplémentaire récolté sur le terrain (matière sèche et matières sèche sans cendres de l'épilithon, comptage de la méiofaune, mesures pigmentaires du biofilm, contenus pigmentaires des rotifères) est estimée à environ 2 semaines.

Le traitement de l'échantillon témoin consiste à mesurer le contenu pigmentaire des rotifères à jeun par HPLC. Cette mesure correspondra au contenu « extra digestif » en pigments des rotifères, après passage du bol alimentaire dans le tube digestif. Il sera ainsi possible de

préciser les mesures de contenus digestifs réelles en déduisant cette mesure à celles *in situ* précédemment réalisées sur les rotifères. La première étape sera d'isoler entre 600 et 1000 individus vivants dans une même journée. Cette opération minutieuse nécessite le travail réuni de 3 personnes qualifiées sur une journée complète, sans interruption. Les rotifères seront ensuite placés dans une boite de pétri contenant de l'eau de Garonne au préalable filtrée sur 0,2 µm pour retirer toute présence d'algues et de bactéries, à température égale à celle du milieu de prélèvement. Les rotifères jeûneront ensuite durant 24 heures, suivant la méthode de Zhou et al. (2009). Enfin, l'échantillon témoin sera traité avec la même méthode que les échantillons directement issus du milieu naturel.

Measuring gut pigment content of bdelloid rotifers in epilithic biofilm by HPLC analysis

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Abstract

In situ gut content of epilithon-associated rotifers of the Garone river (France) were measured by HPLC. Isolating samples of 597 to 946 individuals permitted to detect several pigments among which fucoxanthin, lutein and myxoxanthophyll, characteristic for diatoms, green microalgae and cyanobacteria respectively. Feeding selectivity between green microalgae, diatoms and cyanobacteria was assessed and suggested that, under various conditions of cyanobacteria relative biomass, bdelloids always feed selectively on cyanobacteria. This study, which is to the best of our knowledge the first to report in situ gut contents of epilithon-associated rotifers, raises questions considering that cyanobacteria are known for their toxicity and are considered as less nutritive than diatoms.

Keywords: Bdelloids, Gut contents, HPLC, Periphyton, Pigments, Rotifers, Selectivity

1. Introduction

The epilithic periphyton , also named epilithic biofilm or epilithon, is a complex assemblage of organisms (bacteria, fungi, algae, heterotrophic protozoans, meiofauna and macrofauna) embedded in a mucous matrix of exopolymeric substances together with particulate organic matter and sediment (Lock, 1993; Gaudes et al., 2006; Majdi et al, submitted). Epilithic periphyton grows on any hard substrate submerged in lotic or lentic freshwater. During an annual cycle, the epilithic periphyton of the Garonne river is subjected to alternating disturbed flood periods and low water calm periods. The algal community composition, which is an important contributor of the total epilithic periphyton biomass in the Garonne river is controlled by this seasonal influence (Leflaive et al., 2008; Majdi et al, submitted). Even if diatoms largely dominate the microalgal community, they occur in fluctuating abundances with green algae and cyanobacteria (Eulin and Le Cohu, 1998; Majdi et al, submitted). The detailed taxonomic composition, as observed in 2004 and seasonal succession, as observed in 2008, of the periphytic microalgal community of the Garonne river are given in Leflaive et al. (2008) and Majdi et al. (submitted) respectively.

Meiofauna are by definition motile benthic invertebrates that pass through a 500 µm sieve but are retained on a 42 µm sieve (Giere, 2009). They are considered as influencing microphytobenthic biomass, bacterial activity and organic matter dynamics through bioturbation, grazing and excretion (Borchardt and Bott, 1995; Montagna et al., 1995; Traunspurger et al., 1997; Hakenkamp and Morin, 2000). Rotifers are a permanent and important component of meiofauna associated to epilithic periphyton in the Garonne river (Majdi et al., submitted). Contrarily to monogonont rotifers which feed on a large variety of preys (Ricci and Balsamo, 2000) and to the one bdelloid species known to be carnivorous (Ricci et al., 2001), bdelloid rotifers are known as consumers of small algae, bacteria, and yeasts either by filtering or scraping or browsing (Melone et al., 1998). Most species can consume a wide variety of food items because the size of food seems to be the most discriminating factor (Ricci and Balsamo, 2000). To our knowledge, literature concerning *in situ* studies about rotifers feeding in freshwater ecosystems is very poorly documented (Rundle et al., 2002) and mostly concerns pelagic organisms. This can be explained by the fact that epilithon-associated rotifers are embedded in a complex organic matrix which complicates isolating them. Thus, it is still not known whether epilithon-associated rotifers

selectively feed *in situ* on certain groups of microalgae depending on their relative availability in the periphyton.

The method first introduced by Mackas and Bohrer (1976) to measure zooplanktonic microalgal gut chlorophyll *a* contents by fluorescence from *in situ* samples has been since widely applied on pelagic and benthic organisms, implying both fluorescence and HPLC pigment quantification (i.e. Kleppel and Pieper, 1984; Quiblier et al., 1994). In the present study, we adapted the method described by Buffan-Dubau et Carman's (2000b), who measured biomarker carotenoid pigments in gut contents of marine meiobenthic harpacticoid copepods, for application to epilithon-associated rotifers.

This paper examines the grazing activity of epilithon-associated bdelloid rotifers under field conditions in the Garonne river. The first objective was to test the feasibility of measuring by HPLC method the biomarker carotenoids in gut pigment contents of *in situ* bdelloid rotifers living in a very complex medium such as epilithic biofilm. The second objective was to examine if any selective feeding could be detected.

2. Material and methods

2.1. Study site

The Garonne is the largest river of south-western France. It displays a pluvio-nival flow regime characterized by a spring flood period due to snow-melt followed by a long low-flow period which can remain uninterrupted for the rest of the year (if no stormflow occurs). In the Garonne, alternate cobble bars are frequently found even in channel up to the seventh-order. During low-water periods, a high epilithic biomass can be found on pebbles and cobbles, favoured by low water velocities on the river bed (< 0.5 m s^{-1}) and low turbidity (Boulêtreau et al., 2006; Leflaive et al., 2008). The study site was situated on one of these cobble bars located at 36 km upstream the city of Toulouse (01°17'53"E, 43°23'45"N; elevation 175 m a.s.l., sixth-order) (Fig. 1).

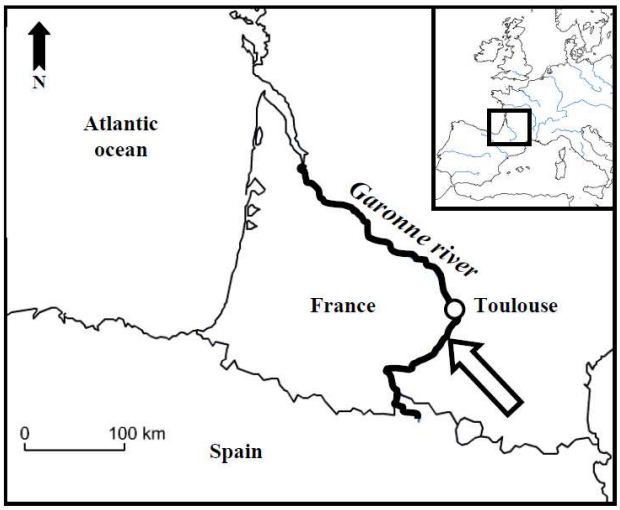

Fig. 1. Location of the study site (white arrow) on the Garonne river.

Samplings from three dates: 19th November 2008, 1st April 2009 and 15th March 2010 were selected from the monitoring campaigns of the periphyton of the Garonne river, presented in Majdi et al. (submitted). These dates were chosen in order to facilitate analysis of rotifer gut contents because they corresponded to the highest densities of rotifers found during the monitoring They also correspond to low-water, moderate discharge (72 to 101 m^3 s^{-1}) and temperate (5.3 to 9.2 °C) periods.

2.2. Epilithon sampling and conservation

At each sampling, 7 immerged cobbles were hand collected using plastic bags to prevent loss of biofilm fractions. For rotifer pigments analyses, epilithon covering three cobbles was immediately collected by scraping-off their upper surface with a scalpel. The epilithon sample was immediately preserved in liquid nitrogen.

For epilithic pigments analyses, four cobbles (four replicas) were transported in a coolbox to the laboratory within 2 h to prevent pigment degradation. At the laboratory, epilithon was then collected by scraping-off the upper surface of each cobble with a scalpel and a toothbrush rinsing with MilliQ water. Then, the four obtained epilithon suspensions were centrifuged (3220 g, 20 min) to obtain pellets which were freeze-dried at -80 °C. Following this, each pellet was weighted and thoroughly homogenized. All Samples were stored at -80 °C until pigment extraction for HPLC analysis. HPLC pigment analyses were performed

within months following samplings. Sampled epilithon areas were photographed and scrubbed surfaces were calculated by computer image analysis, using ImageJ software version 1.38 (Rasband, 1997).

2.3. Rotifer dry mass

Rotifer dry masses were assessed from microscopic pictures. For each sample, all rotifers were counted and 100 individuals were randomly measured, with ImageJ software version 1.38 (Rasband W. S., 1997; ImageJ. Bethesda, MD, U.S.A.: National Institutes of Health). Rotifer wet biomass was calculated according to (Friedrich, 1997) : WM (ng) = 0.26 L W^2 x 1.028. L is the length (µm), W is the width (µm), and 1.028 is the specific gravity. A ratio of 0.1 was used to estimate the dry mass (ng) from the wet mass (Baguley et al., 2004). Mean individual dry mass of rotifers was assessed by dividing the sum of dry masses by the number of individuals measured in each sample.

2.4. Pigment extraction

For epilithic algal pigments extraction, 250 mg of each homogenised pellet were extracted 3 times in a total volume of 25 ml (10, 10, and 5 ml) 98 % cold-buffered methanol (with 2 % of 1 M ammonium acetate) following Buffan-Dubau & Carman (2000a) for 15 minutes at -20 °C. Algal pigment release was favoured at each extraction step by an ultrasonication probe (30 seconds, Sonifier 250A, Branson Ultrasonics corp., 200 Danbury, CT, U.S.A.). Then, 1 ml of the total pigment solution was filtered (0.2 µm PTFE syringe filter) and HPLC analyzed.

For rotifer pigments extraction, for each sample, a fragment of frozen epilithon sample was suspended in tap water in a bucket, using a water jet to facilitate separation of meiofauna from inorganic matter according to the elutriation method (Uhlig et al., 1973). After 2 minutes of decantation, the supernatant containing the organic fraction was sieved trough 40 µm. Because of the very adhesive power of its foot-gland, the rotifer *Proales sp* was not assayed for pigments, but generally, the relative abundance of this species was minor (on average

between 10 and 20 %, not shown). Small groups of 10–40 rotifers were extracted from epilithic biofilm samples under a stereomicroscope (with x 60 magnification) using a needle picker and a petri dish on ice and under minimum light exposure to prevent pigment degradation. Each group of individuals was meticulously removed from the petri dish with a 10 µl pipette and then washed in a cold milliQ water bath to remove biofilm particles as much as possible. Following this procedure, respectively 597, 693 and 946 individuals were photographed for the 3 samples in chronological order, isolated and placed in 500 µl of milliQ water. Rotifers samples were then kept frozen at -80 °C until freeze-drying within a week. Considering the fact that this operation was very time consuming, no replicates have been made. Pigments were extracted from rotifer freeze-drying samples by an ultrasonication cuve, for 90 seconds in 200 µl of 98% cold-buffered methanol (with 2% ammonium acetate 1M) following Buffan-Dubau & Carman (2000a), then by incubating for 20 minutes at -20 °C in darkness. The total pigment solution was then filtered (0.2 µm PTFE syringe filter) and immediately analyzed by HPLC.

2.5. Pigment measurements by HPLC

HPLC pigment analyses were performed using a liquid chromatograph consisting of a 100 µl loop auto-sampler and a quaternary solvent delivery system coupled to a diode array spectrophotometer (LC1200 series, Agilent technologies inc., Santa Clara, CA, U.S.A.). The mobile phase was prepared and programmed according to the analytical gradient protocol described in Barlow et al. (1997). Pigment separation was performed through a C8, 5 µm column (MOS-2 HYPERSIL, Thermo Fisher scientific inc., Waltham, MA, U.S.A.). The diode array detector was set at 440 nm to detect carotenoids, at 665 nm for chlorophylls and at 665 nm for pheopigments (Wright et al., 1991). Data analysis was performed using ChemStation software (version A.10.02, Agilent technologies inc.). Pigments were identified by comparing their retention time and absorption spectra with those of pure standard pigments (DHI LAB products, Hørsholm, Denmark). Each pigment concentration was calculated by relating the peak area of its chromatogram (Fig. 2) with the corresponding area of calibrated standard.

