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# Rôle du mésozooplancton dans un estuaire restauré : l'Escaut

Sophie Chambord

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Université Fédérale



Toulouse Midi-Pyrénées

# THÈSE

En vue de l'obtention du

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Délivré par :

Université Toulouse 3 Paul Sabatier (UT3 Paul Sabatier)

Cotutelle internationale avec l'Université d'Anvers (Belgique)

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Présentée et soutenue par :

**Sophie CHAMBORD**

le jeudi 16 juin 2016

**Titre :**

Rôle du mésozooplancton dans un estuaire restauré : l'Escaut.

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*A ma mère,  
qui n'a jamais cessé de croire en moi !*



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---

*Et voilà, c'est la fin. J'achève l'écriture de ce manuscrit par ce dernier volet : Les remerciements.*

*Par définition une thèse c'est : « une thèse est un mémoire résumant un travail de recherche universitaire, soutenu devant un jury par un étudiant afin d'obtenir un diplôme ou un grade universitaire » (Wikipédia).*

*Cependant, une thèse est bien plus que ça, c'est à la fois un partage, certes beaucoup scientifique, mais aussi humain. En effet, la réalisation d'un tel projet ne se fait pas seule mais en équipe, c'est pourquoi cette partie risque d'être un peu longue. Merci à vous d'avoir la patience et / ou l'intérêt de lire ce passage.*

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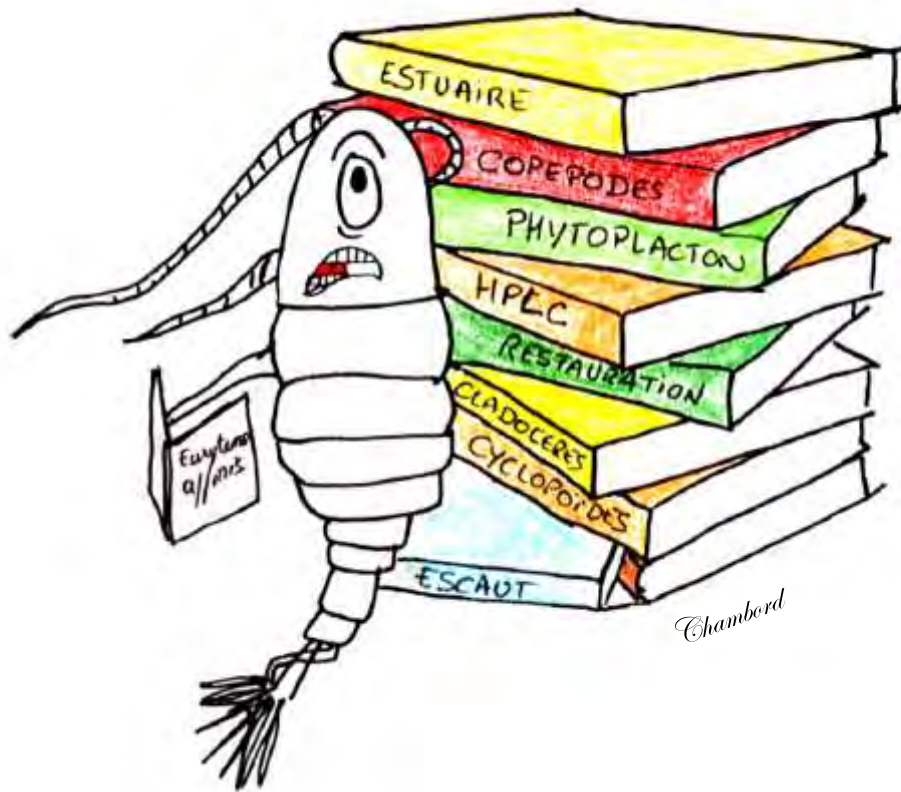
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I. **CHAPITRE I :**  
Introduction

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La gestion des ressources en eau et depuis quelques décennies une préoccupation majeure au niveau mondial (e.g. Ashley et al., 2006, Vörösmarty et al., 2010 ; Conseil mondial de l'eau, 2016). Depuis plusieurs années, les recherches en hydro-écologie et en gestion intégrée de la ressource en eau ont pour but la réduction de l'impact des activités anthropiques sur le fonctionnement écologique des milieux aquatiques. Une bonne compréhension du fonctionnement écologique et donc indispensable pour anticiper l'effet des futurs aménagements sur la qualité de l'eau. La restauration des systèmes aquatiques concerne une grande diversité des personnes impliquées dans diverses activités : agriculture, industrie, navigation et ports, administration, recherche scientifique, associations diverses (pêche, protection de la nature).

Les zones côtières et estuariennes constituent les écosystèmes les plus productifs de la planète (Costanza et al., 1997). A l'interface terre-mer, les estuaires représentent des enjeux contradictoires en raison de leur position stratégique. Ils constituent des écosystèmes particuliers, tout en concentrant des activités socio-économiques considérables (activités portuaires et industrielles (Elliot et Ducrotoy, 1991), agricoles et de loisirs). A ce titre, et afin de favoriser l'implantation de l'activité industrielle les estuaires ont souvent fait l'objet de nombreux aménagements (portuaires, industriels, chenalisation). La pollution et la fragilisation de ces milieux et des écosystèmes en particulier accroît avec le développement des activités qui s'accompagne d'un accroissement de la population et des rejets anthropiques.

## I.1 Le milieu estuarien

## I.2 Le concept « estuaire »

Du latin *aestuarium*, ce terme fait référence aux milieux où les eaux continentales sont soumises à l'influence de la marée avant de rejoindre la mer. D'un point de vue très réducteur il est courant d'assimiler, l'estuaire à l'embouchure d'un cours d'eau se jetant dans la mer. Sa définition est beaucoup plus complexe et fait encore l'objet de débats et de nouvelles propositions (Elliott et McLusky, 2002 ; Potter et al., 2010). La capacité à réunir les notions liées à la distribution de salinité et de densité aux caractéristiques des processus de circulation et de mélange, et aux

limites contrôlant la distribution et le mouvement des mélanges d'eaux, font de la formulation de Pritchard (1967) une des plus reconnues :

*« Une masse d'eau côtière semi-close qui est en libre connexion avec la mer et dans laquelle l'eau de mer est mélangée à une quantité mesurable d'eau douce provenant du drainage continental. » (Pritchard, 1967).*

Toutefois, différents auteurs ont souligné d'autres aspects du milieu estuarien. Les effets des marées sont précisés par Dionne (1963) puis la définition de Pritchard (1967) est reprise et enrichie par Fairbridge (1980).

*« Un estuaire est un bras de mer pénétrant une vallée fluviale jusqu'à la limite amont de propagation de la marée, habituellement divisé en trois secteurs : (a) un bas estuaire ou estuaire marin ; (b) un estuaire moyen, sujet à des importants mélanges entre deux eaux douces et eaux salées ; (c) un haut estuaire ou estuaire fluvial, caractérisé par l'eau douce mais sujet aux actions quotidiennes de la marée. Les limites entre ces trois secteurs sont variables et dépendantes des constantes variations du débit fluvial. » (Fairbridge, 1980).*

Perillo (1995) vient élargir cette définition en faisant référence aux marées et en y ajoutant la biocénose.

*« Un estuaire est une masse d'eau semi-fermée qui s'étend jusqu'à la limite 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 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. » (Perillo, 1995).*

Plus récemment, Potter et al. (2010) ajoutent la possibilité d'une rupture saisonnière de la connexion à la mer et d'une hypersalinisation quand les pertes d'eau par évaporation sont fortes et les apports d'eau douce négligeables. Avec cette définition, le concept d'estuaire s'étend et permet de connecter des estuaires australiens ou sud-africains.

A ceci s'ajoutent des visions juridiques, socio-économiques et administratives (Elliott et McLusky, 2002), ainsi que diverses classifications des systèmes estuariens basées sur des critères physiques (Dyer, 1997), géomorphologiques ou hydrologiques.

En résumé les estuaires sont des milieux en constante évolution, très dynamiques (échanges de flux terre-mer) et très complexes (multi-échelles et les couplages complexes entre ces processus physiques, chimiques et biologiques).

### 1.2.1 Les spécificités des milieux estuariens

Le phénomène de marée, s'exerçant de façon cyclique sur certaines côtes est aujourd'hui bien connu. La marée est causée par l'effet conjugué des forces de gravitation dues à la lune et au soleil, et de la force d'inertie due à la révolution de la terre autour du centre de gravité du système terre-lune. Le débit fluvial, quant à lui, est principalement dépendant de la pluviométrie ainsi que des caractéristiques du bassin versant (taille, présence de barrages, etc). Ces deux forces – marée et débit fluvial – vont s'opposer aux niveaux des estuaires. La physicochimie du milieu estuarien et la distribution spatiale des communautés estuariennes vont être conditionnées en générale par l'émulation des forces hydrauliques provenant du débit fluvial et de la marée.

L'un des premiers facteurs environnementaux à être influencé par cette opposition de forces est la salinité. En effet, la salinité dans les estuaires décroît selon un gradient aval-amont, c'est ainsi que différentes zones ont été définies. Trois zones estuariennes sont définies en fonction de la salinité (Reid, 1961 ; Mouny, Dauvin et al., 1996) : 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.

Basé sur les différents types de circulation des eaux estuariennes, Pritchard (1995), met en place la première classification significative des estuaires, on distingue ainsi les estuaires du plus stratifié au plus homogène (Fig.1).

- Type A : **Estuaire sans mélange ou complètement stratifié**, où les flux dominant sont issus de l'eau douce.
- Type B : **Estuaire à coin salé**, où les gradients verticaux sont importants. Volume d'eau salée < volume d'eau douce. Ce type de comportement correspond aux estuaires à faible marnage, dans lesquels la stratification

verticale est maximale. L'eau douce s'écoule en surface. En profondeur, la masse d'eau forme un coin salé pratiquement stationnaire (Dyer, 1986). La zone de transition entre les deux couches est restreinte avec un fort gradient, rendant difficile le mélange des masses d'eau.

- Type C : **Estuaire partiellement mélangé**. Volume d'eau salée > volume d'eau douce, créant des courants plus forts et des excursions, longitudinales et verticales, plus importantes, des masses d'eau.
- Type D : **Estuaire homogène**, sans gradient de salinité. Les marées sont plus prépondérantes par rapport à la circulation de densité, le mélange se fait sur toute la colonne d'eau. En général, ce type de mélange correspond aux estuaires peu profonds possédant un fort marnage

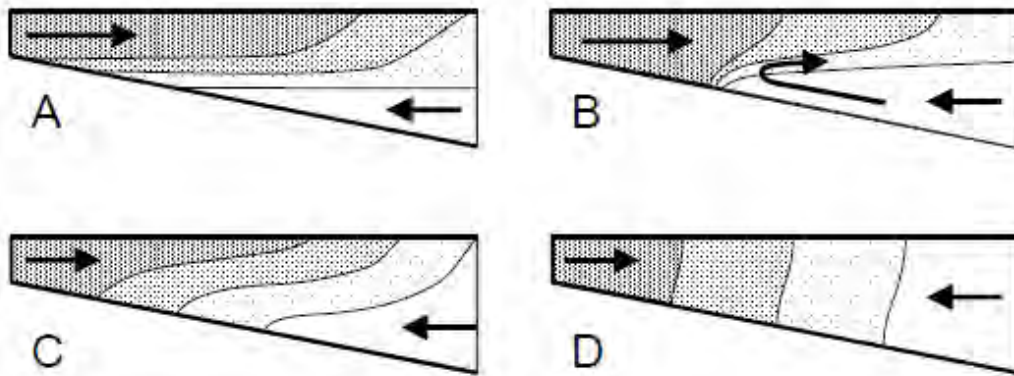


Figure 1 : Représentation schématique des différents types d'estuaires selon Pritchard (1955). L'eau douce est représentée en blanc, l'eau de mer en gris foncé. La gamme de gris correspond au gradient de salinité.

Les deux courants hydrauliques opposés (eaux marines et continentales) forment dans l'estuaire des zones de circulation localisées. Les particules en suspension (matière en suspension = MES) se concentrent pour former le « bouchon vaseux » (Glangeaud, 1938), caractéristique des estuaires dominés par la marée (Wells, 1995). Généralement, il se localise dans la partie aval de l'estuaire à l'amont de l'intrusion saline (Potsma, 1961 ; Allen, 1974 ; Avoine, 1981 ; Castaing, 1981). Sa concentration varie fortement d'un estuaire à l'autre (Uncles et al., 2002a, 2002b). Les MES s'accumulent alors au niveau du point nodal sous trois formes : minérale, organique vivante et organique détritique, et peuvent amorcer un nouveau cycle (Castaing, 1981).

## I.2.2 Caractéristiques biotiques

Les estuaires sont des milieux en constante évolution, très dynamiques et très complexe (partie I- 1.1). Les variations quotidiennes de salinité et de température induisent des grandes difficultés pour les organismes, c'est pourquoi peu d'entre eux sont réellement inféodés à ce milieu. La plupart des espèces trouvées dans les zones estuariennes ne sont que transitoires, et sont natifs soit de la mer soit de l'eau douce. De la même façon que la salinité, un « gradient » se crée au sein des estuaires, d'amont en aval. En effet, les espèces originaires d'eau douce vont petit à petit succomber au bénéfice des espèces euryhalines. En revanche, les espèces typiquement estuariennes vont occuper la zone saumâtre, là où le nombre d'espèces marines et d'eau douce est réduit, mais cette zone reste peu diversifiée (Remane, 1934 ; Telesh et al., 2014, Fig 2).

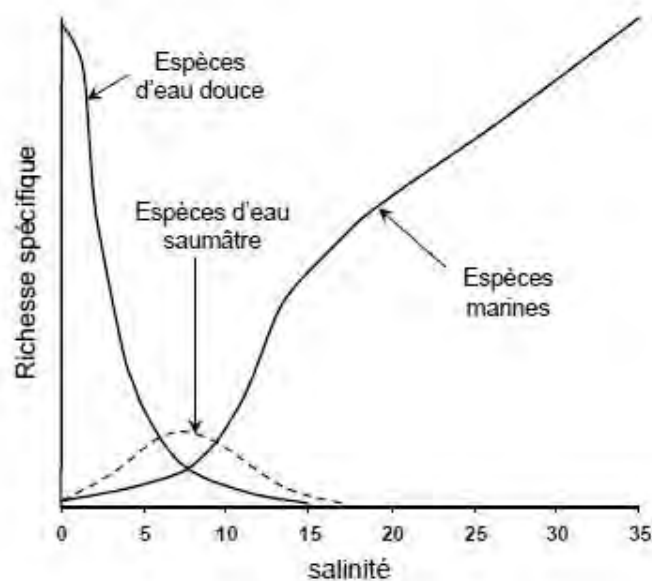


Figure 2 : Répartition de la richesse spécifique sur un gradient de salinité selon Remane (1934).

## I.2.3 Le zooplancton au cœur des réseaux trophiques

### I.2.3.1 Caractérisation du zooplancton

Zooplancton viens du grec *zoo* = « animal » et « *πλαγκτός / plagktós* » = errant, instable. Il constitue le principal compartiment animal aquatique en termes de



biomasse et de diversité à la fois dans les écosystèmes marins mais aussi dans les eaux douces (Mauchline, 1998). Longtemps il a été caractérisé par le fait de ne pas être capable de nager contre – courants. La définition passive du zooplancton a évolué depuis quelque temps, avec notamment les travaux de Michaec et al. (2014) qui ont montré, par exemple qu'*E. affinis* peut nager jusqu'à certaines limites dans un milieu turbulent. De nombreuses espèces viennent agrémenter ce groupe. Le plancton est subdivisé en cinq groupes selon leur taille qui sont (Dussart, 1965) :

- Mégazooplancton (20-200cm)
- Macrozooplancton (2-20cm)
- Mésozooplancton (0.2-2cm)
- Microzooplancton (20-200µm)
- Picozooplancton (0.2-2µm)

#### 1.2.3.2 Le zooplancton estuarien

La composition mésozooplanctonique des zones marines et saumâtres dans les estuaires nord européens est essentiellement constituée de copépodes et de mysidacés (Mees et al., 1993 ; Sautour et Castel, 1995 ; Mouny et al., 2000 ; Mouny et Dauvin, 2002). L'intérêt des écosystèmes estuariens pour les espèces marines est lié à la présence de fortes populations zooplanctoniques, maillons clés permettant le transfert d'énergie du premier niveau trophique à la partie supérieure de la chaîne alimentaire, qui font des estuaires des zones de nurserie idéale pour le développement des larves et des juvéniles de crustacés et de poissons.

Les estuaires sont des lieux de passage d'importantes quantités de matière organique d'origine continentale et océanique transitant dans l'estuaire de l'amont vers l'aval ou de l'aval vers l'amont en fonction des rythmes tidaux et des fluctuations saisonnières des débits fluviaux. Afin d'assurer leurs besoins nutritionnels les organismes zooplanctoniques vont être soumis à ces contraintes, ainsi qu'aux contraintes d'abondance et de qualité du pool nutritif. Ces contraintes nutritionnelles entraînent des adaptations de la part du zooplancton. Il va donc développer des régimes alimentaires « omnivores opportunistes » en fonction de la disponibilité de la ressources (Heinle et al., 1977 ; Gasparini et Castel, 1997, Fockedey et Mees, 1999).

### *1.2.3.3 Les réseaux trophiques estuariens*

D'après David et al. (2006), les réseaux trophiques estuariens sont déterminés par un fonctionnement basé sur des apports allochtones et par la multiplication des maillons trophiques entre la faible biomasse végétale détritique (biomasse macrovégétale, faible en qualité nutritive) et le zooplancton (Heinle et al., 1977 ; Hummel et al., 1988).

Lobry et al. (2008) ont montré l'utilisation optimale de la matière organique (MO) de faible qualité grâce à la l'enchaînement saisonnier d'espèces qui diversifient les flux trophiques et stabilise ainsi le système (fig. 3). Le zooplancton joue un rôle dans le transfert d'énergie (§ I.1.4.2), et, de plus, étant des proies pour d'autres maillons il joue aussi un rôle dans l'échange d'énergie à travers les poissons marins entre l'estuaire et la zone côtière.

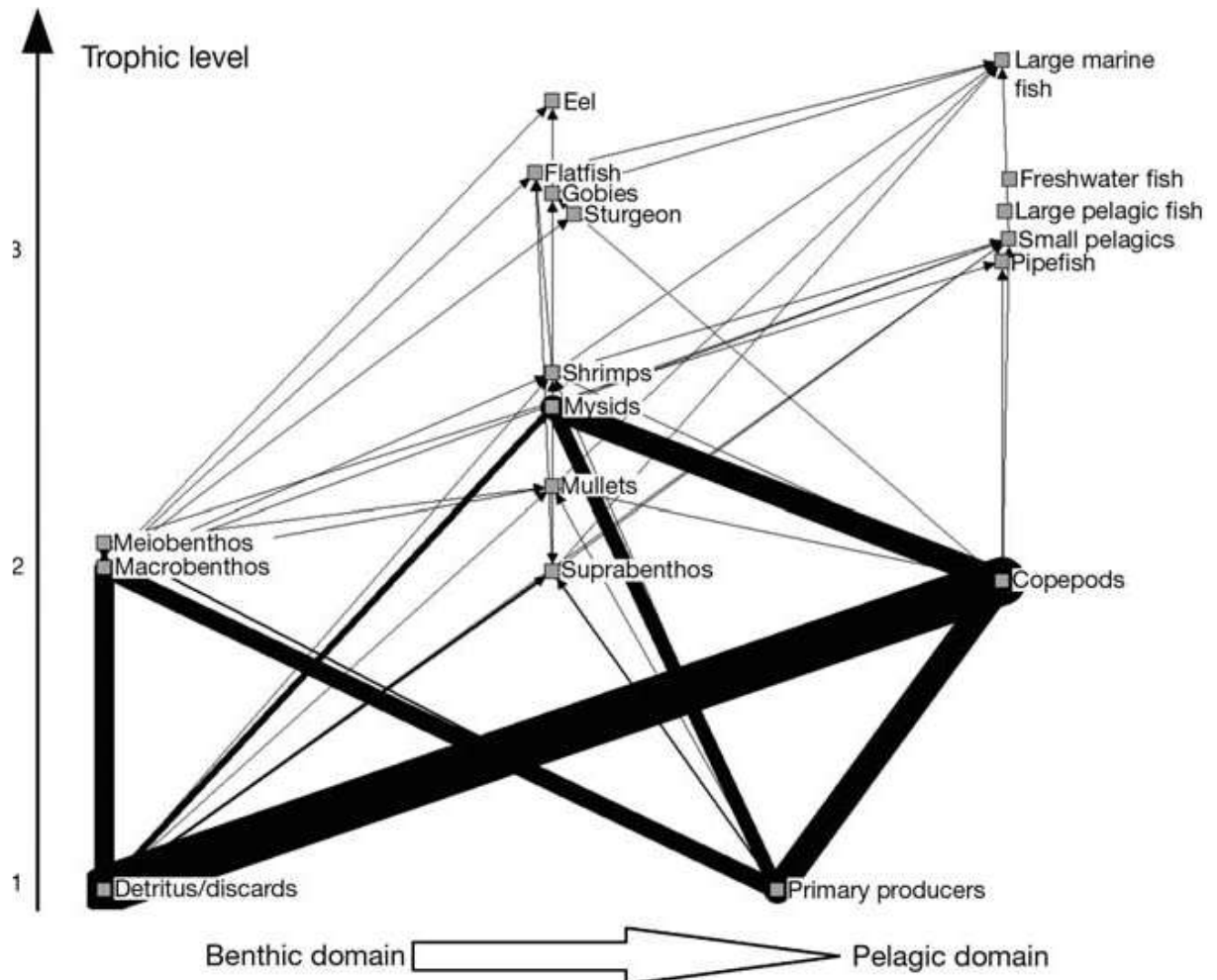


Figure 3 : Réseau trophique de l'estuaire de Gironde. Seuls des flux importants (flèches) et des compartiments (carrés) sont représentés (>90% de la valeur de débit total et 70 % des interactions totales). La largeur de chaque flèche est ajustée à la valeur de débit trophique (Lobry et al., 2008).

### I.3 Cas d'étude : L'Escaut

#### I.3.1 Généralités

L'Escaut, Schelde en néerlandais et Scheldt en anglais, est un fleuve s'étendant sur 355km de long dont le bassin versant couvre une superficie de 21 863 km<sup>2</sup>. Il prend sa source dans le nord de la France à Gouy-le-Câtelet (St Quentin). Avant de se jeter dans la mer l'Escaut traverse deux pays : la Belgique et les Pays-Bas (fig 4a). L'influence de la marée se fait ressentir jusqu'à 160 km de l'embouchure, soit au niveau de Gent, ou elle est arrêtée par des écluses.

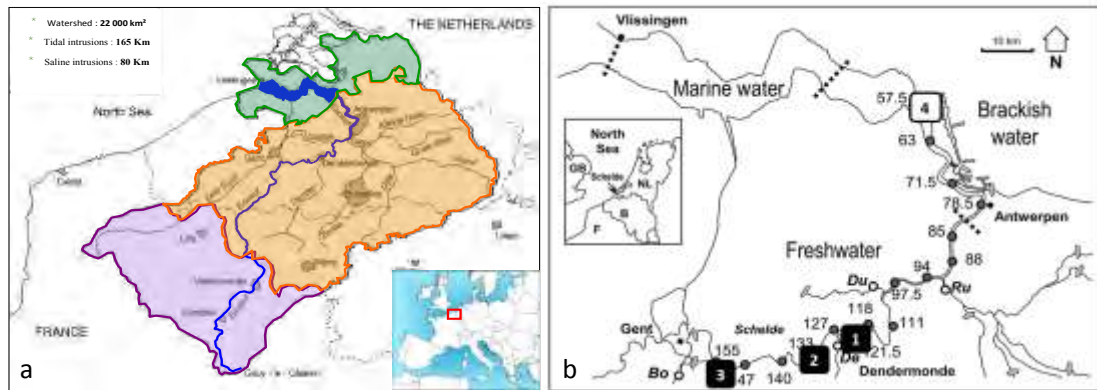


Figure 4 : a) Carte du bassin versant de l'Escaut, b) Map of the Scheldt estuary with OMES sampling stations, designated by their distance, in km, upstream from Vlissingen (mouth). Dotted lines indicate limits between marine water, brackish water and freshwater reaches. Antwerp station considered. Number 1 : Dendermonde, 2: Uitbergen and 3 : Melle.

### 1.3.2 La restauration

Au cours des années, un grand nombre de fonctionnalités écologiques ont connu des altérations voir même des pertes au sein des grands estuaires et ceci est essentiellement dû à un développement des activités anthropiques (e.g. Meire et al., 1992 ; Van Damme et al., 1997 ; Long et al., 2000 ; Verity 2002a, 2002b ; Baird et al., 2004). La restauration de ces écosystèmes irremplaçables est devenue essentielle (Van Damme et al., 2005 ; Istva'novics & Honti, 2012 ; Romero et al., 2016).

L'Escaut est l'un des estuaires le plus suivi au monde, les différents projets et/ou commissions ont permis de mettre en place des mesures restauratrices devant répondre plus efficacement à la dégradation de la qualité écologique de l'Escaut et de son bassin. En effet, depuis environ trente ans, menés par différents pays, de nombreux travaux de restauration ont eu lieu. Avec notamment, la mise en place du traitement des eaux usées sur le bassin versant de l'Escaut (Posel, 2007, Brion et al., 2016).

Au cours de l'histoire, des aménagements de l'Escaut en faveur de la navigation et la protection contre des inondations ont réduit fortement la taille des zones inondables et donc ont causé d'importantes modifications environnementales. En 2005, le plan SIGMA (plan d'aménagement de l'Escaut) est réactualisé afin de prendre en compte à la fois l'aspect sûreté pour la population mais aussi pour la nature. La restitution

d'espaces à la nature est donc engagée par le gouvernement flamand (Cox et al., 2006 ; Jacobs et al., 2008 ; Jacobs et al., 2009).

Le programme scientifique OMES (Onderzoek Milieu-effecten Sigma-plan = « recherche sur les conséquences du plan Sigma ») a été créé en parallèle afin de pallier au manque de connaissances sur la partie supérieure de l'estuaire situé entre Gand et Anvers (« Zeeschelde »). Ce programme commandé par le gouvernement Flamand permet depuis novembre 1995 un suivi conséquent et multidisciplinaire de l'écologie de la partie d'eau douce estuarienne de l'Escaut. A l'heure actuelle, on constate que la restauration du milieu a entraîné une amélioration notable de la qualité de l'eau. Parmi les divers organismes suivis dans le cadre d'OMES, on retrouve le zooplancton.

### 1.3.3 Contexte physico-chimique

#### 1.3.3.1 Régime hydraulique

L'Escaut est de type D (Fig. 1), c'est-à-dire qu'on retrouvera des courants de marées souvent plus importants que les débits fluviaux. En effet, les marées se propagent de Vlissingen jusqu'à la ville de Gand avec des hauteurs respectives de 4 et 2 m et y sont arrêtées par les barrages-écluses de Gentbrugge (Fig. 4b). La figure 5 révèle un faible débit depuis 2004 et notamment dans la partie d'eau douce. L'influence de la marée ainsi que les faibles débits vont induire un temps de résidence variable mais élevé, estimé à 2 ou 3 mois pour la totalité de l'estuaire (Soetaert and Herman, 1995).

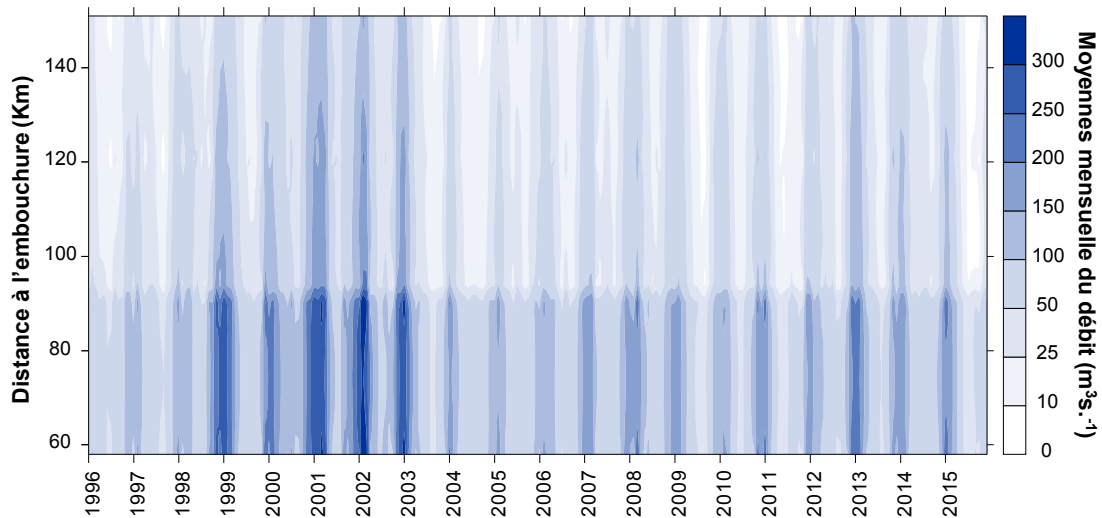


Figure 5 : Moyenne mensuelle des débits dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

### 1.3.3.2 Salinité

Comme vu précédemment, l'Escaut est de type D, il y a donc peu de stratification verticale en termes de salinité. En revanche il y a un fort gradient de salinité au niveau horizontal (Van Eck et al., 1997 ; Baeyens et al., 1998). En fonction des apports hydrologiques issus des débits il y aura des variations de salinité, c'est pourquoi selon la saison au niveau d'Anvers on retrouve de l'eau plus ou moins salée. L'Escaut va donc être subdivisé en trois zones : une zone polyhaline (salinité  $>10$ ), une zone saumâtre ( $0.5 < \text{salinité} < 10$ ) et une zone d'eau douce (salinité  $< 0.5$ ) (fig. 7).

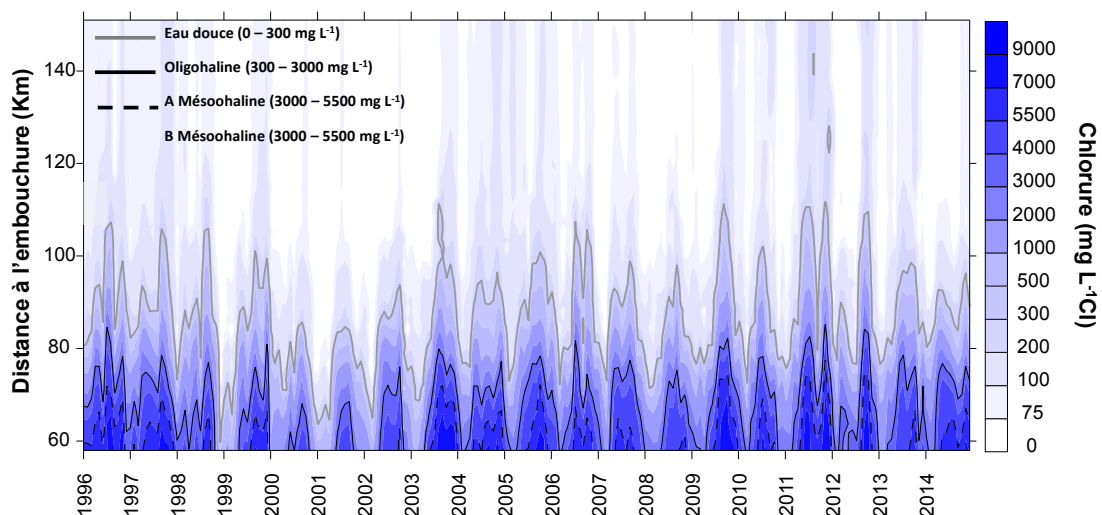


Figure 6 : Chloronité dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

### 1.3.3.3 Concentration en oxygène

L'amélioration de la qualité de l'eau de l'estuaire se révèle notamment par l'augmentation de la concentration en oxygène dissous du milieu. Durant des années la concentration en oxygène a été très faible ( $<0.08 \text{ mg L}^{-1}$ , fig., 7) sur tout le tronçon Grens – Melle. Ensuite, seule une zone de déficit en oxygène aux alentours d'Anvers (km 78) a persisté, résultant des rejets d'eaux usées. Durant la dernière décennie la concentration en oxygène dissous est relativement stable et égale entre l'eau saumâtre et l'eau douce.

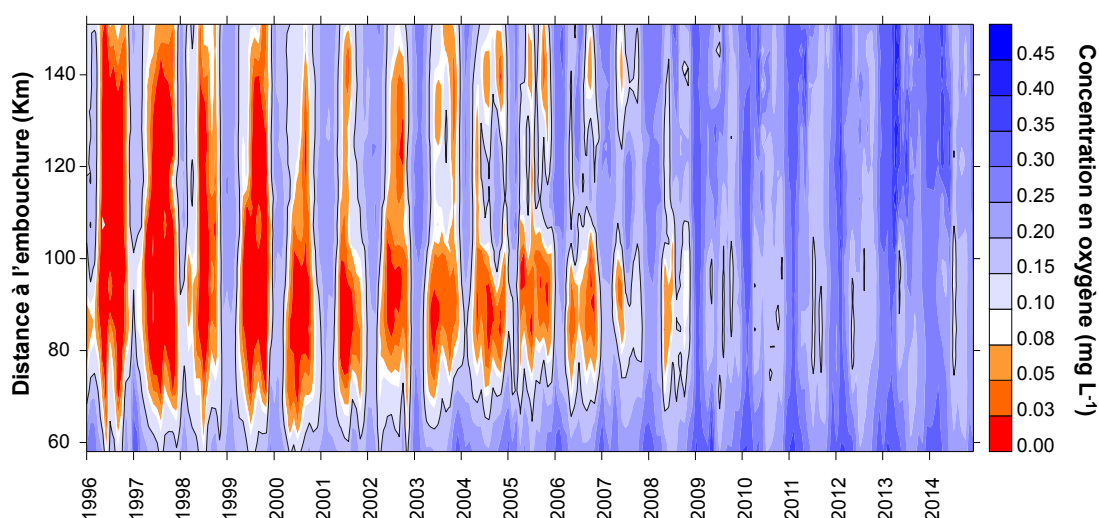


Figure 7 : Concentration en oxygène dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

### 1.3.3.4 Concentration en ammonium

L'azote est un élément essentiel de la matière vivante, pouvant se présenter sous plusieurs formes dans les milieux aquatiques. Ce paragraphe, fait référence à l'azote ammoniacal ( $\text{N-NH}_4$ ), qui est un élément présent dans les milieux aquatiques riches en matières organiques en décomposition. Au-delà d'une concentration de  $2 \text{ mg L}^{-1}$  de  $\text{N-NH}_4$  constitue un élément toxique pour le milieu. La figure 8, montre une concentration supérieure à  $2 \text{ mg L}^{-1}$  dans l'Escaut jusqu'en 2006, puis une forte diminution pour atteindre des valeurs en dessous de  $2 \text{ mg L}^{-1}$  ces dernières années.

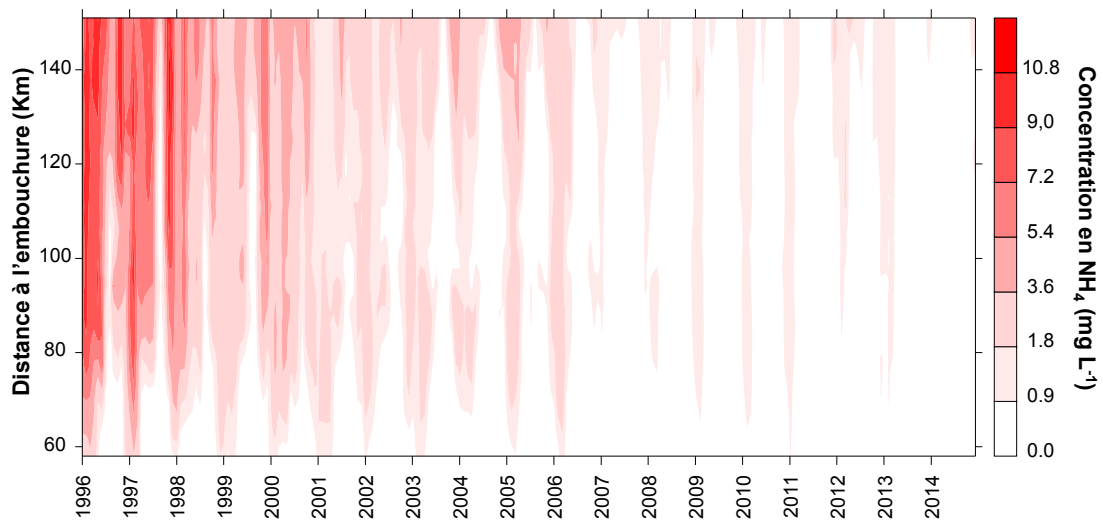


Figure 8 : Concentration en  $\text{NH}_4$  dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

### 1.3.3.5 Matière en suspension

Les matières en suspension totale (MES) sont l'un des facteurs importants du fonctionnement du système estuarien. Dans l'Escaut (fig 9), la majeure partie de la MES est détritique. La dégradation bactérienne de la matière organique contenue dans les MES entraîne des déficits de la concentration en oxygène dissous (Van Damme et al., 2005). La matière détritique, semble également être une source de nourriture, notamment pour le zooplancton (Heinle et al., 1977 ; Harris et al., 1977 ; Castel & Feurtet, 1989).

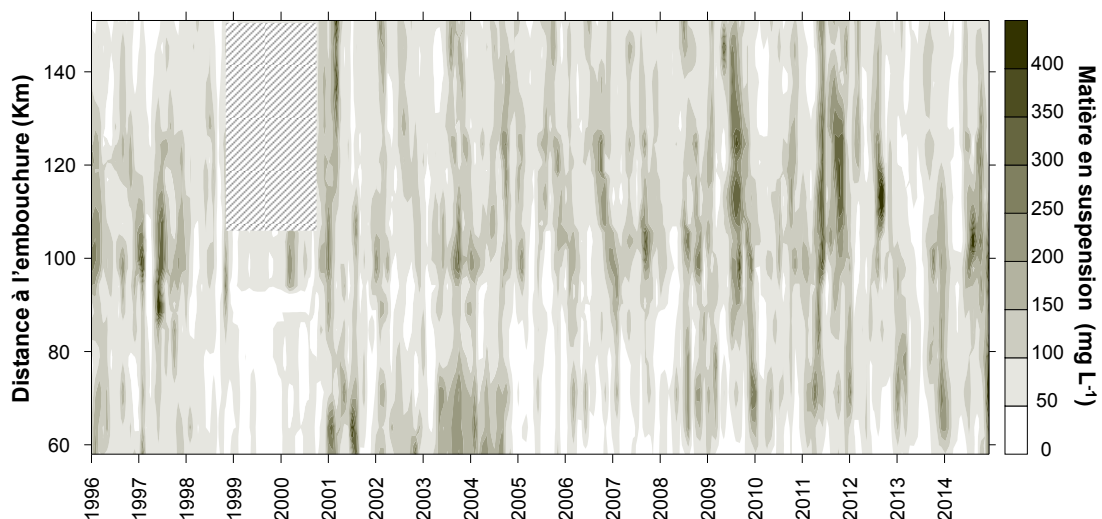


Figure 9 : Concentration de la MES dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.



### 1.3.3.6 Production primaire et biomasse phytoplanctonique

Les travaux de Cox et al. (2009), montrent un changement de milieu d'hypereutrophe à eutrophe dans l'Escaut en quatre étapes. L'étape 1 représente l'état hypereutrophié, caractérisé par des concentrations d'ammonium élevées, l'hypoxie ou l'anoxie du milieu ainsi que l'inhibition de la croissance des algues. La phase de transition (étape2), ou le milieu est passé de l'état hypereutrophié à l'état d'eutrophisation classique. L'étape 3 montre l'état eutrophisé avec les efflorescences algales intenses. La continuation des apports en nutriments et l'activité de broutage du zooplancton peuvent amener à la dernière étape, avec une réduction des efflorescences algales intenses.

Depuis que les concentrations en oxygène se sont améliorées, la production primaire du phytoplancton a pu reprendre. La concentration en Chl *a* en eau douce a augmenté (fig. 10).

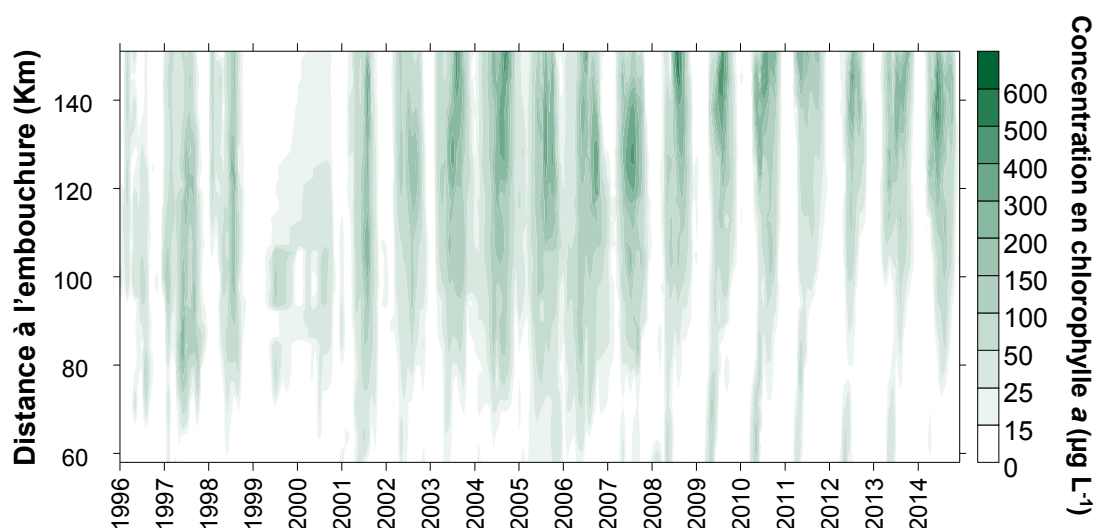


Figure 10 : Concentration de la Chl *a* dans l'estuaire de l'Escaut, de la station de Melle (km 151) jusqu'à Grens (km 51). Données issues du monitoring d'OMES, ECOBE, Université d'Anvers.

Les comptages phytoplanctoniques, réalisés à l'université de Gand (Research Group Protistology and Aquatic Ecology ; PAE), montrent un changement au sein des communautés de phytoplancton. Tout d'abord, il y a une augmentation de la biomasse des chlorophytes depuis 2009, puis un changement au sein de la communauté des diatomées, avec une inversion de dominance entre petites et grandes diatomées. Après une dominance de *Cyclotella* spp. (1996-2002), l'espèce *Actinocyclus normanii* était dominante en 2003-2008 mais depuis 2009 c'est *Cyclotella* spp. qui redevient dominante.

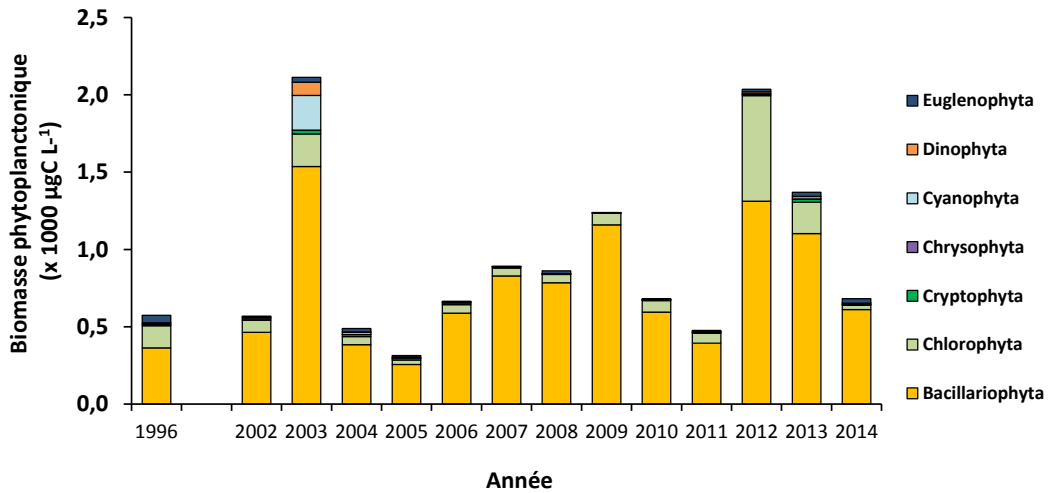


Figure 11 : Biomasse phytoplanctonique de la zone d'eau douce de l'estuaire de l'Escaut en fonction des années. Données issues des travaux de l'université de Gand, PAE.

