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Maïwen Le Coz

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Université de Toulouse

THÈSE

En vue de l'obtention du

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Délivré par :
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Maïwen Le Coz

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Distribution et rôle trophique du zooplancton dans le bassin versant de l'Escaut

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INTRODUCTION GENERALE

1.1 LES BASSINS VERSANTS

En s'écoulant de la source à l'exutoire, les rivières modèlent le relief, façonnent le paysage, mais ont aussi influencé l'histoire humaine. La dépendance de l'Homme envers les rivières s'est traduite par de fortes densités de population aux abords des cours d'eau et des fleuves, qui représentent pour l'Homme non seulement un accès à une ressource vitale, mais fournissent également une multitude de biens et services considérés comme irremplaçables, associés aux activités récréatives, domestiques, industrielles, agricoles ou encore de transport.

Avec les changements démographiques, économiques et technologiques (en particulier au cours des dernières décennies), l'impact des activités anthropogéniques sur les écosystèmes aquatiques continentaux s'est traduit par une dégradation de la qualité des milieux aquatiques et des biens et services qui y sont associés (Vitousek et al., 1997; Millennium Ecosystem Assessment, 2005; Vörösmarty et al., 2010).

La préservation et l'amélioration de la qualité de ces milieux aquatiques sont donc devenues des enjeux majeurs, et passent nécessairement par la compréhension du fonctionnement écologique de ces systèmes. Puisque la biodiversité des écosystèmes aquatiques est intrinsèquement liée aux conditions environnementales et à la qualité de l'eau, l'étude de la distribution et de la variabilité des organismes qui vivent dans ces milieux sont utiles pour comprendre le fonctionnement (et les dysfonctionnements) de l'écosystème.

1.1.1 Fonctionnement écologique des cours d'eau

Bien qu'ils soient souvent représentés comme des éléments linéaires du paysage, les cours d'eau sont organisés en réseaux hydrographiques (Fig. 1). Ceux-ci sont constitués par un ensemble de « segments » drainant le bassin versant, se rejoignant au niveau de confluences, et aboutissant à un exutoire unique rejoignant la mer. La structure de ces réseaux est qualifiée de dendritique (du grec « dendros » : arbre, ramification), et leur géométrie peut être décrite en utilisant leur structure hiérarchique pour définir des ordres. C'est le principe de la classification topologique de Strahler (1957) : l'ordre 1 est défini pour les tronçons les plus en amont, et augmente vers l'aval au fur et à mesure des confluences (Fig. 1).

Les cours d'eau drainent ainsi le bassin versant de la source à l'exutoire de manière unidirectionnelle, et interagissent fortement avec le milieu terrestre sous l'influence des apports terrigènes et de la ripisylve. Les rivières sont aussi des systèmes dynamiques et ouverts soumis

à de multiples contraintes d'origine extérieure comme le contexte climatique, hydrologique et géologique. Par ailleurs, l'influence de la marée et le gradient de salinité dans les tronçons estuariens des cours d'eau constituent une source supplémentaire d'hétérogénéité spatiale et temporelle de ces milieux aquatiques continentaux.

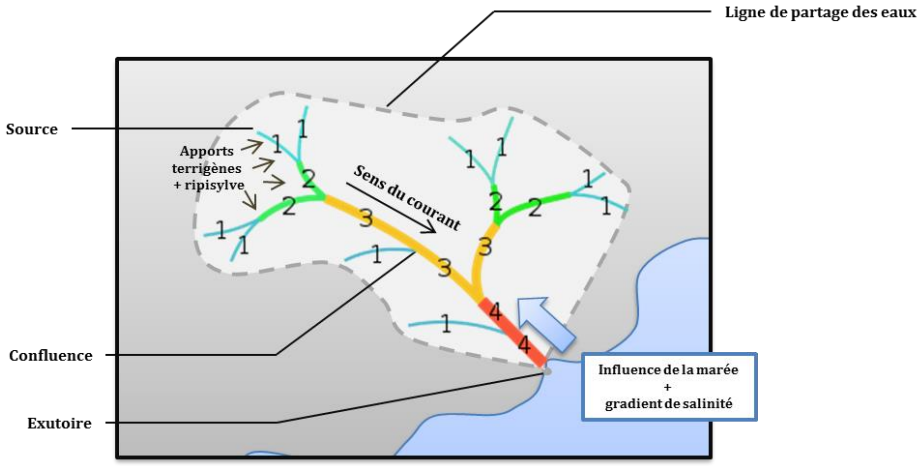

Figure 1. Représentation schématique d'un réseau hydrographique. Les chiffres indiquent les ordres selon la classification de Strahler (1957).

Le fonctionnement d'un écosystème est basé sur des flux et stocks de matière et d'énergie qui transitent dans le système, et sur les interactions entre les compartiments biotiques (les organismes vivant dans le système) et abiotiques. Ces flux de matière et d'énergie sont en grande partie réalisés à travers les interactions trophiques, qui sont fortement influencées par les conditions environnementales et par les différents maillons que constituent les organismes. Elles forment un réseau très complexe et dynamique dans l'espace et le temps (Woodward et al., 2005).

Dans le système pélagique, les producteurs primaires autotrophes (en particulier le phytoplancton) élaborent grâce à la réaction de photosynthèse des substances organiques carbonées, autrement dit de la Matière Organique, et de l'oxygène à partir d'énergie lumineuse, de dioxyde de Carbone et d'eau (Fig. 2, Production). La croissance de ces organismes requiert également des nutriments, comme l'azote et le phosphore, qui vont être utilisés pour la production de protéines et d'acides nucléiques, ou la Silice, utilisée pour la fabrication de squelette siliceux chez certaines espèces (diatomées).

La Matière Organique ainsi formée est utilisée par les consommateurs primaires (dont le zooplancton), organismes hétérotrophes (pratiquant la respiration) qui vont transférer cette matière et donc cette énergie aux niveaux trophiques supérieurs. Environ 10 à 15 % de la production primaire n'est pas consommée directement par le zooplancton, mais transite par la

voie de la boucle microbienne impliquant les bactéries, cillés et flagellés hétérotrophes (Mostajir et al., 2012). Les bactéries hétérotrophes consomment également la matière organique dissoute d'origine détritique ou allochtone et participent ainsi au recyclage des nutriments et de la matière organique.

Le rapport plus ou moins équilibré entre la Production par les organismes autotrophes et la Respiration par les organismes hétérotrophes détermine le fonctionnement trophique de l'Écosystème (rapport P/R) et influence de nombreux processus (Odum, 1971).

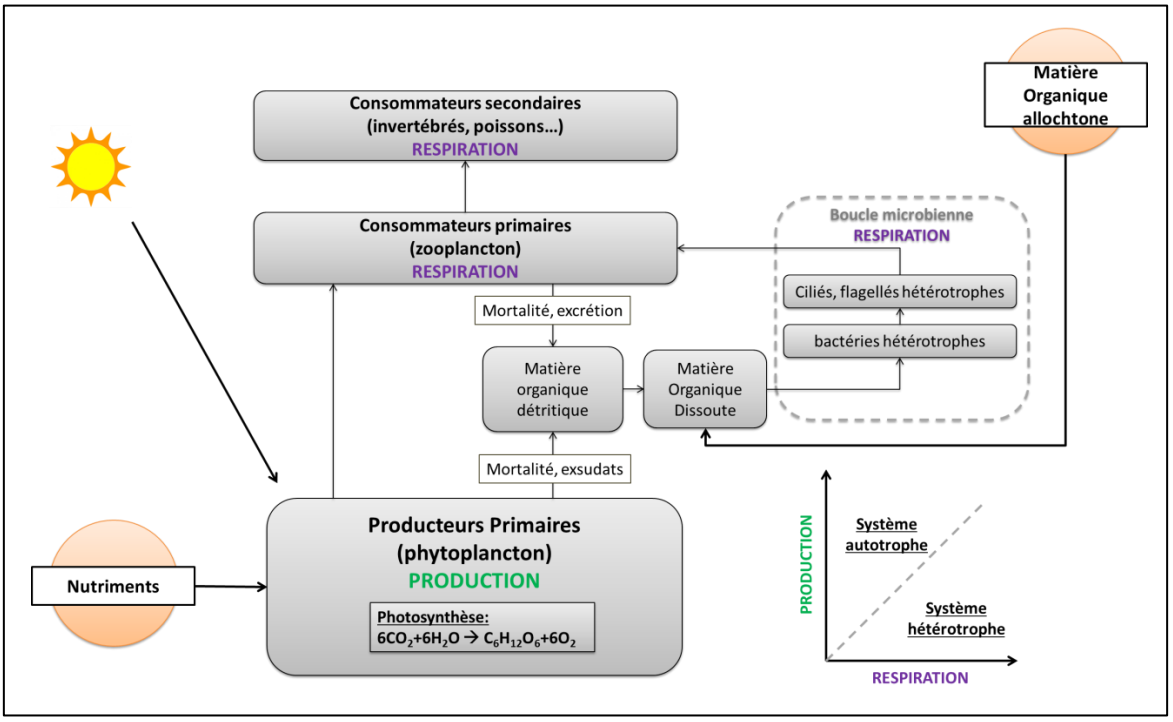

Figure 2. Schématisation simplifiée des réseaux trophiques pélagiques (modifié d'après Amblard et al., 1998).

1.1.2 Concepts décrivant le fonctionnement écologique des cours d'eau

Introduit en 1980, le concept de continuum fluvial (RCC - Vannote et al., 1980) décrit le fonctionnement écologique des cours d'eau de manière longitudinale, en considérant le système de la source à l'embouchure (Fig. 3). Il y est décrit une évolution graduelle des conditions physico-chimiques, et des conditions trophiques (quantité et qualité de la ressource) auxquelles les communautés s'adaptent.

Le concept décrit un fonctionnement hétérotrophe des tronçons en amont, influencés par la ripisylve qui implique un fort ombrage et des apports importants en matière organique

détritique allochtone. Lorsque le cours d'eau s'élargit, la production primaire se développe et les apports détritiques sont moindres, ce qui permet un retour à des conditions autotrophes. Enfin, la turbidité et la profondeur des cours d'eau sont supposées redevenir des facteurs limitant la production primaire en aval ou le système redeviendrait donc hétérotrophe.

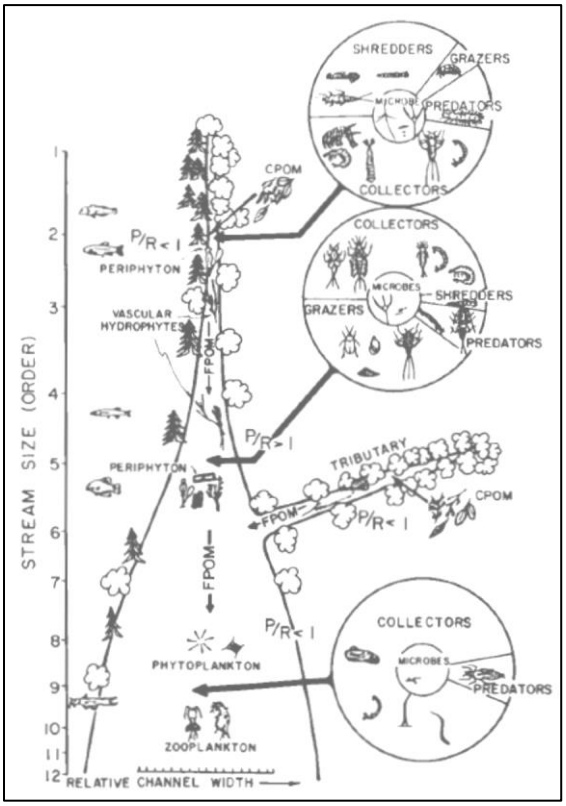

Figure 3. Description des relations entre la largeur du cours d'eau et l'évolution progressive des caractéristiques structurelles et fonctionnelles des communautés, d'après Vannote et al. (1980) : évolution des conditions physico-chimiques et des ajustements biotiques de l'amont (cours d'eau étroit fortement influencé par la ripisylve: rapport P/R<1), vers les tronçons intermédiaires (élargissement du cours d'eau permettant la photosynthèse : rapport P/R>1) puis l'aval du cours d'eau (turbidité liée à la matière organique particulaire et profondeur du cours d'eau limitent la photosynthèse : rapport P/R<1).

Les propriétés physiques et hydrologiques du système influencent donc les modalités de transport, de stockage et d'utilisation de la Matière Organique le long du cours d'eau, qui devient aussi de plus en plus fine en termes de taille de particules. Les communautés biotiques qui se succèdent d'amont en aval sont supposées être adaptées à ces conditions physiques et trophiques, afin d'utiliser de manière efficace la ressource. Dans une logique d'optimisation, les communautés en aval profitent de l'inefficacité des espèces en amont pour un partage équilibré de la ressource et une occupation optimale du gradient des habitats.

Ce concept a été largement discuté, notamment parce qu'il s'appuie sur un modèle d'écosystème naturel non — perturbé, or de nos jours ce type de système est quasiment inexistant, compte tenu des effets de l'anthropisation des bassins versants.

De nombreux travaux sont venus compléter ce concept (Fig. 4), intégrant entre autres les discontinuités (barrages, et confluences ; Ward & Stanford, 1983); les apports de la plaine inondable (Junk et al., 1989), les variations verticales, latérales et temporelles (Ward, 1989), ou encore la production autochtone (Thorp & Delong, 1994). En 2006, Thorp propose un concept ne décrivant pas les rivières comme un continuum, mais plutôt comme un ensemble de patches formés par la géomorphologie et le climat, qui constituent des « zones de processus fonctionnelles » (FPZ pour 'functional process zones' ; Thorp et al., 2006). Ce concept prend en compte la « biocomplexité » spatio-temporelle des réseaux hydrographiques, découlant des interactions dynamiques entre les compartiments biotiques et l'environnement physico-chimique. Il est suggéré que les FPZ comparables peuvent être réparties dans plusieurs parties différentes du réseau hydrographique, et que par conséquent, des communautés associées à des FPZ similaires peuvent être plus comparables entre elles qu'avec des communautés qui leur sont adjacentes, mais issues de FPZ différentes.

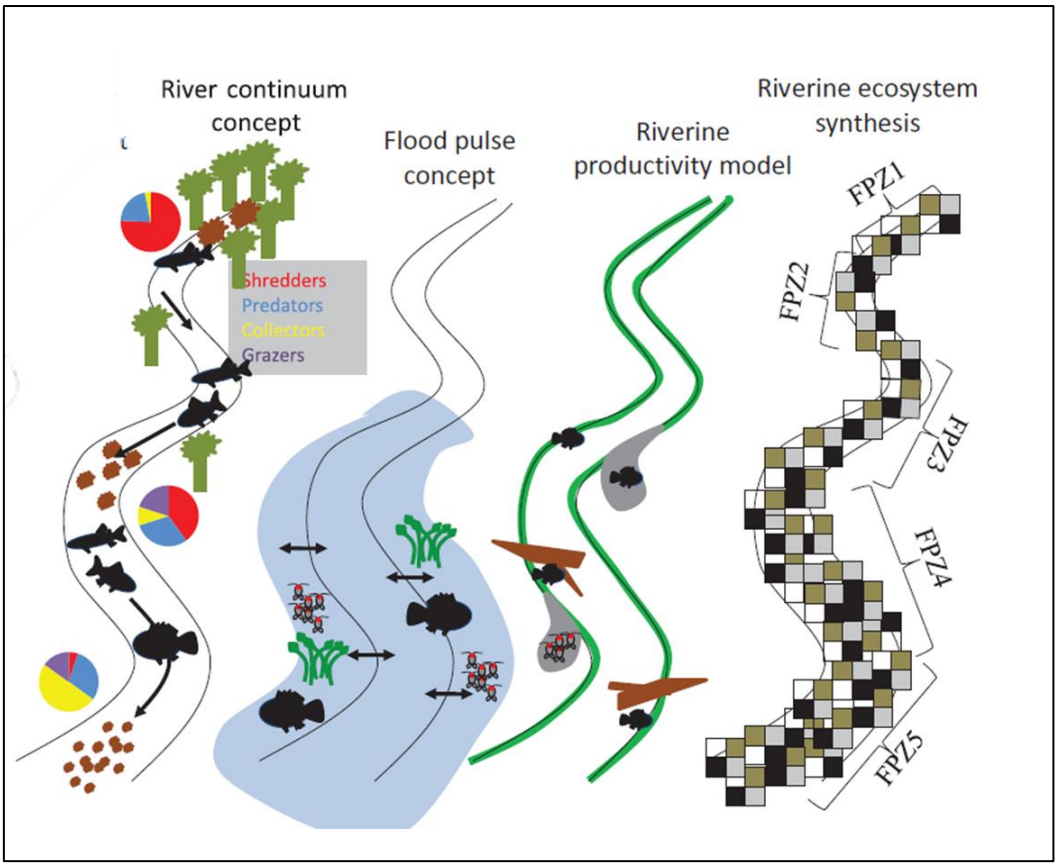

Figure 4. Description selon Humphries et al. (2014) des principaux concepts de fonctionnement des rivières découlant du RCC. De gauche à droite sont représentés: Le River continuum Concept (Vannote et al., 1980), Le Flood Pulse Concept (Junk et al., 1989), le Riverine Productivity Model (Thorp & Delong, 1994), et le Riverine Ecosystem Synthesis (Thorp et al., 2006).

Par ailleurs, le RCC s'applique plus particulièrement à la partie des cours d'eau située entre les têtes de bassin versant et le cours d'eau moyen, et ne prend donc pas en compte les tronçons estuariens, soumis à l'influence de la marée et au gradient de salinité. Pourtant, les variations spatio-temporelles de salinité dans les estuaires influencent fortement la distribution et la composition des communautés qui y vivent. Le long du gradient de salinité (de l'amont vers l'aval), les espèces d'eau douce disparaissent progressivement laissant place à des espèces euryhalines mais typiquement estuariennes et à des espèces d'eau marine. Cette relation entre diversité et gradient de salinité est décrit par Remane (1934) (Fig. 5).

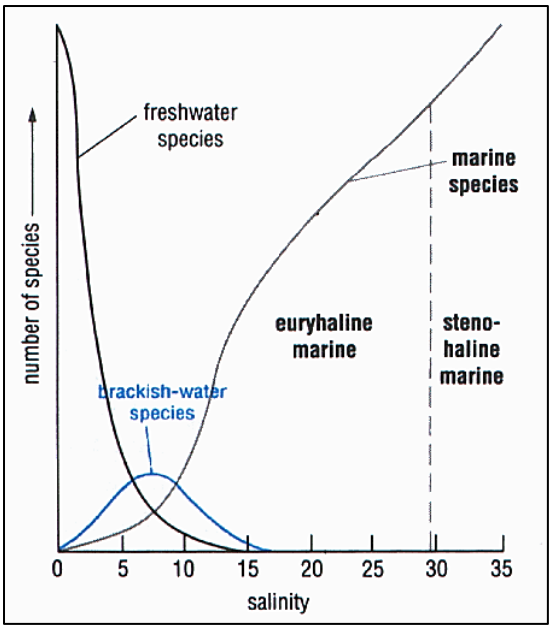

Figure 5. Diagramme de Remane (1934) décrivant l'évolution de la diversité des espèces le long du gradient de salinité.

Dans tous les cas, les organismes vivant dans les réseaux hydrographiques sont soumis à des contraintes particulières, liées à l'hétérogénéité de l'habitat, à l'hydrologie et à la nature dendritique du système, qui influencent la dynamique des populations et la dispersion. De plus, les conditions hydrologiques et physico-chimiques et la composition de la Matière En Suspension (MES) influencent fortement les interactions trophiques qui relient entre eux les différents compartiments. À cela s'ajoutent les activités anthropiques, influençant fortement et de diverses manières le fonctionnement de ces écosystèmes.

1.1.3 Menaces liées à l'anthropisation des bassins versants

Depuis le développement de l'ère industrielle, la croissance démographique et l'intensification des activités anthropiques ont mené à une forte dégradation de la qualité de l'eau. Les écosystèmes aquatiques sont exposés à 5 grandes catégories de menaces qui sont : la pollution des eaux, la dégradation des habitats, la modification des débits, la surexploitation des ressources, et l'introduction d'espèces invasives (Dudgeon et al., 2006). Ces menaces sont liées aux divers impacts des activités humaines, parmi lesquelles les altérations hydrologiques, les rejets de nutriments et de contaminants, plus spécifiquement développés ci-après.

1.1.3.1 Altérations hydrologiques des cours d'eau

Outre le fait qu'elle représente une ressource vitale, l'Homme utilise l'eau douce à de nombreuses fins. On estime à plus de la moitié de l'eau douce accessible comme étant utilisée

par l'Homme, dont plus de 70 % à des fins agricoles (Vitousek et al., 1997; Millennium Ecosystem Assessment, 2005).

Que ce soit pour alimenter les besoins de l'agriculture ou pour fournir de l'énergie hydroélectrique, des réservoirs ont été créés sur les réseaux hydrographiques. Beaucoup de cours d'eau ont aussi été déviés et/ou canalisés à des fins de navigation ou de contrôle des risques d'inondation. Par conséquent, 60 % des cours d'eau dans le monde sont aujourd'hui fragmentés par des barrages et autres infrastructures (Millennium Ecosystem Assessment, 2005).

Ces altérations de l'hydrologie modifient les habitats, affectent la continuité et le fonctionnement écologique des cours d'eau. De plus, la navigation sur les cours d'eau est également une source de perturbation. Le trafic fluvial lui-même altère les habitats en détruisant les macrophytes servant de refuges à certaines espèces (Murphy & Eaton, 1983; Ali et al., 1999), provoque des turbulences qui modifient les habitats des communautés benthiques, et contribue à la remise en suspension des particules, et à modifier la turbidité ou encore la concentration en oxygène dans le milieu (Garrad & Hey, 1987; Anthony & Downing, 2003; Gabel, 2012; Prygiel et al., 2015).

1.1.3.2 Modification des cycles de nutriments

Avec l'accroissement des populations et le développement des techniques modernes d'agriculture, la quantité et la nature des apports de nutriments dans les réseaux hydrographiques ont été fortement modifiés (Garnier et al., 1995; Vitousek et al., 1997; Billen et al., 1999; Galloway & Cowling, 2002; Meybeck, 2003; Smith, 2003). L'utilisation d'engrais pour les cultures, les épandages de fumiers animaux et les rejets d'effluents d'origine domestique ou industrielle émettent dans l'environnement des nutriments, et particulièrement de l'azote et du phosphore. Ceci s'est accompagné de la modification de l'occupation des sols végétalisés au profit de sols cultivés ou urbanisés, favorisant le lessivage des sols et l'exportation de ces nutriments souvent apportés en excès dans les cours d'eau.

De la même manière qu'ils fertilisent les cultures et les végétaux terrestres, les nutriments en excès dans les milieux aquatiques favorisent le développement des algues et des végétaux aquatiques. Ce phénomène d'enrichissement des eaux en nutriments, appelé eutrophisation (du ***grec*** « eu » : bien, vrai, et « trophein » : nourrir), est l'un des facteurs de dégradation de la qualité de l'eau les plus répandus dans le monde (Ryther & Dunstan, 1971; Schindler, 1974; Vitousek et al., 1997; Smith et al., 1999). Non seulement l'eutrophisation conduit parfois à des efflorescences phytoplanctoniques toxiques, mais la prolifération excessive des algues entraine également une réduction de l'accès à la lumière pour les autres organismes aquatiques, et une

diminution de la concentration en oxygène liée à la décomposition de ces végétaux dont la croissance est trop rapide pour être contrôlée par les herbivores. Il en résulte donc souvent une perte de diversité végétale et animale importante (Paerl, 1988; Carpenter et al., 1998; Paerl et al., 2001).

Certaines conséquences liées à ces efflorescences, comme la mort des poissons par asphyxie, les effets sur l'aquaculture ou les activités récréatives ou même dans certains cas la toxicité pour l'Homme, dégradent aussi les biens et services écosystémiques que les cours d'eau fournissent, et ont donc également des impacts économiques importants (Paerl, 1988; Palmstrom et al., 1988; Shumway, 1990; Paerl et al., 2001).

1.1.3.3 Rejets de contaminants

La croissance démographique et le développement des activités humaines ont aussi pour conséquence le déversement de plusieurs types de polluants dans les cours d'eau par le biais de rejets ponctuels (rejets industriels, domestiques, stations d'épuration, etc.) ou diffus (lessivage des sols, retombées atmosphériques) (Novotny, 1995 ; 1999).

La diversité des polluants est très importante. Dans le cadre de cette thèse, on s'intéressera principalement à deux types de contaminants particulièrement persistants dans l'environnement : les métaux (ou éléments traces métalliques, ETM), et les polluants organiques persistants (POP).

Les ETM sont présents sous forme naturelle dans l'environnement. Certains sont qualifiés d'« éléments essentiels » et sont nécessaires au fonctionnement des organismes, mais peuvent avoir un effet toxique à des concentrations trop élevées, alors que d'autres sont dits « non-essentiels » et peuvent être toxiques même à de faibles concentrations. Outre les composés d'origine naturelle, de nombreux secteurs d'activité sont à l'origine d'émission de métaux dans l'environnement. En particulier, les industries minières et de fonderie sont une source importante d'ETM.

Les polluants organiques persistants sont très diversifiés (hydrocarbures, polychlorobiphényles, pesticides…) et peuvent provenir de sources différentes (industrielle, agricole ou domestique). Ce sont des composés chimiques de synthèse, caractérisés par leur toxicité et par une forte persistance dans l'environnement. Particulièrement lipophiles, ils s'accumulent dans les tissus graisseux des organismes et se transfèrent dans les réseaux trophiques où ils sont bio-accumulés et bio-concentrés, et peuvent représenter un risque pour les niveaux trophiques supérieurs, ainsi que pour l'Homme.

Ces polluants, selon leurs propriétés physico-chimiques et leur concentration dans le milieu, peuvent représenter une menace pour les organismes, qui sont exposés à la contamination par voie trophique (ingestion de nourriture), ou bien par diffusion à travers les membranes biologiques (Eggleton & Thomas, 2004). Ils peuvent entrainer des effets létaux ou sub-létaux, conséquences de perturbations physiologiques, métaboliques, ou encore des altérations de la mobilité, du comportement ou de la reproduction (cas des perturbateurs endocriniens). Certains contaminants ont aussi des propriétés mutagènes ou cancérigènes (Vondráĉek et al., 2007; Griffitt et al., 2008; Bihanic et al., 2014; Vignet et al., 2014a, 2014b).

1.1.4 Évaluation et préservation de la qualité de l'eau dans les hydrosystèmes continentaux

L'accroissement démographique ayant participé à la dégradation de la qualité des milieux aquatiques s'est accompagné d'une demande croissante d'eau de bonne qualité (Vörösmarty et al., 2010). Avec la prise de conscience récente de la dégradation des milieux aquatiques continentaux, de leur importance écologique et des enjeux sociétaux, politiques et économiques impliqués, la protection des milieux aquatiques est devenue une préoccupation majeure.

Dans ce contexte, le parlement européen et le conseil de l'Union Européenne ont adopté en 2000 la Directive Cadre sur l'Eau (DCE), « établissant un cadre pour une politique communautaire dans le domaine de l'eau ». Ayant pour objectif la prévention des dégradations, la préservation de la qualité et l'amélioration de l'état des écosystèmes aquatiques (DCE, article 1), elle fixe aux États Membres un objectif d'atteinte de bon état des masses d'eau.

1.1.4.1 Mise en œuvre de la DCE

La mise en œuvre de la DCE s'effectue selon des cycles de gestion d'une durée de 6 ans chacun (Fig. 6). Chaque cycle comporte un état des lieux avec une évaluation de l'état des masses d'eau, et l'élaboration d'un Schéma Directeur d'Aménagement et de Gestion des Eaux (SDAGE), qui définit les objectifs et les mesures à mettre en œuvre (www.developpement-durable.gouv.fr).

Le premier cycle de gestion de la DCE s'est achevé en 2015. Un nouvel état des lieux a été réalisé en 2013, et les programmes de mesures du SDAGE 2016-2021 qui ont été définis courant 2014 sont actuellement mis en œuvre dans le 2eme cycle de gestion de la DCE.

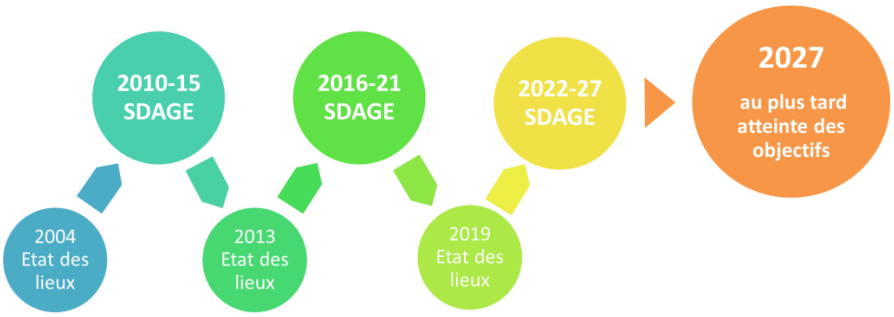

Figure 6. Les cycles de gestion de la DCE (adapté d'après http://www.artois-picardie.eaufrance.fr)

1.1.4.2 Évaluation de la qualité des eaux de surface par la DCE

Pour les eaux de surface, le système d'évaluation de la DCE repose sur 5 classes de qualité qui définissent l'état des masses d'eau : État très bon, bon, moyen, médiocre, ou mauvais (Fig. 7).

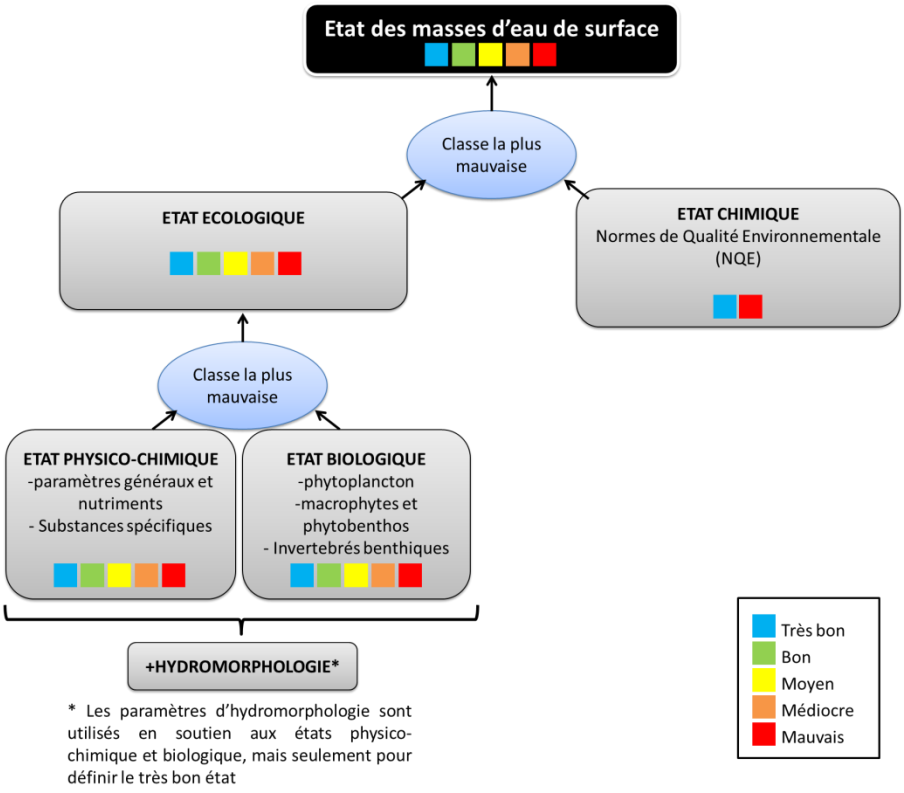

Figure 7. Système d'évaluation de l'Etat des masses d'eau par la DCE. L'état des masses d'eau se décline en 5 classes : très bon (bleu), bon (vert), moyen (jaune), médiocre (orange) et mauvais (rouge), et est basé sur l'état le plus déclassant entre l'état chimique et l'état écologique, lui-même défini à partir de l'état le plus déclassant entre l'état physico-chimique et l'état biologique.

L'évaluation de cet état se fait à partir de l'évaluation de l'État Écologique et de l'État Chimique, sur le principe de l'élément déclassant (one-out/all-out : c'est la classe la plus mauvaise qui détermine l'état général). Ainsi, le bon état des masses d'eau nécessite l'atteinte à la fois d'un bon état chimique et d'un bon état écologique.

Le bon état chimique comporte uniquement 2 classes de qualité (bon ou mauvais), et est évalué à partir de la moyenne annuelle des mesures mensuelles de concentration dans le milieu d'une liste de 41 substances (ou familles de substances). Cette valeur est comparée à une valeur seuil ou « norme de qualité environnementale» (NQE) définie pour chacune des substances concernées.

Conformément à l'article 16 de la DCE (Directive 2000/60/CE), la liste des substances prioritaires est réévaluée tous les 4 ans. Ainsi, 12 nouvelles substances prioritaires ont été introduites et leur NQE établies par la directive de 2013 (Directive 2013/39/UE) ce qui porte le total à 53 substances. Pour ces nouvelles substances les NQE devront être prises en compte à partir de 2018 dans un objectif d'atteinte du bon état en 2027. Par ailleurs des NQE plus strictes ont également été redéfinies dans le directive 2013/39/UE pour 7 des substances déjà inscrites dans la liste prioritaire. Celles-ci doivent être prises en compte à partir de 2015 pour une atteinte du bon état en 2021.

Selon la DCE, l'état écologique correspond à « l'expression de la qualité de la structure et du fonctionnement des écosystèmes aquatiques » (DCE article 2). Tout comme l'état général, l'état écologique se décline suivant les 5 classes de qualité attribuées suivant le principe de l'élément déclassant. Il est estimé sur une période de trois ans (deux avant l'arrêté de 2015) à partir d'indicateurs physico-chimiques, biologiques, et hydromorphologiques.

Il se base donc sur l'état physico-chimique, défini par une gamme de paramètres (concentrations en azote, phosphore, température, pH, concentration en substances spécifiques identifiées par les États membres en quantité significative sur le bassin...), et l'état biologique, qui prend en compte des Éléments de Qualité Biologique (EQB).

Ces EQB sont des paramètres biologiques basés sur les bioindicateurs suivants : diatomées, phytobenthos, macrophytes, macro-invertébrés et poissons. L'utilisation de bioindicateurs est fortement complémentaire des mesures physico-chimiques effectuées dans le milieu. Compte tenu de l'interaction forte entre les organismes aquatiques et l'ensemble des conditions physico-chimiques et biologiques du milieu, les changements de ces conditions liées à la dégradation de la qualité de l'eau ont une influence sur la structure et la composition des communautés. Les

caractéristiques de ces communautés sont donc le reflet de l'état du milieu, et permettent une appréciation synthétique de la qualité de l'eau et des pressions cumulées (Reyjol et al., 2013).

A partir de l'annexe V, chaque état membre doit donc mettre au point des outils de bio-indication pour chaque EQB. Ces indicateurs doivent être scientifiquement pertinents, applicables à grande échelle, et compatibles avec les conditions de la DCE pour permettre une inter-calibration au niveau communautaire. Pour ce faire ils doivent pouvoir être exprimés en termes d' « écart à la référence », c'est-à-dire en fonction du rapport entre l'état observé du cours d'eau et l'état « théorique » qui serait observé sans perturbation anthropogénique. Des valeurs de référence du "bon état" doivent donc être définies pour chaque type de masse d'eau à partir de sites de référence.

Les EQB, qui ont été développés pour les rivières, ne sont pas adaptées pour les masses d'eau fortement modifiées (comme les canaux), et nécessitent de définir des indicateurs adaptés. Ceci illustre bien la difficulté de mettre au point un système d'évaluation sur la base d'indicateurs de manière à prendre en compte à la fois les exigences d'inter-calibration de la DCE et les particularités propres à chaque bassin et à chaque type de cours d'eau. Il est donc nécessaire de comprendre les spécificités de fonctionnement des différents bassins versants. Au cours de cette thèse, nous nous focaliserons plus spécifiquement sur le cas du bassin versant de l'Escaut.

1.1.5 Le cas du bassin versant de l'Escaut

1.1.5.1 Caractéristiques générales

L'Escaut est un fleuve long de 355 km, qui prend sa source à Gouy le Câtelet, en France (49°59′13″N, 3°15′59″E), traverse la Belgique, et se jette dans la mer du Nord au niveau de Flessingue (Vlissingen en néerlandais) aux Pays-Bas (51°25′51″N, 3°31′44″E) (Fig 8). Avec ses tributaires, il draine un territoire d'environ 21 863 km^2 (Meire et al., 2005).

La source de l'Escaut est située à environ 110 m au-dessus du niveau de la mer, ce qui lui procure un dénivelé et des débits relativement lents, mais variables (de moins de 1m^3/s en amont à 125 m^3/s dans l'estuaire). L'estuaire de l'Escaut — c'est à dire la partie sous l'influence de la marée — s'étend jusqu'au niveau de la ville de Gand, en Belgique, à 160 km en amont de l'embouchure, où des écluses mettent fin à l'influence de la marée. L'estuaire de l'Escaut présente la particularité d'être doté d'un tronçon assez étendu d'eau douce, subissant l'influence des courants tidaux. Cette zone estuarienne d'eau douce s'étend sur 35 km entre Gand et Anvers (Meire et al, 2005).

L'Escaut peut être décrit en distinguant plusieurs tronçons : Le Bovenscheldt, qui correspond à la partie d'eau douce non tidale (de la source à Gand), le Zeescheldt qui s'étend de Gand à la frontière entre la Belgique et les Pays Bas, et qui contient la partie estuarienne d'eau douce et un tronçon d'eau saumâtre, et enfin le Westerscheldt, tronçon d'eau saumâtre à marine, qui correspond à la partie néerlandaise de l'estuaire.

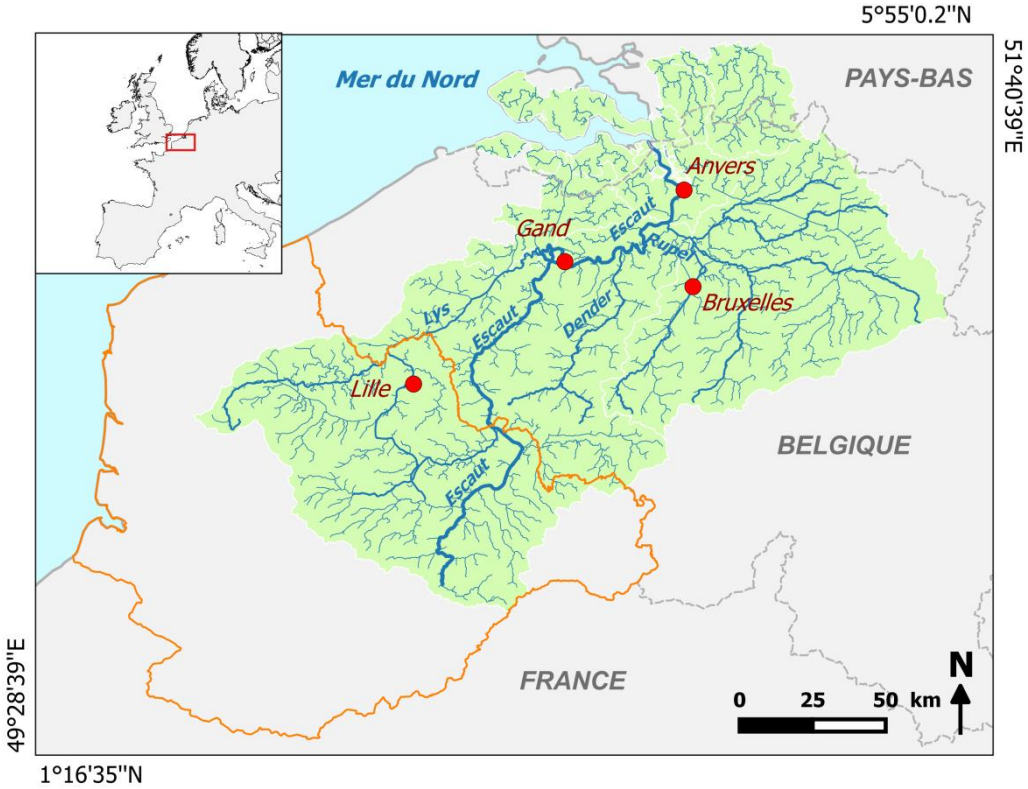

Figure 8. Carte et localisation du Bassin Versant de l'Escaut, transfrontalier entre la France, la Belgique et les Pays-Bas. La ligne orange délimite le bassin Artois-Picardie.