For epilithic algae, pigment concentrations were related to the corresponding area of epilithon scraped. Rotifer specific gut content (Gs) was calculated by dividing the amount of pigments measured in rotifers by the number of individuals and by the mean individual dry mass.

Total chlorophyll *a*, expressed as "Chl *a*-eq" was quantified by the sum of chlorophyll *a*, chlorophyll *a*-like, pheophorbide *a* and pheophytin *a*. "Chl *a*-eq" was considered as a proxy for total algal biomass. Fucoxanthin, lutein and myxoxanthophyll concentrations were used as specific pigments respectively for diatoms, green algae and cyanobacteria biomasses, following Jeffrey et al. (1997). As suggested by Mohamed and Vermaas (2004), myxoxanthophyll is a better indicator of cyanobacteria biomass than zeaxanthin, which is also a pigments of some green algae. Fucoxanthin and chlorophyll *a* pigments that were spectrally similar to but did not have the same retention time as standards were designated "like-pigments", and were quantified using the response factor obtained from standards

2.6. Data analysis

The relative contributions of each microalgal group (*i.e.* diatoms, green algae and cyanobacteria) to total microphytic epilithic biomass (Chl *a* concentration of epilithon samples) were estimated from biomarker carotenoid concentrations using CHEMTAX version 1.95 software (Mackey et al., 1996). This method was described in details in Majdi et al. (submitted).

Feeding selectivity was expressed was expressed as *D* (Jacobs, 1974). *D* is defined as:

$$D = \frac{(r - p)}{(r + p - 2rp)}$$

Where *r* is the proportion of a pigment measured in the body and *p* the proportion of the same pigment in the medium. Proportions are given by the ratio of each pigment to Chl *a*-eq. *D* ranges from -1 to +1 and is symmetrical around zero. *D* = 0 indicates unselective feeding; positive values indicate selection for; and negative values selection against the algae containing the pigment.

3. Results

Pigments detected in rotifers were chlorophylls *c*, fucoxanthin, diadinoxanthin, myxoxanthophyll, zeaxanthin, lutein (not shown), chlorophyll *a*, pheophytin *a*, α and β–carotene and of some unidentified pigments (Fig. 2, Table 1). No pheophorbide has been found in rotifers whereas pheophytin was easily detected at 665 nm in the biofilms (Table 1).

Lowest epilithic biomass, chlorophyll *a* concentration and meiofaunal density were observed in November 2008 (Table 2). Epilithic microalgal community mainly comprised diatoms, green microalgae and cyanobacteria (Table 2). Diatoms dominated epilithic microphytic biomass while proportions of green algae and cyanobacteria were respectively low and very low (Table 2). In November 2008 however, the dominance of diatoms was less important and relative biomasses of green algae and cyanobacteria were higher than those estimated for April 2009 and March 2010.

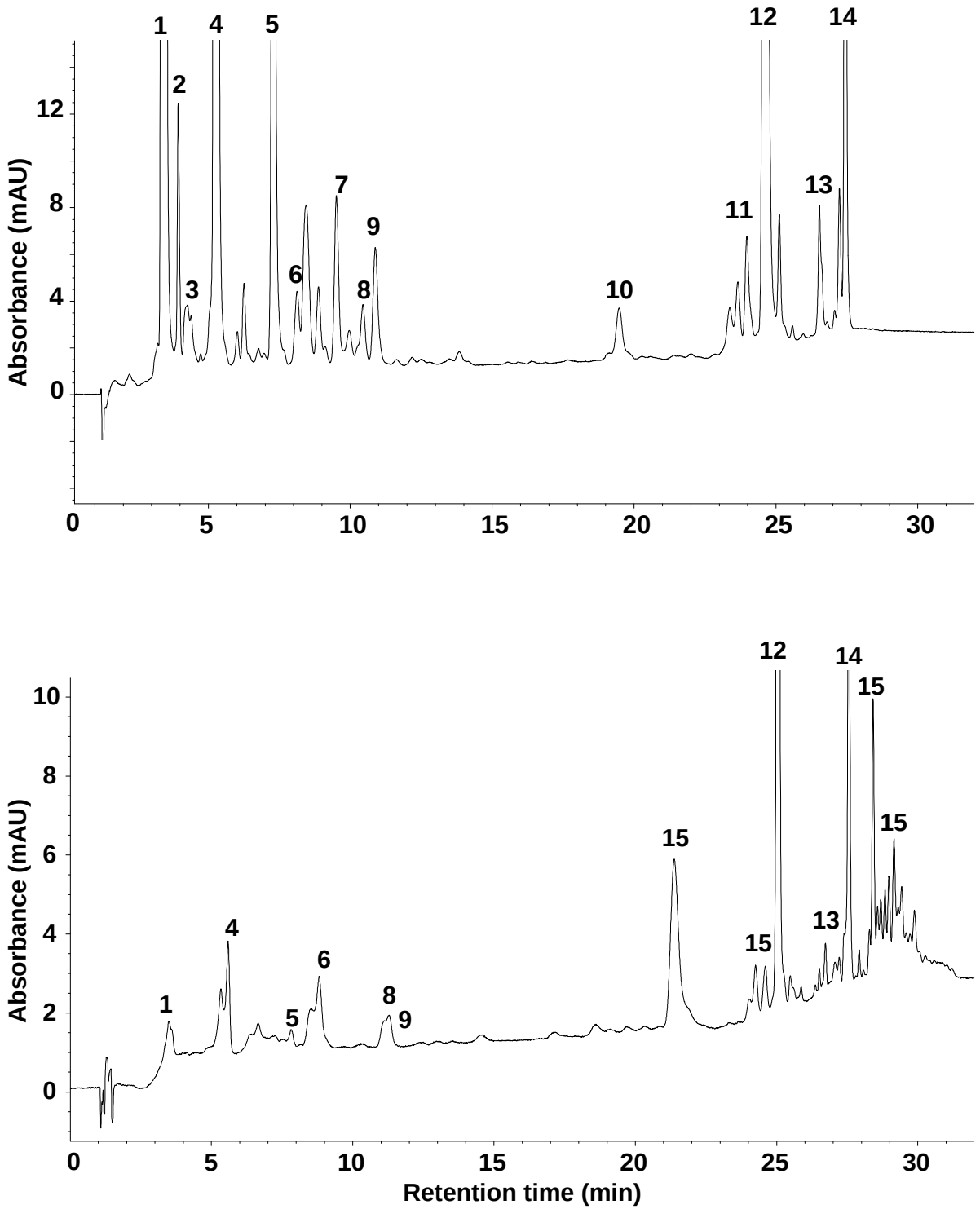

Fig. 2. Examples of HPLC absorbance chromatogram (at 440 nm) for (a) a phototrophic epilithic biofilm sample, (b) a rotifer sample (946 isolated individuals): 1–chlorophylls *c*; 2–fucoxanthin like; 3–pheophorbide *a*; 4–fucoxanthin; 5–diadinoxanthin; 6–myxoxanthophyll; 7– zeaxanthin like; 8–zeaxanthin; 9–lutein; 10–chlorophyll *b*; 11–chlorophyll *a* like; 12–chlorophyll *a*; 13–pheophytin *a*; 14–α and β–carotene; 15–unidentified pigments.

Table 1. HPLC analysis of major pigments in periphyton and rotifer extracts. Pigments are listed following their elution order. Values are means ± SD (n = 4) for periphyton extracts. For rotifer extracts, values were from 597 to 946 individuals; highest and lowest mean concentrations over all samples are given. Likely algal sources were determined for the biomarker pigments according to the literature references listed in the table (Ref): 1, Jeffrey et al (1997); 2, Mohamed and Vermaas (2004). C: cyanobacteria, D: diatoms; GA: green microalgae. Symbols are as follows: *, detected but not quantified; ND, pigment not detected.

No.	Pigment	Periphyton concentration ($\mu g\ m^{-2}$)	Rotifer concentration ($pg\ \mu g_{DW}^{-1}$)	Prob. Source	Ref.
1	Chlorophyll *c*	9.6 ± 1.7 – 100.8 ± 35.3	*		
2	Fucoxanthin like	*	ND		
3	Pheophorbide *a*	0.6 ± 0.1 – 3.8 ± 2.9	ND		
4	Fucoxanthin	35.6 ± 4.0 – 419.7 ± 124.7	0.1 – 0.4	D	1
5	Diadinoxanthin	4.7 ± 0.6 – 77.4 ± 14.0	*		
6	Myxoxanthophyll	0.3 ± 0.1 – 3.0 ± 2.2	0.4 – 0.5	C	2
7	Zeaxanthin like	*	ND		
8	Zeaxanthin	0.8 ± 0.9 – 3.0 ± 1.1	0.1 – 0.2	C, GA	1
9	Lutein	1.1 ± 0.4 – 3.9 ± 2.4	0.001 – 0.008	GA	1
10	Chlorophyll *b*	1.4 ± 0.5 – 5.9 ± 4.4	ND	GA	1
11	Chlorophyll *a* like	*	*		
12	Chlorophyll *a*†	155.2 ± 33.1 – 811.2 ± 266.9	2.0 – 5.4		
13	Pheophytin a‡	2.4 ± 1.1 – 16.9 ± 13.9	0.5 – 0.7		
14	α and β–Carotene	10.4 ± 3.0 – 13.6 ± 2.2	*		

† Chlorophyll *a* quantification = Chlorophyll *a* + Chlorophyll *a* like.

‡ One Pheophytin *a* like compound was additionally detected using the detector, Pheophytin *a* quantification = Pheophytin *a* + Pheophytin *a* like.

Table 2. Biomass, chlorophyll *a* concentrations, relative biomasses of algal groups assessed by CHEMTAX from pigments measurements, density and biomass of meiofauna of the epilithic biofilm (mean values ± SD, n = 4).

		Nov 2008	Apr 2009	Mar 2010
Epilithon ash free dry mass	mg cm^{-2}	2.8 ± 0.5	5.5 ± 0.8	15.7 ± 5.1
Chlorophyll *a* concentration	µg cm^{-2}	1.6 ± 0.3	4.0 ± 0.5	8.1 ± 2.7
Proportion of green algal biomass	%	19.6	5.5	1.8
Proportion of cyanobacterium biomass	%	4.0	0.3	traces
Proportion of diatom biomass	%	76.4	94.2	98.2
Rotifer individual mean dry weight	ng	35.0	45.3	34.6
Density of rotifers	ind cm^{-2}	35.3 ± 35.9	126.3 ± 24.4	83.3 ± 11.7

Specific gut pigment contents (Gs) of rotifers were between 0 and 0.5 ng µgDW^{-1} (Table 1, Fig. 3). Fucoxanthin Gs drastically varied between 0.08 and 0.44 ng μg_{DW}^{-1}. Lutein Gs were low for all samples, varying between 1 and 8 pg μg_{DW}^{-1}. Myxoxanthophyll Gs were the least variable values (between 0.36 and 0.50 μg_{DW}^{-1}) even if cyanobacteria availability in the biofilms (relative biomass) was very variable between samples (Table 2). Overall, these results indicate that rotifers ingested diatoms, cyanobacteria and green microalgae and suggested that diatoms and green microalgae were relatively ingested in lower proportions than cyanobacteria in epilithon.