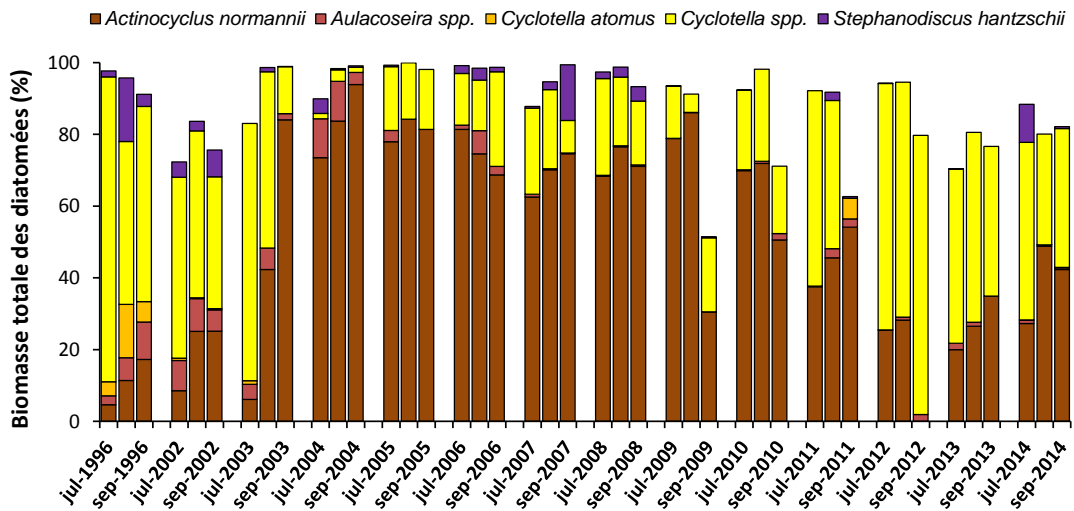


Figure 12 : Contribution à la biomasse phytoplanctonique totale des 5 taxons dominants de diatomées de la zone d'eau douce de l'estuaire de l'Escaut en fonction des années. Données issues des travaux de l'université de Gand, PAE.

### 1.3.4 Le zooplancton de l'Escaut

#### 1.3.4.1 Généralités

Rendant disponible la matière organique issue des producteurs primaires pour les consommateurs secondaires, le zooplancton occupe une position clé au sein des réseaux trophique pélagiques (Sautour et Castel, 1995 ; Tackx et al., 2003; Maes et al., 2005). Wilson (1994) et Appeltans et al. (2003) vont jusqu'à leur allouer un rôle de bioindicateur. Au cours d'une succession de projets, la dynamique zooplanctonique de l'Escaut a pu être suivie. Les études ont débuté à la fin des années 60 avec les travaux de De Pauw (1975), qui a quantifié le zooplancton pendant la période 1967-1969 de Vlissingen à Gand. Durant cette période, la communauté zooplanctonique du tronçon d'eau douce (la Zeeschelde) était dominée par des rotifères, des cyclopoïdes et des cladocères. Le copépode calanoïde *Eurytemora affinis* était une espèce importante dans l'Escaut, observée de l'embouchure jusqu' à Anvers, avec un maximum au printemps dans la zone d'eau saumâtre. Après une longue interruption dans la recherche sur le zooplancton de l'Escaut, Soetaert & Van Rijswijk (1993) ont quantifié les communautés zooplanctoniques entre 1989 et 1991 de Vlissingen à Anvers. Ils observent essentiellement la même composition de la communauté en eau saline et saumâtre que rapportée par De Pauw (1975), mais la zone en amont d'Anvers est caractérisée par une très faible densité de zooplancton. Les travaux qui ont suivi se sont concentrés plus particulièrement sur l'eau saumâtre de l'Escaut. La dominance d'*E. affinis* au printemps dans cette zone est suivie par un déclin de la population d'*E. affinis* pour laisser place au calanoïde *Acartia tonsa* en été (De Pauw, 1975 ; Soetaert et Van Rijswijk, 1993 ; Sautour et al., 1995 ; Tackx et al., 2004 ; Azemar et al., 2007). Avec les travaux de Mialet et al. (2010), on constate que la population d'*E. affinis* a drastiquement étendue sa position dans l'Escaut après 2007. La population d'*E. affinis* s'est également développée dans le tronçon d'eau douce et y est maintenant dominante.

#### 1.3.4.2 *E. affinis*

*E. affinis* (Poppe, 1880), copépode calanoïde, fait partie des espèces l'une des plus étudiées. Pouvant s'acclimater rapidement à des fluctuations de salinité (Roddie et al., 1984, Devreker et al., 2004, 2009 ; Michalec et al., 2010) elle est présente dans un très grand nombre d'estuaires notamment dans l'hémisphère nord, et y est souvent l'une des bases essentielles de leurs chaînes trophiques (Fockedey & Mees, 1999 ; Winkler & Greve, 2004).

Cette espèce est typiquement estuarienne et, est située dans zones oligo-mésohaline à des salinités comprises entre 0 et 18 (Vaupel-Kleine & Weber, 1975 ; Mouny et al., 1998 ; Lawrence et al., 2004).

Différentes études ont révélé la présence de cette espèce dans les eaux douces estuariennes et ce en Amérique du Nord, en Europe ou encore en Asie (Lee, 1999 ; David et al., 2005 ; Mialet et al., 2010). De plus, cette espèce souvent retrouvée dans des zones turbides et ayant la capacité à sélectionner sa ressource alimentaire (Sautour and Castel, 1995 ; Billones et al., 1999 ; Gasparini et al., 1999 ; Tackx et al., 2003) dans un milieu essentiellement détritique, présente un grand intérêt écosystémique. *Eurytemora affinis* domine fortement la communauté mésozooplanctonique de l'estuaire de l'Escaut puisque celui-ci peut représenter, en termes de densité, jusqu'à 70% du mésozooplancton (Chambord et al., accepté). *E. affinis* est par conséquent un maillon essentiel de l'écosystème estuarien de l'Escaut.

### 1.4 Problématique et objectifs de la thèse

#### 1.4.1 Contexte

Le projet OMES a pour but d'aboutir à un modèle d'écosystème qui aidera les décideurs à gérer ce système estuarien (Meire et al., 1997). De façon générale, ce programme de recherche permet l'acquisition de données sur la répartition et le rôle fonctionnel de divers compartiments. Cette acquisition de données multidisciplinaires, comprenant des données biotiques mais aussi des données abiotiques, permet de mettre en avant le suivi suite à la restauration du milieu.

Inscrit dans le projet OMES, le travail réalisé par cette thèse s'inscrit dans la continuité des études déjà démarrées concernant la dynamique de la communauté

mésozooplanctonique de l'estuaire. Ce travail a considéré de nouveaux éléments sur les communautés mésozooplanctoniques dans la partie d'eau douce de l'estuaire, d'une part par une étude des communautés, via le monitoring d'OMES et d'autre part par des expériences *in semi-situ* sur des processus fonctionnels tels que la tolérance à des basses concentrations en oxygène et les relations proie-prédateur.

#### 1.4.2 Objectifs

Afin de répondre aux attentes de ce projet, l'ensemble du travail de thèse s'est articulé autour de quatre principaux objectifs. Les objectifs 1 et 2 sont détaillés dans le chapitre 2.

Objectif 1 : Cet objectif vise à **vérifier les perspectives issues des travaux de Mialet et al. (2010)**, sur la répartition spatiale du mésozooplancton dans l'amont de l'estuaire. Notamment, la durabilité dans le temps de la population d'*E. affinis* installée en eau douce.

Objectif 2 : Une fois la distribution spatiale des communautés mise en avant, cette étude s'est attachée à **déterminer** pour chaque grand taxon zooplanctonique **les facteurs favorisant leur développement** respectif.

Objectif 3 : Suite à la restauration du milieu, la concentration en oxygène a considérablement augmentée. Ce facteur est passé de limitant à optimal pour le développement de certaines espèces. Afin d'estimer **l'impact des faibles concentrations en oxygène** sur les différents taxa de zooplancton, des expériences *in semi-situ* ont été réalisées, les résultats font l'objet du chapitre 3.

Objectif 4 : Le chapitre 4 de cette étude concerne le fonctionnement trophique de l'amont de l'Escaut, via le mésozooplancton et plus particulièrement la **sélectivité de l'espèce dominante *E. affinis***. Et enfin, nous nous sommes intéressés à la caractérisation de **l'impact du broutage** de cette espèce (représentant 70% du mésozooplancton dans la partie eau douce de l'Escaut) **sur le phytoplancton**.

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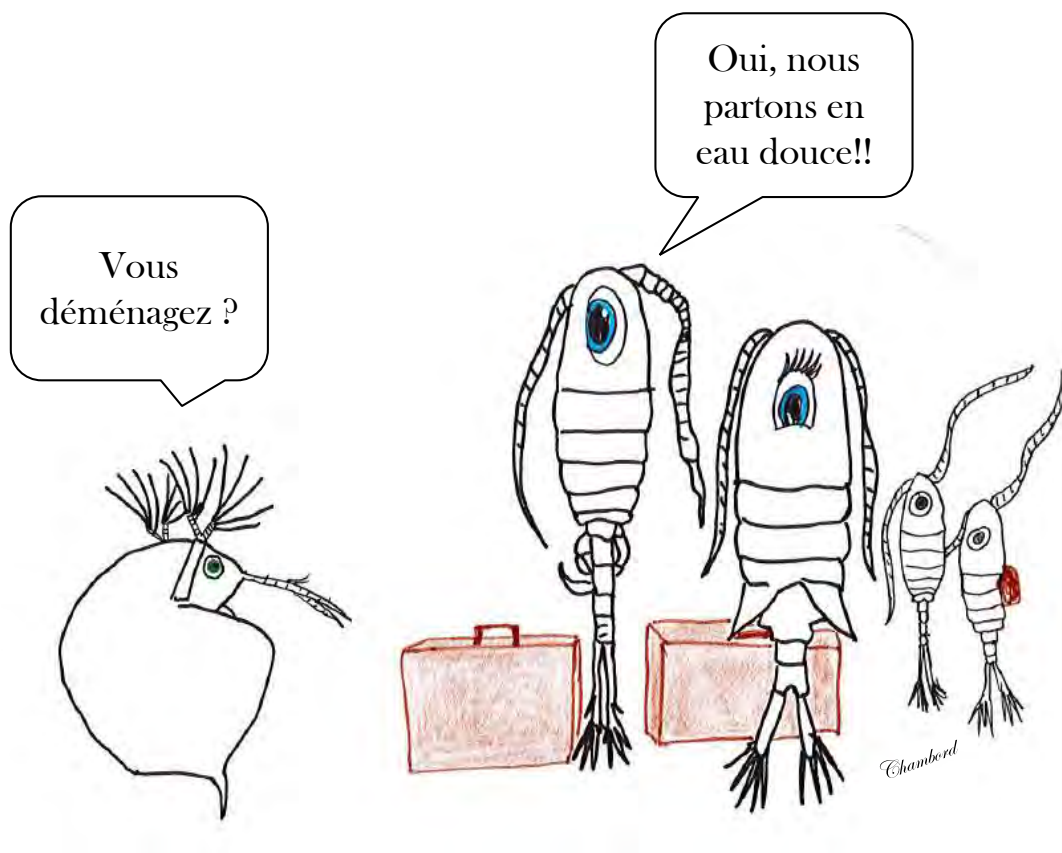
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## II. CHAPITRE 2 :

### Les affinités du mésozooplancton dans l'eau douce de l'estuaire de l'Escaut

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## II.1 Résumé

### II.1.1 Contexte et objectifs

La qualité de l'eau de l'estuaire de l'Escaut s'est considérablement améliorée depuis les années 1990, en particulier en amont. Sachant sa position clé au sein des réseaux trophiques (Tackx et al., 2003 ; Maes et al., 2005b) ainsi que son rôle potentiel de bioindicateur (Appeltans et al., 2003), le zooplancton de l'estuaire est étudié depuis 1996 dans le cadre du programme OMES. Ces études ont permis de révéler des changements dans les communautés zooplanctoniques. En effet, le copépode *Eurytemora affinis*, généralement abondant dans les eaux saumâtres et quasi-absents de l'eau douce avant 2007, s'est depuis sensiblement développé dans les eaux douces (Mialet et al., 2011). Il représente désormais 70 % de l'abondance mésozooplanctonique de l'eau douce. Simultanément, l'abondance des copépodes cyclopoïdes a considérablement diminué dans la zone d'eau douce, tandis que l'abondance des cladocères n'a pas changé.

Objectifs de cette étude :

- 1) L'évolution de la communauté zooplanctonique d'eau douce de l'Escaut, décrite par Mialet et al. (2011) pour la période 2007-2009, à plus long terme
- 2) Une fois le constat confirmé, le second objectif a été d'analyser les facteurs environnementaux ou biologiques qui expliquent le mieux les changements observés sur les communautés zooplanctoniques, et ce sur les trois stations en amont.

### II.1.2 Principaux résultats

Dans la partie d'eau douce de l'estuaire entre 2002 et 2012, la qualité de l'eau a continué à s'améliorer. Depuis 2006, la concentration en oxygène présente une amélioration significativement plus élevée. Les concentrations en NH<sub>4</sub>-N sont significativement plus faibles après 2006 dans les trois stations. A partir de 2008, nous constatons une augmentation des concentrations moyennes annuelles de Cl<sup>-</sup> et SPM. La concentration moyenne annuelle de la Chl *a* est significativement plus faible après 2006 et ce uniquement sur la station 3.

En fonction de ces divers changements environnementaux, nous pouvons classer nos stations selon leur qualité de l'eau. Dendermonde (St 3) est la station ayant la meilleure qualité d'eau, ensuivie d'Uitbergen (St 2) et enfin, de Melle (St 1) avec la qualité d'eau la moins bonne.

En considérant la période 2002-2012, les copépodes calanoides, et plus particulièrement *E. affinis*, sont les plus avantagés par cette amélioration du milieu et ce au détriment des copépodes cyclopoïdes. L'augmentation de l'oxygène, la diminution de la concentration de NH<sub>4</sub>-N ainsi qu'un faible écoulement pendant l'été représentent le « combo idéal » pour expliquer développement d'*E. affinis* dans la partie amont de l'estuaire.

Les changements dans la communauté zooplanctonique ont suivi un gradient induit par l'évolution spatio-temporelle de l'amélioration de la qualité de l'eau. A partir de 2007, la station la plus en aval (St 3) est devenu permissive au développement d'*E. affinis*.

Alors que plusieurs suggestions peuvent être faites pour expliquer la diminution de l'abondance des cyclopoïdes (pâturage concurrentiel, pression de prédation élevée, toxicité du NH<sub>3</sub>-N, sensibilité à l'oxygène, ...), aucune cause évidente de leur déclin ne peut être avancée.

L'abondance de cladocères dans la zone d'eau douce et saumâtre est restée constante.

II.2 Article 1: Mesozooplankton affinities in a recovering freshwater estuary

## Mesozooplankton affinities in a recovering freshwater estuary

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### II.2.1 Abstract

Water quality of the Scheldt estuary (Belgium/The Netherlands) has considerably improved in recent years, especially in the upstream, freshwater reaches. Within the zooplankton community, the copepod *Eurytemora affinis*, typically abundant in brackish water and quasi-absent from freshwater before 2007, has since substantially developed in the latter, where it now represents 90 % of the crustacean mesozooplankton community. Simultaneously, cyclopoid copepods have drastically decreased, while cladocerans abundance did not change.

The aim of this study was 1) to verify if the zooplankton community described for the period 2007–2009 by Mialet et al. (2011) has stabilized until present 2) to look for the environmental conditions favouring *E. affinis* development and causing changes in the upstream freshwater zooplankton community. To this purpose, the 2002-2012 temporal evolution of the zooplankton distribution at three stations in the upstream freshwater Scheldt estuary was analysed.

Water quality remained better after 2007 than before, and some factors revealed continuous improvement in annual mean concentrations (e.g. increase in O<sub>2</sub>, decrease in BOD<sub>5</sub> and NH<sub>4</sub>-N concentration). The increase in oxygen and the decrease in NH<sub>4</sub>-N concentration, together with low discharge during summer were the main environmental factors explaining the development and timing of *E. affinis* in the upstream freshwater reach. In this reach, *E. affinis* maximal abundance is shifted to higher temperatures (summer) compared to its typical maximum spring abundance peak in the brackish zone of the Scheldt estuary and in most temperate estuaries.

The changes in zooplankton community followed a temporal and spatial gradient induced by the spatio-temporal evolution of water quality improvement. The most downstream station (3) became permissive for *E. affinis* development (oxygen concentration > 4 mg L<sup>-1</sup>; NH<sub>4</sub>-N concentration < 2 mg L<sup>-1</sup>, discharge (Q) < 50 m<sup>3</sup> s<sup>-1</sup>) from 2007 onwards, and this station showed the highest *E. affinis* and the lowest cyclopoid abundance. At the more upstream stations *E. affinis* developed later and less strongly, and cyclopoids decreased less in abundance than at station 3.

While several suggestions can be made to explain the decrease in cyclopoid abundance (competitive grazing, high predation pressure, NH<sub>3</sub>-N toxicity, sensitivity to oxygen), no clear cause for their decline could be advanced.

Water quality improvement in the freshwater Scheldt estuary has led to environmental, post-heavy polluted conditions, under which no data on zooplankton populations in this estuary were available. This has permitted to detect a plasticity in the temperature tolerance of *E. affinis*.

**Keywords:**

Scheldt estuary, zooplankton distribution, restoration, *Eurytemora affinis*, oxygen concentration, NH<sub>4</sub>-N concentration.

## II.2.2 Introduction

After decades of ecological degradation, many ecosystems benefit from restoration efforts. Also aquatic ecosystems in general and estuarine ecosystems in particular recovered due to better waste water treatment and habitat restoration. The initial objective of restoration to achieve an original ecological status has often been replaced by a more realistic goal. At present, an ecosystem is considered restored when it is able to sustain itself structurally and functionally and consequently to provide ecosystem services (Borja, 2008a,b; Druschke, 2015). Achievement of this goal for severely and long-term polluted systems is a slow and often fluctuating process, which necessitates long-term monitoring to allow adjustment of the restoration and management strategies when necessary (Borja et al., 2010). Indeed, long-term studies allow the detection of latency of biological responses, as it may take time to observe a recovery or a change of communities (Hawkins et al., 2002; Etcheber et al., 2011). However, long-term studies are quite rare because they are expensive and require a lot of material and human effort (Hawkins et al., 2002).

Monitoring of restoration outputs is also a new experience in science, in the sense that, until a decade or two ago degradation of ecosystems was witnessed, not recovery (e.g. Verity 2002a,b; Kemp et al., 2005, Verity & Borkman, 2010; Langseth et al., 2014). In most cases ecological quantitative monitoring only started after the system was already substantially polluted and consequently little information is available on the pristine or slightly polluted state of system. Hence, it would not be surprising that presently recovering systems face situations that have not been described or quantified yet and reveal some unknown or unexpected ecological relationships. Also, as restoration efforts occur in parallel to ongoing global changes (Anneville et al., 2002, 2009; Verissimo et al., 2013), and estuaries continue to respond to the evolution of multiple user demands, precise effects of environmental factors are often difficult to disentangle.

Many studies on response of estuarine communities focus on large scale aspects, such as hydro-geomorphology, wetland restoration, recovery of top-predator populations (e.g. Orson et al., 1992; Ducrotou & Dauvin, 2008; Beauchard et al., 2011; Maire et al., 2013; Teuchies et al., 2013; Hogg et al., 2014).

Long term monitoring of water quality in estuarine systems are generally focusing on nutrient loads and their management in an eutrophication context. In

general, these studies show that system responses to decreased nutrient loading can differ substantially between systems and between subsystems of an estuary, mainly according to hydrography (e.g. Verity, 2002a,b; Kemp et al., 2005; Boynton et al. 2014; Romero et al., 2016).

However, most papers dealing with estuarine water quality and its effect on pelagic biota report ongoing degeneration of the system rather than restoration, and suggest restoration as a future perspective. For example, Smit et al. (1997) report the evolution in water quality and pelagic community of the Rhine-Meuse Delta after its enclosure in 1970 and give some recommendations (i.e. restoring the estuarine character) for future management. Flaherty et al. (2013) describe the dependence of the nekton community to disturbances of the natural patterns of freshwater delivery to the Florida Bay estuary (USA) by flood-control and water-supply projects and highlight the importance of nekton community monitoring prior to hydrologic manipulations.

Within the pelagic system, phytoplankton and bacteria, as major producers and recyclers, are classically included in biogeochemical studies. Estuarine zooplankton, in spite of being the main trophic link between the primary estuarine resources (i.e. phytoplankton, detritus) and the higher trophic levels (i.e. hyperbenthos, juvenile fishes and some adult fish species) has received little attention. Falcao et al. (2012) report consequences of restoration measures in the Mondego estuary (Portugal) for the zooplankton community. After re-establishment of water circulation between the two branches of this estuary, eutrophication symptoms decreased and higher mesozooplankton density, mainly of estuarine species was observed.

The lack of information on zooplankton response to estuarine restoration is probably due to the minor importance of zooplankton in quantitative energy flow budgets. In addition, contrary to fishes, birds and macrobenthos, the microscopic zooplankton organisms are not readily considered by various stakeholders as a proof of successful management. Also, while phytoplankton can in part be studied by indirect methods (i.e. pigment concentrations, automatic fluorescence monitoring), there are no automated methods used routinely for the evaluation of community composition or activity of zooplankton in estuarine systems. Methods such as Zooscan (Grosjean et al., 2004; Gorsky et al., 2010), applicable in open marine systems or lakes (Schultes et al., 2009; Lelièvre et al., 2012; Marcolin et al., 2013) are

not of use in estuarine systems because of the high suspended matter concentration. Analysing estuarine zooplankton samples thus remains a painstaking task, demanding expertise and patience. Yet, having relatively short lifespan, zooplankton organisms can react rapidly to changing environments (Falcao et al., 2012; Cardoso et al., 2013) and can therefore be considered worthwhile monitoring.

This paper, presents the results of a long-term (11 years) monitoring of the Scheldt estuary, after restoration of water quality from a heavily polluted status since the 1960-1990ties to a less polluted one in the last decades (Heip, 1988; Baeyens et al., 1998; Van Damme et al., 2005; Cox et al., 2009). The Scheldt estuary is a macrotidal estuary covering a marine, brackish and freshwater gradient under tidal influence (Meire et al., 2005; Van Damme et al., 2005) (Fig. 13).

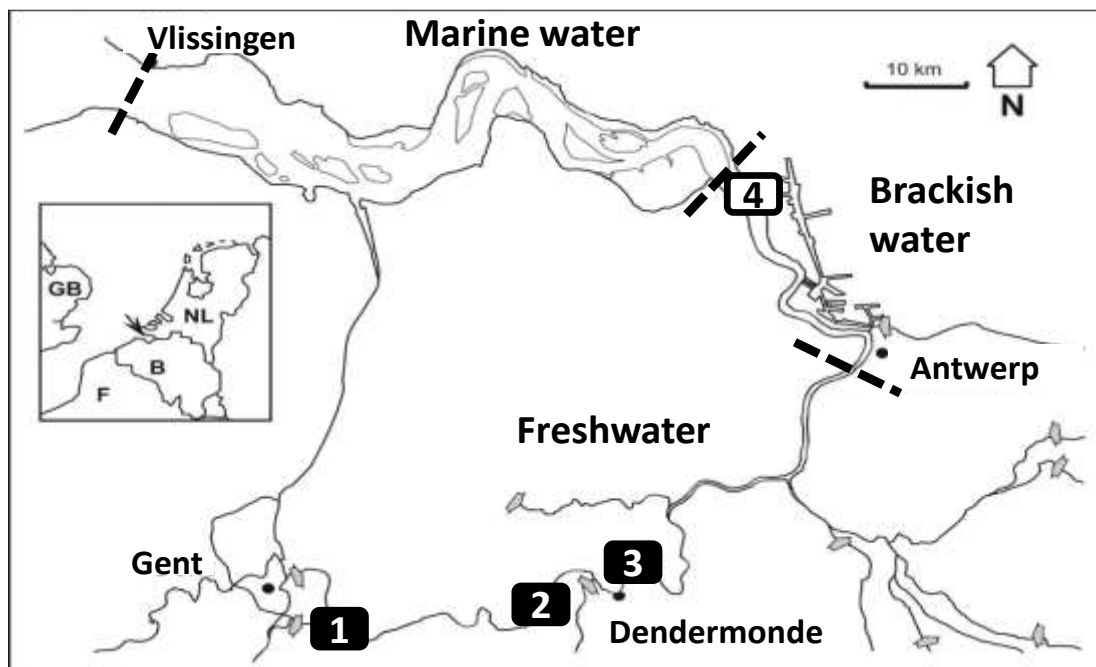


Figure 13 : Map of the Scheldt estuary. Dotted lines indicate limits between marine water, brackish water and freshwater reaches. Freshwater stations considered in this study are represented by black squares: 1- Melle, 2- Uitbergen, 3- Dendermonde. The brackish water station Grens is symbolized by the white square and number 4.

The Scheldt has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands. Its estuary is situated from the mouth at Vlissingen until the city of Ghent, where the tide is stopped by sluices. The tidal reach between the Dutch – Belgian boarder and the city of Ghent, called the Sea Scheldt, covers 110 km of brackish water and 80 km of one of the few remaining freshwater tidal habitats in Europe (Meire et al., 2005; Van Damme et al., 2005). The

main tributaries entering the Sea Scheldt are the Dender, Durme and Rupel. Contrarily to most temperate estuaries, the Scheldt estuary is characterized by vertically well-mixed water flows (Baeyens et al., 1998), generally showing no salinity or current stratification (Heip, 1988).

The Scheldt estuary has historically been one of the most polluted in Europe (Heip, 1988; Meire et al., 2005). Since two decades, European directives, and specifically the 2000 European Water Framework Directive (WFD), have incited important efforts to increase wastewater treatment capacity on the Scheldt basin and reduce pollutant loads, including organic matter, entering the estuary (Brion et al., 2015). In relation to the upstream reach treated in this paper, the Boven Scheldt watershed wastewater treatment capacity was increased from  $2,6 \cdot 10^6$  inhabitant equivalents (IE) in 1986 to  $5,0 \cdot 10^6$  IE in 2014. The capacity on the basin of the Dender, which joins the Scheldt just upstream station 3, increased, during the same period, from  $31,5 \cdot 10^3$  to  $343 \cdot 10^3$  IE (Brion, pers. comm., 2016). As a consequence, oxygen concentrations increased while nutrient concentrations (i.e.  $\text{NH}_4^+\text{-N}$ ,  $\text{PO}_4$ ) decreased concomitantly. Since 2009, the morphology of the estuary has also changed due to its deepening between the mouth and Antwerp harbour, leading to increased salinity of the estuary and an increase of the tidal pumping.

In response to the improved water quality, the zooplankton community experienced important changes (Appeltans et al., 2003; Tackx et al., 2004; Mialet et al., 2010, 2011). Between 1996 and 2006, the brackish water community was dominated by calanoid copepods, with the calanoid copepod *E. affinis* being the most abundant species, especially during spring. The freshwater community, (i.e: upstream of Antwerp) was more diverse, dominated by rotifers, cyclopoid copepods and cladocerans. In 2007, a community shift occurred in the freshwater tidal part with *E. affinis* becoming dominant and reaching higher densities than in the brackish part. Concomitantly, cyclopoid copepods decreased to very low abundances. The abundance of cladocerans in the freshwater and brackish water zone remained constant. In the brackish water zone, no significant fluctuations in zooplankton community composition was observed between 1996 and 2009 (Mialet et al., 2010, 2011). Mialet et al. (2011) report changes in the zooplankton community composition in the freshwater observed during three years (2007-2009). Considering natural variability of zooplankton populations in estuarine systems (e.g. Roman et al., 2005;

David et al., 2005; Feike & Heerkloss, 2008) it is necessary to follow up the zooplankton community for a longer period in order to verify the stability of the new situation. Appeltans et al. (2003) and Mialet et al. (2010; 2011) have evidenced the relationship between oxygen concentration (as proxy for water quality) and *E. affinis* development in the freshwater reach of the Scheldt. In recent years, water quality in the freshwater reach is comparable with than in the brackish water reach, but not better. So, in case the 2007–2009 zooplankton community composition should stabilize, the question arises why *E. affinis* can develop so well in the freshwater reach and why cyclopoid copepods decrease in abundance following water quality improvement.

The objectives of the present study are 1) to verify the zooplankton community situation in the Scheldt upstream freshwater reach considering the 2002-2012 period, including five years before and 5 years after the 2007 community shift, and 2) to analyse which environmental or biological factors best explain the observed changes in comparison to the situation prior to 2007.

### II.2.3 Material and methods

#### II.2.3.1 Monitoring

The OMES project, monitors hydraulics, water chemistry and biology (phytoplankton, zooplankton, fishes) and studies marsh development and interactions between marshes and the pelagic compartment of the Sea Scheldt. The OMES results are used in management sustaining ecosystem models (e.g. Cox et al., 2009; Beauchard et al., 2011). OMES monitoring is conducted monthly since 1996 at 17 stations in the main channel and at the mouths of the main tributaries. Since 2012, sampling frequency was intensified to bi-monthly between March and September. A full description of the OMES project is given in Meire (2005) and Van Damme et al. (2005).

Abiotic parameters were collected according to the OMES protocol (Van Damme et al., 2005). At each station and sampling date, surface water samples were collected in the middle of the estuary using bucket hauls from a vessel. For each sampling date several physico-chemical variables were measured: 5-day biochemical oxygen demand (BOD5) using a WTW OXI 96 oxymeter, pH and temperature using a CONSORT C832 electrode and dissolved oxygen concentration ( $O_2$ ) using a WTW OXI 325, equipped with Clark electrode. Suspended particular matter (SPM) samples were filtered on pre-combusted Whatman GF/C glass fiberfilters. Water samples for quantification of chlorophyll *a* (Chl *a*) concentration was filtered over Whatman GF/F glass fiber filters. Sub-surface water was sampled for the determination of the concentrations of dissolved silica (DSi), chlorine (Cl<sup>-</sup>), ammonium ( $NH_4^+$ -N), nitrates ( $NO_3^-$ -N), nitrites ( $NO_2^-$ -N), orthophosphates ( $PO_4^-$ -P), and total phosphorous (P<sub>tot</sub>) concentrations in the laboratory. Samples were stored at 4°C and analyzed in 24h by colorimetry using a SKALAR SA 5100 segmented flow analyzer. SPM was analyzed by gravimetry, Chl *a* by spectrophotometry. Details of analyzing procedures are given in Van Damme et al. (2005). The Flemish Administration for Waterways and Maritime Affairs (WNZ) provides daily discharge measures (Q) of the tributaries Boven Scheldt, Dender and Rupel.

For this study, the stations Melle (1), Uitbergen (2) and Dendermonde (3) situated at 155, 140 and 121,5 km from the mouth at Vlissingen respectively (Fig 1), were selected as exclusive freshwater stations, with no or very low influence of salinity (maximum salinity observed at the most downstream station 3: 0.58).



In the Scheldt, seasonal variations in discharge and temperature are more marked in the freshwater zone than in brackish and marine areas. Fresh water discharge shows a seasonal variation, with maximum values in March, declining during April and May to summer minima (Meire et al., 2005, Van Damme et al., 2005). Stations 1 and 2 are situated in the freshwater zone with short residence time (monthly mean residence time 0,3 days in winter, 2,3 days in summer) and station 3 is situated at the beginning of the freshwater zone with long residence time (1,0 in winter, 7,1 days in summer). Stations 1 and 2 receive water from the Boven Scheldt, station 3 receives water from both the Dender and Boven Scheldt. In the following, the zone between station 1 and station 3 will be referred to as 'upstream freshwater zone' (UFZ). For comparing with the brackish water reach concerning *E. affinis* seasonal distribution, the most downstream station of the OMES monitoring, Grens at km 58 (station 4) was used.

#### II.2.3.2 Sampling of mesozooplankton

Mesozooplankton was sampled at these 4 stations during the monthly (or bi-monthly, between March and September) sampling from 2002 till 2012 by filtering 50 L of sub-surface water through 50 µm mesh plankton net. Zooplankton was fixed in a formaldehyde solution (4 % final concentration). Species were identified and individuals counted under a stereo- (90x magnification) and microscope (960x magnification).

Organisms were identified mainly at the genus level and when possible at the species level. Afterwards, cladocerans and copepods were grouped at suborder and order levels, respectively, to perform statistical analyses, except for the calanoid *E. affinis* that was kept to species level. Counts were converted to number m<sup>-3</sup>. Abundance data for all months, including those sampled twice, were converted to monthly means.

#### II.2.3.3 Data analyses

Trends of environmental factors reported are tested for significance using a Spearman rank correlation test at p<0.05. Differences between series of observations (for example O<sub>2</sub> concentration at two stations) are considered significant when p<0.05 following Mann-Whitney tests. The term "significant" in the text refers to at least this criterion.

The cumulative percentage distributions of mean abundance of *E. affinis* as a function of temperature classes were computed and fitted using Curve Fitting Toolbox of Matlab Software (Mathworks Inc., Version, 7.5).

For all parametric analyses, normal distribution and homoscedasticity were checked. If not respected, variables were log transformed to achieve normal distribution. Multivariate analyses (RDA and GLM) were performed to describe zooplankton communities' distribution in relation to environmental factors using Canoco software (Ter Braak, 1987, 1994).

To explore the heterogeneity in zooplankton community spatio-temporal distribution, a preliminary detrended correspondence analysis (DCA) analysis was run on the taxa dataset. As the inertia was  $<2.6$ , a linear distribution is expected so a redundancy analysis (RDA) was performed to explore relationships between environmental factors and zooplankton taxa. Significance of factors was tested by Monte Carlo permutation at  $p < 0.05$ .

Then, generalized linear models (GLM, with Gaussian family) followed by Hierarchical partitioning of variance (HP) using R software (R Development Core Team 2008) (Walsh & MacNally, 2004) were used to identify the main specific environmental predictors of *E. affinis*, cyclopoid copepods and cladocerans abundance in time and space. To optimally take into account the seasonality of occurrence of these organisms, these analyses were done by 3 seasons. Spring was considered from March to May, summer from June to September and winter from October to February. A Hierarchical Partitioning was subsequently used to identify the independent contribution of each explanatory variable by reducing collinearity resulting from correlation between variables. This allows ranking the importance of the covariates in explaining the response variable independently of the other covariates. The proportion of deviance explained by each model ( $D^2$ ) was calculated according the formula proposed by Guisan and Zimmermann (2000).

## II.2.4 Results

### II.2.4.1 Temporal evolution of the zooplankton community

Between 2002 and 2007, adult and C5 stages of calanoid copepods were observed in relatively low abundance (with an average 150 ind. m<sup>-3</sup> with a maximum of 2 000 ind. m<sup>-3</sup>) in the UFZ. From 2007 to 2012, the mean annual abundance of calanoid copepods strongly increased a maximum of 11 500 ind. m<sup>-3</sup> at station 3 in 2009 (Fig. 14a). Calanoid copepods abundance was, between 2007 and 2012, higher at station 3 than at station 2 and 1. At the latter station, they became highly abundant (11 000 ind. m<sup>-3</sup>) in 2011 only and dropped to 840 ind. m<sup>-3</sup> the following year.

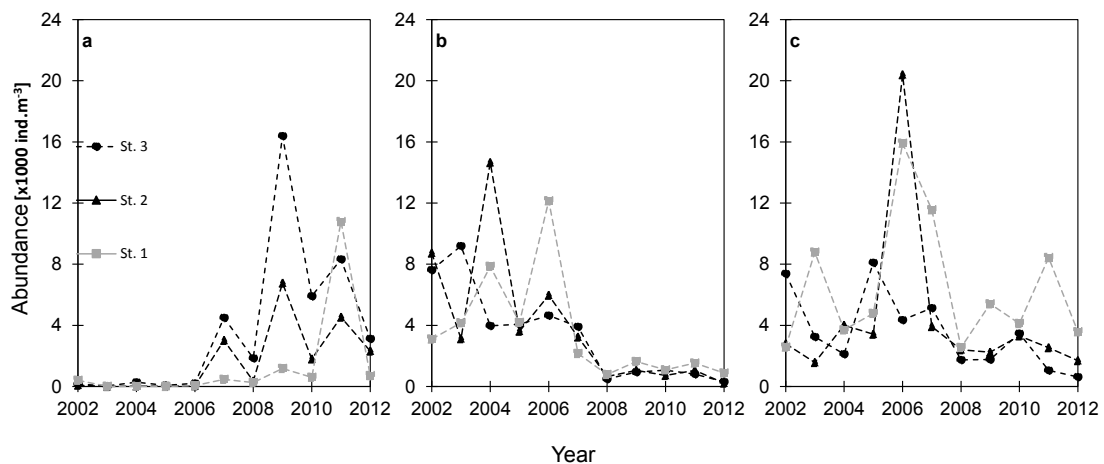


Figure 14 : Evolution of the yearly mean abundance of adult and C5 stages of (a) Calanoid copepods, (b) Cyclopoid copepods and (c) Cladocerans in the Scheldt estuary, at 3 stations. Station 1 (Melle), grey lines), 2 (Uitbergen) and 3 (Dendermonde).

The average annual abundance of adult and C5 stages of cyclopoids (Fig. 14b) varied between 3 000 and 14 700 ind. m<sup>-3</sup> during the period 2002 to 2007, with a maximum of 14 700 ind. m<sup>-3</sup> at station 2 in 2004. Cyclopoid abundance declined sharply after 2007, ranging between 220 and 3900 ind. m<sup>-3</sup>. Cyclopoid abundance fluctuated less after 2007 than before.

Unlike *E. affinis* and cyclopoid abundances, cladoceran mean annual abundance (Fig. 14c) changed little in time during the entire study period with the exception of an mean annual abundance peak (23 400 at station 2 and 15 900 ind. m<sup>-3</sup> at station 1 in 2006). Both before and after 2007, annual mean cladoceran abundance was higher in station 1 than at the two other stations.

The proportion of *E. affinis*, as a percentage of copepod adults and C5, showed a significant increase during the study period in the UFZ of the Scheldt estuary. Indeed, from 4 % in 2002, it increased up to 50 % in 2007 to further increase up to 70-90 % (spearman rank,  $p < 0.05$ ) after 2009 (Fig. 15).

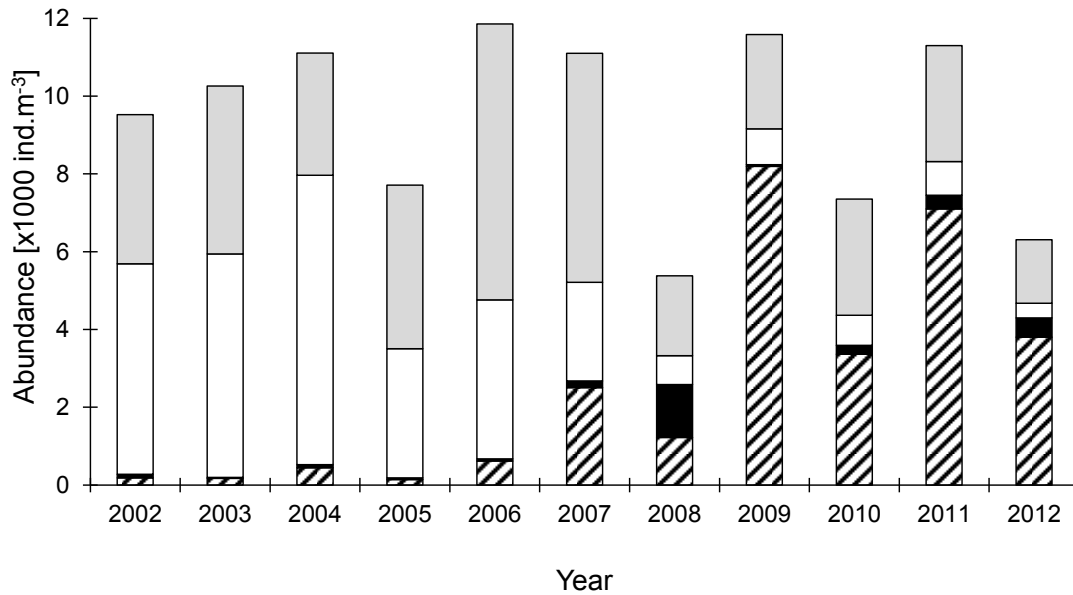


Figure 15: Contribution of different groups to total zooplankton abundance in the upstream freshwater Scheldt from 2002 to 2012.

#### II.2.4.2 Species abundance – season

An increase in the abundance of *E. affinis* (Fig. 16a) has occurred after 2007, especially when temperatures exceeded 15°C. Thus, in the UFZ, *E. affinis* mainly developed during late spring and summer when temperatures are higher than earlier or later in the year. A high cyclopoid copepod abundance was observed during the years before 2007 with an optimum temperature between 18 and 25°C (Fig. 16b), which corresponds to the summer season.

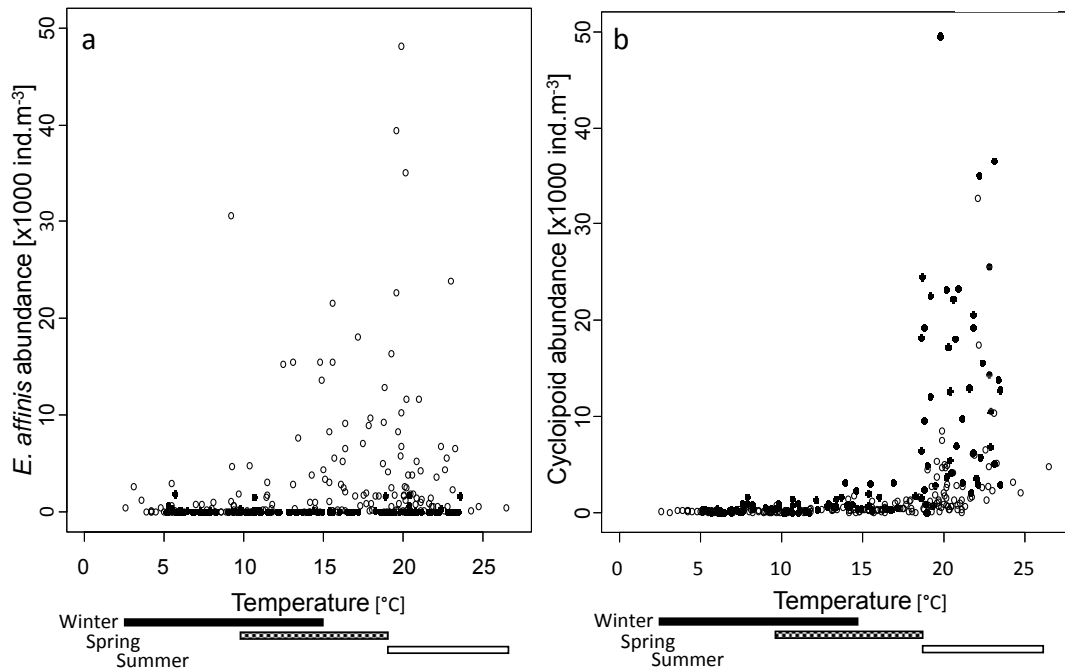


Figure 16 : Relationship between a) *E. affinis* and b) cycloipoid abundance and water temperature at the time of sampling from 2002 to 2012. Bars below the graph indicate temperature range for each season considered.