1.1.5.2 Contexte d'anthropisation dans le Bassin Versant de l'Escaut

Plus de 10 millions de personnes vivent sur le bassin versant de l'Escaut, ce qui équivaut à une densité de population de presque 500 habitants/km^2 (Meire et al., 2005). Les populations sont notamment plus concentrées aux abords des grandes villes, parmi lesquelles on peut citer Lille, Bruxelles, Anvers, ou encore Gand.

Le bassin est caractérisé par un contexte industriel historique, attenant à des secteurs diversifiés, dont l'industrie minière et métallurgique, la chimie, le textile, ou encore l'agroalimentaire. Le Port d'Anvers, de par sa position stratégique, est le deuxième plus grand port d'Europe, et le deuxième complexe pétrochimique le plus important du monde (Commission Internationale de l'Escaut : http://www.isc-cie.org).

Dans cette région caractérisée par de faibles reliefs, le réseau hydrographique a été très fortement sollicité par les activités industrielles, que ce soit pour le transport de matières premières ou de marchandises, ou encore pour l'approvisionnement en eau des industries. De nombreux canaux ont été construits pour la navigation, ce qui a fortement modifié l'hydrologie des cours d'eau. Le trafic fluvial est important et plus de 250 écluses et barrages sont répartis entre le fleuve et ses affluents, la partie de l'Escaut en amont de Gand étant canalisée sur une distance de 138 km.

Le bassin versant de l'Escaut est aussi caractérisé par une surface agricole très importante, avec une prédominance de l'élevage intensif dans la partie nord du bassin et de cultures (en particulier céréalières) dans le sud du bassin. Par conséquent, des apports importants d'azote et de phosphore ont participé à la modification des cycles biogéochimiques dans les milieux aquatiques (Billen et al., 2005, 2009).

Les activités industrielles sont désormais moins importantes qu'auparavant, et certains sites industriels ont été fermés, comme Métaleurop, industrie du Nord de la France destinée à la production de Zinc et de Plomb depuis la fin du XIXè sciècle, et qui a fermé ses portes en 1983. Récemment (depuis quelques décennies), des efforts conséquents ont été engagés en matière de protection des milieux aquatiques, visant à limiter les rejets d'effluents, à améliorer leur traitement, ou à restaurer les zones humides et les cours d'eau.

1.1.5.3 La qualité de l'eau dans le bassin versant

Compte tenu du contexte passé et actuel du territoire drainé par le bassin versant de l'Escaut, et malgré la réduction des activités industrielles, la qualité de l'eau dans le bassin reste préoccupante. De nombreux contaminants organiques se sont accumulés dans le sédiment, et représentent une importante source de pollution, notamment parce qu'ils sont susceptibles d'être relargués dans la colonne d'eau lors de remises en suspension du sédiment.

Le suivi de la qualité de l'eau dans le cadre de la DCE prend, pour le cas du bassin versant de l'Escaut, une dimension transfrontalière. La directive impose dans ce cas aux États Membres concernés une coordination et la production d'un plan de gestion de district hydrographique international (DCE art13). La réalisation de ce plan de gestion coordonné entre la France, la Belgique et les Pays-Bas, constitué des plans de gestion nationaux et d'une partie faîtière, est donc organisée par la Commission Internationale de l'Escaut (partie faîtière du plan de gestion du district hydrographique international de l'Escaut). Un réseau de mesures homogènes de l'Escaut (RHME) mis en place et coordonné par la CIE permet de surveiller de manière homogène la qualité de l'eau de l'Escaut dans les différentes régions qu'il traverse.

Les observations d'un point de vue transfrontalier révèlent une qualité des eaux de surface insuffisante, en lien avec les activités anthropiques passées et actuelles d'origine domestique, agricole ou industrielle. Notamment, les problématiques dans le bassin sont liées à une forte concentration en nutriments, une faible concentration en oxygène dissous, et une pollution par les contaminants métalliques et organiques (HAP, PCB, pesticides…).

La tendance en ce qui concerne la qualité de l'eau est tout de même à l'amélioration (rapport RHME 2011). Pour ce qui est de la DCE en France, les pressions semblent diminuer, et l'État physico-chimique est en voie d'amélioration dans le Bassin Artois-Picardie (Fig. 9 - SDAGE AEAP 2016-2021). Cependant, si le pourcentage des cours d'eau en états mauvais et médiocre diminue au bénéfice des cours d'eau en état moyen, en 2013, seuls 21 % des cours d'eau étaient évalués comme en bon état écologique, et seulement 6 % en bon état chimique.

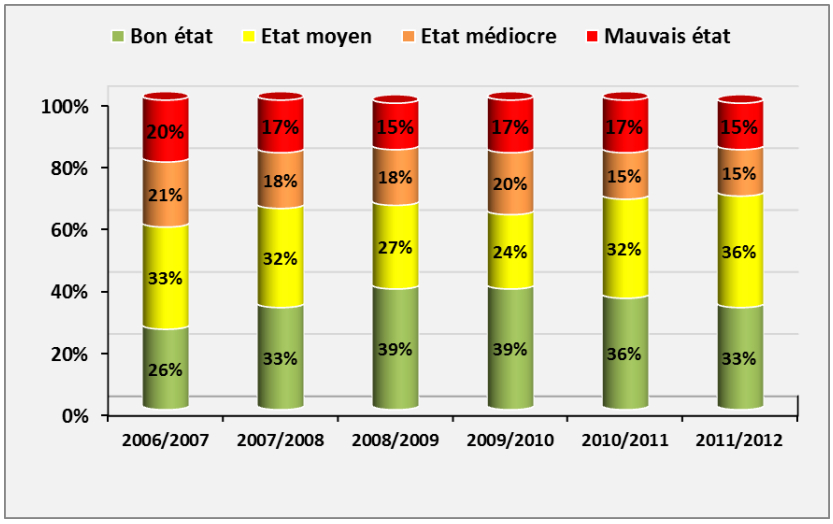

Figure 9. Évolution de l'État physico-chimique des cours d'eau dans le Bassin Artois Picardie lors de la dernière évaluation (SDAGE Artois-Picardie 2016-2021 : http://www.artois-picardie.eaufrance.fr)

Malgré une tendance à l'amélioration de la qualité de l'eau dans le bassin, les combinaisons d'altérations hydrologiques et physico-chimiques rendent difficile l'atteinte du bon état des masses d'eau. Le bassin versant de l'Escaut est donc un système particulier à la fois par sa topographie, sa localisation transfrontalière et son contexte de pollution passé et actuel.

1.2 Le zooplancton

1.2.1 Définition

Le terme plancton (du grec « planktos » : errant) désigne l'ensemble des organismes flottant passivement dans les eaux, ne pouvant se déplacer à l'encontre des courants. Cette définition est donc très large, et ne fait pas de distinction taxonomique. Le plancton végétal est qualifié de « phytoplancton », et le plancton animal, qui fait l'objet de cette thèse, de « zooplancton ».

Le zooplancton est représenté dans la plupart des milieux aquatiques. Il peut être classé par gamme de taille (Table 1), allant, si on exclut les virus, du picomètre (picozooplancton, par exemple les protistes) à plusieurs mètres (megaozooplancton, par exemple certaines méduses). Le mode de vie des organismes permet également de distinguer le méroplancton (qui a un mode de vie planctonique seulement durant certaines parties de son cycle de vie) de l'holoplancton (qui fait partie du plancton de manière permanente).

Les organismes regroupés au sein du zooplancton sont très diversifiés d'un point de vue taxonomique, mais également par leurs caractéristiques morphologiques et écologiques.

Table 1. Classification par taille du plancton, modifié d'après Sieburth et al. (1978).

	femto-plancton	pico-plancton	nano-plancton	micro-plancton	meso-plancton	macro-plancton	mega-plancton
	0.02-0.2µm	0.2-2.0µm	2.0-20µm	20-200µm	0.2-20mm	2-20cm	20-200cm
virio-plancton							
bacterio-plancton							
myco-plancton							
phyto-plancton							
protozoo-plancton							
metazoo-plancton							

1.2.2 Le zooplancton dans les rivières

En milieu lotique, l'hydrologie est considérée comme un des facteurs les plus limitants pour le développement du zooplancton. Les organismes zooplanctoniques étant par définition incapables de contrer les courants, ils requièrent un temps de résidence suffisant des masses

d'eau pour se développer. Ainsi, du point de vue du « concept de continuum fluvial » décrit par Vannote et al. (1980), le zooplancton est supposé se développer plus spécifiquement dans les tronçons en aval des rivières en raison des conditions hydrologiques (temps de rétention plus élevés qu'en amont) et trophiques qui en découlent. L'hydrologie, notamment en cas de faible débit, n'est cependant pas l'unique facteur structurant les communautés zooplanctoniques dans les rivières, et ces systèmes présentant une forte hétérogénéité de conditions exposent les organismes à des conditions de vie très diversifiées, à des échelles variables.

Des abondances importantes de zooplancton ont été observées dans plusieurs rivières (Richardson, 1992; Gosselain et al., 1998; Kobayashi et al., 1998; May & Bass, 1998; Reckendorfer et al., 1999; Baranyi et al., 2002; Lair, 2005). Les espèces de petite taille sont souvent favorisées par les conditions hydrologiques, car leur temps de développement est assez court (1 à quelques jours).

Par conséquent, dans les cours d'eau, le zooplancton est principalement composé de rotifères (Lair, 2006). Les rotifères sont assimilés au micro-zooplancton (Table 1), et forment un embranchement du règne animal très diversifié et cosmopolite. Hormis les rotifères, les crustacés font également partie des organismes zooplanctoniques d'eau douce (Fig. 10). On y distingue les copépodes (principalement Cyclopoïdes, Calanoïdes et Harpacticoïdes), et les Cladocères. Ces derniers peuvent être qualifiés de méso-zooplancton (pour les stades adultes, puisque les copépodes présentent un stade nauplii dont la taille peut être assimilée à du micro-zooplancton). Les rotifères, copépodes et cladocères ont des modes de vie (reproduction, alimentation) et des caractéristiques morphologiques et biologiques très distincts.

Figure 10. Exemples de spécimens de zooplancton du bassin versant de l'Escaut : copépodes calanoïdes (a), copépodes cyclopoïdes (b), cladocères (c : *Chydorus* sp. ; d : *Bosmina* sp.), et rotifères (e : *Brachionus calyciflorus*, f: *Keratella quadrata*). Photos : Akoko Claudine Sossou.

1.2.3 Écologie et rôle fonctionnel dans les écosystèmes pélagiques

Le zooplancton occupe une place centrale des réseaux trophiques pélagiques, assurant le transfert de matière et d'énergie issues des ressources de base (producteurs primaires phytoplanctoniques et éventuels apports allochtones) vers les niveaux trophiques supérieurs (partie 1, Fig. 1). Par conséquent, la structure et le comportement trophique des communautés zooplanctoniques influencent et sont influencés par les autres compartiments du réseau trophique.

D'un point de vue trophique, les communautés zooplanctoniques sont régulées d'une part par la ressource disponible (contrôle de type « bottom-up », des maillons inférieurs du réseau trophique vers les niveaux supérieurs – McQueen et al., 1986). En particulier la composition de la Matière en Suspension (sa proportion en Matière Organique et Inorganique, la quantité et la composition du phytoplancton, des détritus, des micro-organismes…) influence le zooplancton qui y trouve sa nourriture. D'autre part, la pression de prédation que les organismes planctophages exercent sur les communautés (contrôle de type « top-down », c'est-à-dire des maillons supérieurs du réseau trophique vers les niveaux inférieurs — McQueen et al., 1986) est aussi un facteur influençant la structure et la distribution du zooplancton.

De plus, certains contaminants sont associés à la Matière en Suspension au sein de laquelle se nourrit le zooplancton, en particulier les contaminants organiques, du fait de leurs caractéristiques hydrophobes. Les organismes sont donc en contact direct avec ceux-ci, et deviennent un vecteur potentiel des polluants par voie trophique (Cailleaud et al., 2007; Arias et al., 2016; Tlili et al., 2016).

En raison de sa position trophique et de son cycle de vie relativement court, le zooplancton est par ailleurs particulièrement sensible aux variations environnementales et répond rapidement aux changements de conditions de son milieu (Gannon & Stemberger, 1978 ; Mialet et al, 2010, 2011 ; Chambord et al., 2016). Il est donc fortement influencé, de manière directe ou indirecte, par les conditions environnementales, et est fréquemment cité en tant qu'indicateur de pollution. Certaines espèces de rotifères et de cladocères sont aussi très utilisées dans les tests d'écotoxicologie. Par ailleurs, son rôle d'indicateur a été confirmé par plusieurs auteurs, qui déplorent, en particulier concernant les lacs, la non-prise en compte du zooplancton parmi les EQB utilisés pour l'évaluation de la qualité de l'eau dans le cadre de la DCE (Moss, 2007 ; Nõges et al., 2009 ; Jeppesen et al., 2011 ; Haberman & Haldna, 2014).

1.3 PROBLEMATIQUE, OBJECTIFS DE L'ETUDE ET ORGANISATION DU MANUSCRIT

Parce qu'il est souvent considéré comme limité par les conditions hydrologiques, le zooplancton des rivières est généralement moins documenté que celui des autres systèmes aquatiques (lacs, estuaires ou zones côtières et océaniques). En amont du bassin versant de l'Escaut, le zooplancton demeure ainsi mal connu. Les précédentes études sur le zooplancton dans l'Escaut se sont concentrées sur les communautés des tronçons estuariens, et ont mis en évidence des changements dans la structure de ces communautés, parallèlement à l'amélioration récente de la qualité de l'eau (Appeltans et al., Tackx 2005 ; Mialet et al., 2010, 2011 ; Chambord et al., 2016).

Les conditions hydrologiques particulières présentées par le réseau hydrographique de l'Escaut (courants faibles) constituent des conditions favorables au développement du zooplancton. Par ailleurs, le contexte de pollution des cours d'eau dans le bassin versant expose les communautés zooplanctoniques à des conditions environnementales et à une qualité de l'eau variables.

Cette thèse s'inscrit essentiellement dans le projet de recherche BIOFOZI (Biodiversité et Fonctionnalité du Zooplancton : test du potentiel Indicateur de la qualité de l'eau), dont les principaux objectifs reposent sur l'étude des communautés de zooplancton en lien avec les conditions environnementales en amont et en aval du bassin versant de l'Escaut. Ce projet interdisciplinaire implique un partenariat entre les laboratoires EcoLab (Laboratoire d'Ecologie Fonctionnelle - Université Paul Sabatier Toulouse III), LOG (Laboratoire d'Océanologie Et de Géosciences - Université Lille 1), LASIR (Equipe Physico-Chimie de l'Environnement, Université Lille 1), ECOBE (Ecosystem management Group - Université d'Anvers, Belgique), et l'Agence de l'Eau Artois Picardie. Il a été financé dans le cadre d'un appel à projets de recherche sur la biodiversité conjoint du Conseil régional Nord-Pas de Calais et de la Fondation pour la Recherche sur la Biodiversité (FRB), et par l'Agence de l'Eau Artois-Picardie.

L'objectif de ce travail est de fournir une description des caractéristiques du zooplancton du bassin versant de l'Escaut, d'un point de vue structurel (abondance et composition des communautés) et fonctionnel (réponse aux conditions environnementales et efficacité trophique). Dans un contexte où la préservation et l'amélioration de la qualité de l'eau sont des priorités environnementales, l'approche se veut intégrative en prenant en compte les divers aspects de conditions de vie du zooplancton dans une gamme de sites évalués par la DCE comme de qualité variable.

Le manuscrit est articulé en quatre chapitres.

Dans un **premier chapitre**, la distribution longitudinale des communautés zooplanctoniques est décrite à l'échelle du fleuve entier, de la source à l'embouchure de l'Escaut, et en lien avec les conditions physico-chimiques et trophiques. Cette étude confronte la distribution des communautés zooplanctoniques des tronçons successifs avec les différents concepts de fonctionnement écologique des cours d'eau.

Le **deuxième chapitre** considère la variabilité spatiale, saisonnière et interannuelle des communautés dans la partie Amont du bassin versant. Les profils de distribution du zooplancton sont étudiés en lien avec les facteurs physico-chimiques et avec les statuts écologiques de la DCE.

Dans le **troisième chapitre**, les conditions d'habitat du zooplancton sont évaluées en considérant à la fois les facteurs physico-chimiques généraux et les concentrations en contaminants les plus problématiques en termes de qualité de l'eau dans le bassin versant amont (POP, ETM). Il est utilisé une approche combinant des données de physico-chimie, d'état écologique évalué par la DCE, de contamination de l'eau, et d'abondance du zooplancton. L'objectif de cette étude est de caractériser la réponse des communautés zooplanctoniques à ces différentes conditions de vie et aux multiples pollutions, en termes d'abondance et de composition taxonomique.

Le rôle trophique des communautés zooplanctoniques est abordé dans le **quatrième chapitre**. Basée sur une approche expérimentale, cette étude vise à caractériser l'impact et la sélectivité trophique des communautés zooplanctoniques sur les populations de phytoplancton dans plusieurs sites du bassin versant amont et à plusieurs périodes.

Enfin, une **discussion générale** viendra achever ce manuscrit, permettant de synthétiser et de discuter les résultats exposés dans les différents chapitres. Certaines hypothèses y seront plus spécifiquement abordées et pourront donner lieu à la proposition de perspectives.

CHAPITRE I :

DISTRIBUTION LONGITUDINALE DU ZOOPLANCTON DE LA SOURCE A L'EMBOUCHURE DE L'ESCAUT

- ***Article soumis dans Hydrobiologia***

1.1 RESUME DU CHAPITRE

1.1.1 Introduction

La description de la distribution des communautés le long des gradients longitudinaux des cours d'eau a été introduite dans le « River Continuum Concept » (RCC - Vannote, 1980). Le RCC décrit un gradient de conditions physico-chimiques et trophiques auxquelles les communautés sont adaptées de la source à l'embouchure. Cependant, ce concept considère des cours d'eau non-perturbés, et s'applique principalement aux tronçons de la source aux cours d'eau moyens, n'incluant pas les tronçons estuariens sous l'influence de la marée et du gradient de salinité.

Par la suite, l'influence des discontinuités (The Serial Discontinuity Concept; Ward & Stanford, 1983, 1995) et de la plaine inondable (The Flood Pulse Concept ; Junk et al., 1989) ou encore la contribution de la production autochtone (The Riverine Productivity Model; Thorp & Delong, 1994) ont été prises en compte par plusieurs études venues compléter le RCC. Cependant, les tronçons estuariens ne sont jamais explicitement considérés dans ces études.

L'influence du gradient de salinité sur la diversité des espèces a été décrite par Remane (1934), qui met en évidence en mer Baltique une diversité minimale dans la zone de salinité 5-7, ou les espèces d'eau douce sont peu représentées tout comme les espèces marines.

En s'inspirant de ces concepts, élaborés principalement pour les macro-invertébrés, la présente étude a pour objet de décrire pour la première fois la distribution du zooplancton de la source à l'embouchure d'un cours d'eau, l'Escaut, en incluant à la fois les tronçons d'eau douce non tidaux (sans influence de la marée) et les tronçons d'eau douce, saumâtre et saline de l'estuaire, sous l'influence de la marée. L'Escaut est une rivière de plaine, caractérisé par de faibles débits (influencés par la saison) et par l'importance de sa zone estuarienne d'eau douce (Meire et al., 2005).

Dans ce contexte, les objectifs de cette étude sont :

- Décrire, en prenant en compte les plus de 300 km de cours d'eau de Crévecoeur-sur-Escaut (France) à Flessingue (Pays-Bas), **la distribution des communautés zooplanctoniques** en relation avec les **conditions environnementales et trophiques** qui s'appliquent d'amont en aval, l'influence de la présence de **courants tidaux**, et celle du **gradient de salinité**. L'influence des **tributaires**, qui contribuent au profil de distribution d'organismes et des facteurs environnementaux dans le cours d'eau principal (Minshall et al., 1983), est aussi prise en considération.

- Comparer ces profils de distribution pour **deux saisons différentes** (avril 2013 et juin 2015).
- Confronter les résultats obtenus aux différents **concepts écologiques** décrits précédemment.

1.1.2 Principaux Résultats et Discussion :

Cette étude a révélé des abondances substantielles de zooplancton sur l'ensemble du continuum de l'Escaut et des tributaires, y compris dans la partie la plus en amont du bassin versant (eau douce non soumise à l'influence de la marée). Ainsi, en amont de Gand, où l'influence de la marée s'arrête, les abondances ont atteint 6.7×10^6 ind.m^{-3} en avril, et 2.9×10^6 ind m^{-3} en juin. Ces résultats démontrent la capacité du zooplancton à se développer dans les tronçons en amont du bassin versant de l'Escaut, avec des abondances comparables à celles observées dans certains milieux lentiques.

Une classification ascendante hiérarchique des stations, basée sur les communautés zooplanctoniques a permis d'identifier différentes zones successives (en référence aux « functional process zones » définies par le concept de Thorp et al., 2006), principalement liées au **gradient de salinité**, à la **saisonnalité** et à **l'influence de la marée**. Les zones suivantes ont pu être identifiées :

– la **zone non estuarienne d'eau douce**, caractérisée par les températures et concentrations en oxygène les plus élevées, et par des concentrations en chlorophylle *a* également importantes. Les rotifères sont dominants dans cette zone, et on y trouve également des copépodes (principalement cyclopoïdes) et des cladocères.

– la **zone estuarienne d'eau douce**, avec des concentrations en Matière Organique et Inorganique importantes, et les plus fortes concentrations en chlorophylle *a* et phéopigments. Dans cette zone les rotifères sont toujours présents, mais les cyclopoïdes et cladocères sont moins abondants, tandis que les calanoïdes (en particulier *Eurytemora affinis*) et les nauplii de copépodes deviennent plus abondants. Cette zone, caractérisée par une concentration relativement élevée en phytoplancton est caractérisée par une biomasse zooplanctonique importante, ce qui confirme son importance fonctionnelle.

– la **zone estuarienne saumâtre**, où la salinité augmente brutalement, et s'accompagne d'une forte diminution d'abondance et de diversité zooplanctonique, notamment des espèces d'eau douce telles que les rotifères, cyclopoïdes et cladocères. Ces résultats semblent donc cohérents avec le concept de Remane (1934), selon lequel la diversité est minimale dans cette zone de salinité.

– la **zone marine**, avec une forte salinité et une faible température. Dans cette zone on trouve presque exclusivement des calanoïdes, avec des inclusions d'espèces marines, ce qui permet que la diversité augmente à nouveau, comme indiqué par Remane (1934).

Le long du cours d'eau principal, des changements dans les communautés zooplanctoniques ont été observés aux confluences de certains tributaires. Ces changements semblent plutôt associés à des changements d'abondance que de composition taxonomique. L'Escaut peut donc être considéré comme une succession de « Functional Process Zones » (Thorp et al., 2006), présentant des conditions d'habitats différentes pour les communautés zooplanctoniques.

1.2 ARTICLE

Longitudinal distribution of zooplankton along a lowland water course: the Scheldt

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1.2.1 Abstract:

The distribution of zooplankton communities along the entire Scheldt water course, from the source to mouth, including the riverine reaches and the estuary, was investigated to test both the River Continuum Concept (RCC) and Remane's diversity concept.

Substantial zooplankton densities were found all along the water course, not only in the lower reaches as suggested by the RCC. Hierarchical partitioning identified salinity, season and tidal regime as main drivers of the zooplankton communities. Based on the zooplankton communities, several successive functional process zones influenced by different physical and chemical variables and trophic conditions could be distinguished along the Scheldt, in agreement with Thorp et al.'s RES model: the non-tidal riverine, the tidal freshwater, the tidal salinity gradient and the marine zones. Over the entire freshwater reach, rotifers were numerically dominant during both samplings; copepods and cladocerans became more abundant in the downstream reaches in June. Highest total zooplankton biomass and diversity were found in the tidal freshwater reach, lowest in the brackish water reach, in accordance with Remane's concept. An influence of the tributaries on zooplankton communities is suggested, directly by import or dilution of zooplankton communities or through changing environmental conditions.

Keywords: Zooplankton distribution; River continuum concept; Remane's diagram; freshwater tidal estuary.

1.2.2 Introduction

From source to mouth, river systems are characterized by a number of changes in geomorphological, hydrological, physical, chemical, and biological features. The understanding of the distribution of communities along longitudinal gradients, especially for macrobenthic species, resulted in the development of important theoretical ecological concepts, such as the Remane's diagram (Remane, 1934) and the River Continuum Concept (Vannote et al., 1980).

Based on a study of invertebrates in the Baltic Sea, Remane (1934) described the diversity of macrobenthos along a salinity gradient, showing a minimum of diversity in the 5–7 salinity zone, where neither freshwater nor marine species are well represented. This concept only considers the influence of salinity on communities and does not involve other abiotic and biotic factors. A more integrated approach was developed in the River Continuum Concept (RCC, Vannote et al., 1980), describing the occurrence of aquatic species along a continuous gradient of physical and chemical characteristics, in particular concerning trophic resources, to which biotic communities are adjusted from headwaters to mouth. The RCC, developed for pristine systems, has been applied by its authors (Minshall et al., 1983) to four rivers from their source to the most downstream station unaffected by pollution and impoundments. As such the RCC is limited to freshwater riverine systems, and does not involve estuarine reaches, and therefore the influence of salinity or tidal effects.

The RCC has paved the way for many studies on aquatic communities along the longitudinal gradients and opened the discussion on the importance of various aspects to be taken into consideration. Minshall et al. (1985) proposed a broader consideration of the RCC, including aspects such as morphology, climate and the influence of tributaries. Most important criticisms and additions to the original RCC concept concern how to take into account (human induced) discontinuities (The Serial Discontinuity Concept; Ward & Stanford, 1983, 1995); the lateral floodplain pulse influence (The Flood Pulse Concept; Junk et al., 1989) and the role of local primary production and dissolved matter from riparian vegetation in creating local patchiness in conditions for community development and functioning of lotic systems (The Riverine Productivity Model; Thorp & Delong, 1994). Also the role of human induced discontinuities and climatic and temporal variability on system structuring and functioning has been discussed (Ward, 1989; Brierley et al., 1999). We refer the reader to the "Riverine Ecosystem Synthesis" by Thorp et al. (2006) and the recent synthesis by Humphries et al. (2014) which also introduces the River Wave Concept in an attempt to integrate the existing models into a simple, unifying one. This concept suggests that variations in the river flow influence the location and the source of allochtonous and autochthonous inputs and their storage and transport. The flow variations

are described as wave-shaped, and suggest that ecosystem processes differ according to the position on the wave, considering that these flow waves have a major role in river ecosystem processes.

Although most of the RCC related papers consider rivers from source to mouth, the estuarine reach is rarely explicitly considered. Yet, the whole system includes the river reach without influence of the tide and the estuary, with influence of the tides and salinity. It should be stressed that the effective limits of the tidal influence can be situated in the freshwater reach of the continuum (Fairbridge, 1980; Perillo, 1995; Elliott & McLusky, 2002).

In this study, we consider the entire system, including both the riverine upstream reaches and the estuary. We use the term "river" for freshwater non-tidal reaches and the term "estuary" for freshwater, brackish water and marine reaches under tidal influence. We use the term "water course" for the entire longitudinal transect running from the source to the mouth, which avoids talking "a priori" about a continuum or a discontinuum.

In both estuarine and riverine studies, the focus was mainly on the distribution of benthic macroinvertebrates and fish communities (e.g. Remane, 1934; Burton & Odum, 1945; Gerking, 1945; Minshall, 1968; Angermeier & Karr, 1983; Rice et al., 2001; Torgersen et al., 2006; Tornwall et al., 2015). Much less attention was given to zooplankton, despite its close relationships with the pelagic environment and its importance in the food web.

For estuarine systems, some studies have revised Remane's (1934) diagram and taken into account zooplankton. Contrarily to Remane's benthic diversity minimum observed in the Baltic between 5–7 salinity, Telesh et al. (2011) observed, in the same system, a diversity maximum in this salinity range for phyto-and microzooplankton (ciliates and rotifers). For metazooplankton (crustaceans), they observed a decrease in diversity with salinity. Whitfield et al. (2012) critically consider the applicability of Remane's concept for true estuaries. According to the data reviewed by these authors, zooplankton diversity tends to increase, towards the mouth. This increase is not necessarily related to the direct effect of the salinity gradient on organisms as suggested by Remane (1934), but to the contribution of marine and brackish species, and to the synergetic influence of co-varying physical and chemical factors (e.g. temperature).

The RCC (Vannote et al., 1980) suggests that riverine zooplankton develops mainly in the lower reaches because water residence times are too short to allow substantial plankton development in upstream rivers, and with few exceptions (Basu & Pick, 1997; Casanova & Henry, 2004), zooplankton abundance and biomass are generally reported to be higher close to the mouth than upstream (Saunders & Lewis, 1989; Vásquez & Rey, 1989; de Ruyter van Steveninck et al., 1992;

Kim & Joo, 2000; Akopian et al., 2002; Burger et al., 2002). However, in low-flowing systems, zooplankton development is probably not limited by current velocity, and thus may be dependent of other environmental factors (Reynolds, 2000). Also, in estuarine reaches the back and forward movement of water masses created by the tide increases residence times, which may influence the development conditions for planktonic organisms, passively transported with the currents. Moreover, several planktonic species are known to use vertical migration combined with tidal currents to stay within the estuary (Morgan et al., 1997; Devreker et al., 2008).

There seems to exist a segregation in the literature between papers following the RCC (Vannote et al., 1980) which, as stated above, pay little attention to estuaries, and the papers following Remane's (1934) concept, which focus precisely on the salinity gradient encountered in estuaries. Not only is the focus on different reaches of the lotic system; Remane's paper and following focus on diversity and its relation to salinity; while in the RCC approach, the main focus is on energy/matter transport and system functioning, and diversity is treated as a consequence of these. Neither approaches explicitly consider the presence or not of a tidal influence.

This study is one of the first that describes the distribution of zooplankton along the entire longitudinal gradient of a typical lowland river and its estuary. Lowland rivers are characterized by a flat topography and consequent low discharge, depending on the season. Many lowland rivers and estuaries have been substantially modified to support land drainage, navigation, or flow regulation, and therefore display particular characteristics concerning hydrology, nutrient status, resources supporting secondary producers development and resulting biotic communities (Baldwin & Mitchell, 2000; Franklin et al., 2008). The influence of tributaries is also considered here: according to their size, their discharge and the abundance of pelagic organisms they carry, confluence with a tributary can either increase pelagic organisms abundance in the main course, or decrease it by dilution phenomena (Minshall et al., 1983).

Zooplankton communities are known to depend on abiotic factors such as hydrology and physical and chemical factors (Ferrari et al., 1989; Thorp et al., 1994; Marneffe et al., 1996; Basu & Pick, 1997; Kim & Joo, 2000; Kimmel & Roman, 2004; Dodson et al., 2005; Mialet et al., 2011). The influence of biotic processes is also recognized: predation (Stibor, 1992; Akopian et al., 1999, 2002; Shurin, 2001), but also bottom-up processes, since zooplankton is controlled by food availability and quality (Stemberger & Gilbert, 1985; Giani, 1991; Walz, 1997; Nejstgaard et al., 2001). Zooplankters are suspension feeders with various degrees of selectivity capacity (Allan et al., 1977; DeMott, 1986; Knisely & Geller, 1986; Kirk, 1991). In general, live

phytoplankton and micro-organisms are considered as higher quality food than detritus (DeMott, 1988, 1995; Müller-Solger et al., 2002), and important inorganic matter concentrations can hamper feeding efficiency (Gasparini et al., 1999; Tackx et al., 2003). So, the concentration of suspended particulate matter (SPM), the contribution of organic matter (OM) to SPM and the contribution of live phytoplankton to OM can be considered to represent zooplankton feeding conditions.

The aim of this study is to describe the pattern of occurrence of zooplankton communities along the river/estuary watercourse of the Scheldt water course and analyze which factors influence community composition and biomass development of the zooplankton. The observed patterns will be discussed in relation to relevant aspects of the above mentioned concepts on river—estuary continua.

1.2.3 Material and Methods

1.2.3.1 Study area

The river Scheldt has its source in Northern France near Gouy (49° 59′ 13″ N 3° 15′ 59″ E) at only 110 m above sea level and runs through Belgium and flows into the North Sea at Vlissingen, the Netherlands (51° 25′ 51″ N 3° 31′ 44″ E) (Fig.1). Along its 355 km length, it drains a watershed of 21.863 km^2, which is characterized by dense population, and historical intensive agricultural and industrial activities, resulting in important nutrient loading, although recent improving of water quality (Billen et al., 2005; Cox et al. 2009).

Although almost the whole river is canalized (Hoffmann & Meire, 1997; Meire et al., 2005), the Scheldt estuary is one of the few European estuaries still having an extensive freshwater tidal zone (salinity <0.5) (Meire et al., 2005). Being rain-fed, discharge is highly variable depending on the season (Heip, 1988; Soetaert & Herman, 1995).

From the upstream reaches till the mouth, the Scheldt can be divided in four zones based on tidal influence and average salinity: 1) the non-tidal, freshwater part of the Scheldt, which runs from Gouy to Gent, is called Bovenscheldt (B7-B1 in Fig.1); 2) the freshwater tidal reaches (between Gent and Antwerp, stations Z5-Z2) 3) the brackish water tidal reaches (between Antwerp and Bath, stations Z1, W5) and 4) the tidal brackish–marine part (salinity> 5) called Westerscheldt and extending from Bath (at the Dutch-Belgium border) to Vlissingen (at the mouth) (stations W4-W1). Reaches 2 and 3, on Belgian territory, together constitute the Zeescheldt.

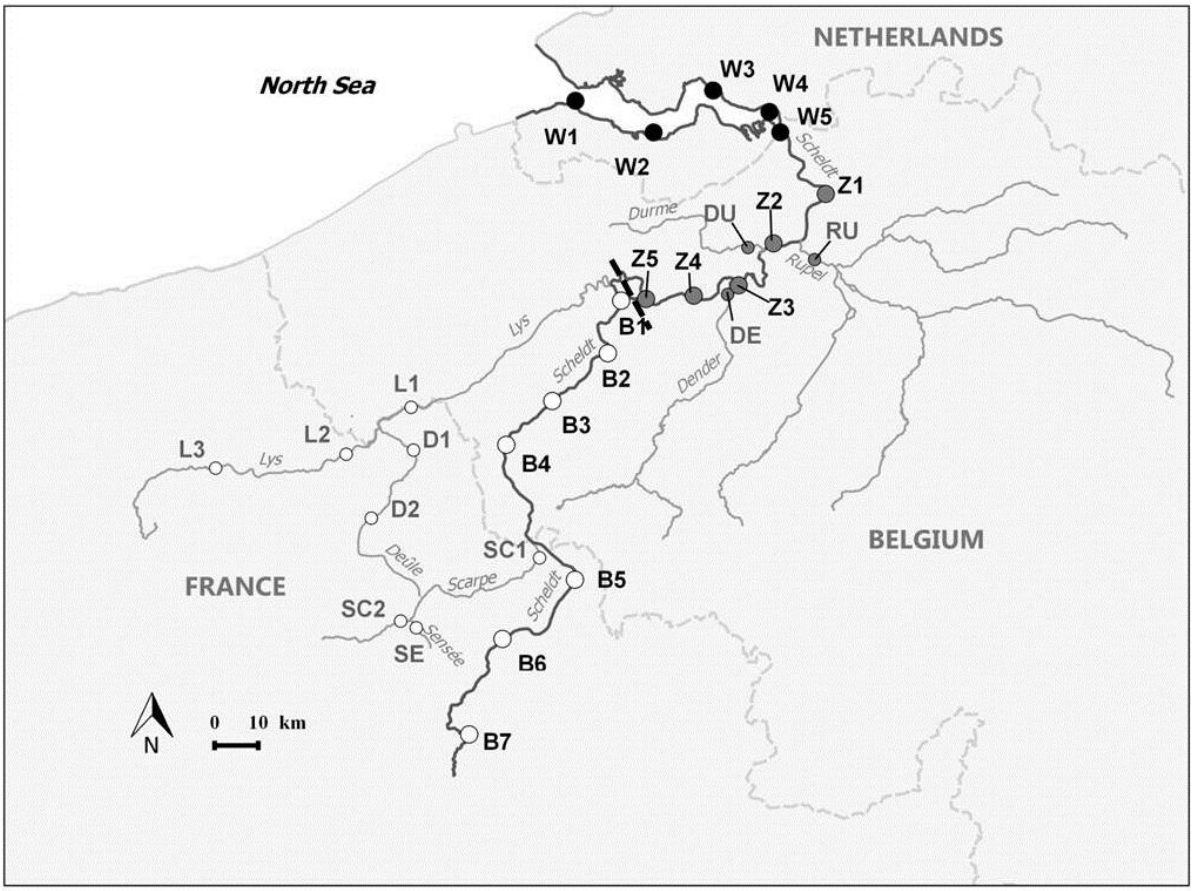

Fig.1. The Scheldt and the Rupel, Durme, Dender, Lys, Deûle, Scarpe and Sensée tributaries. (Black circles are for Westerscheldt, Grey circles for Zeescheldt, White circles for Bovenscheldt. Tributary stations are represented by smaller symbols). The end of tidal influence delimiting the river and the estuary is represented by dotted line. See in Table 1 for abbreviations.

Sampling took place from the 22th to the 30th April 2013 and from the 15th to the 25th June 2015, as representative of spring and early summer seasons, corresponding to the onset of the low flow season (April 2013) and after several weeks of low flow (June 2015) on 23 sites from near the source to the mouth of the Scheldt (Fig. 1, Table 1). As the aim of the study is to compare two different seasonal situations, not the years, the sampling periods will be reffered to as April and June in the following. Sampling sites were situated on the main water course Scheldt and on the Scarpe, Dender, Rupel and Durme as tributairies, and also on the Deûle-Lys watershed, joining the main Scheldt at station B1.

Table 1. Code, location, waterbody and distance from the mouth of the sampling sites

	Station code	location	waterbody	distance from the mouth (km)
Westerscheldt				
	W1	Breskens (ND)	Scheldt	0
	W2	Terneuzen (ND)	Scheldt	23
	W3	Hansweert (ND)	Scheldt	43
	W4	Schaar Van Ouden Doel (ND)	Scheldt	56
	W5	Zandvliet (ND)	Scheldt	57
Zeescheldt				
	Z1	Antwerp (BE)	Scheldt	78
	Z2	Temse (BE)	Scheldt	99
	Z3	Dendermonde (BE)	Scheldt	121
	Z4	Uitbergen (BE)	Scheldt	138
	Z5	Melle (BE)	Scheldt	151
Bovenscheldt				
	B1	Bovenschelde (BE)	Scheldt	167
	B2	Zingem (BE)	Scheldt	174
	B3	Berchem (BE)	Scheldt	193
	B4	Warcoing (BE)	Scheldt	208
	B5	Fresnes-sur-Escaut (FR)	Scheldt	249
	B6	Neuville-sur-Escaut (FR)	Scheldt	274
	B7	Crévecoeur-sur-l'Escaut (FR)	Scheldt	309
tributaries				
	RU	Boom (BE)	Rupel	98
	DU	Durme (BE)	Durme	114
	DE	Dender (BE)	Dender	136
	L1	Wervicq (FR)	Lys	232
	L2	Erquinghem-Lys (FR)	Lys	254
	L3	Aire-sur-la-Lys (FR)	Lys	289
	D1	Wambrechies (FR)	Deûle	252
	D2	Don (FR)	Deûle	273
	SC1	Nivelle (FR)	Scarpe	239
	SC2	Brebières (FR)	Scarpe	281
	SE	Férin (FR)	Sensée	281

1.2.3.2 Environmental variables:

Temperature, conductivity, pH and dissolved oxygen concentration (O_2) were measured *in situ* using a multi parameter sensor (WTW, Multi 3430). Conductivity was used a proxy for salinity, as salinity data were not available for all stations. Conductivity and salinity have been shown to

be significantly correlated in the Scheldt (r= 0.994; n= 205). Suspended Particulate Matter (SPM) concentrations were quantified by filtration of 250 to 1200 ml of water, depending on the SPM concentration, onto pre-weighed Whatman GF/C. Filters were dried (45 °C) during several days, briefly cooled in a desiccator and weighed. The same filters were burned at 500 °C for 5 hours, and re-weighed to obtain Ash concentration. Organic Matter (OM) concentrations were calculated as the difference between SPM and Ash concentrations.