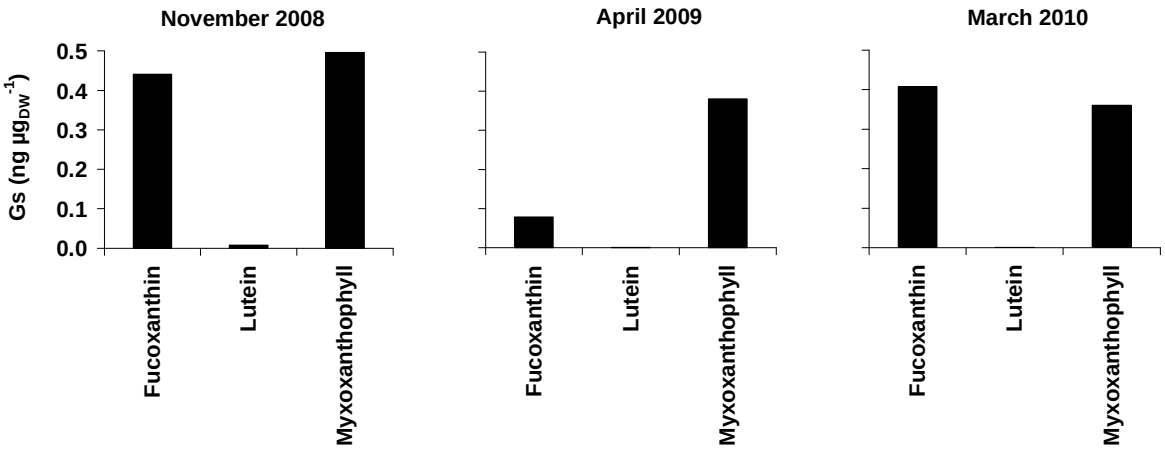

Fig. 3. Rotifer specific pigment contents (Gs) in diatoms (fucoxanthin), green algae (lutein) and cyanobacteria (myxoxanthophyll) in the epilithic biofilm of the Garonne river.

Biomarker pigments to Chl *a* ratios were compared between biofilm and rotifer samples to examine the feeding behaviour of rotifers in the epilithon (Fig. 4). The same pattern was found for the three samples analysed. *D* values were negative for fucoxanthin and for lutein with a similar degree for both pigments (between -0.9 and -0.6) suggesting that rotifers tended to avoid to ingest diatoms and green microalgae. Inversely, for myxoxanthophyll, *D* was highly positive (between 0.6 and 0.99) suggesting that rotifers fed selectively on epilithic cyanobacteria.

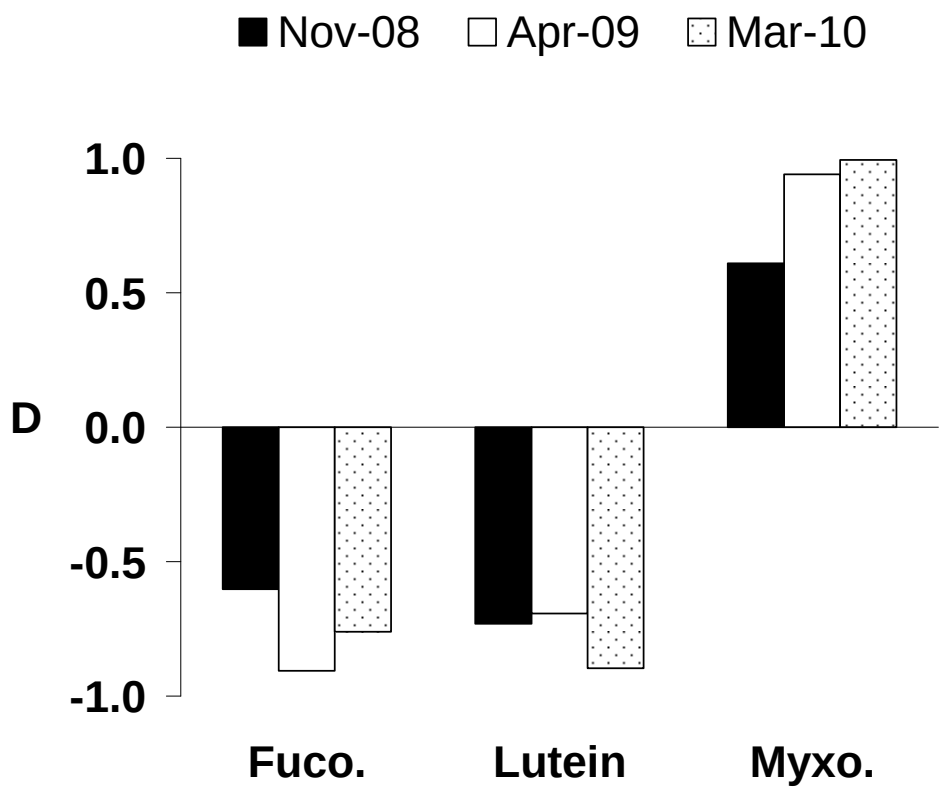

Fig. 4. Jacobs' (1974) index *D* values for epilithic rotifers for November 2008, April 2009 and March 2010. Microphytic biomarker pigments indicate feeding on diatoms (fuco: fucoxanthin), green microalgae (lutein) and cyanobacteria (myxo: myxoxanthophyll).

4. Discussion

The purpose of this study was to apply for the first time, the method of gut pigment HPLC analyses of microphytic biomarker pigments to epilithon-associated rotifers and examine their trophic behaviour under different conditions in the epilithon. These conditions were represented by the availability of the different microalgal groups.

4.1. HPLC analysis method for quantification of gut pigments of epilithon-associated rotifers

Ou results have shown that it is feasible to obtain quantifiable chromatogram peaks for both marker Chl *a* and marker pigments (fucoxanthin, myxoxanthophyll and lutein) by isolating 600 to 1000 individuals of bdelloid rotifers from biofilm samples. To the best of our knowledge, this is the first report of *in situ* gut pigment content of epilithic rotifers. Quiblier et al. (1994) already performed HPLC measurements on microzooplanktonic organisms taken *in situ*, but considered a whole zooplankton fraction which size was comprised between 80–315 µm. While the rotifer isolation procedure developed in this study is time consuming and requires a great precision and patience, it is feasible to obtain a sample within a total of 10–40 hours of isolation, be it divided in several sessions.

The stable isotope quantification could be an alternative way to inquiry about rotifer diet, but this method requires as many individuals per analysis as the HPLC analysis presented in this study (Goldfinch and Carman, 2000). Moreover, contrarily to stable isotopes, HPLC analysis allows distinguishing between the different microalgal types ingested by rotifers, even if they are not assimilated. Therefore, it represents a good way to compare concentrations of the main microalgal groups between epilithon-associated organisms and epilithon.

Present gut pigment contents could have been underestimated, due to their potential degradation during their passage through the gut. Indeed, Chl *a*:total pheopigment ratios in rotifers were on average 16 ± 3 % of those in the epilithic biofilm (Table 1) for all the samples. However, experimentation on carotenoid pigments degradation showed no distinct difference between the destruction rates of carotenoid and xanthophyll pigments, although

myxoxanthophyll (a xanthophyll pigment) was more labile than other cyanophyte carotenoids (Leavit, 1988). Considering this, an underestimation of myxoxanthophyll concentration in gut measurements of the present study would not have changed the clear trend showed by the results.

Pandolfini et al. (2000) measured variable breakdown of pigments during gut passage, depending on pigment type and on mesozooplankton grazer, *i.e.* cladocerans and copepods. In consequence, this author recommends caution in using gut pigments as indicators of cladocerans and copepods diets. However, previous feeding studies on microzooplankton (*i.e.* ciliates and heterotrophic flagellates) and epilithon-associated harpacticoid copepods suggested that carotenoids (fucoxanthin, zeaxanthin and β–carotene) were not significantly degraded during the gut passage and that fucoxanthin gut content constitute a good quantitative biomarker of diatom ingestion (Mostajir et al., 1998; Buffan-Dubau and Carman, 2000b). Mostajir et al. also found also found that pheophytin accounted for almost 100 % of the recorded pheopigments in microzooplakton. The same pattern was found in the present gut measurements, with no carotenoid-like pigments detected in rotifers and only pheophytin *a* as pheopigment (Table 1, Fig. 2b). Moreover, Strom et al. (1998) observed in protozoan cultures that large species (> 80 µm, which corresponds to rotifer size) degraded carotenoids less efficiently than the small (< 25 µm) ones. These two arguments support the hypothesis that the main concentrated carotenoid pigments detected in rotifer gut contents in the present study were likely not submitted to a significant degradation during the gut passage.

As for planktonic and benthic copepods, some carotenoid pigments could be used as photo-protectors (Hairston, 1976; Buffan-Dubau and Carman, 2000b) by rotifers. Thus it is possible that some carotenoid pigments were partly issued from the rotifer's body rather than from their gut content. Thus, a control measurement of pigment content issued from analyses of starved rotifer samples has to be done to correct quantification of gut pigments.

4.2. Cyanobacteria as a part of rotifer diet

Analyses using Jacobs' (1974) index *D* values showed that the relative concentration of myxoxanthophyll was higher in rotifer samples than in epilthic biofilm samples whereas fucoxanthin and lutein were relatively less concentrated in rotifer samples (Fig. 4). These

results suggest that rotifers fed highly selectively on cyanobacteria. This was observed for all samples. Previous studies showed that diatoms, with their high EPA (eicosapentaenoic acid, 20 : 5ω3) content, are generally considered as high-quality foods whereas cyanobacteria, with their both low EPA and P content, are low-quality food for zooplanktonic organisms, including rotifers (Gulati and DeMott, 1997). Thus, the question rises why rotifers would prefer to feed on cyanobacteria rather than on diatoms.

To our knowledge these results are the first field measurements that indicate that epilithon-associated rotifers ingest cyanobacteria. They are also the first field data supporting the hypothesis that meiofauna can feed on cyanobacteria *in situ*, even select them. Indeed, previous studies indicated that meiofauna organisms feed mainly on diatoms and on green microalgae (Buffan-Dubau et al., 1996; Buffan-Dubau and Carman, 2000b; Goldfinch and Carman, 2000). Furthermore, Buffan-Dubau & Carman (2000b) indicated that epilithon-associated harpacticoid copepods avoid to ingest cyanobacteria in marine superficial sediments. Thus, these results raise questions about the reasons for such a diet. A previous taxonomic study of epilthic microphytes indicated that three taxa of filamenteous cyanobacteria can be found in the epilithic biofilm studied in the present work (Table 3). Cyanotoxins produced by *Lyngbia sp.* and *Phormidium sp.* are know for their toxic effect (UNESCO, 1999). There is a report of toxic effects of *Lyngbya sp.* on planktonic rotifers (Snell, 1980) and some cyanobacteria can increase or decrease growth of planktonic rotifer populations in freshwater (Starkweather and Kellar, 1983; Weithoff and Walz, 1995). However, *Brachionus calyciflorus*, a freshwater planktonic rotifer, is known to be able to feed partially on cyanobacteria in laboratory experiments (Starkweather and Kellar, 1983). Moreover, filamentous cyanobacteria may cause severe inhibition of zooplankton filter-feeding process by mechanical interference, but rotifers are in general less sensitive to this interference than larger invertebrates (Lampert, 1987). These characteristics might be involved in the apparent selective behaviour of rotifers in the epilithic biofilms.

Table 3. Most microphytobenthic abundant taxa determined in epilithic biofilms of the Garonne river from February 2005 to February 2006 at the study site, associated with their equivalent spheric diameter (ESD) and their morphological type (C: colonial, F: filamentous, FU: fixed unicellular, FrU: free unicellular, NF: non filamentous). In bold, species which represent more than 5 % of total epilithic microphytobenthic biomass in at least one biofilm sample. Data issued from Leflaive et al. (2008).