Distributions of mean abundance of *E. affinis* were symmetrical; with all values of  $R^2$  for Gaussian distributions exceeding 0.98 (Table 1). *E. affinis* shows a clear gradient in temperature of occurrence at the different stations (Fig. 17), with values of  $\mu$  ranging from 9.56°C at the brackish station 4 until 18.58°C at station 1.  $\mu$  values for all UFZ stations were significantly higher than the  $\mu$  value from the brackish water station 4 ( $p < 0.05$ ). Standard deviations in the first three stations were not statistically different, which means that the three Gaussian distributions are shifted by  $\mu$  values that are significantly different ( $p < 0.05$ ). However, in the most upstream station 1 both  $\mu$  and sigma are significantly different from the other distributions ( $p < 0.05$ ). The temperature range (i.e. variance) at Melle, the most upstream station, is smaller than the other stations.

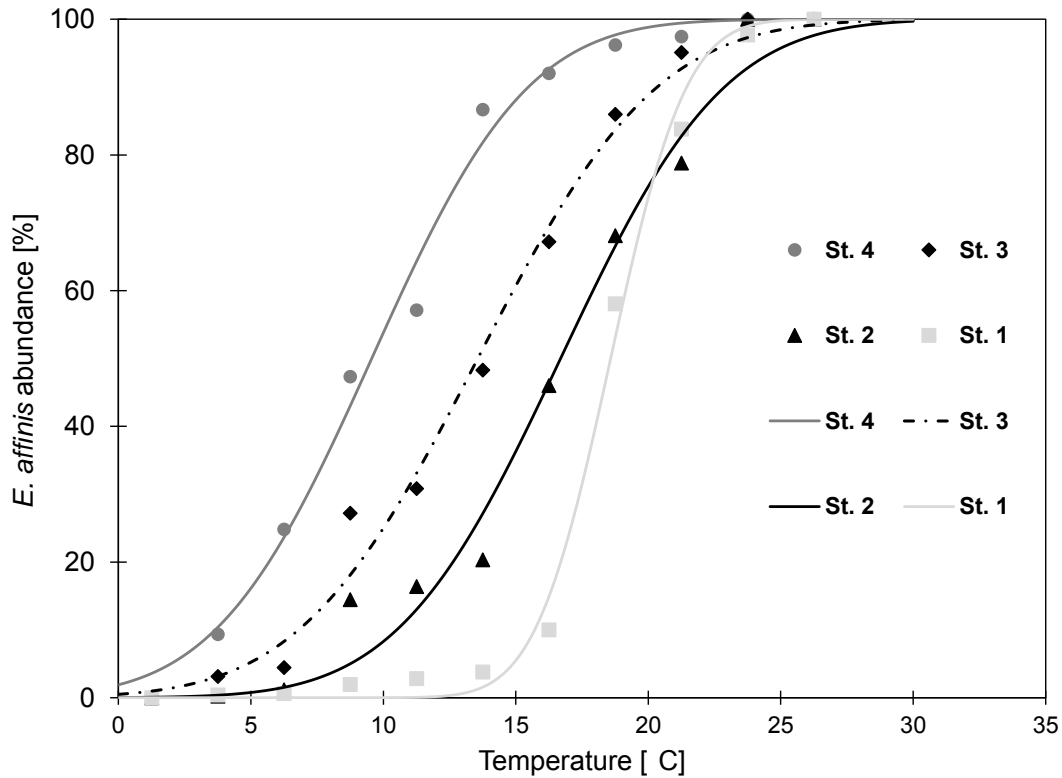


Figure 17 : Cumulative distributions of average abundance of *E. affinis* as a function of temperature classes at the three freshwater stations (1,2,3) and at a brackish water station (4, Grens). The fitted parameters and their associated statistics are shown in Table 1.

Table 1: Values of the fitted parameters of the normal cumulated density function of average abundance of *E. affinis* and their associated statistics (see also Fig. 5).

	Brackish water		Freshwater	
	Grens	Dendermonde	Uitbergen	Melle
R <sup>2</sup>	0.9923	0.9916	0.9859	0.9952
μ	9.561	13.56	16.69	18.58
sigma	4.611	5.281	4.827	2.239
μ Coefficients (95% confidence bounds)	9.044-10.08	12.96-14.17	15.96-17.43	18.26-18.89
Sigma Coefficients (95% confidence bounds)	3.874-5.348	4.425-6.136	3.783-5.872	1.79-2.688

#### II.2.4.3 Water quality evolution

The temporal evolution of the main water quality parameters shows a similar trend during the study period for the three stations (Fig. 18). Between 2002 and 2007, environmental factors characterising water quality in the UFZ showed a downstream-upstream gradient, water quality being worst at station 1 and best at station 3, with station 2 in between. Since 2008, a gradual increase in chlorinity was measured for all 3 upstream freshwater stations, which slightly dropped again in 2012. Some

environmental factors representing water quality remained rather stable after 2007, others continued to improve. Annual mean O<sub>2</sub> concentration was significantly higher since 2006 at all three stations than before, while annual mean BOD<sub>5</sub> decreased significantly from 2006 onwards and mean concentrations of NH<sub>4</sub>-N were significantly lower at all three stations after 2006 than before. Annual mean concentrations of CL and SPM increased from 2008 onwards. No significant changes in annual mean Chl *a* concentration were observed at stations 1 and 2 over the study period, but at station 3, Chl *a* concentration was significantly lower after 2006 than before. DSi and PO<sub>4</sub>-P concentrations stayed rather stable at all three stations (Fig. 18). Water quality was best in the most downstream station 3, worst in the most upstream station 1.

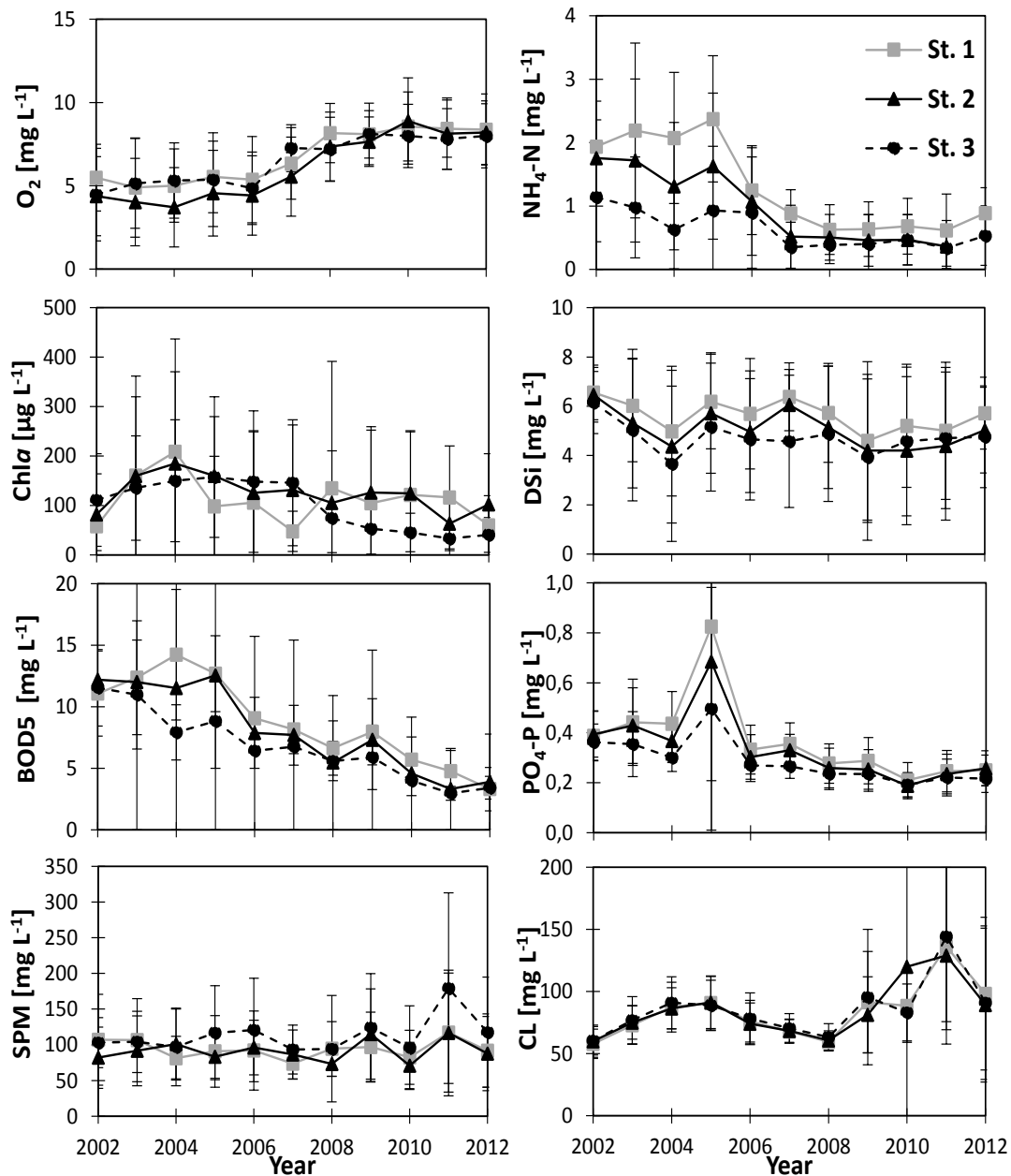


Figure 18 : Evolution of environmental factors in the Scheldt estuary from 2002 to 2012, showing annual mean values. Melle (St1), Uitbergen (St2) and Dendermonde (St3). Vertical bars are standard deviations.

#### II.2.4.4 Coupling zooplankton species distribution and environmental factors

The first two axes of the RDA analysis accounted for 35 % of the variance of the zooplankton spatio-temporal distribution that was significantly related to (in decreasing significance): temperature, oxygen, BOD5, CL, NH<sub>4</sub>-N, discharge, Chl *a*, SPM, NO<sub>3</sub>-N, NO<sub>2</sub>-N, DSi and SO<sub>4</sub> (Fig. 19; Table 2).



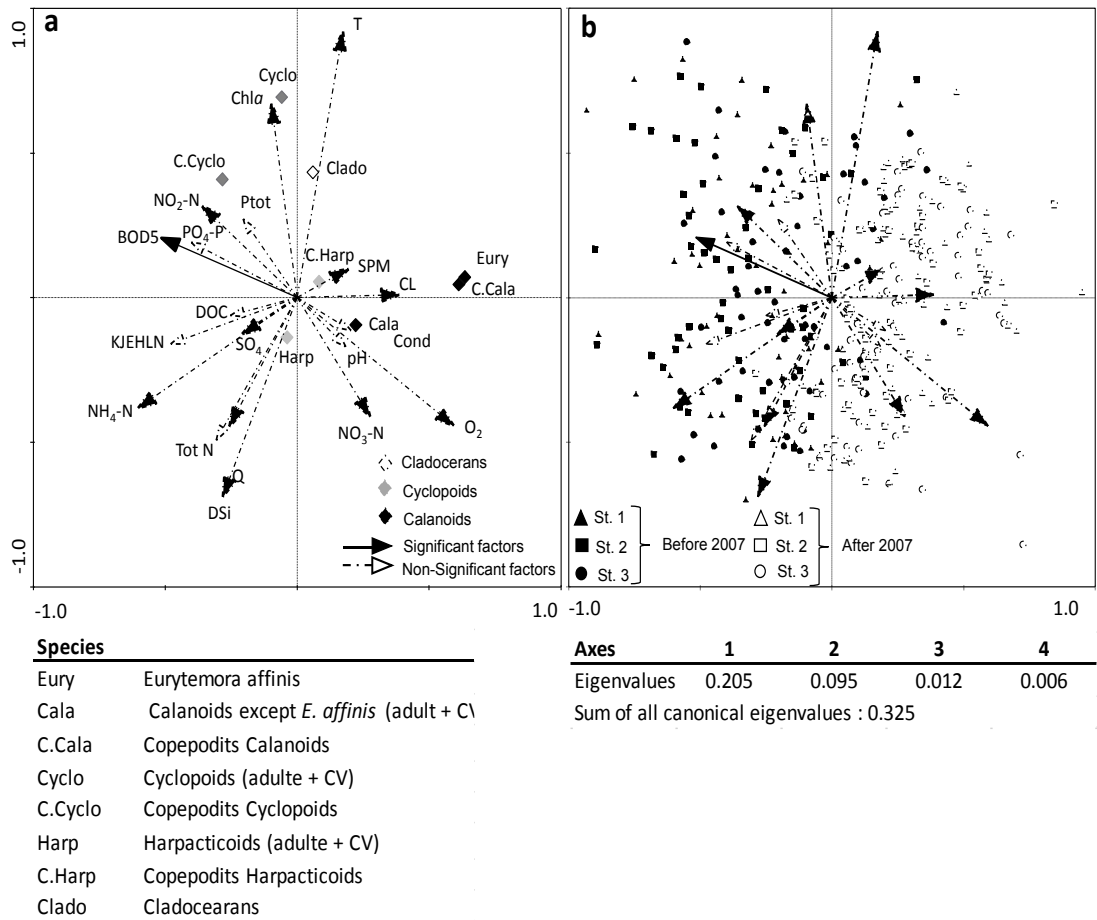


Figure 19 : Redundancy analysis (RDA): taxa-environmental factors 1,2 (a, b) biplots in the upstream freshwater Scheldt for the 2002-2012 period.

Table 2: Results of the redundancy analysis (RDA) testing the effects of abiotic factors on the abundance distribution of mesozooplankton in the upstream freshwater Scheldt. Factors are listed by their eigenvalues ( $\lambda$ ), i.e., the contribution of each factor to the explanation of zooplankton abundance, without covariability (see Methods). \*, \*\*, \*\*\* indicate factors that were statistically significant (Monte Carlo permutation test at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  respectively).

RDA conditional effects			
Factors	$\lambda$	F-value	p-value
T	0.1	43.72	0.001***
O <sub>2</sub>	0.09	35.42	0.001***
BOD5	0.03	11.16	0.001***
CL	0.02	8.97	0.001***
NH <sub>4</sub> -N	0.01	6.4	0.001***
Q	0.02	5.86	0.001***
Chl $a$	0.01	5.41	0.003**
SPM	0	3.41	0.010**
NO <sub>3</sub> -N	0.01	2.53	0.040*
NO <sub>2</sub> -N	0	3.02	0.014*
Dsi	0.01	2.6	0.031*
SO <sub>4</sub>	0	2.43	0.042*
PO <sub>4</sub> -P	0.01	2.01	0.079
DOC	0.01	1.53	0.153
pH	0	1.63	0.155
Ptot	0	1.31	0.235
Ntot	0	1.29	0.262
KJEHLN	0	1.15	0.312
Conductivity (Cond)	0	1.13	0.361
Sum of all $\lambda$	0.32		

The geometric representation of zooplankton abundances and environmental factors in the plan defined by the first two axes indicates that the distribution of *E. affinis*, calanoid copepodites, and to a lesser extent calanoid adults/C5 are associated with oxygen concentration, NO<sub>3</sub>-N, CL and SPM, and perpendicular to the temperature and Chl *a* vectors (Fig. 19a). Most other environmental variables are in opposite direction towards the left hand side of the biplot. Cyclopoid adults/C5 and copepodites, as well as cladocerans are associated with increasing temperature and Chl *a* concentration (Fig. 19a). A clear separation on the second and third axes of the RDA between samples before and after 2007 was highlighted (Fig.19b), the latter

being situated at the opposite of poor water quality indicators (NH<sub>4</sub>-N, NO<sub>2</sub>-N, and BOD5).

In order to eliminate the seasonal influence, GLM analysis aiming at detecting the specific factors influencing *E. affinis*, cyclopoids and cladocerans specifically, were run for each season separately. In view of the change in timing of *E. affinis* maximal abundance between the UFZ and the brackish water zone and between stations within the UFZ, the complete spring-summer period was also considered. While showing the complete results in Table 3. For clarity, only the seasons of maximum abundance of each zooplankton taxa, and factors being significant predictors at a significance level of at least  $p < 0.01$  (ANOVA) are discussed here.

Table 3 : Results derived from the hierarchical partitioning and generalized linear models performed on *E. affinis*, cyclopoids copepods and cladoceran abundance responses, during the three seasons considered and during the combined spring-summer season. Variables shown for each multiple regression were kept after a stepwise procedure of variable selection. The relative independent contribution of each environmental predictor is given as a percentage of the total independent contribution. Significance following ANOVA testing is indicated as \*, \*\*, \*\*\* for  $p < 0.05$ , 0.01 and 0.001 respectively. Minus signs between brackets show negative regression coefficients in the GLM analysis. The D-squared of generalized linear models is the equivalent of the R-squared value of linear models that measures the proportion of variation that the model accounts for. AIC is a model comparison test, to select the "best" model. Ni: not included in the model, ns: not significant.

<i>Eurytemora affinis</i>			Cyclopoids				Cladocerans			
Spring	Summer	Spring-Summer	Winter	Spring	Summer	Spring-Summer	Winter	Spring	Summer	Spring-summer
53.99	53.66	47.04	45.32	50.77	50.42	47.75	49.99	59.05	31.41	24.23
ni	ni	ni	16.09***	25.01***	22.75***	6.91***	ni	ni	78.55***	ns
20.57*** (-)	ns	5.43 ** (-)	13.77*** (-)	21.35*** (-)	4.89*	16.67***(-)	32.84***(-)	12.68***(-)	19.13*	ni
5.89*** (-)	10.48** (-)	5.45* (-)	39.52***	44.26***	ni	20.76***	ns	30.50***	ni	31.96***
ni	7.29***	9.3*	4.6* (-)	ni	ni	ni	ni	ni	ni	ni
ni	ni	ni	ni	ns	ni	3.06***(-)	21.82***(-)	ns	ni	ni
49.81*** (-)	6.47*** (-)	18.84*** (-)	ni	ns	ni	ni	ni	ns	ni	ni
ni	8.01*	ni	19.53**	ni	ni	ni	15.53***	18.92**	ns	32.23***
ni	ni	ni	ni	ni	ni	ni	ns	ns	ni	8.65**
23.71**	36.58***	27.84***	ni	ni	23.74*** (-)	19.56***(-)	3.44**	ni	ns	ni
ni	25.28***(-)	22.94* (-)	6.45* (-)	ni	16.72*	11.71*	ni	ni	ni	ni
ni	ni	6.16*** (-)	ni	9.37** (-)	ni	5.24***(-)	26.37***(-)	10.47**(-)	ni	ni
ni	ns	4**	ni	ni	26.74*** (-)	ns	ns	27.50***(-)	2.31**(-)	23.37***(-)
ni	5.86**	ni	ns	ns	5.54* (-)	ni	ni	ns	ni	ni

During spring and summer, the two most important seasons for *E. affinis*, the selected GLM model explained 53.99 and 53.66 % of the total deviance of the *E. affinis* abundance respectively (Table 3). The best predictors were the NH<sub>4</sub>-N (negative) and O<sub>2</sub> concentrations (positive), which independently contributed to 49.81 % (spring) and 6.47 % (summer) for NH<sub>4</sub>-N and 23.71 % (spring) and 36.58 % (summer) for O<sub>2</sub>. Discharge was a significant negative predictor of the *E. affinis* abundance in spring and when considering the spring and summer period together, but contributed less than O<sub>2</sub> or NH<sub>4</sub>-N. Cyclopoid copepods were mainly abundant in summer. In this season, DSi and O<sub>2</sub> concentrations were the most significant negative predictors of cyclopoid abundance, independently contributing to 26.74 %, 23.74 %, 26.74 %, 23.74 %, 26.74 %, 23.74 %, 26.74 %, 23.74 %, 26.74 %, 23.74 %, 26.74 %, 23.74 %.

while BOD<sub>5</sub>, PO<sub>4</sub>-P and discharge were significant positive predictors of cyclopoid abundance, explaining 22.75 %, 16,72 and 4,89 % of the deviance, respectively. The selected model explained 50.42 % of the total deviance of the cyclopoid abundance (Table 3). When considering spring and summer together, O<sub>2</sub> concentration and discharge were significant negative predictors for cyclopoid abundance (19,67 and 16,67 % respectively), while Chl *a* concentration (20,76 %), PO<sub>4</sub>-P concentration (11,71 %) and BOD (6,91 %) were the strongest positive predictors of cyclopoid abundance.

Like cycloids, cladoceran abundance was most important in summer. GLM showed the importance of two major factors explaining cladoceran abundance in summer, namely BOD<sub>5</sub> (78.5 %) and discharge (19.13 %). The selected model explained 52.62 % of the total deviance of cladoceran abundance. Considering the spring–summer period, Chl *a* concentration (31,96 %) and NO<sub>2</sub>-N concentration (32,23 %) predicted cladocerans abundance positively, while Dsi concentration predicted it negatively (23,37 %). Discharge was no significant predictor of cladocerans abundance during the spring–summer period.

*E. affinis* abundance at all three UFZ stations, plotted as a function of the three main predictors (O<sub>2</sub> and NH<sub>4</sub>-N concentration, and discharge) resumes the development conditions for this copepod *E. affinis* could develop high abundances (>3000 ind m<sup>-3</sup>) in a space limited between O<sub>2</sub> >4mg L<sup>-1</sup> and NH<sub>4</sub>-N concentrations < 2 mg L<sup>-1</sup>. However, between 1 and 2 mg NH<sub>4</sub>-N L<sup>-1</sup>, abundance values > 3 000 ind. m<sup>-3</sup> were rare. This was also the case at discharge values > 50 m<sup>3</sup> s<sup>-1</sup> (Fig. 20).

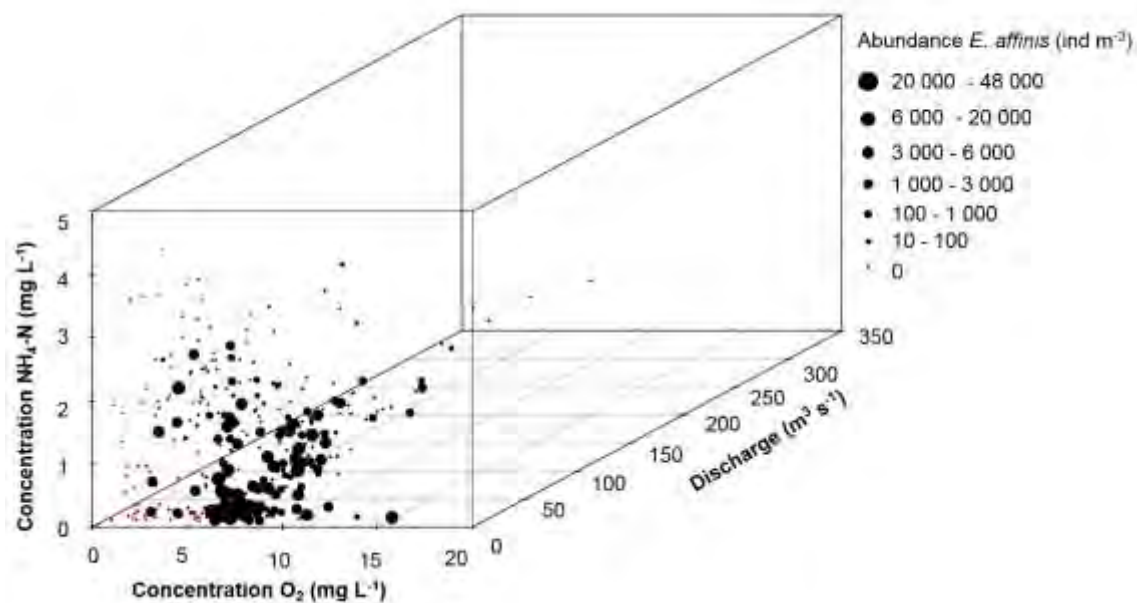


Figure 20 : Distribution of *E. affinis* abundance in the upstream freshwater Scheldt estuary between 2002 and 2012 as a function of O<sub>2</sub>, NH<sub>4</sub>-N concentrations and discharge.

## II.2.5 Discussion

### II.2.5.1 Temporal evolution of water quality

The gradual and obvious trend towards a better water quality of the Scheldt already observed in the nineties (Van Damme et al., 2005, Mialet et al., 2010) has continued in recent years. The major changes observed in this study are a decrease in concentrations of NH<sub>4</sub>-N, BOD<sub>5</sub>, PO<sub>4</sub>, NO<sub>2</sub> and an increase in O<sub>2</sub> concentrations. While before 2007 yearly mean concentrations of water variables typical of poor quality such as NH<sub>4</sub>-N, BOD<sub>5</sub>, PO<sub>4</sub>, and NO<sub>2</sub> were significantly higher at the most upstream site (station 1) than downstream (station 3), values became more similar between the 3 stations after 2007. We here consider these main changes in environmental conditions only as a background to interpret the evolution of the zooplankton community. Details and in depth discussion about two decades of water quality evolution in the Sea Scheldt, and comparison with other European estuaries, will be presented in another paper (Maris et al., in press).

*II.2.5.2 Possible causes of E. affinis' abundance increase in the UFZ*

The 2010–2012 monitoring period showed that *E. affinis* has continued to increase its presence in the UFZ, becoming highly dominant within the copepod community since 2009. *E. affinis* has previously been reported as a 'freshwater-invader' in various types of freshwater habitats in North America, Asia and Europe within the past century (Bowman & Lewis, 1989; Lee et al., 2003, for a summary), probably due to its ability to cope with low salinity by adapting its osmoregulatory system rapidly (Lee, 1999; Lee et al., 2012). As such, the colonization of the freshwater area of the Scheldt by *E. affinis* is not surprising. Yet, it raises a number of interesting questions with regard to the consequences of restoration measures upon the zooplankton community abundance and composition, especially when considering the strong decrease in cyclopoid abundance that has occurred concomitantly. During the seasons of highest abundance of *E. affinis* (spring and summer), oxygen and NH<sub>4</sub>-N concentrations contributed most in explaining the variation in its abundance. Appeltans et al. (2003) and Mialet et al. (2010; 2011) highlighted that *E. affinis*' abundance in the upstream Scheldt (i.e. salinity < 0.5) is conditioned by oxygen concentration. Between 1996 and 2009, *E. affinis* was only abundant at salinity > 0.5 when O<sub>2</sub> concentration in the upstream area was > 4 mg L<sup>-1</sup> and > 1.3 mg L<sup>-1</sup> in the middle part of the Scheldt (km 82–110).

The GLM analysis has shown that, discharge also (negatively) contributes to explaining *E. affinis* development in the UFZ, but less strongly than oxygen and NH<sub>4</sub>-N concentration. Nevertheless, low summer discharge and hence longer residence times (2,3- 7,1 days) during summer may have favored *E. affinis* the development in the UFZ in addition to improved water quality. In a situation of particularly low spring flow, Kimmel et al. (2006) observed a rise in the abundance of *E. affinis* in freshwater areas of the Chesapeake Bay during the spring.

From 2007, oxygen concentration in all 3 UFZ stations has been, on most occasions, higher than 4 mg L<sup>-1</sup>, the threshold value advanced by Mialet et al. (2011) as permissive for *E. affinis* development. With regard to oxygen concentration, the UFZ was suitable for the development of *E. affinis* since 2007. In concert with increasing oxygen concentrations, ammonia concentrations have decreased in the UFZ over the study period. Months with NH<sub>4</sub>-N concentrations > 1 to 2 mg L<sup>-1</sup> are mainly winter months, as mineralisation is slower at low temperature. In the brackish water area, where *E. affinis* has always been abundant, ammonia concentration has

always been lower than  $1 \text{ mg L}^{-1}$  (OMES, unpublished data). In the UFZ, 2007 was the first year with no (station 3) or few (stations 2, 1) months with  $\text{O}_2 < 4 \text{ mg L}^{-1}$  and/or  $\text{NH}_4\text{-N} > 1\text{-}2 \text{ mg L}^{-1}$  between June and October (not shown). This could explain the quite sudden development of *E. affinis* in the UFZ from this year onwards. The fact that  $\text{O}_2$  and  $\text{NH}_4\text{-N}$  conditions enabling the development of *E. affinis* occurred later during long periods at stations 2 and 1 than at station 3 may also explain why *E. affinis* developed later and less importantly at these two former stations, especially at station 1, than at station 3. As to seasonality, months with  $\text{NH}_4\text{-N}$  concentrations  $> 1 \text{ mg L}^{-1}$  are winter months, as mineralisation is slower at low temperature.

This suggests that temperature could be the limiting factor for *E. affinis* development in the UFZ. However, comparison with *E. affinis* abundance  $> 2\,900 \text{ ind. m}^{-3}$  in brackish waters (i.e. station 4) shows that *E. affinis* can be present earlier in the year since there, its peak abundance was reached during March (at a temperature of  $9.8^\circ\text{C}$ ), while in the UFZ, *E. affinis* abundance peaked between March and June. In most temperate estuaries, *E. affinis* is known as a spring species (Heinle & Flemer, 1975; Sautour & Castel, 1995; Gasparini et al., 1999; Devreker et al., 2009). Apparently, its development in the UFZ implied a change in temperature distribution from a temperature corresponding to the yearly median population abundance of  $9.56^\circ\text{C}$  in brackish water to  $13.56\text{-}18.58^\circ\text{C}$  in UFZ. In the UFZ, the decrease in discharge from spring to summer has probably also contributed to *E. affinis* maximum development during summer rather than spring. Within the UFZ, a tendency for later and less strong *E. affinis* development was manifested in upstream direction, as shown in Fig. 5. This could also reflect the shorter residence times in upstream direction, hampering *E. affinis* development upstream. Although chlorinity has increased in the UFZ since 2009, potentially favouring *E. affinis* development, chlorinity in the former remained much lower than in brackish water zone (OMES, unpublished data).

Although no increase in the mean annual Chl *a* concentration was observed over the study period, primary production has increased in the UFZ following water quality improvement (Cox et al., 2009). Chl *a* concentration is generally higher in the UFZ of the Scheldt than in the brackish water zone (Van Damme et al., 2005). Hence, a higher amount of phytoplankton available might also have stimulated *E. affinis* to

extend upstream. *E. affinis* has indeed been reported to benefit from food concentration to increase its salinity tolerance (Lee et al., 2013; Hammock et al., 2015).

#### II.2.5.3 Possible cause of the evolution in cycloipoid and cladoceran abundance

Contrary to *E. affinis*, cyclopoid mean annual abundances were lower after than before 2007. Thus, it seems that cyclopoids did not benefit from the improved water quality in the UFZ.

During the season of their highest abundance (i.e. summer), cyclopoids were mostly associated with BOD5 and negatively related to DSi and O<sub>2</sub> concentrations. The negative correlation with DSi is a seasonal phenomenon as the summer peak of cyclopoids occurs each year between the fall of Dsi concentration caused by diatom blooms and the rise of the DSi concentration in autumn (not shown). The fact that UFZ residence times are increased during summer (up till a mean of 2,31 days at station 1 and 2 and 7,08 days at station 3) probably profited cyclopoid development during this season both before and after the 2007 shift.

A possible explanation for the decrease in cyclopoid abundance is a direct or indirect negative impact of *E. affinis* on other zooplankton species. While the invasion by *E. affinis* into freshwater systems has been well documented, few studies have considered the parallel evolution of the whole mesozooplankton community. From those rare studies, results suggest that, in most systems where *E. affinis* or other *Eurytemora* spp. co-occur with other zooplankton species, *E. affinis* is highly dominant (Counahan et al., 2005; Vad et al., 2012). Predation by *E. affinis* on (young) cyclopoids and cladocerans could reduce the size of these populations. Nevertheless, this seems rather unlikely, as fatty acid analysis carried out on *E. affinis* females collected from the 0.5-1.8 salinity range in the Scheldt during 2007-2008 suggested a dominant contribution of phytoplankton versus terrestrial or heterotrophic inputs in *E. affinis*' diet (Mialet et al., unpublished). Another fatty acid analysis performed on *E. affinis* sampled in 2005 for the same season and area (Van den Meersche et al., 2009) reflected a slightly more carnivorous but still phytoplankton dominated regime for *E. affinis*. Grazing experiments conducted in 2013-2014 also showed an important grazing activity by *E. affinis* (Chambord et al., unpublished).



Contrary to the observed increasing trend of *E. affinis* abundance and decreasing trend of cyclopoids abundance over the study period, the cladoceran abundance did not show any significant temporal trend in any of the 3 stations studied. Cladocerans, like cyclopoids, were found to be mostly associated with BOD5 and Chl *a* concentration and seem to thrive well in poor water quality conditions. But, in contrast to cyclopoid copepods, cladocerans seem to have been unaffected by the improvement in water quality in the UFZ. Cladocerans have the ability to produce extracellular haemoglobin (Hb) at low oxygen concentrations (Sell, 1998; Pirow et al., 2001 and earlier references therein), which explains they can thrive in eutrophic and oxygen poor environments such as the freshwater Scheldt before 2007. In copepods, the presence of this haemoglobin is scarcely documented. As far as we know, it has only been reported in a few species collected from mud dwelling and borrow habiting harpacticoids (Fox, cited by Green, 1957) and in parasitic siphonostomatoids of deep hydrothermal vent environments (Sell, 2000). Given the importance of eutrophication issues worldwide, studies about oxygen metabolism and oxygen tolerance have mainly dealt with hypoxia. Physiological studies on differences in low oxygen tolerance and metabolism between cladocerans and copepods and between calanoid and cyclopoid copepods are thus clearly needed.

An indirect consequence of high oxygen concentration is the formation of unionized ammonia (NH<sub>3</sub>-N), which is more toxic than NH<sub>4</sub>-N for organisms. Arauzo and Valladolid (2003) have found that, in waste water treatment ponds, a population of 2 zooplankton species (the cladoceran *Moina micrura* and the rotifer *Brachionus rubens*) showed high mortality rates during periods of increased pH and temperature leading to the formation of NH<sub>3</sub>-N. In the Scheldt UFZ, following the increase of primary production pH has risen between 2002 and 2008 (Cox et al., 2009), but combined with the NH<sub>4</sub>-N concentration decrease, calculated NH<sub>3</sub>-N concentrations were around 0,01 mg L<sup>-1</sup> and decreasing to 0,001 mg L<sup>-1</sup> between 2002 and 2012. These concentrations are way below the NH<sub>3</sub>-N LC<sub>50</sub> values for crustaceans which vary from 0,3– 4,4 mg L<sup>-1</sup> (Ostrensky et al., 1992).

While fish populations have been re-colonizing in the freshwater Scheldt (Breyne et al., 2011), there is no obvious reason why cyclopoids would be more heavily predated than *E. affinis*. This copepod has been shown to be a major food source for (larvae and/or adults of) sprat (*Sprattus sprattus*), herring, goby, smelt

(*Osmerus eperlanus*), flounder and striped bass in several estuarine systems (Nobriga, 2002, Martino and Houde, 2010, Mehner, 2011), among which the brackish water Scheldt (Maes et al., 2005). Nevertheless, Lankov et al. (2010) found that in the Gulf of Riga, where the zooplankton community included the cladoceran *Bosmina longispina*, rotifers *Keratella cochlearis* and *K. quadrata* and the copepod *E. affinis*, several fish species such as adult sprat, juvenile smelt and the three spined stickleback *Gasterosteus aculeatus* were mainly feeding on *B. longispina* rather than on other species. But they also highlighted that the zooplankton abundance dynamics did not reflect fish predation selectivity, suggesting that fishes may switch prey as a function of the zooplankton community composition. Byron et al. (1984), report that, in the oligotrophic Lake Tahoe (USA), the cladoceran *Bosmina longirostris* is more heavily predated by the omnivorous calanoid copepod *Epischura nevadensis* than the calanoid copepod *Diaptomus tyrrellii*. Viitasalo and Rautio (1998), observed escape reactions of cladocerans and *E. affinis* to mysid predation and suggested that copepods have higher escape abilities and need to be actively attacked while cladocerans can be collected by filter-feeding.

Competition for resources could also explain the observed changes in zooplankton distribution. Mialet et al. (2011) studied the entire OMES sampling transect from Ghent to the Dutch/Belgium border and found that cyclopoids are positively related to Chl *a* concentration. This could however be due to a spatial effect, as Chl *a* concentration in the freshwater zone, where cyclopoids were abundant before 2007, was always higher than in the brackish water zone. Yet, in the present study, considering only the upstream freshwater zone, cyclopoids are also associated with the Chl *a* concentration. Before 2007, cyclopoids in the UFZ reached highest abundance ( $> 10\ 000\ \text{ind. m}^{-3}$ ) during June-August at Chl *a* concentrations between 100-280  $\mu\text{g L}^{-1}$ . After 2007 during these months, the Chl *a* concentration decreased to median concentrations of 89  $\mu\text{g L}^{-1}$  at station 3, 202  $\mu\text{g L}^{-1}$  at station 2 and 192  $\mu\text{g L}^{-1}$  at station 1. It is possible that phytoplankton biomass has become too low to enable cyclopoids feeding efficiently, at least at station 3 and this decrease could be caused by *E. affinis* grazing, but phytoplankton biomass decrease cannot explain cyclopoid abundance decrease at the more upstream stations. The decline in phytoplankton biomass at station 3 may also be induced by an increased turbidity due to increased tidal pumping in the last years (OMES, unpublished data). In addition, other food

resources than phytoplankton need to be considered. BOD<sub>5</sub>, indicative of detritus and the associated microbial community (e.g. bacteria) was one of the significant predictors for cyclopoid abundance. Some rotifer species, such as *K. cochlearis*, *Filinia longiseta*, *Lepadella* sp., which can be abundant in the Scheldt, can efficiently consume bacteria (Bogdan & Gilbert, 1984; Sanders et al., 1989; Ooms-Wilms et al., 1995). Cladocerans feed on bacteria with more or less limited effect (Borsheim & Andersen, 1987; Güde, 1989). Cyclopoids are known for their omnivory and can thus affect the bacterial abundance (Dobberfuhl et al., 1997). In the UFZ the decrease of BOD<sub>5</sub> may have resulted from a decrease of microbial abundance, representing a resource limitation for cyclopoids. *E. affinis*, being mainly herbivore, would rather benefit from increased primary production.

The results of this study support the importance of long-term monitoring programs. Indeed, the long-term monitoring of the restoring Scheldt estuary has highlighted the response of the mesozooplankton taxa to water-quality improvement. The overall trend is that the reduction of organic load enabled the upstream Scheldt estuary to change from a hypereutrophic system in which phytoplankton primary production was inhibited to a eutrophic system with substantial increase in primary production (Cox et al., 2009). Subsequently, these changes led to suitable conditions for *E. affinis* in this stretch of the estuary. It is worth noting that not only the re-oxygenation of the system (i.e. O<sub>2</sub> concentrations > 4 mg L<sup>-1</sup>) improved the colonization of *E. affinis* in the upstream freshwater Scheldt, but also a decrease of NH<sub>4</sub>-N concentration below a threshold of 1 mg L<sup>-1</sup>. As far as we are aware, the studies on the Modego (Falcao et al., 2012) and the Scheldt (Mialet et al., 2011) zooplankton communities are pioneer studies reporting the evolution of an estuarine zooplankton community in parallel to water quality restoration. In the Scheldt estuary, the change of the freshwater reach from hypereutrophic to eutrophic has created conditions under which no previous data on zooplankton have been published. The oldest zooplankton data for the freshwater Scheldt cover the 1967-'69 period (De Pauw 1975). During this period, oxygen concentration during spring in the freshwater reach was below the 4 mg L<sup>-1</sup> limit for *E. affinis* to develop in this area. At that time, *E. affinis* was mainly found in the brackish part of the Scheldt estuary, as in the 1996-2007 period. Hence, the oxygenation of the upstream Scheldt being an environmental situation closer to pristine conditions than ever inventoried in Scheldt zooplankton

studies, it seems possible that *E. affinis* is not an invader but in fact a returning fugitive. It would thus be interesting to investigate how far improved water quality was also involved in other freshwater systems invaded by *E. affinis*. The change in environmental conditions has also permitted to discover a capacity of *E. affinis* to adapt to temperature ranges for maximum development. This copepod, so far considered as a typical spring species, can reach higher abundances during summer in the Scheldt UFZ than ever observed during spring in the brackish water zone.

Explaining the observed negative effect of the improved water quality on cyclopoids since 2007 is less obvious.

It is important to realize that total mesozooplankton abundance is not higher after 2007 than before. Only the composition of the mesozooplankton community has changed. Does this composition represent a better functioning ecosystem? It will have to be verified in how far the present, apparently stabilizing mesozooplankton community assures its trophic function (i.e. to shuttle energy from the primary producers and the detritus to be higher trophic levels) before the dominant taxa occurring in this community (*E. affinis*) can be evaluated as a water quality indicator.

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# III. CHAPITRE 3 :

## Tolérance du zooplancton estuarien à de faibles concentrations en oxygène

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### III.1 Résumé

#### III.1.1 Contexte et objectifs

Comme nous avons pu démontrer dans le premier chapitre, d'importants changements de la qualité de l'eau sont survenus dans l'estuaire de l'Escaut (Van Damme et al., 2005 ; Cox et al., 2009 ; Mialet et al., 2011). De plus, nous avons constaté l'enrichissement en oxygène dans la partie amont de l'Escaut. Entre 1996 et 2006, la communauté zooplanctonique était dominée par les copépodes cyclopoïdes et les cladocères. Depuis 2007, un changement communautaire a eu lieu, le copépode calanoïde *E. affinis* s'y est fortement développé au détriment des cyclopoïdes. Issu de ce constat un intérêt particulier est porté sur ce copépode : *E. affinis*. Ce copépode printanier domine une grande partie des zones d'eau saumâtre de l'hémisphère Nord (Lee, 1999). 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 2007 (chap.1), on observe l'espèce de façon permanente et en grande abondance dans le tronçon d'eau douce en amont de l'Escaut.

L'objectif de cette étude a été de tester expérimentalement la tolérance aux faibles concentrations d'oxygène, pour *E. affinis* mais aussi pour les copépodes cyclopoïdes. Pour cela les espèces zooplanctoniques ont été incubées dans de l'eau naturelle durant 2 à 24h et selon un gradient de concentration en oxygène dissous. A chaque échantillon, les organismes morts ont été comptés sous binoculaire et la totalité de l'échantillon a été fixé au formol afin d'être conservé pour le comptage de l'abondance totale.

### III.1.2 Principaux résultats

Entre 2 et 4,5 h d'incubation les taux de mortalités des calanoïdes et les cladocères ont diminué avec l'augmentation de la concentration en oxygène entre 0,5 et 7,22 mg L<sup>-1</sup>. Le taux de mortalité des cyclopoïdes ne variait pas avec la concentration en oxygène et, était inférieure à celle des calanoïdes et des cladocères.

Les conséquences de cette différence de tolérance pour une faible concentration d'oxygène entre les taxa de zooplancton sont discutées en relation avec le développement des communautés de zooplancton dans l'Escaut, et des estuaires en général.

Nos résultats sur la tolérance à faible concentration d'oxygène de trois ordres différents de zooplancton, combinées à l'évolution de la communauté de zooplancton dans l'estuaire de l'Escaut suivantes amélioration de la qualité de l'eau et l'augmentation de la concentration en oxygène associée, montrent clairement que la tolérance à l'hypoxie est un facteur important dans la détermination de la composition des communautés zooplanctoniques dans l'eau douce de l'Escaut.