Water samples were also filtered on Whatman GF/C filters for the determination of pigments (chlorophyll *a* (Chl*a*) and phaeopigment) concentrations, which were then extracted in 2% ammonium acetate solution and quantified by reversed phase HPLC, following the method of Wright & Jeffrey (1997). Details of the methodology are given in Majdi et al. (2011). The contribution of phytoplankton biomass to OM and SPM was estimated by calculating phytoplankton Carbon biomass using C : Chl*a* ratio of 30 and multiplying by 2 to convert C into dry weight.

pH and phaeopigment concentrations were not measured during the April 2013 sampling.

1.2.3.3 Zooplankton sampling and analysis

At each site, 50 to 250 liters of sub-surface water was collected with a pump and filtered over a 50 µm mesh plankton net. The zooplankton thus collected was narcotized with carbonated water and fixed with formaldehyde (4% final concentration), and finally stained with erythrosine in the laboratory.

Subsamples were taken for counting and identification of organisms in a counting wheel under a binocular microscope, to obtain zooplankton abundances at each site, and at the most precise taxonomic level possible. The minimum number of individuals counted was 100 per sample.

Zooplankton dry weight biomass was calculated from abundances, using individual biomass values from Dumont et al. (1975), and Hawkins & Evans (1979). Following values were used: rotifer and nauplius: 0.5 µg animal^{-1}; adult copepods: 10 µg animal^{-1}; copepodite: 5 µg animal^{-1}; cladocerans: 1 µg animal^{-1}.

1.2.3.4 Data analysis

Spearman rank correlation was used to detect relations among the environmental variables, zooplankton data and between variables and the distance from the mouth. Differences between series of values (e.g. temperature between the two sampling series) were tested by Mann–Whitney at $P<0.05$.

The distribution of zooplankton biomass was compared between the two sampling occasions using Kolmogorov–Smirnoff at P<0.05.

1.2.3.5 Spatio-temporal structuring

Firstly, similarity and dissimilarity in patterns of taxa abundances between all samples (including both sites and periods), were assessed by conducting a hierarchical ascendant classification using Euclidian distance and Ward agglomerative method.

Subsequently, multivariate analyses were performed to relate zooplankton distribution to environmental factors (temperature, conductivity, pH, oxygen concentration, SPM and OM, Chl*a* and phaeopigment concentrations). Selected data were centered and standardized by species. Abundances were log(x+1) transformed, in order to approach a normal distribution. A detrended canonical correspondence analysis (DCCA) with detrending by segments was first conducted using the CANOCO software package, version 4.5 (ter Braak et al., 1987; ter Braak, 1994), in order to determine the method of constrained ordination to use (Lepš & Šmilauer, 2003). Since the value of the length of gradients was less than 3.0, species were considered to have a linear response model and Redundancy Analyses (RDA) were then performed to investigate the relationships between environmental factors and taxa composition. A Monte Carlo test was applied (999 permutations) to test the statistical significance of variables to explain the ordination (P <0.05).

Also Generalized Linear Models (GLM, with Gaussian family) were conducted using R (R. Core Team, 2013) to identify the main environmental predictors of zooplankton biomass. Variables selected by the model were then subjected to Hierarchical Partitioning analysis (HP; Walsh et al., 2008) to estimate the independent contribution of each in explaining the biomass.

In order to assess tributaries effect on zooplankton taxonomic composition in the main course, the Bray–Curtis and Sørensen's similarity indices (Sørensen, 1948; Bray & Curtis, 1957) were calculated for each couple of consecutive sites along the water course.

1.2.4 Results

For clarity, the results and discussion sections will first consider the main water course (Scheldt) and afterwards the tributaries and their influence on the main water course.

1.2.4.1 Physical and chemical variables:

Using Melle (station Z5) as a reference site, the average daily discharge during sampling period was 28.03 m^3 sec^{-1} in April 2013 and only 3.32 m^3 sec^{-1} in June 2015 (Fig.2). Also the average daily discharge differed significantly in the month before the sampling ($P<0.01$).

Both in April and in June, temperature gradually decreased from upstream to downstream ($P<0.01$ and $P<0.05$, Fig. 3a), with systematically higher values in June that in April.

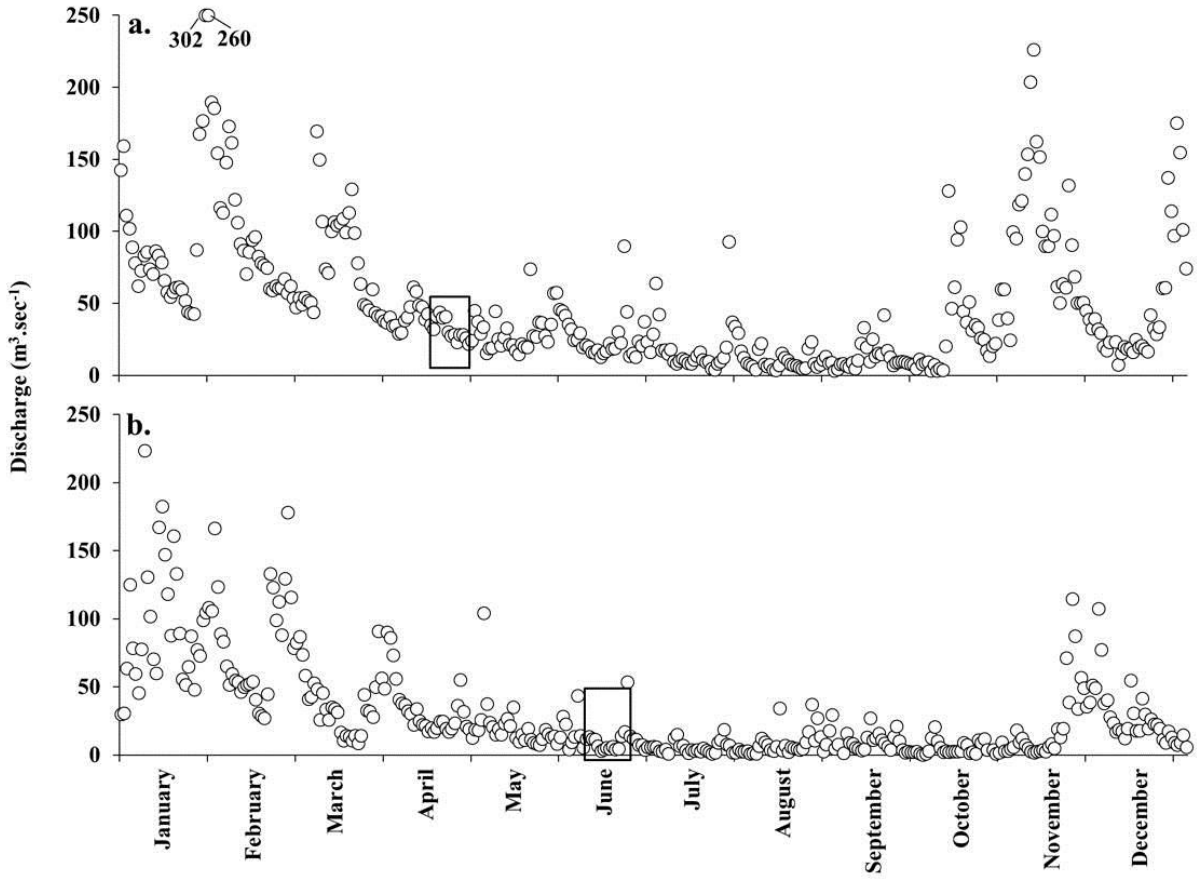

Fig.2 Average monthly discharge at Melle (Z5) in 2013 (a) and in 2015 (b). Frames represent sampling periods

The pH decreased from approximatively 8.2 to 7.7 downstream the freshwater reaches (from B7 to B1), and increased again to 8.2 in the polyhaline region of the estuary (Fig. 3b). Conductivity, reflecting the salinity gradient, displayed an increase from station Z1 downwards ($P<0.01$), reaching a maximum of 39 mS cm^{-1} at the mouth both in April and June (Fig.3c). In April, O_2 concentration was highest in the Bovenscheldt and the Westerscheldt with minimum values around the Zeescheldt, and was, on the contrary, lowest in the Bovenscheldt in June.

SPM and OM concentrations were both higher in June than in April ($P<0.01$ and $P<0.05$; Fig.3e, f), contrarily to O_2 and Chl*a* concentration values, which were generally higher in April (respectively $P<0.01$ and $P<0.05$; Fig. 3d,g). In June 2015, a sharp increase of SPM, OM, Chl*a* and

phaeopigment concentrations was observed at Z5, and the 3 highest values of Chl*a* and phaeopigments were displayed in the freshwater tidal zone (stations Z3 to Z5).

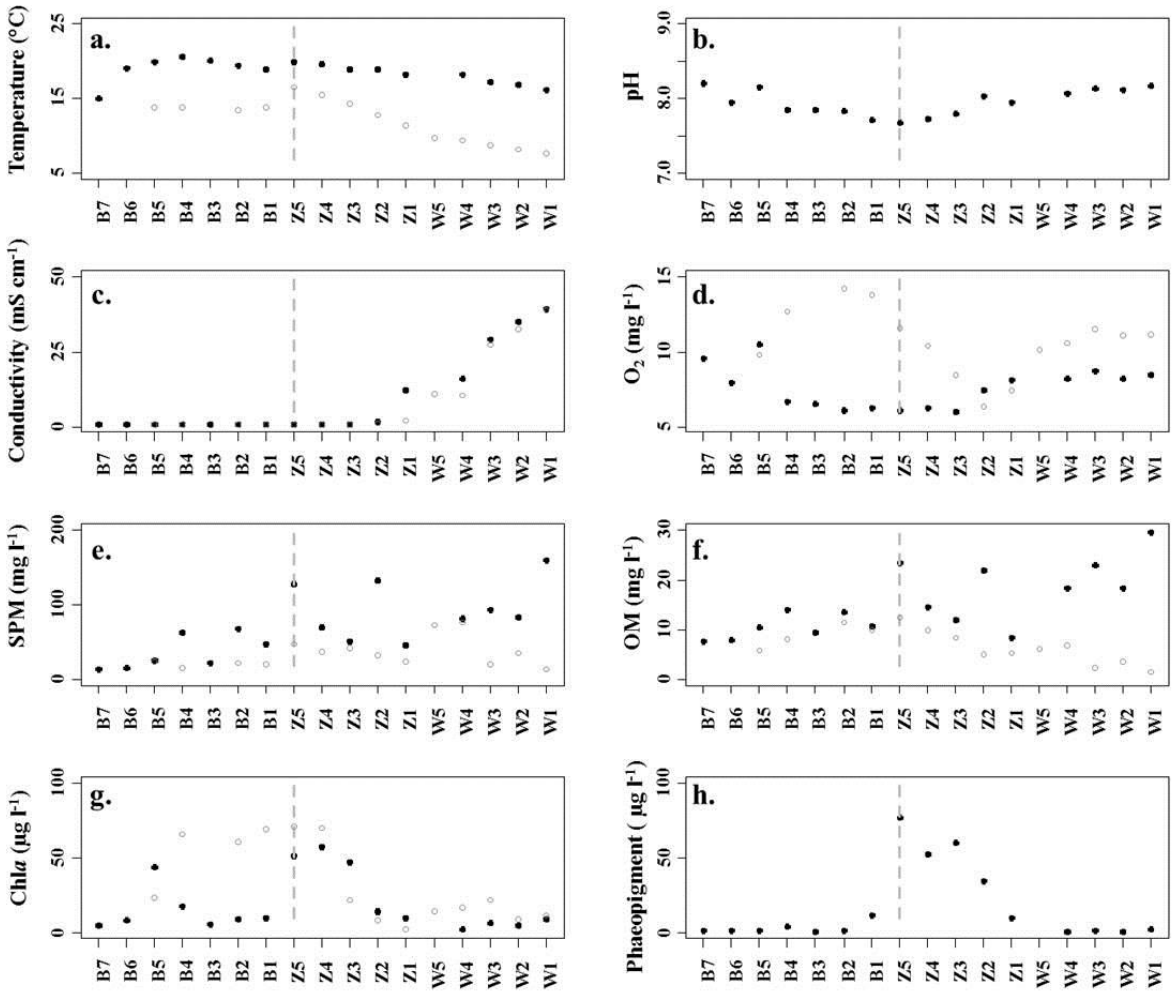

Fig.3. Temperature (a.), pH (b.), conductivity (c.), dissolved oxygen (d.), SPM (e.), Organic Matter (f.) Chl*a* (g.) and phaeopigment (h.) concentrations as a function of the distance from the mouth (W1) at each sampling site of the Scheldt continuum. Empty circles: April 2013, black circles: June 2015. The dotted line represents the end of the tidal influence at 150 km from the mouth

1.2.4.2 Zooplankton composition

41 taxa were identified in April and 43 in June (Table 2) in the Scheldt water course. In the freshwater riverine and estuarine reaches, the number of taxa varied between 5 and 23 taxa both in April and in June, and was significantly lower the brackish-marine reach (Z1-W4) where only 5 taxa were observed in April and June (Fig 4). This sharp decrease of diversity occurred in April between Z1 and W5, while in June diversity dropped upstream, between Z2 and Z1 where salinity was lower than in April. An increase in diversity was observed in the marine part of the Westerscheldt, reaching 9 to 11 taxa in April and June respectively. Maximal diversities were found at B5 (23 taxa) in April and in June.

Table 2. Zooplankton taxa observed in the Scheldt continuum and tributaries, their abbreviations, as employed in ordinations, and their percentage of total abundance in both sampling campaigns

		April 2013	June 2015
Rotifera			
Bdelloida	*(Bde)*	0.41%	0.27%
Monogononta			
Asplanchna sp.	*(Asp)*	0.97%	0.04%
Brachionus angularis (Gosse, 1851)	*(Ban)*	4.62%	3.44%
Brachionus calyciflorus (Pallas, 1766)	*(Bca)*	50.56%	4.66%
Brachionus diversicornis (Daday, 1883)	*(Bdi)*	-	0.01%
Brachionus leydigii (Cohn, 1862)	*(Ble)*	0.44%	<0.01%
Brachionus quadridentatus (Hermann, 1783)	*(Bqu)*	0.03%	0.10%
Brachionus rubens (Ehrenberg, 1838)	*(Bru)*	-	0.01%
Brachionus urceolaris (Müller, 1773)	*(Bur)*	0.66%	0.02%
Cephalodella sp.	*(Cep)*	0.08%	0.66%
Euchlanis sp.	*(Euc)*	0.06%	0.22%
Filinia longiseta (Ehrenberg, 1832)	*(Flo)*	4.09%	0.06%
Kellicotia sp.	*(Kel)*	0.10%	-
Keratella cochlearis (Gosse, 1851)	*(Kco)*	8.70%	10.40%
Keratella quadrata (Müller, 1786)	*(Kqu)*	6.35%	1.50%
Lecane sp.	*(Lec)*	0.01%	0.01%
Lepadella sp.	*(Lep)*	0.02%	-
Monomatta sp.	*(Mon)*	-	0.01%
Notholca sp.	*(Not)*	0.27%	0.03%
Polyarthra sp.	*(Pol)*	9.78%	4.14%
Synchaeta sp.	*(Syn)*	3.78%	24.37%
Trichocerca sp.	*(Trc)*	-	0.01%
Trichotria sp.	*(Trt)*	<0.01%	15.86%

Copepoda		April 2013	June 2015
Eurytemora affinis (Poppe, 1880)	*(Eaf)*	0.41%	0.65%
Pseudocalanus elongatus (Boek, 1864)	*(Pel)*	-	<0.01%
Temora longicornis (Müller, 1792)	*(Tlo)*	0.01%	-
Acartia sp.	*(Aca)*	<0.01%	<0.01
Centropages hamatus (Lilljeborg, 1853)		<0.01%	-
other Calanoida	*(Cal)*	0.04%	0.06%
adult Harpacticoida	*(Har)*	<0.01%	0.04%
adult Cyclopoida	*(Cyc)*	0.08%	0.70%
Harpacticoida copepodite	*(Har.c)*	<0.01%	0.03%
Cyclopoida copepodite	*(Cyc.c)*	0.51%	1.67%
Calanoida copepodite	*(Cal.c)*	0.28%	0.76%
Nauplii	*(Nau)*	7.39%	25.30%
Cladocera			
Alona sp.	*(Alo)*	0.02%	0.12%
Bosmina sp.	*(Bos)*	0.12%	2.58%
Ceriodaphnia sp.	*(Cer)*	<0.01%	0.05%
Chydorus sp.	*(Chy)*	0.07%	0.77%
Daphnia sp.	*(Dap)*	0.02%	0.38%
Illyocryptus sp.	*(Ill)*	<0.01%	<0.01
Macrotrix sp.	*(Mac)*	<0.01%	-
Pleuxorus sp.	*(Ple)*	-	<0.01%
other cladocerans	*(Cla)*	-	0.25%
Other			
Echinodermata		-	0.19%
Oikopleura		-	0.40%
Tintinnida		-	0.15%
Polychaeta		0.01%	0.07%
Cirripedia nauplii		<0.01%	-
Gastropoda larvae		<0.01%	-
Bivalvia		0.10%	-

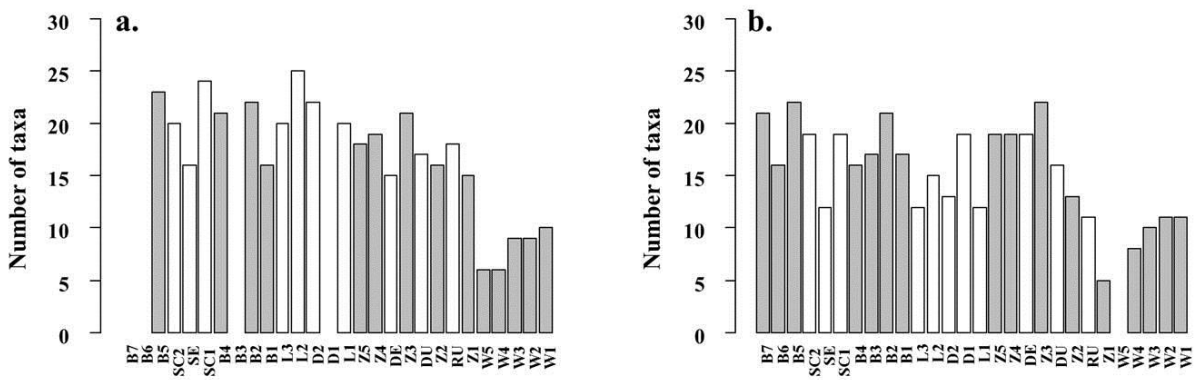

Fig.4. Number of taxa identified in April 2013 (a.) and in June 2015 (b.), at each sampling site of the Scheldt continuum (grey) and in tributaries (white)

The average zooplankton abundance observed was 5.49 × 10^5 ind.m^{-3} in April and 3.10 × 10^5 ind.m^{-3} in June (Fig.5). Overall, rotifers accounted for 92% of total zooplankton abundance in April. The major part of this abundance was due to *Brachionus calicyflorus*, and to a lower extent to: *Keratella cochlearis*, *Keratella quadrata*, *Filinia longiseta, Polyarthra* sp., *Synchaeta* sp. and *Brachionus angularis*. The maximal rotifer abundance in April was 4.57 × 10^6 ind. m^{-3} (station B1: Zingem).

In June, rotifers represented 66% of total abundance, and reached their highest abundance at station Z3 (Dendermonde), with 1.21 × 10^6 ind. m^{-3}. They were in majority represented by *Trichotria* sp. and *Synchaeta* sp., followed by *Brachionus*, *Keratella*, and *Polyarthra* species.

Copepods (including all stages), accounted for only 7% of total zooplankton abundance in April, reaching 2.6 × 10^5 ind. m^{-3} at Z2 whereas in June, they represented a higher part of zooplankton abundance: 30% of total zooplankton with a maximum of 4.26 × 10^5 ind.m^{-3} at Z3.

Finally, cladocerans were the least abundant group, representing less than 1% of zooplankton abundance in April and only 3% in June. Abundances were much higher in June reaching 30 × 10^3 ind.m^{-3}, compared to maximally 5.77 × 10^3 ind.m^{-3} in April.

The zooplankton community in the upstream freshwater reaches of the Scheldt from B1 to B6 was clearly dominated by rotifers (Fig.5). In these freshwater parts, cladocerans were scarce (0.17% of total abundance in April and 5% in June), and copepods (0.8% of upstream zooplankton abundance in April and 22% in June) were principally represented by cyclopoid species.

In the estuarine fresh- brackish water Zeescheldt (stations Z1 to Z5) rotifers were still dominating (respectively 75 and 64% of total zooplankton in April and June) and copepods were

dominated by calanoids and reached their highest abundance along the continuum (2.59 × 10^5 ind.m^{-3} in April and 4.26 × 10^6 ind.m^{-3} in June).

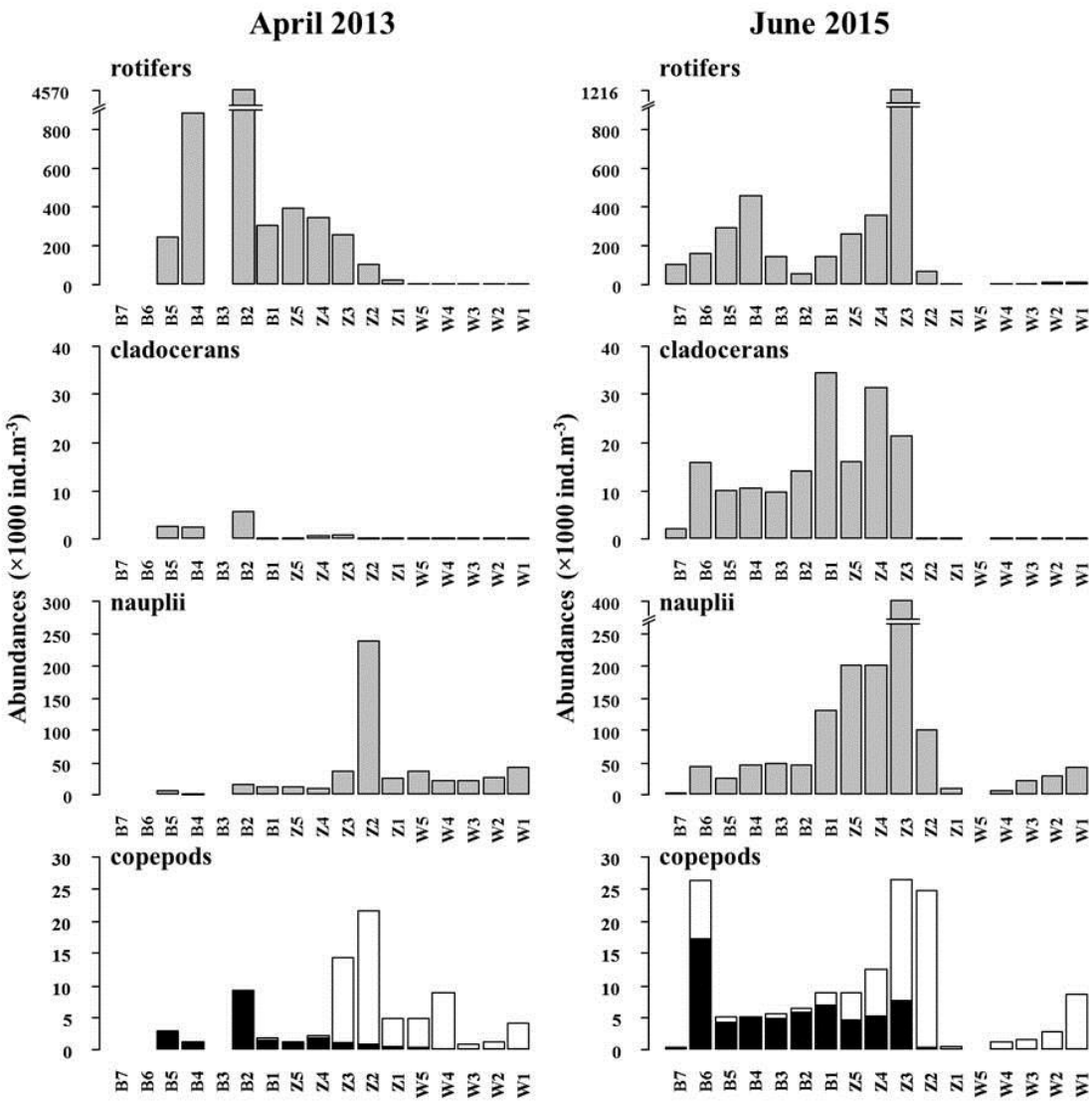

Fig.5. Abundance of rotifers, cladocerans, copepod nauplii, copepod copepodites and adults (white: calanoids, black: cyclopoids, grey: harpacticoids) in April 2013 (left) and in June 2015 (right), at each sampling site of the Scheldt continuum

From Z1 to the mouth of the Westerscheldt, rotifers were much less abundant or absent (April), and practically exclusively represented by the genus *Synchaeta* sp. (June). Copepods dominated the assemblage. These were represented by three taxa of calanoids: *Eurytemora affinis, Pseudocalanus elongatus*, and *Acartia* sp.. Harpacticoids were also present in the Westerscheldt (up to 3.1 × 10^3 ind.m^{-3} at W1 in June), while they were quasi absent from the upstream reaches of the Scheldt. Cladocerans were absent from the Westerscheldt. Marine planktonic organisms

such as echinoderms larvae, appendicularians and tintinnids were observed in low abundances (423 to 4.35 × 10^4 ind. m^{-3}) (they are therefore not represented in Fig.5).

The distribution of rotifers, calanoids, cyclopoids and nauplii (Fig.5) did not differ significantly along the main water course (Kolmogorov-Smirnov, $p>0.05$) between April and June. Only cladocerans were differently distributed ($p<0.05$), showing high abundances between B1 and Z3 in June, which was not the case in April.

1.2.4.3 Zooplankton spatio-temporal structuring

In the cluster analysis, Westerscheldt samples were clearly separated (cluster I) (Fig. 6). The remaining samples (clusters II-V) were divided in two clusters, separating April sites (clusters II + III) and June sites (clusters IV + V). At the next level non tidal sites (cluster II; cluster V) are separated from the tidal sites (cluster III; cluster IV).

As the cluster analysis clearly separated the Westerscheldt sites from the other study sites, Redundancy Analyses (RDA) were conducted on the dataset excluding Westerscheldt samples in order to avoid the influence of conductivity on the ordinations and to favor detection of other factors differentiating zooplankton communities in the brackish-freshwater reaches (Z1 and upstream including tributaries).

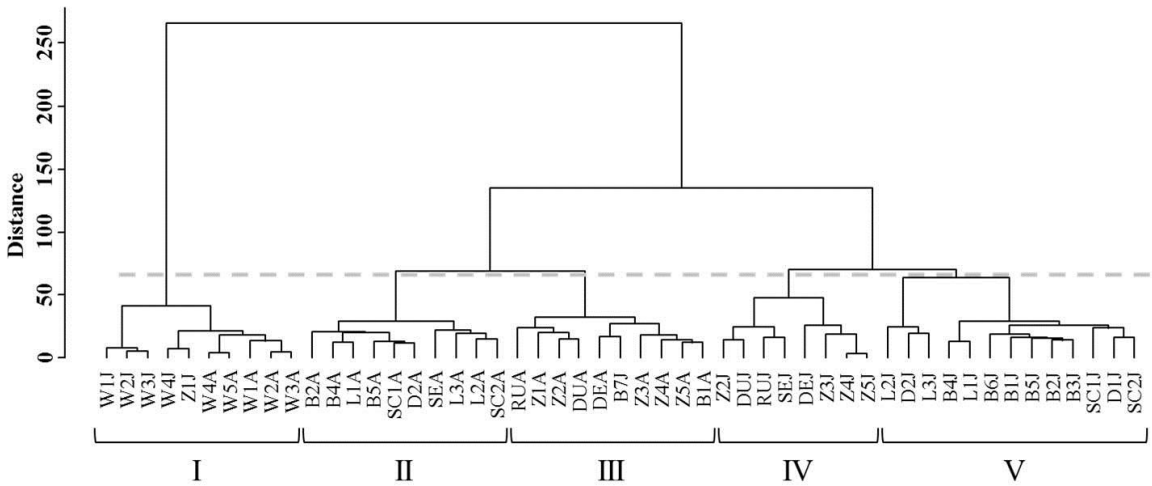

Fig.6. Dendrogram of the hierarchical classification of sampling sites for April 2013 and June 2015 samplings. Site codes are followed by the letter A for the April 2013 campaign, and by the letter J for the June 2015 campaign. The dotted line represent the cut-off level choosed for describe the dendrogram.

RDA on samples of both sampling occasions explained 31.8% of the total variance in zooplankton abundance data (Fig.7). O_2, Chl*a,* OM concentrations, and temperature were significant factors structuring the zooplankton community (Monte-Carlo P <0.05). The ordination separated April and June samples along the first axis, and tidal and non-tidal sites along the second one. While the seasonality was represented by an opposition between high temperatures in June and high Chl*a* and O_2 concentrations in April, the factors characterizing tidal sites were mainly OM and SPM vectors which were opposed to the higher oxygen concentrations of upstream sites. April was mainly associated with rotifer taxa, in particular the most abundant ones, while June samples were rather associated with copepods and cladocerans. Tidal sites, on the positive part of the second axis were associated with calanoids, especially *E. affinis* and nauplii.

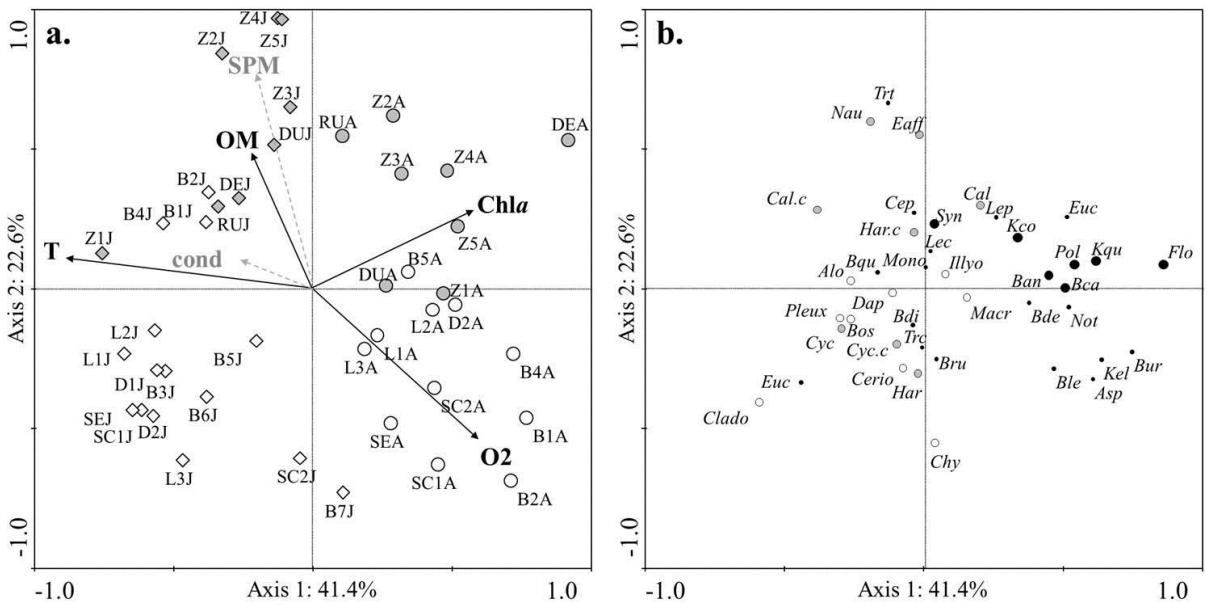

Fig.7. Ordinations of RDA analysis: Axis 1 and 2 biplots for: (a) environmental variables (T: Temperature, cond: Conductivity, SPM: Suspended Particulate Matter, OM: Organic Matter, Chla: chlorophyll a, O2: Oxygen concentration) and sampling sites, excluding the Westerscheldt (circles: April 2013, diamonds: June 2015, grey: Zeescheldt and its tributaries, white: Bovenscheldt and its tributaries), and (b) taxa (grey circles are for copepods, white for cladocerans and black for rotifers. The bigger symbols represent the most abundant taxa). For taxa abbreviations see Table 2. Full arrows represent significant environmental factors, dotted arrows non-significant factors (Monte Carlo permutations test, P <0.05)

1.2.4.4 Zooplankton biomass and trophic conditions:

Total zooplankton biomass was not differently distributed along the main water course between the two sampling occasions (Kolmogorov-Smirnov, P>0.05). Both in April and June, zooplankton biomass varied irregularly along the course, and no correlation was found with the distance from the mouth (P >0.05; Fig.8a,b). Extreme high values were seen, particularly at B2 in April, due to a rotifer peak (and mainly to *Brachionus calyciflorus*, accounting for 74% of total zooplankton biomass), and at Z3 in June, with a majority due to *Trichotria* sp. (51% of total zooplankton biomass), followed by nauplii (16%) and *Eurytemora affinis* (10%). In general rotifers dominated the biomass in the Bovenscheldt, and copepods in the Zeescheldt and the Westerscheldt. Over the total course, copepods were important contributors to biomass, representing 18% in April and 47% in June.

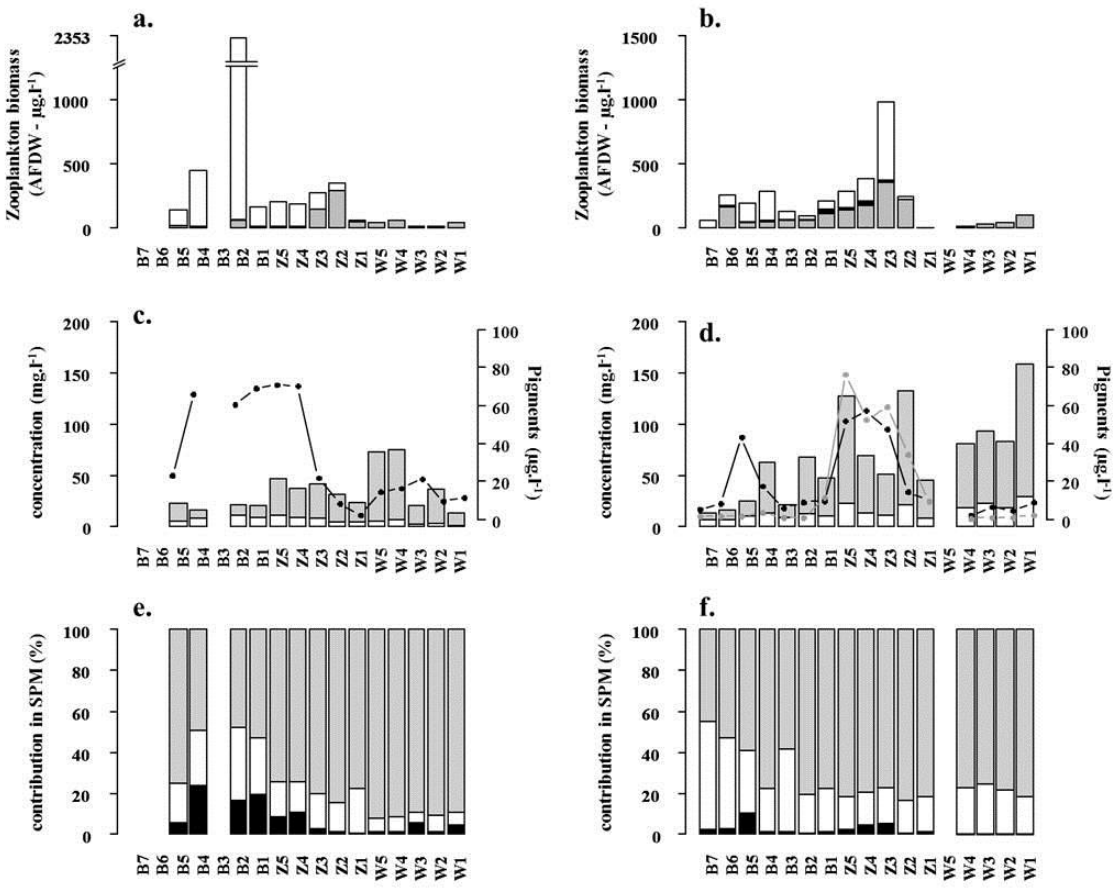

Fig.8. Zooplankton biomass and trophic conditions at each sampling site of the Scheldt continuum: zooplankton biomass estimations for April 2013 (a) and June 2015 (b) respectively (bar plots; copepods (all stages) in grey, cladocerans in black and rotifers in white); (c.) and (d.): SPM (grey and white), OM (white), and pigment concentrations (black line for Chla and grey line for pheopigments); (e.) and (f.): percentage of phytoplanktonic (black), organic white), and inorganic grey) matter in the SPM composition

Trophic conditions did also vary along the main course. In April, SPM concentrations were highest in the brackish reach, Chl*a* concentrations were higher in the upper freshwater reaches (from B4 to Z4, Fig.8c) than downstream ($P<0.05$).

Also phytoplankton contribution to SPM was maximal in this reach (Fig.8e). In June, SPM concentration varied irregularly over the continuum (Fig.8d). Inorganic matter contribution to SPM was higher from Z5 to the mouth than upstream in both April and June ($P<0.01$ in April, $P<0.05$ in June; Fig.8e,f).

Trophic conditions also differed between the two sampling periods. Both SPM and OM concentrations were higher in June than in April ($P<0.05$ and $P<0.001$ respectively). However, the OM/SPM ratio along the water course was not significantly different between April and June ($P>0.05$). Phytoplankton concentration and contribution to SPM was maximal in the upstream riverine and estuarine reaches in April, while it peaked at B5 and in the estuarine freshwater zone in June (Fig. 8e,f)

GLM analyses conducted on zooplankton total and taxonomic group biomasses using the same environmental factors and excluding the Westerscheldt samples (Table 3) showed conductivity as a negative predictor of total zooplankton biomass while Chl*a* concentration was a positive predictor, not only for total biomass but also for rotifer biomass. Oxygen concentration was found as a negative predictor of total zooplankton biomass, and of each studied group. Rotifer, cladoceran and cyclopoid biomasses were negatively influenced by conductivity. SPM concentration, which was a negative driver for rotifer and cyclopoid biomasses, resulted being a positive driver for calanoids. Finally, OM concentration was a positive driver for cyclopoids and a negative one for cladocerans.

Table 3 Summary of the generalized linear models (GLM) analysis and Hierarchical partitioning performed on zooplankton biomass in both April 2013 and in June 2015. 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. 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

	D^2	predictor	coeficient	%	p.value (chi^2)
total biomass	**32.95%**	temp			
		conductivity	-	46%	**
		oxygen	-	14%	
		SPM			
		OM			
		chl a	+	40%	**
rotifers biomass	**50.45%**	temp			
		conductivity	-	41%	***
		oxygen	-	5%	
		SPM	–	6 %	
		OM			
		chl a	+	48 %	***
cladocerans biomass	**46.89%**	**temp**	+	20%	**
		conductivity	-	60%	***
		oxygen	-	16%	
		SPM			
		OM	–	3 %	
		chl a			
cyclopoids biomass	**54.69%**	temp			
		conductivity	-	59%	***
		oxygen	-	11%	
		SPM	-	10%	*
		OM	+	20%	
		chl a			
calanoids biomass	**22.01%**	temp			
		conductivity			
		oxygen	-	61%	**
		SPM	+	39%	
		OM			
		chl a			

1.2.4.5 Tributaries

Some values of zooplankton abundance in tributaries were strongly different from those observed in the main stream (Fig 9). In particular, some tributaries displayed much higher or lower values than the site upstream of the confluence, and may thus create an input or a dilution of organisms. Besides differences in abundance between tributary and main course, the discharge of both will also influence the tributary effect observed.