Taxa	ESD (µm)	Type
Cyanobacteria		
Lyngbya sp.	3.1	F
Phormidium sp.	2.7	F
Phormidium uncinatum Gomont ex Gomont	8.0	F
Diatoms		
***Achnanthes biasolettiana* Grunow**	7.4	FU
Achnanthes sp.	7.1	FU
***Amphora libyca Ehernberg* (syn. *A.opulata* (Kützing) Schoeman & Archibald)**	12.5	FrU
Amphora ovalis (Kützing) Kützing	39.4	FrU
Cocconeis pediculus Ehrenberg	11.2	FU
***Cocconeis placentula* Ehrenberg**	20.6	FU
Cymbellaistula (Ehrenberg) Kirchner	10.5	C
***Cymbella helvetica* Kützing**	17.6	C
Cymbella lanceolata (Ehrenberg) Van Heurk	17.6	C
Cymbella minuta Hilse ex Rabenhorst	7.4	C
Cymbella prostata (Berkeley) Grunow	11.5	C
Cymbella tumida (Brébisson) Van Heurk	11.5	C
Cymbella tumidula Grunow	9.8	C
***Diatoma ehrenbergii* Kützing**	24.0	C
Diatoma mesodon (Ehrenberg) Kützting	12.5	C
Diatoma tenuis var. elongatum Agardh	9.6	C
***Diatoma vulgaris* Bory**	19.0	C
***Ellerbeckia arenaria* (Moore) Crawford**	30.7	F
Fragilaria capucina Desmazières	6.9	C
Fragilaria capucina var. vaucheriae (Kützing) Lange-Bertalot	9.2	C
Fragilaria ulna (Nitzsch) Lange-Bertalot	13.2	C
Gomphoneis minuta (Stone) Kociolek & Stoermer	13.2	C
Gomphonema olivaceum (Hronemann) Brébisson	10.6	C
Gyrosigma attenuatum (Kützing) Rabenhorst	18.8	FrU
***Melosira varians* Agardh**	19.2	F
Navicula capitatoradiata Germain	10.0	FrU
Navicula cryptotenella Lange-Bertalot	7.2	FrU
Navicula lanceolata (Agardh) Ehrenberg	11.0	FrU
***Navicula tripunctata* (O.. Müller) Bory**	11.1	FrU
Nitzschia heufleriana Grunow	14.4	FrU
***Nitzschia dissipata* (Kützing) Grunow**	12.1	FrU
Nitzschia frustulum (Kützing) Grunow	7.8	FrU
Nitzschia sp. 1	7.7–10.2	FrU
Nitzschia sp. 2	7.7–10.2	FrU
Rhoicosphenia abbreviata (C. A. Agardh) Lange-Bertalot	13.2	FU
Green algae		
Cladophora sp.	16.1	F
Closterium sp.	11.3–19.5	NF
Cosmarium sp.	16.2	NF
Rhizoclonium sp.	16.1	F
Stigeoclonium sp.	7.3	F
Ulothrix sp.	7.3	F
Ulothrix zonata Kützing	23.7	F

A hypothesis is that they could select algae mainly according to their size which might be the primary factor influencing selective feeding as previously suggested by (Ricci and Balsamo, 2000) for lotic rotifers. Hansen et al. (1994) showed from Rothhaupt's work (1990) that the size (given in Equivalent Spheric Diameter, ESD) ratio between 5 species of the genera *Brachionus* (planktonic rotifers) and their optimal preys was comprised between 13.5:1 and 21:1. In the present study, mean rotifer ESD varied between 110 and 120 µm. Considering the range of this ratio, the optimal prey ESD corresponding to the present rotifers would be comprised between 5 and 9 µm. Excepted for *Phormidium uncinatum*, which was rarely found in the algal community (not shown), epilithic cyanobacterial ESD of the Garonne river were in majority ≤ 3.1 µm whereas diatom and green algal ESD ranged between 4–40 µm (Table 3). Among all microalgal taxa found in this biofilm, diatom ESD values were in range of the theoretical optimal prey ESD for epilithic rotifers. Thus, considering Hansen et al. (1994), diatoms algae, which also dominated the epilithic microphytic biomass, should better fit to rotifer diet than cyanobacteria. However, the optimal prey ESD for meiobenthic rotifers was assessed by Hansen et al. with only one monogonont genera (*Brachionus sp.*). Pagano (2008), using natural phytoplankton assemblages in experiments, showed a clear preference of *Brachionus calyciflorus* for algae which ESD was comprised between 11–18 µm rather than smaller ones as Hansen et al. suggested. Baer et al. (2008), analysing size selective feeding of a different *Brachionus* species on polystyrene latex beads from 1.6 to 20 µm in diameter, found a preference for a diameter of 4.5 µm. These are examples of different preferred sizes found for one single genera of planktonic rotifer. Thus, knowledge about rotifer size preferendum is lacking and the present study did not allow to conclude about the reason of this selective behaviour.

A simple regression indicated a significant and negative correlation ($p < 0.05$) between relative biomass of cyanobacteria in the epilithon the values of selectivity index *D* (issued from myxoxanthophyll relative concentrations). This would suggest that rotifers more intensively selected cyanobacteria when their contribution to total microphytic biomass was the lowest. However, the validity of this relation is limited, considering that it was based on only 3 samples. Further measurements will be made to verify this trend. Quiblier et al. (1994) and Scheda and Cowell (1988), suggested a high grazing impact of freshwater planktonic rotifers on phytoplankton. It would be interesting to assess in which extent epilithon-associated bdelloids are involved in regulating epilithic cyanobacteria, by laboratory experiments.

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Conclusion générale et perspectives

1. Etude de la réponse du zooplancton à la restauration de l'estuaire de l'Escaut

Ce travail s'insère en partie dans la continuité des travaux de Nathalie De Pauw (1975) et de Frédéric Azémar (2007) qui, par une approche taxonomique ont décrit la répartition spatiotemporelle et l'écologie du mésozooplacton de l'estuaire de l'Escaut. Le suivi de la qualité de l'eau et de la répartition du mésozooplancton de l'estuaire de l'Escaut, financé par le gouvernement flamand dans le cadre du programme OMES depuis le mois de novembre 1995 constitue un jeu de données conséquent sur le plan spatial (16 stations sur environ 150 km de cours d'eau) et temporel (14 années d'échantillonnages mensuels). L'intérêt d'un tel patrimoine scientifique est en partie illustré par ce travail de thèse qui a permis d'observer d'importantes modifications physicochimiques et biologiques survenues au sein de l'estuaire.

La tendance graduelle et flagrante vers une amélioration de la qualité de l'eau, déjà constatée il y a quelques années (Van Damme et al., 2005), a été confirmée par l'étude présentée dans le chapitre 1. Elle fait suite notamment à la réduction des apports en matière organique du bassin versant et en particulier en provenance de Bruxelles par le Rupel. En parallèle à la physicochimie du milieu, la répartition spatiale et temporelle du zooplancton a montré des changements importants mais plus abruptes. Premièrement constatée en 2001, l'intrusion printanière des copépodes calanoïdes en eau douce, plus particulièrement celle d'*Eurytemora affinis*, s'est subitement amplifiée depuis 2007 jusqu'à la fin de la période étudiée. La capacité de ce copépode calanoïde à envahir rapidement les milieux d'eau douce a été précédemment démontrée dans d'autres milieux côtiers de l'Amérique du Nord et d'Asie orientale (Lee, 1999). Cette étude a montré que, la qualité de l'eau peut être considérée comme un facteur jouant un rôle essentiel dans l'installation des populations de calanoïdes (*E. affinis* et autres espèces). L'abondance des populations de cyclopides a inversement chuté en eau douce durant la même période, suggérant une interaction importante entre les deux groupes, dont les répartitions spatio-temporelles ne se chevauchaient pas auparavant (Azémar, 2007). A notre connaissance, la littérature sur la compétition entre calanoïdes et cyclopides en milieu estuarien est peu documentée. Les expérimentations en mésocosmes de Soto et Hurlbert (1991) n'ont pas permis de tirer des caractéristiques générales sur la compétition entre les deux groupes et suggèrent qu'elle dépendrait fortement des espèces prises comme sujets. Des expérimentations en milieu contrôlé pourraient permettre d'étudier les interactions entre les

espèces de copépodes dominants observés (les calanoïdes *Acartia tonsa* et *Eurytemora affinis*, les cyclopides *Cyclops vicinus vicinus* et *Acanthocyclops trajani*) afin de mieux comprendre les mécanismes qui ont conduit aux changements constatés. Les interactions directes, comme la prédation sur les œufs et stades naupliens, devront être étudiées séparément des interactions indirectes, comme l'efficacité de prédation sur les proies autotrophes ou hétérotrophes. Ce type d'étude ferait intervenir de nombreuses combinaisons de situations et leurs réplicas associés. Il serait donc raisonnable de l'entreprendre sur des supports de taille réduite (microcosmes) afin qu'elle soit réalisable. D'autre part, cette étude traite les données issues de prélèvements uniquement effectués en surface. Or, les interactions biotiques potentiellement mises en jeu dans les changements observés ne se limitent pas à la colonne d'eau. Comme énoncé dans le chapitre 1, Feike et al. (2007) remarquent que la rapide diminution des abondances des copépodes calanoïdes (*Eurytemora affinis* et *Acartia tonsa*) de la lagune de Darß-Zingst (Allemagne) fait immédiatement suite à l'introduction d'une espèce invasive de polychètes benthiques. Les auteurs suggèrent une diminution des oeufs de durée des calanoïdes par une prédation ou une bioturbation causée par ce polychète. Afin de comprendre les interactions possibles entre compartiment pélagique et benthique, l'intérêt de suivre l'évolution des communautés benthiques de l'estuaire de l'Escaut est donc justifié.

L'étude présentée dans le chapitre 2 suggère, à travers des régressions multiples de type PLS, un rôle important de la concentration en oxygène dissous dans l'établissement des populations d'*E. affinis* dans le tronçon d'eau douce de l'estuaire. Une concentration seuil, évaluée à 1,3 mg l^{-1}, semble être une condition nécessaire à cet établissement. En revanche, si les analyses multivariées sont un outil pratique pour traiter les données récoltées dans un milieu aussi complexe et changeant qu'un estuaire, elles n'en restent pas moins limitées, notamment du fait de la covariabilité des facteurs environnementaux entre eux. De même, ces analyses se basent essentiellement sur des corrélations entre des facteurs environnementaux et des abondances d'organismes, qui ne constituent pas une preuve de liens de cause à effet. Ainsi, pour évaluer plus précisément la tolérance d'*Eurytemora affinis* à l'hypoxie, des expérimentations en laboratoire seront nécessaires. Sa tolérance à la salinité et à la température et leurs effets sur la fertilité et les stages de développement larvaires ont essentiellement été traités dans les travaux de Devreker et al. (Devreker et al., 2004; Beyrend-Dur et al., 2009; Devreker et al., 2009). Des expérimentations basées sur le même principe, avec la concentration en oxygène dissous comme facteur contrôlé pourraient constituer une clé pour une meilleure compréhension du mode d'adaptation d'*E. affinis* en eau douce.

2. Test du modèle de sélectivité trophique dans les biofilms épilithiques de la Garonne

Les mesures effectuées récemment dans l'estuaire de l'Escaut (cf. chapitre 3) n'ont pas permis d'obtenir une calibration concluante pour le modèle de sélectivité proposé par Tackx et al. (2003). L'hypothèse retenue comme la plus probable pour expliquer ce phénomène est un changement du régime trophique d'*E. affinis*, qui consommerait désormais plutôt du microzooplancton hétérotrophe. En effet, l'utilisation du modèle de sélectivité trophique basée sur des mesures pigmentaires n'est plus possible si le copépode ne se nourrit plus de façon sélective sur le phytoplancton. Une utilisation basée sur une quantification intestinale des proies hétérotrophes serait alors nécessaire s'il sélectionnait particulièrement les organismes hétérotrophes. Le dosage des acides gras polyinsaturés (PUFAs) des contenus digestifs permettrait de quantifier les proies préférentielles du copépode (Kainz et al., 2004) et ainsi, de tester à nouveau le modèle de sélectivité. Un régime plus bénéfique en termes de coût et de gain pourrait en effet être à l'origine de la tendance d'*E. affinis* à se déplacer vers la zone d'eau douce de l'estuaire, bien que la qualité de l'eau n'y est pas meilleure qu'en aval.

Dans la dernière partie de la thèse, nous avons réussi à quantifier des pigments biomarqueurs dans les contenus digestifs des rotifères associés à l'épilithon de la Garonne. Ceci permet de tracer leur ingestion et leur comportement trophique potentiellement sélectivif *in situ*. Le rôle du meiobenthos dans le fonctionnement trophique des biofilms est peu étudié, en grande partie à cause des difficultés techniques liées à l'isolation des organismes. L'application de la technique des pigments biomarqueurs sur des organismes de petite taille (50–200 µm) pourra donc contribuer à avancer dans l'étude des relations trophiques au sein des biofilms. Bien que, dans les biofilms épilithiques de la Garonne, le comportement sélectif des rotifères envers les cyanobactéries n'ait pas été démontré en totalité dans le chapitre 4, les résultats le suggèrent fortement. Nous pouvons donc considérer que les trois conditions nécessaires au test du modèle de sélectivité trophique (Tackx et al., 2003) sur le méiobenthos des biofilms de la Garonne ont été validées. L'étude est donc potentiellement réalisable. Cependant, une mesure des pigments biomarqueurs dans le contenu digestif de tels organismes représente en moyenne une semaine de temps de travail, en prenant en compte les sorties sur le terrain, la temps nécesssaire pour isoler suffisamment d'organismes dans un échantillon concentré en matière organique comme le biofilm épilithique et l'analyse HPLC. L'étude des contenus digestifs sur la totalité du gradient du ratio « Chl *a* / MS », basée sur approximativement une

vingtaine de mesures, requiert donc un investissement en temps de travail assez conséquent. D'autres méthodes, telles que l'analyse des contenus en isotopes stables ou en acides gras sont aussi couramment utilisées pour les études trophiques, toutefois, dans le cas de la méiofaune, elles ne constituent pas des alternatives moins exigeantes en termes de temps de travail requis.