Le développement d'*E. affinis* dans la zone amont n'a été possible que depuis l'amélioration de la qualité de l'eau et donc l'augmentation de la concentration en oxygène dissous. Une fois cette condition réalisée *E. affinis* pouvait se développer en amont, il est rapidement devenu une espèce dominante, comme observé dans plusieurs autres systèmes (Counahan et al 2005; Vad et al 2012). Davidson et al. (2000), avec leur étude sur les communautés de zooplancton dans divers habitats des plaines inondables de la rivière Atchafalaya (USA), ont aussi observé une forte dominance de quelques taxa zooplanctoniques sur des sites bien oxygénés, par rapport à des sites plus riches en détritiques, où l'abondance du zooplancton était plus faible, mais plus diversifiée et représentant uniformément divers taxons. Ainsi, il peut être envisagé que la dominance de *E. affinis* dans l'Escaut en amont n'est peut-être pas typique pour cette espèce, mais illustre un modèle plus général de la diversité de la communauté par rapport aux conditions environnementales.

III.2 Article 2 : Estuarine zooplankton tolerance for low oxygen concentration

## Estuarine zooplankton tolerance for low oxygen concentration

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### III.2.1 Abstract

Water quality in the Scheldt estuary (Belgium/The Netherlands) has improved since two decades. Oxygen concentration has increased, especially in the upstream freshwater tidal reach. The zooplankton community in this area was dominated by cyclopoid copepods and cladocerans between 1996 and 2006. Since 2007, a change in community composition occurred: the calanoid copepod *E. affinis* developed strongly, and cyclopoid copepods decreased in abundance.

The present study experimentally tested the tolerance of calanoid copepods (in casu *E. affinis*) and cyclopoid copepods for low oxygen concentrations.

Zooplankton was incubated in the laboratory in Scheldt water gradient of oxygen concentration from 0.5 to 7.22 mg L<sup>-1</sup> during 2 to 24 h. At each sampling, dead organisms were counted under binocular microscope and the total sample was fixed with formalin afterwards for counting of total abundance.

Between 2 and 4,5 h of incubation calanoid and cladoceran mortality rates decreased with increasing oxygen concentration between 0.5 and 7.22 mg L<sup>-1</sup>. Cyclopoid mortality rate did not vary with oxygen concentration and was lower than that of calanoids and cladocerans.

The consequences of this difference in tolerance for low oxygen concentration between zooplankton orders is discussed in relation to zooplankton community development in the Scheldt, and estuaries in general.

**Key words:**

Low oxygen, estuary, zooplankton, *Eurytemora affinis*, cyclopoid copepods.

### III.2.2 Introduction

As an essential element to most metazoans, oxygen concentration in aquatic systems has an important influence on the composition of communities and their spatio-temporal distribution. In both marine and freshwater systems, low oxygen concentrations can occur either by natural causes or by eutrophication. Concerns about the influence of hypoxia on organisms, their distribution and the resulting functioning of the ecosystem lead to many studies, but these are mainly focused on fish and large – sized crustaceans (e.g. Wu et al., 2002; Moyson et al., 2004; Greg et al., 2014; Bonvillain et al., 2015). Research on the response of zooplankton to hypoxia concerns mainly coastal shelf regions and estuarine bays, in which distinct low oxygen concentration strata develop during at least a part of the year such as, for instance, the coast of central Peru, Puget Sound (USA) or Chesapeake Bay (USA) (Criales-Hernández et al., 2008; Elliot et al., 2013; Keister and Tuttle, 2013). Among the dominant taxa of such systems, the calanoid copepod *Acartia tonsa* is one of the most studied in relation to its hypoxia tolerance and vertical positioning in relation to the oxycline. Field observations have shown that summer hypoxia (generally considered as  $< 2 \text{ mg L}^{-1}$ ) in the lower part of the water column negatively affects the abundance of this species. Both laboratory experiments and field data indicate that, while hypoxia seems to increase mortality of *A. tonsa* nauplii, its effect on copepodites seems sublethal. Nevertheless, anoxia does have a negative effect on *A. tonsa* abundance, either through increased exposure to predation following vertical anoxia avoidance, or through physiologic weakening caused by increased energy requirements, resulting in lower somatic growth and egg production (Marcus et al., 2004, 2005, Richmond et al., 2006; Elliot et al., 2013a, b).

Studying the northern Gulf of Mexico zooplankton community structure in relation to environmental conditions, including summer hypoxia, Elliot et al. (2012) found a differentiation in hypoxia effects on the abundance of taxa. One group of taxa composed of the calanoid copepods *Acartia* spp., cladocerans, copepod nauplii, harpacticoids and gelatinous medusa was most abundant at high temperature (29–32°C), low to intermediate salinities (12–33) and conditions with less than 30 % of the water column being hypoxic. Another group of dominant taxa, composed of the calanoid copepods *Centropages* spp., *Temora* spp., *Paracalanus* spp., the cyclopoid copepods *Oithona* spp and *Corycaeus* spp. and salps were associated with higher

salinities (>27) and temperatures between 28-31°C and were most abundant when 40 % of the water column was hypoxic. Looking at the effect of hypoxia on the total zooplankton community of a sub-estuary of Puget Sound, Keister and Tuttle (2013) showed that oxygen concentration in this system does not play a major role in structuring the community taxonomic composition, but influences the vertical positioning in the water column of several species. Meroplanktonic organisms, larvae and the copepod *Paracalanus parvus* were generally found above the oxycline, while polychaetes and the calanoid copepod *Metrida pussilus* occurred below the oxycline, and the vertical positioning of the cyclopoid *Oithona similis* showed no relation to the oxycline. So, while the literature on marine and estuarine zooplankton tolerance and response to anoxia suggest that the response of zooplankters is taxa specific, and can vary according to the type of ecosystem, suggesting the capacity for low oxygen concentration avoidance and tolerance of sub-lethal concentration for several copepod species, at least from the copepodite stage onwards.

Hypoxia being wide spread in freshwater systems, the tolerance for hypoxia has been quite extensively studied for cladocerans as they occur in many low oxygenated systems and often during warm, hypoxic periods. All branchiopoda have hemoglobin (Terwiller and Ryan, 2001) and many cladocerans (e.g. *Daphnia*, *Bosmina*, *Chidorus*, *Simocephalus*, *Moinia*) are known as tolerant to rather severe hypoxia, because of their capacity to adjust the level of hemoglobin (Hb) in the haemolymph (e.g. Fox, 1948, 1957; Svetlichny et Hubareva, 2002; Pirow et Buchen, 2004). However, little information is available about Hb in copepods. To the best of our knowledge, it has only been reported in a few parasites (Fox, 1957) and mud dwelling and borrow habiting harpacticoids (Fox, 1957; Green, 1959) and in some taxa from hydrovent environments (Hourdez et al., 2000; Sell, 2000).

The present study was inspired by changes occurring in zooplankton community composition in the freshwater part of an estuary, harboring both cladocerans and copepods as main mesozooplankters. The Scheldt estuary (Belgium/The Netherlands) covers a total salinity gradient including a 60 km freshwater stretch under tidal influence (Fig. 1) (Meire et al., 2005; Van Damme et al., 2005). During the second half of the 20<sup>th</sup> century, the Scheldt was heavily polluted. Besides chemical pollution, organic matter and nutrients represented the main pollution arising from discharge within the heavily habituated and cultured drainage basin (Baeyens et al.,

1998; Van Damme et al., 2005). This pollution led to very low oxygen concentrations, mainly in the freshwater area between km 95 – km 164. (km 90; Fig. 21). Oxygen saturation levels in the surface water were lower than 40 % during the major part of the year. Since the 1980ties measures were taken to decrease pollution in the catchment and the Scheldt has been recovering.

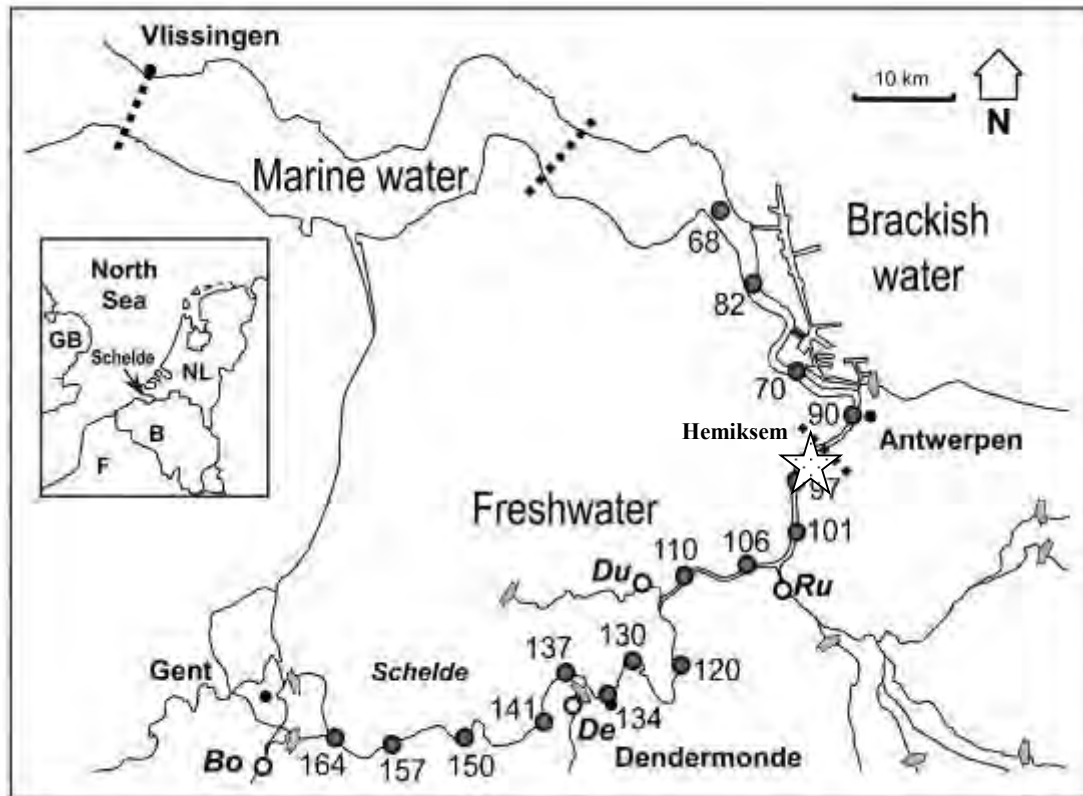


Figure 21 : Map of the Scheldt estuary with OMES sampling stations, designated by their distance, in km, upstream from Vliissingen (mouth). Dotted lines indicate limits between marine water, brackish water and freshwater reaches. Hemiksem station considered in this study is represented by the star.

Between 2000 and 2003, the upstream Scheldt gradually changed from hypereutrophic, heterotrophic system to eutrophic, autotrophic system (Cox et al., 2009). Lower organic and nutrient loads led to an improving oxygen concentration and strong phytoplankton primary production, which was, prior to the switch, hampered by too low oxygen concentrations or high ammonia concentrations. The substantial increase in oxygen concentration in the freshwater reach, co-occurred with a decrease in  $\text{NH}_4$  and  $\text{NO}_3$  concentration, and an initially strong increase in chlorophyll concentration. Detailed descriptions of the evolution of the Scheldt system can be found in Meire et al. (2005), Van Damme et al. (2005), Cox et al. (2009), Mialet et al. (2011).

Earlier studies have shown the freshwater area of the Scheldt to be very poor in zooplankton and hyperbenthos (Soetaert et Van Rijswijk, 1993). A comparison of the spatial distribution of *E. affinis* in the Ems (The Netherlands), the Gironde (France) and the Scheldt estuary, showed that in the two former estuaries, the dominant copepod *E. affinis* abundance peaked between 0 and 6 salinity, while in the Scheldt its maximum abundance was observed between 10–12 salinity. This ‘displacement’ was attributed to the very low oxygen concentration in the 0–2 salinity zone of the Scheldt (km 70–160) compared to the other two estuaries, where this salinity range apparently corresponded to the optimum for *E. affinis* (Soetaert et Van Rijswijk, 1993; Sautour et Castel, 1995). Appeltans et al. (2003), reported that the Scheldt spring *E. affinis* population reached higher abundance (320–14 000 ind. m<sup>-3</sup>) in the 0–2 salinity zone during 1996–‘97 compared to 1989–‘90, when its abundance was between 0 and 160 ind. m<sup>-3</sup>. This increase was explained by the improved water quality and higher spring oxygen concentrations in this area (3.52–7.05 mg L<sup>-1</sup>). In the following years, *E. affinis* was sporadically observed in the freshwater stretch in variable abundances. Mialet et al. (2010), demonstrated that the presence and abundance of *E. affinis* in the Scheldt freshwater stretch during the 1996–2007 was related to the oxygen concentration in the upstream Scheldt. *E. affinis* could be abundant upstream if the oxygen concentration there was > 4 mg L<sup>-1</sup>. Mialet et al. (2011) reported a remarkable change in the zooplankton community in parallel with water quality improvement. Between 1996 and 2006, the brackish water stretch was dominated by calanoid copepods, with *E. affinis* as dominant species, mainly occurring in spring. The freshwater zooplankton community was more diverse, with a dominance of several cyclopoid copepod species and cladocerans, and few calanoid copepods (Tackx et al., 2004; Mialet et al., 2011). From 2007 onwards, a strong development of *E. affinis* took place in the freshwater stretch. At the same time, cyclopoid copepods decreased strongly in abundance, which resulted in a 50–90 % *E. affinis* contribution to the freshwater copepod community, whereas before, it represented maximally 25 %. Cladoceran average yearly abundance remained unchanged over time (Mialet et al., 2011; Chambord et al., accepted).

Considering that on the one hand, cyclopoids and cladocerans were abundant in the upstream freshwater stretch of the Scheldt estuary prior to water quality improvement, and on the other hand, there was a clear association between oxygen

concentration and *E. affinis*' abundance in this zone, we formulated the hypothesis that cyclopoids and cladocerans have a higher tolerance for low oxygen concentration than the calanoid *E. affinis*. This hypothesis was tested by exposing concentrated natural Scheldt zooplankton communities to a gradient of oxygen concentrations and observing the mortality of the different orders of zooplankters. Evidently, besides oxygen concentration, other environmental variables (e.g. NH<sub>4</sub> concentration, pH...) changed in parallel in the Scheldt estuary. However, multivariate analysis has shown oxygen concentration to be a main driver of changes occurring in the zooplankton community (Chambord et al., accepted, chapter II) and hence, its specific influence was experimentally tested.

### III.2.3 Material and methods

The Scheldt has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands. Its estuary is situated from the mouth at Vlissingen until the city of Gent, where the tide is stopped by sluices (Fig. 21). Contrarily to most of the other temperate estuaries, the Scheldt estuary is characterized by vertically well mixed water flows (Baeyens et al., 1998), inducing most of the time no salinity or current stratification (Heip, 1988).

50 L of Scheldt water was collected in Hemiksem (51°08'35.49"N; 4°19'48.56"E) situated in the freshwater tidal area of the estuary (Fig 21). At the same occasion, zooplankton was collected by submerging a 50 µm net counter current for 10 to 30 minutes according to the prevailing current. The collected zooplankton was transferred to a 1 L container and water and zooplankton were transported to the laboratory. In the laboratory, series of 1 L bottles were filled up till 970 mL with Scheldt water, filtered on a 50 µm mesh. These bottles were bubbled with nitrogen during various periods to obtain a gradient in oxygen concentration. Per oxygen concentration, 5 replicate bottles were used for incubation during 2, 4, 6, 18, and 24h. A first series of 4 experiments was realized between 2 and 5 April 2014 and a second series of 5 experiments between 9 and 13 July 2014. The 9 experiments covered an oxygen concentration range between 1 and 8 mg L<sup>-1</sup>. Within one experiment, the difference between minimum and maximum oxygen concentration was between 0.5 and 2 mg L<sup>-1</sup>.

The zooplankton was concentrated over a 50  $\mu\text{m}$  mesh, and 30 mL aliquots were added to each experimental bottle at  $t_0$  of the experiment. Oxygen concentration was measured in each bottle with an oxygen sensor (model: WTW Oxi323 with a WTW Cellox325 sensor) at  $t_0$  and  $t_{\text{end}}$  of the experiment.

At each incubation time, the zooplankton of 5 bottles was separately collected with a 50  $\mu\text{m}$  mesh and concentrated in 60 mL. From these concentrates, 6 mL subsamples were analyzed under binocular microscope to count the dead individuals, distinguishing adults of calanoids, cyclopoids and cladocerans. Afterwards, the 6 mL subsamples were fixed with formalin (4 % final concentration) for subsequent counting of the total abundance of each zooplankton order under binocular microscope.

Mortality was calculated as the number of dead organisms of each group observed at each sampling, expressed as percentage of the total abundance of each group in each bottle. Mortality rate was calculated as mortality per hour, also expressed in percentage of total abundance. Oxygen concentration on axes in the graphs are mean values between  $t_0$  and  $t_{\text{end}}$  in each experimental bottle.

### III.2.4 Results

#### III.2.4.1 Incubation time

The mortality of calanoids, cyclopoids and cladocerans did not show any tendency with incubation time as can be seen from two examples representing an experiment in the low oxygen concentration range (1,6 – 2,7  $\text{mg L}^{-1}$ ) and one in a higher oxygen concentration range (3,6 – 4,9  $\text{mg L}^{-1}$ ) (Fig. 22a, b). So, independent of the oxygen concentration, the mortality occurring at the longer incubation times had already occurred earlier.

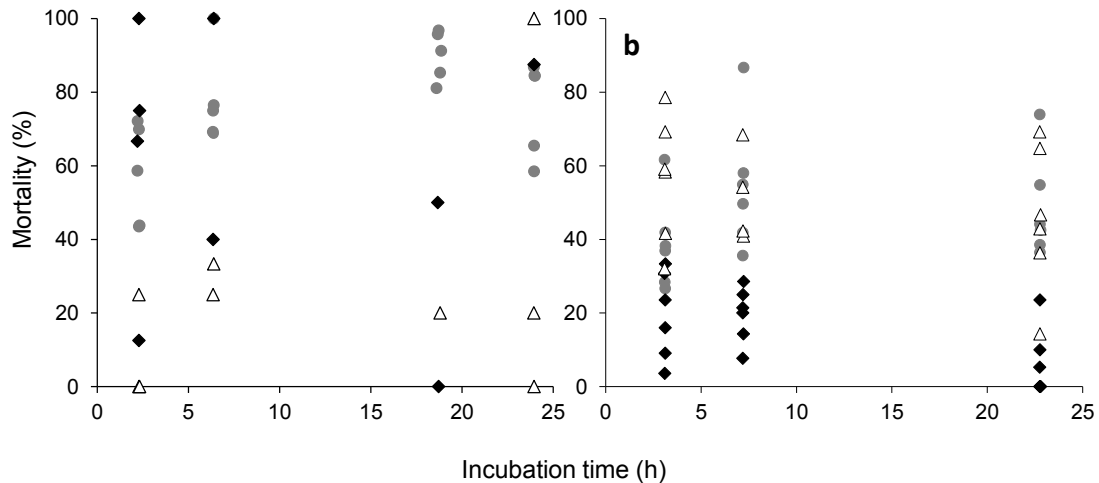


Figure 22 : Examples of mortality obtained for calanoids (grey circles), cycloids (black diamonds) and cladocerans (white triangles) as a function of incubation time. a) experiment run at 1,6-2,7 mg L<sup>-1</sup> and b) experiment run at 3,6-4,9 mg L<sup>-1</sup> oxygen concentration.

Consequently, for further analysis, only the results obtained at incubation times between 2 and 6 h were considered.

#### III.2.4.2 Density of organism

Because in 2014, the Scheldt freshwater zooplankton community was already strongly dominated by *E. affinis*, we had to substantially concentrate the natural zooplankton in order to have a sufficient number of observations on cycloids and cladocerans in each experimental bottle. Bottles in which any of these were below 4 ind. L<sup>-1</sup> have been discarded from the analysis. The number of individuals observed (dead and alive) varied between 4–50 cycloids, 15–156 calanoids and 4–81 cladocerans. The high abundance of total individuals incubated might have led to stressful experimental conditions. To verify that this potential stress was not the main driver of mortality, we analyzed mortality rates for each order as a function of the number of individuals incubated, considering the three orders together. No trend between the number of individuals incubated and mortality rate was found for any of the orders (Fig. 23).



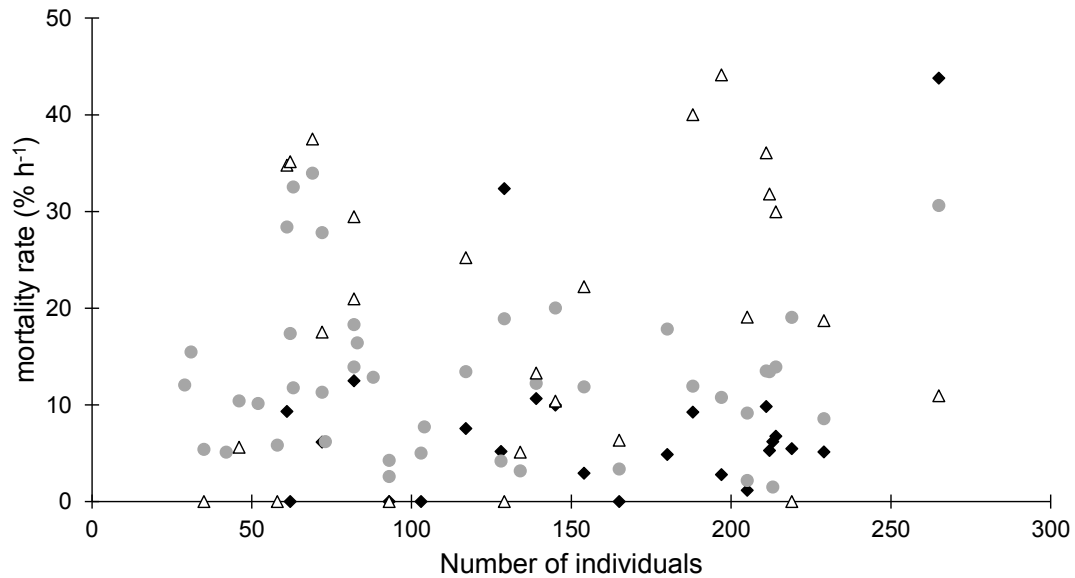


Figure 23 : Mortality rate of calanoids (grey circles) cyclopoids (black diamonds) and cladocerans (white triangles) as a function of number of individuals incubated, considering the three groups together.

Mortality rates of both calanoids and cladocerans decreased significantly (spearman rank,  $p= 6.78e-07$  and  $p= 0.00018$  respectively) with oxygen concentration (Fig 24). The mean mortality rate observed within the  $0.5-7.22 \text{ mg L}^{-1}$  oxygen concentration range covered by the experiments did not differ significantly between calanoids and cladocerans (Mann Whitney,  $p= 0.1011$ ). Cyclopoid mortality rates, on the contrary, did not vary significantly with oxygen concentration (spearman rank,  $p= 0.191$ ), and were significantly lower than those of calanoids and cladocerans (Mann Whitney,  $p= 0.00094$  and  $p= 0.00812$  respectively) (Fig. 24).

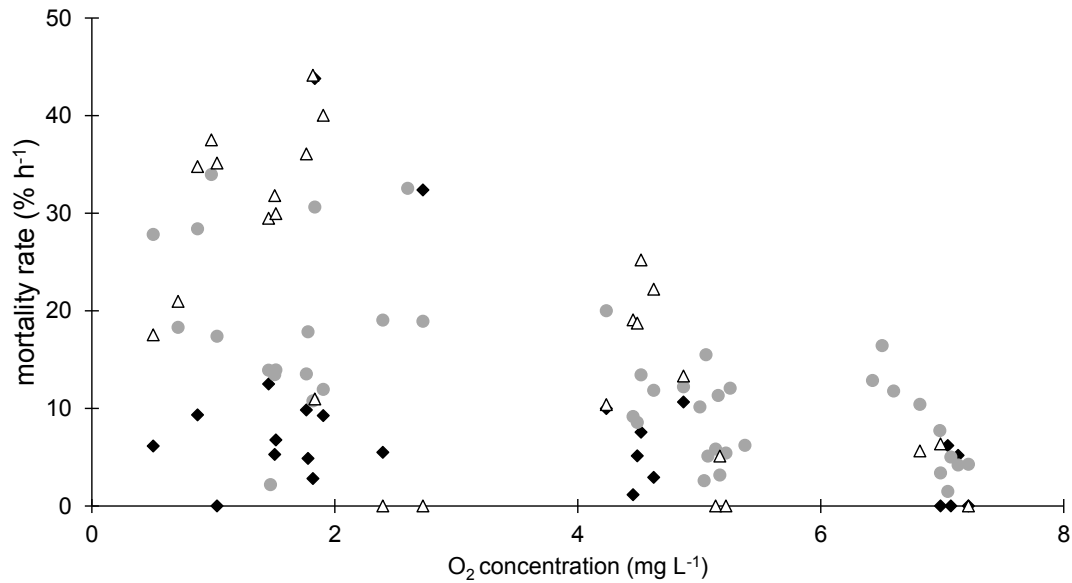


Figure 24 : Mortality rate of calanoids (grey circles) cyclopoids (black diamonds) and cladocerans (white triangles) as a function of oxygen concentration. Oxygen concentrations on x axis are mean values between  $t_0$  and  $t_{end}$  for each experimental bottle.

### III.2.5 Discussion

We used visual observation of dead animals rather than staining with dyes. Several authors have successfully used neutral red in zooplankton studies in estuarine, calanoid dominated systems (e.g; Tang et al., 2006; Kimmel et al., 2009a, b). Bickel et al. (2009) stained dead copepods and cladocerans from freshwater lakes with satisfactory efficiency using aniline blue. Nevertheless, in preliminary tests, we were not able to find a stain that colored calanoids, cyclopoids and cladocerans with similar efficiency. Dressel et al. (1972) also report variable results in staining various planktonic crustacean taxa.

Our results showed a clear decrease of calanoid mortality rates with increasing oxygen concentration but no relation between cyclopoid mortality rates and oxygen concentration. In our quantitative analyses, no distinction between taxa was made within the orders of copepods. However, since 2009, *E. affinis* on average accounts for 85 % of calanoid abundance in the upstream freshwater zone of the Scheldt, so the obtained results validate the hypothesis that cyclopoids have a higher tolerance for low oxygen concentrations than the calanoid *E. affinis*. Cladocerans, on the contrary, show a similar dependence of their mortality rate on oxygen concentration as

calanoids, and had statistically the same average mortality rate. So for this order, the hypothesis was rejected.

As explained in the introduction, most studies on low oxygen concentration tolerance are carried out in the context of oxygen minimum layer occurrence, either in coastal or estuarine systems. Stalder and Marcus (1997) compared hypoxia tolerance for 3 calanoids (*Labidocera aestiva*, *Acartia tonsa* and *Centropages hamatus*) under laboratory conditions, but few studies have specified differences in low oxygen concentration tolerance between different orders of zooplankters. In their Gulf of Mexico study, the groups of zooplankters found by Elliot et al. (2012) as occurring under more or less hypoxic conditions include either both calanoids and cladocerans or calanoids and cyclopoids.

Keister and Tuttle (2013) showed that in Hood canal, a sub-basin of Puget sound (USA), the calanoid *Paracalanus parvus* was generally found above the oxycline, while another calanoid, *Metridia pussilus* occurred below the oxycline. Their observation that the vertical positioning of the cyclopoid *Oithona similis* showed no relation to the oxycline is in line with our findings of independence of mortality rates of cyclopoids to oxygen concentration, but it should be mentioned that the only cyclopoid species present in the Scheldt water used for the experiments was *Acanthocyclops trajani*.

In the eutrophic Naka-umi lake (Japan) lake, Chang et al. (2013) studied zooplankton abundance and composition in an area where oxygen-rich water was infiltrated in the bottom to reduce eutrophication. The mesozooplankton dominating calanoid during spring, *Acartia hudsonica*, showed a ten-fold increase in abundance at the oxygenated site compared to a control, non-oxygenated site. During summer, no significant effect of oxygen addition was observed on the abundance of the dominant mesozooplankter, the cyclopoid *Oithona* spp. These results also agree with our results showing cyclopoids to be less sensitive to oxygen concentration than calanoids. In fact, *Oithona* spp. have been suggested as indicators of eutrophication and low oxygen concentration by several authors (Paffenhöfer, 1993; Roman and al., 1993, Richard and Jamet, 2001). Keister and Tuttle (2013) explain the tolerance of *Oithona* spp. for low oxygen concentrations by its food preference for flagellates, which can dominate over diatoms in eutrophied areas (Uye, 1994) and the fact that *Oihona* carries its eggs and can thus avoid their sedimentation to hypoxic layers.

Our results showing a higher hypoxia tolerance for cyclopoids than for calanoids explains why the former were abundant in the upstream Scheldt prior to water quality improvement, but they do not explain the strong decrease in cyclopoid abundance since 2007. A detailed multifactor analysis of the zooplankton community changes in the upstream Scheldt and their possible causes is discussed in Chambord et al. (accepted).

It is also surprising that cladocerans which are known as tolerant for low oxygen concentration, in our experiments display a similar mortality rate as calanoids. The dominant cladoceran taxa in the experiments were *Bosmina longirostris* and *Daphnia galeata*. *Daphnia obtusa*, *Chydorus sphaericus* and *Ceriodaphnia quadrangula* were also present, but less abundant.

*B. longirostris* is known as typical for eutrophic, so oxygen poor systems and also to be more tolerant than other cladocerans to a large number of stresses including changing environmental conditions (Adamczuk, 2016). However, Bosminidae are suspected to include cryptic species (Taylor, 2002) and it is possible the *B. longirostris* having developed in the oxygenated freshwater Scheldt are a subspecies which has adapted to high oxygen concentration and lost its tolerance for lower oxygen concentration. Heisey and Porter (1977) showed that *Daphnia galeata*, the other dominant cladoceran in our experiments is less tolerant to environments with low oxygen concentration than *Daphnia magna*. This low tolerance of *D. galeata* is possibly due to a lower hemoglobin content.

Several authors suggest that hypoxia avoidance and/or differences in tolerance for hypoxia could have important implications for zooplankton community composition, positioning in space and trophic functioning (e.g. Sedlacek et Marcus, 2005; Richmond et al., 2006; Criales-Hernandez et al., 2008; Elliot et al., 2012). However, the development of clear scenarios on this topic is hampered by an important variability of oxygen related behavior and tolerance among zooplankton taxa and among development stages (Keister et Tuttle, 2013).

Our results on low oxygen concentration tolerance of three different orders of zooplankters, combined with the evolution of the zooplankton community in the Scheldt estuary following water quality improvement and associated oxygen concentration increase, clearly show that hypoxia tolerance is an important factor in

determining zooplankton community composition in the freshwater Scheldt estuary. The development of *E. affinis* in this area was only possible since oxygen concentrations in the upstream area permitted it. Once *E. affinis* could develop upstream, it rapidly became a dominant species, as observed in several other systems (Counahan et al. 2005; Vad et al., 2012). Davidson et al. (2000), studying the zooplankton communities in various floodplain habitats of the Atchafalaya river (USA), also observe strong dominance of a few zooplankton taxa in well oxygenated sites, compared to more detritus rich sites, where zooplankton abundance was lower, but more diverse and evenly representing various taxa. So it can be envisaged that the dominance of *E. affinis* in the upstream Scheldt is perhaps not typical for this species, but a more general pattern of community diversity in relation to environmental conditions.

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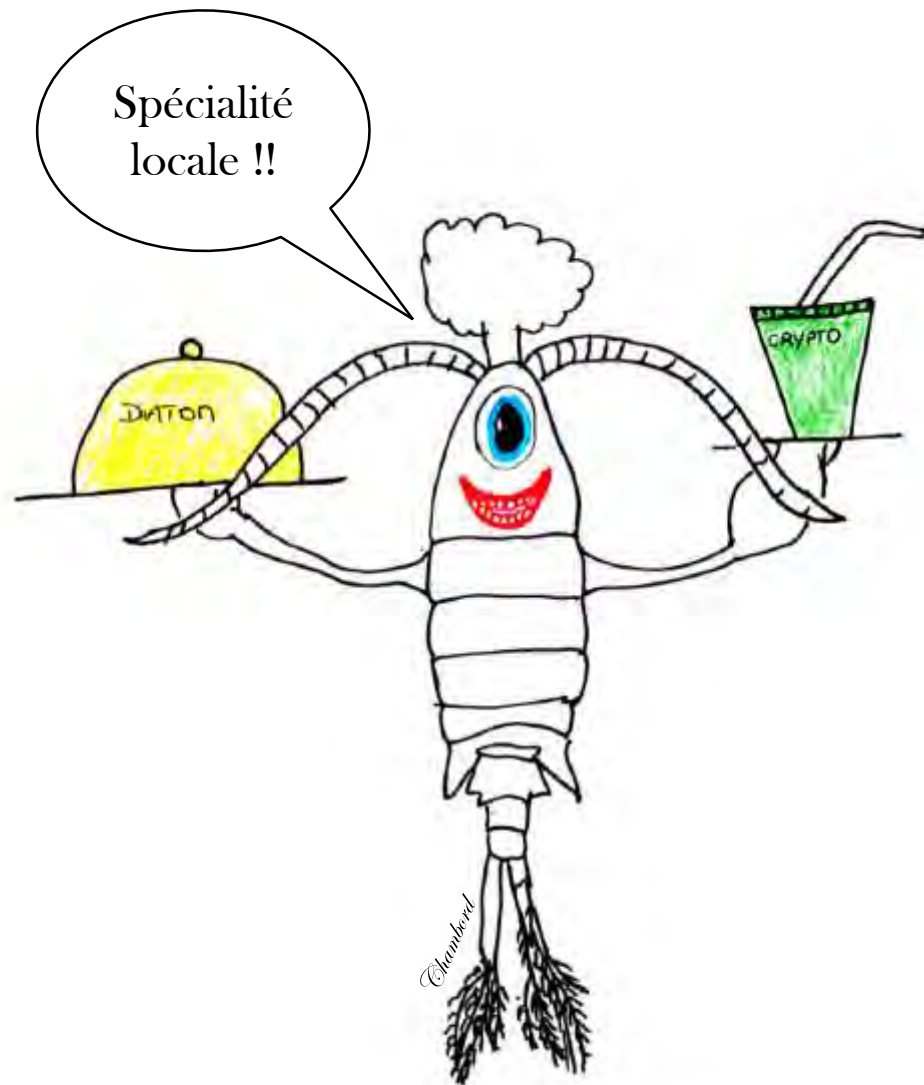
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# IV.

# CHAPITRE 4 :

Sélectivité trophique du copépode calanoïde  
*Eurytemora affinis* en eau douce dans  
l'estuaire de l'Escaut

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## IV.1 Résumé

### IV.1.1 Contexte et objectifs

L'amélioration de la qualité de l'eau dans l'estuaire de l'Escaut, et plus particulièrement dans le tronçon d'eau douce, s'est manifestée à travers certains changements des facteurs physico-chimiques, tels que l'augmentation des concentrations en oxygène parallèlement à la diminution des concentrations en  $\text{NH}_4^+$ . La production primaire ayant augmenté, la silice est récemment devenue limitante pour la production des diatomées.

Le suivi de l'abondance et de la composition des communautés de zooplancton depuis 1996, dans le cadre du projet OMES, montrait à l'origine une communauté constituée principalement en eau saumâtre par les copépodes calanoïdes (dominée par *E. affinis*), et par les rotifères et les cladocères en amont, dans le tronçon d'eau douce.

Un changement important est survenu en 2007, avec le développement d'*E. affinis* en eau douce. Depuis, *E. affinis* est présent en abondance élevée dans ce tronçon, et est devenu largement dominant en termes d'abondance au sein de la communauté de copépodes, tandis que les copépodes cyclopoïdes ont fortement diminué.

Malgré la faible contribution du phytoplancton vivant à la matière en suspension estuarienne, l'importance de l'alimentation herbivore du zooplancton estuarien a été démontrée à plusieurs reprises (Gasparini et al., 1999 ; Tackx et al., 2003). Considérant la diminution des concentrations en silice dans la partie amont de l'estuaire de l'Escaut, la question de la capacité de la communauté de zooplancton dominée par *E. affinis* à contrôler de potentiels blooms phytoplanctoniques non-diatomiques se pose.

Cette étude a pour objectif de quantifier la pression de prédation du zooplancton sur le phytoplancton. A cet effet, des expériences d'incubation ont été réalisées. De l'eau naturelle de l'Escaut filtrée à 250  $\mu\text{m}$  a été incubée avec et sans ajout d'*E. affinis* adultes et CV. Les pigments algaux ont ensuite été quantifiés par HPLC afin de déterminer l'impact des copépodes sur le phytoplancton.

Les résultats de ces expériences d'incubation ont été comparés à ceux obtenus par quantification des pigments intestinaux d'*E. affinis*.

#### IV.1.2 Principaux

Les expériences d'incubation suivies de la quantification pigmentaire par HPLC ont révélé une sélection des diatomées par *E. affinis*. A l'inverse, une augmentation de la quantité d'algues vertes a été observée, une croissance semblant stimulée par la présence d'*E. affinis* dans les flacons de broutage.

La méthode des contenus stomacaux a livré des résultats différents, avec une sélection des cryptophytes. Cependant, les deux méthodes ont abouti à des résultats comparables pour ce qui est des taux d'ingestion de chlorophylle *a* par *E. affinis*.

Nos résultats montrent un impact limité de la population d'*E. affinis* sur le stock phytoplanctonique et les diatomées dans le tronçon d'eau douce de l'Escaut estuarien, impliquant que la nourriture n'est pas un facteur limitant pour *E. affinis*. Par conséquent le zooplancton n'est probablement pas limitant pour le développement des niveaux trophiques supérieurs dans la partie d'eau douce de l'estuaire.

Compte tenu des différents résultats obtenus par les expériences d'incubation et la méthode des contenus stomacaux, l'impact d'*E. affinis* sur le phytoplancton non-diatomique est moins évident.

Les expériences d'incubation de microzooplancton (50-250µm) ont montré un impact surtout sur les phéopigments, suggérant une consommation importante des détritiques. L'impact du microzooplancton sur le phytoplancton vivant n'a pas démontré de tendance régulière de sélectivité, soulignant la nécessité d'études supplémentaires à ce sujet.

IV.2 Article 3: Feeding selectivity of the calanoid copepod *Eurytemora affinis* in  
freshwater of the Scheldt

## Feeding selectivity of the calanoid copepod *Eurytemora affinis* in freshwater of the Scheldt estuary

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#### IV.2.1 Abstract

Water quality in the Scheldt estuary has substantially improved since two decades, especially in the upstream, freshwater zone. The main effects in this regard are the improving oxygen concentration and in parallel, a reduction of  $\text{NH}_4^+$  concentration. Primary production has increased, and recently, silica is becoming occasionally limiting for diatom production.

Zooplankton abundance and community composition has been monitored since 1996 in the frame of the OMES project, between Gent and the Belgian-Dutch boarder. The original spatial zooplankton distribution, showed mainly calanoid copepods (dominated by *E. affinis*) in the brackish water stretch and rotifers, cyclopoid copepods and cladocerans in the upstream, freshwater stretch. In 2007, an abrupt change was observed, as *E. affinis* developed massively in the freshwater area. Since then, *E. affinis* has maintained high abundance upstream and has become strongly dominant within the copepod community. Cyclopoids, on the other hand, have very strongly decreased in abundance.

Despite the small contribution of live phytoplankton to estuarine SPM, several studies indicate a substantial herbivorous feeding mode of estuarine zooplankton (Gasparini et al., 1999; Tackx et al., 2003). In view of the decreasing silica concentrations in the upstream Scheldt, the question arises in how far *E. affinis* as the dominate species of the zooplankton community will be capable of controlling potential non-diatom phytoplankton blooms.

This study therefor focused on the quantification of zooplankton feeding on phytoplankton. Incubation experiments using natural Scheldt water  $< 250 \mu\text{m}$  with and without addition of adult and CV *E. affinis* in natural Scheldt water were realized. HPLC quantification of algal pigments showed that *E. affinis* selected mainly diatoms. Green algae, on the other hand, showed growth –stimulated by the presence of *E. affinis* in the grazing bottles. This method was compared with the results of HPLC gut content quantification of *E. affinis*, which revealed, on the contrary to the incubation results, a selectivity for cryptomonads. *E. affinis* ingestion rates on Chl *a*-(eq) obtained by both methods were comparable. The *E. affinis* population has a limited impact on the standing stock of phytoplankton and of the dominating diatoms in the freshwater Scheldt. This means that the *E. affinis* population is not food limited, and that the zooplankton is probably not limiting the potential for higher trophic level

development in the freshwater Scheldt. Considering the different results obtained on grazing on non-diatom phytoplankton taxa with incubation and gut pigment content method, the *E. affinis* impact on non-diatom phytoplankton is less clear. Also the grazing activity of the microzooplankton community ( $t_c < 50 \mu\text{m}$  and  $t_{\text{microzoo}} < 250 \mu\text{m}$ ) was quantified by 4 incubation experiments using natural Scheldt water. The impact of the microzooplankton community was mainly on pheopigments, suggesting an important consumption of detritus. Microzooplankton grazing on live phytoplankton taxa showed no regular selectivity pattern, but more experiments are needed on this topic.

**Key words:**

Trophic selectivity, zooplankton, phytoplankton, *E. affinis*, Scheldt estuary.



## IV.2.2 Introduction

Estuaries are perceived as highly productive ecosystems because they are often nutrient rich and have multiple sources of organic carbon, including riverine and waste inputs and autochthonous primary production by vascular plants, macroalgae, phytoplankton, and benthic microalgae to sustain populations of heterotrophs.

The special status of estuaries with high concentrations of suspended matter can interfere with the feeding of pelagic organisms (Hart, 1988; Kirk and Gilbert, 1990; Jack et al., 1993; Miquelis, 1996). Indeed, in estuaries, zooplankton faces an important constraint to ensure its nutritional needs. The pool of potential food sources consisting mainly of detrital POM of low nutritional quality (Heinle et al., 1977) can hinder the selectivity of the zooplankton for potential preys (i.e. high quality food), such as phytoplankton and microzooplankton (Gasparini and Castel, 1999). In the freshwater part of the Scheldt mesozooplankton is since 2007 dominated by the calanoid copepod: *Eurytemora affinis* (Mialet et al., 2011; Chambord et al., accepted). *E. affinis* is known as being generally herbivorous (Gasparini and Castel, 1997) or opportunistically omnivorous (Hoffman et al., 2008) and its food ingestion is highly efficient even in hyper turbid environments such as estuaries (DeMott, 1988; Tackx et al., 1995, 2003). Tackx et al. (2003), demonstrated that *E. affinis*, in the brackish zone of the Scheldt estuary, exerted a higher feeding pressure on phytoplankton than on the total particulate matter.

Selection between detritic organic material and phytoplankton preys has been studied in several laboratory experiments, where copepods often showed a preference for phytoplankton (DeMott, 1988, 1995; Paffenhofer & Van Sant, 1985). Some studies highlight that zooplanktonic copepods are able to detect and subsequently select their preys by chemoreception (Poulet et Ouellet, 1982; Hammer et al., 1983; Martel et al., 2006).

The ecosystem of the Scheldt estuary has been studied during the last years, especially the freshwater part. The freshwater tidal part extending over more than 80 km is one of the few remaining freshwater tidal habitats in Europe (Meire et al., 2005; Van Damme et al., 2005). The Scheldt estuary has historically been one of the most polluted in Europe (Heip, 1988; Meire et al., 2005). Since the 1980s, waste water treatment and regulation of pollutant discharge by industries led to an important improvement of the water quality (Van Damme et al., 2005; Cox et al., 2009). As a

consequence, oxygen concentrations increased while nutrient concentrations (i.e.  $\text{NH}_4^+$ ,  $\text{PO}_4^-$ ) decreased concomitantly. Since 2009, the morphology of the estuary has also changed due to its deepening between the mouth and Antwerp harbour, leading to an increase of the tidal pumping and increased salinity and SPM concentration in the upstream reaches (OMES, unpublished data).