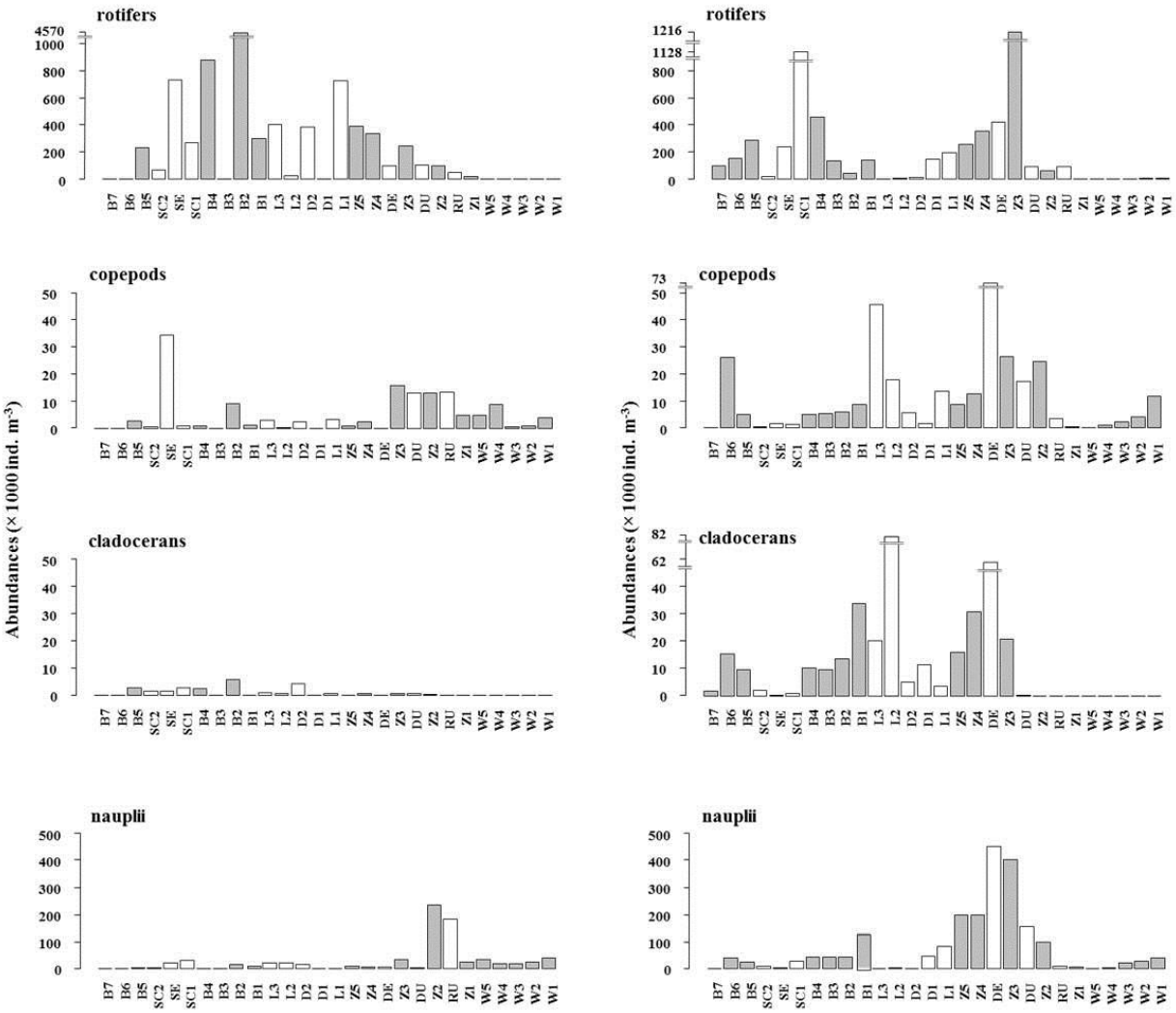

Fig.9. Zooplankton abundance in the stations of the main course (grey) and tributaries (white)

For example, in June, the Scarpe, which flows in the Bovenscheldt between B5 and B4, had higher zooplankton abundance at station SC1 than the main Scheldt at B5 (+253%) mainly because of rotifers (Fig.9), and rotifers abundance was also higher at the downstream station B4 than at B5. On the contrary, in the same season, the Durme displayed much less zooplankton abundance than the main course upstream of their confluence (-84%). However, the abundances observed downstream of the confluence were not systematically consistent with a pattern of

increase or decrease suggested by tributary abundances. For example, despite the fact that the Dender carried a high abundance of cladocerans in June compared to Z4, these were less abundant downstream the confluence at Z3.

Bray–Curtis indices values between two consecutive sites along the main water course not interrupted by a tributary were generally > 50%, showing that zooplankton communities were rather similar. Exceptions were B4-B2 and B2-B1 in April, B7-B6 and W4-W3 in June (Table 4), but low values (below 50% similarity) were particularly observed between the sites upstream and downstream from the Rupel and the Durme both in April and in June, and the Scarpe in April. On the contrary, despite the confluences with the Dender in April and with the Scarpe in June, the values of Bray-Curtis similarities were above 50% between upstream and downstream the confluence, indicating more similar communities. Sørensen's index values were generally high (often >80%), indicating few changes in taxonomic composition along the water course, the only value below 50% being between Z1 and W5 in April. Sørensen's were not lower when confluence was localized between two sites.

Table 4. Values of Bray Curtis and Sorenson's similarity indices calculated from zooplankton abundances for each pair of sites "upstream-downstream" along the water course of the Scheldt in April 2013 and in June 2015. When a tributary flows in the main course between the two sites of a pair, its name is indicated into brackets and the value is in bold.

April 2013		Bray-Curtis	Sorenson	June 2015		Bray-Curtis	Sorenson
				B7-B6		0.23	0.72
				B6-B5		0.59	0.85
B5-B4	(Scarpe)	**0.17**	**0.91**	B5-B4	(Scarpe)	**0.62**	**0.80**
				B4-B3		0.54	0.86
B4-B2		0.32	0.88	B3-B2		0.67	0.85
B2-B1		0.11	0.74	B2-B1		0.52	0.77
B1-Z5		0.87	0.88	B1-Z5		0.71	0.94
Z5-Z4		0.89	0.86	Z5-Z4		0.76	1.00
Z4-Z3	(Dender)	**0.73**	**0.80**	Z4-Z3	(Dender)	**0.51**	**0.88**
Z3-Z2	(Durme)	**0.45**	**0.81**	Z3-Z2	(Durme)	**0.19**	**0.69**
Z2-Z1	(Rupel)	**0.23**	**0.84**	Z2-Z1	(Rupel)	**0.10**	**0.56**
Z1-W5		0.58	0.40				
W5-W4		0.72	1.00	Z1-W4		0.73	0.80
W4-W3		0.84	0.55	W4-W3		0.39	0.55
W3-W2		0.88	0.83	W3-W2		0.84	0.92
W2-W1		0.74	0.62	W2-W1		0.73	0.86

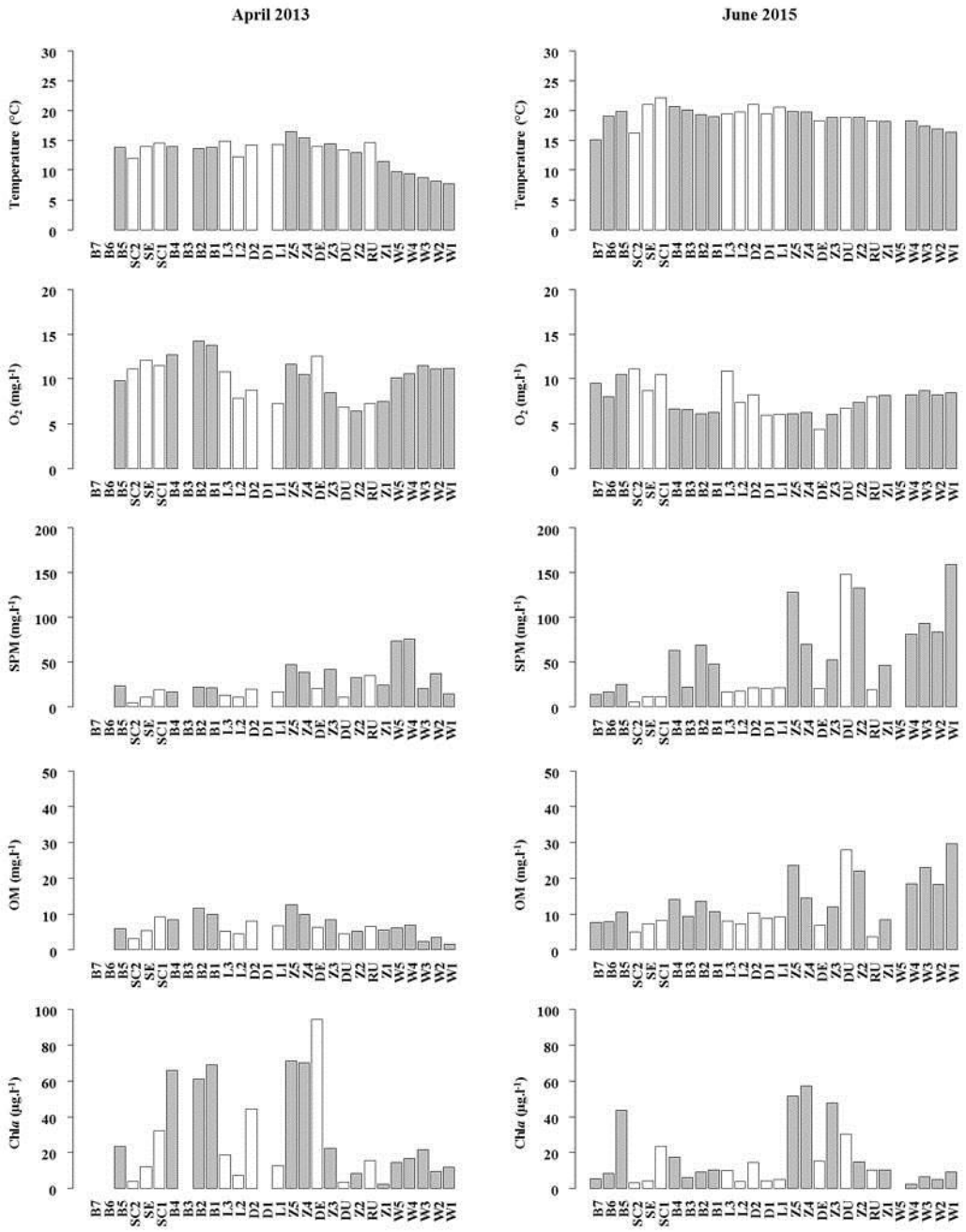

Fig.10 Temperature, oxygen SPM OM and Chla concentrations in the stations of the main course (grey) and tributaries (white) in April (left) and June (right)

Environmental variables also differed in the tributaries and in the main course (Fig.10). Both in April and June, the Lys had lower values of SPM, OM and Chl*a* than the main course upstream (B1), but no consequent decrease was observed downstream at Z5. On the contrary, in 2015, the Durme was carrying an important concentration of OM and SPM, and an increase was shown downstream the confluence (Z2).

1.2.5 Discussion

Inspired by the concepts developed following Remane's (1934) paper and the RCC (Vannote et al. 1980), this study examined the zooplankton composition, abundance and biomass from near the source to the mouth of a lowland river, the Scheldt, and related them to the physical and chemical variables and trophic conditions. The Scheldt having an entire gradient from the river to the fresh, brackish and marine tidal zones offered a unique opportunity to consider the continuity (or discontinuity) between the riverine (non-tidal) and the estuarine tidal reaches, as well as between the freshwater and the salinity-gradient tidal reaches.

In the following, various aspects concerning the zooplankton community composition and spatio-temporal distribution are discussed as they arose from the cluster analysis (Fig. 6) and can be interpreted by the basic data and other multivariate analysis performed. When relevant, the results will be situated within the existing concepts on river continua.

Hydrological conditions are considered as an important factor for zooplankton development: in particular residence times, or inversely current velocity can be a limiting factor for phytoplankton and zooplankton growth (Saunders & Lewis, 1988; Baranyi et al., 2002; Lair, 2006). According to Rzoska (1978), current velocities higher than 0.4 m sec^{-1} do not permit plankton development. However, low-flow systems present residence times sufficient for plankton development (Saunders & Lewis, 1988). Given the residual current velocities found in the upstream freshwater channelized reach of the Scheldt (0.06 m sec^{-1} at B5 in April), zooplankton development would not be limited by current velocity. Our results showed indeed that the entire system, including the upstream riverine sites supported zooplankton development, with substantial abundance and biomass. In the riverine sites upstream from Gent, including tributaries, zooplankton abundances reached on average 6.7×10^6 ind.m^{-3} in April and 2.9×10^6 ind.m^{-3} in June. These are even within the range reported for lentic systems: zooplankton abundance in Lake Blaarmeersen (Belgium) reached a maximum of 2×10^6 ind.m^{-3} in early summer 2003 (Van Gremberghe et al., 2007), and in the Loosdresht lakes (Netherlands) they reached of 22×10^6 ind.m^{-3} in summer 1983 (Gulati et al., 1992). Pace (1986) found average annual densities ranging between 10^5 and 10^6 ind.m^{-3} in Quebec lakes (Canada). Substantial zooplankton abundance has been observed in other low-flow rivers. In the non-tidal low flowing Nakdong River (Korea), Kim & Joo (2000) found a longitudinal pattern of increasing abundance downwards, reaching up to 2.2×10^6 ind.m^{-3} near the mouth due to higher residence times due to a dam at the mouth. Akopian et al. (2002) found in the upper Seine 0.15×10^6 ind.m^{-3} and increasing densities downwards, due to the confluence with the Marne reservoir and to the increase of residence times.

Salinity was considered as the main structuring factor for macrobenthos diversity in salinity–gradient systems in Remane (1934) and following papers (De Jonge, 1974; Ysebaert et al., 1993; Laprise & Dodson, 1994; Bruyndoncx et al., 2002; Piscart et al., 2005; Telesh & Khlebovich, 2010; Telesh et al., 2011). In this study, **conductivity** (as a proxy of salinity), also played a major role. In the Westerscheldt, where salinity strongly increased, the dominant zooplankters were calanoid copepods, with only 3 taxa (*Temora longicornis, Centropages hamatus* and *Acartia* spp.) present. Rotifers were only represented by the genus *Synchaeta* in June, and cladocerans and cyclopoids were absent. These taxa, which are typical of freshwater, are also negatively influenced by conductivity according to the GLM: despite the removing of Westerscheldt stations in the analysis, the remaining salinity differences were strong enough to be an important driver in the community composition. As a result, conductivity was the main negative driver for zooplankton biomass, in the GLM run on the total dataset. The paucity of taxa observed in the Westerscheldt also resulted in significant lower biomass there than in the more upstream reaches. The aspect of salinity as a diversity driver will be discussed below.

Apart from the effect of salinity on the Westerscheldt zooplankton composition, hierarchical classification based on zooplankton composition showed also **the importance of seasonality,** mainly influenced by differences in temperature and oxygen, Chl*a* and OM concentration between April and June. Crustacean abundances were associated to June samplings, but the higher temperatures in June had no significant impact on zooplankton biomass, except for cladocerans (Table 3). Oxygen concentration, associated with April samples, was, somewhat counter-intuitively, a negative driver for total zooplankton biomass. In our study, oxygen concentration was never below 4.35 mg l^{-1}, which could explain the absence of harmful effects on zooplankton communities since the threshold of hypoxia is generally considered at 2 mg l^{-1} (Roman et al., 1993; Vanderploeg et al., 2009; Ekau et al., 2010). In the Zeescheldt, Mialet et al. (2010, 2011) have shown that *E. affinis* can develop in the freshwater reach when oxygen concentration is > 4 mg l^{-1}. It is thus likely that, under non-limiting O_2 concentrations, other factors prevail as drivers for zooplankton abundance and composition. However, in our results, O_2 concentration was a negative predictor, suggesting that zooplankton was favored by low O_2 concentrations or by other factors, associated with oxygen concentrations. The first assumption could be explained by the possibility that low but non-lethal oxygen concentrations can alter trophic interactions and have in particular effects on predators, as demonstrated Breitburg et al. (1997). Fish are more sensitive than zooplankton to hypoxia, and it was, for example, observed that zooplankton in Lake Erie (USA) can use the hypoxic zone as a refuge to avoid predation (Vanderploeg et al., 2009). A1so, several of the rotifer species occurring in the Scheldt are known to be tolerant to low oxygen concentrations (eg. Bērziņš & Pejler, 1989b).

Factors negatively related to O_2 concentration in the June RDA ordination are SPM, OM, and Chl*a* concentrations (Fig. 7), so that the second assumption might be explained by trophic conditions.

Trophic conditions were represented in this study by the concentration and composition of SPM (SPM, OM and Chl*a* concentrations in April and June; phaeopigment concentration in June). RDA and GLM showed their influence in differentiating seasons and reaches in which different zooplankton taxa occurred.

Chl*a* concentration, as a proxy of phytoplankton biomass, was higher in April than in June, and was a positive predictor of total biomass and also of rotifers, according to GLM. In particular, Chl*a* was associated with most abundant rotifer taxa, as shown by RDA analysis. In April, O_2, OM and Chl*a* concentrations all reached maximum values in the Bovenscheldt (B stations), where also maximum abundance of the dominant rotifer taxa occurred. In June, OM showed high concentration from the Zeescheldt downwards, and Chl*a* and phaeopigment concentrations peaked specifically in the Zeescheldt (maximum at Z4). In the freshwater tidal reach, concentration of phaeopigments was even higher than those of Chl*a*, suggesting high phytoplankton mortality in this reach. Muylaert et al. (2000) have shown that in the freshwater tidal reach of the Scheldt, riverine phytoplankton declined after import into the estuary where it was replaced by autochthonous, estuarine populations.

The freshwater tidal reach also displayed the lowest O_2 concentration. Even after considerable water quality improvement (Van Damme et al., 2005; Cox et al., 2009) the Z2-Z4 reach of the Zeescheldt still sporadically shows oxygen minima during warm periods, as a consequence of a production/respiration ratio <1 (Cox et al., 2009). Under these conditions, rotifer abundance peaked somewhat more downstream, at Z3.

Considering the two sampling campaigns together in the GLM analysis, high Chl*a* concentrations especially favored the development of rotifers, which are known to be mainly controlled by bottom-up processes, and highly dependent of food resources. Their growth rate can increase strongly and rapidly in high food concentration conditions (Hansen et al., 1997; Walz, 1997). However, Chl*a* was not a predictor for other zooplankton groups. Cyclopoid copepods were rather favored by OM and thus possibly (also) by its detrital part. SPM concentration, highest in the Westerscheldt, and to a lesser extent in the Zeescheldt, where calanoids dominated, had a positive relation to calanoid biomass. Zooplankton will evidently not purposely eat inorganic particles, but can be hampered in its selection of (live or dead) organic food. GLM analyses including OM / SPM and Chl*a* / OM ratios showed the OM / SPM to be positive driver for cyclopoids and a negative one for calanoids (not shown). A high Chl*a* / OM-ratio was a positive

driver for total biomass and rotifers. In the Scheldt, cyclopoids occur mainly between stations B7 and Z4 where OM / SPM ratios were high.

The freshwater tidal zone (sites Z3, Z4, Z5) was characterized by high SPM and Chl*a* concentrations, with low OM/SPM ratios, but high Chl*a*/OM ratios. The Zeescheldt reach in fact displayed a particular zooplankton community composition, constituted mainly by calanoids, especially *Eurytemora affinis*, (representing 82% of total adult calanoids in June). *E. affinis* is known as a typical species of turbid estuarine environments (Castel & Feurtet, 1989; Castel & Veiga, 1990; Escaravage & Soetaert, 1993; Soetaert & Van Rijswijk, 1993; Sautour & Castel, 1995; Gasparini et al., 1999; Tackx et al., 2003; Winkler et al., 2003; David et al., 2005; Devreker et al., 2010). This species has been shown to be able to select phytoplankton within the turbid conditions prevailing in the brackish zone of both the Elbe (Germany) and the Scheldt estuary (Gasparini et al., 1999; Tackx et al., 2003). The abundance of *E. affinis* in the Scheldt upstream freshwater has increased recently following the improvement of water quality, in parallel with the decrease of cyclopoid abundance (Mialet et al., 2010, 2011; Chambord et al., 2016). The present study thus confirms the upstream expansion of *E.affinis* observed in these previous studies.

The resulting total zooplankton biomass in the Scheldt transect during early summer peaked in the tidal freshwater Zeescheldt, despite low-but not limiting- oxygen concentrations in this reach. Clearly, under such non limiting O_2 concentrations, favorable trophic conditions (high OM and/or Chl*a* concentrations) are important drivers for the spatial distribution of zooplankton biomass along the Scheldt transect.

Tributaries

The above results highlight the partitioning of the Scheldt main course in successive reaches subject to different physical and chemical variables and trophic conditions, influencing the zooplankton communities, in accordance to the riverine productivity model (Thorp & Delong, 1994). However, along the water course, confluences with several tributaries cause disruptions. The effects of confluences have been shown to create spatial discontinuities and have important impact on the latter (Rice et al., 2001, 2006; Kiffney et al., 2006; Thorp et al., 2006). In the Scheldt, flow regulation and water deviation for safety reasons or navigation purposes have led to important hydrological changes. In particular it induces important variations in tributary discharge and thus on their impact on the main stream (Meire et al., 2005).

Obviously, the impact of tributary confluences on zooplankton communities of the main course is highly dependent of the discharge. Since the discharge vary between Scheldt tributaries and in time, the contribution of confluences to main course community structure is difficult to quantify.

Bray-Curtis similarity index values within reaches non-interrupted by a tributary confluence indicated both consistency and changes in the zooplankton composition along the main course. Changes along the main course can be explained by changes in salinity (e.g. W4-W3) or other environmental conditions (eg. geomorphologic conditions between the main course at B7 and the channel at B6). The distance between two consecutive sampling sites may also decrease the similarity between communities (eg. B2-B4). However, important changes in zooplankton composition were most frequently, but not always, observed when a tributary joined between two sites, indicating a probable impact of tributaries on zooplankton. However, even when low Bray-Curtis similarities were observed, Sørensen's index was high. As the Sørensen's index takes only occurrence into account, while the Bray-Curtis index uses abundance data, the effect of confluences on zooplankton composition seems, in some cases, more implying changes in abundances than in taxonomic composition (e.g. the Scarpe in April, Table 4).

Rice et al. (2006) explained that tributaries contribute to important ecological changes along water courses by modifying physical, chemical and geomorphological characteristics, nutrient and organisms loads. In the Rhine, tributaries contributed not only to a substantial input of zooplankton, but also to variable nutrient and SPM loads (de Ruyter van Steveninck et al., 1992). It is also possible in the present case that environmental variables of the main course were modified by the inputs of tributaries, since results also showed different physical and chemical characteristics in some tributaries compared to the main course. The disturbance created by confluences themselves (mixing of waters, turbulence, local circulations etc.) may also change environmental conditions for zooplankton in these areas.

The **influence of the tide** was reflected in the hierarchical classification which distinguished freshwater sites between tidal and non-tidal ones. The tidal effect was not unfavorable for zooplankton since high abundance and biomass was found in the freshwater tidal reach (Z3–Z5). In particular, as described above, the calanoid copepod *Eurytemora affinis* had its maximal abundance in these sites. Indeed, the freshwater tidal zone of the Scheldt is rather a productive area for phytoplankton (Muylaert et al., 2000), despite the turbidity imposed by the tide. In the present study, the freshwater tidal reach is also characterized by the highest Chl*a* concentrations, despite high SPM and low oxygen concentrations, due to a favorable photic zone/mixing depth ratio (Muylaert et al., 2005). This illustrates the functional importance of this reach, which yet received less attention than riverine freshwater systems or brackish - marine

ones. It also arguments the necessity to restore and preserve these freshwater tidal reaches which have unfortunately disappeared from many estuaries.

The Scheldt longitudinal structure and processes can be viewed as a succession of "functional process zones", as described by Thorp et al. (2006) in the Riverine Ecosystem Synthesis (RES). The RES describes river networks as discontinuous systems, structured in "functional process zones" where the type of habitat defines ecosystem functions on which depend species density and diversity. In the present study, different functional zones could be identified: the upstream freshwater non-tidal reach dominated by rotifers, the freshwater tidal zone, where copepods and especially *Eurytemora affinis* dominated and where rotifers were still occurring, the brackish–marine zone, where only calanoids species and some marine taxa (appendicularians, echinoderms…) were found. In this study, zooplankton diversity in the estuarine Scheldt main course was minimal in the brackish tidal zone, in accordance with Remane's concept. Diversity slightly increased in the marine Scheldt waters as compared to the brackish zone, but was much higher in the freshwater reaches, reaching a maximum in the tidal freshwater reach. Soetaert & Van Rijswijk (1993), also found, in a 1989–1991 monitoring of the zone W1-Z1, a higher diversity in mesozooplankton in the marine than in the brackish water stretch in the months April and June (17 vs 8 and 18 vs 11 taxa respectively). In a detailed inventory of the zooplankton in 2009 at stations W5, Z1 and Z4, Azémar et al. (unpublished) observed 5 taxa at the brackish station W5, and 9 and 14 taxa at the freshwater tidal stations at Z1and Z4 respectively. So our results are in accordance with Remane's concept based on benthic taxa. It is indeed generally assumed that freshwater system present higher diversity levels than marine ones (Bloom et al., 2013; Dudgeon et al., 2015). However, it should be realized that comparisons of diversity along entire watercourse gradients, as established in this study, are rather rare in literature, and suffer from a differentiation in taxonomic skills which can probably be related to the existing segregation between scientist studying marine/estuarine and those studying freshwater, non-tidal systems. In our study, rotifers were mostly determined at species level, while Soetaert & Van Rijswijk (1993) only distinguished rotifers as one taxon, which likely reduced their estimation of diversity in the brackish reaches as compared to ours. Inversely, we did not distinguish typical marine taxa such as Bryozoa, Echinoderma, Noctiluca and Oicopleura, and did not differentiate further than genus level for the copepod *Acartia* spp. as did Soetaert & Van Rijswijk (1993) and probably underestimated diversity in the marine reach.

Telesh et al. (2011) found an exponential decrease of metazooplankton diversity (considering copepods and cladocerans) along the salinity gradient of the Baltic Sea. When also considering ciliates in the diversity estimation, these authors add another 164 planktonic species, peaking at salinity 4–8, overlapping with Remane's (1934) diversity minimum salinity range. This again

illustrates the delicacy of considering diversity without a very precise framework on taxonomic resolution and uniformed definitions of (planktonic) group taxonomic composition.

In conclusion, this study showed the existence of four successive functional zones for zooplankton:

-**The freshwater non-tidal zone** (B7-B1), with higher temperature and O_2 concentration. Chl*a* concentration was also high, in particular in April. Rotifers constituted the majority of zooplankton abundance, and copepods (mainly nauplii and cyclopoids) and cladocerans were also found.

-**The freshwater tidal zone** (Z5-Z2), with highest Chl*a* and phaeopigments concentrations, and important OM and SPM concentrations. It's the zone of maximal zooplankton biomass: high abundances of rotifers and copepods calanoids *Eurytemora affinis* and nauplii were found, while cyclopoids and cladocerans decreased.

-**the brackish zone** (Z1-W5), where salinity sharply increased. Zooplankton abundances and diversity decreased. Freshwater species (rotifers, cyclopoids and cladocerans) dropped.

-**the marine zone** (W4-W1), characterized by high salinities and low temperatures. Zooplankton is principally represented by calanoids, mixed with inclusions of marine species, increasing diversity.

Existing concepts have essentially been developed for benthic organism, on pelagic components and organisms. The demonstration that zooplankton abundance and diversity was highest in the tidal freshwater zone demonstrated the potential of combining riverine and estuarine studies, and the ecological importance of such transition zones and their adequate management.

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CHAPITRE II :

COMPOSITION DES COMMUNAUTES ZOOPLANCTONIQUES DANS LE BASSIN VERSANT EN AMONT D'UN FLEUVE DE PLAINE (L'ESCAUT) : VARIABILITE SPATIALE, INTERANNUELLE ET SAISONNIERE.

- ***Article soumis dans Freshwater Biology***

2.1 RÉSUMÉ DU CHAPITRE:

2.1.1 Introduction

Le **chapitre 1** a mis en évidence des abondances importantes de zooplancton dans l'ensemble des tronçons de l'Escaut, y compris dans la partie amont de son bassin versant.

En raison de son rôle clé dans les réseaux trophiques pélagiques et de sa sensibilité aux conditions environnementales, le zooplancton peut être considéré comme un bon indicateur de la qualité de l'eau, et plusieurs auteurs déplorent le fait qu'il ne soit pas inclus parmi les Éléments de Qualité Biologiques utilisés pour la détermination de l'État Écologique de la Directive Cadre sur l'Eau (Moss, 2007 ; Nõges *et al.*, 2009 ; Jeppesen *et al.*, 2011 ; Haberman & Haldna, 2014). L'étude des interactions entre les communautés zooplanctoniques et les facteurs environnementaux qui influencent leur variabilité est donc importante pour la compréhension du fonctionnement des écosystèmes.

Dans les tronçons estuariens de l'Escaut, de précédentes études ont montré des changements dans les communautés zooplanctoniques parallèlement à l'amélioration de la qualité de l'eau (Appeltans et al, 2003 ; Mialet et al., 2010, 2011 ; Chambord et al., 2016). En revanche, le zooplancton des cours d'eau en amont du bassin versant de l'Escaut demeure peu documenté. Cette région à la frontière entre le nord de la France et la Belgique est sujette à de multiples sources de pollution. Les cours d'eau y constituent un réseau dense de canaux, et sont classifiés comme de qualité variable (de mauvais à bon) selon la Directive Cadre sur l'Eau.

2.1.2 Objectifs

En s'appuyant sur un ensemble de **stations évaluées par la Directive Cadre sur l'Eau**, l'étude vise à décrire la **variabilité spatiale, interannuelle et saisonnière** des communautés zooplanctoniques du bassin versant et à identifier les **facteurs généraux (c'est à dire physico-chimiques sans prendre en compte les polluants) influençant cette variabilité**. 18 stations ont été échantillonnées lors de 5 campagnes d'échantillonnage réparties entre avril 2013 et juin 2015.

Dans un premier temps, la variabilité spatiale, temporelle et interannuelle du zooplancton a été caractérisée en utilisant la méthode de Souissi et al. (2001) et Anneville et al. (2002), couplant classification hiérarchique des échantillons et identification des taxons indicateurs des classes ainsi obtenues (par calcul d'indices IndVal).

Une fois les profils de variabilité du zooplancton identifiés, des analyses multivariées ont été utilisées pour identifier les potentiels facteurs physico-chimiques pouvant expliquer cette variabilité.

Les différents profils de distribution spatiale ont ensuite été plus spécifiquement étudiés : les profils de distribution des communautés zooplanctoniques ont été mis en relation avec l'état écologique des stations, et avec les profils de variabilité environnementale.

2.1.3 Principaux Résultats et Discussion

Les résultats, en incluant l'ensemble des stations et des périodes d'échantillonnage, confirment l'abondance importante des communautés zooplanctoniques, principalement dominées par les rotifères (92 % de l'abondance totale) dans la partie amont du bassin versant de l'Escaut, avec une moyenne globale de $4.33 \times 10^5 \pm 9.84 \times 10^5$ ind.m^{-3}.

La principale source de variabilité des communautés zooplanctoniques au sein de la zone d'étude a été identifiée comme étant la saisonnalité, puisque les communautés des échantillons des mois d'avril 2013, 2014 et 2015 se distinguaient nettement de celles des échantillons de septembre 2014 et de juin 2015. Les facteurs influençant cette différenciation étaient les concentrations en oxygène et chlorophylle *a* (indicateur de biomasse phytoplanctonique), associées aux échantillons du printemps, et en opposition à la température, caractérisant les échantillons prélevés en été.

Les taxons identifiés comme plutôt caractéristiques du printemps étaient les rotifères, et en particulier les taxons majoritairement abondants représentés par les brachionidés (*Brachionus* sp., *Keratella sp.*), tandis qu'en association avec les mois d'été et les températures les plus élevées, des rotifères moins abondants (*Euchlanis* sp. ou *Trichocerca* sp.) et des copépodes caractérisaient plutôt les assemblages.

Hormis l'influence des facteurs associés à la saisonnalité, les échantillons présentaient une hétérogénéité en termes de composition des communautés, ne permettant de détecter aucun profil de variabilité interannuelle ou spatiale.

Le zooplancton des échantillons issus des campagnes de printemps présentait autant de similarités/dissimilarités au sein de chaque campagne d'avril (2013, 2014, 2015) qu'entre elles. Le même constat a pu être dégagé à partir des échantillons de septembre 2014 et juin 2015, suggérant une ré-occurrence des profils de distributions décrits ci-dessus lors des trois printemps consécutifs, et lors des deux étés consécutifs.

L'étude de la variabilité spatiale du zooplancton montre également une importante hétérogénéité des communautés dans le bassin versant au cours d'une même période. Les sites les plus proches n'étaient pas particulièrement plus similaires en termes de composition zooplanctonique, mais parfois présentaient plus de similarité avec des sites plus éloignés et non connectés d'un point de vue hydrologique. Le même constat a été fait en s'appuyant sur les facteurs environnementaux. Le fait que les stations hydrologiquement connectées ne présentent pas systématiquement des communautés zooplanctoniques plus similaires suggère un rôle limité de l'hydrologie et une contribution plus importante des conditions locales.

La corrélation entre les profils de variabilité spatiale de la composition du zooplancton et de ceux des facteurs environnementaux s'est révélée significative pour les périodes d'échantillonnage d'avril 2014, septembre 2014 et avril 2015. Cependant, il n'a pas été possible d'identifier de facteurs influençant clairement cette variabilité parmi ceux pris en compte dans cette étude, à savoir : la température, la conductivité, et les concentrations de Matières en suspension, Matière Organique, oxygène dissous, chlorophylle *a*, Phosphore total, Nitrite, Nitrate et Orthophosphate. Ceci suggère la contribution d'autres facteurs expliquant une partie de la variabilité du zooplancton.

De même, aucune relation entre les profils de distribution du zooplancton et les statuts écologiques (DCE) des stations n'a pu être mise en évidence.

2.2 ARTICLE :

Zooplankton composition in the upstream Basin of a lowland water course (the Scheldt): spatial, inter-annual and seasonal variability.

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2.2.1 Abstract:

The upstream part of the Scheldt drainage basin, at the border of France and Belgium, is constituted by an extensive network of low-flow channelized rivers, of variable Ecological Status, according to the Water Framework Directive assessment. This paper reports a first investigation on the zooplankton communities within these rivers. Zooplankton was sampled between April 2013 and June 2015 at 5 sampling occasions, at 18 sites. The total abundance of rotifers, copepods and cladocerans varied from 2×10^4 to 7×10^6 ind.m^{-3}, with rotifers contributing to 92 % of total abundance considering all samples.

Seasonal, inter-annual and spatial patterns of zooplankton distribution were studied using a method combining hierarchical classification and assessment of indicator species. A seasonal variability clearly emerged: spring communities were characterized by a set of abundant taxa of rotifers associated with high oxygen and chlorophyll *a* concentrations, while in summer rotifer abundance decreased in favor of crustaceans, in relation to temperature increase. The typical rotifer dominated spring communities were reoccurring among years.

Zooplankton community distribution was not related to the WFD Ecological Status. Except for variables associated with seasonality, environmental conditions did not allow to explain the heterogeneity in zooplankton communities. Only higher temperatures in some June samples, favorable to copepods, were creating some dissimilarity within summer communities, which suggest the importance of taking pollutant concentrations into account.

Keywords: Zooplankton distribution; Scheldt watershed; Water Framework Directive; IndVal; spatio-temporal patterns

2.2.2 Introduction

Zooplankton communities are closely related to their environment, since they are influenced by a complex combination of hydrological, physico-chemical and biotic factors (Laprise & Dodson, 1994; Beaugrand, Ibañez & Reid, 2000; David *et al.*, 2005; Mialet *et al.*, 2010, 2011; Chambord *et al.*, 2016). Zooplankton plays an essential role in energy fluxes by ensuring the transfer between the phytoplanktonic primary producers and higher trophic levels (Carpenter, Kitchell & Hodgson, 1985; Park & Marshall, 2000; Tackx *et al.*, 2003). Through the trophic cascade, its dynamics, community composition and resulting biomass can have repercussions on other ecological compartments, either on prey (e.g. phytoplankton) or predators (e.g. fish) (McQueen *et al.*, 1989; Beaugrand *et al.*, 2000; Carpenter *et al.*, 2001; Sommer & Sommer, 2005).

Because they display rapid growth, and respond rapidly to environmental changes, zooplankton organisms are considered as good indicators of water quality (Gannon & Stemberger, 1978; Stemberger & Lazorchak, 1994; Attayde & Bozelli, 1998; Xu, Jørgensen & Tao, 1999; Reynolds, 2003) and several zooplankton taxa are widely used in ecotoxicological studies (Buikema, Geiger & Lee, 1980; Nikunen & Miettinen, 1985; Weber, 1993; Snell & Janssen, 1995; Kwok *et al.*, 2015). Considering its important role in pelagic food webs and its sensitivity to environmental changes, the integration of zooplankton among biological quality elements used for the evaluation of water quality of the Water Framework Directive (WFD) has been strongly recommended, in particular for lakes assessment (Moss, 2007; Nõges *et al.*, 2009; Jeppesen *et al.*, 2011; Haberman & Haldna, 2014).

Determining the factors controlling zooplankton abundance, diversity, and distribution patterns is thus important to understand the functioning of aquatic ecosystems. For these reasons, zooplankton spatial, seasonal and inter-annual variability have been investigated in lakes, marine and estuarine systems (Kratz, Frost & Magnuson, 1987; Arnott *et al.*, 1999; Mackas & Tsuda, 1999; Beaugrand *et al.*, 2000; Li, Gargett & Denman, 2000; David *et al.*, 2005; Roman *et al.*, 2005; Molinero *et al.*, 2006; Anneville *et al.*, 2007). However, few studies focused on zooplankton of freshwater riverine systems since zooplankters are generally considered to be poorly represented, due to short residence times, unfavorable to zooplankton development (Vannote *et al.*, 1980; Baranyi *et al.*, 2002; Lair, 2006). Yet, relatively important zooplankton populations have been reported in some rivers (Saunders & Lewis, 1989; Sterner *et al.*, 1996; Pourriot, Rougier & Miquelis, 1997; Kobayashi *et al.*, 1998).

The Scheldt, which has been subject to heavy organic pollution, was considered as one of the most polluted estuaries in Europe, and has benefited from restoration measures since the 80's, resulting in substantial water quality improvement (Van Damme *et al.*, 2005; Meire *et al.*, 2005;

Cox *et al.*, 2009). Scheldt zooplankton monitored since 1996 has shown substantial changes in community composition in parallel to water quality improvement. In particular, the estuarine copepod *Eurytemora affinis* has developed in the freshwater reach of the estuary, concomitant with a decrease of cyclopoid abundance. These changes were mainly explained by an increase in oxygen concentration and a decrease in NH_4^+ concentration (Mialet *et al.*, 2010, 2011; Chambord *et al.*, 2016).

Zooplankton in the freshwater non-tidal Scheldt has hitherto been poorly documented (Lebon, 1997). Located on the border between Northern France and Belgium, the watercourses composing the upper Scheldt watershed are also highly polluted, in particular resulting from historical industry, agricultural land-use and urbanization (Van Damme *et al.*, 2005; Billen, Garnier & Rousseau, 2005; Boughriet *et al.*, 2007; Lesven *et al.*, 2009; Net *et al.*, 2015 a b). These different sources load the watershed with nutrients and toxic substances, which results in variable water quality in these watercourses, which is reflected in the WFD classification of rivers (Prygiel & Coste, 1993; Rabodonirina *et al.*, 2015). The Scheldt takes its source at low elevation (97 m) and the low-elevation river network which drains its watershed has been strongly canalized for navigation purposes, resulting in low-flow conditions (about 1 m^3sec^{-1}) which provide suitable conditions for zooplankton development (Le Coz et al., Unpub. data).

In this context, this study aims to provide a first inventory of zooplankton communities in the upper Scheldt watershed: an area of highly variable water quality, according to the WFD evaluation criteria. In the present paper, we investigate 1) how does the zooplankton community distribution relate to the WFD scores for these water courses? 2) which environmental factors best explain the spatio-temporal zooplankton community distributions? 3) Do environmental factors and zooplankton community composition result in similar spatial patterns? To do this, 18 sites spread over the watershed were sampled at five occasions between April 2013 and June 2015. The data were analyzed by the method of Souissi *et al.* (2001) and Anneville *et al.*, (2002), combining hierarchical partitioning and assessment of indicator species (by IndVal index calculation) to investigate the pattern of taxa associations. The main environmental factors which could influence these patterns of assemblage have then been investigated.

2.2.3 Methods

2.2.3.1 Study area

The Scheldt has its source in Northern France near Gouy (49° 59′ 13″ N 3° 15′ 59″ E), runs through Belgium and flows into the North Sea at Vlissingen, the Netherlands (51° 25′ 51″ N 3°

31′ 44″ E) (Fig.1). The study area is a part of the Scheldt watershed located on the French-Belgian border. It encompasses the freshwater non-tidal part of the Scheldt, upstream from Gent, and some tributaries being part of its watershed: the Lys and its tributary Deûle, the Scarpe and its tributary Sensée (Fig.1). Some sites which were not located in the Scheldt watershed but on the Sambre (Jeumont), the Aa (St-Momelin and St-Folquin) and the Bergues channel (Cappelle la Grande) were also sampled for comparison purposes. Five sampling campaigns were conducted between April 2013 and June 2015.