Si la poursuite des travaux sur la sélectivité trophique des rotifères des biofilms de la Garonne s'avère concluante (cf. Chapitre 4), il serait intéressant de déterminer la pression de broutage effectuée par ces organismes sur la biomasse épilithique des cyanobactéries. Cette étude serait réalisable expérimentalement, en exposant des rotifères benthiques de la Garonne à des concentrations différentes de cyanobactéries des genres *Lyngbia sp.* et *Phormidium sp.* Il serait ainsi possible de distinguer la contribution biotique et abiotique dans la limitation de la biomasse cyanobactérienne de ces biofilms. Finalement, il sera intéressant de comparer le régime alimentaire des rotifères avec celui des autres organismes meiobenthiques dominants : les nématodes. Cela fait l'objet de la thèse de Nabil Majdi (en cours).

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ANNEXE

Meiofauna in epilithon: a temporal monitoring in the Garonne river (SW France)

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SUMMARY

1. Although meiofauna are abundant, diverse and functionally important, little is known about the factors influencing their seasonal dynamics in lotic epilithon. The purpose of this study was to examine the temporal dynamics of epilithic meiofauna over long time periods with regard to the influence of main environmental parameters.

2. Temporary and permanent meiofauna densities were monitored in the epilithon of the Garonne river (SW France) through a 15 months (2004–2006) and an 18 months (2008–2010) weekly sampling campaigns. Abiotic factors, total epilithic biomass, microalgal biomass and density of trichopter larvae were concomitantly monitored and associated to the distribution of meiofauna through canonical ordination to reveal the main factors affecting their temporal dynamics.

3. Nematodes and rotifers dominated the permanent meiofauna while chironomids dominated the temporary meiofauna. Overall, our study evidenced that meiofauna were an abundant—up to 319 nematodes, 127 rotifers and 32 chironomids per cm^2—and a persistent component of river epilithon throughout seasons and years, thus deserving more attention in future benthic studies.

4. Meiofauna were strongly associated to epilithic biomass variations. They were also positively influenced by long undisturbed periods whereas drastically reduced by floods. Nevertheless, permanent meiofauna appeared resilient to floods—particularly to stormflows.

5. Important decreases in meiofaunal density during early summer were not linked to floods and occurred concomitantly to abundance peak of trichopter larvae, suggesting either a direct or an indirect negative effect of trichopter larvae on meiofauna.

6. The potential impact of the self-generated detachment of the epilithon as well as the potential impact of cyanobacteria, diatoms and green algae availability on meiofauna was discussed.

Introduction

The epilithon, also named epilithic biofilm or epilithic periphyton is a complex assemblage of organisms (bacteria, fungi, algae, heterotrophic protozoans, meiofauna and macrofauna) embedded in a mucous matrix of exopolymeric substances together with particulate organic matter and sediment (Lock, 1993). Epilithon grows on any hard substrate submerged in lotic and lentic freshwater. When enough light is available, photoautotrophic organisms generally dominated the epilithon in terms of biomass (Peterson, 1996). Thus, in large—not shaded by riparian forest—and shallow rivers harbouring cobble banks such as the Garonne, epilithon can constitute the main site of primary production (Ameziane, Dauta & Le Cohu, 2003). Epilithon has been increasingly investigated in fluvial ecosystems because of its important influence on biogeochemical processes (Battin *et al.*, 2003) and its role in secondary production (Fuller, Roelofs & Fry, 1986), decomposition (Ford & Lock, 1987) and nutrient retention (Sauvage *et al.*, 2003; Teissier *et al.*, 2007).

Meiofauna are motile benthic invertebrates defined as passing through a mesh of 500 µm and retained on a mesh of 44 µm. Meiofauna can be divided in two functional groups depending on life cycle characteristics: the permanent meiofauna remain in meiofaunal size range all life long whereas the temporary meiofauna—mainly insect larvae in freshwater—pass only earlier larval stages in this size range (Giere, 2009). Meiofaunal organisms are abundant in streams (Hakenkamp & Morin, 2000). With a high production/biomass ratio, they may contribute substantially to benthic secondary production (Bergtold & Traunspurger, 2005; Stead, Schmid-Araya & Hildrew, 2005). Meiofauna are expected to regulate microalgae, bacterial activity and organic matter dynamics through bioturbation, grazing and excretion (Borchardt & Bott, 1995; Hakenkamp & Morin, 2000; Traunspurger, Bergtold & Goedkoop, 1997). Moreover Schmid-Araya *et al* (2002) point out the importance of meiofauna as intermediates in stream food webs. Consequently the accuracy of the benthic food web is greatly improved when meiofauna are considered (Schmid-Araya & Schmid, 2000). Nevertheless, most benthic studies in riverine environment remain focused on micro and/or macrofauna (e.g. Feminella & Hawkins, 1995; Lock *et al.*, 1984), thus leading Giere (2009) to plead recently that “the meiofauna ecology of large rivers requires urgent investigation”.

Despite the richness of biotic interactions occurring in lotic epilithon, available studies taking into account meiofauna generally focus on interstitial, neglecting epibenthic meiofauna (Robertson, Rundle & Schmid-Araya, 2000). So far, only a few studies underlined meiofauna

ecology in lentic (Hillebrand *et al.*, 2002; Höckelmann, Moens & Jüttner, 2004; Peters *et al.*, 2007) and lotic (Gaudes *et al.*, 2006; Mathieu *et al.*, 2007; Sabater *et al.*, 2003) epilithon. The long-term dynamics of meiofauna in lotic epilithon are particularly poorly understood as available field studies investigated rather limited time periods. Thus, the purpose of this study was to follow the long-term dynamics of epilithic meiofauna in seeking to address the following questions:

1. In which extent are meiofauna a persistent and abundant component of river epilithon?
2. What are the main biotic and/or abiotic factors influencing epilithic meiofaunal dynamics?

Methods

Study site

The Garonne is the largest river of south-western France with a drainage basin of 57000 km² and a length of 647 km. It displays a pluvio-nival flow regime characterized by a spring flood period due to snow-melt followed by a long low-flow period which can remain uninterrupted for the rest of the year (if no stormflow occurs). In the Garonne, alternate cobble bars are frequently found even in channel up to the seventh-order. During low-water periods, a high epilithic biomass can be found on pebbles and cobbles, favoured by low water velocities on the river bed (< 0.5 m s^{-1}) and low turbidity (Boulêtreau *et al.*, 2006; Leflaive *et al.*, 2008). The study site is situated on one of these cobble bars located at 36 km upstream the city of Toulouse (01°17’53”E, 43°23’45”N; elevation 175 m a.s.l.) where the Garonne is of sixth-order (Fig. 1).

Environmental parameters

Mean Daily Discharge (MDD) was supplied by a gauging station of the French water management authority (DIREN Midi-Pyrénées, Marquefave station) located at 10 km upstream the study site with no tributary and no dam between the gauging station and the study site. The Mean Weekly Discharge (MWD) before each sampling was considered for statistical analysis. Days After Flood (DAF), which were effective days after last flood (MDD > 300 m^3 s^{-1}) were calculated for each sampling and considered for statistical analysis. DAF reflected also the duration of undisturbed periods. Global daily radiations were provided by a

weather station of the French meteorological agency (Météo France, Lherm station) located at 8 km north-west from the study site. These global daily radiations were converted into daily integrated Photosynthetically Active Radiation (PAR) according to Steeman-Nielsen (1975). As for discharge, daily PAR was converted in Weekly PAR (WPAR) for statistical analyses. During the first sampling campaign (C1), temperature, conductivity, pH and dissolved oxygen concentration were measured at each sampling in the water column using respectively a Lf 95 conductimeter, a pH320 pH-meter and an OXI320 oximeter (WTW, Weilheim, Germany). During the second sampling campaign (C2), these parameters were measured every 30 minutes with an automated multi-parameter probe (YSI 6000, YSI inc., Yellow springs, OH, U.S.A.) which was permanently settled at 5 cm above the streambed at the study site. Captors were cleaned and calibrated monthly to avoid any loss of accuracy. Annual mean values and ranges of these abiotic parameters are listed in Table 1.

Sampling

The first sampling campaign (C1) lasted from December 2004 to February 2006 with 44 samplings. Concerning the second sampling campaign (C2), 51 samplings were conducted from September 2008 to March 2010. Samplings were weekly conducted depending on hydrological conditions. All samplings were performed at the same location. At each sampling, 12 cobbles were hand-collected underwater using plastic bags to prevent any epiliton detachment during removal. To take into account water level changes, cobbles were collected at either 45 m or 33 m from a reference point located on the riverside, so that water height above cobbles remained always comprised between 30 and 50 cm. Collected cobbles were transported to the laboratory within 2 h in a cool box to prevent eventual predation, grazing and/or pigment degradation. Then, epilithon was gathered by scraping the upper surface of each cobble with a scalpel and a toothbrush. The scraped epilithon was finally rinsed with MilliQ water and the obtained 12 epilithon suspensions were used for meiofaunal counts, algal pigment analyses and epilithic ash-free dry mass (AFDM) measurements (four replicates for each parameter). In order to obtain quantitative results, epilithon areas scraped were photographed and scrubbed surfaces were calculated using ImageJ software version 1.38 (Rasband, 1997). It has to be noticed that as C1 supplied other studies about epilithon (Boulêtreau, 2007; Leflaive *et al.*, 2008), AFDM was recorded at each of the 44 samplings. Algal pigments were measured for 24 of the 44 samplings. Meiofauna were determined for 17

of the 44 samplings. During C2, AFDM, algal pigments and meiofauna were recorded at all 51 samplings.

Epilithic meiofauna and macrofauna

For each sampling, the organic fraction of four epilithon suspensions were separated from the mineral fraction, using a differential flotation technique (Hodda & Abebe, 2006) with LUDOX HS-40 (Sigma-Aldrich corp., St. Louis, MO, U.S.A.). Then, LUDOX was discarded (sieved on 63 µm for C1 samples and on 40 µm for C2 samples). Epilithon samples collected on sieve were then preserved with formaldehyde (5% final concentration) and stained with rose bengal. In each sample, at least 200 invertebrates were identified and counted in a Dolfuss cell under a stereomicroscope with 60x magnification. The distinction between permanent meiofauna, temporary meiofauna and macrofauna was taxonomically based: nematodes, tardigrades, rotifers, harpacticoid copepods, oligochaetes and hydrachnidia were considered as permanent meiofauna whereas plecopter and chironomid larvae were considered as temporary meiofauna. Trichopter larvae were considered as macrofauna. The resilience of permanent meiofauna—time required for population densities to reach pre-flood densities (Schmid-Araya, 1994)—was also estimated during C2 after 2 stormflows (23 January 2009 and 15 January 2010, both MDD = 462 m^3 s^{-1}) and after the last flood of the spring snow-melt flood period (12 April 2009, MDD = 330 m^3 s^{-1}).

Epilithic microalgae

Algal pigments: at each sampling, four epilithon suspensions were centrifuged (3220 g, 20 min) and obtained pellets were freeze-dried. Following this, pellets were weighted and thoroughly homogenized. Each homogenized pellet was precisely weighted (250 mg). Algal pigments were then extracted three times (15 min at -20 °C) with a total of 25 ml (10, 10, and 5 ml) 98% cold-buffered methanol (with 2% of 1 M ammonium acetate) following Buffan-Dubau & Carman (2000b). Algal pigment release was favoured at each extraction step by an ultrasonication probe (Sonifier 250A, Branson Ultrasonics corp., Danbury, CT, U.S.A.). One ml of the pigment solution so obtained was then filtered on 0.2 µm PTFE syringe filter and analyzed using a liquid chromatograph consisting of a 100 µl loop auto-sampler and a quaternary solvent delivery system coupled to a diode array spectrophotometer (LC1200 series, Agilent Technologies inc., Santa Clara, CA, U.S.A.). The mobile phase was prepared

and programmed according to the analytical gradient protocol described in Barlow, Cummings & Gibb (1997). Pigment separation was performed through a C8, 5 µm column (MOS-2 HYPERSIL, Thermo Fisher Scientific inc., Waltham, MA, U.S.A.). The diode array detector was set at 440 nm to detect carotenoids, and at 665 nm to detect chlorophylls and pheopigments (Wright *et al.*, 1991). Data analysis was performed using ChemStation software (version A.10.02, Agilent Technologies inc.). Pigments were identified by comparing their retention time and absorption spectra with those of pure standards pigments (DHI LAB products, Hørsholm, Denmark). Each pigment concentration was calculated by relating its chromatogram's peak area with the corresponding area of calibrated standard.