In response to the improvement of water quality, the zooplankton community has experienced significant changes (Appeltans et al, 2003; Tackx et al., 2004; Mialet et al, 2010, 2011). Rotifers, and cladocerans and cyclopoid copepods represented the dominant taxa of the freshwater community (i.e upstream of Antwerp) between 1996 and 2006. Since 2007, a different zooplankton community has developed in the tidal freshwater reach with *E. affinis* becoming dominant and achieving higher densities than before in the brackish part. Concomitantly, cyclopoid copepods decreased to very low abundances. Only the abundance of cladocerans in fresh and brackish waters has remained constant. So, particular attention has been paid to the study of the zooplankton community (Mialet et al., 2011; Chambord et al., accepted). Furthermore, in the Scheldt estuary, the phytoplankton community has also changed. The biomass of diatoms and green algae has been increasing since 2006 and a change in the diatom community composition has been noted. Indeed, since 2009 the trend of the variation of the biomass of two taxa of diatoms has been reversed, i.e. *Actinocyclus normanii*, the most abundant species before 2009, decreased while the genus *Cyclotella* spp. increased in abundance, inducing a change in size of diatoms (PAE-OMES data).

Predator-prey relationships between zooplankton and phytoplankton which in estuarine areas plays a key role in the transfer of energy between primary producer and higher trophic levels (Steele, 1974, Tackx et al., 2003; Maes et al., 2005b). They determine the potential contribution of primary production to higher trophic levels, and in estuaries, the efficiency of the uptake of primary produced material also influences the loss of primary production through export.

Climate change, and other impacts caused by humans can result in changes in the aquatic environments and thus in the configuration of food webs (e.g. Elliott & Quinto, 2007). Thus, a fundamental understanding of the mechanisms behind energy transfer through the food web is essential for sustainable management of biological resources. Diatoms were long time considered as not always dominant, but at least, an

important food source for copepods (e.g. Irigoien et al., 2000; Pasternak & Schnack-Schiel., 2001). However, the role of diatoms as the dominant food for copepods was challenged in the late 1990s when negative effects of certain diatom species on egg production and hatching success were found (Miralto et al., 1999; Turner et al., 2001). More recent findings contradict, however, these studies (Dutz et al., 2008; Jonasdottir et al., 2011). Selective feeding can have profound consequences for food fluxes (Kagata and Ohgushi 2011). For example, zooplankton selective grazing affects nutrient recycling through the removal of nutrient-rich species (Löder et al. 2011).

In the Scheldt estuary, the reduction of organic load enabled the upstream Scheldt estuary to change from a hypereutrophic system in which phytoplankton primary production was inhibited to a eutrophic system with substantial increase in primary production (Cox et al., 2009). During the last twenty years, substantial Chl *a* concentrations were observed, especially in the upstream reach (Fig. 25).

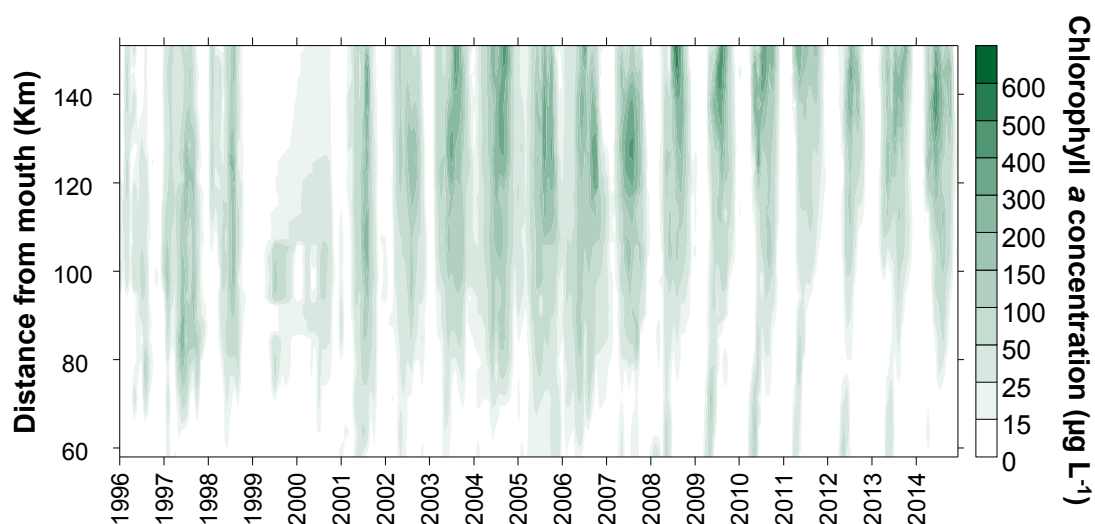


Figure 25 : Chl *a* concentrations in the Scheldt estuary, from station Melle (km 151) till Grens (km 51). OMES monitoring data, ECOBE, University of Antwerp.

During the same periods, low concentrations of dissolved silica (Dsi) became more frequent and were observed over of longer durations (Fig 26).

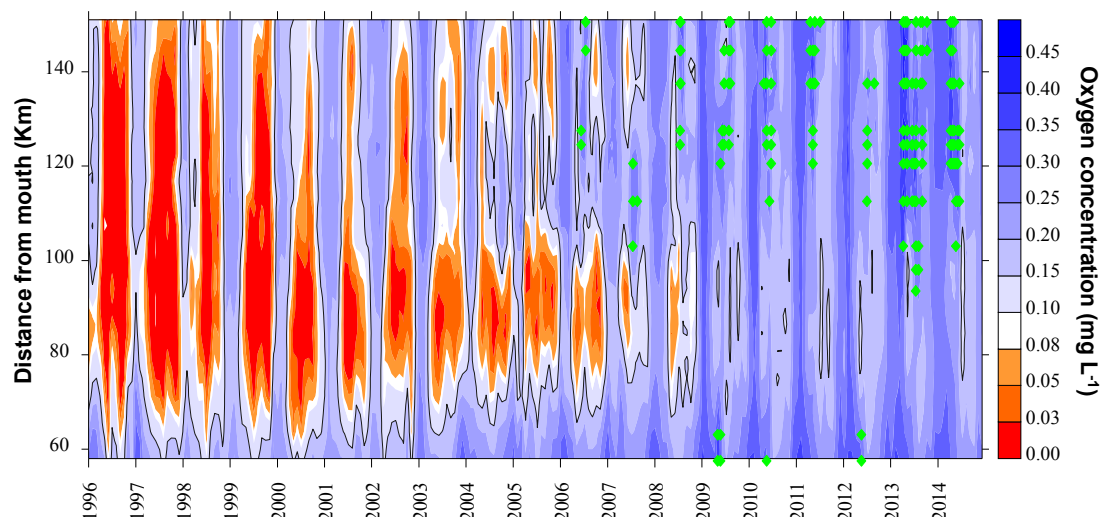


Figure 26 : Oxygen concentrations in the Scheldt estuary, from station Melle (km 151) till Grens (km 51). The green stars indicate situations during which Dsi is potentially limiting diatom development. OMES monitoring data, ECOBE, University of Antwerp.

Copepods are often reported to selectively feed on diatoms, and this is also the case for *E. affinis*, which has become dominant in the freshwater estuary since 2007. If Dsi limitation should limit diatom development, it is likely that non-diatom phytoplankton taxa, less consumable by a copepod dominated community, could become dominant. Also the recently increasing SPM concentration could enhance the development of non-diatom phytoplankton, as cryptomonads (Lionard et al., 2005).

The goal of the present study was to determine the impact of *E. affinis* on the present Scheldt phytoplankton community. Thus, in this paper we set out to answer the following questions:

- 1) What are the phytoplankton taxonomic groups which are selected by *E. affinis* as trophic sources, in the freshwater reach of the Scheldt estuary?
- 2) Considering decreasing silica concentrations in the upstream Scheldt, which could induce a decrease of diatom abundance, the question arises as how far the *E. affinis*-dominating the zooplankton community could be able to control potential non-diatom phytoplankton blooms.

The study of the diet of planktonic organisms in estuarine environments has been addressed using different methods: experiments by incubating predators and preys under controlled conditions or simulated *in situ* conditions (Gasparini and Castel, 1999; Winkler and Greve, 2004; David et al., 2006), trophic biomarker analyses such as pigments (Islam et al., 2005) fatty acids (Bodineau et al., 1998, Casvol et al., 2015)

and isotope ratios (Gladishef et al., 2016). All these techniques provide different approaches to trophic relationship study.

In the present study, we combined two complementary methods (i.e; experimental and field studies). First, grazing experiments using incubation of *E. affinis* in natural Scheldt water, combined with HPLC quantification of algal pigments in microcosm water incubated with and without *E. affinis*. Secondly, gut pigment content analyses on adult *E. affinis*. Copepods for gut pigment analyses and location.

### IV.2.3 Material and methods

#### IV.2.3.1 Study site

The Scheldt has its source in the North of France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands. Its estuary is situated from the mouth at Vlissingen until the city of Gent, where the tide is stopped by sluices (Fig. 27). Its four main tributaries are Boven Scheldt, Dender, Durme and Rupel. All the samples for this study were collected at a fixed station (Hemiksen, 51°14431 NS, 4°34605 OE) during two campaigns, one in April 2013 (9 experiments) and one in April 2014 (4 experiments) (Fig 27).

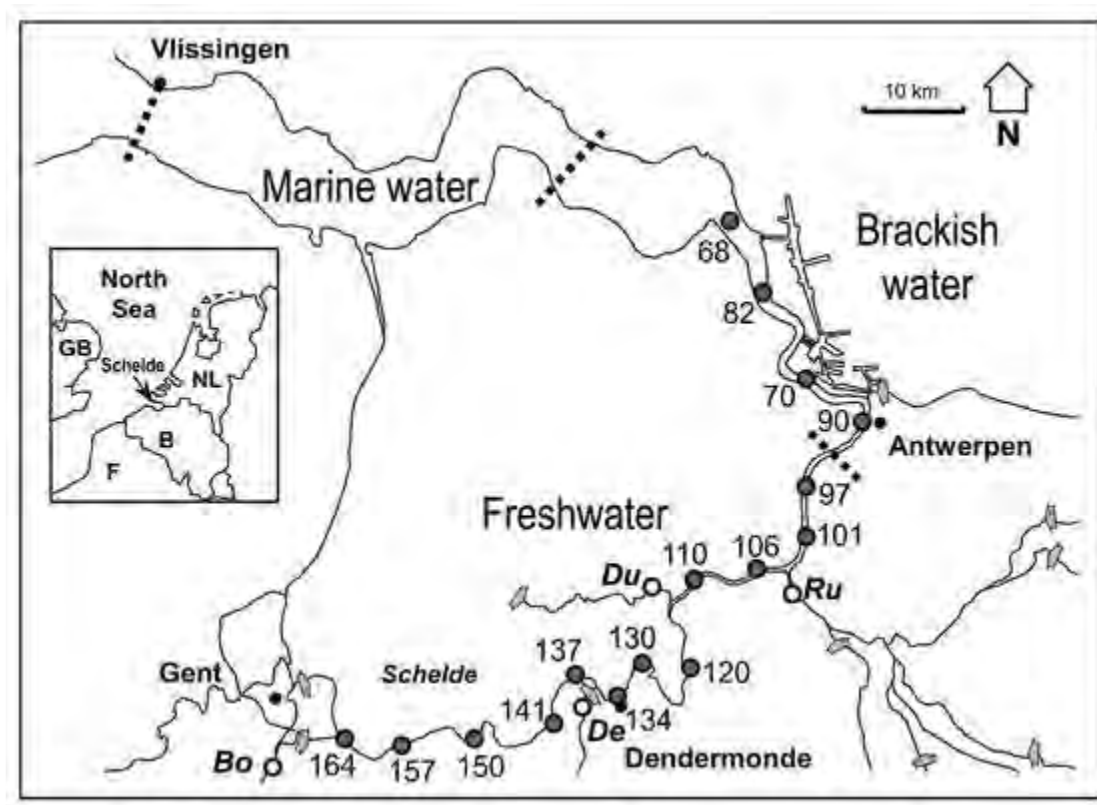


Figure 27 : Map of the Scheldt estuary with OMES sampling stations, designated by their distance, in km upstream from Vlissingen (mouth). Dotted lines indicate milits between marine water, brackish water and freshwater reaches. Sampling station Antwerpen indicated.

#### IV.2.3.2 Experimental procedure

##### IV.2.3.2.1 Field work

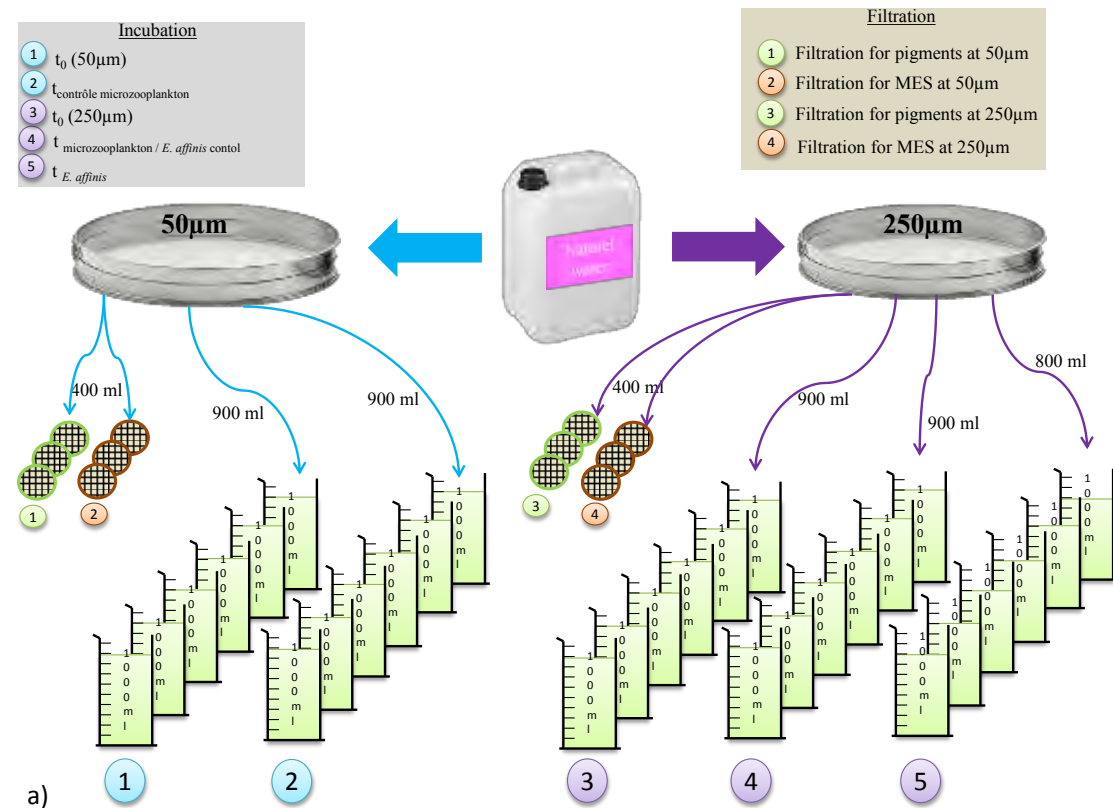
Conductivity, temperature and salinity of the natural water were measured at each sampling time using a Multi parameter sensor (WTW, Multi 3430). Water samples of 250- 450 ml, depending on the SPM load, were filtered on pre -weighed Whatman GF/C filters (DW filter) for the quantification of SPM and OM concentration in the water at the time of sampling.

For the incubation experiments, a concentrated zooplankton sample was collected at the beginning of each experiment by leaving a 150 µm mesh plankton net counter current at sub-surface for 15 minutes. The collected zooplankton was brought into a 1 liter container filled with of natural Scheldt water. At the same time, 50 L of natural water were also taken at surface and both zooplankton and water were transported to the laboratory.

For the gut pigment analysis, zooplankton was collected by filtering of sub-surface water with 150  $\mu\text{m}$  mesh plankton net. The collected zooplankton was immediately frozen in liquid nitrogen. These frozen samples were stored at  $-80^\circ\text{C}$  upon arrival in the laboratory until *E. affinis* sorting for gut pigment analyses.

#### IV.2.3.2.2 Incubation experiments

For logistic reasons, the both sets of incubation experiments (9 experiments in April 2013 and 4 in April 2014) (fig. 28a,b) were conducted in the ECOBE laboratory at Antwerp University.



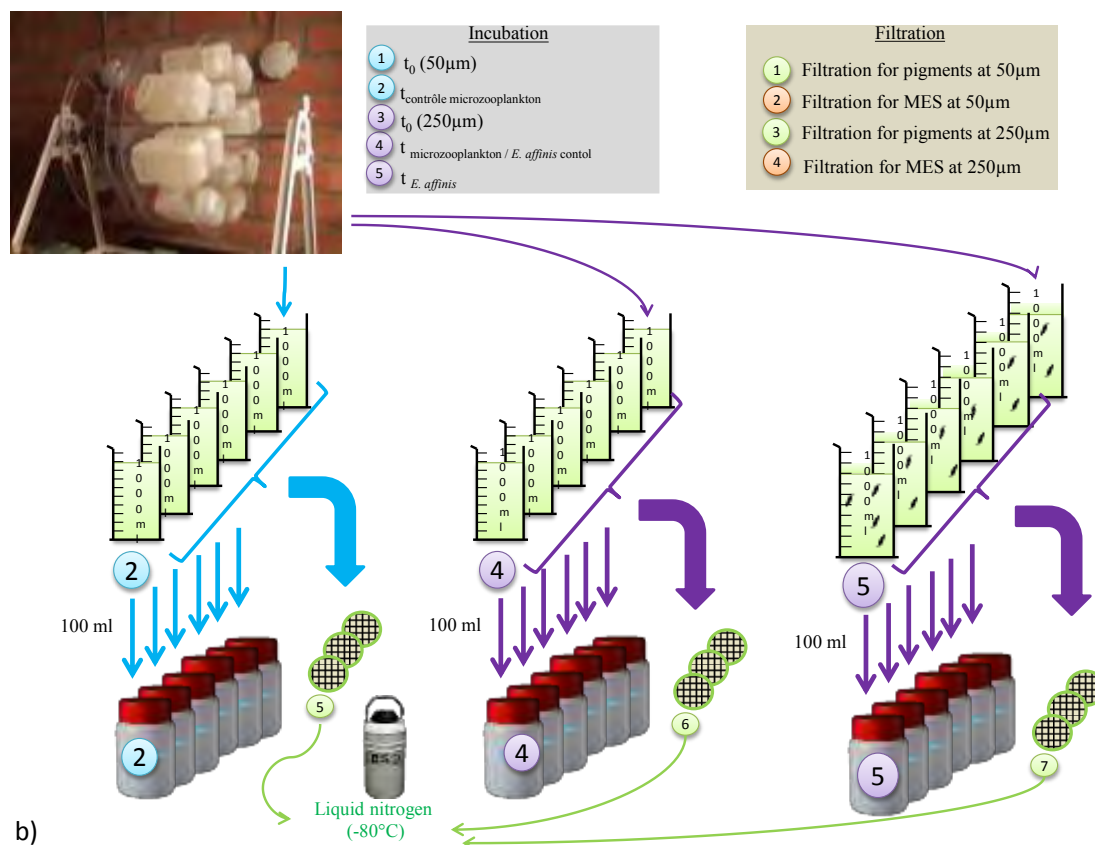


Figure 28: Experimental design for incubation experiments. a) at the beginning of each experiment b) at the end of each experiment.

Living adults and CV (stage V for copepodites) individuals of *E. affinis* to be used in the experiments were carefully isolated from filtered water samples under stereomicroscope (90x magnification) using an ophthalmic surgical clamp and 6x 50 individuals and were brought into < 100 ml volumes of natural water filtered on 250  $\mu$ m.

9 replicates per experiment were realized for 2013 experiments (18 for 2014 experiments). The natural water was filtered on 250  $\mu$ m to remove zooplankton. Each microcosm (1 L polycarbonate bottle) was filled with 900 ml of this filtered water at time zero. For each experiment, there were 9 (18 for 2014) microcosms: 3  $t_0$  (6 for 2014) replicates and 3 (6 for 2014) control replicates (tc) containing only the filtered water, and, 3 (6 for 2014) treatment replicates (tz) 50 *E. affinis* per microcosms using 100 ml natural water filtered to adjust to the same 900 ml level as the control microcosms.



Once individuals introduced into the  $t_z$  microcosms, each bottle was closed and placed in a rotating incubator (2rpm) to prevent SPM sedimentation during the incubation (for 24 hours, in the dark). In 2013, the temperature was 11°C (as in the naturel environment). Due to technical problems, temperature was 19°C in 2014 whereas it averaged 11.7 °C in the field. At the beginning of the experiment ( $t_0$ ), 250 ml water samples were collected from the  $t_0$  bottles and filtered on to Whatman GF/C glass fiber filters to measure the phytoplankton pigment concentration present in the Scheldt water at the start of the experiment. Filters were then stored in liquid nitrogen for further HPLC pigment analyses. At the end of the incubation, between 200 and 450 ml water samples collected from each bottle were filtered on GFC filters and these filters were also stored in liquid nitrogen prior to HPLC analysis. As in our experiments, the Scheldt water was filtered over 250  $\mu\text{m}$ , it is likely that a substantial microzooplankton community was present in both control and grazing bottles. To verify for the intensity of microzooplankton feeding on phytoplankton, for each experiments microcosms filled with the water filtered over 50  $\mu\text{m}$ , were also incubated: 3  $t_0$ ,  $t_c$  and  $t_z$  replicates. Sampling was carried out as described above and samples were stored in liquid nitrogen. Upon arrival in the EcoLab laboratory, all frozen samples were stocked in a -80 deepfreezer.

For SPM and OM quantification, filters were dried (45 °C), weighed for SPM and re-weighed to obtain Organic Matter (OM) concentrations.

#### IV.2.3.3 HPLC protocol

Extraction of individuals for the gut content as well as HPLC analysis were carried out at the Ecolab laboratory in Toulouse.

##### IV.2.3.3.1 Pigment extraction

For phytoplankton pigment extraction, each filter was extracted 2 times in a total volume 10 ml (5 and 5 ml) 98% cold-buffered methanol (with 2% of 1 M ammonium acetate) following Mialet et al. (2013) for 15 minutes at 20°C in darkness. Algal pigment release was favoured at each step by 60 seconds an ultrasonication

probe (Branson ultrasonic bath model 5810). Then, 1 ml of the total pigment solution was filtered (0.2 µm PTEE syringe filter) and HPLC analysed.

#### IV.2.3.4 Gut pigment measurements

*E. affinis* individuals (adults and CV) were isolated from the frozen samples using a needle picker, clamp and petri dish on ice and under minimum light exposure to prevent pigment degradation. Each individual was meticulously washed in a cold milliQ water bath to remove organic or sediment particles as much as possible and finally 30 individuals were placed in 10 µl of milliQ water. Following this procedure, when it was possible, 6 replicates were made (3 replicates of females and 3 replicates of males). Pigments were extracted from *E. affinis* samples by ultrasonication (Fisher scientific, FB15046), for 120 seconds in 300 µl of 98 % cold-buffered methanol (with 2 % ammonium acetate 1M) following Buffan-Dubau and Carman (2000a) and were then incubation for 15 minutes at 20°C in darkness. The total pigment solution was then filtered (0.2 µm PTEE syringe filter) and HPLC analysed.

##### IV.2.3.4.1 HPLC pigment quantification

HPLC analyses of *E. affinis* gut pigments and phytoplankton pigments 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, USA). 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, USA). The diode array detector was set at 440 nm to detect carotenoids, at 665 nm for chlorophylls and pheopigments (Wright et al., 1991). 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 with the corresponding area of calibrated standard.

#### IV.2.3.5 *Suspension and organic matter*

Filters for SPM quantification were dried at 60 °C for 24 h, cooled in a desiccator and weighed (DW<sub>sample</sub>) on a Mettler balance (precision: mg). SPM (dry weight) concentration was calculated as the difference between DW sample and DW filter.

The same filters were afterwards burned at 500 °C in a Muffler oven, cooled in a desiccator and reweighed for Ash weight (AW). The concentration of the organic matter (OM) in the samples was calculated as SPM-AW and expressed in mg L<sup>-1</sup>

#### IV.2.3.6 *Data analyses*

Incubation experiments. Differences in mean pigment concentrations between control ( $t_c$ ) and bottles with zooplankton ( $t_z$ ) are considered significant when  $p < 0.05$  following Mann-Whitney tests.

For each pigment, clearance- and ingestion rates were calculated according to Frost (1972).

The grazing coefficient,  $g$  (h<sup>-1</sup>) for each experimental bottle was calculated as:

$$g = \frac{1}{(t_2 - t_1)} \times \ln \left( \frac{\bar{t}_c}{t_z} \right)$$

- $t_1$  is the time zero,  $t_2$  the time end of the experiment (T°C)
- $\bar{t}_c$ : mean pigment concentration ( $\mu\text{g L}^{-1}$ ) at the end of the experiment in the control bottles,
- $t_z$ : pigment concentration ( $\mu\text{g L}^{-1}$ ) at the end of the experiment in each of the experimental bottles.

Clearance rate ( $F$ , ml ind.<sup>-1</sup> h<sup>-1</sup>) was calculated from each experimental bottle as:

$$F = \frac{V \times g}{N}$$

where  $V$  is the volume (ml) of water of the experimental bottles and  $N$  is the number of copepods in the experimental bottles ( $t_z$ ).

Ingestion rate ( $I$ , ng ind.<sup>-1</sup>h<sup>-1</sup>) of *E. affinis* on each pigment was calculated as:

$$I = C \times F$$

with  $C$ , the concentration ( $\mu\text{g L}^{-1}$ ) in the experimental bottle, detailed below.

$k$ : growth coefficient (h<sup>-1</sup>):

$$k = \frac{1}{(t_2 - t_1)} \times \ln \left( \frac{\overline{t_c}}{t_0} \right) \quad (1)$$

$\overline{t_0}$ : mean pigment concentration ( $\mu\text{g L}^{-1}$ ) in the replicate  $t_0$  bottles (2)

$$C = \frac{\overline{t_0} \times \left( \frac{e^{(k-g)(t_2-t_1)} - 1}{k-g} \right)}{(t_2 - t_1) (k - g)} \quad (3)$$

Community ingestion rate ( $C$   $\mu\text{g ind.}^{-1}\text{h}^{-1}$ ) was calculated by multiplying individual ingestion rates by *E. affinis* adult and CV abundance in the field.

The clearance rate of the microzooplankton ( $t_0 < 50 \mu\text{m}$  and  $t_{\text{microzoo}} < 250 \mu\text{m}$ ), was also calculated following Frost, 1972, adapted as:

$$F = \frac{V_1}{t \times V_2} \times \ln \left( \frac{\overline{t_c}}{t_2} \right) \quad \text{ml L}^{-1}\text{h}^{-1} \quad (4)$$

with :  $V_1$  : incubation volume in microcosm (ml)  $V_2$ : 900 ml (4).

The use of  $Q_{10}$  has been necessary to correct the 2014 temperature difference between the incubation conditions and the field temperature at the time of sampling.

A  $Q_{10}$  of 2,5 (Durbin and Durbin, 1992; David et al., 2006) was therefore applied to the calculated clearance and ingestion rates as:

$$F_2 = F_1 \times Q_{10}^{\left(\frac{T_2 - T_1}{10}\right)}$$

- **F1** is the measured clearance or ingestion rate ( $I_1$ ) at temperature  $T_1$  (where  $T_1 > T_2$ ).  $T_1$  is the 2014 laboratory incubation temperature.
- **F2** is the measured clearance or ingestion rate ( $I_1$ ) at temperature  $T_2$  ( $T_2$  is the natural water temperature).

For gut pigment content results, the ingestion rate ( $I$ ) of *E. affinis* on phytoplankton was calculated by multiplying gut content (ng of pigment ind.<sup>-1</sup>) by the Gut Clearance Rate (GCT), using the regression:  $GCT = 0.0117 + 0.001794 T$  (°C) (Dam and Peterson, 1988). Clearance rate ( $F$ ) on each pigment was calculated by dividing the ingestion rate by the pigment concentration, measured in the water at the time of sampling.

#### IV.2.4 Results:

##### IV.2.4.1 Concentration and composition of the suspended matter

SPM concentration in the experiments varied from  $18.03 \pm 6.63 \text{ mgL}^{-1}$  to  $178.11 \pm 52.10 \text{ mgL}^{-1}$ , OM concentration between  $5.65 \pm 0.86$  and  $27.75 \pm 31.49 \text{ mgL}^{-1}$ . The SPM was composed for minimum 14.53 and maximum 31.87% of organic matter. Chl *a* concentration varied between  $1.53 \pm 0.09$  to  $3.83 \pm 0.51 \text{ } \mu\text{g L}^{-1}$ . Estimating live phytoplankton contribution to OM as:  $\text{Chl } a \times 30 \times 2$  (Lionard et al., 2008; OMES, PAE), the contribution of phytoplankton was between 0.4 and 2.11 % (fig. 29).

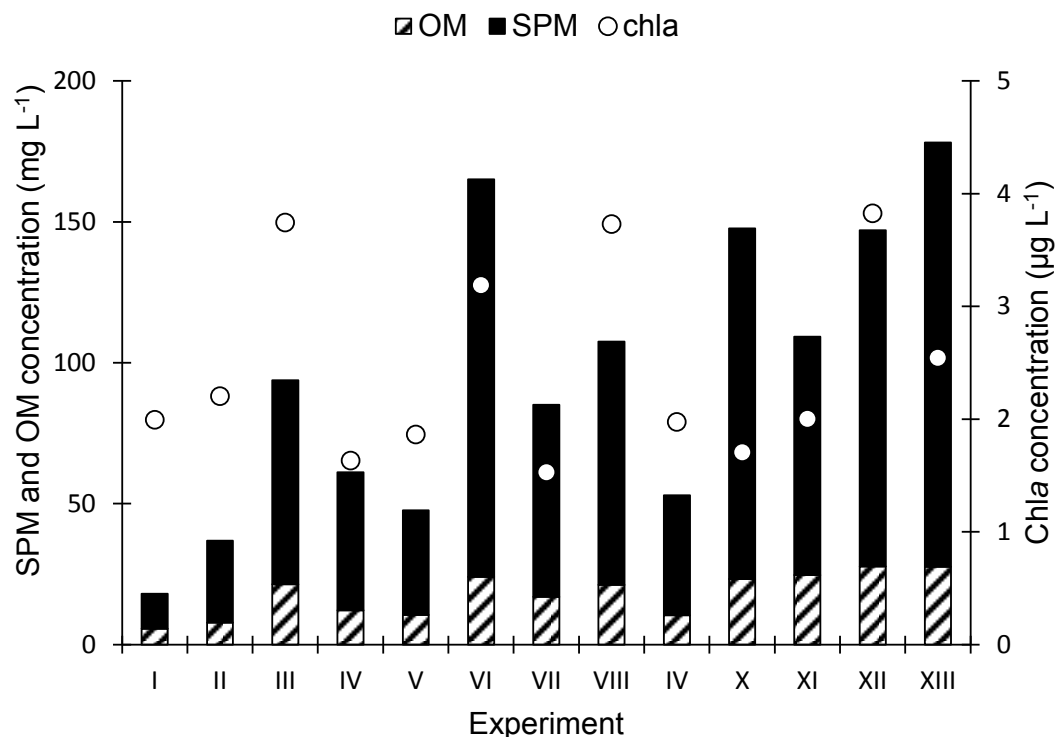


Figure 29 : Mean concentrations of SPM and OM in each experiment at  $t_0$ . Symbols: Chl  $a$  concentration

There was a significant correlation ( $p < 0,001$ ) between the SPM and OM concentrations in the natural filtered (on 250µm mesh) Scheldt water which was used for the incubation experiments at  $t_0$  (Fig. 30a). However, there was no significant correlation between Chl  $a$  and OM concentrations at  $t_0$  ( $p > 0,05$ , Fig 30b).

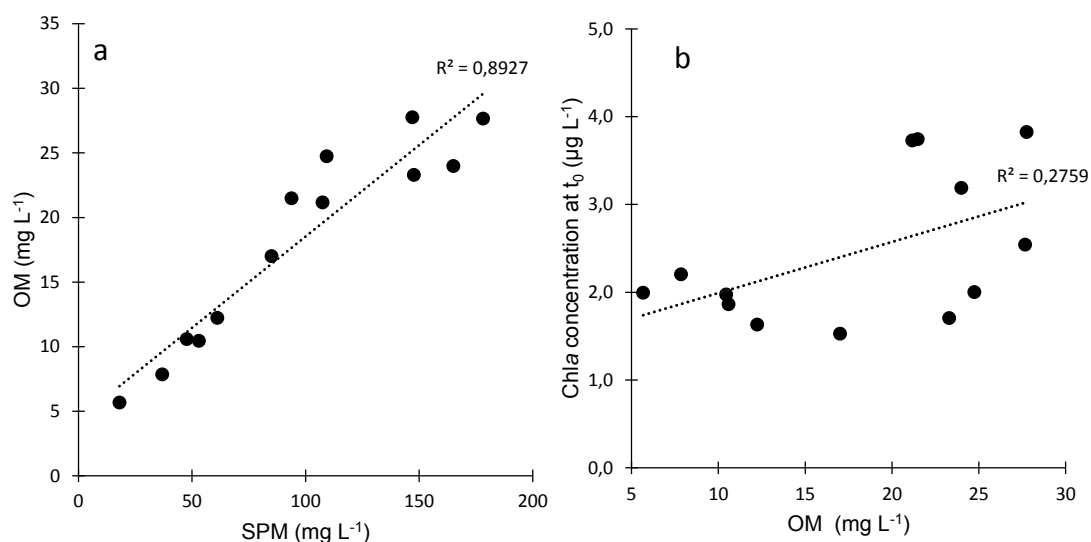


Figure 30 : Average concentrations of OM ( $n=3$ ) as a function of average concentrations of SPM ( $n=3$ ) (a) and average concentration Chl  $a$  as a function of OM concentrations (b) at  $t_0$  in natural Scheldt water used for the incubation experiments.

#### IV.2.4.2 Pigment composition

The biomarker pigments which were detected in microcosm water were (A) chlorophyll *c*, fucoxanthin, diadinoxanthin and diatoxanthin, (B) alloxanthin and an unidentified pigment which is probably monadoxanthin, (C) lutein and violaxanthin, (D) zeaxanthin (Fig. 31a). These can be considered as biomarker pigments for the following phytoplankton taxa: diatoms (A), cryptophytes (B), green algae (C), and cyanobacteria/green algae (D) (Jeffrey et al., 1997).

Gut content analyses of *E. affinis* indicated that astaxanthin was the most concentrated pigment in all samples (Fig. 31b). This pigment is present in the body tissues of copepods (Matsuno, 1989). Cryptophytes (B) and diatoms (A) were represented by the biomarker pigments monadoxanthin and alloxanthin, and diatoxanthin respectively. 26 % of the gut content analyses revealed the presence of diatoxanthin.

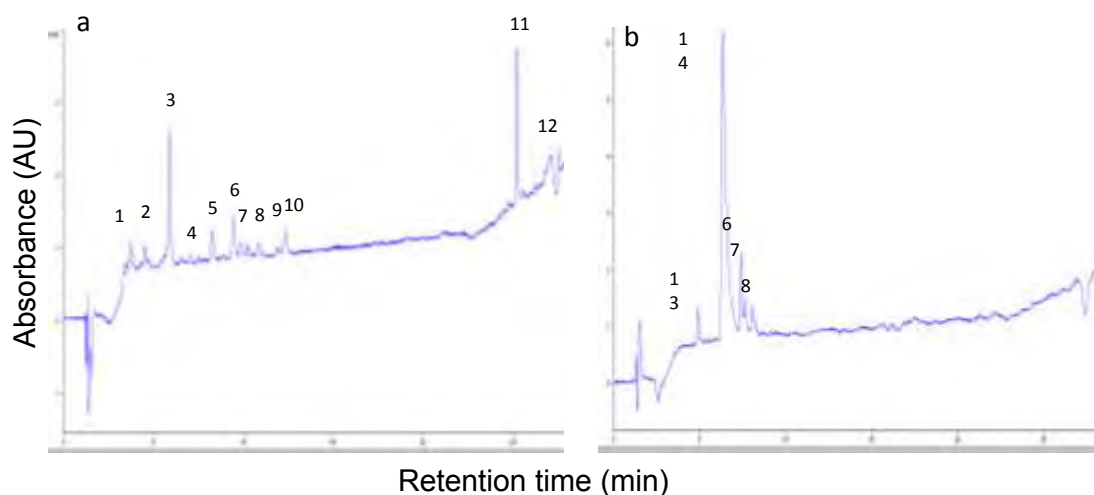


Figure 31 : Examples of HPLC absorbance chromatograms obtained at 440nm from samples. (a) incubation experiment, (b) gut content. 1: chlorophyll *c*; 2: pheophorbide *a*; 3: fucoxanthin; 4: violaxanthin; 5: diadinoxanthin; 6: possibly monadoxanthin; 7: alloxanthin; 8: diatoxanthin; 9: zeaxanthin; 10: lutein; 11: chlorophyll *a*; 12:  $\beta$ -carotene; 13: pheophorbide coeluted with unidentified pigment 14: asthaxanthin.

At  $t_0$ , in water samples, the more important biomarker pigment was fucoxanthin (mean concentration =  $1.15 \pm 0.44 \mu\text{g L}^{-1}$ ) (Fig. 32). Chl *a* concentration ranged from  $1.53 \pm 0.09$  to  $3.83 \pm 0.51 \mu\text{g L}^{-1}$ .

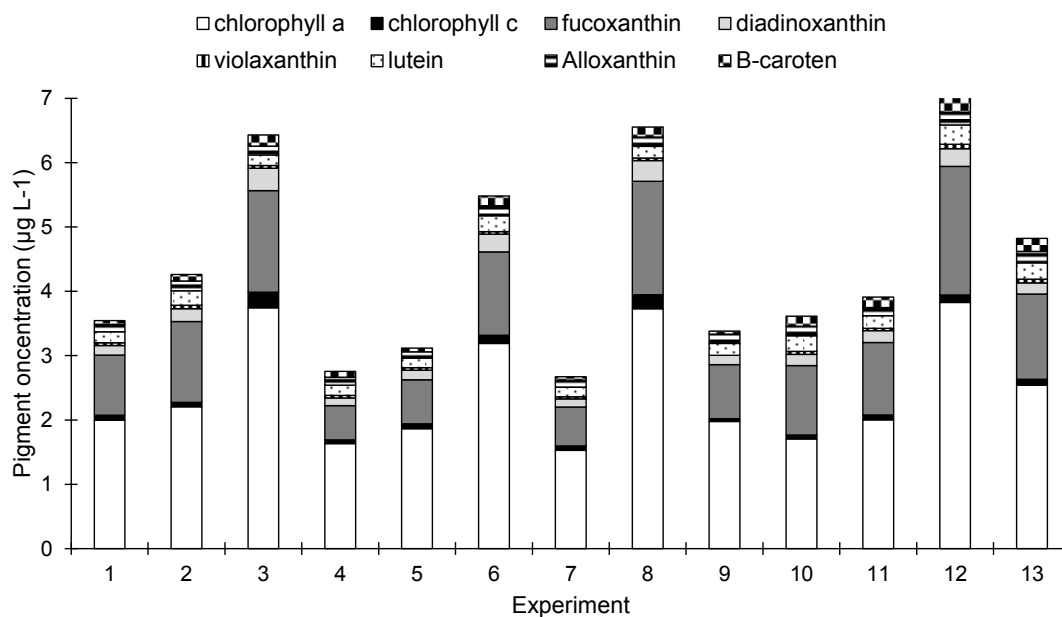


Figure 32 : Marker pigments mean concentration, in the natural Scheldt water used for incubation experiments at  $t_0$  in all experiments (n=13).

Fucoxanthin concentrations were correlated to the concentrations of Chl *a* ( $p=0.034$ ), showing that diatom constituted the major constituent of the phytoplankton community (Fig. 33).

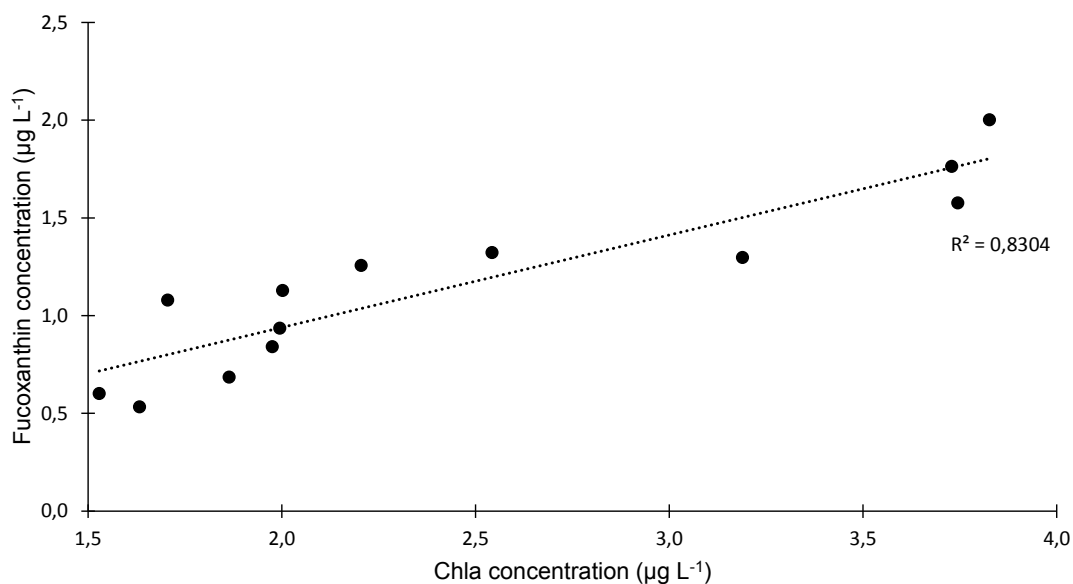


Figure 33 : Fucoxanthin concentration as a function of chlorophyll *a* concentration in natural Scheldt water at  $t_0$ .

Although, the diatom pigments, fucoxanthin was not detected in gut contents, diatoxanthin was present (fig.30), indicating that diatoms were ingested by the copepod. Besides, the biomarker pigments originated from cryptophytes (peak: 8, 9,



Fig.31) were also found in gut contents (while green algal pigments (peak: 4, 9, 10, Fig.31) were not), indicating that the copepod also ingested cryptophytes, but likely no green algae.

#### IV.2.4.3 Clearance rates in incubation experiments with *E. affinis*

Over the 13 incubation experiments run with *E. affinis*, Chl *a* had a significant lower mean concentration ( $p < 0.05$ ) in the experimental bottles than in the control bottles in 61 % of the cases at the end of the experiment. This difference was also observed for the mean concentrations of the diatom pigments fucoxanthin, diadinoxanthin and Chl *c* in 54 % of the cases. In contrast, the mean concentrations of alloxanthin (a cryptophyte marker pigment) and lutein (a chlorophyte marker pigment) lutein were significantly higher in the experimental bottles than in the control bottles in 69 and 46 % of the experiments respectively. Other pigments showed less significant or repetitive differences in mean concentration between control and experimental bottles (Table 4).