The sampling dates and stations are presented in Table 1. Most of the considered sites are monitored for water quality in the frame of the WFD. Their ecological states, provided by the Artois-Picardie Water Agency (http://www.eau-artois-picardie.fr), are given in Table 1.

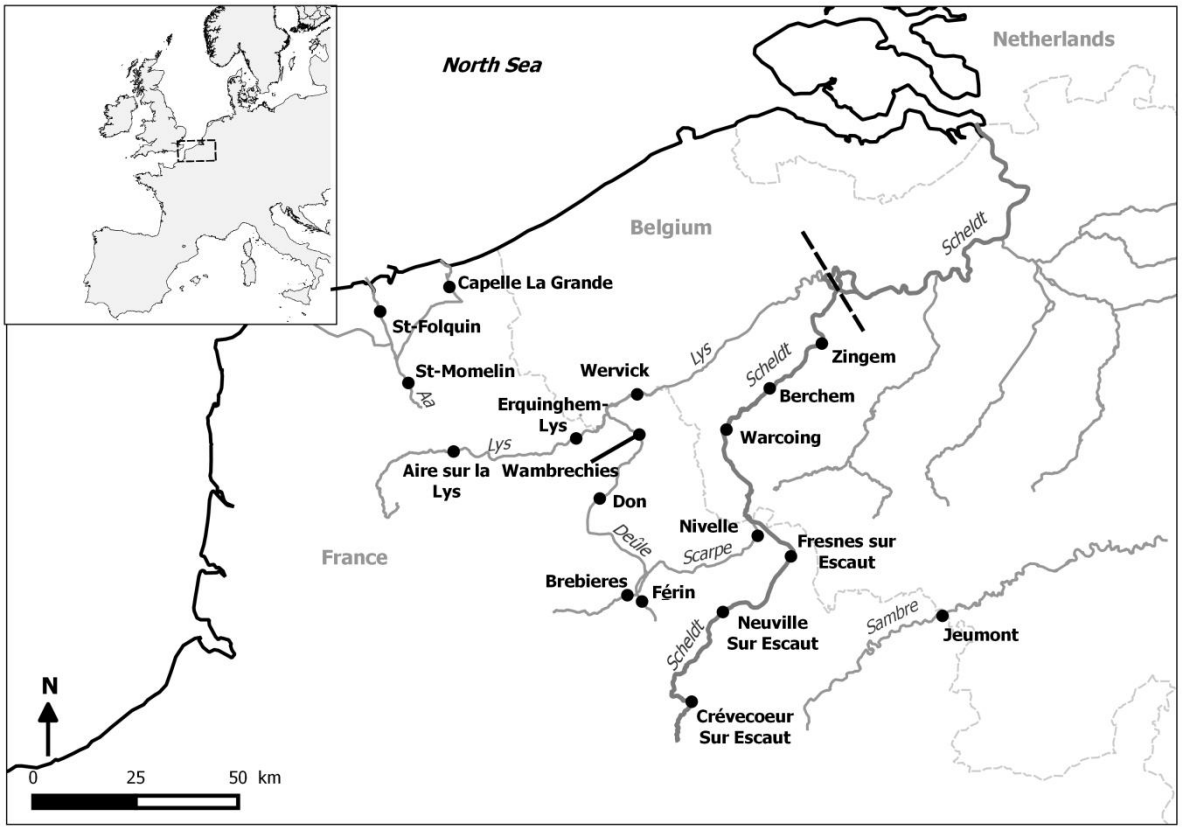

Fig.1: Location of sampling sites in the upstream basin of the Scheldt. The black dotted line is the limit of the tidal influence

Table1: Sampling stations, codes, 2014 Ecological Status, and periods.

Station name	station code	2014 Ecological Status WFD	April 28-30th 2013	April 7-10th 2014	September 2-7th 2014	April 16-23th 2015	June 15-19th 2015
Jeumont	**JEU**	**3 - moderate**	×	×	×	×	×
Fresnes-sur-Escaut	**FSE**	**3 - moderate**	×	×	×	×	×
Brebières	**BRE**	**5 - bad**	×	×	×	×	×
Nivelle	**NIV**	**4 - poor**	×	×	×	×	×
Férin	**FER**	**2 - good**	×	×	×	×	×
Erquinghem-Lys	**EL**	**4 - poor**	×	×	×	×	×
Wervicq	**WER**	**5 - bad**	×	×	×	×	×
Aire-sur-la-Lys	**ASL**	**3 - moderate**	×	×	×	×	×
Don	**DON**	**4 - poor**	×	×	×	×	×
Warcoing	**WAR**		×	×	×	×	×
Zingem	**ZIN**		×	×	×	×	×
St.-Folquin	**SF**	**2 - good**	×				
Capelle-La-Grande	**CLG**	**5 - bad**	×				
St.-Momelin	**SM**	**2 - good**	×				
Wambrechies	**WAM**	**5 - bad**		×	×	×	×
Crèvecœur sur Escaut	**CSE**	**3 - moderate**		×	×	×	×
Neuville sur Escaut	**NSE**	**3 - moderate**		×	×	×	×
Berchem	**BER**			×	×	×	×

2.2.3.2 Zooplankton sampling

Fifty liters of sub-surface water were collected at each site using a pump and filtered through 50 µm mesh plankton net. The collected zooplankton was narcotized with carbonated water and fixed with formaldehyde (4 % final concentration), and finally stained with erythrosine in the laboratory.

Subsamples were taken for counting and identification of organisms at the most precise taxonomic level possible under binocular microscope, to obtain zooplankton abundances at each site. The minimum number of individuals counted was 100 per sample.

2.2.3.3 Statistical analysis

In order to exclude the influence of rare taxa, only taxa present in more than 5% of the observations were retained for statistical analysis.

A Principal Component Analysis (PCA) was applied to the log-transformed data. PCA scores were then extracted from the first Principal Components (PCs) accounting for more than 90% of total variance, in order to obtain linearly independent variables. A hierarchical classification based on Ward's method (Ward, 1963) was applied to the PCA scores using the Euclidian distance as in Anneville *et al.*, (2002), in order to obtain clusters of samples based on their zooplankton communities.

The indicator species index (IndVal) proposed by Dufrêne & Legendre (1997) was used to identify the indicator taxa and the significant assemblages for each cluster of habitat obtained from the hierarchical classification.

IndVal were calculated for each taxon in each cluster at each hierarchical level and combine the measures of Specificity (A) and of Fidelity (B) of that taxon *i* for a cluster *j*, following the formulae:

$$IndVal_{ij}=A_{ij} \times B_{ij} \times 100$$

The specificity A_{ij} of a taxon *i* for a cluster *j* is given as:

$$A_{ij}= Nindividuals_{ij} / Nindividuals_{i}$$

Where $Nindividuals_{ij}$ is the mean number of induviduals of the taxon *i* in the sites of cluster *j*, and $Nindividuals_{i}$ is the sum of mean number of individuals of the taxon *i* in all clusters.

And the fidelity B_{ij} as:

$$Bij= Nsites_{ij} / Nsites_{i}$$

Where $Nsites_{ij}$ is the number of sites of the cluster *j* where the taxon *i* is present, and $Nsites_{i}$ is the total number of sites in the cluster *j*.

Taxa whose IndVal value was above 25% for a cluster were retained in the presented results (Souissi *et al.*, 2001; Anneville *et al.*, 2002).

2.2.3.4 Environmental data

Temperature, conductivity and dissolved oxygen concentration (O_2) were measured *in situ* using a multi parameter sensor (WTW, Multi 3430). Suspended Particulate Matter (SPM) concentrations were quantified by filtration of 250 to 1200 mL of sub-surface water, depending on the SPM concentration, onto pre-weighed Whatman GF/C filters (porosity: 1.2µm). Filters were dried (45 °C) during 24 hours, briefly cooled in a desiccator and weighed. The same filters

were burned at 500 °C for 5 hours, and re-weighed to obtain ash concentration. Organic Matter (OM) concentrations were calculated as the difference between SPM and ash concentrations.

110 to 1080 mL water samples were also filtered on Whatman GF/C filters for the determination of chlorophyll *a* (Chl*a*) concentrations, which were then extracted in 2 % ammonium acetate solution and quantified by reversed phase HPLC, following the method of Wright & Jeffrey (1997). Details of the methodology are given in Majdi *et al.* (2011).

To identify the environmental conditions characteristics of each cluster obtained from the hierarchical classification described above, we used box-and-whiskers plots for the environmental factors. In order to determine if environmental conditions differed between clusters, for each environmental variable, clusters were compared to each other using one-way analysis of variance (ANOVA) when data respected normality and homoscedasticity (respectively tested by Shapiro-Wilk and Bartlett tests at P=0.05). If not, a non-parametric Kruskal-Wallis ANOVA by ranks was performed.

Then, a multivariate Discriminant Analysis was performed to identify the environmental variables that best discriminate the clusters. This approach searches for linear combinations of quantitative variables (ie. physico-chemical parameters in the present study) that provide the best separation among predefined groups (ie. Clusters of samples in the present study), by maximizing the ratio of interclass variance over total variance. A Monte-Carlo test with 1000 permutations was conducted to assess the significance of variables.

For the April 2014, September 2014, April 2015 and June 2015 sampling periods, Total Phosphorus (Ptot) concentrations were determined with the ammonium molybdate spectrophotometric method, and Nitrite (NO_2^--N), Nitrate (NO_3^--N) and Orthophosphate (HPO_4^{2-}-P) concentrations were determined by Ion Chromatography analyses (Dionex Ics-5000+, Dionex Corp., Sunnyvale, CA, USA), onto 100 mL samples of water filtered through 0.2 µm cellulose nitrate filters. In order to assess the contribution of nutrients in discriminating zooplankton clusters, and the potential information gain they provide, a second Discriminant Analysis was run after addition of the nutrient data to the previous set of environmental factors. No nutrient data were available for April 2013.

All statistical analyses were conducted using R (R. Core Team, 2013), (packages 'ade4', "vegan" and "indicspecies").

2.2.3.5 Assessment of spatial variability

In order to assess spatial distribution patterns of zooplankton in the river network without temporal influence, hierarchical classifications were also conducted separately for each sampling campaign, following the same procedure than for the overall dataset.

The same procedure was used to investigate spatial patterns of environmental factors (conductivity, temperature, Chl*a*, O_2, SPM, OM/SPM, Ptot, NO_2^--N, NO_3^--N and HPO_4^{2-}-P concentrations) separately for each campaign excluding April 2013 for which nutrient data were not available.

For each sampling occasion, Mantel tests (Mantel, 1967) were used to investigate correlations between the two sets of distance-based data (zooplankton abundance and environmental factors.

The relations between WFD ecological states of sites and, on the first hand, the clustering of stations based upon zooplankton community composition, and on the second hand, the clustering of stations based upon environmental factors were tested using a chi-square test of independence. This test was run only for April and September 2014 samples to fit with the year of WFD data.

2.2.4 Results:

2.2.4.1 Zooplankton composition

A total of 41 taxa were identified at the highest possible taxonomic level across all 18 stations and 5 sampling periods (Table 2).

Rotifers dominated the communities with 92 % of total abundance, over all sites and sampling occasions. Copepods represented 6% and cladocerans 1%. The maximal total abundance was reached at Cappelle-La-Grande (CLG) in April 2013, with more than 7×10^6 ind. m^{-3} (Fig 2). Considering all samples, the most abundant taxon was the rotifer *Brachionus calyciflorus*, representing 33 % of the total assemblage. *Polyarthra* sp. represented 14%, *Keratella* sp. 19 % and *Synchatea* sp. 12 %. Copepod nauplii were also an important part of the community, accounting for 5 % of total abundance, and for 80 % of copepod abundance.

However, the proportion of each group fluctuated among sampling periods (Fig 2). While rotifers represented 88 to 97% of total abundance in April samplings, in September they accounted for only 57% while nauplii then represented 34%, other copepod stages 5% and cladocerans 3%.

Rotifers were significantly less abundant in September 2014 (on average 4.5×10^4 ind.m^{-3}; Mann & Whittney, P<0.001) and significantly more abundant in April 2013 (on average 1.2×10^6 ind.m^{-3}) than on other sampling occasions (on average 2.5×10^5 ind.m^{-3} for April 2014, April 2015 and June 2015).

Crustacean (copepods all stages and cladocerans) mean abundance varied between 1 (April 2014) and 5×10^4 ind.m^{-3} (June 2015).

Abundances were also substantially variable between stations within each sampling occasion. For example, in June 2015, total abundance per station varied between 2×10^4 and 100×10^4 ind. m^{-3}.

Table 2. Zooplankton taxa observed in the upstream Scheldt basin and their percentage of total zooplankton abundance, considering all sites and sampling occasions.

	% abundance		% abundance
Rotifera			
Brachionus calyciflorus	37.60%	*Cephalodella sp.*	0.11%
Polyarthta sp.	12.32%	*Lepadella sp.*	0.08%
Synchatea sp.	11.54%	*Kellicotia sp.*	0.06 %
Keratella cochlearis	11.43%	*Lecane sp.*	0.04 %
Keratella quadrata	6.89 %	*Brachionus quadridentatus*	0.04 %
Brachionus angularis	4.37 %	*Brachionus rubens*	0.03 %
Filinia longiseta	3.31 %	*Monomata sp.*	0.02 %
Bdelloids	3.05 %	*Trichotria sp.*	0.02 %
Asplanchna sp.	0.82 %	*Harpacticoids copepodites*	0.01%
Brachionus urceolaris	0.36 %	*Keratella valga*	0.01%
Brachionus leydigii	0.35%	*Trichocerca sp.*	0.01 %
Notholca sp.	0.12%	*Brachionus diversicornis*	<0.01
Euchlanis sp.	0.12 %	*Testudinella sp.*	<0.01
		Mytilina sp.	<0.01
Copepoda		**Cladocera**	
Nauplii	4.93%	*Bosmina sp.*	0.51%
Cyclopoids copepodites	0.87%	*Chydorus sphaericus*	0.35%
Cyclopoids adults	0.17%	*Cladocerans (other)*	0.06%
Calanoids copepodites	0.13%	*Scapholeberis sp.*	0.01%
Calanoids adults	0.09%	*Illiocryptus sp.*	0.01%
Harpacticoids adults	0.02%	*Pleuxorus sp.*	<0.01
Eurytemora affinis	<0.01	*Daphnia sp.*	0.08%
		Alona sp.	0.05%

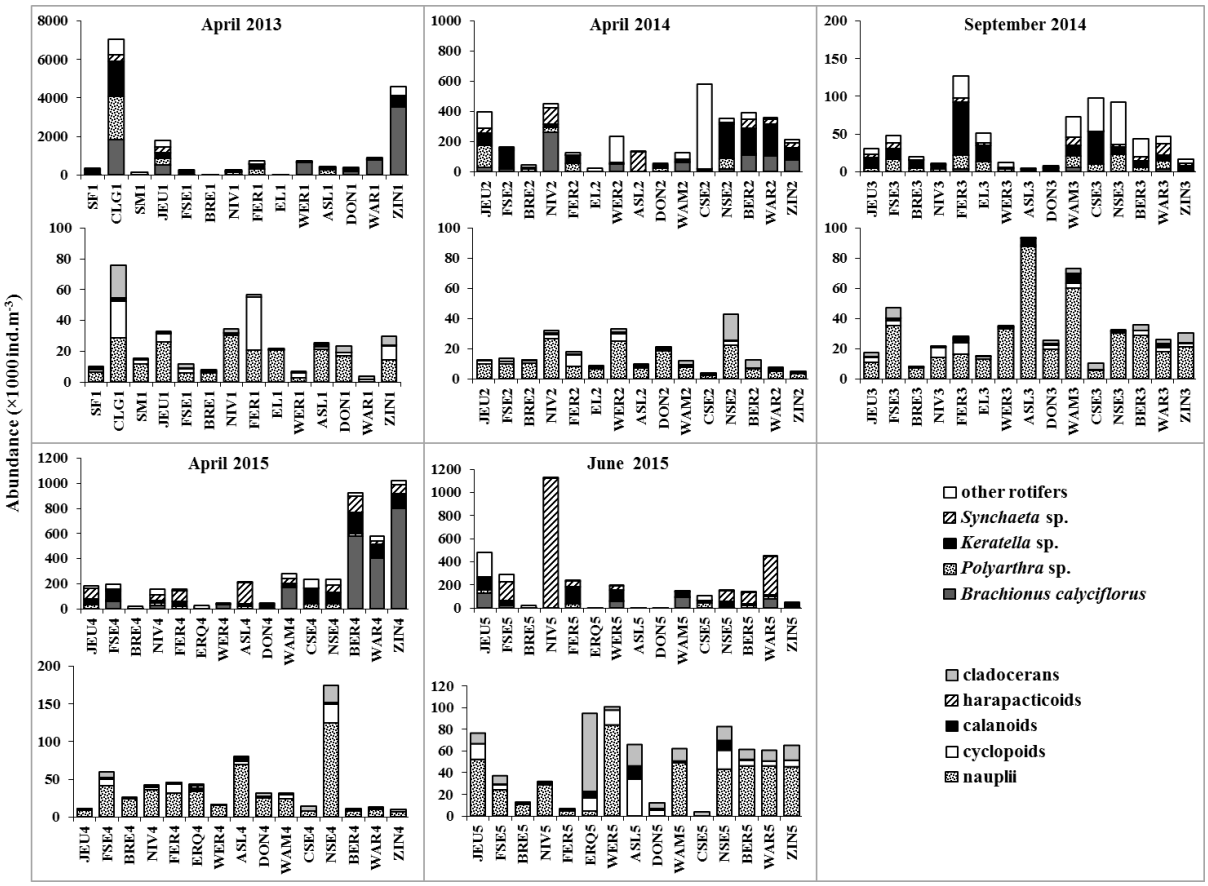

Fig.2: Abundance of rotifers (upper figure), and crustaceans (lower figure) for all sampling stations and at each sampling occasion.

2.2.4.2 Community clusters and distribution patterns

At each cut-off level of the hierarchical classification (Fig. 3), each cluster was characterized by taxa (Table 3) chosen so that each of these taxa presented its maximal indicator value for a cluster (Dufrêne & Legendre, 1997).

The first cut-off level separated mainly April samples (cluster A) from those of June and September (cluster B).

Cluster A showed high (>25 %) and significative IndVal values for the following rotifers: *Brachionus calyciflorus* (95 %), *Keratella quadrata* (95 %), *Filinia longiseta* (93 %), *Polyarthra* sp. (91 %), *Brachionus angularis* (88 %), *Brachionus urceolaris* (84 %), *Keratella cochlearis* (81 %), *Asplanchna* sp. (81 %), *Notholca* sp. (77 %), *Brachionus leydigii* (62 %). In April (2013, 2014 and 2015), these taxa represented more than 84 % of total abundance. IndVal values of calanoid copepodites (75 %), *Alona* sp. (56 %), *Euchlanis* sp. (52 %) and *Brachionus quadridentatus* (51 %) were significative for the cluster B regrouping June and September samples. Besides June

and September samples, the cluster B also contained two April samples: ASL 2 and ASL4 (Aire sur la Lys), which were closely associated with ASL3.

The second level distinguished, within the cluster B, three June samples: Erquinghem (ERQ), Aire (ASL), and Don (DON) (cluster B1). Calanoid adults and cyclopoid (adults and copepodites) copepods, as well as *Daphnia* sp., presented high and significative IndVal values for cluster B1 (respectively 94 %, 80 %, 64 % and 93 %). Other samples of the cluster (B2) were characterized by Nauplii (56%).

The third cut-off separated the April samples in two clusters: A1 and A2. 3 taxa showed high IndVal for cluster A1: Bdelloids (86 %), *Brachionus rubens* (83 %), and *Trichotria* sp. (45 %). All April 2013 samples were included in A2, while April 2014 and 2015 samples were distributed in clusters A1 and A2.

At the fourth cut-off level, B2 was divided in two groups (B2A and B2B), and at the fifth level, A2 formed the two groups A2A and A2B. B2A was characterized by *Trichocerca* sp. (100 %) and *Lecane* sp. (93%), and A2B by *Kellicotia* sp. (50 %).

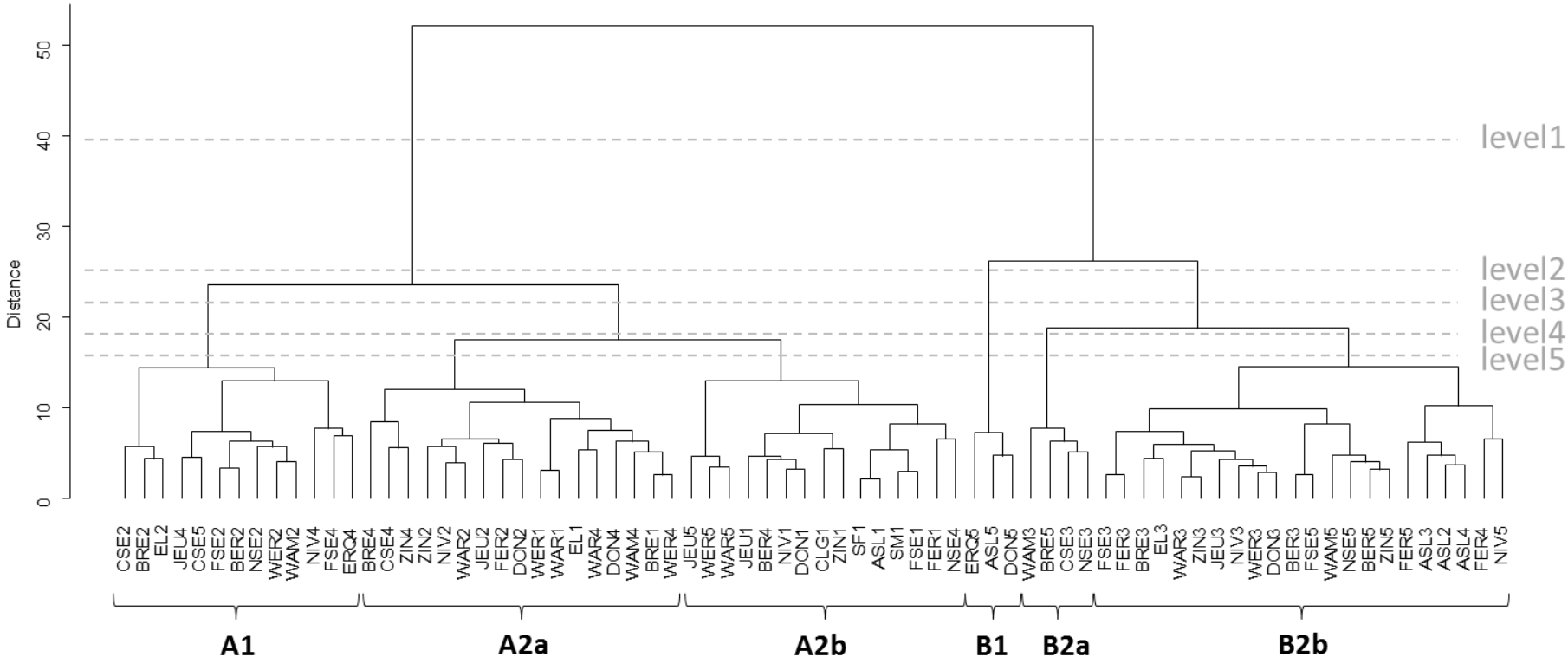

Fig.3: Dendrogram resulting from the hierarchical classification of samples, and the 5 hierarchical levels giving from 2 to 6 groups of samples.

Table 3: Indicator species of clusters and their Specificity, Fidelity, and Indval value (only those> 25% are presented). Cluster where a species has the maximal IndVal value are in bold.

	level 1: 2 clusters				level 2 : 3 clusters				level 3: 4 clusters				level 4: 5 clusters				level 5: 6 clusters			
	cluster	**Specificity**	**Fidelity**	**INDVAL**	**cluster**	**Specificity**	**Fidelity**	**INDVAL**	**cluster**	**Specificity**	**Fidelity**	**INDVAL**	**cluster**	**Specificity**	**Fidelity**	**INDVAL**	**cluster**	**Specificity**	**Fidelity**	**INDVAL**
Asplanchna sp.	A	0.96	0.84	**81.01**	A	0.95	0.84	80.63	A2	0.86	0.88	75.40	A2	0.84	0.88	73.43	A2B	0.73	0.93	68.23
B. angularis	A	0.88	1.00	**88.47**	A	0.87	1.00	87.22	A2	0.77	1.00	77.43	A2	0.73	1.00	73.12	A2B	0.75	1.00	74.71
B.calyciflorus	A	0.98	0.98	**95.38**	A	0.96	0.98	93.98	A2	0.90	0.97	87.05	A2	0.89	0.97	86.43				
B. leydigii	A	0.99	0.62	**61.66**	A	0.99	0.62	61.60												
B. quadridentatus	B	0.83	0.62	**51.36**					B2	0.65	0.65	42.64								
B. rubens	A	0.99	0.31	30.74					A1	0.98	0.85	**83.32**	A1	0.98	0.85	83.26	A1	0.97	0.85	82.44
B. urceolaris	A	0.99	0.84	**83.64**	A	0.95	0.84	79.83												
Bdelloids									A1	0.86	1.00	**86.27**								
Euchlanis sp.	B	0.65	0.79	**51.90**																
F.longiseta	A	0.99	0.93	**92.72**	A	0.99	0.93	92.65	A2	0.95	0.91	85.79	A2	0.94	0.91	85.18	A2B	0.89	0.93	82.80
K.cochlearis	A	0.81	1.00	**80.89**	A	0.79	1.00	79.07	A2	0.58	1.00	57.72					A2B	0.57	1.00	57.12
K.quadrata	A	0.95	1.00	**95.12**	A	0.94	1.00	94.19	A2	0.84	1.00	83.51								
Kellicotia sp.	A	0.96	0.51	49.19													A2B	0.68	0.73	**50.00**
Lecane sp.	B	0.92	0.41	37.97	B2	0.93	0.46	42.72					B2A	0.93	1.00	**93.16**	B2A	0.92	1.00	92.33
Notholca sp.	A	0.96	0.80	**76.82**	A	0.96	0.80	76.47												
Polyarthra sp.	A	0.91	1.00	**91.39**	A	0.90	1.00	90.49	A2	0.82	1.00	82.15								
Trichocerca sp.													B2A	1.00	1.00	**100.00**	B2A	1.00	1.00	**100.00**
Trichotria sp.									A1	0.84	0.54	**45.29**								
Calanoid adult	B	0.90	0.69	62.17	B1	0.94	1.00	**93.65**	B1	0.92	1.00	92.15	B1	0.89	1.00	88.51	B1	0.87	1.00	87.27
Calanoid cop.	B	0.83	0.90	**74.64**																
Cyclopoid adult					B1	0.80	1.00	**80.12**	B1	0.72	1.00	72.37	B1	0.70	1.00	70.04	B1	0.62	1.00	62.08
Cyclopoid cop.					B1	0.64	1.00	**63.52**	B1	0.56	1.00	55.94	B1	0.52	1.00	52.45	B1	0.43	1.00	43.05
Nauplii					B2	0.56	1.00	**56.25**												
Alona sp.	B	0.70	0.79	**55.54**																
Daphnia sp.					B1	0.93	1.00	**93.22**	B1	0.91	1.00	90.51	B1	0.88	1.00	88.30	B1	0.86	1.00	86.10

2.2.4.3 Environmental characteristics of zooplankton-based clusters

The Ecological states of sites, according to the WFD assessment of water quality, was not significantly related with their reparation in clusters (chi-squared test, P>0.05).

ANOVA and Kruskal-Wallis tests showed that conductivity, SPM and OM/SPM ratio did not differ according to clusters (Fig. 4). The factors which significantly differed between clusters were: temperature, Chl*a* and O_2 (P<0.05). ANOVA and Kruskal-Wallis tests run on the dataset without April 2013 resulted in non-significant difference of NO_2^--N, NO_3^--N, HPO_4^{2-}-P and Ptot between clusters.

Temperature was lower in samples of Cluster A (P<0.001) than in the other clusters. In particular the cluster B1 presented high temperatures ranging from 19.5 to 21 °C, while temperature reached, on average, 14.5 °C (±2) in cluster A and 18 °C (±2) in B2.

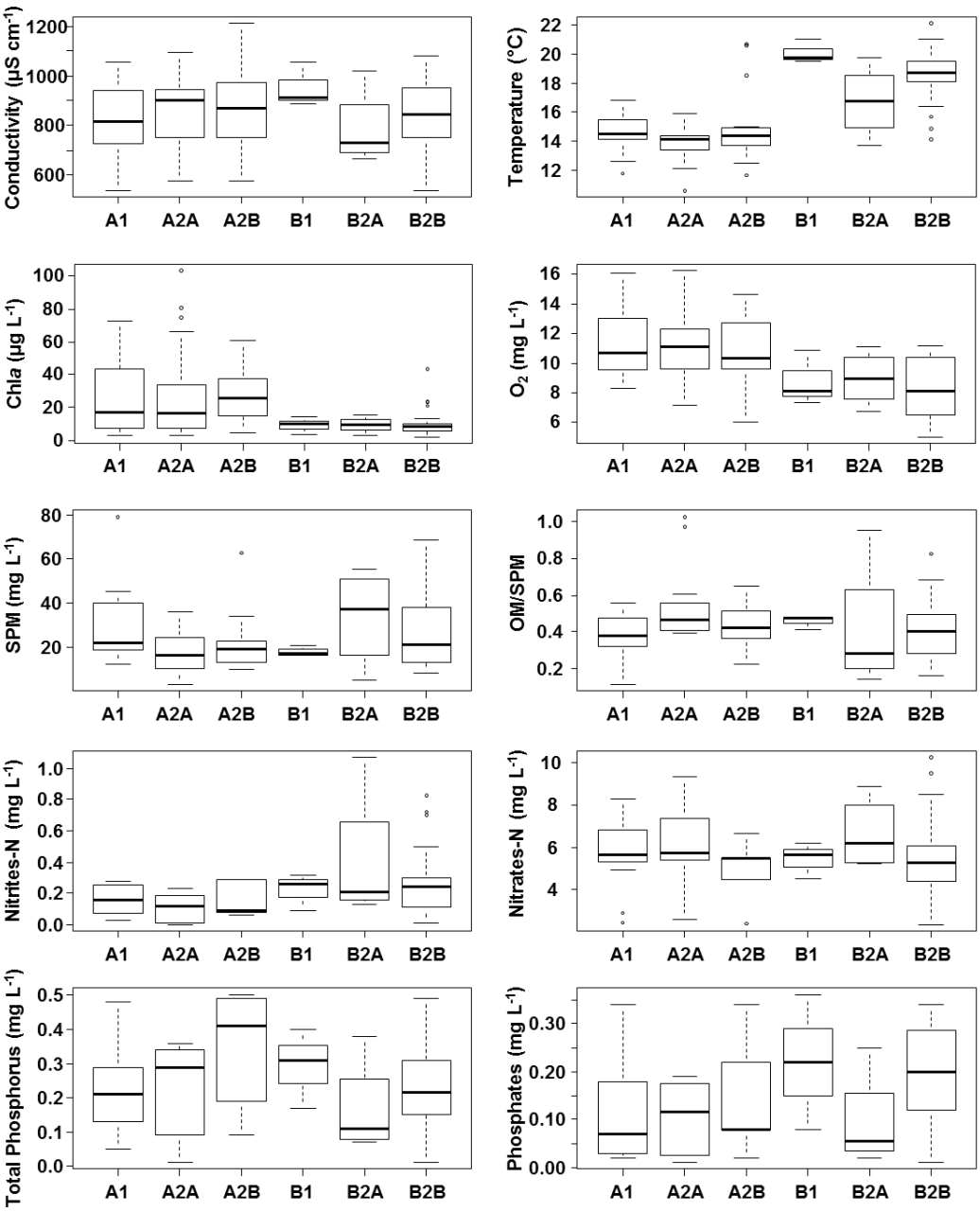

Fig 4. Box-and-whisker plots describing the distribution of environmental conditions within clusters (confidence interval =95%). April 2013 samples were excluded from figures related to nutrient concentrations.

Contrarily, both Chl*a* and O_2 concentrations were higher in cluster A than in cluster B ($P<0.01$ for both).

A2B samples differed significantly from all other samples in nitrate concentrations, with an average of 16.52 (±12.82) mg L^{-1}, while it was only 7.01 (±6.09) mg L^{-1} in cluster B.

The Discriminant Analysis (Fig.5a) displayed five functions and was validated by Monte Carlo permutation resulting in a P. value of 0.0009. The two first axes accounted for 51 % and 23 % of variability between clusters.

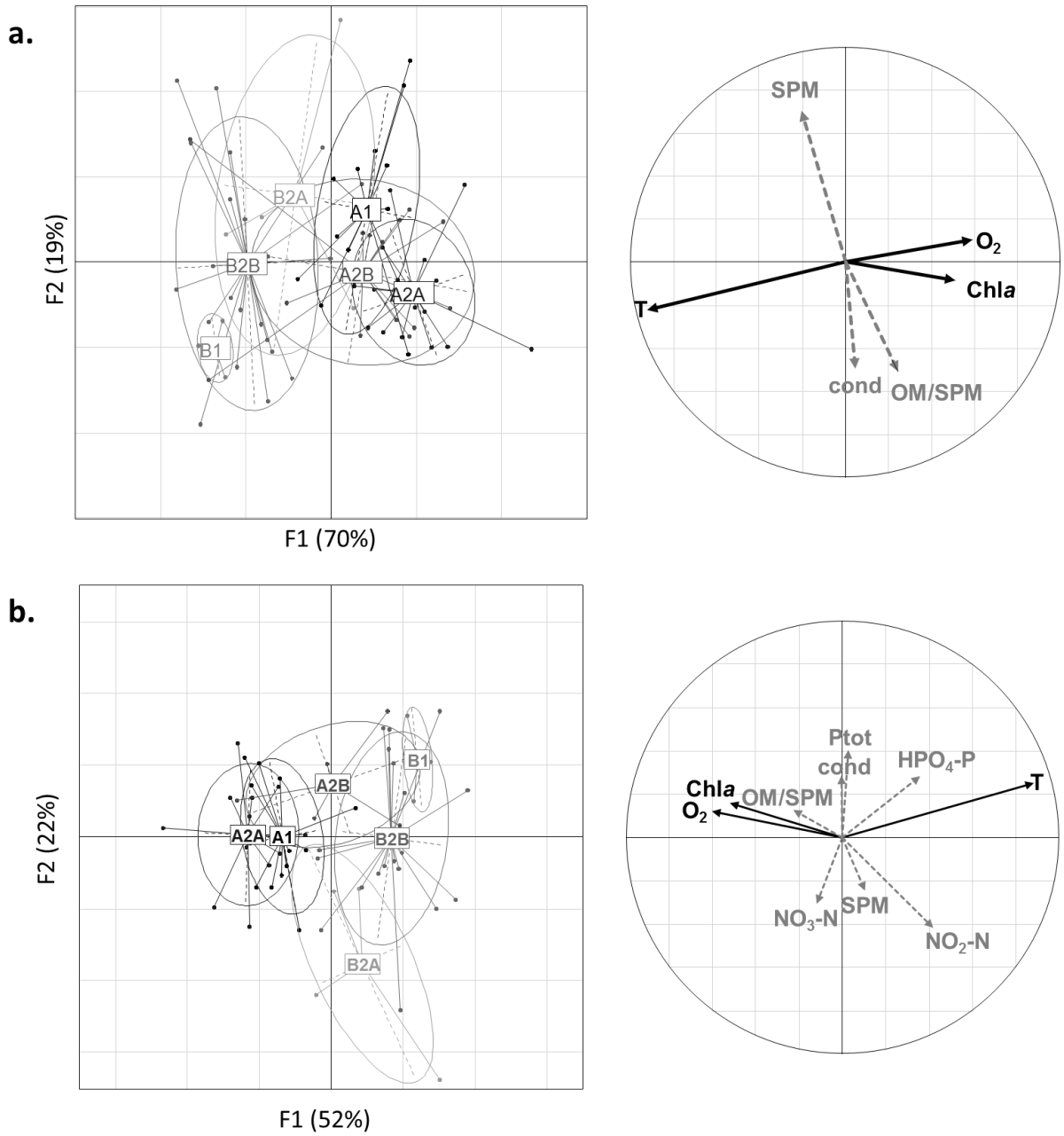

Fig.5: Projection of clustered samples and correlation circles of the environmental variables resulting from the first (a) and second (b) discriminant analyses: conductivity (cond), temperature (T), SPM, OM/SPM, Chl*a*, O_2. Nutrient concentrations were included in the second analysis, from which April 2013 samples were removed (b).

F1 axis was mainly determined by the opposition of temperature associated with clusters B, and Chl*a*, O_2 and Nitrate concentration, associated with clusters A. F2 axis was mainly determined by Nitrate concentrations associated with A2B cluster.

Clusters A1, A2A and A2B presented an important overlap, and their centroids were relatively close in the negative part of F1, as it is the case also for B1, B2B and B2A in the positive part.

The second Discriminant Analysis (Fig. 5b), including nutrient concentrations as variables, and excluding April 2013 samples, provided an ordination which was quasi-identical to the first one, with an opposition of spring samples still influenced by O_2 and Chl*a*, and summer samples associated to temperature. The Nutrients, which were more associated with the F2 axis but were not significantly different between clusters, did not clearly separate clusters along the F2 axis. The Monte Carlo permutation resulted also in a P. value of 0.0009.

2.2.4.4 Assessment of spatial patterns of zooplankton distribution and environmental conditions

The 5 hierarchical classifications conducted for each sampling campaign provided 5 seasonal dendrograms based on distribution of zooplankton communities (Fig. 6a).

The sample clustering showed that the similarities between zooplankton communities, at different sites of the study area were most of the time not more important between successive sites than among all sites of a same watercourse. The stations were also often more similar with distant sites than with geographically or hydrologically adjacent sites.

For example, Jeumont (JEU) station, which is situated in an off-center region comparatively to other sites, and which is not hydrologically connected with any of them since it is located in the Meuse basin, always presented similarities with other samples for its zooplankton composition.

However, Zingem (ZIN), Berchem (BER) and Warcoing (WAR), three successive sites along the Scheldt watercourse, were represented in the same cluster in April 2014, September 2014 and April 2015, and thus presented more similar zooplankton communities among them than compared with other sites. A similar close clustering was found for Neuville-sur-Escaut (NSE) and Fresnes-sur-Escaut (FSE) in April 2014, September 2014, April 2015 and June 2015.

The sample clustering based on environmental factors (Fig.6b) often presented similarities between hydrologically connected sites. Some sites were always represented in the same clusters, such as Wambrechies (WAM) and Wervick (WER), Erquinghem-Lys (ERQ) and Don (DON), Jeumont (JEU) and Neuville-sur-Escaut (NSE) or Warcoing (WAR) and Zingem (ZIN). As for zooplankton-based clustering, sites were sometimes more similar with distant sites than with the nearest ones. For example, Aire-sur-la-Lys (ASL) and Jeumont (JEU) in September 2014

and June 2015 are found in the same cluster, while station Erquinghem-Lys (ERQ), near to ASL is not clustered with this station.

Mantel tests revealed that zooplankton spatial patterns were significantly correlated with environmental spatial patterns in April 2014, September 2014 and April 2015 ($P<0.05$). The correlation was on the contrary not significative in June 2015 ($P>0.05$). However, Kruskal-Wallis tests did not allow identifying environmental factors significantly different between zooplankton-based clusters, except Chl*a* concentration in April 2014. Coefficients of Variation were important for most environmental factors (see Appendix 1 in supporting information). It can be noticed that, even if not significantly different, environmental conditions were not completely homogeneous among zooplankton-based clusters.