Algal cultures and chemotaxonomy: As reported by Leflaive *et al.* (2008), microalgal groups inhabiting the epilithon of the Garonne river were mainly diatoms, green algae and cyanobacteria. Thus, a green algae species, *Pediastrum boryanum* (Turpin) Meneghini (strain Pedbo01) and a diatom species, *Nitzschia palea* (Kützing) W. Smith (strain Nitpa01) were isolated from the epilithon of the Garonne river and maintained on Combo medium (Kilham *et al.*, 1998) at 18 °C (light:dark 16:8, 45 µmol m^{-2} s^{-1}). An aliquot of each algal culture (10 mL) was filtered on 0.7 µm glass fiber filter (Glass fibre GF/F, Whatman, Clifton, NJ, U.S.A.) and algal pigments were extracted and analysed from the filters following the same procedure than epilithon samples. Concerning cyanobacteria, pigment ratios calculated by Schlüter *et al* (2006) for *Synechococcus leopoliensis* (University of Toronto Culture Collection strain 102) were considered. The biomarker pigment ratio to chlorophyll *a* (Chl *a*) obtained permitted to supply the initial matrix needed to run the CHEMTAX analysis (Table 2). In knowing biomarker pigment ratios to Chl *a* in epilithon samples, CHEMTAX version 1.95 software (Mackey *et al.*, 1996) estimated the relative contribution of each microalgal group to Chl *a* content of epilithon samples. Biomass of diatoms, green algae and cyanobacteria so obtained were then expressed as Chl *a* equivalents and were considered as biotic factors in canonical ordination analyses.

Epilithic biomass and autotrophic index

At each sampling, four epilithon suspensions were dried at 105 °C for 18 h, weighted and then combusted (450 °C) for 8 h to determine the ash-free dry mass (AFDM) content of the epilithon. The Autotrophic Index (AI) which was defined as AFDM/Chl *a* ratio (Greenberg, Trussell & Clesceri, 1985) was also determined. AI is used to describe changes in the trophic nature of the epilithic community: an AI < 100 indicates an epilithon dominated by

microalgae, whereas an AI > 400 indicates an epilithon dominated by heterotrophs and/or organic detritus. Otherwise, 100 < AI < 400 indicates that the epilithic community is balanced between heterotrophs and autotrophs (Ameziane *et al.*, 2002; Biggs & Close, 1989).

Data analysis

Mann-whitney U tests, Spearman rank tests and simple linear regressions were performed with STATISTICA software (version 8.0, Statsoft inc., Tulsa, OK, U.S.A.). Temporal trends in the distribution of epilithic meiofaunal community were analyzed considering biotic and abiotic factors through canonical ordination analyses with CANOCO software (version 4.5, Biometris, Wageningen, The Netherlands). Taxa abundances were log (x + 1) transformed prior to the analysis. The modality of taxa distribution was first analyzed by a detrended correspondance analysis (DCA) using detrending by segments and down-weighting for rare taxa. As the total inertia observed was less than 2.6, a predominance of linear species response curves could be expected (Ter Braak, 1987; 1994). Then, a redundancy analysis (RDA) in which the ordination axes were constrained to be linear combinations of provided environmental factors was used to investigate the relationships between these factors and the temporal variations of meiofaunal abundances. Forward selection of environmental factors served to select those most closely associated with the temporal distribution of meiofaunal taxa, and to quantify their relative importance. The statistical significance was tested with Monte Carlo permutation tests (499 unrestricted permutations, $P < 0.05$) and a Bonferroni correction was applied. For each analysis, the variance explanation attributed to each environmental factor (marginal effects), and their additional variance explained when included in the model (conditional effects) were also shown.

Results

Temporal dynamics

Epilithon: C1 displayed a 9 month-long uninterrupted low flow period (from July 2005 to March 2006) allowing epilithic biomass to reach its highest values (i.e. 139.4 g AFDM m^{-2}) observed over the two campaigns. During C2, stormflows in January 2009, November 2009 and January 2010 interrupted the low flow period and prevented such a long term AFDM development. AFDM decreased strongly just after spates (> 300 m^3 s^{-1}) whereas between spates, AFDM remained relatively stable during summer–autumn while it increased clearly during winter (Fig. 2a,b). Moreover, sudden downfalls of epilithic biomass were not linked to floods and occurred for both sampling campaigns during July. The autotrophic index (AI) was generally comprised between 100 and 400 (n = 75, mean = 194, C.I. 95 %: 157–231) indicating that epilithon was commonly balanced between heterotrophs and phototrophs. However, epilithon tended towards a heterotrophic structure when AFDM was low: AI exceeded 400 only when AFDM < 23 g m^{-2} (Fig. 3c). This notably happened during spring snow-melt floods and during July for both C1 and C2.

Microalgae: A strong correlation was found between fucoxanthin (i.e. the main diatom biomarker pigment) and Chl *a* concentrations (Fig. 3a) as well as between fucoxanthin and AFDM (Fig. 3b). Diatoms clearly dominated epilithic microalgal assemblages in terms of biomass (Fig. 2c,d and Table 3). They particularly dominated during winter. The annual mean relative biomass of diatoms was higher during C2 than during C1 (Mann–Whitney U test; P = 0.006). Green algae contributed less than diatoms to algal biomass. The annual mean relative biomass of green algae was higher during C1 than during C2 (Mann–Whitney U test; P = 0.03). Cyanobacteria were minor contributors to algal biomass (Table 3). However, during July they peaked up to 21 % of the relative algal biomass for both C1 and C2.

Meiofauna: Nematodes and rotifers clearly dominated permanent meiofauna in terms of density—they both represented more than 97 % of the annual mean density of permanent meiofauna. Chironomids dominated temporary meiofauna. Oligochaetes, tardigrades, harpacticoid copepods, hydrachnidia and plecopters were less represented (Table 4). Oligochaete and nematode densities were significantly (Mann-Whitney U test, P < 0.05) higher during C1 than during C2 (Table 4). Like AFDM, nematode and rotifer densities

decreased strongly after floods. Nevertheless, it must be emphasized that some sudden density decreases, which occurred during July and October, were not linked to spates (Fig. 2e,f). During C2, the resilience of nematodes, rotifers, oligochaetes and tardigrades was quicker after stormflows than after snow-melt floods (Table 4).

Macrofauna: As a general trend, trichopter larvae density peaked during July and October (Fig. 2g,h). Density peaks were concomitant to a diminution of AFDM, Chl *a* and density of nematodes and rotifers (Fig. 2a–f). trichopter larvae were significantly (Mann-Whitney U test, $P < 0.01$) more abundant during C1 than during C2 (Table 4).

Influence of biotic and abiotic factors on the temporal dynamics of meiofauna

The percentages of explained variance (i.e. sum of all canonical eigenvalues) were 91% for C1 and 51% for C2.

First sampling campaign (C1): when considering the importance of the variables themselves without co-variability of other ones—as estimated by their conditional effects—Days After Floods (DAF) and dissolved oxygen concentration (O_2) influenced significantly meiofaunal distribution (Monte Carlo permutation test, $P < 0.05$) during C1 (Table 5). O_2 was positively correlated to diatom biomass (Diatoms), AFDM and conductivity in Fig. 4a. However, these factors appeared less important in the conditional effects—which are established after removing their covariance with O_2—but remained positively correlated to O_2 (Spearman rank, $P < 0.05$, $n = 17$). The first axis of the RDA, explaining 39.7% of the variance, represented a mix of several factor effects, however cyanobacterial biomass (Cyano) appeared more correlated to this axis than other factors (Fig. 4a). The second axis, explaining 25.4% of the variance, was on the one hand clearly and positively correlated to factors linked to algal biomass like O_2, AFDM, diatom and green algal biomass (GreenAlg) together with DAF. On the other hand, this axis was negatively correlated to factors linked to seasonality like temperature (T) and WPAR. Meiofaunal taxa were mainly distributed along this second axis (Fig. 4a). Moreover, all taxa were spread in the left part of the biplot. Nematodes, tardigrades and rotifers were grouped and positively related to algal biomass and DAF whereas plecopters, harpacticoid copepods, and hydrachnidia were rather positively related to T, AI, trichopter larvae density (Tricho) and WPAR. Oligochaetes and chironomids occupied an intermediate position along axis 2 and followed no clear pattern.

Second sampling campaign (C2): T, WPAR, DAF, green algal and cyanobacterial biomass significantly influenced meiofaunal distribution. Furthermore, T, green algal and cyanobacterial biomass appeared as highly significant factors (Table 5). DAF was less explicative than during C1 while T became the most important factor explaining meiofaunal distribution. AFDM and diatom biomass were positively correlated with DAF in Fig. 4b. Diatom biomass was positively correlated with green algal biomass and negatively correlated with cyanobacterial biomass (Spearman rank, $P < 0.05$, $n = 51$). The first axis, explaining 27.4% of the variance, represented a seasonality pattern in involving factors like T, WPAR, trichopter larvae density and green algal biomass. The second axis, explaining 10.5% of the variance, represented algal availability together with DAF in being on the one hand positively correlated to DAF, AFDM, diatom biomass and O_2 and on the other hand negatively correlated to cyanobacterial biomass and AI. The distribution of meiofaunal taxa was close to the one observed during C1, except that chironomids were more related to green algal biomass and that oligochaetes followed the distributional trend of nematodes and rotifers (Fig. 4b).

Discussion

Mature epilithon harbours more meiofauna

This field study provides the first evidence that meiofauna may constitute an abundant and persistent component of river epilithon. Maximum and annual mean density values provided in this study were comparable to those previously reported by Gaudes *et al.* (2006) and Sabater *et al.* (2003) in attached epilithon of the Llobregat river, Catalonia, NE Spain. However, this study is the first long-term field study considering meiofauna in lotic epilithon and showing that meiofauna persistently inhabit this epilithon throughout the seasons and years.

Nematodes were significantly more abundant during C1 than during C2 (Table 4). This difference can likely be attributed to the 9 month-long undisturbed period encountered during C1, which lead AFDM and nematode density to reach their highest values. DAF reflected the maturation degree reached by the epilithon (i.e. its age and thickness) in being related to the duration of undisturbed periods. Thus, the strong positive influence of DAF on

nematode and rotifer densities evidenced the positive impact of mature epilithon on main permanent meiofaunal taxa: nematodes and rotifers (Table 5 and Fig. 4).

Meiofaunal resilience after flood disturbance

Biggs & Close (1989) state that the epilithon is detached by shear stress, substratum instability and abrasive effects of suspended solids during flood events. Our results confirm this finding as epilithic AFDM was clearly decreased by floods. Meiofauna density was associated to such AFDM variations and was also dampened by floods. Thus, meiofauna were probably swept away with epilithon when flood occurred. This result confirms findings of Robertson *et al.* (1997) and Palmer *et al.* (1996) who show that flow can strongly influence interstitial meiofauna in superficial sediments of rivers.

Nevertheless, nematodes, rotifers, tardigrades and oligochaetes appeared resilient to floods and particularly to stormflows (Table 4). Their resilience times were comparable with shortest resilience times estimated by Robertson, Lancaster & Hildrew (1995) for lotic epibenthic microcrustaceans (< 57 days). These short resilience times after stormflows could arise from river morphology: the Garonne river harbours numerous side arms where water flow velocity remains usually lower during floods, and where woody debris and leaf litter accumulate. These habitats could, as for macrofauna (Rempel, Richardson & Healey, 1999), favour recolonization of meiofauna after flood disturbance following a 'refuge as habitat' strategy (Golladay & Hax, 1995; Robertson *et al.*, 1995). However, the resilience times observed after spring snow-melt flood were longer than after stormflows (Table 4). A possible explanation for this difference might be that snow-melt flood periods showed numerous higher flood peaks than stormflow events. In the Garonne, snow-melt floods can deeply scour and submerge proximate floodplain (Chauvet & Décamps, 1989), thus reducing the availability of eventual refuges for meiofauna. Another possible explanation for this difference might be that an additional type of disturbance retards meiofaunal resilience after spring floods.