Table 4 : Results of the 13 grazing experiments (from I to IX in 2013 and from X to XIII in 2014) with *E. affinis*. Mean concentrations of each pigments (n=3 for 2013; n=6 for 2014  $\pm$  standard error in annexe1) at t0, in

# CHAPITRE IV : Sélectivité trophique du copépode calanoïde *Eurytemora affinis* en douce dans l'estuaire de l'Escaut

control (tcontrol) and treatment (tzoo) microcosms. Mean ingestion and clearance rates (n=3 for 2013; n=6 for 2014 ± standard deviation in annex1)

Mean t <sub>g</sub> (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	β-carotene	Chlorophyll c	Chlorophyll a	Phaeophytin a	Phaeophorbide a	Zeaxanthin
I	0.925	0.046	0.146	0.037	0.169	0.113	0.062	0.081	1.994	0.511	0.190	0.041
II	1.256	0.058	0.199	0.068	0.222	0.153	0.101	0.068	2.204	0.631	0.258	0.052
III	1.577	0.042	0.349	0.057	0.164	0.138	0.174	0.243	3.745	0.419	1.537	0.052
IV	0.532	0.040	0.117	0.083	0.162	0.120	0.093	0.059	1.633	0.318	1.958	0.037
V	0.685	0.039	0.150	0.065	0.148	0.097	0.059	0.075	1.864	0.255	1.539	0.045
VI	1.297	0.043	0.274	0.084	0.244	0.155	0.152	0.127	3.189	0.481	1.828	0.091
VII	0.601	0.034	0.127	0.069	0.150	0.107	0.053	0.069	1.529	0.264	1.226	0.141
VIII	1.763	0.042	0.320	0.052	0.181	0.142	0.159	0.219	3.729	0.375	1.162	0.073
IX	0.841	0.185	0.145	0.064	0.185	0.142	0.050	0.045	1.975	0.494	0.188	0.064
X	1.079	0.046	0.173	0.147	0.244	0.149	0.154	0.063	1.705	0.582	0.474	0.114
XI	1.129	0.037	0.182	0.112	0.196	0.126	0.163	0.075	2.002	0.461	0.285	0.061
XII	2.001	0.069	0.273	0.228	0.301	0.195	0.301	0.116	3.826	0.949	0.429	0.143
XIII	1.323	0.057	0.176	0.196	0.254	0.176	0.203	0.091	2.542	0.690	0.391	0.121

Mean tcontrol (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	β-carotene	Chlorophyll c	Chlorophyll a	Phaeophytin a	Phaeophorbide a	Zeaxanthin
I	0.456	0.037	0.104	0.082	0.111	0.076	0.067	0.059	1.331	0.167	1.406	0.027
II	0.551	0.033	0.084	0.079	0.117	0.080	0.042	0.046	1.007	0.177	1.660	0.033
III	1.594	0.029	0.288	0.053	0.120	0.106	0.090	0.359	3.386	0.265	1.315	0.041
IV	0.602	0.039	0.112	0.076	0.146	0.100	0.077	0.061	1.524	0.272	1.875	0.046
V	0.748	0.048	0.130	0.061	0.145	0.098	0.060	0.067	1.761	0.218	1.397	0.044
VI	1.875	0.046	0.225	0.075	0.188	0.126	0.104	0.130	2.579	0.360	1.564	0.065
VII	0.692	0.051	0.118	0.060	0.189	0.106	0.075	0.082	1.929	0.307	1.448	0.063
VIII	1.956	0.044	0.331	0.059	0.183	0.125	0.094	0.235	3.611	0.319	1.209	0.069
IX	0.943	0.116	0.131	0.074	0.222	0.130	0.068	0.042	1.911	0.467	0.362	0.064
X	0.877	0.034	0.116	0.124	0.180	0.114	0.069	0.065	1.391	0.235	0.265	0.092
XI	1.063	0.032	0.141	0.101	0.187	0.098	0.125	0.074	1.891	0.440	0.253	0.061
XII	1.917	0.072	0.305	0.186	0.283	0.152	0.210	0.143	3.873	0.766	0.424	0.116
XIII	1.550	0.046	0.249	0.162	0.260	0.130	0.202	0.104	2.753	0.680	0.359	0.097

Mean tzoo (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	β-carotene	Chlorophyll c	Chlorophyll a	Phaeophytin a	Phaeophorbide a	Zeaxanthin
I	0.428	0.039	0.096	0.070	0.116	0.083	0.081	0.080	1.400	0.227	1.553	0.056
II	0.472	0.033	0.076	0.091	0.115	0.088	0.054	0.033	0.978	0.195	1.783	0.034
III	0.885	0.037	0.214	0.056	0.166	0.150	0.194	0.109	2.410	0.475	2.183	0.053
IV	0.439	0.036	0.078	0.072	0.142	0.095	0.070	0.033	1.235	0.281	1.826	0.044
V	0.554	0.045	0.107	0.055	0.147	0.096	0.061	0.047	1.418	0.209	1.645	0.039
VI	0.846	0.040	0.161	0.075	0.199	0.150	0.108	0.074	1.879	0.588	1.785	0.072
VII	0.452	0.036	0.084	0.065	0.155	0.114	0.040	0.046	1.367	0.222	1.246	0.054
VIII	0.853	0.045	0.176	0.043	0.188	0.123	0.176	0.088	1.774	0.519	1.511	0.071
IX	0.709	0.059	0.094	0.072	0.199	0.146	0.078	0.031	1.538	0.463	0.285	0.064
X	0.871	0.038	0.120	0.147	0.217	0.154	0.128	0.039	1.503	0.510	0.369	0.092
XI	0.658	0.034	0.097	0.096	0.173	0.127	0.148	0.033	1.294	0.432	0.228	0.071
XII	0.972	0.046	0.128	0.156	0.225	0.141	0.219	0.049	2.002	0.763	0.407	0.106
XIII	1.033	0.037	0.150	0.154	0.240	0.166	0.194	0.056	1.850	0.713	0.397	0.105

Mean F (ml ind. <sup>-1</sup> .h <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	β-carotene	Chlorophyll c	Chlorophyll a	Phaeophytin a	Phaeophorbide a	Zeaxanthin
I	0.065	-0.682	0.090	0.194	-0.017	-0.064	-0.136	-0.238	-0.021	-0.280	-0.066	-0.525
II	0.166	0.003	0.110	-0.135	0.031	-0.077	-0.123	0.358	0.052	-0.033	-0.055	0.026
III	0.582	-0.216	0.294	-0.047	-0.316	-0.341	-0.751	1.188	0.334	-0.533	-0.498	-0.251
IV	0.325	0.072	0.359	0.049	0.033	0.063	0.099	0.610	0.208	-0.025	-0.029	0.063
V	0.301	0.062	0.193	0.106	-0.002	0.022	-0.003	0.377	0.216	0.046	-0.161	0.145
VI	0.783	0.154	0.329	-0.003	-0.050	-0.174	0.015	0.558	0.322	-0.873	-0.121	-0.087
VII	0.422	0.344	0.331	-0.052	0.199	-0.042	0.634	0.609	0.354	0.321	0.156	0.168
VIII	0.818	-0.023	0.628	0.318	-0.023	0.018	-0.617	0.979	0.701	-0.471	-0.218	-0.005
IX	0.280	0.333	0.027	0.106	-0.107	-0.120	0.297	0.214	0.008	0.008	0.241	0.011
X	0.016	-0.022	-0.001	-0.060	-0.071	-0.119	-0.258	0.231	-0.016	-0.324	-0.137	0.010
XI	0.217	-0.024	0.173	0.024	0.048	-0.112	-0.056	0.348	0.178	0.029	0.054	-0.051
XII	0.304	0.201	0.395	0.079	0.115	0.073	-0.004	0.464	0.294	0.008	0.024	0.039
XIII	0.195	0.093	0.249	0.030	0.050	-0.089	0.023	0.271	0.175	-0.003	-0.036	-0.009

Mean I (ng ind. <sup>-1</sup> .h <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	β-carotene	Chlorophyll c	Chlorophyll a	Phaeophytin a	Phaeophorbide a	Zeaxanthin
I	0.041	-0.067	0.009	0.008	-0.006	-0.008	-0.014	-0.023	-0.080	-0.105	-0.070	-0.033
II	0.124	0.000	0.013	-0.011	0.004	-0.011	-0.018	0.016	0.048	-0.030	-0.059	-0.002
III	0.694	-0.008	0.081	-0.003	-0.053	-0.049	-0.138	0.195	1.012	-0.255	-0.918	-0.013
IV	0.151	0.003	0.034	0.004	0.005	0.006	0.007	0.027	0.294	-0.010	0.050	0.002
V	0.182	0.002	0.024	0.006	-0.002	0.002	-0.001	0.021	0.347	0.010	-0.256	0.005
VI	0.826	0.006	0.070	0.000	-0.012	-0.027	-0.005	0.055	0.774	-2.495	-0.242	-0.008
VII	0.220	0.012	0.034	-0.004	0.030	-0.008	0.028	0.033	0.488	0.077	0.183	0.014
VIII	1.023	-0.001	0.151	0.014	-0.004	0.002	-0.108	0.134	1.842	-0.205	-0.288	-0.003
IX	0.217	0.039	0.039	0.001	0.020	-0.016	-0.008	0.011	0.373	0.004	0.004	-0.001
X	0.0034	-0.0019	-0.0022	-0.0109	-0.0187	-0.0201	-0.0372	0.0114	-0.0529	-0.1667	-0.0601	0.0001
XI	0.1826	-0.0010	0.0220	0.0021	0.0065	-0.0146	-0.0112	0.0179	0.2687	0.0036	0.0115	-0.0044
XII	0.4223	0.0110	0.0723	0.0143	0.0259	0.0055	-0.0041	0.0360	0.8106	0.0020	0.0077	0.0046
XIII	0.2070	0.0042	0.0358	0.0038	0.0086	-0.0181	0.0035	0.0195	0.3777	0.0073	-0.0174	-0.0038

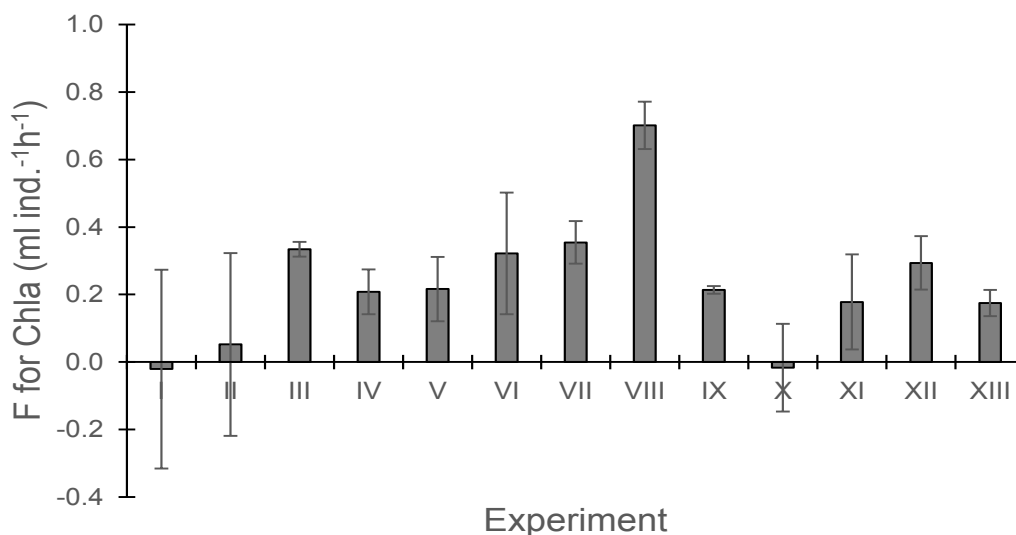


Figure 34 : *E. affinis* adults mean clearance rates (n=3 for 2013; n=6 for 2014 ± standard error in annexe 1) calculated from Chl *a* concentrations representing the total phytoplankton biomass. Vertical lines show standard deviation.

Clearance rates measured in term of Chl *a* concentrations ranged from  $-0.02 \pm 0.29$  to  $0.7 \pm 0.07$  ml ind.<sup>-1</sup>h<sup>-1</sup> (Fig.34), and tented to be higher for Chl *a* concentrations between 1.5 and 3.8  $\mu\text{g L}^{-1}$ .

*E. affinis* clearance rates calculated from fucoxanthin concentrations (representing diatoms), ranged from  $0.02 \pm 0.24$  to  $0.81 \pm 0.07$  ml ind.<sup>-1</sup>h<sup>-1</sup>. Clearance rates from alloxanthin concentrations, (representing cryptophyceae), and from lutein concentrations (representing chlorophyceae), were often negative, i.e. between  $-0.34 \pm 0.02$  and  $0.15 \pm 0.46$  ml ind.<sup>-1</sup>h<sup>-1</sup>;  $-0.31 \pm 0.12$  and  $0.19 \pm 0.10$  ml ind.<sup>-1</sup>h<sup>-1</sup>, respectively. The highest clearance rate from fucoxanthin values occurred at Chl *a* concentrations of 1.53  $\mu\text{g L}^{-1}$  and, between 3.18 and 3.83  $\mu\text{g L}^{-1}$  ( $p < 0.05$ , Fig 35).

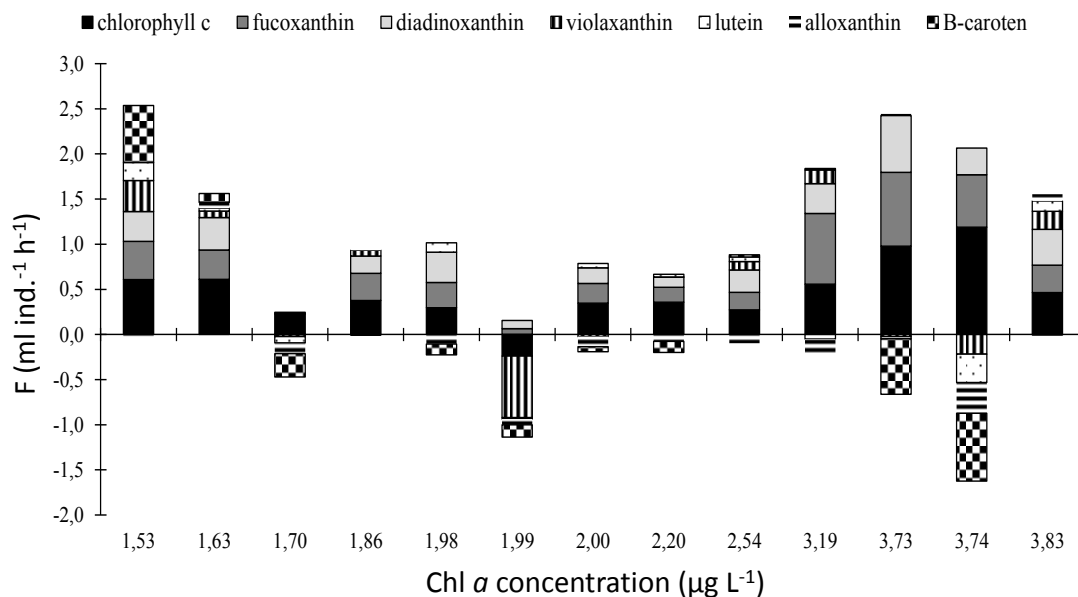


Figure 35 : *E. affinis* adult mean clearance rates (n=3 for 2013; n=6 for 2014  $\pm$  standard error in annexe 1) measured from each biomarker pigment concentration for the grazing incubation experiments. X axis: Chl *a* concentration in water, at  $t_0$  for each experiment.

#### IV.2.4.4 Comparaison of ingestion rate results between both methods

In incubation experiments, the *E. affinis* ingestion rates calculated from Chl *a* concentrations varied from  $-0.08 \pm 0.29$  to  $1 \pm 0.07 \text{ ng ind.}^{-1} \text{ h}^{-1}$  (Fig 36). Among the ingestion rates which were calculated from biomarker pigment concentrations, those calculated from fucoxanthin concentrations were generally the highest ( $p < 0.05$ ) excepted for two Chl *a* mean concentrations ( $3.18 \pm 0.40$  and  $3.8 \pm 0.51 \mu\text{g L}^{-1}$ ).

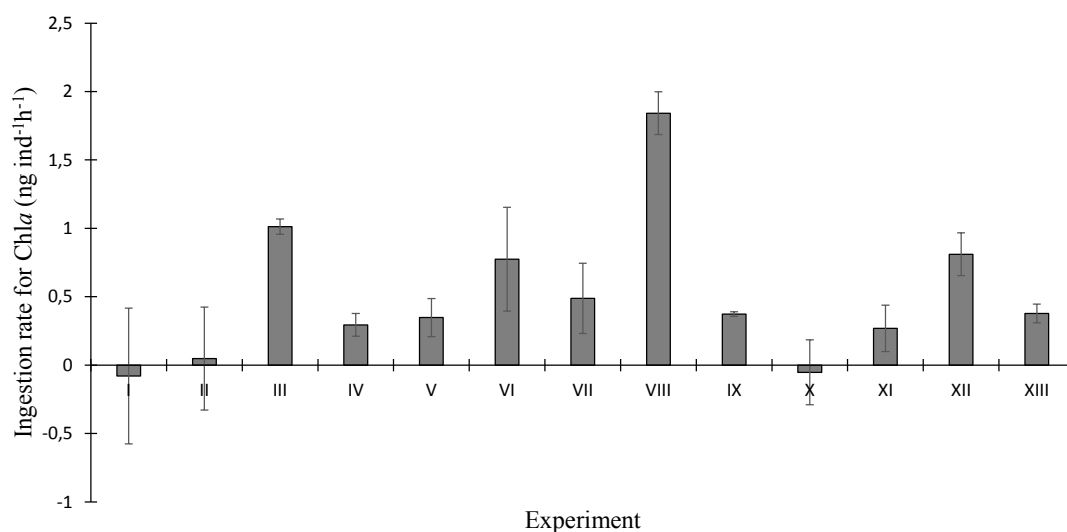


Figure 36 : *E. affinis* adult mean Chl *a* ingestion rate n=3 for 2013; n=6 for 2014  $\pm$  standard error in annexe 1) (in all experiments. Vertical lines show standard deviation.

In order to facilitate comparison of the results obtained by the two methods, the ingestion rates obtained from gut pigment content measurement of *E. affinis* are represented together with the ingestion rates from the incubation experiments (Fig. 37). Since only three biomarker pigments were quantified in the pigment gut contents; i.e. alloxanthin representing cryptophyceae, diatoxanthin representing diatoms, the comparison was made for these three markers. Pheophorbide *a* represented the ingestion of total biomass (Fig. 31b).

Mean ingestion rates calculated from pheophorbide *a* concentrations in gut contents were not significantly different than the ingestion rates from Chl *a* concentrations calculated from the incubation experiments ( $p=0.49$ ). For gut content analyses, ingestion rates from alloxanthin concentrations ranged between 0.01 and 0.12  $\text{ng ind.}^{-1}\text{h}^{-1}$  for Chl *a* concentrations comprised between 1.99 and 2.54  $\mu\text{g L}^{-1}$  whereas they were negative for the incubation experiments. Ingestion rates from diatoxanthin concentrations were comprised between 0.03 and 0.08  $\text{ng ind.}^{-1}\text{h}^{-1}$ .

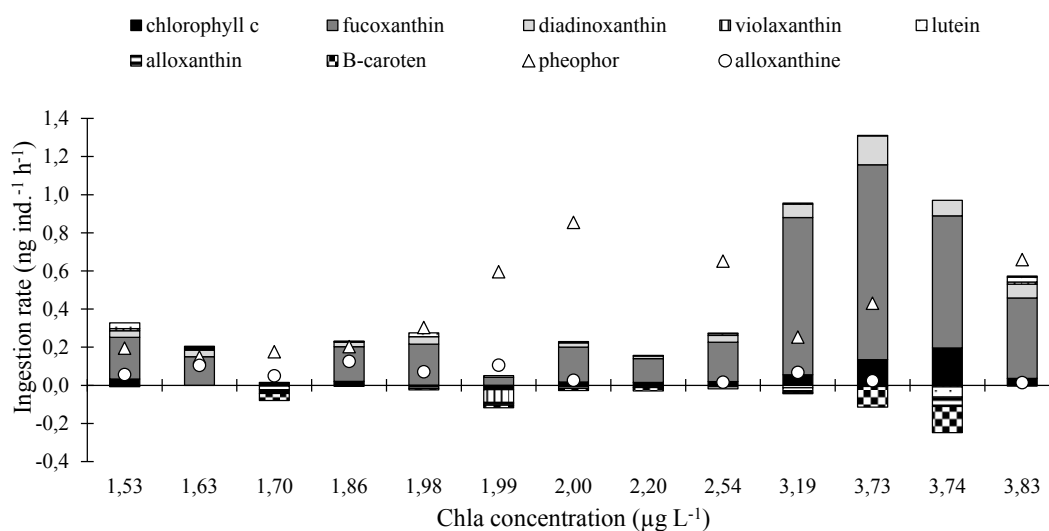


Figure 37 : *E. affinis* adult mean ingestion rates  $n=3$  for 2013;  $n=6$  for 2014  $\pm$  standard error in annexe 1 for incubations, and  $n=6$  for 2013 and 2014 for gut contents calculated from the different pigment concentrations, obtained from incubation experiments (bars) and from gut content analyses (symbols). X axis: Chl *a* concentration, at  $t_0$  of each experiment.

To detect any potential selective uptake of diatoms or cryptophyceae by the copepod *E. affinis*, the fucoxanthin / Chl *a* ingestion rate ratios (i.e. respective biomass proportions of diatoms and Chl *a* to total phytoplankton in the incubation experiments) were plotted against fucoxanthin / Chl *a*-eq concentration ratios (i.e. respective biomass proportions of diatoms and Chl *a*-eq to total phytoplankton in

copepod gut contents), calculated from the natural water used in the incubation experiments at  $t_0$  (Fig. 38a). All the values of the fucoxanthin /Chl *a*-eq ingestion rate ratios were systematically above the bisectrix of the graph representing the expected increase of these ingestion rate ratios as a function of fucoxanthin/Chl *a* concentration ratios when diatom ingestion by *E. affinis* is non selective. The same analysis was made for the alloxanthin / Chl *a* ratio obtained from ingestion rates calculated from gut content analyses and from the natural Scheldt water samples. Most ratios (7/10) from concentrations in the gut contents were also above the bisectrix (Fig. 38b). This shows that *E. affinis* consumed diatoms and cryptophyceae in disproportion to their abundance in the natural water, selecting them above other phytoplankton taxa.

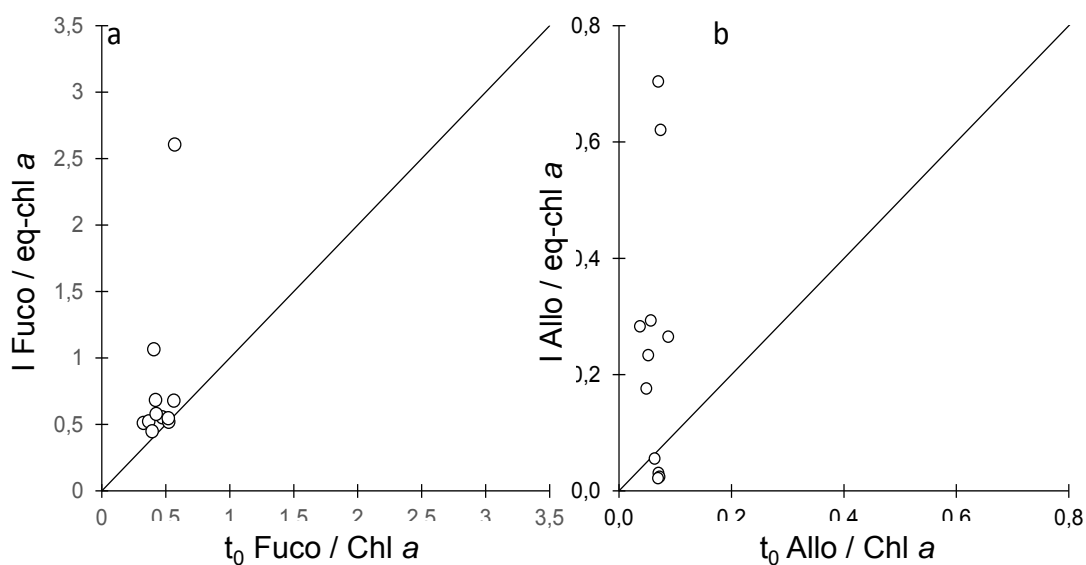


Figure 38 : Comparison of pigment proportions in *E. affinis* ingestion rate obtained from incubation experiments (a) and from gut contents (b) with the same proportions in the feeding medium.

#### IV.2.4.5 *E. affinis* impact on phytoplankton

Both methods used for *E. affinis* grazing quantification showed that, under natural feeding conditions, i.e. in the freshwater Scheldt, this species selected diatoms within the phytoplankton community. In the incubation experiments, ingestion rates of *E. affinis* were between 0.05 – 1.5 ng ind.<sup>-1</sup>L<sup>-1</sup> (mean around 0.6 ng ind.<sup>-1</sup>h<sup>-1</sup>) (Table 4). The 2013-2014 abundance of adults and CV *E. affinis* in the freshwater Scheldt in April was 6390±3521 ind. m<sup>-3</sup> (Table 5). This means that the adult and CV *E. affinis* population removed 3.65% of the phytoplankton standing stock d<sup>-1</sup>, and 2.06% of the diatom standing stock d<sup>-1</sup>. *E. affinis* adult and CV dry weight (males and females averaged) was 10-14 µg ind<sup>-1</sup> in 2010 (Lambert, 2012). Considering a mean value of

12  $\mu\text{g ind.}^{-1}$ , the adults and CV daily ration (DR) in terms of Chl *a* / DW is 0.12  $\mu\text{gChl } a \mu\text{gDW d}^{-1}$ . Applying the same DR to the younger development stages, the total consumption of phytoplankton stock by adults, copepodite and nauplii of *E. affinis* was 4,5 %  $\text{d}^{-1}$ .

Table 5 : *E. affinis* adult and CV abundance in April 2013-2014, and calculated grazing impact on the total phytoplankton and on the diatom stock.

	Adult + CV	Copepodites	Nauplii	Total population
Abundance april 2013-2014 (ind. $\text{m}^{-3}$ )	6390±3 521	1 596±401	18 733± 11 111	26719
Ingestion ( $\mu\text{g L}^{-1}\text{d}^{-1}$ )	0.014	0.006	0.0006	0.0206
DW ( $\mu\text{g ind.}^{-1}$ )	12	5	0.5	
% of total phytoplankton consumed $\text{d}^{-1}$	3.65	0.39	0.46	4.5

#### IV.2.4.6 Microzooplankton experiments

For the total of the 4 experiments, only 16% of the pigments quantified showed a significant lower mean concentration in the microcosms at  $t_{\text{microzoo}}$  than in the microcosm at  $t_c$ . These significant differences concerned concentrations of diatoxanthin (a diatom marker pigment), zeaxanthin (a chlorophyte marker pigment) and 2 pheopigments (pheophorbide *a*, and pheophytin *a*) in experiment 1, only pheophorbide *a* in experiment 2, fucoxanthin (a diatom marker) and a violaxanthin (a chlorophyte pigment marker) in experiment 3. No significant grazing activity was detected in experiment 4. (Table 6). Consequently, the results of the microzooplankton grazing experiments were rather variable.

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Table 6 : Results of the 4 grazing experiments with microzooplankton. Mean concentrations of each pigment (n=6 ± standard error in annexe2) at t<sub>0</sub>, in control (tcontrol) and treatment (tzoo) microcosms. Mean clearance rates (F) and ingestion rates (I). (n=6± standard deviation in annexe 2).

Mean t <sub>0</sub> (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	0.970	0.042	0.150	0.115	0.208	0.123	0.127	0.064	1.575	0.463	0.343	0.083
XI	1.022	0.034	0.178	0.112	0.167	0.102	0.165	0.070	1.870	0.465	0.245	0.056
XII	2.050	0.050	0.308	0.196	0.330	0.206	0.276	0.130	3.640	0.920	0.430	0.106
XIII	1.744	0.058	0.292	0.230	0.308	0.166	0.238	0.134	2.952	0.868	0.562	0.110

Mean tcontrol (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	0.977	0.040	0.158	0.127	0.188	0.127	0.083	0.043	1.315	0.298	0.267	0.087
XI	1.102	0.035	0.182	0.110	0.168	0.107	0.110	0.072	1.918	0.367	0.237	0.058
XII	2.340	0.068	0.350	0.217	0.310	0.212	0.230	0.168	4.222	0.845	0.502	0.107
XIII	1.728	0.050	0.277	0.192	0.280	0.142	0.227	0.100	2.873	0.863	0.490	0.117

Mean tzoo (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	0.875	0.035	0.117	0.123	0.178	0.113	0.070	0.063	1.392	0.233	0.267	0.092
XI	1.063	0.032	0.140	0.102	0.188	0.098	0.125	0.075	1.892	0.440	0.253	0.062
XII	1.917	0.072	0.303	0.187	0.283	0.152	0.212	0.143	3.873	0.767	0.425	0.117
XIII	1.553	0.046	0.250	0.162	0.260	0.132	0.203	0.103	2.753	0.678	0.360	0.098

Mean F (ml L <sup>-1</sup> h <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	6.163	7.254	16.070	1.769	3.293	5.780	10.092	-19.002	-2.390	13.715	0.217	-2.495
XI	2.807	5.356	18.778	5.319	-5.028	7.883	-4.731	0.068	2.172	-7.893	-1.691	-2.069
XII	11.220	-1.212	8.660	8.694	5.604	19.150	7.671	11.005	5.659	5.457	10.217	-2.365
XIII	6.792	7.322	5.641	10.006	4.858	4.829	8.205	0.018	3.551	14.628	17.696	9.714

Mean I (ng L <sup>-1</sup> h <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	5.108	0.258	2.042	0.159	0.533	0.663	0.849	-1.218	-4.213	3.761	0.003	-0.247
XI	1.600	0.248	2.485	0.580	-0.983	0.371	-1.565	-0.203	0.781	-4.273	-2.426	-0.173
XII	19.904	-0.335	2.197	1.434	1.392	2.981	1.041	0.971	16.192	4.132	3.556	-0.503
XIII	8.869	0.220	1.384	2.430	1.064	0.551	1.211	-0.186	6.153	8.970	7.074	0.896

Clearance rates measured varied from 0.02 – 19.15 ml L<sup>-1</sup> h<sup>-1</sup>, which means that, during the 24 h experiments, the microzooplankton grazed between 0.48 and 460.00 ml L<sup>-1</sup> d<sup>-1</sup> i.e. 4.8 to 46 % of certain phytoplankton taxa. No significant grazing on Chl *a* was detected, positive microzooplankton grazing was most frequently observed on pheopigments, this suggests that microzooplankton fed mainly on detritus.

## IV.2.5 Discussion:

### IV.2.5.1 SPM concentration and composition in the experimental water

In the water Scheldt water samples used for the grazing experiment, the SPM, in term of dry weight, was dominated by organic detritus, while live phytoplankton concentration, estimated from Chl *a* concentrations accounted for only a small share of SPM dry mass. The importance of OM in the SPM pool was evidenced by the significant correlation between OM and SPM, and the small fraction of live phytoplankton within this organic matter by the lack of such a correlation between Chl *a* and OM.



#### IV.2.5.2 Zooplankton feeding activity: methodological considerations

Despite the small contribution of live phytoplankton to estuarine SPM, several studies indicate a substantial herbivorous feeding mode of estuarine zooplankton (Gasparini et al., 1999; Calbet et al., 2000; Tackx et al., 2003). For this reason, and in view of the questions posed in the PhD work, we have opted for methods focusing on the quantification of zooplankton feeding on phytoplankton.

Numerous techniques exist to quantify grazing activity of pelagic or benthic organisms on phytoplankton. The use of radioactive markers (Haney, 1971, Daro, 1978) and later of stable isotope markers (e.g. Minagawa et Wada, 1984; Wada et al., 1991; Grey and Jones, 1999) and the quantification of pigments by fluorescence microscopy (Gasparini et Castel, 1997) or by quantification in gut content extracts (Mackas et Bohrer, 1976) were developed. Also, fatty acids as a food quality indicators and trophic tracers, can be analyzed in zooplankton (David et al., 2006; Gladyshev et al., 2016). More recently, the detection of trophic DNA has been developing quickly (e.g. Pommier et al., 2010).

The incubation method (Gauld, 1951) consists of creating a (preferably statistically significant) difference in concentration of the potential food items present in the medium between control and experimental microcosms and can in principle be adapted to all potential zooplankton preys which are quantifiable. This method was chosen for this study because of its feasibility in the field and in the laboratory conditions offered by EcoLab, ECOBE and LOG. An advantage of the incubation method is that it allows incubation of natural water samples, and hence the possibility to quantify zooplankton feeding activity and selectivity in quasi- natural circumstances (see further). Also, the incubation method allows to measure zooplankton grazing activity over relatively long periods, which is advantageous because most zooplankters are known have strong feeding rhythms (e.g. Daro, 1985; 1988 Sellner et al., 1994; Calliari et Antezana, 2001 ; Pagano et al., 2006).

A disadvantage of the incubation method is that, to arrive at significant food concentration differences between control and experimental bottles, the abundance of zooplankton has to be increased in comparison to the natural abundance of zooplankton in the field. For example, Lionard et al. (2005), did not find any significant grazing by *E. affinis* or on freshwater Scheldt phytoplankton in incubations using 20-40 *E. affinis* adults in a 1L volume of water during 24 h. In our experiments, 50 adult *E. affinis* were incubated in 900 mL of Scheldt water, whereas, field

abundance of *E. affinis* in the freshwater Scheldt seldom exceeded 7 individuals per liter. This concentrating of zooplankton can lead to a decrease in feeding activity measured, both with incubation time and with abundance of zooplankton organisms used (Roman et Rublee, 1980; Tackx et Polk, 1986, see production of particles).

These methods are also difficult and time consuming, especially when applied to zooplankton grazing experiments in natural water samples, which require working with several replicates because of natural variability of both the phytoplankton communities and the zooplankton feeding activity.

Besides the incubation method, we used the quantification of copepod gut pigment contents (Mackas et Bohrer, 1976). Pigments in copepod gut extracts were originally quantified by fluorimetry (e.g. Mackas et Boher, 1976; Gasparini et Castel, 1999) which allowed to quantify Chl *a* and its degradation pigments, and to calculate grazing on the total phytoplankton stock in the feeding medium. Measuring gut extracted pigments with HPLC, as applied in the Scheldt measurements, allows to quantify grazing activity on both the total phytoplankton community and on specific groups of phytoplankton, by the quantification of marker pigments, such as, for example, fucoxanthin for diatom and alloxanthin for cryptomonads (e.g. Everitt et al., 1990; Descy et al., 1999, Buffan –Dubau & Carman, 2000; Lemaire et al., 2002; Goffart, 2010; Oechsler-Christensen et al., 2011). A major advantage of gut pigment measurement is that the pigments are natural tracers of the feeding activity of the zooplankters in the field. However, the study of the pigment contents in the natural environment involves taking into account their degradation products. Besides abiotic degradation such as photo-oxydation, the pigments are subject to biological pressure that causes the same effects. These pressures are either natural cell senescence, or consumption by bacteria or even grazing by zooplankton. Many studies have shown the occurrence of these degradation pigments or pheopigments mainly for chlorophyll products (Head et al., 1992; Barlow et al., 1995; Cariou-LeGall et al., 1995; Llewelyn et al., 1996). Two groups of pheopigments are observed: chlorophyllide formed under the action of very active chlorophyllase during cell senescence and more particularly in some algal groups (diatoms) and pheophorbide associated with consumption activities by heterotrophic organisms. Carotenoids are degraded by the same processes, but the products are not necessarily identifiable (Descy et al., 1999, Antajan et Gasparini, 2004). Our HPLC analyses the phytoplankton taxa identified in

water of the Scheldt agree with previous reports of HPLC analysis in the freshwater Scheldt (Muyllaert et al., 2000; Lionard et al., 2008).

We chose fucoxanthin pigment as diatom biomarker. The other diatom marker present in the samples was diadinoxanthin. The strong correlation between fucoxanthin and diadinoxanthin in the ingestion rates calculated from the incubation experiments, (p-value = 0.002, Fig. 39) enforces the validity of the selection for diatoms deduced from our results.

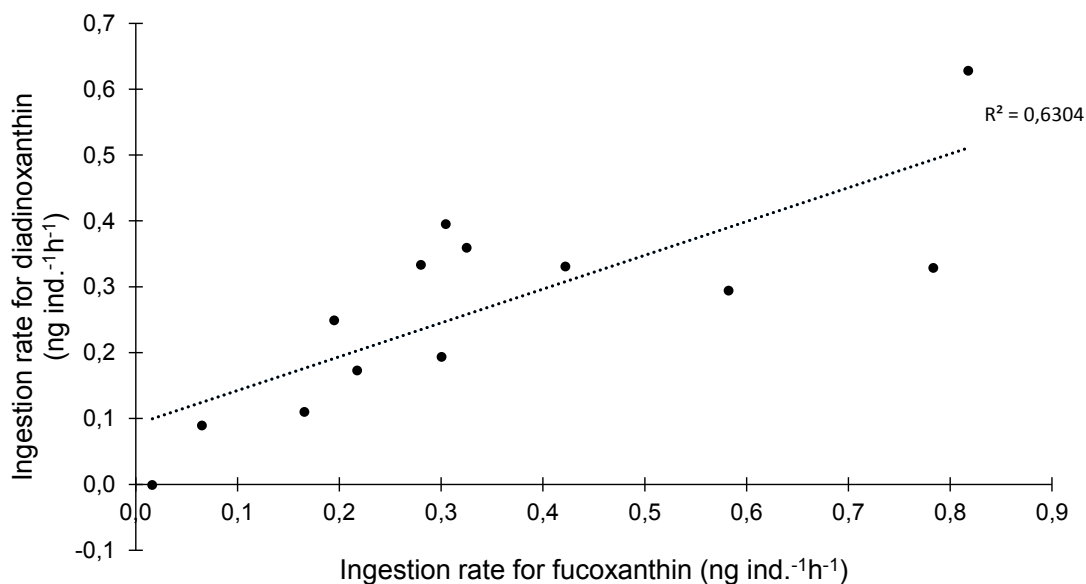


Figure 39 : Ingestion rate for diadinoxanthin as a function ingestion rate for fucoxanthin.

Ingestion rates calculated from marker pigments for chlorophytes and cryptomonads were much less significant and often negative. This aspect will be developed below.

#### IV.2.5.3 Clearance and ingestion rate:

To the best of our knowledge, this study provides the first application of HPLC quantification of phytoplankton biomass in zooplankton grazing experiments. The main advantage is that this allows to detect selective grazing activity on a certain type of algae, without necessarily having a significant concentration difference in Chl *a*-eq. concentration between control and experimental bottles (Leising et al., 2005). In our experiments, 32 % of the pigments quantified showed a significant difference in final concentration between control and experimental bottles. Fucoxanthin, the diatom pigment marker, revealed significant grazing activity in 54 % of the experiments with *E. affinis*. As in our experiments, diatoms were strongly

dominating the phytoplankton community, a significant grazing activity was also detected on Chl *a* concentration in 62 % of the experiments.

A surprising result is the absence from the guts of *E. affinis*. This contrasts with the significant ingestion rates calculated from diatom marker pigments for the incubation experiments. Indeed, according to the method which was used (i.e. incubation or gut content analysis) two different phytoplankton taxa were revealed as selected: diatoms for incubation experiment and cryptophyceae for gut content of *E. affinis*.

Pandolfini et al. (2000), showed that pigment processing during gut passage can be different between zooplankton species/groups (e.g. a copepod vs. a cladoceran) while Quiblier-Lloberas et al. (1994) and Oechsler-Christensen et al. (2011) did not find any significant differences between the zooplankton species tested. In *E. affinis* collected from the Scheldt, alloxanthin, was measured in the gut contents, but little or no other pigments. Similar results were found in two Wisconsin lakes the for copepods *Diaptomus minutus* and in lake of Esch-sur-Sûre for *Eudiaptomus gracilis* (Descy et al., 1999; Pandolfini et al., 2000).

#### IV.2.5.4 Negative clearance rates

For alloxanthin and lutein, negative clearance rates occurred in several incubation experiments. Production of algae in the grazing bottles as compared to the control bottles has been quite frequently reported in previous incubation experiments (e.g. Roman et Rublee, 1980; Tackx et Polk, 1986; Nejstgaard et al., 1997, 2001; Leising et al., 2005).

This phenomenon can, in principle, be caused by two processes:

- 1) Algal growth in the grazing bottles which can be stimulated by zooplankton excretion (Lehman, 1980; Roman et Rublee, 1980; Tackx et Polk, 1986)
- 2) Grazing pressure exerted on algae by microzooplankton is lessened in the grazing bottles because the mesozooplankton incubated feeds (also) on the microzooplankton (Nejstgaard et al., 2001),

#### IV.2.5.4.1 Algal growth in the grazing bottles can be stimulated by zooplankton excretion

For the occurrence of negative clearance rates in our experiments, the first explanation seems unlikely. Firstly, the experiments were run in the dark, which makes phytoplankton production unlikely. Yet, it could be possible that cells having stored enough light energy prior to the experiment, were stimulated in division by a nutrient boost (Vyverman, pers. comm.).

Total N excretion rates reported in literature for copepods the size of *E. affinis* are maximally around  $1 \mu\text{g N ind}^{-1} \text{ day}^{-1}$  (Saba et al., 2011, Alcaraz et al., 2013). Knowing that concentrations of  $\text{NO}_3\text{-N}$ , the most likely form of N to be rapidly taken up by the phytoplankton, in the Scheldt at the time of the experiments were around  $4,8 \mu\text{g ml}^{-1}$ , the N addition by copepod excretion could amount to maximally 1,25 %.

$\text{PO}_4\text{-P}$  excretion rates reported in literature for copepods of the size of *E. affinis* are maximally  $0,003 \mu\text{g ind}^{-1} \text{ day}^{-1}$  (Saiz et Alcaraz, 1992; Alcaraz et al., 2013). In our experiments, 50 *E. affinis* adults were incubated during around 24 h in 900 ml of natural Scheldt water. This means that  $0,15 \mu\text{g -P}$  was added in each bottle through copepod excretion bottle, i.e.  $0,0002 \mu\text{g ml}^{-1}$ . In April 2013,  $\text{PO}_4\text{-P}$  concentration at Antwerp was minimally  $0,1 \mu\text{g ml}^{-1}$ . So the increase in P concentration caused by copepod excretion in our experiments was maximally 0,2%.

Ning et al., 2013, report P excretion rates of a copepod / cladoceran community in Meiliang lake (China) of  $0,20\text{-}0,57 \text{ mg P (g DW)}^{-1} \text{ h}^{-1}$ . The mean DW of adult *E. affinis* (males and females) is  $12 \mu\text{g ind}^{-1}$  (Lambert, 2012). So *E. affinis* P excretion during the 24 h incubation would have been between 58 and  $153 \text{ ng P ind}^{-1} \text{ day}^{-1}$ . The 50 *E. affinis* adults incubated would thus, have added, between 2,9 and  $7,7 \mu\text{g P}$  per bottle. This amounts to  $0,003\text{-}0,009 \mu\text{g P ml}^{-1}$  which corresponds to a P concentration increase between 3-9 %.

Knowing that the  $\text{PO}_4\text{-P}/\text{NO}_3\text{-N}$  ratio on a weight basis in the Upstream Scheldt in April 2013 was maximally 0,04, which is below the Redfield ratio of 0,08 (Redfield, 1934). P is probably the relative limiting nutrient. So, an increase of 9 % could have stimulated phytoplankton production. It should be realized however, that the P-excretion values reported by Ning et al, 2013, are for a mixed copepod/ cladoceran community, and are much higher than the values given by Saiz et Alcaraz (1992) and Alcaraz et al. (2013), which would not lead to a substantial P enrichment. As moreover the incubation took place in the dark, it is difficult to consider nutrient

enrichment, a major factor leading to phytoplankton production in the incubation experiment.