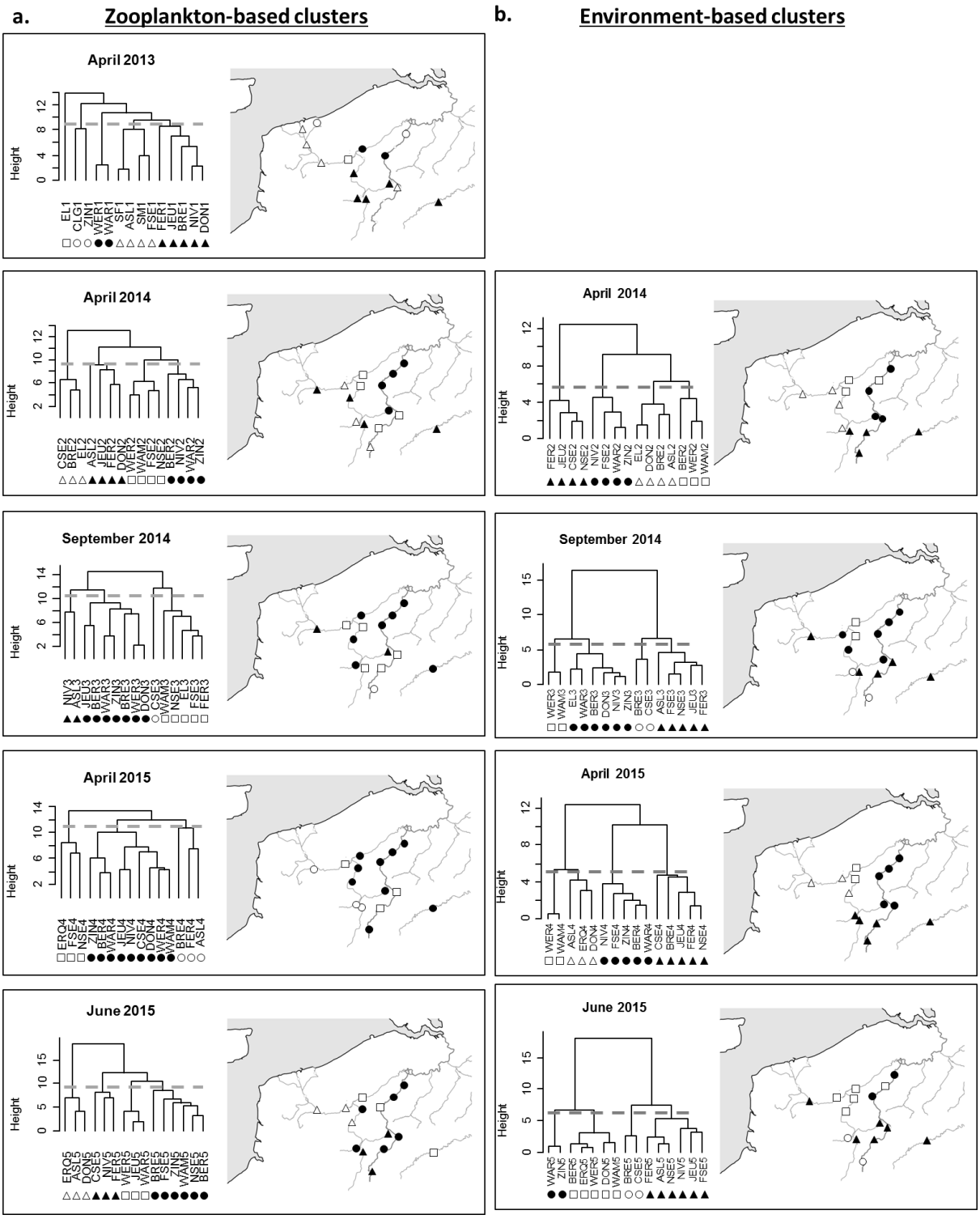

Fig.6: Dendrogram resulting from the hierarchical classification of samples for each sampling campaign, based on zooplankton composition (left) and to physico-chemical factors (right) and localisation of clusters in the study area.

2.2.5 Discussion

Zooplankton communities of the riverine upstream reaches of the Scheldt watershed displayed substantial abundances showing that the prevailing hydrologic lowland circumstances (residence times) permit zooplankton development in these upstream basin rivers, contrarily to the assumption that planktonic organisms develop only in the lower reaches of rivers (Vannote *et al.*, 1980). The zooplankton communities were widely dominated by rotifers (92% considering all samples and all sampling occasions together), while crustaceans (copepods and cladocerans) were scarce. Lebon (1997) also found a clear dominance of rotifers (and ciliates) in the Scarpe and the Petite Sensée, situated in the study area and influenced by eutrophication. Similar patterns of relative composition of zooplankton communities have been described in other rivers: rotifers abundance represented 85% of zooplankton abundance in the Waikato River (New Zealand; Burger, Hogg & Green, 2002), 84% in the Danube floodplain (Austria; Baranyi *et al.*, 2002) and 99% in the Po river (Italy; Ferrari, Farabegoli & Mazzoni, 1989). Rotifers often dominate zooplankton assemblages in freshwaters because they have a parthenogenic reproduction mode and a high growth rate, allowing them to develop in systems with relatively short residence times, such as river reaches. Through their reproduction mode, rotifers can optimize development in favorable conditions, and they can cover a wide range of environmental conditions, since they present a wide tolerance to temperature (Bērzinš & Pejler, 1989), oxygen concentrations (Bērziņš & Pejler, 1989), turbidity (Kirk & Gilbert, 1990), or even to certain pollutants (Buikema, Cairns & Sullivan, 1974; Hanazato & Yasuno, 1990).

Besides this general pattern, the results of the present study showed an average total zooplankton abundance of 4.33×10^5 ind. m^{-3}, $\pm 9.84 \times 10^5$ (including all sampling occasions and locations), a value which is comparable to those observed in other riverine systems cited above (Ferrari *et al.*, 1989; Gosselain *et al.*, 1998; Baranyi *et al.*, 2002). Zooplankton showed an important variability across samples, both in terms of total abundance and in terms of community composition. In some cases, rotifers were the minority (2.2% in June 2015 at ASL), or adult copepods were completely absent from some samplings (for example at ZIN in April 2015).

The use of the IndVal method coupled to a hierarchical classification allowed an investigation of the different taxa characterizing these patterns of variability relying on environmental conditions, and the results provided a frame to explore seasonal, inter-annual, and spatial variability within zooplankton communities.

The seasonal variability of zooplankton distinctly emerged, opposing spring (April 2013; 2014; 2015) and summer communities (September 2014; June 2015), and was mainly related to

higher temperature in summer, and to indicators of spring phytoplankton development (higher O_2 and Chl*a* concentrations). Even if rotifers were found in majority at all seasons, the results suggested a seasonal succession in zooplankton composition: while April months were characterized by high indicator values for rotifers, and mainly Brachionids (*Brachionus* sp., *Keratella* sp.), in summer, indicator taxa included both rotifers (*Euchlanis* sp or *Trichocerca* sp.), and crustaceans (copepods and cladocerans), and the rotifer contribution to total zooplankton abundance was less important.

Temperature is an important factor for structuring zooplankton communities (Mikschi, 1989; Pinel-Alloul *et al.*, 1999; Tackx *et al.*, 2004). The association of copepods with high temperature was here confirmed by their high indicator value for the warmest samples—displayed at ERQ, ASL and DON in June. Crustaceans spring development onset being later than that of rotifers (Allan, 1976; Herzig, 1983; De Ruyter van Steveninck, Zanten & Admiraal, 1990; Pace, Findlay & Lints, 1992), they are often more abundant in summer, benefiting also from the decrease of the flow in some systems. The increase of crustacean abundance can, in addition, have a negative influence on rotifers, the former suppressing the latter by trophic competition, predation, or mechanical interference (Stemberger & Evans, 1984; Gilbert, 1988; Marneffe, Descy & Thomé, 1996; Kumar & Rao, 2001; Baranyi *et al.*, 2002).

The seasonal differences observed in zooplankton communities may also be related to those of phytoplankton, since Chl*a* and O_2 concentrations significantly differed between spring and summer. A Chl*a* peak in spring followed by a summer decline of phytoplankton biomass is frequently observed in rivers, and associated to grazers (Billen, Garnier & Hanset, 1994; Gosselain, Descy & Everbecq, 1994; Gosselain *et al.*, 1998; Garnier, Billen & Coste, 1995). This seasonal succession could be compared with the clear-water phase observed in lakes (Lampert *et al.*, 1986; Sommer *et al.*, 1986; Talling, 2003; Arhonditsis *et al.*, 2004), except that in lotic systems, hydrological conditions generally prevail in phytoplankton regulation (Billen *et al.*, 1994; Sterner *et al.*, 1996; Lair, 2006). However, in the low elevation canalized rivers of the studied upstream Scheldt watershed, the influence of hydrological constraints may be less important. In the Scarpe, the main controlling factors of phytoplankton dynamics were shown to be temperature and nutritive conditions (Noppe *et al.*, 1999). Grazer dynamics and their impact on phytoplankton may also be considerable in this type of low-flow systems.

Beyond the seasonal classification, the heterogeneous mix of samples within clusters did not permit to detect any pattern of spatial of inter-annual distinction.

Between-years zooplankton variations were not clearly detected in the present study, but within the April clusters, the 2013 samples were all included in the same group (A2) probably because they

displayed particularly high rotifer abundances (1.3×10^6 ind.m^{-3} in average). However, among the environmental factors studied, which were obviously not exhaustive, we could not identify one which was significantly different between these clusters. Contrarily to April 2013 samples, 2014 and 2015 spring samples were well mixed within cluster A, and the seasonal pattern of successive development of rotifers and crustaceans was recurring during the months of April across the studied years. When inter-annual variations are observed in zooplankton communities, they are generally associated to climatic and environmental year-to-year variations (George & Harris, 1985; Herzig, 1987; Romare *et al.*, 2005). It is thus likely that these conditions were rather similar between years.

In the same way that samples of different years were distributed independently in different clusters, geographically close, hydrologically connected, or localized along a same watercourse sites were not particularly found in a common cluster. Some samples seemed to be more similar for zooplankton even if they were far from each other or sampled in different years. The understanding of zooplankton community structure was thus difficult, since biotic or abiotic factors resulting in the zooplankton structure observed could not be identified in the light of the physico-chemical factors investigated in the present study.

Considering seasonal clusters (separately for each sampling occasion), few associations were detected among geographically close or hydrologically connected sites. Some reaches seemed to present similar zooplankton communities; however, these "low heterogeneity zones" did not exceed more than 2 or 3 consecutive stations of a river reach. Moreover, some sites were more similar with geographically far sites than the closer ones (considering the hydrological distance between sites). The environmental-based clustering presented the same characteristics, and was in addition related to zooplankton spatial patterns for most of cases. Even if a direct influence of environmental factors on zooplankton communities could not be clearly identified, sites presenting more similar environmental conditions were rather characterized by similar zooplankton communities, whether they were hydrologically connected (e.g. ZIN-BER-WAR in September 2014 and April 2015) or not (ASL-NSE in September 2014; JEU-CSE in April 2015).

In the "River Ecosystem Synthesis", species communities in a river network are described to be more similar with communities of similar "Functional Process Zones" (influenced by hydrogeomorphologic conditions and physico-chemical habitats) than with the adjacent communities (Thorp, Thoms & Delong, 2006). The concept of "Functional Process Zones" has already been used to describe the distribution of zooplankton from the source to the mouth of the Scheldt, and, at this scale (300 km hydrological distance), identified the upstream non-tidal watershed of the Scheldt as characterized by high temperature, O_2 and Chl*a* concentrations and dominated by rotifers (Le Coz et al., Unpub. data). However, the present results suggest a finer scale for zooplankton structuring, since similar zooplankton communities seem to occur in distant areas within the upstream river reaches, and even

across different years, while different communities were found in adjacent stations during single sampling campaigns. This highlights the probable importance of local conditions, and can raise a question already asked by Jenkins & Buikema (1998) “Do similar zooplankton develop in similar conditions?”. The dissimilarity in zooplankton communities between nearest sites is probably also favored by low flow conditions (about $1m^3$ sec^{-1},Prygiel & Coste, 1993). Indeed, the effect of connectivity and directionality in river networks has recently been shown to be less important than local environmental factors for zooplankton regulation at low flow (Zhao *et al.*, 2017).

SPM concentration, OM/SPM ratio and conductivity displayed no significant difference from one cluster to another, and could not explain the heterogeneity of zooplankton within spring or summer clusters. The discriminant analysis illustrated this by an important overlap of clusters within each season, despite the representation which maximizes inter-groups variability inherent to this analysis. Even if SPM composition and resource availability may play a major role in structuring zooplankton communities, other factors not included in the present study, such as predation or pollution may also influence zooplankton distribution in the watershed and contribute to the unexplained part of zooplankton heterogeneity (McQueen, Post & Mills, 1986; Hanazato, 2001; Reissig *et al.*, 2006; Jeppesen *et al.*, 2011; Souza Costa *et al.*, 2016).

The water quality in the study area is highly variable according to the WFD evaluation system, ranging from good to bad ecological qualities across the basin. Zooplankton distribution was not related to that of the WFD ecological states classes. The classification resulting from WFD assessment in the study area takes into account several sources of pollution. Agriculture and urbanization in the basin have led to an important load of nutrients, even if the situation tends to improve thanks to waste water treatment (Billen *et al.*, 2005). However, the present results showed that zooplankton heterogeneity could not be clearly explained by nutrient concentrations.

Zooplankton is related to nutrient cycles, through grazing on phytoplankton, and regenerating nutrients. However, these interactions are complex and involve a number of influencing variables, as illustrated in the RIVERSTRAHLER model applications (Billen *et al.*, 1994; Garnier *et al.*, 1995, 2005). In the Seine for example, phytoplankton dynamics simulations, using the RIVERSTHRALER model, showed that the implementation of nutrient limitation in the model could not improve the accuracy of the simulation, because of the high nutrient concentration in the Seine watershed (Billen *et al.*, 1994). Garnier *et al.*, (1995) argue that in river systems, nutrients are probably not limiting due to continuous inputs by soil leaching and anthropogenic releases. In this case, a more important impact of top-down regulation of phytoplankton by zooplankton is suggested. This may also be true in the Scheldt watershed, since nutrient loads are also important in the basin (Billen *et al.*, 2005; Thieu, Billen & Garnier, 2009). High nutrient concentrations have also been suggested to improve zooplankton top-down control of phytoplankton in the Scheldt estuary (Muylaert, Sabbe & Vyverman,

2000). Investigations on trophic interactions with phytoplankton communities may thus help in explaining zooplankton dynamics in the Upper-Scheldt watershed.

Nutrient loads are not the only reason of variable WFD classification in the basin. The diversity of anthropogenic disturbances and thus of pollution sources in the studied rivers may contribute to zooplankton heterogeneity. In particular, chemical states of all the sites considered are classified as in bad quality, since the area is subject to an important load of POPs and metallic pollution, due to past and present anthropogenic activities (Prygiel *et al.*, 2000; Charriau *et al.*, 2009; Lesven *et al.*, 2009; Louriño-Cabana *et al.*, 2011; Sanctorum *et al.*, 2011; Rabodonirina *et al.*, 2015). The effect of these diverse sources of pollutants should thus be investigated more accurately, regarding the sensitivity of zooplankters to toxic substances.

In conclusion, the physico-chemical factors investigated in the present study demonstrated that seasonality was the main factor leading to zooplankton community diversification across the study area, showing spring communities dominated by rotifers and summer-autumn communities with more importance of crustaceans. The April samples of the 3 successive years clustering together suggest that the spring situations are little variable among years. Since the present study only allowed detecting different "spring" and "summer-autumn" situations, more frequent samplings could improve the understanding of zooplankton temporal dynamics.

Little geographical association of zooplankton communities among adjacent or close sampling sites was observed. On the contrary, some sites, situated far apart, or even on different rivers, harbored comparable zooplankton communities, with some of them characterized by indicator taxa, suggesting the influence of sufficiently similar local conditions to favor similar zooplankton communities despite distance and a reduced role of hydrological connectivity.

Whatever the cause of this heterogeneity, these findings indicate the necessity for zooplankton monitoring networks in apparently rather physico-chemically homogeneous riverine reaches to be spatially dense. The distribution of zooplankton community seems not related to Ecological quality according to the WFD assessment, so more accurate investigations on pollution sources, as well as on trophic interactions are needed.

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Appendix 1: Mean and coefficient of variation of each environmental factor for each zooplankton-based cluster of each sampling campaign, and Kruskal-Wallis p. value.

		Conductivity (µS.cm^{-1})	Temperature (°C)	SPM (mg.L^{-1})	Chl*a* (µg.L^{-1})	O_2 (mg.L^{-1})	Nitrites -N (mg.L^{-1})	Nitrates -N (mg.L^{-1})	Phosphates -P (mg.L^{-1})	Total Phosphorus (mg.L^{-1})	OM/SPM
April 2014	mean-cluster1	787.50	13.78	17.14	14.72	10.29	0.18	6.54	0.09	0.12	0.57
	CV-cluster1	23.06	2.40	86.31	89.94	10.29	37.48	47.39	98.60	74.03	50.30
	mean-cluster2	851.75	15.20	29.56	21.53	10.13	0.19	5.79	0.18	0.29	0.38
	CV-cluster2	12.64	8.06	31.50	51.32	20.75	45.76	10.80	88.09	70.95	19.02
	mean-cluster3	734.33	12.97	27.81	6.14	10.44	0.18	6.93	0.15	0.15	0.38
	CV-cluster3	13.66	10.70	53.60	40.06	12.39	54.72	19.76	70.97	57.99	17.29
	mean-cluster4	974.25	14.78	41.46	82.97	11.99	0.21	4.68	0.14	0.36	0.39
	CV-cluster4	8.28	9.00	61.03	17.11	10.38	15.92	29.95	25.42	19.94	22.01
	global mean	**836.96**	**14.18**	**28.99**	**31.34**	**10.71**	**0.19**	**5.99**	**0.14**	**0.23**	**0.43**
	K-W p.value	0.13	0.12	0.25	**0.01**	0.26	0.95	0.16	0.77	0.13	0.48
September 2014	mean-cluster1	810.29	18.30	22.67	4.84	7.65	0.47	5.18	0.22	0.23	0.44
	CV-cluster1	17.88	5.20	58.52	77.26	23.21	56.81	40.67	41.04	50.37	48.20
	mean-cluster2	836.20	18.56	34.21	10.02	7.71	0.32	5.23	0.15	0.17	0.28
	CV-cluster2	24.82	5.62	43.18	42.64	24.01	136.84	11.75	82.31	76.74	29.36
	mean-cluster3	887.00	19.30	35.15	14.68	8.91	0.25	4.70	0.21	0.20	0.37
	CV-cluster3	10.04	7.33	76.11	84.66	35.71	140.86	38.10	53.87	14.14	45.95
	mean-cluster4	669.00	13.70	55.50	9.68	9.57	0.13	7.05	0.02	0.07	0.15
	CV-cluster4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	global mean-	**800.62**	**17.47**	**36.88**	**9.81**	**8.46**	**0.29**	**5.54**	**0.15**	**0.17**	**0.31**
	K-W p.value	0.86	0.69	0.27	0.18	0.80	0.33	0.7631	0.64	0.69	0.38
April 2015	mean-cluster1	888.89	14.20	22.69	30.15	12.63	0.03	5.71	0.09	0.26	0.50
	CV-cluster1	20.11	10.23	36.10	62.58	23.97	129.35	34.65	83.08	50.75	15.47
	mean-cluster2	855.00	14.73	23.13	26.44	12.92	0.10	6.28	0.07	0.20	0.35
	CV-cluster2	14.47	2.07	83.97	85.76	19.60	60.83	12.69	96.44	47.25	70.20
	mean-cluster3	802.67	15.00	21.17	11.40	10.92	0.03	7.96	0.05	0.14	0.56
	CV-cluster3	10.72	4.37	112.53	75.51	2.07	121.24	21.08	108.25	105.70	72.12
	global mean-	**836.80**	**15.35**	**25.97**	**19.45**	**11.23**	**0.11**	**6.37**	**0.09**	**0.19**	**0.43**
	K-W p.value	0.49	0.42	0.71	0.33	0.64	0.20	0.22	0.74	0.30	0.64

CHAPITRE III :

REPONSE DES COMMUNAUTES ZOOPLANCTONIQUES A LA VARIABILITE DE LA QUALITE DE L'EAU ET AUX CONTAMINATIONS MULTIPLES DANS LES COURS D'EAU DU BASSIN AMONT DE L'ESCAUT

- ***Article en préparation***

3.1 RÉSUMÉ DU CHAPITRE:

3.1.1 Introduction

Depuis 2000 la Directive Cadre sur l'Eau impose aux États Membres de l'Union Européenne l'évaluation de l'État Écologique des cours d'eau, basé sur un ensemble d'indicateurs physico-chimiques et biologiques. Les Éléments de de Qualité Biologique (EQB) utilisés pour la détermination de l'Etat Ecologique incluent le phytoplancton, le phytobenthos, les macrophytes, les macro-invertébrés benthiques et les poissons. Le zooplancton en revanche n'en fait pas partie.

Pourtant plusieurs auteurs ont critiqué cette omission, car le zooplancton présente un fort potentiel indicateur (Moss, 2007 ; Nõges et al., 2009 ; Jeppesen et al., 2011 ; Haberman & Haldna, 2014). Il est représenté dans la quasi-totalité des milieux aquatiques, occupe une position centrale dans les réseaux trophiques pélagiques, et est particulièrement sensible aux conditions environnementales, répondant rapidement aux perturbations (Gannon et Stemberger 1978 ; Stemberger & Lazorchak, 1994 ; Attayde & Bozelli, 1998 ; Xu, Jørgensen & Tao, 1999 ; Reynolds, 2003 ; Mialet et al., 2010 ; 2011 ; Chambord et al., 2016).

Le **chapitre 2** a démontré que la variabilité spatiale du zooplancton est corrélée aux conditions physico-chimiques, mais cette variabilité restait difficile à expliquer en se basant sur un ensemble de facteurs généraux, sans prise en compte des polluants. Les conditions de vie pour le zooplancton sont probablement également influencées par d'autres facteurs, comme la qualité chimique de l'eau.

Dans la partie en amont du bassin versant de l'Escaut, les cours d'eau présentent un niveau important de contamination, lié aux rejets industriels et urbains de métaux traces (ETM — Boughriet *et al.*, 2007a, b ; Louriño-Cabana *et al.*, 2011, Charriau *et al.*, 2009) et de polluants organiques persistants (POP — Sanctorum *et al.*, 2011 ; Net *et al.*, 2015 a, b ; Rabodonirina *et al.*, 2015). Cette pollution multiple, se superposant à la variabilité des facteurs environnementaux autres dans le bassin versant, crée probablement des conditions de vie variables pour le zooplancton de ces cours d'eau.

3.1.2 Objectifs

En s'appuyant sur la question posée par Jenkins et Buikema (1998) : « des communautés similaires se développent-elles dans des conditions similaires ? », cette étude vise à évaluer si

l'abondance et la composition des communautés zooplanctoniques sont plus similaires dans les stations du bassin présentant des conditions environnementales similaires.

Afin de répondre à cette question, les données d'abondance et de composition du zooplancton ont été couplées à des données de physico-chimie, d'état écologique évalué par la DCE et de contamination de l'eau. En considérant trois périodes d'échantillonnage (avril 2014, septembre 2014 et avril 2015), la méthode suivante a été employée :

Dans un premier temps, les stations ont été classées selon leurs similarités en termes de conditions environnementales. Plusieurs critères ont été successivement utilisés pour définir la notion de « conditions environnementales » :

- L'**État Écologique** des stations (DCE),
- Les **conditions physico-chimiques** « générales » (MES, MO/MES, concentrations en Chl*a*, oxygène et nutriments),
- Le **niveau de contamination** de l'eau : Celui-ci a été défini plus spécifiquement à partir des principales sources de contamination de la région : Polychlorobiphényles (PCB), Hydrocarbures Aromatiques Polycycliques (HAP), di-2-ethylhexyl phtalate ester (DEHP), et les trois métaux lourds Cadmium, Plomb et Zinc. Ces différents polluants ont également été pris en compte à plusieurs niveaux de précision (contamination totale, contamination aux métaux, contamination aux POP, et contamination individuelle).

Dans un second temps, la **réponse du zooplancton** à ces différentes conditions environnementales a été testée, en comparant l'abondance (à l'aide de tests de Mann & Whitney et de Kruskal-Wallis) et la composition taxonomique (à l'aide de tests ANOSIM et PERMANOVA) des communautés zooplanctoniques dans les stations ainsi classifiées.

Enfin, les contributions relatives des concentrations en polluants et des facteurs environnementaux autres à expliquer la variabilité du zooplancton ont été évaluées par une partition de la variance.

3.1.3 Principaux résultats et Discussion :

Bien que les stations étudiées se répartissent en 4 classes d'État Écologique, aucune différence n'a pu être établie entre les communautés zooplanctoniques de ces différentes classes.

La classification des stations basée sur les **conditions physico-chimiques** a révélé des profils de distribution « régionalisés » au sein de la zone d'étude, avec des stations classées par zone

géographique (Nord/Sud-Est/Sud-Ouest). L'analyse RDA utilisant ces conditions comme variables explicatives a montré une différenciation des stations de septembre 2014, mais n'a pas permis de mettre en évidence de différenciation entre les échantillons d'avril 2014 et avril 2015, indiquant la ré-occurrence des profils environnementaux en avril 2014 et 2015. Seule la classification d'avril 2015 a montré une réponse significative des rotifères à la classification basée sur les paramètres physico-chimiques en termes de composition taxonomique.

Contrairement à la classification des sites basée sur les conditions physico-chimiques, la classification basée sur la contamination n'a pas donné de profil régionalisé, mais plutôt des conditions hétérogènes au sein du bassin. Cette fois, l'ordination issue de la RDA basée sur les contaminants séparait clairement les 3 différentes périodes d'échantillonnage, suggérant que la contamination contribuait potentiellement à la dissimilarité entre les communautés d'avril 2014 et avril 2015.

En comparant les communautés zooplanctoniques des différentes classes de condition créées à partir des contaminants, les réponses suivantes ont pu être identifiées :

- Un effet significatif de la contamination aux POP sur la composition taxonomique des rotifères et des copépodes en septembre 2014,
- Un effet significatif de la concentration en DEHP sur l'abondance des cladocères en septembre 2014,
- Un effet significatif du zinc sur l'abondance des cladocères en avril 2014,
- Une influence croisée significative des POP et ETM sur la composition taxonomique des cladocères en avril 2015 (bien que l'influence des POP et des ETM n'aient pas présenté d'influence séparément).

Outre ces réponses significatives, quelques tendances non-significatives ont pu être dégagées : un effet des concentrations en HAP particulaire sur les compositions taxonomiques copépodes en avril 2014 et sur les rotifères en avril 2015, du DEHP sur la composition des copépodes en septembre 2014, de l'ensemble des métaux sur la composition des cladocères en avril 2014 et sur l'abondance des rotifères et des cladocères en septembre 2014, et un effet des concentrations de Plomb sur la composition des rotifères et cladocères en avril 2014, et sur l'abondance de ces deux groupes en septembre 2014.

La partition de variance indique que les contaminants contribuaient à expliquer environ 7 % de la variance du zooplancton (probablement en partie assimilée aux différences inter-annuelles), tandis que les facteurs physico-chimiques autres en expliquaient 14 %, et que 5 % étaient attribués à la contribution des deux.

La part non-expliquée de variabilité du zooplancton restait donc très importante (74 %). Globalement, les réponses du zooplancton aux paramètres physico-chimiques et aux polluants étaient assez faibles, en raison probablement de l'hétérogénéité de nos données, qui pourraient être réduites par un effort d'échantillonnage plus important.

Par ailleurs, le fait que les rotifères dominent l'assemblage pourrait être associé à une plus forte tolérance de ces organismes à la pollution, favorisés par leur petite taille, leur stratégie de reproduction et leur compétitivité (Odum, 1985, Havens & Hanazato, 1993; Hanazato, 2001).

Enfin, la distribution du zooplancton est régulée par de multiples facteurs biotiques et abiotiques et par leurs interactions. La liste des facteurs utilisés dans cette étude est loin d'être exhaustive, et l'influence de la prédation et de l'hydrologie, et de certains contaminants, comme les résidus médicamenteux, ont probablement aussi un impact sur la structure des communautés.

Néanmoins, cette étude a permis de démontrer que bien que le zooplancton ne réponde pas aux variations de la qualité de l'eau telle qu'elle est évaluée par le Directive Cadre Eau (ie. Les États Écologiques des stations), la pollution des cours d'eau semble influencer l'abondance et la composition du zooplancton. Les résultats obtenus, même s'ils nécessitent d'être renforcés, traduisent la complexité liée à la fois aux effets directs et indirects des polluants sur les communautés dans leur globalité, et à l'effet des pollutions multiples telles qu'elles existent dans le milieu naturel. La surveillance des communautés zooplanctoniques fournit donc une information d'un point de vue fonctionnel.

3.2 ARTICLE:

Zooplankton communities' response to variability of water quality and multiple contaminations in the upper Scheldt basin watercourses.

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3.2.1 Abstract

The Scheldt upstream watershed is an area of high historical pollution due to urbanization, industrialization and agriculture. As a consequence, rivers of the drainage basin present high metallic (ETM) and Persistent Organic Pollutants (POP) contamination. This multiple contamination, combined with general conditions' variability (non-contamination related environmental parameters), results in variable ecological status as determined by the Water Framework Directive (WFD) assessment, and probably led to an important variability of habitat conditions for zooplankton communities of the drainage basin.

In order to investigate if zooplankton communities are more similar in similar environmental conditions, we tested the response of zooplankton communities in terms of abundance and composition to several types of condition. Using a set of 18 stations sampled at 3 occasions (April 2014, September 2014 and April 2015), we classified sites according to their ecological status (WFD), general environmental parameters, and contamination data. A bottom-up (unsupervised) approach combining principal component analysis and hierarchical classification was used to classify sites according to their general parameters, while for ecological status and contamination data, sites were classified using a top-down (supervised) approach. Zooplankton communities were then compared among sites groups thus created in terms of community composition and abundance.

The results of variation partitioning showed that general environmental parameters explained approximatively 14% of zooplankton variability while contaminants explained 7% and both explained together 5%. No difference in zooplankton abundance and composition was found between the sites presenting different ecological status, and the classification of sites according to general environmental conditions only allowed to differentiate rotifers taxonomic composition in April 2015. Different trends were also found for zooplankton response to contamination, even if these observations were poorly significant and not systematic. Effects of grouped POPs, DEHP, zinc and combined ETM and POPs levels were in particular identified.

Despite an important variability of zooplankton response to these conditions of water quality, the present study illustrate the complexity of interactions and the integrated and functional response of zooplankton to habitat conditions, reflecting in particular multiple pollutions and indirect effects of contamination on communities.

3.2.2 Introduction:

Among the anthropogenic threats on aquatic systems, agriculture, industry and urbanization contribute to the release of pollutants in soils and atmosphere, which, by leaching or atmospheric deposition, are found in watercourses. Implemented in 2000, the Water Framework Directive (WFD, 2000) imposes the evaluation of Ecological Status for water bodies, based on a set of biological and physico-chemical indicators, supported by chemical and hydromorphological data upon Member States of European Union. The biological quality elements (BQE) used in the WFD evaluation include phytoplankton, phytobenthos, macrophytes, benthic macroinvertebrates and fish. Despite its key functional role in pelagic food webs, zooplankton is not monitored in the frame of the WFD, which is regrettable according to several authors (Moss, 2007; Nõges et al., 2009; Jeppesen et al., 2011; Haberman & Haldna, 2014).

Zooplankton is represented in quasi in all aquatic systems and occupies a crucial role in pelagic systems, as a link for energy transfer between phytoplankton primary producers and higher trophic levels. Being very sensitive to environmental conditions and responding rapidly to perturbations (Gannon & Stemberger, 1978), it is therefore frequently used in ecotoxicological studies (Buikema, Geiger & Lee, 1980; Nikunen & Miettinen, 1985; Weber, 1993; Snell & Janssen, 1995; Kwok et al., 2015). However, ecotoxicological tests alone are not always relevant for estimations of the ecological risk, since inter and intra species differences in response to a perturbation can lead to consequences at the community level, implying mechanisms such as predation, competition, and trophic cascade effects (Cairns, 1983; Clements, 2000; Fleeger, Carman & Nisbet, 2003). Completing species-level ecotoxicological studies with a more ecological perspective at the community level is therefore encouraged (Prygiel *et al.*, 2000; Rohr, Kerby & Sih, 2006).

In the upper Scheldt watershed, metallurgical industrial activities have led to high soil contamination by atmospheric fallout of Cadmium (Cd), lead (Pb) and zinc (Zn) (Sterckeman *et al.*, 2000; Lesven *et al.*, 2009). Rivers of the upper Scheldt basin consequently present contamination of sediments at toxic levels (Boughriet et al., 2007b a; Louriño-Cabana et al., 2011). Polychlorobiphenyles (PCB), Phthalic Acid Esters (PAE) and Polycyclic Aromatic Hydrocarbons (PAH) have also been shown as highly concentrated in the sediments, the dissolved phase and associated to SPM in the region's river network. These substances are in particular found near agglomerations where they mainly originate from atmospheric deposition or combustion processes (Charriau et al., 2009; Sanctorum et al., 2011; Net et al., 2015b a; Rabodonirina et al., 2015). PCBs, PAEs and PAHs are Persistent Organic Pollutants (POP), and are toxic, hydrophobic, and particularly persistent in the environment. Because of their tendency

to bioaccumulate they can easily transit in the food-chain (Geyer et al., 2000; Goerke et al., 2004; Kelly et al., 2007; Bettinetti & Manca, 2013).

The contamination of sediments by POPs and metals can affect benthic and sediment dwelling organisms (Prygiel et al., 2000; De Lange et al., 2004; De Jonge et al., 2008; Josefsson et al., 2011). In the region, the important human activity and the low relief led to channelization and navigation of the river network. It is subject to an important fluvial traffic (approximatively 30 ± 10 boats per day in certain channels; Prygiel et al. 2015). Hence, contaminants can arrive in the water column by runoff from the basin, atmospheric deposal or resuspension of sediments due to boat passage (Lesven et al., 2009), and pelagic communities may also be affected by the contamination.

The effects of POPs and trace metals on zooplankton species are relatively well documented through ecotoxicological studies, and have been shown for example to affect population densities, mating success, or feeding behavior of organisms (Hjorth, Forbes & Dahllf, 2008; Berrojalbiz et al., 2009; Seuront, 2011; Michalec et al., 2013; Viaene et al., 2015). Zooplankton community dynamics are complex and regulated by a set of biotic and abiotic factors in interaction (Pinel-Alloul, 1995; Sterner et al., 1996; Pinel-Alloul et al., 1999; Lair, 2006; Bertani, Ferrari & Rossetti, 2012). Communities of the upper Scheldt watershed are subject to a strong seasonality, in interaction with food resources (ie. Phytoplankton), but the spatial patterns within the watershed is still poorly understood (Le Coz et al., Unpub. data). In a more general way, spatial patterns of zooplankton communities have been seldom documented in river networks, in particular in upstream reaches where they are considered to be poorly represented due to hydrological constraints. Zhao *et al.* (2017) found that in riverine systems, zooplankton variability is mainly related to local environmental factors during low flow conditions, contrarily to high flow conditions, when zooplankton variability is more related to hydrological features. In a low-flowing system such as the Scheldt upstream basin, it is thus likely that environmental conditions have a prevailing influence on zooplankton community variability.

The environmental variability in the upstream Scheldt basin, and the multiple contamination related to the different pollutants described above, may create variable habitat conditions for zooplankton communities, in superposition with the variability of non–pollution related environmental conditions. Jenkins & Buikema (1998), comparing the composition and functions of zooplankton communities developing in a set of similar new ponds, raised the question: “do similar zooplankton develop in similar sites?”. They found that in ponds presenting similar physico-chemical conditions, zooplankton communities were structurally and functionally different.

In the present study, we consider the question if natural zooplankton communities' abundance and composition are similar in sites presenting similar environmental conditions of the upper drainage basin of the Scheldt. To answer this question, we defined environmental conditions at several levels: the Ecological Status as determined by the WFD assessment, the general physico-chemical conditions (represented by the factors: Chl*a*, SPM, OM/SPM, oxygen and Nutrient concentrations), and the water contamination. We considered contamination at several hierarchical levels, focusing on the main potentially harmful substances in the area (PCB, PAH, DEHP and trace metals Cd, Zn, Pb). The response of zooplankton communities to different conditions thus defined was investigated, in terms of abundance and taxonomic composition. Also the contribution of pollutant concentrations in explaining zooplankton variability relatively to physico-chemical factors is studied.

3.2.3 Material and Methods

3.2.3.1 Study Area

Samples were collected in 15 sites situated in the transboundary area of France and Belgium and spread on the following watercourses: the Scheldt, the Scarpe, the Sensée, the Lys, the Deûle, and the Sambre (Fig.1). These sites are all located on channelized watercourses, except Crévecoeur sur Escaut (CSE). Except for the Sambre, these rivers belong to the Scheldt watershed.

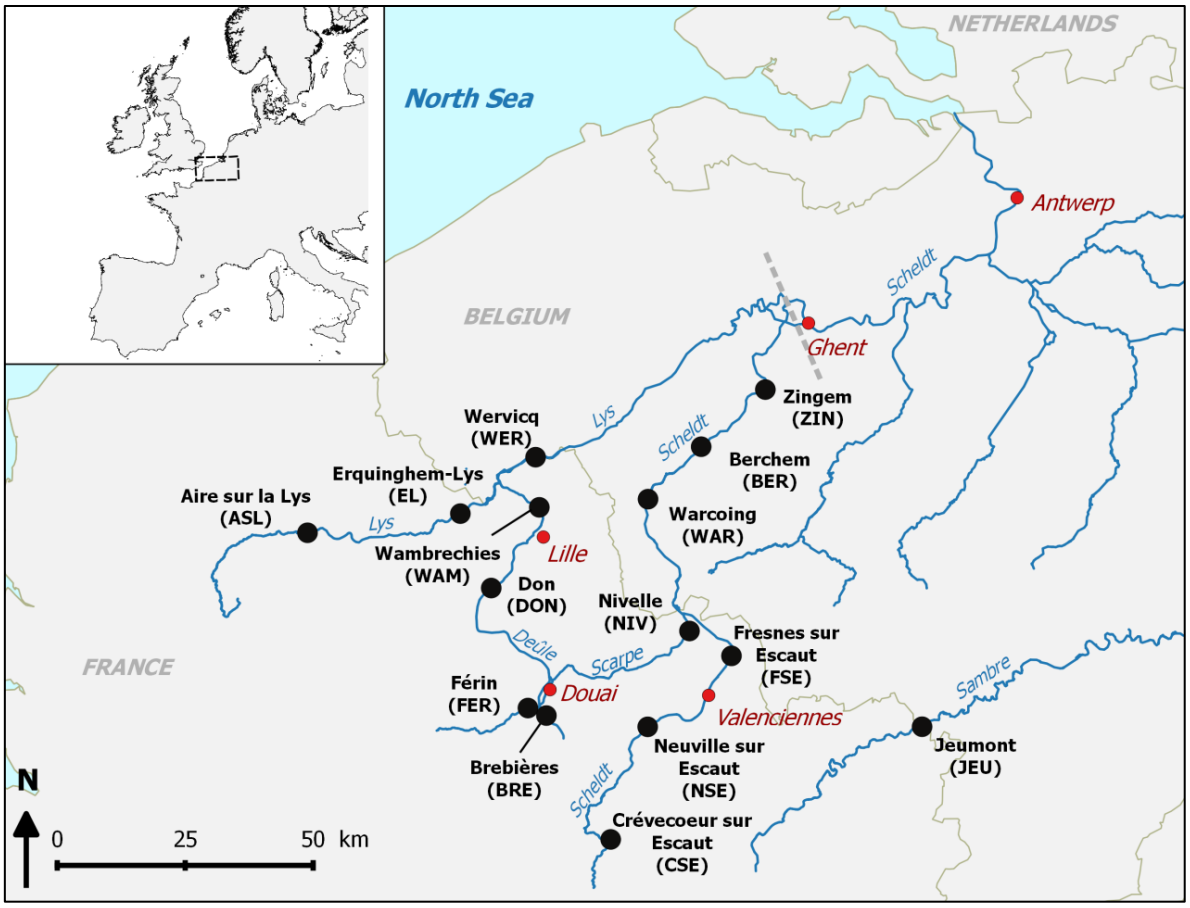

Fig. 1. Location of sampling sites in the Scheldt watershed and their codes as they are used in the following maps. The grey dotted line represents the limit of the tidal influence. Cities are indicated in red color.

The Scheldt takes its source in Northern France (49° 59’ 12,95" N 3° 15’ 59,40" E), and flows 355 km downwards in the North Sea in Netherlands, after crossing Belgium. The Scheldt Basin have been subject to high levels of pollution, since it is a territory densely populated (Van Damme *et al.*, 2005; Billen, Garnier & Rousseau, 2005; Meire *et al.*, 2005; Boughriet *et al.*, 2007a; Charriau *et al.*, 2009; Lesven *et al.*, 2009; Net *et al.*, 2015b), of important past and present industrial and agricultural activities (Billen *et al.*, 2005; Lesven *et al.*, 2009). As a consequence, the WFD classifies most of the studied watercourses as in moderate to bad Ecological Status.