Macrofaunal grazing and epilithon self detachment constraints

One of the main interesting results of the present study is that several important decreases in epilithic AFDM, algal biomass, rotifer and nematode densities did not result from flood events (Fig. 2 a–f). These decreases occurred mainly during early summer (July 2005 and July

2009), although environmental factors (e.g. low flow, high PAR and high temperature) were expected to favour the development of a high epilithic biomass. During these periods it was also observed that trichopter larvae and hydrachnidia were particularly abundant (Figs. 2g,h and 4a,b). This is consistent, knowing that hydrachnidia are predators and/or parasites of freshwater insects (Di Sabatino, Gerecke & Martin, 2000). It is also known that insect larvae peak in density during summer because of appropriate water temperature (Morin & Dumont, 1994). Furthermore trichopter larvae can strongly reduce epilithon biomass by grazing especially when water temperature is high (Feminella & Hawkins, 1995; Hillebrand, 2009). Therefore, it would seem that during July, the abundance increase of trichopter larvae together with a temperature-dependant increase of their metabolism would likely constitute a short-term 'extreme grazing pressure' which leads to a drastic clearance of epilithic surfaces. We indeed observed that cobbles were cleaned of epilithon while being crowded with insect larvae during July. The question remains, if meiofauna partly disappeared by being ingested together with the epilithon, or by escaping knowing that meiofauna can actively drift between sediment and water (Smith & Brown, 2006; Swan & Palmer, 2000). Trichopter density peaked also during October (particularly in 2005 and 2008). However, in October, the potential related impact on epilithon and its associated meiofauna appeared less important than during July.

Boulêtreau *et al.* (2006) show that the epilithon of the Garonne river crumble into free-floating fractions resulting from a temperature dependant bacterial degradation of deeper senescent algal layers of the epilithon during summer–autumn low flow periods. This self-generated detachment was thus pointed out as enhancing meiofaunal drift process, as free-floating epilithon fractions were reported as being densely inhabited by meiofauna (Gaudes *et al.*, 2006; Sabater *et al.*, 2003). Thus, the self-generated detachment could likely also contribute to reduce the density of meiofauna in attached epilithon fractions during summer–autumn periods.

The Autotrophic Index (AI) indicated that the epilithic microbial community was dominated by heterotrophic microorganisms during July when epilithic AFDM was low and probably submitted to both grazing and self-generated detachment. These disturbances likely favour the heterotrophic compartment of epilithon (e.g. bacteria, ciliates) over microalgae. This would be partly consistent with previous studies indicating that bacteria which are abundant and diversified in the epilithon of the Garonne river (Lyautey *et al.*, 2005) are crucial early colonizers for the establishment of the epilithon after disturbances (Lyautey *et al.*, 2010). It would be also in agreement with Hillebrand *et al.* (2002) who show that

macrofaunal grazing impact epilithic microalgae while it tend to favour the growth of ciliates and bacteria during field experiments in a mesoeutrophic lake and a coastal marine site. However, it was not previously reported that the presence of macrofaunal grazers could meiofaunal densities to such a degree as observed in our study.

Influence of microalgal biomass

AFDM, diatom and green algal biomass were important factors influencing positively nematode and rotifer densities during both sampling campaigns (Table 5 and Fig. 4). Due to their high content of polyunsaturated fatty acids (Phillips, 1984) and the lack of cellulose in their cell walls, diatoms represent a high-quality food resource for primary consumers (e.g. Buffan-Dubau & Carman, 2000a; Goedkoop & Johnson, 1996). Diatoms also produce exopolymeric substances which consolidate the mucus matrix of the mat and stimulate bacterial growth (Underwood, Paterson & Parkes, 1995). Our study did not permit to separate the bottom–up effect from the micro-habitat effect of diatom biomass on nematodes and rotifers. However, Mathieu *et al.*(2007) suggest that dominant nematode species feed on epilithic diatoms in artificial biofilms. Recent quantitative feeding studies in the epilithon of the Garonne river confirm this suggestion (Majdi et al., unpubl. data). While Höckelmann *et al.* (2004) report that cyanobacterial mats can attract and shelter meiofauna, we found no evidence of cyanobacterial attractiveness in this study. Future research should address trophic behaviour to detail the functional role of meiofauna within river epilithon.

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Tables

Table 1 Annual mean and range of abiotic parameters. C1: first sampling campaign. C2: second sampling campaign.

	C1 (2004–2006)			C2 (2008–2010)		
Abiotic parameter	Annual mean	Min	Max	Annual mean	Min	Max
Temperature (°C)	14.1	2.9	23.3	14.4	3.5	23.0
Oxygen (mg l^{-1})	11.8	6.9	24.4	11.6	7.4	17.9
Conductivity (µS cm^{-2})	206.3	134.0	278.0	262.7	183.0	333.0
pH	8.1	7.3	8.7	7.6	7.0	8.8
MDD (m^3 s^{-1})	105.2	25.0	608.0	125.1	18.0	814.0
PAR (E m^{-2})	24.3	1.8	56.2	24.1	1.4	57.4

Table 2 CHEMTAX input pigment ratio matrix. Ratios were calculated considering fucoxanthin (Fuco), lutein (Lut), violaxanthin (Viola), diadinoxanthin (Diad), zeaxanthin (Zea), β-carotène (β-car), chlorophyll *b* (Chl *b*) and chlorophyll *c* (Chl *c*) concentrations versus chlorophyll *a* (Chl *a*) concentrations from corresponding microalgal cultures. For green algae and diatoms these ratios were calculated from pure cultures of respectively *P. boryanum* and *N. palea*. For cyanobacteria, pigment ratios are from *S. leopoliensis* (Schlüter *et al.*, 2006).

		Biomarker pigment ratios to Chl *a*								
Algal group	Species	Fuco	Lut	Viola	Diad	Zea	β-car	Chl *a*	Chl *b*	Chl *c*
Green algae	*P. borianum*		0.143	0.049		0.014	0.043	1	0.088	
Diatoms	*N. palea*	0.477			0.102		0.002	1		0.121
Cyanobacteria	*S. leopoliensis*					0.411	0.011	1		

Table 3 Relative annual mean contribution (%) of each microalgal group to total chlorophyll *a* content of the epilithon. C1: first sampling campaign. C2: second sampling campaign.

	C1 (2004–2006)		C2 (2008–2010)	
Algal group	Relative biomass	(%)	Relative biomass	(%)
Green algae	26.2		15.5	
Cyanobacteria	3.2		2.2	
Diatoms	70.7		82.3	

Table 4 Annual mean and maximum density of invertebrates inhabiting the epilithon of the Garonne river during C1: first sampling campaign and C2: second sampling campaign. Significant density differences among each taxa were showed between C1 and C2 (Mann-Whitney U test * $P < 0.05$ and ** $P < 0.01$). During C2, the Resilience After Stormflows (RAStorm) and the Resilience After Snow-melt flood (RASnow) were expressed as the range of days (between two consecutive samplings) needed by a population to reach pre-flood densities.

		C1 (2004–2006)		C2 (2008–2010)				U test
		Density (ind cm^{-2})		Density (ind cm^{-2})		Resilience (day)		
Epilithic invertebrate taxa		Annual mean	Max	Annual mean	Max	RAStorm	RASnow	
Permanent meiofauna	Nematodes	52.5	319.0	14.7	104.0	58–65	148–156	*
	Rotifers	26.1	127.0	38.4	126.0	50–58	>340	
	Oligochaetes	1.9	7.7	0.4	4.2	55–62	142–148	**
	Harpacticoids	0.1	0.8	0.3	1.5	–	–	
	Tardigrades	0.1	4.0	0.1	0.8	47–55	>340	
	Hydrachnidia	0.04	0.2	0.03	0.6	–	–	
Temporary meiofauna	Chironomids	7.7	28.0	8.6	32.0	–	–	
	Plecopters	0.1	0.3	0.4	3.8	–	–	
Macrofauna	Trichopters	4.5	25.0	0.7	3.3	–	–	**

Table 5 Marginal and conditional effects resulting from the redundancy analyses (RDA) of meiofaunal taxa densities. Environmental factors are listed in order of their eigen-values (λ) reflecting the importance of their contribution to explain the temporal distribution of meiofaunal taxa. The sum of all λ—percentage of explained variance—was also provided. Significant factors * ($P < 0.05$, Monte Carlo permutation test) and highly significant factors ** ($P < 0.0038$, Monte Carlo permutation test with Bonferroni's correction) were identified. Biotic factors considered in the RDA were: biomass of diatoms (Diatoms), green algae (GreenAlg) and cyanobacteria (Cyano); Total epilithic biomass (AFDM), Autotrophic Index (AI) and density of trichopter larvae (Tricho). Abiotic factors considered were: water temperature (T), Mean Weekly Discharge (MWD), Days After Flood (DAF), Weekly Photosynthetically Active Radiation (WPAR), pH, dissolved O_2 (O2) and conductivity (Cond).

RDA C1 (2004–2006)					RDA C2 (2008–2010)				
Marginal effects		Conditional effects			Marginal effects		Conditional effects		
Factors	λ	Factors		λ	Factors	λ	Factors		λ
DAF	0.19	DAF	*	0.19	T	0.19	T	**	0.19
T	0.17	O2	*	0.18	GreenAlg	0.13	GreenAlg	**	0.07
Cyano	0.16	T		0.08	MWD	0.11	Cyano	**	0.07
Diatoms	0.15	Cyano		0.07	WPAR	0.11	WPAR	*	0.05
Cond	0.15	GreenAlg		0.06	pH	0.09	DAF	*	0.03
WPAR	0.14	Cond		0.06	O2	0.09	AFDM		0.03
AFDM	0.13	Tricho		0.05	Diatoms	0.07	Tricho		0.02
GreenAlg	0.12	MWD		0.05	Tricho	0.06	O2		0.01
O2	0.12	WPAR		0.04	DAF	0.05	MWD		0.01
Tricho	0.10	pH		0.04	AFDM	0.05	Diatoms		0.01
pH	0.09	AI		0.04	AI	0.04	Cond		0.01
MWD	0.06	AFDM		0.03	Cond	0.03	pH		0.01
AI	0.04	Diatoms		0.02	Cyano	0.02	AI		0
		Sum of all λ		0.91			Sum of all λ		0.51

Figure legends

Fig. 1 Location of the study site (white arrow) on the Garonne river.

Fig. 2 Epilithic ash-free dry mass (AFDM) temporal dynamics (mean, n = 4, ± SE) and Mean Daily Discharge (MDD) during (a) the first sampling campaign (C1) and (b) the second sampling campaign (C2). Stars show spate periods where MDD > 300 m^3 s^{-1}. Numbers in X axes indicate the months of the year. Temporal dynamics of epilithic chlorophylle *a* concentrations (mean, n = 4) during (c) C1 and (d) C2 considering the relative contribution of each microalgal group. Temporal dynamics (mean, n = 4, ± SE) of nematode and rotifer densities in epilithon during (e) C1 and (f) C2. For clarity reasons, density values were linked excepted during spate period. Temporal dynamics (mean, n = 4, ± SE) of trichopter larvae densities in epilithon during (g) C1 and (h) C2.

Fig. 3 (a) Correlation between fucoxanthin and chlorophyll *a* (n = 75, P < 0.0001, R^2 = 0.97) and (b) between fucoxanthin and epilithic ash-free dry mass (AFDM) (n = 75, P < 0.0001, R^2 = 0.74). (c) Autotrophic Index (AI) versus AFDM (n = 75).

Fig. 4 Redundancy analysis biplots (axis 1 and 2) showing the distribution of meiofaunal taxa densities according to environmental parameters for (a) the first sampling campaign (C1) and (b) the second sampling campaign (C2). Ordination axes were rescaled to range from -1 to 1. Slim dotted arrows are non-significant factors (Monte Carlo permutation test, P > 0.05). Bold arrows represent significant factors (Monte Carlo permutation test, P < 0.05). The eigen-values (λ) are indicated for main ordination axes. Environmental factor abbreviations are provided in Table 5. Meiofaunal taxa abbreviations: Nematodes (Nema), rotifers (Roti), tardigrades (Tardi), harpacticoid copepods (Cop), Oligochaetes (Oligo), hydrachnidia (Hydra), Chironomids (Chiro), and plecopters (Pleco).