#### IV.2.5.4.2 Grazing pressure exerted on algae by microzooplankton can be lessened in the grazing bottles because the mesozooplankton incubated feeds (also) on the microzooplankton

Microzooplankton feeding on phytoplankton as the cause of negative clearance and ingestion rates occurring in our experiments was also possible. Microzooplankton is known to feed on rather small cells (e.g. Muylaert et al., 2000; 2005) and in our experiments, negative clearance rates occurred mainly for alloxanthin and lutein, marker pigments for cryptophyceae and green algae, which are, generally, smaller than most diatoms.

It should be realized that feeding by the mesozooplankton organism incubated on microzooplankton happens not only in the case that negative clearance rates were observed at the end of the experiments, but can also lead to an underestimation of (positive) clearance rates calculated for the mesozooplankton studied. Reducing the microzooplankton predation pressure on phytoplankton in the grazing bottles versus the control bottles means that the average phytoplankton concentration in the grazing bottles during the experiment is higher than calculated by comparison with the control bottles at the end of the experiment following Frost (1972).

Muylaert et al. (2000), report the existence of substantial populations of heterotrophic nanoflagellates (HNAN) in the freshwater Scheldt estuary. Microscopic analysis showed most flagellates containing pigments to belong to autotrophic or weakly heterotrophic taxa, which led Muylaert et al., 2000 to conclude that most HNAN in the freshwater Scheldt are bacterivorous rather than algivorous. Also ciliates, among which oligotrichs, known to feed on bacteria, HNAN and small algae, were observed in the upstream Scheldt in the Muylaert et al. (2000) study. Lionard et al. (2005), quantified microzooplankton grazing on the spring phytoplankton community in the upper Scheldt estuary by comparing algal pigment concentrations in an incubation experiment using natural Scheldt water filtered through 20  $\mu\text{m}$  (control) and 200 $\mu\text{m}$  (with microzooplankton). They report that the 20-200  $\mu\text{m}$  fraction contained mainly rotifers, and that grazing impact of the microzooplankton could remove between 33 and 84 % of the phytoplankton standing stock day<sup>-1</sup>. Grazing by rotifers did not show a selectivity for any algal groups.

In the 2014 experiments run to quantify microzooplankton grazing activity, microzooplankton community clearance rates measured varied from 0.06 - 19 ml L<sup>-1</sup> h<sup>-1</sup>, which means that, during the 24 h experiments, the microzooplankton grazed between 1.44 and 456 ml L<sup>-1</sup> d<sup>-1</sup> i.e. 0.144 to 45, 60 % of certain phytoplankton taxa. These values are in the same range as those reported by Lionard et al. (2005) (33-84 % d<sup>-1</sup>), but their results consider the microzooplankton grazing pressure on the total phytoplankton community. In our experiments, no significant grazing on Chl *a*, representing the total phytoplankton biomass, was ever detected. As positive microzooplankton grazing was most frequently observed on pheopigments, this suggests that microzooplankton fed mainly on detritus.

For mesozooplankton predation on microzooplankton to have a substantial impact on the estimation of mesozooplankton grazing on phytoplankton, the former has to be substantial. However, in most estuarine feeding experiments, *E. affinis* is shown to be mostly herbivorous. Fatty acid analysis carried out on *E. affinis* females collected from the 0.5-1.8 salinity range in the Scheldt during 2007-2008 suggested a dominant contribution of phytoplankton versus terrestrial or heterotrophic inputs in *E. affinis*' diet (Mialet et al., unpublished). Another fatty acid analysis performed on *E. affinis* sampled in 2005 for the same season and area (Van den Meersche et al., 2009) reflected a slightly more carnivorous but still phytoplankton dominated regime for *E. affinis*. Also in the St Lawrence estuary, fatty acid analysis have shown that both the North Atlantic of *E. affinis* clade, living in the brackish waters and the Atlantic clade, living in the lower salinity range of the estuary feed mainly on phytoplankton (Cabrol et al., 2015; Pommier et al., 2010). This suggests an efficient transfer of diatom fatty acids to *E. affinis* in this estuary. Yet, *E. affinis* is known to be able of carnivorous feeding. In the Gironde estuary, where SPM is even very heavily loaded with inorganic and highly degraded organic material, Gasparini and Castel (1997) observe that *E. affinis* switches to heterotrophic preys at high SPM concentrations, but that nanoplankton remains its bulk food. In the same estuary, David et al. (2006), observe that *E. affinis* herbivorous feeding does not cover its energy requirements and suggest it supplements these with protozoa.

IV.2.5.5 Comparison with other studies

In our experiments, the ingestion rates obtained in terms of Chl *a* ranged from 0,05-1,84 ng ind.<sup>-1</sup>h<sup>-1</sup>, with an average of 0,64 ± 0,50 ng ind.<sup>-1</sup>h<sup>-1</sup>. Taking into account a GCR of 1,99 h<sup>-1</sup> (see material and methods), this amounts to a gut content of 0,32 ± 0,25 ng ind.<sup>-1</sup>. Gut pigment content values for pheophorbide were between 0,15 and 0,86, with a mean value of 0,41 ± 0,25 ng ind.<sup>-1</sup>. So both methods applied yielded similar ingestion rates for Chl *a*. Oechsler-Christensen et al. (2011), compared ingestion rates for different pigments from gut pigment contents of 3 marine copepods with results from incubation experiments using microscopic cell counts to quantify grazing selectivity, and also found that the two methods gave similar results. Mialet et al. (2010), measured Chl *a*-eq pigment contents of 0,67 ± 0,57 ng ind.<sup>-1</sup> for *E. affinis* adults in the brackish –freshwater fringe of the Scheldt. Gasparini et al. (1999) and Tackx et al. (2003) observed *E. affinis* Chl *a* –eq gut contents of 1,03 ± 0,38 ng ind.<sup>-1</sup> for *E. affinis* females in the brackish reach of the Scheldt. So the values obtained in this study are comparable to those of Mialet et al. (2010).

Because of little available experimental data on estuarine zooplankton feeding, we have compared the Chl *a*-eq. gut contents measured in our study with those of copepods of similar size in various environments in order to situate our results.

Table 7 : Comparison of gut pigment content between different copepod taxa.

Copepod species	Size (µm)	Individual dry weight (µg ind. <sup>-1</sup> )	Gut content (ng Chl <i>a</i> ind. <sup>-1</sup> )	Environment	Source
<i>Eurytemora affinis</i>	800 - 1000	09 -12	0,15-0,86	Freshwater estuary	This study
<i>Pseudodiaptomus hessei</i>		8.4-16.39	0,16-1,63	Sundays River estuary	Jerling & Woodlridge 1991 ; Kibirige & Perissinotto 2003
<i>Acartia natalensis</i>		1.65	0,12-0,45	Sundays River estuary	Kibirige & Perissinotto 2003 ; Woodlridge & Bailey, 2015
<i>Pseudocalanus sp</i>			0.1 - 0.75	Oresund	Nicolajsen et al., 1983
<i>Centropages hamatus</i>			0.1 - 0.8	Oresund	Nicolajsen et al., 1983
<i>Calanus helgolandicus</i>	3800-3900	265,04	0.09-0.28	Celtic sea	Williams & Robins, 1982
	1600 - 2000	23.2 - 50,8 (female)	0.3-1.9 (female)	New York shelf waters / New york Bight	Dagg et Grill, 1980Smith & Lane, 1987
<i>Centropages typicus</i>					
	890-1120	23.2-41.8 (female)	0.3-1.9 (female)	New York shelf waters / New york Bight	Dagg et Grill, 1980Smith & Lane, 1987
<i>Temora longicornis</i>	990 - 1300		0.1-1.4	Marine plankton	Wang, 1986 ; Dam et Peterson, 1991
	91 µm	7.97 (female)	0.06-0.19	Marine plankton / Narragansett Bay, Rhode Island	Durbin & Durbin, 1981 ; Kiorboet Tiselius, 1987
<i>Acartia tonsa</i>					
	740 µm	6.03 (male)		Narragansett Bay Rhode Island	Durbin & Durbin, 1981

As can be seen form table 7, our results on gut pigment contents for *E. affinis* fall within the range reported for other copepod species. In our study, measurements of *E. affinis* grazing activity on total phytoplankton by both methods gives statistically the same results. However, both these measurements are considerably lower than the *E. affinis* gut pigment content values measured in the Scheldt brackish water zone by Gasparini et al. (1999) and Tackx et al. (2005). This could be due to the fact that the measurements in brackish water only considered female *E. affinis*, which are known



to feed at a higher rate than males and also, that Chl *a* –eq pigment gut contents by Gasparini et al. (1999), also used in Tackx et al. (2005), were in part made by a fluorimeter, which probably overestimates in comparison to HPLC.

#### IV.2.5.6 Impact of *E. affinis* grazing on phytoplankton

Our results show that the impact of *E. affinis* grazing on the total spring phytoplankton population was very low. The low grazing impact of *E. affinis* on the phytoplankton could also explain why Lionard et al. (2005), using relatively low abundance of animals in their incubation experiments did not measure a significant impact of adult and CV copepods (*E. affinis* and cyclopoids) on the Scheldt phytoplankton.

To verify the realism of our results, we converted the  $\mu\text{g Chl } a$  – ingestion rates to carbon ingestion rates, using a Chl *a* –C conversion of min 10 and max 30 (Lionard et al., 2008; PAE- OMES unpublished results) and for zooplankton, and a DW-C conversion of 45 %. A mean ingestion rate of  $0,6 \text{ ng Chl } a \text{ ind}^{-1} \text{ h}^{-1}$  for *E. affinis* adults and CV, with a mean DW of  $12 \mu\text{g ind}^{-1}$ ; corresponds to  $0.14\text{-}0.42 \mu\text{g C} / 5,4 \mu\text{g C d}^{-1}$ ; i.e. a daily ration (DR) of 2.5-7.8 %.

These values fall within the range of Daily Ration values found in the literature for copepods (e.g. Calbet et al., 2000).

The extrapolation of the DR calculated for adults and V stages to copepodite and nauplii might represent an underestimation of the population impact, as younger stages, which except for N1-2, usually feed at a higher rate than adults.

As the phytoplankton is strongly dominated by diatoms, grazing pressure on these is also low. So it is unlikely that *E. affinis* will overexploit the diatom stock, nor that the development of *E. affinis* is limited by phytoplankton food availability. While cryptomonads appeared ingested in some of the gut pigment contents showing alloxanthin, this pigment was produced in the experimental bottles as compared to the controls in several incubation experiments. The impact calculated from the gut content derived alloxanthin ingestion rates is  $<1\% \text{ d}^{-1}$ , which renders it unlikely that strong grazing pressure is exerted by *E. affinis* on cryptophytes. Lutein, a marker for chlorophytes, was never found in the gut, and was often produced in the experimental incubation bottles. As we have shown above, nutrient enrichment is unlikely as a

cause of the stimulated production of some phytoplankton taxa in the experimental bottles.

So, even the fact that phytoplankton ingestion measures in this study seems to satisfy energetic requirements of *E. affinis*, and that both the methods used give comparable results, which are confirmed by the literature comparison, it seems worthwhile to verify if any *E. affinis* feeding activity on microzooplankton occurs in Scheldt water in future incubation experiments.

The results of the microzooplankton experiment also suggest the need for follow up, as the measure clearance rates on the phytoplankton community are sometimes substantial (up to 19 ml L<sup>-1</sup>h<sup>-1</sup>). The problem is that the results of these experiments were very variable: the pigments on which a significant effect by microzooplankton feeding was measured, were variable from one experiment to the other, and in one experiment, no effect was detected at all. In this work, we only conducted 4 microzooplankton experiments, so clearly, more are needed to evaluate the trophic role of microzooplankton in the Scheldt.

While this method allowed to distinguish taxonomic groups selected by *E. affinis*, or the microzooplankton community, it is not sufficiently precise to detect if the change occurring in the phytoplankton community (an increase in small diatom taxa (*Cyclotella* spp.) and a decrease in the big diatom species (*Actinocyclus normanii*) is caused by *E. affinis* selective grazing. Several studies have shown that HPLC analysis could be associated with optical microscopy to identify the dominant species (Havskum et al., 2004; Lampert, 2001). A comparative study by Roy et al. (1996) showed that data provided by microscopy allow to go beyond the HPLC in the specific determination except for very small cells.

During our experimentation, 100 ml samples of all bottles were fixed with formalin and kept for microscopic analysis. This work is presently being carried out as part of Master thesis, as it was too time consuming to be realized within the time limit of this thesis.

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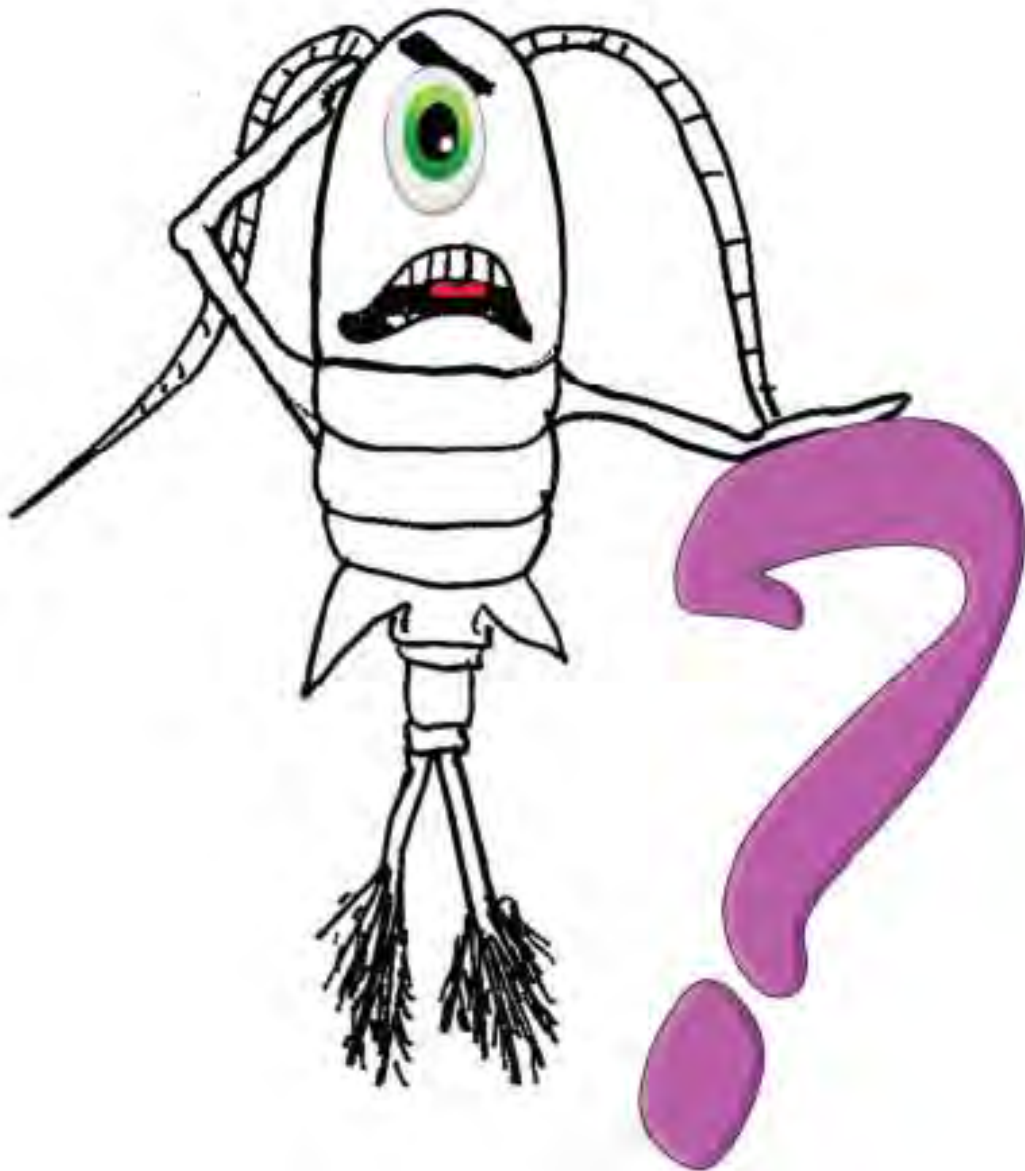
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V.

## CHAPITRE 5:

Synthèse, discussion générale et  
perspectives

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## V.1 Synthèse

Les communautés zooplanctoniques de l'estuaire de l'Escaut en eau saumâtre ont fait l'objet de plus d'attention que celles de la zone d'eau douce (De Pauw, 1973 ; De Pauw, 1975 ; Soetaert et Van Rijswijk, 1993 ; Tackx et al., 1995 ; Irigoien et al., 2000 ; Tackx et al., 2004 ; Tackx et al., 2005 ; Azémar et al., 2007, Mialet et al., 2010). Les travaux de thèse d'Azémar (2007) et de Mialet (2010) soulignent l'importance de la zone d'eau douce, qui héberge des communautés zooplanctoniques plus abondantes et plus diversifiées que la zone d'eau saumâtre.

La restauration de l'Escaut a conduit à des modifications de la distribution spatiale et temporelle du zooplancton. Mialet et al. (2010 ; 2011) rapportent l'installation de la population du copépode calanoïde *Eurytemora affinis*, dominant jusqu'en 2007 en eau saumâtre, dans le tronçon d'eau douce de l'Escaut, et une forte diminution des abondances des copépodes cyclopoïdes dans ce même tronçon. Le premier objectif de ces travaux de thèse était de vérifier la stabilité de cette 'nouvelle' communauté zooplanctonique en étudiant la communauté méso-zooplanctonique en relation avec les facteurs environnementaux après ajout de données complémentaires concernat les années 2010-2012. Ce travail a aussi permis de mettre en évidence la structure spatio-temporelle du méso-zooplancton dans la zone d'eau douce. L'abondance des cladocères a révélé une stabilisation avec un pic d'abondance en 2006 à Dendermonde et Uitbergen. Les copépodes calanoïdes, dominés par *E. affinis*, se sont introduits dans la zone d'eau douce tout d'abord au printemps 2001, pour connaître un accroissement spectaculaire en 2007. Bien que moins marqué à Melle, l'augmentation de l'abondance d'*E. affinis* a été croissante depuis 2007 (cf. chapitre 1). En effet, l'espèce est aujourd'hui plus abondante dans la zone d'eau douce que dans la zone d'eau saumâtre. Parallèlement, les cyclopoïdes ont continué à diminuer en abondance.

Vu sa dominance, nos recherches sur la nutrition du zooplancton de l'Escaut se sont concentré sur *E. affinis*. Les deux méthodes utilisées ; des expériences d'incubation utilisant de l'eau naturelle de l'Escaut et des adultes + CV d'*E. affinis*, et des analyses pigmentaires des contenus stomacaux donnent des taux d'ingestion comparables sur le phytoplancton total ( $0.55 \pm 0.51$  ng ind.<sup>-1</sup> jour<sup>-1</sup>). Le dosage des pigments par HPLC a été appliqué aussi bien à la quantification de la concentration du phytoplancton dans les expériences d'incubation qu'aux analyses des contenus

pigmentaires stomacaux. Ceci a permis, grâce aux pigments biomarqueurs, de détecter la sélectivité de broutage sélectif sur des groupes d'algues, mais les résultats des deux méthodes diffèrent. Les incubations relèvent une sélectivité pour les diatomées, tandis que les contenus stomacaux indiquent un broutage sur les cryptophycées.

L'impact du broutage de la population d'*E. affinis* sur la communauté phytoplanctonique semble peu importants : 4.5% jour<sup>-1</sup>. Des expériences de broutage de la communauté microzooplanctonique ont été entamées, mais les résultats sont à ce jour difficilement exploitable.

## V.2 Discussion générale et perspectives

### V.2.1 Morphométrie et trade-off

Aujourd'hui la biodiversité est une préoccupation partagée notamment entre scientifiques et gestionnaires. Il est donc important de comprendre l'influence des facteurs environnementaux sur la distribution des espèces. Dans le tronçon d'eau saumâtre de l'Escaut, *E. affinis* atteignait son maximum d'abondance au printemps (avril-mai) tandis que les cyclopoïdes, dominant en eau douce, montraient leur pic d'abondance en été. Il y avait donc une ségrégation spatiale et temporelle entre les deux taxons. Nous avons pu constater que le pic d'abondance d'*E. affinis* en eau douce se décale dans le temps et arrive de plus en plus tard (juin). Ce décalage pourrait entraîner une « compétition » entre ces deux taxa. De plus, comme il a été souligné dans cette étude (chapitre I), la concentration en oxygène est un facteur environnemental prépondérant dans la structure des communautés méso-zooplanctoniques de l'Escaut. Afin d'évaluer plus précisément la tolérance d'*E. affinis* à l'hypoxie, des expériences en laboratoire ont été réalisées (chapitre II). Nos résultats ont montré une nette diminution des taux de mortalité chez les calanoïdes avec l'augmentation de la concentration en oxygène, mais pas de relation entre les taux de mortalité des cyclopoïdes et la concentration en oxygène.

Etant donnée la forte relation de la distribution spatiale d'*E. affinis* avec les variables environnementales, son identification aisée, sa position clé dans le réseau trophique estuarien (Fockedey et al., 1999), et sa dominance des communautés de zooplancton dans la plupart des estuaires de l'hémisphère nord (Mialet et al., 2010, Chapitre 1), l'espèce est une bonne candidate au rôle d'indicateur de la qualité

écologique des estuaires (Ben Radhia-Souissi, 2010). Certaines études, menées sur la Gironde, ont montré que les variations morphométriques seraient une réponse à un compromis entre l'énergie dépensée dans l'osmorégulation et celle dépensée dans le développement et la reproduction (Feurtet et al., 1987). Il est alors possible de penser que le repositionnement d'*E. affinis* vers les eaux douces, son optimum écologique dans d'autres estuaires et probablement initial, lui permettent d'optimiser son développement et sa reproduction grâce à un effort d'osmorégulation moindre, autorisant l'allocation d'une plus grande quantité d'énergie vers les compartiments reproductif et de croissance (Cody, 1966). Dans ce contexte, Lambert (2012) a réalisé une étude afin de vérifier si les caractéristiques morphométriques (taille et poids) d'*E. affinis* dénotent des différences d'état physiologique entre les copépodes des différentes zones halines de l'estuaire, dans le but de mieux comprendre les raisons du repositionnement spatial de l'espèce. De plus, cette étude permet de vérifier la pertinence de l'utilisation des caractères morphologiques d'*E. affinis* pour en faire un indicateur écologique. Cette étude a montré à la fois une augmentation de la taille de la population chez les adultes + CV, mais aussi du poids individuel dans l'eau saumâtre entre 2002-2010. *E. affinis* n'étant présent en eau douce que depuis 2007, seulement deux années ont été analysées.

L'étude de traits morphométriques peut permettre de voir si l'amélioration de la qualité de l'eau dans l'estuaire peut être mise en parallèle avec une amélioration de la condition d'*E. affinis*. Afin d'estimer l'évolution physique de la population en eau douce et ainsi essayer d'analyser l'«état de santé» (via les traits morphométriques) de cette population, la continuité des travaux de Lambert (2012), serait à envisager. Les histoires de vie évoluent en réponse à des pressions sélectives de l'environnement, mais sont limitées par la variance génétique et l'histoire phylogénétique (Partridge et Harvey 1988; Ben Radhia-Souissi, 2010). Toutes les caractéristiques du cycle de vie qui déterminent le succès de reproduction ne peuvent être maximisées simultanément ; tout investissement dans un seul trait de l'histoire de vie peut se faire au détriment des autres (Stearns 1989, 1992). C'est pourquoi il serait intéressant de coupler des analyses de traits morphométriques avec des analyses sur les trade-off entre le nombre et la taille des œufs chez ce copépode.

### V.2.2 Tolérance des taxons

Lors de nos expériences de tolérance envers des basses concentrations en O<sub>2</sub>, seul *E. affinis* était identifié à l'espèce, alors que pour les cyclopoïdes le mélange naturel d'espèces a été utilisé. Sachant que les tolérances pour la concentration en oxygène varient selon l'espèce, il serait intéressant de refaire des expériences en identifiant les individus jusqu'à l'espèce. Le changement de la communauté zooplanctonique a été très abrupt : entre 2006 et 2008, *E. affinis* est devenu très dominant et les cyclopoïdes ont quasiment disparu. Ceci a posé des problèmes dans les tests. Afin d'analyser en même temps les tolérances d'*E. affinis* et des cyclopoïdes, il faudrait soit une veille pendant la période printemps-été afin d'obtenir une communauté suffisamment mixte (cyclopoïdes – *E. affinis*), soit maintenir en culture des cyclopoïdes.

### V.2.3 Allelopathie ?

Nous n'avons pas pu identifier le(s) facteurs environnementaux responsable(s) de la diminution des abondances des copépodes cyclopoïdes en parallèle du développement d'*E. affinis* en eau douce de l'Escaut. Les rares études qui discutent l'abondance d'autres taxons zooplanctoniques dans des milieux où *E. affinis* est présent, suggèrent que cette espèce est souvent le taxon dominant. C'est le cas, par exemple, dans le tronçon amont de la rivière Ohio (USA) (Counahan et al., 2005), dans des lacs au nord de la Hongrie (Vad et al., 2012) et dans la partie fluviale de l'estuaire de la Gironde (France) (Dinaud, 2015). Une possibilité non-étudiée est l'existence d'effets allélopathiques. Folt & Goldman (1981) ont en effet démontré que le copépode *Epischura nevadensis* du lac Tahoe (USA) excrète des substances chimiques qui réduisent l'activité de filtration de son compétiteur *Diatomus tyrelli*. Bien que l'allélopathie soit essentiellement considérée comme associée à des plantes terrestres et des macrophytes, elle a également été démontrée chez des coraux, des éponges et des ascidies (Jackson & Buss, 1975 ; Maida et al., 2001 ; Singh & Thakur, 2016). Il est curieux que l'étude de Folt & Goldman (1981), n'ait pas eu de suite par des études sur l'allélopathie dans le monde zooplanctonique, même si le phénomène est bien connu pour des espèces phytoplanctonique (e.g. Van Wichelen et al., 2012 ;

Allen et al., 2014, Leflaive et al., 2014 ; Poulson –Ellenstad et al., 2014 ; Qui et al., 2014).

Les individus d'*E. affinis* dans l'Escaut sont souvent infectés d'épibiontes (Lambert, 2012). Une étude préliminaire a permis d'identifier des ciliés et des champignons comme étant des épibiontes d'*E. affinis* dans l'Escaut. Des études, ont aussi montré la présence d'algues épibiontes sur le zooplankton (e.g. Sirnadel et al., 1997). On pourrait donc se demander si certaines de ces associations ou intégrations de composés allélopathiques dans les tissus et / ou carapaces des copépodes aurait pu se produire et ainsi lui conférer des capacités allélopathiques ?

#### V.2.4 Rôle trophique d'*E. affinis*

Les expériences de broutage ont montré une sélectivité d'*E. affinis* adultes et CV pour les diatomées, mais un faible impact sur le stock de phytoplancton (4,5 % du stock au mois d'avril 2013-2014). Les rations journalières en termes de carbone calculé à partir de nos résultats sont conformes aux valeurs de la littérature pour les copépodes calanoïdes. Mais la fourchette de ces valeurs est très large et ne permet pas de tirer des conclusions claires sur la suffisance de la nutrition herbivore pour ce copépode. Malgré de nombreuses indications révélant qu'*E. affinis* dans l'Escaut est essentiellement herbivore, il nous semble indiqué de vérifier dans quelle mesure cette nourriture pourrait être complétée par d'autres ressources. Notamment considérant le problème des productions de chlorophycées dans les microcosmes expérimentaux, il serait intéressant de vérifier si *E. affinis* n'exerce pas également une activité carnivore dans les eaux douces de l'Escaut.

#### V.2.5 Comparaison inter-estuariers :

Le complexe d'espèces *E. affinis* est présent dans la plupart des estuaires tempérés Européens et Nord-Américains. Winkler et al. (2011) ont montré que les *E. affinis* présents dans l'Escaut et la Seine appartiennent au même clade. *E. affinis* a montré une réactivité à l'amélioration de la qualité de l'eau dans l'Escaut (Appeltans et al., 2003 ; Mialet et al., 2010; 2011). De plus, l'espèce *E. affinis* figure parmi les quelques modèles biologiques mis en avant dans le cadre de plusieurs programmes de



recherche menés dans l'estuaire de la Seine (e.g. Mouny, 1998 ; Souissi et al., 2007 ; Devreker et al., 2009 ; Souissi et al., 2010).

Les estuaires de L'Escaut et de la Seine sont comparables sur le plan climatique, hydrodynamique et concernant leurs problématiques multi-usages (activités portuaires importantes, aménagements) (Dauvin & Destroy, 2006 ; GIP Seine-aval, 2010). Pourtant, des études récentes menées dans le cadre du projet 'ZooSeine' ont montré que l'abondance et la diversité du zooplancton (copépodes, cladocères, rotifères) sont plus élevées dans l'Escaut que dans la Seine, surtout sur le tronçon d'eau douce (Azémar et al., 2011). Les quantifications des abondances du zooplancton dans la Seine ont été réalisées sur la base d'échantillonnages à plusieurs profondeurs (Devreker et al., 2011, Schmitt et al., 2011), tandis que dans l'Escaut, les échantillonnages pour le zooplancton sont faits en surface. Ce facteur devra être pris en compte lors des comparaisons inter-estuaires, car les abondances peuvent être fortement affectées par la profondeur (Mouny & Dauvin, 1996 ; Devreker et al., 2008 ; Schmitt et al., 2011).

L'abondance et la diversité du zooplancton sont d'une part déterminées par le potentiel de production offert par le milieu : la disponibilité de ressources alimentaires (MES assimilable, phytoplancton, proies animales) et les conditions physico-chimiques du milieu (température, hydrodynamisme, qualité de l'eau). D'autre part, la production de zooplancton réalisée entrera de façon plus ou moins importante dans le réseau trophique, par la prédation exercée par l'hyperbenthos et les poissons, ou par la décomposition des organismes morts par d'autres causes (qualité de l'eau insuffisante, par exemple). Le stock observé in situ (= abondance numérique ou exprimé en biomasse) est le résultant de ces flux.

Une comparaison inter-estuaires des stocks zooplanctoniques observés permettrait de tester certaines hypothèses qui se situent dans le contexte du contrôle des stocks/productions par le 'bottom-up/top down' (McQueen et al., 1989). Une étude préliminaire de la concentration et composition de la matière en suspension (MES) montre que la matière organique dans l'Escaut contient plus de phytoplancton vivant que celle de la Seine. Au vu des connaissances sur la sélectivité des copépodes estuariens envers le phytoplancton (Gasparini et al., 1999 ; Tackx et al., 2003; Azémar, 2007 ; Chapitre III), cette comparaison permettrait de tester l'hypothèse

selon laquelle la composition de la matière en suspension est un facteur expliquant le plus grand 'succès' du zooplancton dans l'Escaut que dans la Seine.

Cette hypothèse pourrait être testée par des mesures de production du zooplancton dans le milieu naturel (en excluant les prédateurs), en comparant ce 'potentiel de production' dans la Seine et dans l'Escaut. En partant du cas le plus probable que la production est plus faible dans la Seine que dans l'Escaut, il s'agira essentiellement de détecter si l'ajout de phytoplancton augmente la production du zooplancton dans la Seine. Dans ce cas, la disponibilité de phytoplancton est le facteur limitant la production du zooplancton dans la Seine. Dans le cas contraire, la qualité de l'eau pourrait être responsable d'une faible production du zooplancton dans la Seine.

#### V.2.6 Le méso-zooplancton dans les estuaires mais aussi dans d'autres milieux :

Le zooplancton peut aussi être observé dans d'autres milieux que les zones pélagiques des estuaires, des océans et des mers. Dans certains milieux, comme dans les lacs, le zooplancton peut être abondant ou encore se 'cacher' auprès des végétations macrophytes et/ou litières. Cela est traditionnellement considéré comme une action de refuge contre la prédation, mais la possibilité d'un aspect trophique n'a presque pas été considérée.

Jusqu'à présent les études sur le compartiment mesofaunistique se sont essentiellement concentrées sur l'étude des assemblages mésoplanctoniques des zones pélagiques que ce soit en milieu lacustre, estuarien ou marin. Ces études se sont focalisées sur la distribution et la structuration des assemblages et le rôle trophique du mésozooplancton. Le couplage des habitats benthiques et littoraux ainsi que le rôle fonctionnel des communautés mésobenthiques ont été largement sous-explorés. Pourtant, l'étude du fonctionnement des communautés benthiques est particulièrement importante pour comprendre les mécanismes de réponses des communautés pélagiques dans la mesure où ces deux types d'habitat sont interconnectés et structurés par des échanges de matière et d'énergie, primordiaux pour le fonctionnement biogéochimique des écosystèmes. Par exemple, les habitats pélagiques constituent une source de matière organique pour les habitats benthiques via la sédimentation du plancton (Covich et al., 1999) et de proies pour les invertébrés benthiques (e.g. MacIsaac et al., 1992). En interceptant la lumière et les nutriments,

les réseaux trophiques pélagiques ont une incidence sur les processus benthiques (Sand-Jensen and Borum, 1991; Vadeboncoeur et al., 2002). La zone benthique fournit également des proies pour les prédateurs pélagiques comme les poissons (e.g. Stein et al., 1995) et des habitats refuges (e.g. Cáceres, 1997). Elle est le siège de nombreux processus biologiques permettant la remobilisation de nutriments via les processus métaboliques des organismes benthiques et via la reminéralisation de la matière organique (Vanni, 1996). Le rôle de la meiofaune dans la reminéralisation de la matière organique a été suggéré à de nombreuses reprises. De nombreux travaux en milieux marins et en zones pélagiques d'eau douce soulignent le lien trophique entre la mésofaune et les communautés microbiennes associées à la matière organique et les macroinvertébrés détritivores. (e.g. Kemp 1990; Golladay & Hax 1995; Robertson, Lancaster & Hildrew 1995; Freckman et al. 1997; Hakenkamp & Morin 2000; Schmid-Araya & Schmid 2000; Swan & Palmer 2000). Malgré cette évidence et de nombreux appels à développer des études sur le rôle fonctionnel de la mésofaune dans le réseau trophique hétérotrophe, très peu d'études ont été réalisées sur le sujet. Dans le cadre de ma thèse et en collaboration avec les membres du laboratoire qui travaillent sur les réseaux hétérotrophes, j'ai réalisé une expérimentation qui avait pour objectif d'identifier le rôle de la mésofaune dans la dégradation de la matière organique (Annexe 1). Nous supposons que la mésofaune influence la structure des assemblages microbiens associés à la matière organique par son activité de broutage et par conséquent leur contribution aux processus de dégradation de la matière organique. En interaction avec le compartiment macrobenthique, nous supposons que l'introduction de la mésofaune dans le réseau trophique hétérotrophe influe sur des interactions trophiques entre les organismes. Ceci pourrait être lié à une augmentation de l'efficacité des processus de dégradation de la matière organique via des effets additifs ou de complémentarité (ex : facilitation ou partitionnement de la ressource) entre compartiments, ou encore à une diminution des taux de dégradation de la matière organique via des interactions négatives entre compartiments, telles que la compétition ou la prédation. Notre étude confirme que la mésofaune peut jouer un rôle important dans le réseau hétérotrophe aquatique, notamment via son interaction avec les communautés microbiennes et de macroinvertébrés déchetiers.

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# VI. ANNEXES

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## VI.1 Annexe 1

Tableau 8 : Results of each 12 grazing experiments (from I to IX in 2013 and from X to XIII in 2014) with *E. affinis*. Standard deviation for concentration of each pigments (n=3 for 2013; n=6 for 2014) at t0, in control (tcontrol) and treatment (tzoo) microcosms. Standard deviation for ingestion and clearance rates (n=3 for 2013; n=6 for 2014)

Standard deviation t <sub>0</sub> (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
I	0.179	0.005	0.035	0.010	0.013	0.026	0.018	0.036	0.394	0.146	0.096	0.005
II	0.087	0.008	0.022	0.003	0.013	0.019	0.021	0.009	0.380	0.092	0.052	0.011
III	0.400	0.001	0.081	0.015	0.027	0.012	0.073	0.045	1.063	0.119	0.277	0.007
IV	0.065	0.011	0.012	0.021	0.012	0.021	0.028	0.027	0.260	0.061	0.262	0.006
V	0.024	0.004	0.001	0.010	0.001	0.004	0.010	0.007	0.101	0.034	0.042	0.011
VI	0.098	0.009	0.013	0.001	0.031	0.027	0.028	0.019	0.398	0.034	0.326	0.008
VII	0.098	0.011	0.018	0.003	0.016	0.017	0.007	0.006	0.091	0.042	0.046	0.110
VIII	0.108	0.007	0.024	0.003	0.007	0.011	0.004	0.011	0.088	0.039	0.083	0.028
IX	0.069	0.016	0.016	0.007	0.022	0.002	0.010	0.006	0.118	0.035	0.013	0.013
X	0.093	0.005	0.018	0.011	0.022	0.012	0.018	0.007	0.150	0.128	0.118	0.006
XI	0.041	0.002	0.010	0.004	0.008	0.009	0.018	0.010	0.127	0.032	0.076	0.007
XII	0.149	0.008	0.019	0.012	0.050	0.019	0.055	0.016	0.244	0.052	0.057	0.020
XIII	0.263	0.007	0.039	0.026	0.055	0.026	0.029	0.012	0.387	0.127	0.008	0.023

Standard deviation control (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
I	0.078	0.012	0.023	0.008	0.015	0.016	0.018	0.025	0.317	0.053	0.314	0.002
II	0.066	0.003	0.017	0.001	0.003	0.008	0.005	0.012	0.128	0.018	0.060	0.001
III	0.083	0.001	0.013	0.010	0.020	0.004	0.026	0.033	0.204	0.017	0.024	0.000
IV	0.045	0.007	0.004	0.010	0.002	0.010	0.013	0.022	0.143	0.030	0.256	0.006
V	0.017	0.004	0.012	0.010	0.004	0.014	0.005	0.002	0.164	0.036	0.303	0.005
VI	1.034	0.006	0.004	0.008	0.020	0.017	0.012	0.016	0.122	0.131	0.111	0.011
VII	0.048	0.002	0.033	0.004	0.015	0.008	0.011	0.011	0.138	0.014	0.174	0.004
VIII	0.037	0.002	0.008	0.008	0.029	0.003	0.019	0.028	0.148	0.026	0.068	0.022
IX	0.073	0.014	0.013	0.013	0.005	0.013	0.023	0.005	0.122	0.059	0.005	0.005
X	0.070	0.003	0.009	0.009	0.013	0.004	0.009	0.003	0.097	0.028	0.016	0.004
XI	0.106	0.002	0.026	0.012	0.015	0.017	0.017	0.012	0.220	0.050	0.034	0.005
XII	0.198	0.008	0.035	0.020	0.028	0.024	0.044	0.023	0.428	0.059	0.056	0.017
XIII	0.203	0.008	0.020	0.020	0.033	0.015	0.033	0.014	0.366	0.112	0.061	0.011

Standard deviation tzoo (µg L <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
I	0.026	0.096	0.019	0.020	0.032	0.019	0.035	0.032	0.410	0.065	0.478	0.044
II	0.093	0.007	0.010	0.006	0.019	0.019	0.030	0.011	0.249	0.079	0.378	0.016
III	0.086	0.001	0.010	0.009	0.021	0.003	0.009	0.027	0.053	0.174	0.095	0.003
IV	0.090	0.005	0.005	0.003	0.012	0.015	0.013	0.002	0.082	0.042	0.155	0.010
V	0.068	0.007	0.012	0.002	0.030	0.007	0.009	0.013	0.137	0.013	0.024	0.010
VI	0.043	0.007	0.009	0.004	0.028	0.007	0.040	0.002	0.332	3.941	0.305	0.011
VII	0.036	0.005	0.008	0.014	0.017	0.033	0.011	0.016	0.297	0.018	0.197	0.010
VIII	0.061	0.006	0.018	0.007	0.020	0.004	0.013	0.019	0.124	0.087	0.100	0.018
IX	0.011	0.013	0.013	0.012	0.015	0.013	0.014	0.017	0.017	0.023	0.040	0.016
X	0.113	0.007	0.017	0.020	0.025	0.021	0.012	0.002	0.238	0.064	0.034	0.010
XI	0.062	0.003	0.010	0.006	0.020	0.008	0.021	0.001	0.150	0.079	0.026	0.010
XII	0.099	0.005	0.018	0.010	0.029	0.029	0.026	0.002	0.173	0.066	0.033	0.005
XIII	0.143	0.003	0.025	0.016	0.032	0.015	0.016	0.003	0.079	0.106	0.045	0.018

Standard deviation F (ml ind. <sup>-1</sup> h <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
I	0.059	0.895	0.204	0.316	0.296	0.242	0.414	0.406	0.295	0.263	0.313	0.710
II	0.191	0.211	0.137	0.064	0.176	0.220	0.677	0.347	0.270	0.439	0.215	0.439
III	0.093	0.020	0.045	0.162	0.129	0.022	0.045	0.239	0.022	0.336	0.042	0.061
IV	0.200	0.129	0.067	0.045	0.081	0.159	0.187	0.056	0.066	0.153	0.082	0.233
V	0.122	0.159	0.110	0.035	0.195	0.074	0.140	0.304	0.096	0.063	0.015	0.248
VI	0.051	0.175	0.055	0.056	0.142	0.047	0.410	0.031	0.180	1.798	0.163	0.142
VII	0.078	0.141	0.092	0.208	0.108	0.284	0.264	0.326	0.219	0.079	0.152	0.189
VIII	0.072	0.131	0.103	0.174	0.103	0.032	0.069	0.226	0.070	0.161	0.064	0.258
IX	0.015	0.130	0.130	0.166	0.076	0.092	0.181	0.011	0.050	0.138	0.248	0.100
X	0.117	0.164	0.123	0.123	0.110	0.124	0.095	0.053	0.130	0.128	0.084	0.100
XI	0.097	0.075	0.112	0.061	0.123	0.058	0.135	0.039	0.120	0.144	0.101	0.134
XII	0.093	0.095	0.136	0.062	0.136	0.224	0.115	0.033	0.079	0.076	0.075	0.045
XIII	0.142	0.065	0.180	0.095	0.130	0.088	0.077	0.053	0.039	0.139	0.110	0.171

Standard deviation I (ng ind. <sup>-1</sup> h <sup>-1</sup> )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
I	0.037	0.102	0.022	0.013	0.040	0.023	0.034	0.037	0.496	0.110	0.208	0.050
II	0.144	0.009	0.016	0.005	0.027	0.027	0.047	0.014	0.376	0.155	0.171	0.020
III	0.084	0.001	0.011	0.009	0.024	0.004	0.011	0.020	0.056	0.207	0.100	0.004
IV	0.082	0.005	0.005	0.003	0.012	0.016	0.014	0.002	0.084	0.044	0.155	0.009
V	0.063	0.006	0.012	0.002	0.030	0.007	0.008	0.014	0.139	0.014	0.025	0.010
VI	0.033	0.007	0.009	0.004	0.033	0.008	0.050	0.001	0.380	4.417	0.340	0.014
VII	0.033	0.004	0.008	0.015	0.015	0.033	0.008	0.014	0.256	0.016	0.177	0.016
VIII	0.056	0.006	0.019	0.007	0.017	0.004	0.019	0.010	0.157	0.079	0.098	0.016
IX	0.010	0.013	0.013	0.011	0.014	0.014	0.012	0.017	0.024	0.024	0.028	0.016
X	0.114	0.007	0.019	0.020	0.026	0.021	0.016	0.002	0.238	0.085	0.041	0.010
XI	0.059	0.003	0.011	0.006	0.019	0.008	0.022	0.001	0.141	0.073	0.025	0.009
XII	0.092	0.004	0.016	0.010	0.027	0.030	0.029	0.001	0.156	0.066	0.030	0.006
XIII	0.118	0.003	0.019	0.016	0.029	0.016	0.014	0.003	0.069	0.090	0.042	0.018

## VI.2 Annexe 2

Tableau 9 : Results of each 4 grazing experiments with microzooplankton. Standard deviation for concentration of each pigments (n=6) at  $t_0$ , in control (tcontrol) and treatment (tzo) microcosms. Standard deviation for ingestion and clearance rates (n=6)

Standard deviation $t_0$ ( $\mu\text{g L}^{-1}$ )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	0.086	0.004	0.014	0.007	0.026	0.005	0.026	0.006	0.272	0.090	0.070	0.014
XI	0.125	0.003	0.021	0.013	0.014	0.013	0.019	0.010	0.179	0.052	0.018	0.004
XII	0.066	0.006	0.010	0.013	0.018	0.009	0.020	0.000	0.166	0.053	0.003	0.012
XIII	0.305	0.002	0.047	0.037	0.056	0.027	0.050	0.037	0.506	0.103	0.126	0.023

Standard deviation $t_{\text{control}}$ ( $\mu\text{g L}^{-1}$ )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	0.031	0.003	0.006	0.006	0.012	0.010	0.012	0.006	0.084	0.029	0.045	0.009
XI	0.072	0.003	0.008	0.004	0.006	0.007	0.011	0.006	0.167	0.045	0.014	0.006
XII	0.072	0.010	0.015	0.008	0.011	0.021	0.038	0.020	0.150	0.022	0.036	0.007
XIII	0.211	0.006	0.027	0.027	0.032	0.033	0.024	0.014	0.336	0.070	0.039	0.012

Standard deviation $t_{\text{zo}}$ ( $\mu\text{g L}^{-1}$ )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	0.071	0.003	0.010	0.008	0.014	0.005	0.009	0.003	0.098	0.029	0.015	0.006
XI	0.106	0.002	0.027	0.012	0.015	0.017	0.016	0.007	0.221	0.050	0.008	0.006
XII	0.199	0.008	0.035	0.020	0.029	0.024	0.043	0.006	0.429	0.059	0.065	0.016
XIII	0.203	0.009	0.020	0.020	0.032	0.014	0.033	0.012	0.366	0.113	0.082	0.011

Standard deviation F ( $\text{ml ind.}^{-1}\text{h}^{-1}$ )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	4.205	3.818	4.345	3.448	3.896	2.177	5.953	2.156	3.650	5.966	2.739	3.032
XI	5.512	2.846	14.321	6.157	4.265	11.159	7.099	8.437	6.683	6.192	7.141	4.494
XII	5.933	5.754	6.644	5.913	5.675	8.169	9.679	9.455	6.327	3.959	7.411	8.195
XIII	6.152	9.483	3.742	6.275	5.347	5.709	9.014	7.085	6.052	8.295	7.372	5.458

Standard deviation I ( $\text{ng ind.}^{-1}\text{h}^{-1}$ )												
Experiment	Fucoxanthin	Violaxanthin	Diadinoxanthin	Diathoxanthin	Lutein	Alloxanthin	B-carotene	Chlorophyll c	Chlorophyll a	Pheophytin a	Pheophorbide a	Zeaxanthin
X	3.557	0.136	0.486	0.404	0.729	0.247	0.543	0.167	5.409	2.014	0.842	0.278
XI	4.740	#DIV/0!	1.391	0.675	0.713	0.783	1.133	0.514	10.210	2.787	0.598	0.260
XII	9.307	0.318	1.647	0.960	1.492	1.192	2.385	1.131	19.988	3.089	2.649	0.818
XIII	10.265	0.473	1.044	0.790	1.669	0.788	1.713	0.771	18.692	6.366	3.324	0.544

VI.3 Annexe 3

## **Two freshwater meiofaunal crustaceans affects leaf breakdown by microbial and macroinvertebrate decomposers**

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**Submitted**

## Summary

1. Leaf litter processing is a fundamental ecosystem process in freshwaters performed by a diverse range of decomposers. The meiofauna are an important constituent of aquatic heterotrophic assemblages which can provide a trophic link between plant detritus and associated microbial and macroinvertebrate communities, but their contribution to leaf breakdown remains poorly understood.
2. In this study, we designed experiments to evaluate the potential contribution of freshwater meiofaunal crustaceans (assemblage of one cladoceran and one copepod taxa) to leaf litter breakdown. We considered that interactions with the two main biological compartments involved in this process, microbial decomposers (fungal hyphomycetes) and macroinvertebrate detritivores (a trichopteran and gammarid amphipod) would influence the contribution of meiofaunal assemblages.
3. The contribution of meiofauna was evaluated in laboratory trials with six treatments where fungi were always present (presence/absence of meiofauna crossed with either detritivore presence/absence) and effects determined after 6 and 13 day incubations. We hypothesized that through their feeding activity, meiofauna influences the structure of fungal assemblages and consequently, fungi-mediated leaf litter breakdown. Meiofauna were predicted to change the way energy from organic matter moved through the food web. This was expected to vary according to the trophic relationships with each detritivore, reflecting either positive (e.g. complementarity) or negative (e.g. predation) interactions.
4. The presence of leaf-associated meiofauna enhanced fungi-mediated leaf mass loss by 62% and by trichopteran-driven leaf mass loss by 22% while no significant effect was observed on amphipod-driven leaf mass loss. Moreover, the presence of meiofauna led to strongly increased production of fine particulate organic matter, particularly for treatments with fungi (+637%). The leaf consumption rate by both detritivores tended to decrease in presence of meiofauna. Nonetheless, this effect was only significant at 13 days for the amphipod (-61%), even though a positive effect of meiofauna occurred at 6 days for this detritivore. The lower amphipod consumption rate likely resulted because of a feeding switch from detritus to meiofauna, evidenced by the strong reduced abundances of cladocerans in this treatment.
5. Our study provides evidence that meiofauna can play a significant role in the detrital food web of streams and rivers. Meiofauna contribute to detrital processing by interacting with microbial communities and macroinvertebrates shredders, which can be positive or negative depending on the trophic relationships. Therefore, meiofauna add complexity to detrital food webs by increasing vertical diversity and modulating biotic interactions.