Samplings were conducted at 3 campaigns: in April 2014 (7th -10th), September 2014 (2nd-7th) and April 2015 (16th-23rd).

3.2.3.2 Zooplankton Sampling

For zooplankton sampling, 50 liter of subsurface water was sampled using a pump system, and filtered through a 50 µm mesh net. The organisms collected were then narcotized with carbonated water and fixed with formaldehyde solution (4% final concentration). In the

laboratory, samples were stained with erythrosine and counted under a binocular microscope (magnification × 90). A minimum of 100 individuals was counted.

3.2.3.3 Environmental parameters

Temperature, dissolved oxygen concentration (O_2), and conductivity were measured *in situ* using a multi parameter sensor (WTW, Multi 3430). Suspended Particulate Matter (SPM) concentrations were quantified by filtration of 250 to 1000 mL of sub-surface water, depending on the SPM concentration, onto pre-weighed Whatman GF/C filters (porosity size: 1.2 µm). Filters were dried (at 45 °C) during 24 hours, briefly cooled in a desiccator and weighed. The same filters were burned at 500 °C for 5 hours, and re-weighed to obtain ash concentration. Organic Matter (OM) concentrations were calculated as the difference between SPM and ash concentrations.

200 to 600 ml water samples were filtered on Whatman GF/C filters for the determination of chlorophyll a (Chl*a*) concentrations, which were then extracted in 2 % ammonium acetate solution and quantified by reversed phase HPLC, following the method of Wright & Jeffrey (1997). Details of the methodology are given in (Majdi *et al.*, 2011).

100 ml of water was sampled and filtered through 0.2 µm cellulose nitrate filters in order to determine Total Phosphorus (Ptot), Nitrite (NO_2^--N), Nitrate (NO^{3-}-N), and Phosphate (PO_4^{3-}-P) concentrations by Ion Chromatography analyses (Dionex Ics-5000+, Dionex Corp., Sunnyvale, CA, USA).

3.2.3.4 Water chemistry

Samples for characterization of pollutant concentrations were taken at the same sampling dates. The following metals and persistent organic pollutants (POPs) were investigated:

Water sampling were performed at 30 cm depth under the surface using 1L PTFE bottles for trace metal analyses and 2.5 L amber glass bottles for organic micropollutants. All bottles were previously cleaned with detergent (Decon, UK) in case of organic micropollutants, 10% ultrapure nitric acid in case of trace metal analyses and then rinsed thoroughly with ultrapure water (Millipore, Milli-Q gradient, σ 18.2 MΩ cm).

Water samples dedicated to trace metal analyses were filtered on site using cellulose acetate filters (0.45 µm porosity, Sartorius, Germany) and were immediately acidified at 2% (v/v) using nitric acid (67–69 %, optima grade, Fisher Scientific).

- **metal concentrations:**

Concentrations of dissolved elements in waters were determined by using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS, X Series Thermo elemental) for Cadmium (Cd), lead (Pb) and zinc (Zn). The ICP-MS spectrometer was calibrated using standard solutions, and blank corrections were applied when necessary. For the purposes of quality control, a riverine water standard reference material (SLRS-4) was analyzed.

Analyses of organic micropollutants were conducted using a Varian 3900 Gas Chromatograph (GC) equipped with a deactivated fused-silica guard column (5 m, 0.25 mm i.d.) and a fused-silica Phenomenex XLB capillary column (60 m length, 0.25 mm i.d., 0.25 mm film thickness, Phenomenex) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). Helium was used as carrier gas at a constant flow rate of 1 $mL.min^{-1}$. Oven programmation was optimized specifically for each targeted analytes, and quantification was performed in the single ion storage (SIS), MS/MS or multiple reaction monitoring (MRM).

- **Polycyclic Aromatic Hydrocarbons (16 PAHs):**

Naphthalene, acenaphtylene, acenaphtene, fluorene, phenanthrene, anthracene, pyrene, fluoranthene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi]perylene, indeno[1,2,3-cd]pyrene.

- **Polychlorobiphenyls (28 PCBs)**

8; 18; 28; 44; 52; 66; 77; 81; 101; 105; 114; 118; 123; 126; 128; 138; 153; 156; 157; 167; 169; 170; 180; 187; 189; 195; 206; 209.

- **Phthalates (1 PAEs)**

di-2-ethylhexyl phthalate ester (DEHP)

POPs were quantified in the dissolved phase, and are expressed in $\mu g.l^{-1}$. For PAHs, the concentrations in the particulate phase (associated to SPM) were also investigated, and are expressed in $\mu g/mg_{SPM}$. For clarity, the POP concentrations of the dissolved phase will be indicated by a 'd" (PAH.d; PAE.d; PCB.d) and those of the particulate phase by a "p" (PAH.p). A dataset composed of 69 compounds was therefore obtained.

3.2.3.5 *Data analysis*

a. Defining "similar sites"

Since the aim of this work was to compare zooplankton communities between "similar sites", the first step was to define groups of sites which could be considered as similar in terms of environmental conditions. Several "levels" have been investigated, presented in Fig 2.

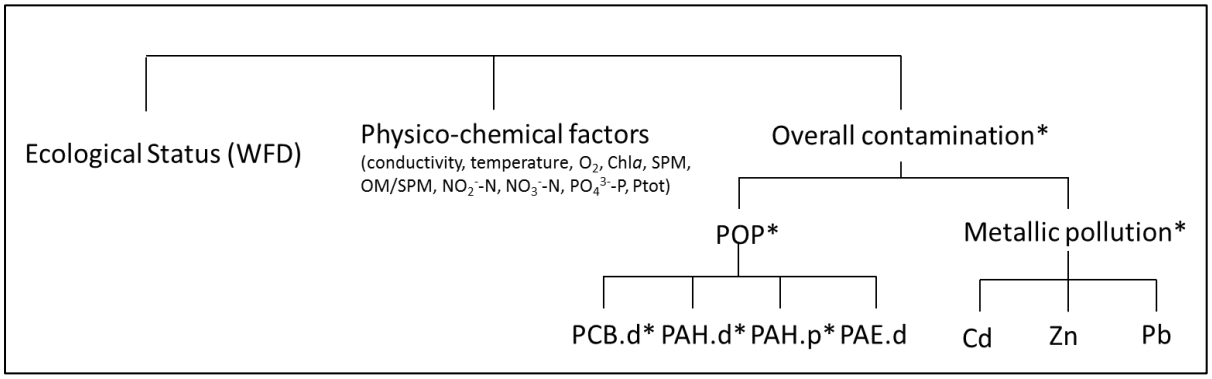

Fig. 2. Diagram of the different levels of conditions investigated. Levels marked by an asterisk are based on sums of various sub-components.

Two different methods were used to classify the sites (Fig 3):

-The classification of sites according to physico-chemical factors was done using a "bottom-up", or unsupervised approach. A Principal Component Analysis (PCA) was applied to standardized environmental data, and the first principal components explaining more than 90% of total variance were used as linear independent variables to conduct an ascendant hierarchical classification (ACH) based on Ward's method and using Euclidian distance. The clusters therefore obtained represented groups of "similar" sites according to physico-chemical factors.

-A "top-down" or supervised approach was used to classify sites according to their Ecological Status and to their contaminant concentration (at all "contamination levels"). The Ecological Status of the WFD being categorized in 5 classes, this classification was directly used to compare zooplankton between classes. WFD classification was available only for the year 2014.

However, other levels necessitated to define categories of value of component concentrations. The categorization of samples for these levels can be decomposed in two steps:

Step 1: coding data

The chemical data comported an important number of non-detected values (below the limit of quantification or detection).

The thresholds of the SEQ-Eau grid were used for coding data. The SEQ-Eau (i.e. Water Quality Evaluation System) is a grid providing thresholds for pollutants concentrations used in French water quality assessment system (Oudin & Maupas, 2003).

Each value of contaminant concentration was coded 1 if the value was non-detected of below the lowest threshold of the SEQ-Eau (threshold excellent-good status), 2 if the concentration ranged between the lowest and the highest threshold (threshold moderate to bad status), and 3 if the value was higher than the more declassifying threshold of the grid.

This step provided for each substance a classification of sites from which clusters could be compared in terms of the abundance and composition of their zooplankton community. However, investigations on the effect of PAH.p, PAH.d, PCB.d, POP, Metallic pollution and overall contamination were all based on several substances classification, and thus necessitated another step of codification described in the following.

Step 2: classification of groups of contaminants

Certain levels of contamination necessitated taking into account a set of substances (marked by an asterisk in Fig 2): PAH.p, PAH.d, PCB.d, POP, Metallic pollution and overall contamination. For these levels, the codes (calculated in step 1) of all substances considered were summed. The score obtained can be expressed in percentage of maximal theoretical contamination (sum of codes of all substances / 3 × number of substances, since 3 is the maximal value of a code).

A binary transformation was applied to these scores: stations presenting a score lower than the median of scores were coded 1, and all the samples with scores higher than the median were coded 2 (following Hamadou et al. 2001).

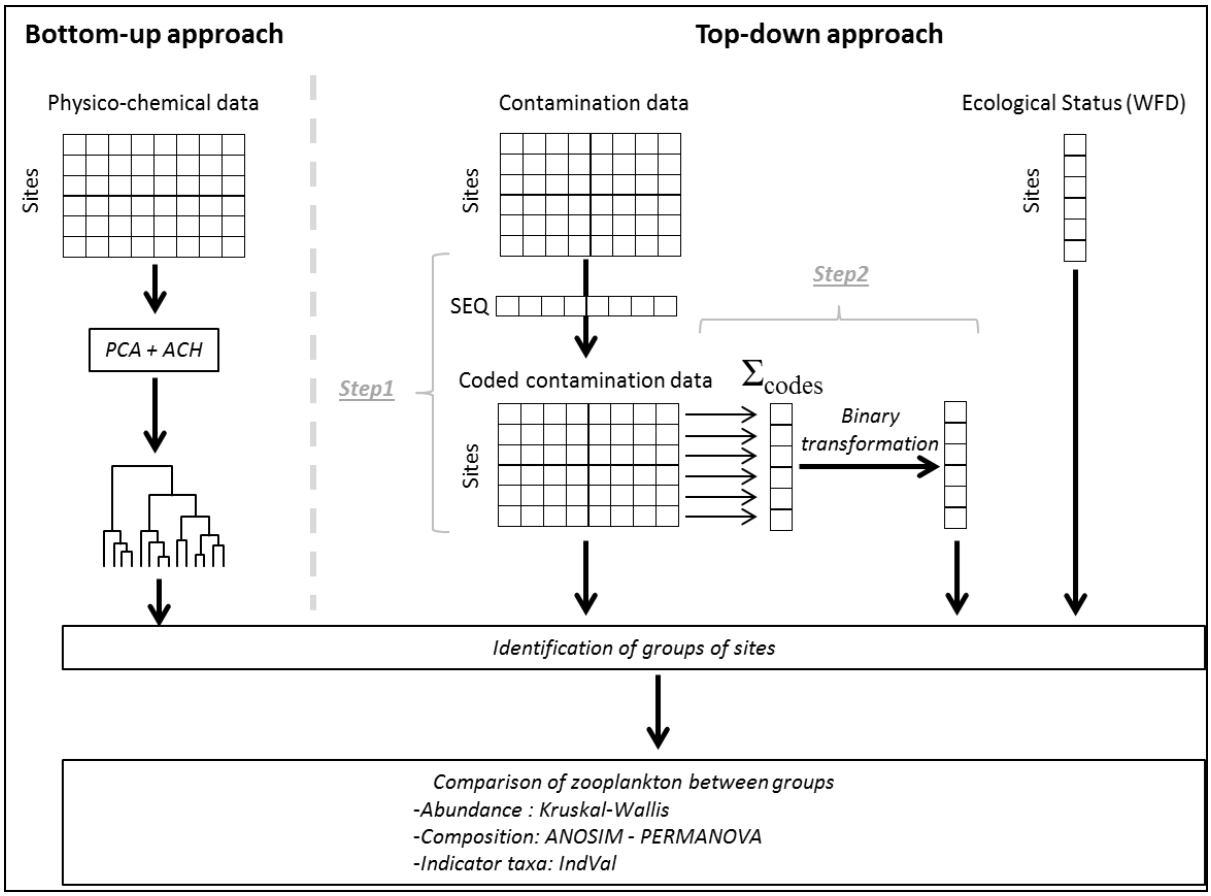

Fig. 3. Diagram summarizing the different steps used to define sites classification. See text for more details.

b. comparing zooplankton communities between groups of similar sites

Once sites were distributed in different clusters for the different levels described in Fig 2, the next step consisted in comparing the zooplankton communities between different groups thus created. Analyses were conducted considering all zooplankton taxa, and also separately for rotifers, copepods, and cladocerans.

Zooplankton abundance was compared between the different groups of sites using Mann & Whitney tests, or Kruskal-Wallis tests when more than 2 different classes of sites.

PERMANOVA (permutational multivariate analysis of variance) and ANOSIM (Analyses of similarities) with 9999 permutations were performed on Bray–Curtis matrix of zooplankton abundance (previously log (x+1) transformed) to investigate the taxonomic differences between different groups of sites defined. Both are permutation methods based on resemblance of samples, but are not redundant (Anderson & Walsh, 2013). While PERMANOVA is considered as more powerful, ANOSIM which is rank-based is more sensitive to heterogeneity and to the correlation structure of the data. Indicator species were identified using Indicator Value (IndVal)

method described by (Dufrêne & Legendre, 1997). IndVal allows identifying taxa which are mostly found in a group of sites (measured by Specificity A), and are represented in a high number of the sites which constitute this group (measured by Fidelity B).

The IndVal thus combines taxa abundances with their relative frequency of occurrence, and can be calculated for a taxon i and a cluster j, following the formulae:

$$IndVal_{ij}=A_{ij} \times B_{ij} \times 100$$

With the specificity A_{ij} of a taxon i for a cluster being:

$$A_{ij}= Nindividuals_{ij} / Nindividuals_{i}$$

Where $Nindividuals_{ij}$ is the mean number of individuals of the taxon i in the sites of cluster j, and $Nindividuals_{i}$ is the sum of mean number of individuals of the taxon i in all clusters.

And the fidelity B_{ij} being:

$$B_{ij}= Nsites_{ij} / Nsites_{i}$$

Where $Nsites_{ij}$ is the number of sites of the cluster j where the taxon i is present, and $Nsites_{i}$ is the total number of sites in the cluster j.

c. Determining the part of variability explained by contaminants relatively to global physico-chemical factors.

Once effects of different levels of contamination were studied, the overall contribution of contaminants relatively to physico-chemical global factors (O_2, conductivity, temperature, Chl*a*, SPM, OM/SPM, and nutrients) in explaining zooplankton variability was assessed. This part of the study will consider all three sampling occasions together, in order to obtain an overall image of the contaminants / physico-chemistry interactions with zooplankton.

First, a redundancy analysis (RDA) was conducted to assess relation between global physico-chemical factors and zooplankton. Forward selection was used to test which factors explained a significant proportion of the taxa variance, and the significance of the model was tested by 999 Monte-Carlo permutations.

The same procedure was run using contamination data at the lowest level: PAH.p, PAH.d, DEHP.d, PCB.d, Cd, Zn and Pb scores.

A partitioning of Variance (Borcard, Legendre & Drapeau, 1992; Borcard, Gillet & Legendre, 2011) was then conducted to quantify which part of the zooplankton variability was explained by

physico-chemical factors, which part was explained by contaminants, which part by a combination of both and which part remained unexplained.

All statistical analysis were conducted with R (R. Core Team, 2013), using the following packages: 'ADE4', "packfor", "vegan", and "indicspecies".

3.2.4 Results

3.2.4.1 Zooplankton community composition

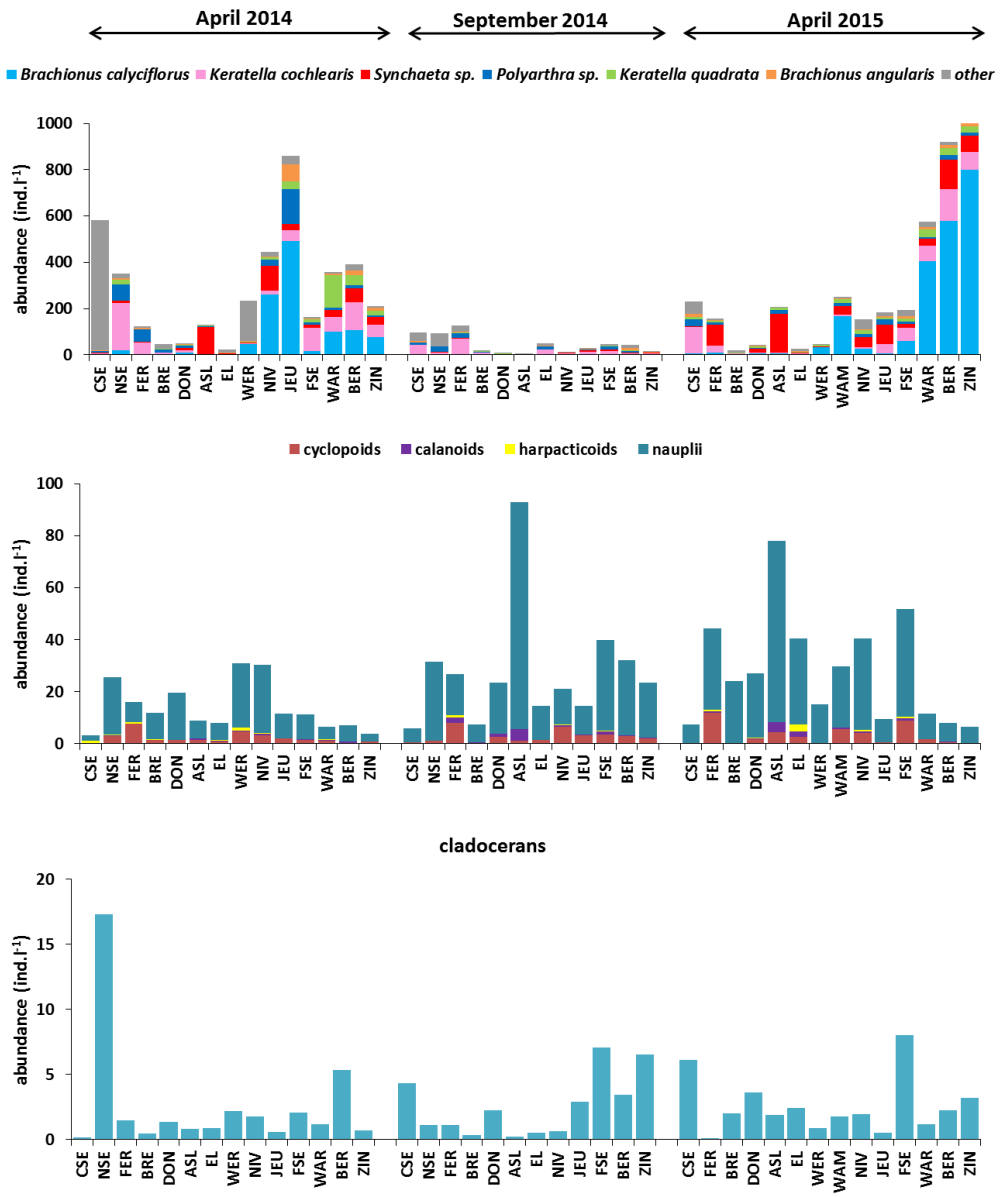

Fig. 4. Abundance of rotifers, copepods and cladocerans at each sampling station in April 2014, September 2014 and April 2015.

A total of 38 zooplankton taxa were identified considering the 3 sampling occasions. 3 taxa were considered as rare and removed for analysis. Mean total abundance reached 299 ±235 ind.l^{-1} in April 2014, 75.81 ± 40.57 ind.l^{-1} in September 2014 and 317.68 ± 312.27 ind.l^{-1} in April 2015, reflecting a high variability between both sampling occasions and sites (Fig 4). When considering all samples of all sampling occasions, the coefficient of variation of total zooplankton abundance reached 105%. It was 79% in April 2014, 68% in September 2014 and 100% in April 2015.

Rotifers were much less abundant and their contribution to total abundance was less important in September (60% of total on average while 92% on average in April). The rotifer community was composed for 85% by a set of 6 majoritarian taxa (*Brachionus calyciflorus*, *Keratella cochlearis*, *Synchaeta* sp., *Polyarthra* sp., *Keratella quadrata*, *Brachionus angularis*).

Crustaceans were mainly represented by copepod nauplii and cyclopoids (respectively 85 and 11 % of copepod abundance).

3.2.4.2 Zooplankton community response to different classifications levels

For the different types of environmental conditions considered, zooplankton response in terms of abundance and taxonomic composition of the different groups (rotifers, copepods and cladocerans) are summarized in the Table 1 presenting the results of Kruskal-Wallis, Mann & Whitney, ANOSIM and PERMANOVAs tests. The results are then presented separately for the different types of environmental condition considered in the following sub-parts.

Table 1. Results of Kruskal-Wallis, ANOSIM and PERMANOVA tests conducted for comparing zooplankton communities between different clusters of sites. (P-C is for physico-chemical based clusters, E.S. for ecological states, total for total contamination). When results are significant, the statistic is indicated in red, and the P value is coded as: **P<0.01; *P<0.05; · P<0.1.

	P-C	E.S.	total	POP	PAH.p	PAH.d	PCB.d	DEHP.d	metals	Cd	Pb	Zn
total zopolankton						***April 2014***						
Kruskal -Wallis statistics	**4.72·**	1.7189										
Mann Withney U			31	31	22	15			22	26	25	13
ANOSIM R stat.	0.03	-0.06982	0.03207	0.03207	0.0243	0.1			0.03401	-0.06997	0.1108	-0.1724
PERMANOVA F.	1.0446	1.0154	1.0851	1.0851	1.359	1.0998			0.8006	0.77606	**1.9031·**	0.55277
rotifers												
Kruskal -Wallis statistics	3.92	2.4552										
Mann Withney U			31	31	24	14			22	25	24	15
ANOSIM R stat.	0.125	-0.1148	0.1283	0.1283	0.03887	0.1167			-0.04276	-0.1195	0.06373	-0.1515
PERMANOVA F.	0.98725	1.3365	1.7118	1.7118	0.98832	1.1508			0.60978	0.46896	**1.8985.**	0.53277
copepods												
Kruskal -Wallis statistics	0.52	2.7924										
Mann Withney U			22	22	12	13			20	25	12	13
ANOSIM R stat.	1.8502	0.1006	-0.1331	-0.1331	0.0758	0.04804			0.05345	0.102	0.04118	-0.1745
PERMANOVA F.	1.8502	1.2928	-0.31905	-0.31905	**2.4176·**	1.2407			1.8661	**2.4381·**	0.88109	0.57435
cladocerans												
Kruskal -Wallis statistics	0.19	0.3829										
Mann Withney U			27	27	28	10			14	17	24	**3***
ANOSIM R stat.	-0.09	0.01657	-0.05248	-0.05248	0.04568	-0.03137			**0.1691·**	0.02915	0.201	-0.2079
PERMANOVA F.	0.50656	-0.08551	0.19811	0.19811	1.7144	0.91217			0.72111	0.93543	**3.5508·**	0.41848
						September 2014						
total zopolankton												
Kruskal -Wallis statistics	**2.9·**	-0.08551										
Mann Withney U			17	20	20	21		9	26		26	
ANOSIM R stat.	-0.0212	-0.03001	-0.1171	**0.1834·**	-0.2953	-0.04044		0.1728	-0.005556		-0.005556	
PERMANOVA F.	1.4451	0.91191	0.32832	**3.2195***	0.5887	1.3193		0.67954	1.6458		1.6458	
rotifers												
Kruskal -Wallis statistics	1.4835	5.8269										
Mann Withney U			21	28	18	21		14	**30·**		**30·**	
ANOSIM R stat.	0.06175	-0.1429	-0.1207	**0.2903***	-0.1681	0.07353		-0.01471	-0.02593		-0.02593	
PERMANOVA F.	0.80209	0.80419	0.67094	**3.7366****	0.39396	1.0892		0.10941	1.9175		1.9175	
copepods												
Kruskal -Wallis statistics	0.0593	1.1308										
Mann Withney U			21	12	17	16		11	19		19	
ANOSIM R stat.	-0.0341	0.1309	-0.09677	0.05069	-0.1396	-0.136		**0.2849·**	-0.1019		-0.1019	
PERMANOVA F.	1.2089	1.0819	0.10292	**3.292***	0.83688	1.6911		2.0129	0.076435		0.076435	
cladocerans												
Kruskal -Wallis statistics	0.0593	3.7731										
Mann Withney U			22	22	12	15		**28***	**29·**		**29·**	
ANOSIM R stat.	0.06359	-0.003601	-0.1244	-0.1539	-0.1206	-0.1765		0.06985	0.1648		**0.1648·**	
PERMANOVA F.	**2.5103·**	0.78823	0.38561	0.40129	0.80778	1.1779		1.5953	2.1856		2.1856	
						April 2015						
total zopolankton												
Kruskal -Wallis statistics	**5.12·**	0.2667										
Mann Withney U	0.07337	**0.1834·**	20	21	18	18	22		31	12	31	
ANOSIM R stat.			**0.1834·**	-0.004608	0.009718	-0.02206	0.1491		-0.07171	0.04353	-0.07171	
PERMANOVA F.	**1.7887·**	1.1946	1.1946	0.83727	1.4364	0.64742	0.5641		0.5983	0.54256	0.5983	
rotifers												
Kruskal -Wallis statistics	3.8914	0.4167										
Mann Withney U			19	20	19	13	19		30	13	30	
ANOSIM R stat.	**0.13·**	0.1171	0.1171	0.0341	0.1506	0.02941	0.04653		0.002907	0.006219	0.002907	
PERMANOVA F.	**2.6657***	1.6935	1.6935	1.1669	2.2121.	0.56248	0.59613		0.82015	0.80167	0.82015	
copepods												
Kruskal -Wallis statistics	1.62	0.0667										
Mann Withney U			26	29	23	28	25		17	11	17	
ANOSIM R stat.	-0.06036	0.1373	0.1373	-0.002765	-0.1098	0.04228	0.2251		-0.02132	0.05473	-0.02132	
PERMANOVA F.	0.38954	0.033754	0.033754	-0.024042	-0.12231	1.0043	0.57783		0.75121	0.32065	0.75121	
cladocerans												
Kruskal -Wallis statistics	1.1829	1.6667										
Mann Withney U			34	27	29	25	12		34	11	34	
ANOSIM R stat.	-0.06627	0.09124	0.09124	0.04516	-0.02235	0.05882	0.2555		-0.09738	0.2612	-0.09738	
PERMANOVA F.	1.7189	1.8653	1.8653	0.46218	1.9562	0.17355	0.00998		0.69185	2.3692	0.69185	

a. Ecological Status

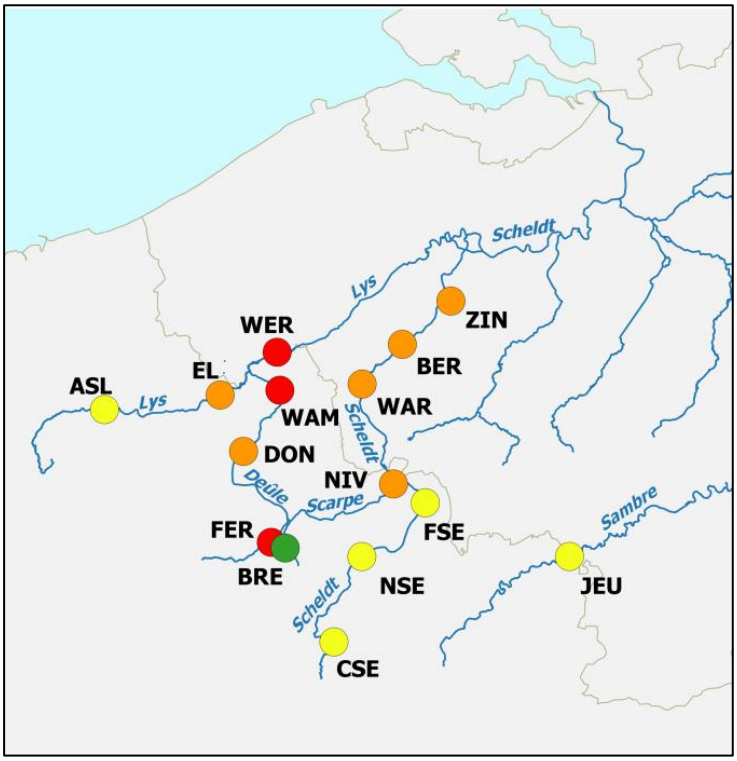

Fig. 5. Ecological states of sampling sites according to the Water Framework Directive assessment (good status in green; moderate status in yellow, mediocre status in orange, bad status in red) for the year 2014.

The Ecological Status as evaluated by the WFD for 2014 displayed 4 classes within the studied sites, ranging from good to bad Ecological Status (Fig 5). The very good Ecological Status was not displayed among the studied sites. Only one site was considered as in "good Ecological Status". 5 were moderate, 6 mediocre and 3 bad.

No significant difference between zooplankton abundance (Kruskal-Wallis, $P>0.05$) or composition (ANOSIM: $P>0.05$, PERMANOVA: $P>0.05$) was found among the Ecological States of sites, neither in April 2014 nor in September 2014 (Fig.6).

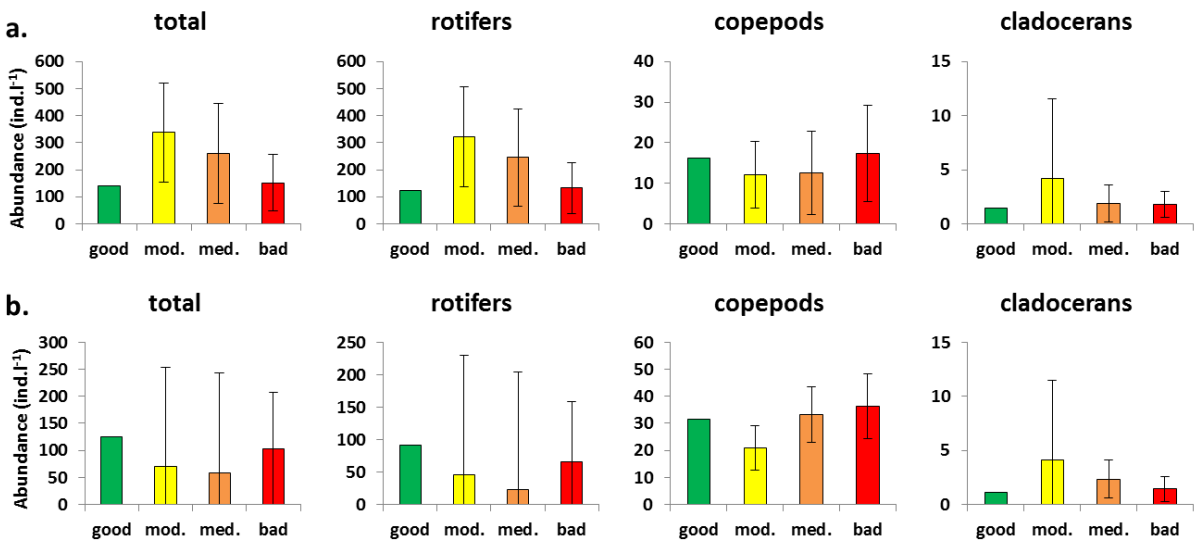

Fig. 6. Mean abundance of total zooplankton and of each zooplankton group for stations grouped per WFD Ecological Status, in April 2014 (a) and September 2014 (b). The color code corresponds to the one used in Fig. 5. Thin vertical bars represent standard deviations.

b. physico-chemical parameters

The bottom-up procedure used to classify sites according to physico-chemical conditions provided 3 clusters for April 2014, 2 for September 2014 and 3 for April 2015 (Fig 7).

The clustering obtained presented a regionalized pattern, discriminating downstream Scheldt sites from others. In both April 2014 and April 2015, a regionalization also occurred between the west part of the sampling area and the eastern part. The re-occurrence of the April pattern was associated to the influence of the same factors according to the PCA from which clusters derived (Fig. 7b). The Northern part is associated with high Chl*a* and O_2 concentrations, the eastern part with high OM contribution to SPM and the western part with inorganic pollution (NO_2^-, NO_3^- PO_4^{3-}). In September, the North was rather characterized by Organic Matter and organic pollution while the south part was rather marked by higher O_2, Chl*a* and SPM concentrations.

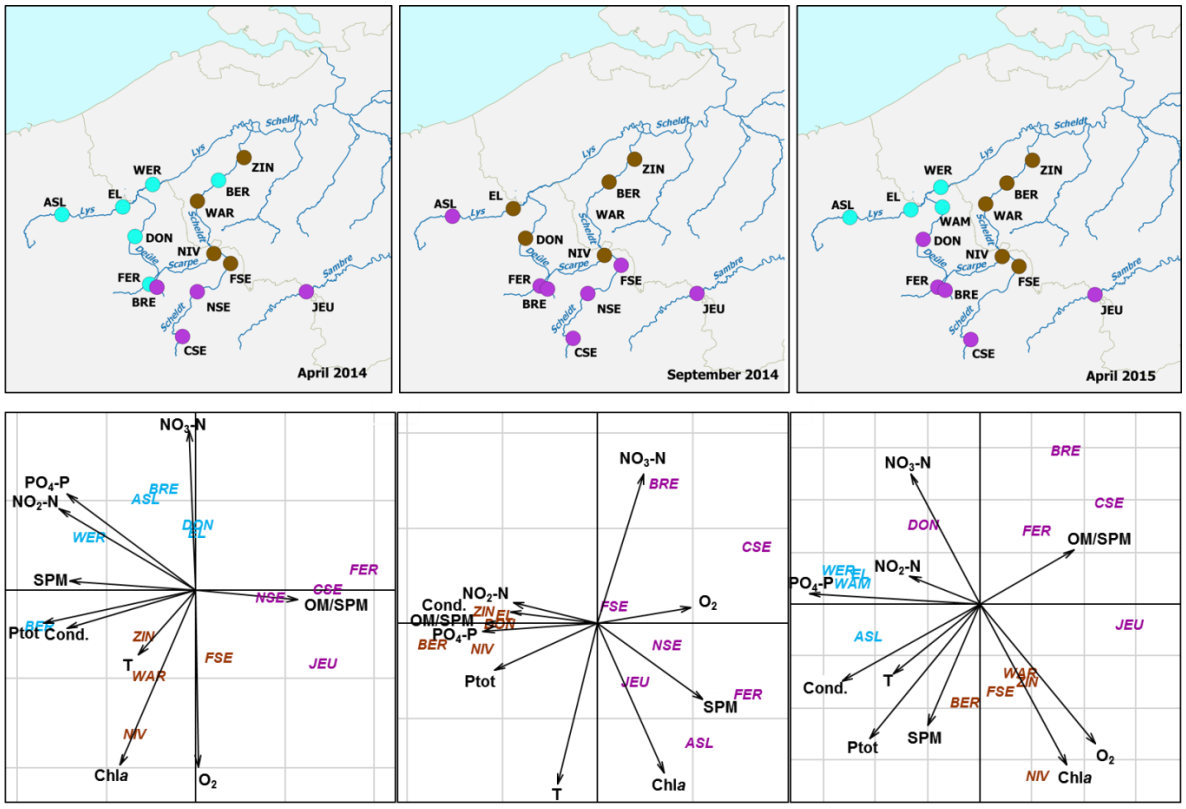

Fig. 7. Classification of sampling sites according to the physico-chemical based clustering obtained by bottom-up approach in April 2015, September 2014 and April 2015, and the corresponding biplots of PCA used for this classification.

In April and in September 2014, total zooplankton abundance tended to differ among groups of sites (Fig 8), even if this result was not significant at α=0.05 according to the Kruskal-Wallis test (respectively P=0.094, P=0.088 and P=0.077 for the 3 successive sampling occasions, Table 1). In April 2015, rotifers tended to be more abundant in northern sites with higher Chl*a* and O_2 concentrations (Mann & Whitney test, P=0.054).

PERMANOVA tests detected a difference in zooplankton community composition and in particular of rotifers (P=0.03) according to the classification of April 2015 samples.

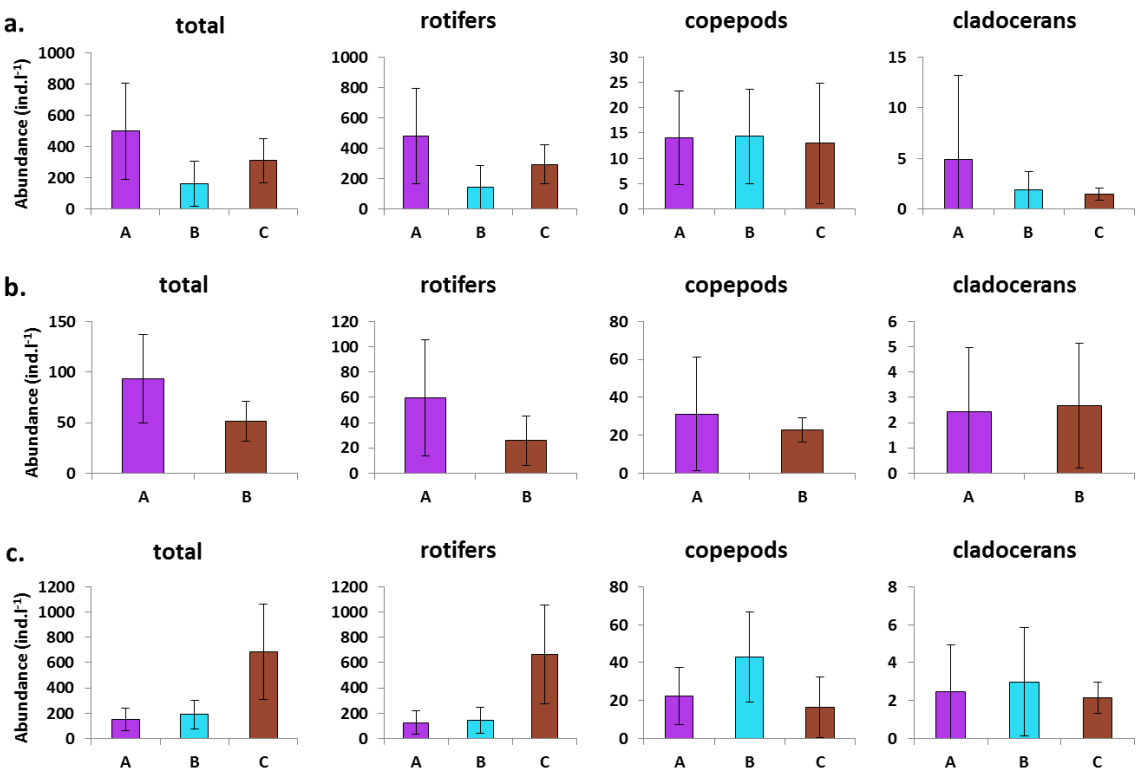

Fig. 8. Mean abundance of total zooplankton and of zooplankton groups in the different categories based on physico-chemical conditions in April 2014 (a), September 2014 (b), April 2015 (c). The color code corresponds to the one used in Fig. 7. Thin vertical bars represent standard deviations.

c. Contaminants

i. Overall contamination

The overall contamination level ranged between 37 and 67% of the maximal potential level of contamination (43 to 52% in April 2014; 37 to 51% in September 2014; 48 to 67% in April 2015). The levels of contamination were different according to sampling periods, so the median used to separate high and low values of contamination were different (46% in April 2014, 43% in September 2014, 53% in April 2015). The repartition of high and low scores among sites was also differing according to the sampling occasion, as shown in Fig 9. Contrarily to the pattern obtained from classification based on physico-chemical parameters, few regional patterns could be detected. It can however be noticed that the Deûle samples always displayed high level of overall contamination.