Fig. 1

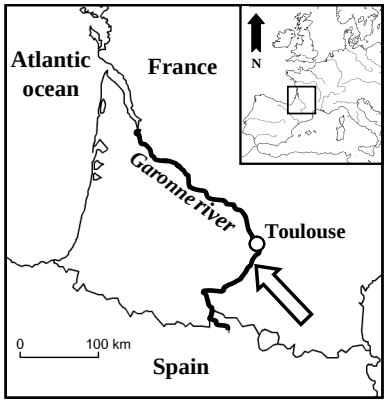

Atlantic ocean
France
N
Garonne river
Toulouse
0
100 km
Spain

Fig. 2

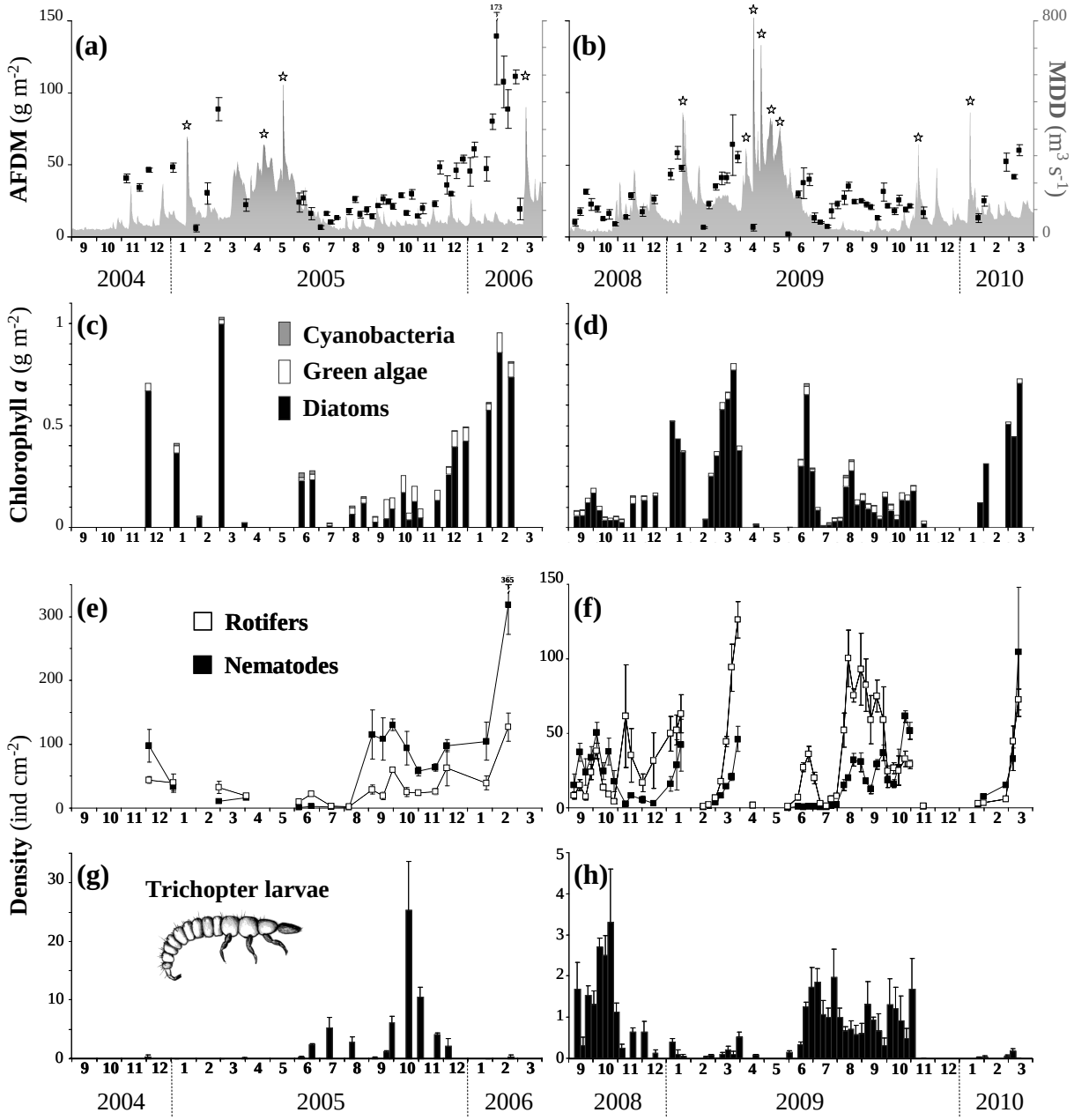

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
AFDM (g m-2)
MDD (m3 s-1)
Chlorophyll a (g m-2)
Density (ind cm-2)
Cyanobacteria
Green algae
Diatoms
Rotifers
Nematodes
Trichopter larvae
2004
2005
2006
2008
2009
2010

Fig. 3

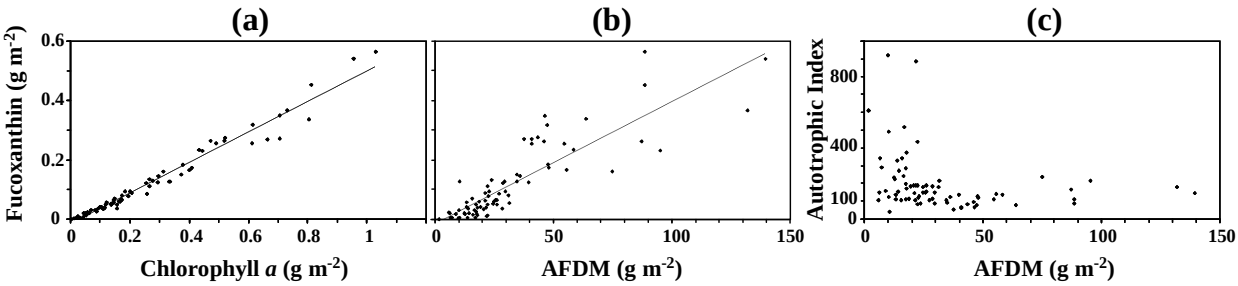
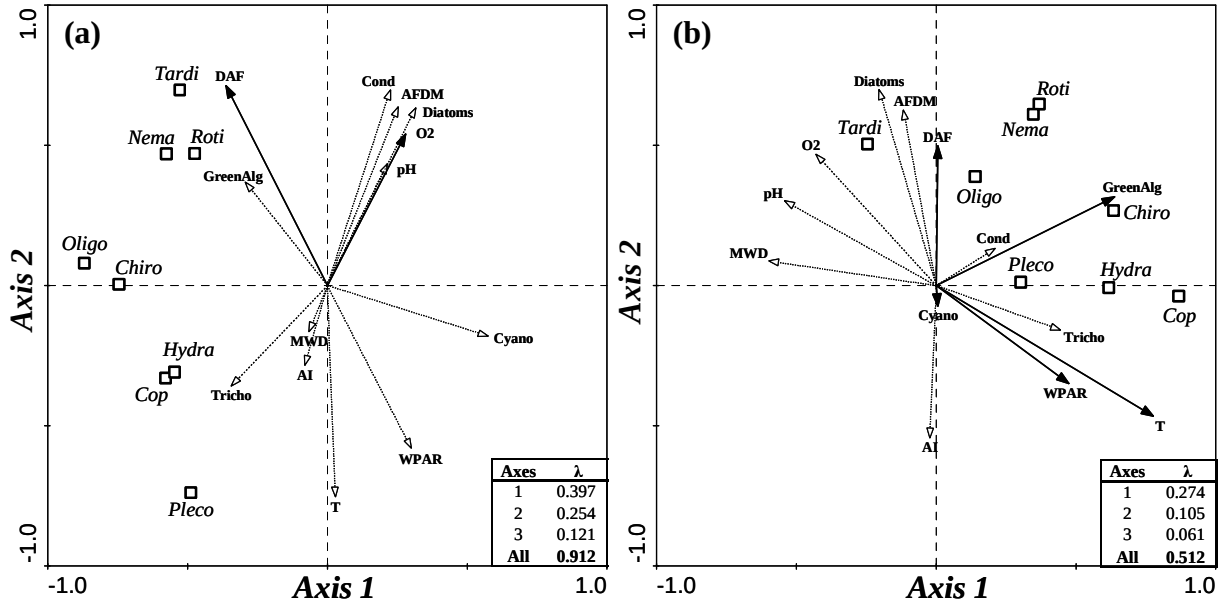

(a)
(b)
(c)
Fucoxanthin (g m-2)
0
0.2
0.4
0.6
0
0.2
0.4
0.6
0.8
1
Chlorophyll a (g m-2)
0
50
100
150
AFDM (g m-2)
Autotrophic Index
0
100
400
800
0
50
100
150
AFDM (g m-2)

Fig. 4


(a)
Tardi
DAF
Cond
AFDM
Diatoms
O2
Nema
Roti
GreenAlg
pH
Oligo
Chiro
Cyano
Hydra
MWD
AI
Cop
Tricho
WPAR
Pleco
T
Axes λ
1 0.397
2 0.254
3 0.121
All 0.912
Axis 2
Axis 1
1.0
-1.0
(b)
Diatoms
AFDM
Roti
Tardi
Nema
DAF
O2
Oligo
GreenAlg
pH
Chiro
Cond
MWD
Pleco
Hydra
Cyano
Cop
Tricho
WPAR
T
AI
Axes λ
1 0.274
2 0.105
3 0.061
All 0.512
Axis 2
Axis 1
1.0
-1.0

Author: Benoît Mialet

Title: Response of zooplankton to improving water quality in the Scheldt estuary and test of a trophic selectivity model.

Supervisor: Michèle Tackx

Defence place and date: Toulouse, December 17th, 2010

Abstract: The response of the crustacean zooplankton community to improving water quality in the Scheldt estuary was studied (1996-2009). From 2007 onwards, calanoids moved the bulk of their population from the brackish water reach to the freshwater reach, while cyclopoids strongly decreased in freshwater. Contrarily to cyclopoids and cladocerans, calanoids spatio-temporal distribution responded positively to an improvement in water quality. Our findings suggest that the trophic regime of *E. affinis* shifted from autotrophic to heterotrophic organisms. With a view to testing a trophic selectivity model in a benthic ecosystem, the selective behaviour of bdelloid rotifers of the Garonne periphyton (France) was analysed. Results suggested that they fed selectively on cyanobacteria, witch was, to the best of our knowledge, the first evidence of a selective behaviour of rotifers on cyanobacteria in this medium.

Keywords: zooplankton, estuary, Scheldt, restoration, *Eurytemora affinis*, gut contents, HPLC, periphyton, rotifers, selectivity

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Discipline: Functional ecology

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Auteur : Benoît Mialet

Titre : Réponse du zooplancton à la restauration de l'estuaire de l'Escaut et test d'un modèle de sélectivité trophique.

Directeur de thèse : Michèle Tackx

Lieu et soutenance : Toulouse, le 17 décembre 2010

Résumé : La réponse de la communauté de crustacés zooplanctoniques de l'estuaire de l'Escaut à l'amélioration de la qualité de l'eau a été étudiée (1996-2009). A partir de 2007, on observe un déplacement des calanoïdes de l'eau saumâtre vers l'eau douce, associée à une forte diminution des cyclopides en eau douce. Contrairement aux cyclopides et aux cladocères, la distribution spatio-temporelle des calanoïdes a répondu positivement à une amélioration de la qualité de l'eau. Nos résultats suggèrent un basculement du régime trophique d'*Eurytemora affinis* des organismes autotrophes vers des organismes hétérotrophes. Enfin, en vue de tester un modèle de sélectivité trophique en milieu estuarien dans un écosystème benthique, le comportement sélectif des rotifères du périphyton de la Garonne (France) a été analysé. Les résultats ont suggéré une sélection positive envers les cyanobactéries qui, en notre connaissance, a été mise en évidence pour la première fois dans ce milieu.

Mots clés: zooplancton, estuaire, Escaut, restauration, *Eurytemora affinis*, contenu digestif, HPLC, périphyton, rotifères, sélectivité.

Discipline : Ecologie fonctionnelle

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