**Key-words:** Meiofauna; functional role; leaf breakdown; trophic interactions; aquatic hyphomycete; *Gammarus pulex*; *Sericostoma personatum*.



## Introduction

Freshwater meiofauna are typically defined as invertebrates passing through a 0.5 mm sieve (Allan and Castillo 2007), and encompass a diverse group including copepods, nematodes, rotifers, ostracods and cladocerans (Bund & Davids 1993). It is now recognized that the meiofauna compartment contributes significantly to the biodiversity of freshwater ecosystems in terms of species richness and abundance (Robertson, Rundle & Schmid-Araya 2000) and potentially plays a wide range of ecological roles in a variety of ecosystem processes (e.g. Freckman *et al.* 1997; Schmid-Araya & Schmid 2000). Freshwater meiofauna are also known to be abundant and diverse in association with leafy and woody debris (e.g. Golladay & Hax 1995; Casas 1997; Swan & Palmer 2000; Robertson & Milner 2001). Consequently, they may contribute to leaf matter breakdown, although their role is poorly understood.

Aquatic ecosystems receive substantial amounts of allochthonous leaf litter coming from the riparian vegetation along the watershed. These inputs of leaf litter and its breakdown, by providing energy source for biota, play pivotal roles in aquatic food webs (e.g. Cummins *et al.* 1989; Graça 1993; Webster, Wallace & Benfield 1995; Wallace & Webster 1996; Suberkropp 1998). Consequently, leaf breakdown has been recognized as a critical process in the functioning of aquatic environments including streams, littoral zones of lakes, and coastal marine environments (Webster & Benfield 1986; Boulton & Boon 1991) and as such, as a putative indicator of ecosystems integrity (Gessner & Chauvet 2002). Leaf breakdown involves organisms ranging from bacteria and fungi to invertebrate detritivores commonly referred to as shredders (Fig. 1A). Bacteria have been rapidly disregarded because the relative importance of bacteria to leaf breakdown may be substantially less than of fungi in terms of standing biomass (Findlay & Arsuffi 1989; Baldy, Gessner & Chauvet 1995). Fungi, mainly aquatic hyphomycetes, contribute to leaf breakdown in two ways: (i) they are responsible for a significant fraction of total leaf breakdown and (ii) fungal colonisation of leaves enhances litter nutritional value and palatability to shredding invertebrates through mycelial biomass and enzymatic metabolization of leaf tissue into more assimilable compounds (e.g. Rounick & Winterbourn 1983; Suberkropp 1998; Bärlocher 2005). Invertebrates have an important effect on the leaf breakdown through direct shredding of leaves and by feeding on fungi, which in turn influences microbial assemblages associated with decaying leaves (Arsuffi & Suberkropp 1989; Suberkropp 1992; Graça 1993).

While numerous studies have documented the role of fungi and shredding invertebrates in leaf breakdown, little information is available regarding the role of meiofauna in the

decomposition process. Due to their abundances, the fauna from this food web compartment may significantly contribute to leaf breakdown. In many ecosystems (e.g. marine, soil and freshwater pelagic systems) meiofauna is already assumed to be a significant component of the heterotrophic assemblage representing a trophic link between detritus, its associated microbial community and larger macroinvertebrates (e.g. Kemp 1990; Golladay & Hax 1995; Robertson, Lancaster & Hildrew 1995; Freckman *et al.* 1997; Hakenkamp & Morin 2000; Schmid-Araya & Schmid 2000; Swan & Palmer 2000). Thus, in their study on the impact of harpacticoid copepod upon detritally associated bacteria, Perlmutter & Meyer (1991) were the first to have designated meiofauna as “microdetritivores” clearly suggesting their role in the detrital dynamics in streams. Previous studies on the role of meiofauna in leaf litter breakdown were focused on temporary meiofauna such as early-instar chironomids and on interactions with microbial communities, notably bacteria associated with decaying leaves. Even if it is not clear if meiofauna feed on detritus itself, such studies reported that lotic meiofauna feed on the associated biofilm (e.g. Fenchel 1970; Hargrave 1972; Meyer-Reil & Faubel 1980). Thus, Palmer *et al.*, (2000) reported an increase in temporary meiofauna abundance (i.e. early instar chironomids, nematodes and oligochaetes) in the leaf packs with highest microbial biomass (i.e. bacteria and fungi). Moreover, it has been shown that meiofauna may discriminate between various groups of bacteria and microfungi (e.g. Dash & Cragg, 1972; Carman & Thistle, 1985). Due to their feeding activity, meiofauna may influence microbial assemblages associated with decaying leaves and consequently the quality and availability of organic matter and the microbial-mediated leaf breakdown. Despite this and previous recommendations for more experimental work on this topic (e.g. Palmer *et al.* 1997), no study has so far examined the effects of meiofauna-fungi interactions on the leaf litter breakdown.

Also, there is still a limited knowledge of meiofauna-macrofauna interactions in freshwater ecosystems. Yet, potential trophic relationships between both biological compartments may have important ecological consequences (e.g. influencing macroinvertebrates-mediated leaf breakdown), but the nature of such relationships remains unclear. Lancaster & Robertson (1995) reported the presence of meiofauna in the diet of several predator invertebrates. Only one study reported some meiofauna taxa within the diet of leaf-associated stoneflies (Feminella & Stewart 1986), suggesting trophic relationships between macroinvertebrate shredders and meiofauna associated with leaves. A potential competition between meiofauna and macroinvertebrates shredders for food resources has been proposed in a few studies,

suggesting that meiofauna and macroinvertebrates operate “in parallel” at the primary consumer-level of the food chain (Van de Bund & Davids 1993).

Traditionally, studies on biodiversity effects on leaf litter breakdown have been focused at within-trophic level (i.e. at the litter, microbial or detritivore invertebrates level). However all individuals within an ecosystem are connected via vertical (i.e. complexity across trophic levels) and horizontal (diversity within a trophic level) linkages (Gessner et al., 2010), for example within the food web. Several studies have attempted to integrate the role of diversity variations among trophic levels (i.e. vertical diversity) in ecosystem functioning (e.g. Duffy et al., 2007; Srivastava et al., 2009; Jabiol et al., 2013). These studies, mainly on trophic cascades, have evidenced that changes in vertical diversity could enhance or reduce diversity effects on leaf litter breakdown particularly in the case of predator effects, by altering patterns of competitive dominance among detritivores species. The inclusion of the meiofauna compartment in heterotrophic food web studies (Fig. 1B) may substantially increase the structuration of interactions already known between the microbial, detritivore and predator compartments, and consequently, our understanding of the complex relationships between diversity and ecosystem functioning. In such food web, meiofauna contribution to leaf breakdown may be additive to contribution of others compartments (i.e. fungi and detritivores invertebrates) or non-additive including facilitation (i.e. meiofauna may enhance the contribution of other compartments), resource partitioning (meiofauna may use different food types than detritivores invertebrates) and antagonistic interactions (e.g. competition, predation).

In this study we aimed to evaluate the potential contribution of assemblages of two meiofaunal crustaceans to leaf litter breakdown. We focused on two taxa of permanent meiofauna, one species of cladoceran (i.e. *Chydorus sphaericus*) and one species of cyclopoids (i.e. *Cyclops bohater*), previously observed in leafy detritus collected from headwater forested streams. Similar colonization of leaf packs by microcrustaceans including copepods and cladocerans has been already reported (Gaudes et al. 2009). *C. sphaericus* is the most common of all Cladocera, often attributed to littoral areas near the bottom sediments (e.g. Evans and Stewart, 1997) and macrophyte-dominated habitats (Basinska, 2014). Therefore, *C. sphaericus* is probably better adapted to detrital food sources compared to large-bodied pelagic cladocerans (Vijverberg and Boersma, 1997). *C. sphaericus* has been described as filter feeder for small particles of algae and detritus and scraper feeder (or sweeper) of detritus and diatoms (Fryer, 1968; Lair, 1990; de Eyto, 2000). *C. bohater* is a

cyclopoid copepod reported living along the plant-covered banks of stagnant and slow-flowing bodies of water and in the clay-pit (Wierzbicka, 1974). Even if, cyclopoid copepods are abundant in aquatic ecosystems, practically nothing is known of their food and feeding mechanisms. Cyclopoid copepods are able to utilise a much broader food spectrum. Some freshwater cyclopoid copepods are reported predator feeding (e.g. *Macrocyclops albidus*, *Mesocyclops leuckarti*) or herbivorous (e.g. *Eucyclops agilis*, *Acanthocyclops bisetosus*) feeding on a variety of algae (e.g. unicellular diatoms, filamentous algae) and protists (Fryer, 1959). They probably switch opportunistically between food sources (crustaceans and rotifers to protists, phytoplankton and bacterial aggregations and detritus) as availability changes within their habitat (Santer et al., 2006).

We designed laboratory experiments on conditioned leaves to test the hypotheses that meiofauna contributes to leaf litter breakdown via interactions with main biological compartments associated with this process i.e. aquatic hyphomycetes and invertebrate detritivores. We predicted that meiofauna would influence fungal assemblages and fungal-mediated leaf breakdown through their feeding activity. Concerning interactions with invertebrate detritivores, we used two shredder species with different feeding strategies i.e. the amphipod *Gammarus pulex* as opportunistic shredder and the trichoptera *Sericostoma personatum* as selective shredder (Colas et al. 2013). We tested several hypotheses regarding the role of meiofauna in the breakdown of leaf litter: 1) meiofauna facilitates the decomposition of leaf litter through positive effects on other food web compartments; 2) predation by *G.pulex* dampens the contribution of meiofauna; and 3) competition for resources by meiofauna causes a decrease in the contribution of macroinvertebrates detritivores to leaf breakdown

## **Materials and methods**

### **Experimental set up**

#### *Biological material collection*

Senescent alder (*Alnus glutinosa* Gaertn.) leaves were collected from trees just before abscission in autumn 2013 and air-dried in the laboratory. Leaf discs 14 mm in diameter were cut in the alder leaves avoiding the major veins. 76 sets of 25 leaf discs were randomly selected, lyophilised (i.e. “freeze-dried”) and weighted to the nearest 0.01 mg. Each set of leaf discs was enclosed into a mesh bag (10 x 10 cm, 500 µm mesh size). Leaf bags were then incubated in a reference forested headwater stream located in the Pyrenees Mountains (south-west France) to allow fungal colonisation. Bags were retrieved after 15 days, returned to the

laboratory and rinsed with stream water to remove fine particulate matter. The remaining leaf material after conditioning stage of 4 sets of 25 leaf discs was preserved at -20°C and later lyophilised and weighed to the nearest 0.01 mg to estimate the initial dried mass introduced in the experimental units. 30 L of water was collected for the laboratory experiments, filtered (Whatman®, 0.45 µm pore size) and kept at 4°C in the dark until use.

The selection of meiofauna taxa has been performed based on previous observations on meiofauna assemblages identified from leaves samples collected from three streams located in the Pyrenees Mountains. Two taxa were chosen according their abundance within leaves samples and their resistance to laboratory conditions: *Chydorus sphaericus* (O.F. Müller, 1976; cladocerans) and *Cyclops bohater* (Kozminski, 1933; copepods) named hereafter *Chydorus* and *Cyclops*, respectively. Organisms of both taxa were sampled using a plankton net (50 µm mesh size) in two ponds near the laboratory. The ponds are situated in a forest, and hence contain high amounts of leaf litter as well as high abundance of meiofauna, which allows collecting more individuals than in streams. Organisms were individually sorted and counted in the laboratory. Ovigerous females were systematically removed to avoid potential nesting. Then, 36 sets of 20 ( $\pm 3$ ) individuals *Cyclops* and 40 ( $\pm 4$ ) *Chydorus* were randomly selected and starved prior to the experiment by putting them in plastic container with 500 ml of filtered stream water at 10°C without food during 24h.

Two species of shredder invertebrates with different feeding habits were used: the amphipod *Gammarus pulex* (L.) and the trichoptera *Sericostoma personatum* (Spence), named hereafter *Gammarus* and *Sericostoma*, respectively. Individuals were sampled in a reference headwater stream located in the Pyrenees Mountains, sorted and counted at the laboratory. Particular attention was taken to select individuals of a same size class (8-9 mm for *Gammarus*; 10-11mm for *Sericostoma*). For *Gammarus*, females were systematically removed. Individuals were then starved prior to the experiment by putting them in plastic container with 500 ml of filtered stream water at 10°C without food for 24 h.

#### *Experimental design*

Contribution of meiofauna to leaf breakdown were evaluated by using feeding assays (Elger and Lemoine, 2005; Colas et al., 2016). Six treatments were used (Table 1) and two incubation times (i.e. 6 and 13 days). For each treatment and incubation time, six replicates were realized using a permuted block randomisation (detailed on block randomisation is available in the Supporting information, Appendix A). For each replicate, one set of 25 leaf

discs was introduced into a plastic container with 300 ml of filtered water. For treatments with shredder invertebrates, one individual of *Gammarus* (i.e. FG and FGM) or *Sericostoma* (i.e. FS and FSM) was randomly assigned to experimental unit. Similarly, for treatments with meiofauna (i.e. FM, FGM and FSM), one set of organisms was randomly assigned to treatments with meiofauna and experimental unit. The 72 experimental units were placed for 6 and 13 days in a thermostatic chamber ( $10 \pm 1^\circ\text{C}$ ) under a daily photoperiod of 10 h light and 14 h dark. At the end of each experiment, for each replicate, ten leaf discs were randomly selected. Five discs were immediately introduced into Erlenmeyer flasks containing 20 mL of filtered water for the sporulation experiment (see section 2.2.4). Five discs were preserved at  $-80^\circ\text{C}$  until ergosterol analysis. The remaining leaf discs were preserved at  $-20^\circ\text{C}$  and later lyophilised prior to weighing. Then, meiofauna organisms were removed individually from leaves and counted in order to determine survival and preserved in 4% formalin. Invertebrates were frozen at  $-20^\circ\text{C}$  and later lyophilised.

### Variables measured

#### *Leaf litter decomposition*

For each replicate, the remaining exposed discs were lyophilised and weighed to the nearest 0.01 mg. The weights of leaf discs used for ergosterol analysis and sporulation experiment were added to the remaining mass of the corresponding set of leaf discs. Leaf litter decomposition was expressed as the percentage of mass remaining after subtracting fungal biomass (as estimated by ergosterol contents, see Section 2.2.5). Additivity between meiofauna and macrofauna was tested for each shredder specie by extracting the contribution of each compartment coming from treatment FG and FS (i.e. the loss of dry mass, expressed in percentage) and by comparing the sum of each contribution to the joint contribution coming from treatments FGM and FSM. To formulate this hypothesis, if there is additivity between compartments it means that:

(1)

$$LDM_{FGM \text{ or } FSM} = IC_{Fungi} + IC_{meiofauna} + IC_{macrofauna}$$

with:

$$IC_{Fungi} = LDM_F$$

$$IC_{mesofauna} = LDM_{FM} - LDM_F$$

$$IC_{macrofauna} = LDM_{FG \text{ or } FS} - LDM_F$$

Where LDM is the loss of dry mass corresponding to each treatment.

#### Consumption rates

At the end of the experiments, invertebrates were weighed to the nearest 0.01 mg and relative consumption rates (RCR), expressed in mg leaf mg invertebrate<sup>-1</sup> day<sup>-1</sup>, were calculating following Maltby (1992) using Eq.(2):

(2)

$$RCR = \frac{[(DM_1 \times C) - DM_2]}{W \times T}$$

where  $DM_1$  is the initial mass of the leaf discs (in mg) exposed to invertebrates,  $DM_2$  the remaining weight of the leaf discs (in mg) at the end of the experiments,  $W$  the dry weight of invertebrates (in mg) and  $T$  the exposure time (in days). For each treatment, the initial weight of leaves was corrected for non-consumptive weight loss by a correction factor (C) according Eq. (3):

(3)

$$C = \frac{\sum(DM_b / DM_a)}{6}$$

where  $DM_a$  and  $DM_b$  are the initial and final dry weights (mg) of control leaf discs used to estimate the mass loss not caused by invertebrate consumption. For treatment FG and FS (i.e. fungi x invertebrate), leaf discs of treatment F (i.e. fungi) were used as control. For treatment FGM and FSM (i.e. fungi x meiofauna x invertebrate), leaf discs of treatment FM (i.e. fungi x meiofauna) were used as control.

#### *Fine particulate organic matter (FPOM) production*

At the end of experiments, water from the experimental units (i.e. 300 ml ) was filtered on a 0.45 µm pore size, 25 mm diameter, nitrate cellulose membrane (Whatman®). The membrane was first washed with pure water, dried at 80°C and weighed to the nearest 0.001 mg. After filtration, the membrane was dried at 80°C for 12 h and weighed to determine the mass of FPOM produced.

### *Fungal assemblages*

Erlenmeyer flasks containing five discs and 20 mL of filtered water were placed on an orbital shaker (100 rpm) for 48h at 10°C to induce fungal sporulation. After incubation, the leaf discs were removed and the conidial suspension was poured into 50-ml centrifuge tube, which was rinsed in the flask with distilled water (3 x 2 ml) to dislodge remaining attached conidia. All rinse water was collected in the tube, and the volume was adjusted to 30 ml with distilled water and 2 ml of 37% formalin. Conidial suspensions were stored in the dark until analysis. The five leaf discs were frozen at -20°C, lyophilised and then weighed to the nearest 0.01 mg. Before conidial identification, Triton X-100 solution (5%) was added to the suspensions, which were then shaken on a magnetic stirrer for 10 min to ensure a uniform distribution of conidia. An aliquot of the suspensions was filtered (membrane filter, 5 µm porosity, 25 mm diameter, Millipore, Bedford, MA, USA), stained with Trypan blue (0.1% in 60% lactic acid), counted and identified under microscope at 200-400x (Bärlocher, 2005 ; Gulis et al. 2005). For each species, the sporulation rate (conidia mg<sup>-1</sup> leaf day<sup>-1</sup>) was determined.

### Fungal biomass

Ergosterol was extracted from leaf discs and quantified as previously described (Gessner and Schmitt, 1996). Briefly, the leaf discs were lyophilised, weighed to the nearest 0.1 mg and heated in 5 ml of alkaline methanol (KOH, 8 g L<sup>-1</sup>) for 30 min at 80°C. The extract was purified by solid-phase extraction on cartridges (Waters Oasis HLB, 60 mg, cm<sup>3</sup>). Ergosterol was separated by reversed phase high performance liquid chromatography on C18 and quantified by measuring absorbance at 282 nm. Ergosterol was converted to fungal biomass using a conversion factor of 5.5 mg ergosterol g<sup>-1</sup> mycelial dry mass (Gessner & Chauvet 1993).

### Data analyses

Multiple comparisons were performed to test the hypotheses stated in the introduction concerning interactions between meiofauna and fungi or invertebrates compartments. Leaf litter breakdown and FPOM production have been compared between treatments F and FM (fungi-meiofauna hypothesis), treatments FG and FGM or treatments FS and FSM (invertebrates-meiofauna hypotheses) using analyses of covariance (ANCOVA). Two-way analyses of variance (ANOVA) have been used to compare fungal biomass, conidia production and relative consumption rates between treatments with and without meiofauna. At the end of experiments, meiofauna abundance has been compared between experimental



units with and without macrofauna using Student's t-test in order to test the hypothesis of invertebrate predation on meiofauna. Additivity between meiofauna and invertebrates has been tested by comparing leaf litter breakdown of treatments (FGM and FSM) and the sum of independent contribution of each compartment (IC) calculated for each replicate coming from treatments F, FM, FG and FS using Student's t-test. For all parametric analyses, normal distribution and homoscedasticity were respected. R software (R Development Core Team 2008) was used for all statistical analyses.

## Results

### Leaf mass loss

The dry mass remaining of alder leaves was significantly lower (Fig. 2;  $F_{3,20} = 16.7$ ,  $p < 0.01$ ) in the without-detrivore treatment with meiofauna (FM) than in the treatment without meiofauna (F), with percentage remaining at 13 d being 66% and 79%, respectively. The remaining dry mass was not different between treatments with (FGM) and without (FG) meiofauna in presence of *Gammarus* (Fig. 2; 76 and 75 %, respectively). Conversely, when *Sericostoma* was present, the dry mass remaining was significantly lower (Fig. 2;  $F_{3,20} = 23.6$ ,  $p < 0.001$ ) in treatment with meiofauna (FSM; 56%) than without (FS; 64%).

### FPOM production

The amount of FPOM released was significantly higher (Fig. 2;  $F_{3,20} = 29.7$ ,  $P < 0.001$ ) in treatments with meiofauna (FM: 0.017 and 0.028 mg at 6 d and 13 d respectively) compared to the treatment with fungi alone (F: 0.0036 and 0.0038 at 6 d and 13 d, respectively). Similarly, when *Gammarus* was present, the amount of FPOM released was significantly higher (Fig. 2;  $F_{3,20} = 3.3$ ,  $P < 0.05$ ) in treatment with meiofauna (FGM; 0.028 mg) than without (FG; 0.017), particularly at 6 d. In contrast, the FPOM production was not different between treatments without (FS: 0.032 and 0.045 mg at 6 d and 13 d, respectively) and with meiofauna (FSM: 0.043 and 0.047 mg at 6 d and 13 d, respectively) for *Sericostoma* (Fig. 2).

### Leaf consumption by detritivores

The relative consumption rates of *Gammarus* showed a non-significant increase in presence of meiofauna at 6 d, but was significantly depressed by 61% under the effect of meiofauna at 13 d (Fig. 3A;  $F_{3,21} = 17.8$ ,  $p < 0.01$ ). The relative consumption rates of *Sericostoma* were not significantly different between treatments even though a slight decrease in presence of meiofauna was observed at both 6 and 13 d (Fig. 3B).

### Fungal community structure, activity and biomass

The fungal richness based on sporulating species varied between 3 and 4. The dominant species was *Alatospora acuminata* Ingold accounting for a total of 92.2 ( $\pm 3$ ) % on average, with *Tetraladium marchalianum* De Wild, *Heliscus ludunensis* Saccardo & Therry and *Tetracladium setigerum* (Grove) Ingold being less abundant. The total sporulation rates were not different between treatments (Fig. 4). Except for *T. marchalianum* (TEMA) for which sporulation rates were significantly lower in treatment with meiofauna than with fungi only ( $F_{3,21}=4.3$ ,  $p < 0.01$ ), sporulation rates of species present did not differ between treatments. In absence of detritivores, mycelial biomass was significantly higher in treatments with meiofauna compared to treatment without at 13 d (Fig. 4;  $F_{3,21}=11.8$ ,  $p < 0.01$ ). The presence of both *Gammarus* and meiofauna induced a significantly lower mycelial biomass (Fig. 4;  $F_{3,21}=18.6$ ,  $p < 0.01$ ) while no difference were found in presence of both *Sericostoma* and meiofauna (Fig. 4).

### Meiofauna survival

At both 6 ( $t(9.84 \text{ df}) = 7.20$ ,  $p < 0.001$ ) and 13 days ( $t(9.81 \text{ df}) = 17.3$ ,  $p < 0.001$ ), the abundance of *Chydorus* was significantly lowered by the presence of *Gammarus* (Fig. 5A). The abundance of *Chydorus* was not significantly affected by the presence of *Sericostoma* at 6 (Fig. 5A;  $t(7.45 \text{ df}) = 1.88$ ,  $p > 0.05$ ) and 13 days (Fig. 5A;  $t(9.9 \text{ df}) = -0.26$ ,  $p > 0.05$ ). The abundance of *Cyclops* did not significantly differ between treatments with and without *Gammarus* at 6 days (Fig.5B;  $t(9.90 \text{ df}) = 2.21$ ,  $p = 0.05$ ) but was significantly different between these two treatments at 13 days (Fig. 5B;  $t(9.9 \text{ df}) = 2.7$ ,  $p = 0.02$ ). Like for *Chydorus*, the abundance of *Cyclops* was not significantly affected by the presence of *Sericostoma* at 6 (Fig.5B;  $t(5.45 \text{ df}) = 0.67$ ,  $p > 0.05$ ) and 13 days (Fig.5B;  $t(6.34 \text{ df}) = 1.50$ ,  $p > 0.05$ ). Table 2 summarizes the main results about comparisons between treatments without or with meiofauna.

### Functional additivity

The sum of individual contributions (IC) of *Gammarus* and meiofauna to litter breakdown did not significantly differ from the breakdown when all decomposers occurred together (JC) at 6 d (Fig. 5A;  $t(7.33 \text{ df}) = 1.15$ ,  $p > 0.05$ ). This also held for *Sericostoma* at 6 days (Fig. 5B;  $t(9.5 \text{ df}) = 0.12$ ,  $p > 0.05$ ) and at 13 days (Fig. 5B;  $t(7.6 \text{ df}) = 0.5$ ,  $p > 0.05$ ), suggesting additivity between meiofauna and *Sericostoma*. Conversely, IC and JC were significantly different for treatments with *Gammarus* at 13 days (Fig. 4A;  $t(5.8 \text{ df}) = 3.15$ ,  $p < 0.05$ ).

Indeed, the sum of individual contributions of *Gammarus* and the other decomposers to litter breakdown (IC) was significantly higher than breakdown when all decomposers occurred together at 13 d suggesting no additivity between meiofauna and *Gammarus*.

## Discussion

Our study is the first to provide experimental evidence of direct and indirect interactions between meiofauna and the two main decomposer compartments, i.e. macroinvertebrates and fungi, and the consequences of such interactions on the leaf litter breakdown. Indeed, the presence of meiofauna enhanced leaf breakdown mediated by leaf-shredding invertebrates and fungi by 22% and 62% respectively, and consequently led to increased food availability (via FPOM) to other organisms (e.g. collector invertebrates). While meiofauna contribute to leaf breakdown, their role varied with the presence of two leaf-shredding invertebrates species due to complex trophic interactions such as resource switching and complementarity.

The reduced leaf mass in treatment with meiofauna compared to treatment with fungi alone can be related to direct consumption of leaves by meiofauna or alternatively meiofauna may facilitate greater fungal biomass by bioturbating the leaf surface or by preferentially consuming bacteria, thereby reducing competition for resources. While there is no clear evidence that meiofauna feed directly on leaf material, some studies reported their feeding preference for small particles of detritus and FPOM-associated microflora (Perlmutter and Meyer, 1991; Sherr & Sherr 1994; Snyder & Hoch 1996; Mikola & Setälä 1998; Ribblett *et al.* 2005; Basinska *et al.* 2014). For instance, in a laboratory experiment on the response of three chydorid species to food, de Eyto and Irvine (2001) evidenced that *C.sphaericus* populations are able to utilise detritus (i.e. FPOM) for growth and reproduction. Some studies emphasized that meiofauna may enhance detritus mineralization presumably due to high rates of bacterial turnover in response to grazing pressure by consumers (e.g. Perlmutter & Meyer, 1991; Sherr & Sherr, 1994; Snyder & Hoch, 1996; Mikola & Setälä, 1998). However, nothing is known about the feeding pressure of meiofauna on leaf-associated fungi while fungi are clearly the predominant microbes on coarse particulate organic material (i.e. leaf litter and woody debris) in streams (Findlay *et al.* 2002). In our study, the feeding effect of meiofauna led to an increased fungal biomass despite no significant change in fungal richness. In addition, sporulation rates of *Tetracladium marchalianum* were significantly depressed by 35% on average in presence of meiofauna, suggesting that meiofauna specifically affected some fungal species, either directly or indirectly. This result raises questions about feeding preferences of meiofauna, while more information on the diet and feeding behaviour of

meiofauna is still needed. Nonetheless, few studies have already reported some species of meiofauna to be able to discriminate among various groups of microfungi (e.g. Dash & Cragg 1972; Carman & Thistle 1985). The selective feeding of leaf-shredding invertebrates on leaf-colonizing fungi has been abundantly documented (e.g. Arsuffi & Suberkropp 1989; Graça, Maltby & Calow 1994; Rong, Sridhar & Bärlocher 1995). That such feeding preferences on fungi also occur in meiofauna species is expectable. This nevertheless deserves to be confirmed by additional laboratory studies as *Tetracladium marchalianum* was overall marginally present on the leaf discs in contrast to *Alatospora acuminata* but whose sporulation rate did not differ in presence of meiofauna. Another important question, not dealt with here, is whether meiofauna choose food items to fulfil specific nutrient requirements (Hakenkamp & Morin 2000). Fungal conditioning and nutrient content enrichment (i.e. P content) of decaying leaves are known to positively influence macroinvertebrate survival and/or growth rates (e.g. Maltby 1999; Graça et al. 2001; Danger et al. 2013). Whilst studies on this topic are still rare, some research has suggested similar mechanisms such as significant effects of biofilm composition and quality of organic matter on copepod reproduction (Brown et al. 2003) and on leaf-associated meiofauna assemblages (e.g. Lenting, Williams & Fraser 1997; Palmer et al. 2000).

The co-occurrence of meiofauna and the opportunistic shredder *Gammarus* (Usseglio-Polatera et al. 2000; Colas et al. 2013) did not induce any significant change in leaf breakdown. Nonetheless, the omnivorous *Gammarus* unsurprisingly switched food resources in the presence of meiofauna, with the resulting predation leading to drastically lower densities of the cladoceran *C. sphaericus*. Several studies already reported the herbivore/predator plasticity of *Gammarus* spp. (e.g. MacNeil, Dick & Elwood 1997; Felten et al. 2008; Colas et al. 2014), probably linked to its feeding apparatus able of coping with a wide variety of foodstuffs (MacNeil et al. 1997). Previous studies have shown predation of *Gammarus* spp on meiofauna, particularly cladoceran species (Hutchinson 1937; Kortelainen 1991). Surprisingly, the reduced leaf consumption by *Gammarus* did not lead to slower leaf breakdown, likely due to compensatory mechanisms that can dampen or even reverse the top-down predator effects predicted by the trophic cascade concept (Gessner et al. 2010; Majdi et al. 2014). For instance, microbial decomposers might process litter more efficiently once released from grazing pressure by leaf-shredding macroinvertebrates (e.g. Mancinelli, Costantini & Rossi 2002). Conversely, in the presence of the caddisfly *Sericostoma*, the meiofauna were able to enhance breakdown rates. Despite increased leaf mass loss, FPOM

production did not change suggesting that the meiofauna may use this resource when a non-predatory shredder is also consuming the same source of detritus (resource partitioning). The complex trophic interactions between meio- and macrofauna as described above may thus have important consequences for the way organic matter is transferred through the food webs. While some authors have proposed that meio- and macrofauna may operate “in series” in a linear food chain (Strayer 1991) or “in parallel” at the primary consumer level of the food chain (Van de Bund & Davids 1993), our study shows that the nature of interactions between meio- and macrofauna and their impact on ecosystem processes are species-dependent. In in-stream conditions, where the species mixture and their interactions are more important than in our experiments, this species dependency probably leads to even more complex trophic webs.

This paper has focused on the role of meiofauna in leaf breakdown. This process plays a pivotal role in food webs of a wide variety of aquatic ecosystems and, as such, has been identified as a putative indicator of ecosystem integrity (e.g. Wallace & Webster 1996; Gessner & Chauvet 2002; Hieber & Gessner 2002). Although involved mechanisms need to be further explored by additional experiments, results of our study provides evidence that meiofauna may contribute to leaf breakdown probably by trophic interactions with leaf-decaying microbial and macroinvertebrates biota. In this present paper, we have chosen to use a controlled laboratory setting because this study was designed to test the hypothesis that meiofauna could alter leaf breakdown mediated by fungi and leaf-shredding invertebrates. Nonetheless, trophic interactions identified in this study may not occur or to less extent in-field conditions. For instance, top-down effects of gammarids on meiofauna may be dampen in field conditions by the presence of refugia habitats for meiofauna and the availability of prey alternatives for gammarids. Thus, further experiments are need under laboratory and in-stream conditions to increase understanding of organic matter processing and trophic interactions between all compartments involved in this process. More specifically, a greater appreciation for the contribution of meiofauna may help to improve methods assessing detrital processing in the field and experimental mesocosms. Currently, the arbitrary mesh sizes typically used in the assessment of leaf breakdown rates based on leaf bags (e.g. Graça et al., 2005) may obfuscate the relative importance of microbes and meiofauna detritivores to rates of leaf breakdown. To cover this gap, a third type of leaf bags with an intermediate mesh size (e.g. from 500 to 1000  $\mu\text{m}$ ) could be proposed. Such an approach should aim to identify meiofauna taxa assemblages and determine the extent of their colonization of leaf surfaces together with their contribution to leaf breakdown rate as already suggested by Gaudes et al.

(2009). In addition, a comparison of the role of meiofauna between various aquatic ecosystems should provide interesting insights with, for instance, an increased contribution of meiofauna being expected in lentic ecosystems such as lakes, pools and downstream reaches of streams and rivers.

### **Conclusion**

Results of this study provide evidence that meiofauna contribute to the detrital process in aquatic ecosystems. Although the mechanisms involved need to be elucidate by further experiments, meiofauna may facilitate fungal-mediated breakdown in addition to their own detrital consumption. This study evidences for complex trophic interactions between meiofauna and leaf-shredding invertebrates (i.e., facilitation, resource partitioning and /or predation) suggesting that the role of meiofauna to leaf breakdown varied according the presence of different shredder species. Therefore, meiofauna can change the way energy from organic matter moved through the food web depending on trophic relationships with the microbial and macrofaunal assemblages associated with decaying leaves. Because the meiofauna compartment increases the food web complexity of heterotrophic assemblages, their consideration is crucial for a comprehensive understanding of organic matter and nutrients dynamics in aquatic ecosystems.

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# Rôle du méso-zooplancton dans un estuaire en voie de restauration : l'Escaut.

**Résumé :** L'estuaire de l'Escaut est un estuaire en voie de restauration. L'étude s'intéresse à l'écologie de la communauté zooplanctonique dans le tronçon d'eau douce de l'Escaut, où, suite à l'amélioration de la qualité de l'eau le copépode calanoïde *Eurytemora affinis* est devenu dominant depuis 2007 et les copépodes cyclopoïdes ont diminué en abondance.

Nous avons cherché à trouver quels facteurs environnementaux expliquent ce changement de la composition de la communauté zooplanctonique. Les résultats d'analyses RDA et GLM entre les abondances des taxons zooplanctoniques et les facteurs environnementaux montrent un lien étroit entre l'augmentation de l'abondance d'*E. affinis* et l'augmentation des concentrations en oxygène ainsi que la diminution des concentrations en N-NH<sub>4</sub>. En fait, le tronçon amont de l'estuaire est devenu 'permissive' pour le développement d'*E. affinis* à partir où la concentration en O<sub>2</sub> a dépassé le seuil de 4 mg L<sup>-1</sup> et la concentration en N-NH<sub>4</sub> est restée en dessous de 2 mg L<sup>-1</sup>. La cause du déclin en abondance des cyclopoïdes reste à trouver.

Dans l'Escaut, le phytoplancton est fortement dominé par les diatomées, mais la concentration en Si dissoute s'avère parfois limitant. La question se pose sur quelles composantes de la communauté phytoplanctonique le zooplancton dominant se nourrit. La sélectivité de broutage d'*E. affinis* a été quantifiée à l'aide d'expériences d'incubation et de quantification de contenu pigmentaire à l'aide d'HPLC. *E. affinis* sélectionne des diatomées au sein de la communauté phytoplanctonique et en moindre mesure des cryptomonades. L'impact de la population d'*E. affinis* sur le stock de phytoplancton – et sur les diatomées dominantes – est < 4.5 % jour<sup>-1</sup>, ce qui implique que dans le tronçon d'eau douce de l'Escaut le zooplancton n'est pas limité par la nourriture et ne présente pas de limitation pour le développement des niveaux trophiques supérieurs. Certains taxons phytoplanctoniques (chlorophycées, par exemple) sont apparemment stimulés en croissance par la présence d'*E. affinis* dans les bouteilles expérimentales et l'impact précis d'*E. affinis* sur le phytoplancton non-diatomées est moins clair.

L'activité de broutage du microzooplancton a également été testée avec des expériences d'incubation. Son impact sur la communauté phytoplanctonique est variable en intensité et en sélectivité, nécessitant plus d'expérimentation.

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

# Role of zooplankton in a restoring estuary: the Scheldt

**Abstract:** The Scheldt is an estuary on way of recovery. The study concerns the ecology of the zooplankton community in the freshwater reach of the estuary. In parallel to water quality improvement, the copepod *Eurytemora affinis* has become dominant since 2007 and abundance of cyclopoid copepods has decreased. We tried to find out which environmental factors had caused these changes in the zooplankton community composition. The results of RDA and GLM analysis between the abundance of zooplankton taxa and the environmental factors showed a strong link between *E. affinis* abundance and the increasing O<sub>2</sub> concentration, but also the decreasing NH<sub>4</sub>-N concentration. The upstream Scheldt became permissive for *E. affinis* development as soon as oxygen concentration was above the threshold level of 4 mg L<sup>-1</sup> and the NH<sub>4</sub>-N concentration remained below 2 mg L<sup>-1</sup>. The cause of the decrease in cyclopoid abundance remains unclear.

The phytoplankton community of freshwater Scheldt is strongly dominated by diatoms, but the dissolved silica concentration could become limiting for their development. The question arises on which phytoplankton taxa the dominant zooplankton feeds.

Grazing selectivity of *E. affinis* adults and CV was measured by incubation experiments using natural Scheldt water and by gut pigment content quantification. Phytoplankton taxa concentration was quantified by HPLC. *E. affinis* selects diatoms and sometimes cryptophytes. The impact of the *E. affinis* population on the phytoplankton standing stock is < 4.5% d<sup>-1</sup>, which means that the zooplankton community is not food limited and hence does not present a limitation to the development of higher trophic levels. The grazing activity of the microzooplankton community has also been measured by incubation experiments. Its impact on the freshwater Scheldt phytoplankton community is variable in intensity and in selectivity, and clearly needs further investigation.

**Key words:** zooplankton, estuary, Scheldt, restoration, *Eurytemora affinis*, gut pigment content, HPLC, trophic selectivity.