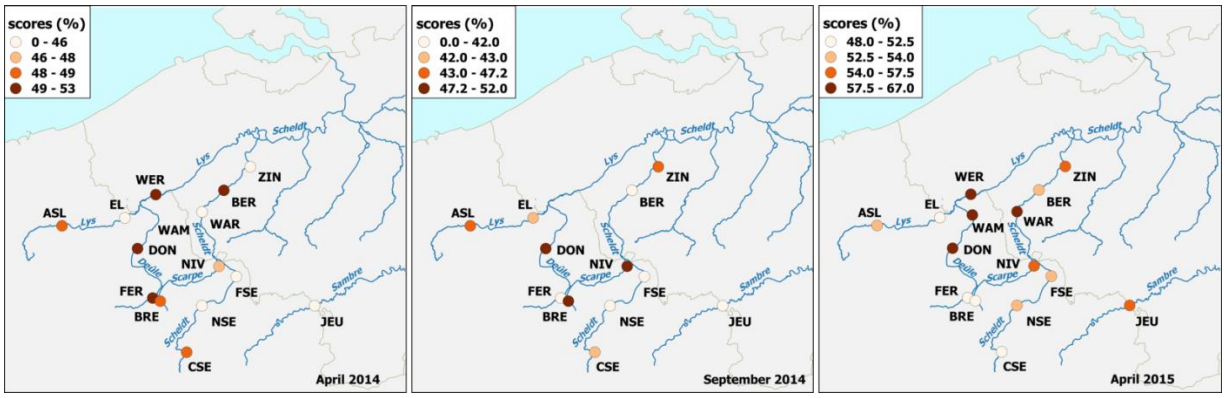

Fig. 9. Total contamination level of sites (score in % of maximal potential contamination) for the 3 sampling campaigns.

After the binary classification step, most of sites were differently classified according to sampling campaigns. Only Don (DON) and Aire-sur-la-Lys (ASL) were always belonging to the more contaminated stations and only Fresnes-sur-Escaut (FSE) was always classified within the less contaminated sites (Table 1). No clear effect of the classification of total contamination on zooplankton abundance and composition of the different zooplankton groups were detected.

Table 2. Codes attributed to samples for the different contamination steps. (Color code: green = 1; yellow = 2, red= 3).

	April 2014										September 2014										April 2015									
	total	**POP**	PAH.p	PAH.d	PCB.d	DEHP.d	**metals**	Cd	Pb	Zn	**total**	**POP**	PAH.p	PAH.d	PCB.d	DEHP.d	**metals**	Cd	Pb	Zn	**total**	**POP**	PAH.p	PAH.d	PCB.d	DEHP.d	**metals**	Cd	Pb	Zn
Crévecoeur sur Escaut	2	2	2	1	1	3	1	1	1	2	2	1	1	1	1	2	1	2	1	2	1	1	1	2	2	3	1	1	1	2
Neuville sur Escaut	1	1	2	1	1	3	1	1	1	3	1	1	1	1	1	3	2	2	2	2										
Férin	2	2	2	1	1	3	2	1	2	3	2	2	1	2	1	3	1	2	1	2	1	1	1	1	1	3	1	1	1	2
Brebières	2	2	2	1	1	3	1	1	2	2	1	1	1	1	1	3	2	2	2	2	1	1	1	1	1	3	1	2	1	2
Don	2	2	2	1	1	3	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	1	2	3	2	2	2	2
Aire sur la Lys	2	2	1	2	1	3	1	1	2	2	2	2	1	1	1	3	2	2	2	2	2	1	2	1	1	3	2	2	2	2
Erquinghem-Lys	1	1	1	1	1	3	1	2	1	2	2	1	2	1	1	2	1	2	1	2	1	1	1	1	1	3	1	2	1	2
Wervicq	2	2	2	2	1	3	2	2	2	2											2	2	2	2	1	3	2	2	2	2
Wambrechies																					2	2	2	2	1	3	2	2	2	2
Nivelle	1	1	1	2	1	3	2	2	2	2	2	2	1	2	1	2	2	2	2	2	2	2	2	1	1	3	2	2	2	2
Jeumont	1	1	2	1	1	3	1	1	2	2	1	1	1	1	1	2	2	2	2	2	2	1	1	1	2	3	2	2	2	2
Fresnes sur Escaut	1	1	1	1	1	3	1	1	2	2	1	1	1	1	1	2	1	2	1	2	1	1	1	1	1	3	1	2	1	2
Warcoing	1	1	1	1	1	3	2	2	2	2											2	2	2	2	1	3	1	2	1	2
Berchem	2	2	1	2	1	3	2	2	1	3	1	1	1	1	1	2	1	2	1	2	1	1	1	1	1	3	1	2	1	2
Zingem	1	1	1	1	1	3	2	2	2	2	2	2	2	2	1	2	1	2	1	2	2	2	2	1	1	3	1	2	1	2

ii. Persistent Organic Pollutants (POPs)

Only 3 PCBs were detected among the 28 investigated (compounds 28, 101 and 153). Also, 5 PAHs were never detected in the samples (dibenzo[a,h]anthracene in the dissolved and particulate phases, indeno[1,2,3-cd]pyrene in the dissolved and particulate phases, and

dissolved acenaphtene). Those substances were thus all coded as "1" by the SEQ-Eau classification. Their contribution in total contamination is not taken into account in the following results to avoid diluting other compound effects.

Since among the 32 substances used to calculate overall contamination, 29 belonged to POPs, classifications of POPs were rather similar to those of overall contamination. In April 2014, it was even exactly the same. In September, only Crévecoeur-sur-Escaut and Erquinghem- Lys sites were different, and in April 2015, Aire-sur-la-Lys and Jeumont were the only sites presenting different classification between overall contamination and POPs contamination.

PAH.p, PAH.p, PCB.p and DEHP.p were however displaying different patterns between them, and also according to the sampling occasion. While PCB.d concentrations were always lower than the threshold of good status of the SEQ-Eau in April and September 2014, for DEHP.d, all values were on the contrary higher than the bad status threshold in April 2014 and 2015.

The classification of the sites for the different steps of contamination has been summarized in Table 2. As for total contamination, no difference was found between zooplankton abundance of the different POP classes for April months. However, in September 2014 zooplankton taxonomic composition was significantly different between classes (PERMANOVA, $P<0.05$), rotifers taxonomic composition was different between high and low pollution levels according to ANOSIM ($P<0.05$) and PERMANOVA tests ($P<0.05$), and copepods taxonomic composition was different between high and low pollution levels (PERMANOVA, $P<0.05$).

PAH.p contamination level probably affected rotifer taxonomic composition, as quasi-significative results for PERMANOVA were found in April 2015 ($P=0.0758$), and copepods taxonomic composition since as quasi-significative results for PERMANOVA were found in April 2014 ($P=0.0643$). However, except for these results, only DEHP.d contamination level provided significant results in discriminating zooplankton communities: cladocerans were significantly affected by DEHP.d contamination level (Mann-Whitney test; $P<0.05$). The abundance of cladocerans was higher at intermediate contamination levels of DEHP.d (3.5 ± 2.4 ind.l^{-1}) than at high level (0.7 ± 0.5 ind.l^{-1}).

iii. Metallic contamination

Classification of sites based on metallic contamination was also differing between sampling periods, but some sites were always displaying high (Nivelle, Don and Wervicq) or low (Crévecoeur-sur-Escaut, Erquinghem-Lys and Fresnes-sur-Escaut) metallic contamination. In September 2014 and April 2015, zinc was presenting an intermediate classification according to the SEQ-Eau at all sites. In September 2014, Cadmium was also classified in intermediate class

for all sites. Among metals, only Zn was found as exceeding the threshold of bad classification of the SEQ.

Metallic total contamination scores also showed no significant results for the different tests of zooplankton communities' comparison. However, rotifers and cladocerans presented slightly higher abundances at low contamination level in September 2014 (Mann & Whitney $P<0.1$). On average, 63.5 ±40.5 ind.l^{-1} rotifers and 3.8 ±2.7 ind.l^{-1} cladocerans were observed in less contaminated sites, while 27.4 ±32.8 ind.l^{-1} rotifers and 1.2 ±1.1 ind.l^{-1} clacocerans were found at sites with higher contamination.

Few differences could be detected among zooplankton according to the level of Cadmium contamination, since only quais-significant PERMANOVA was found for copepods in April 2014 ($P<0.1$).

In April 2014, despite the few number of samples classified as highly Zinc contaminated (N=3), cladocerans were significantly more abundant these sites (on average 295.5±312 ind.l^{-1}) than at intermediate Zinc level sites (on average 260±147 ind.l^{-1}), (Mann and Whitney, $P<0.05$)

In September 2014 rotifer and cladoceran abundance tended to be slightly lower at sites with intermediate level of lead contamination than at low lead level sites (Mann and Whitney; $P<0.1$), and PERMANOVA were quasi-significant for total zooplankton, rotifers and copepods in April 2014 ($P<0.1$).

The PERMANOVA analysis conducted on POP and metals (Table 3) detected a combined effect of POP and metallic contaminations were also found to significantly influence cladocerans taxonomic composition in April 2015.

Table 3. Summary of results of PERMANOVA analysis for total zooplankton, rotifers, copepods and cladocerans community composition, based on Bray-Curtis dissimilarities of log transformed data. df= degrees of freedom; MS= mean square; P= level of significance.

	April 2014					September 2014					April 2015				
	df	*SS*	*MS*	*F*	*P*	*df*	*SS*	*MS*	*F*	*P*	*df*	*SS*	*MS*	*F*	*P*
All taxa															
POP	1	0.03723	0.03723	0.08293	0.412	1	0.07997	0.07997	3.093	**0.021**	1	0.03343	0.03343	0.85995	0.565
Metals	1	0.03781	0.03781	0.08422	0.41	1	0.03292	0.03292	1.2734	0.259	1	0.02734	0.02734	0.70337	0.696
POP × Metals	1	0.01427	0.01427	0.03179	0.912	1	0.00863	0.00863	0.3338	0.938	1	0.06304	0.06304	1.62173	0.136
Residuals	10	0.35964	0.03596			8	0.20683	0.02585	0.62991		10	0.38874	0.03887	0.75843	
Total	13	0.44895	1			11	0.32835	1			13	0.51256	1		
Rotifers															
POP	1	0.05185	0.05185	1.64997	0.146	1	0.09658	0.09658	3.9092	**0.013**	1	0.03844	0.03844	1.16843	0.361
Metals	1	0.03205	0.03205	1.01993	0.421	1	0.03861	0.03861	1.5629	0.178	1	0.0258	0.0258	0.78422	0.567
POP × Metals	1	0.01719	0.01719	0.54692	0.786	1	0.02221	0.02221	0.8991	0.393	1	0.0405	0.0405	1.23118	0.299
Residuals	10	0.31426	0.03143	0.75661		8	0.19764	0.02471	0.55667		10	0.32898	0.0329	0.7585	
Total	13	0.41535	1			11	0.35504	1			13	0.43373	1		
Copepods															
POP	1	-0.0108	-0.0108	-0.3067	0.987	1	0.05471	0.05471	2.63731	**0.083**	1	-0.0019	-0.0019	-0.0231	0.977
Metals	1	0.05593	0.05593	1.59251	0.269	1	-0.0011	-0.0011	-0.0539	0.943	1	0.08905	0.08905	1.10696	0.34
POP × Metals	1	-0.002	-0.002	-0.0578	0.932	1	0.00135	0.00135	0.06517	0.903	1	0.034	0.034	0.42267	0.661
Residuals	10	0.35119	0.03512	0.89063		8	0.16595	0.02074	0.75127		10	0.80448	0.08045	0.86907	
Total	13	0.39432	1			11	0.2209	1			13	0.92568	1		
Cladocerans															
POP	1	0.02115	0.02115	0.18048	0.81	1	0.02721	0.02721	0.38751	0.8	1	0.04332	0.04332	0.5523	0.632
Metals	1	0.08422	0.08422	0.71861	0.526	1	0.11847	0.11847	1.68736	0.204	1	0.04035	0.04035	0.5143	0.645
POP × Metals	1	0.02505	0.02505	0.21371	0.792	1	-0.0022	-0.0022	-0.0308	0.973	1	0.3001	0.3001	3.8257	**0.021**
Residuals	10	1.17203	0.1172	0.89986		8	0.56168	0.07021	0.79649		10	0.78441	0.07844	0.67148	
Total	13	1.30245	1			11	0.70519	1			13	1.16818	1		

3.2.4.3 Indicator taxa

The Indicator Value method allowed identifying certain taxa, characteristic of certain contamination levels (Table 3). *Brachionus Leydigii*, *Cephallodella* sp., *Asplanchna* sp. and *Euchlanis* sp., all displayed significant IndVals for low contamination level of several types of pollutants (*B.leydigii*, *Cephalodella* sp. and *Asplanchna* sp. for total contamination, *Euchlanis* sp. and *Cephalodella* sp. for POP, *Cephallodella* sp. and *Asplanchna* sp. for PAH.p, and *Euchlanis* sp. for lead). On the contrary, another set of species showed significant IndVals for higher levels of contamination: Calanoids for POPs, PAH.d, total metals and Cadmium, *Alona* sp. for PAH.p, Harpacticoids for DEHP.d and *Kellicotia* sp. for zinc contamination.

Table 4. List of the indicator species recorded in the different classes of contamination, their Indicator Value and the sampling occasion for which they were detected.

level of classification	class	Indicator taxa	IndVal	sampling occasion
physico-chemical factors	class 2	*Daphnia* sp.	0.871	(September 2014)
	class 3	*Brachionus leydigii*	0.962	(April 2014)
WFD ES	class 2+4+5	Cyclopoid copepods	0.989	(April 2014)
overall contamination	class 1	*Brachionus leydigii*	0.904	(April 2014)
		Cephalodella sp.	0.868	(April 2015)
		Asplanchna sp.	0.865	(April 2015)
POPs	class 1	*Brachionus leydigii*	0.904	(April 2014)
		Euchlanis sp.	0.948	(September 2014)
		Cephalodella sp.	0.903	(September 2014)
		Calanoid copepods	0.906	(April 2015)
	class 2	Calanoid copepods	0.948	(September 2014)
		Brachionus leydigii	0.775	(September 2014)
		Alona sp.	0.807	(April 2015)
PAH.p	class 1	*Asplanchna* sp.	0.918	(April 2015)
		Cephalodella sp.	0.888	(April 2015)
PAH.p	class 1	*Cephalodella* sp.	0.906	(September 2014)
	class 2	Calanoid copepods	0.922	(April 2014)
PCB.d				
DEHP.d	class 3	Harpacticoid copepods	0.97	(September 2014)
metals	class 2	Calanoid copepods	0.901	(April 2014)
Cd	class 2	Calanoid copepods	0.91	(April 2014)
Pb	class 1	*Euchlanis* sp.	0.882	(April 2014)
Zn	class 3	*Kellicotia* sp.	0.91	(April 2014)

3.2.4.4 Relation between environmental conditions and zooplankton spatio-temporal variation

After exploring separately the roles of contaminants and other physico-chemical parameters on abundance and taxonomic composition of total zooplankton and of zooplankton groups, the following results will address the influence of environmental and chemical variables on zooplankton communities' distribution using Redundancy Analysis and variation partitioning.

The first two axes of the Redundancy Analysis using only physico-chemical parameters explained together 25.6% of the total variance of the data (Fig 10). A forward selection procedure determined that temperature; Chl*a*, OM/SPM and NO_3^--N were significative explanatory variables. The variability between sampling occasions was clearly visible on the RDA ordination, separating April samples from September on the first axis, associated to an opposition between temperature and Chl*a* factors.

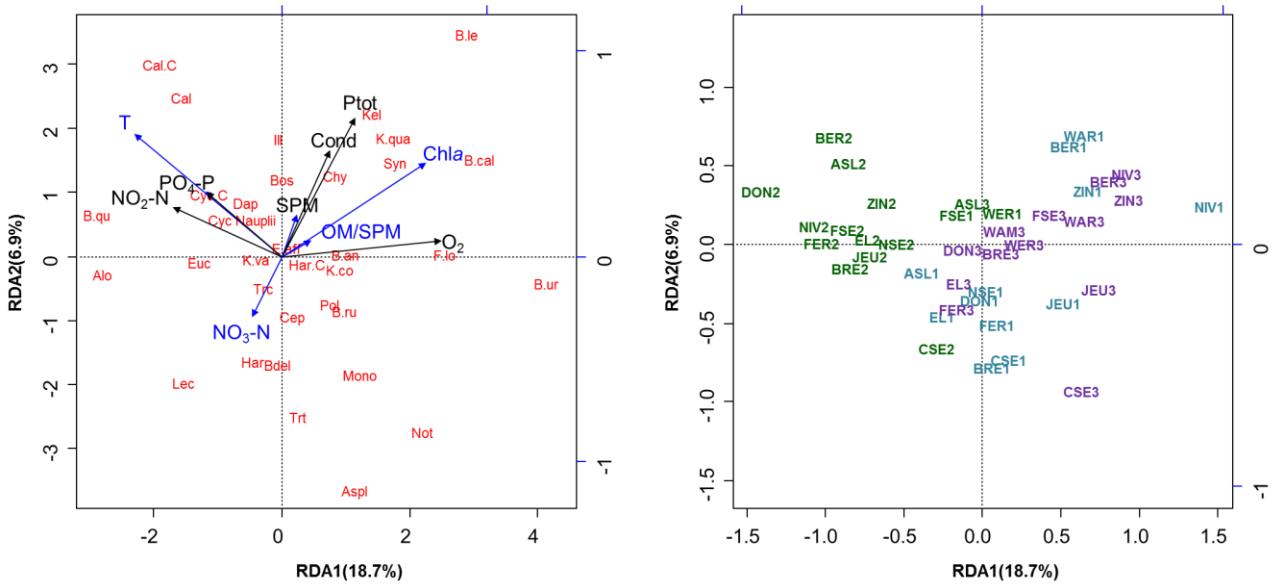

Fig. 10. Ordination of RDA analysis based on global physico-chemical factors. Axis 1 and 2 biplots for environmental variables and taxa (left) and plot for sampling sites (right), considering all sampling periods.

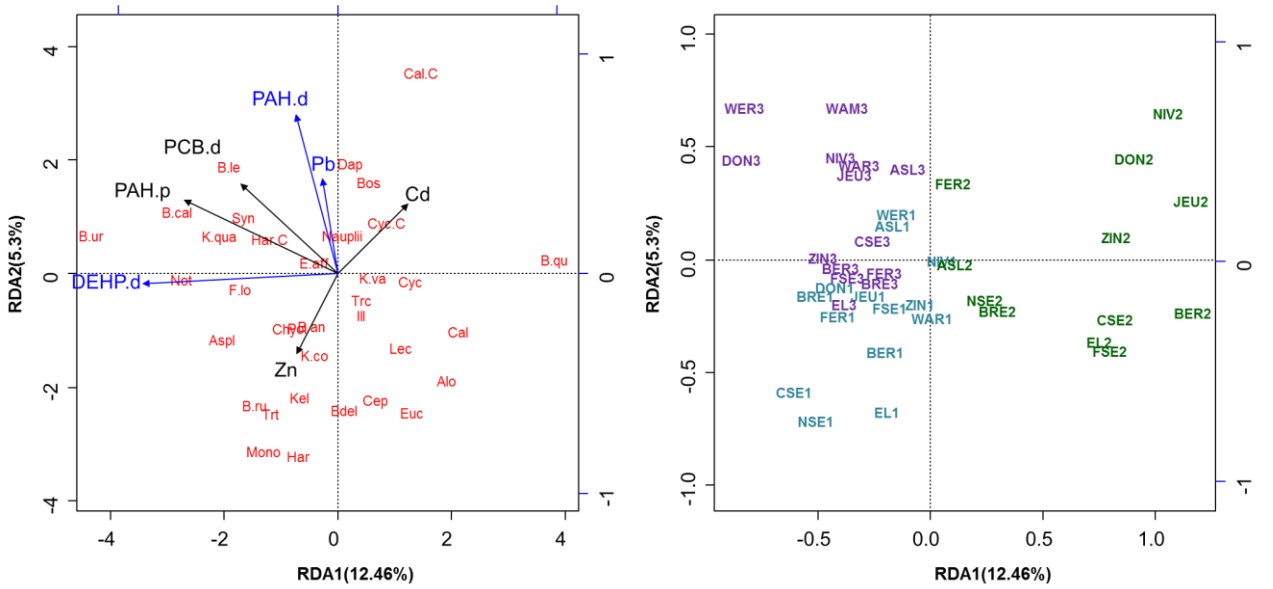

Fig. 11. Ordination of RDA analysis based on contaminants. Axis 1 and 2 biplots for environmental variables and taxa (left) and plots for sampling sites (right), considering all sampling periods.

The RDA using contamination scores as explanatory variables explained for the two first axis 17.8% of total inertia, and PAH.p, DEHP.d and Pb were the factors identified as significant (Fig 11). The differentiation of September samples was also strongly marked along the first axis with

DEHP.d related to April samples. The second axis was associated for its negative part with April 2014 samples while the positive part was associated with April 2015 samples mainly influenced by PAH.d and Pb factors.

The partition of variance (Fig 12) revealed that zooplankton variance was explained for 14.1% by physico-chemical parameters, for 6.9% by contaminants and for 5.2% by the combination of both. 73.9% of total zooplankton variance remained unexplained.

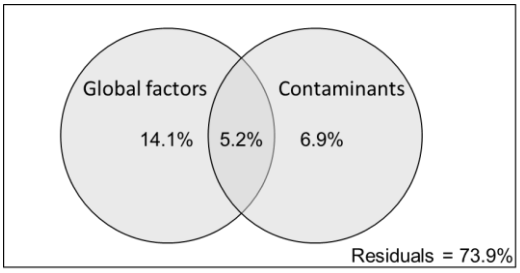

Fig. 12. Variation partitioning between physico-chemical parameters and contaminant models for zooplankton communities considering all samples of all sampling occasions.

3.2.5 Discussion

While physico-chemical measures provide a punctual picture of water quality, living organisms reflect the complexity of biotic and abiotic interactions occurring in an ecosystem, and can provide a more integrative response of multiple stressors at different timescales. WFD Ecological status is therefore partly based on a set of bioindicator taxa (ie. BQE), which are complementary to physico-chemical evaluation of water quality, but zooplankton is not considered among them. The present study aimed to test the response of zooplankton community (in terms of abundance and composition) to different environmental conditions, considering the physico-chemical conditions, the water quality as defined by Ecological Status, and several levels of different contaminants.

Due to its dependence on hydrological conditions, zooplankton has been less studied in riverine networks than in lakes, (Baranyi et al., 2002; Lair, 2006; Zhao et al., 2017). Yet zooplankton has been found to be well represented in certain river systems (Ferrari, Farabegoli & Mazzoni, 1989; De Ruyter van Steveninck et al., 1992; Thorp et al., 1994, 1994; Gosselain et al., 1998; Reckendorfer et al., 1999; Lair, 2005, 2006; Zhao et al., 2017). In the study area, situated in the low elevation upper Scheldt river basin, hydrological conditions are favorable to zooplankton development (Prygiel & Coste, 1993; Le Coz et al, Unpub. data). Considering all sampling

occasions and all stations of the present study, zooplankton abundance ranged between 28 and 1027 ind.l^{-1}. Variability of abundance was also important within each sampling occasion (coefficients variation ranging between 68 and 100%). One of the objectives of this work was to identify the contribution of water quality in explaining this variability in abundance and community composition. Specifically, we set out to identify the contribution of different sources influencing water quality in explaining zooplankton abundance and community composition. This approach logically led us to consider the question: 'Do similar zooplankton communities develop in similar sites?" (Jenkins & Buikema, 1998).

The studied stations presented variable Ecological States, which, according to the WFD, reflect "an expression of the quality of the structure and functioning of aquatic ecosystems". The results showed that zooplankton did not respond to Ecological Status as evaluated by the WFD, neither in abundance nor in the community composition. Since Ecological Status is based on both BQE and physico-chemical parameters, the reason of the variability in Ecological States classification, and in particular of the declassification of certain sites could be related to different sources. In addition, the Chemical States of the studied sites, which, such as the Ecological Status, allows characterizing the water quality in the frame of the WFD, is particularly bad in all sites considered in this study (http://www.eau-artois-picardie.fr) and could also be related to multiple sources of pollution.

The first difficulty when treating this question "Do similar zooplankton develop in similar sites?"was to define "similar conditions" for zooplankton, focusing on water quality. Zooplankton is regulated by multiple biotic and abiotic factors interacting together, and the identification of explanatory variables of zooplankton heterogeneity is complex and scale dependent (Pinel-Alloul, 1995; David et al., 2005).

Community responses to contaminants are known to vary with environmental conditions: toxicity and bioavailability of contaminants are, for example, dependent of physico-chemical conditions (Pelletier *et al.*, 1997; Clements, Hickey & Kidd, 2012). It was thus imperative to interpret zooplankton response to contamination in parallel with the influence of physico-chemical conditions on communities. To do this, we combined two different approaches to classify samples based on physico-chemical conditions (bottom-up approach) and based on semi-quantitative contamination data (top-down approach).

physico-chemical conditions

Physico-chemical conditions occurring across the study area presented a pattern of regionalization which was re-occurring in April campaigns. The RDA conducted on all sampling

occasions revealed a strong differentiation of September samples, mainly related to an opposition between temperature and Chl*a*, but did not strongly differentiate April 2014 samples from April 2015 samples. However, zooplankton response was not identical for both April months. The zooplankton (and in particular rotifer) taxonomic composition was more affected by physico-chemical conditions in April 2015 than in April 2014. However, little influence of environmental conditions could be detected in explaining within sampling occasion spatial patterns. Zooplankton distribution patterns have been shown previously to be related to environmental patterns (Le Coz et al., Unpub data). However, the factors explaining this heterogeneity could not be identified, suggesting an influence of other variables not measured in our studies.

Contamination conditions

Results showed that, contrarily to classification based on physico-chemical parameters, clustering of sites based on their contamination was not regionalized, or at the most in 2 or 3 neighboring stations. For example, the Deûle River presented more contamination, probably because of the high industrialization and urbanization historic of the area (Boughriet et al., 2007b a; Lesven et al., 2009; Louriño-Cabana et al., 2011). The level of contamination was also different between sampling occasions, and the regional patterns were less similar between the two April months than those obtained from physico-chemical parameters. This pattern was clearly observed with the RDA, which clearly separated the three sampling campaigns, and contrarily to the physico-chemical-based RDA, also separated April 2014 samples from April 2015 ones. Zooplankton responses were also not re-occurring for the different sampling periods.

While few results were significative due to a low number of stations and to a high heterogeneity of the data, some contaminants were found to have an effect on zooplankton communities. At high POPs contamination level, changes in taxonomic community composition were observed for rotifers and copepods. However, few impacts of individuals POPs were found: only cladocerans were significantly less abundant at high DEHP contamination sites. Yet, even if DEHP, which is widely used as plasticizer, is often found as the dominant phthalate in aquatic environments (Fromme et al., 2002), several bioassays on Daphnids have been conducted and showed no acute toxicity (see Staples et al. 1997 for a review). A decrease of abundance in presence of contamination could be related to direct effect of this contaminant (i.e. lethal effect), or to indirect effects, which may traduce changes in ecosystem functions, such as nutrient cycles or oxygen dynamics, in particular through the trophic cascade (alteration of competition or predation interactions) (Fleeger et al., 2003). So, indirect effects can either decrease or increase abundance, or change community structure, and the effect detected through single species in

ecotoxicological studies could differ from those observed in the natural environment. Effects of contaminants on community composition can lead to important changes and have even been compared to the effect of a selective predator, modifying inter-species interactions within communities (Rohr et al., 2006).

Concerning metallic contamination, the only significant observations were a change in taxonomic composition of copepods at high Cadmium levels, and of cladocerans with the combined effect of POP and metallic contamination. Rotifers are generally found to be more tolerant than copepods and cladocerans to metallic contamination. The fact that an effect of combined POPs and metallic contamination was found on cladoceran taxonomic composition, while it was not observed for both taken separately suggests an interactive effect of both contaminants, illustrated the importance of assessment of multiple stressors effect, which can display synergetic or antagonist effects (Folt et al., 1999; Hanazato, 2001; Jara & ArnguizAcua, 2013).

According to Odum (1985), stresses in ecosystems involve a decrease of diversity, with a development of r-strategists. For zooplankton communities, it results in a dominance of rotifers, which are less affected by chemical contamination, because their resilience allows them to develop rapidly after a punctual perturbation, and because their less tolerant competitors are disfavored. Moreover, rotifers present an important diversity and are likely to contain both sensitive and tolerant species, the latter logically becoming the dominant species (Havens & Hanazato, 1993; Hanazato, 2001). In the present study, a few taxa largely dominated the assemblage (*Brachionus calyciflorus*, *Keratella cochlearis*, *Synchaeta* sp., *Polyarthra* sp., *Keratella quadrata*, *Brachionus angularis*), and may be more tolerant to contamination. For example, *Keratella* and *Brachionus* species have already been shown to indirectly benefit for metal contamination in experimental conditions (Jak, Maas & Scholten, 1996). It was also found in the Lower Basin of the Salado River (Argentina) that rotifers were more tolerant to metallic contamination than copepods and cladocerans, resulting in a community composition showing rotifer dominance (Gagneten & Paggi, 2009).

Contribution of the studied environmental conditions to zooplankton heterogeneity

Contaminants explained a minor but non negligible part of variability (7%, according to the variance partitioning) which is mainly related to inter-campaign differences. The contaminants influence seems to interact with the more important physico-chemical factors in structuring zooplankton communities, since more than 5% of the data variability was explained by the combination of physico-chemical global factors and contaminants. Contaminants may also explain a part of the inter-annual zooplankton variability, according to the RDAs results which

separated April 2014 and April 2015 samples better when using contaminants as explanatory variables than when using only physico-chemical parameters.

An important part of zooplankton variability in the upstream Scheldt watershed remains unexplained, since this study did not take into account certain variables such as predation or hydrological data for example, which are known to be important drivers of zooplankton community structure. In addition, many other compounds could affect biotic communities, such as drug residues, or pesticides. Pesticides can have directs and indirect effects on zooplankton communities (Relyea, 2009), and have been detected in the particulate phase in the studied sites (Net et al., 2015b). Drug residues have also been detected in the dissolved and particulate phases (Net et al., 2015b) and could also have directly or indirectly influenced zooplankton communities (Cleuvers, 2003). The present study focused on principal threatening compounds of the study area listed in the WFD priority substances (WFD Annex X), but further investigations could be conducted on several other compounds to refine these results.

Even if global factors explained a greater part of zooplankton heterogeneity, the role of water contamination also played a role in community abundances and composition. Such effects on community structure of zooplankton could have top-down and bottom-up consequences on other trophic levels of the pelagic food-chain and in trophic functions of pelagic ecosystems. Moreover, it is known that zooplankton plays an important role in contaminant cycling and in the transfer of POPs in the food web, by the processes of bioconcentration or bioaccumulation (Berrojalbiz et al., 2009; Bettinetti & Manca, 2013; Everaert et al., 2015).

Conclusion

In conclusion, the present study did not allow stating clearly that similar zooplankton occur in similar conditions in the rivers of the Scheldt Watershed, whatever the type of "environmental conditions" we considered. Since zooplankton communities distribution patterns are complex, and respond to a wide range of biotic and abiotic factors, the first difficulty in this study was to define the sense of "similar conditions" for zooplankton communities. By considering a set of physico-chemical parameters, coupled to contamination data, more than 70% of zooplankton variability origin was still non-identified.

This study was conducted in a highly anthropized area, exposed to several sources of pollution, and a particular focus was applied to contamination by several key substances. The responses identified in zooplankton abundance and compositions were reflecting the heterogeneity of conditions displayed in the watershed. Consequently, these preliminary results showed high variability, and could be straightened by improving sampling efforts. The present results

allowed identifying some responses in zooplankton communities, even if these responses were non–systematic, and differed according to the sampling season. Different zooplankton groups did not respond to water quality as represented by WFD Ecological Status. However, contaminants explained approximatively 7% of zooplankton variability, and some specific responses were identified for several levels of contamination. Effects were found for metallic contaminants, POPs, and for the combination of both.

Even if difficult to interpret, the detected community-level responses are more relevant than single species responses, since they reflect the high level of complexity, induced by the complexity of inter-species interactions, and of direct, indirect and multiple stressor effects. As an important part of zooplankton community variability remains unexplained, the use of this compartment as an indicator of water quality seems limited. However, zooplankton monitoring can be useful as reflecting water quality occurring in natural ecosystem in a more functional aspect (control of algal blooms by grazing activity, for example), and could be complementary with more structural indicators of water quality. In particular, long term monitoring of zooplankton could allow detecting changes in communities resulting from water quality improvement, with a possible reappearance of certain taxa sensitive to pollution.

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CHAPITRE IV :

ACTIVITE ET SELECTIVITE TROPHIQUE DE LA COMMUNAUTE ZOOPLANCTONIQUE DES COURS D'EAU DE LA PARTIE AMONT DU BASSIN VERSANT DE L'ESCAUT

- ***Article en préparation***

4.1 RÉSUMÉ DU CHAPITRE:

4.1.1 Introduction et objectifs

Le rôle du zooplancton dans les réseaux trophiques pélagiques a fait l'objet de nombreuses études et son importance a été démontrée dans plusieurs types de systèmes, tels que les lacs (Lampert et al., 1986 ; Lair & Ali, 1990 ; Gulati et al., 1992 ; Davis et al., 2012 ; Wigdahl-Perry et al., 2015), les océans et zones côtières (Landry & Hasset 1982 ; Sherr & Sherr, 2002 ; Calbet & Landry, 2004 ; Strom et al., 2007) et les estuaires (Tackx et al., 1990 ; Sellner et al, 1993 ; Sautour et al., 2000, Lionard et al, 2005). Cependant, le zooplancton a fait l'objet de beaucoup moins d'intérêt dans les rivières, où il est supposé être peu représenté en raison des contraintes hydrologiques.

L'amont du bassin versant de l'Escaut est constitué d'un réseau dense de cours d'eau canalisés, parmi lesquels certains ont été rapportés lors de l'état des lieux qui a eu lieu en 2013 dans le cadre de la DCE comme étant potentiellement eutrophes, c'est-à-dire que la concentration en nutriments présente un risque de prolifération algale, mais que celle-ci n'est pas observée (Comité de Bassin Artois Picardie, 2013).

Au cours des **chapitres 1 et 2**, il a été mis en évidence une communauté zooplanctonique importante dans ces cours d'eau, dominée par les organismes de petite taille (rotifères et copépodes nauplii), et sous l'influence de conditions environnementales très variables et complexes (**Chapitres 2 et 3**). Une sélectivité de prédation par le zooplancton peut induire des changements importants dans la communauté phytoplanctonique.

L'objectif de cette étude est de **quantifier l'impact de la prédation** de cette communauté zooplanctonique (plus particulièrement la fraction entre 50 et 250 µm) sur le stock naturel de phytoplancton et de détecter une potentielle **sélectivité dans cette activité de broutage**. Pour ce faire, de l'eau naturelle filtrée à 50 µm a été incubée parallèlement à de l'eau naturelle filtrée à 250 µm, et les concentrations pigmentaires ont été quantifiées par HPLC, au début et à la fin de l'expérience, dans les deux types de traitement. La sélectivité trophique a pu être étudiée grâce à la prise en compte de plusieurs pigments marqueurs issus de différents groupes algaux.

4.1.2 Principaux résultats et discussion

L'étude a permis de mettre en évidence un impact du broutage du zooplancton sur le phytoplancton, à hauteur de 23 à 228 % du stock de chlorophylle brouté par jour, et de 9 à 42 % de sa production. Les calculs de taux de filtration (mesure de la pression de prédation) sur les

différents pigments marqueurs de groupes algaux ont permis de mettre en évidence une pression de prédation plus importante sur les chlorophytes que sur les autres groupes de phytoplancton, donc une sélection des chlorophytes. Les cryptophytes sont également sélectionnés, mais en moindre mesure, tout comme les diatomées. Une possible pression de prédation sur les cyanophytes est évoquée par un impact détecté sur un pigment marqueur de ce groupe, la zéaxanthine, mais demande à être confirmée par des identifications microscopiques, puisque la zéaxanthine peut également être trouvée chez les chlorophytes. Des réponses différentes entre les pigments marqueurs des diatomées (chlorophylle *c* et fucoxanthine) suggèrent également une sélectivité au sein des diatomées.

La comparaison du pourcentage de stock brouté et du pourcentage de « croissance » des différents pigments phytoplanctoniques indique que le phytoplancton croit suffisamment pour que le stock moyen ne soit pas surexploité par le broutage.

Des taux de filtration négatifs ont été obtenus sur certains pigments, traduisant une quantité plus importante de pigments en présence de de zooplancton > 50 µm. Il est donc probable que la fraction de zooplancton comprise entre 50 et 250 µm se nourrisse également des micro-organismes de taille inférieure à 50 µm (bactéries, ciliés, nanoflagellés hétérotrophes), qui sont eux-mêmes des prédateurs du phytoplancton. Par cascade trophique, le zooplancton entre 50 et 250 µm aurait donc également un rôle de préservation de la communauté phytoplanctonique.

4.2 ARTICLE:

Grazing and selectivity patterns of zooplankton community in the upstream non-tidal rivers of the Scheldt watershed.

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4.2.1 Abstract

While freshwater upstream rivers are generally considered not to support high plankton abundance, the low flow upstream Scheldt watershed displays substantial abundances of rotifers-dominated zooplankton. To investigate their grazing impact on the phytoplankton communities, grazing experiments have been carried out in situ at 14 occasions covering 6 stations and 4 periods from April 2014 to June 2015.

Natural water filtered on 50µm was incubated together with water filtered on 250µm and phytoplankton pigment concentrations quantified by HPLC were compared in both treatments to assess the impact of the 50–250µm fraction of zooplankton on phytoplankton.

Zooplankton daily removed 23 to 228% of the phytoplankton stock, and 35 to 83% of its growth. While exerting substantial grazing pressure, zooplankton did not seem to overexploit the natural phytoplankton communities as phytoplankton growth was generally higher than grazing upon it. Clearance rates measured on pigment markers of different phytoplankton taxa showed a more important grazing pressure on chlorophytes and cryptophytes and probably on cyanophytes than on diatoms.

Negative zooplankton grazing rates suggested a substantial impact of micro-organisms on phytoplankton, limited by zooplankton in the >50µm fraction. Hence, it seems zooplankton >50µm not only predates on phytoplankton, but also prevents phytoplankton depletion by microzooplankton predation.

Keywords: zooplankton grazing; phytoplankton; trophic selectivity; rotifers; HPLC; Scheldt watershed.

4.2.2 Introduction:

Zooplankton–phytoplankton trophic links are since long the subject of studies demonstrating the pivotal role of zooplankton in pelagic food webs (Sterner, 1989; Kiørboe, 1998; Tackx et al., 2003; Calbet & Landry, 2004). By feeding on phytoplankton, zooplankton organisms exert a top down pressure on their composition and biomass (McQueen et al., 1986, 1989; Sommer & Sommer, 2005; Hillebrand et al., 2007; Van Gremberghe et al., 2008).

Zooplankton grazing activity has been studied in many different systems, and its impact on phytoplankton reported in literature is variable but relatively well documented. In lakes, seasonal successions in phytoplankton communities are related to dynamics of large crustaceans, and particularly of cladocerans (Lampert et al., 1986; Sommer et al., 1986; Sterner, 1989; Ekvall et al., 2014). Lair & Ali (1990) also report the important role of rotifers in clearing phytoplankton of eutrophic lakes. In oceans and coastal areas, microzooplankton (ciliates, heterotrophic flagellates, copepod nauplii, etc.) is considered as the main predator of phytoplankton, exerting a grazing pressure higher than that of meso-zooplankton (Landry & Hassett, 1982; Sherr & Sherr, 2002; Calbet & Landry, 2004; Strom et al., 2007).

In rivers, grazing quantifications have focused principally on estuarine reaches where significant micro- and meso- zooplankton grazing impact have been shown on phytoplankton communities (Tackx et al., 1990; Sellner et al., 1993; Sautour et al., 2000; Murrell et al., 2002; Tan et al., 2004; Lionard et al., 2005; Quinlan et al., 2009). Freshwater non-tidal rivers however have been poorly studied. Gosselain et al. (1998b) studied grazing impact on phytoplankton by a rotifer - dominated zooplankton community during summer in the Meuse River (Belgium) and found daily predation rates ranging from 1 to 113% d^{-1} of the phytoplankton standing stock, while in the Moselle river (Germany, Luxembourg and France), grazing was lower (1–15% d^{-1}; Gosselain et al., 1998a).

Upstream riverine reaches of watercourses are often considered to support only low plankton abundances since residence times are generally short and thus may not allow its development. The upstream part of the Scheldt watershed presents low-flow (<1m^3 s^{-1}; Prygiel & Coste, 1993), due to low relief of the watershed and important hydromorphological alterations (channelization, enlarged watercourses, deviations), providing favorable conditions for phytoplankton development (Lebon, 1997; Noppe et al., 1999). In addition, intense agriculture and urbanization cause important nutrient inputs (Billen et al., 1985, 2005; Autreaux Noppe, 2000; Thieu et al., 2010), and most of the water courses in the upstream Scheldt are thus considered (according to the Water Framework Directive, WFD) as potentially eutrophic, meaning that despite the absence of phytoplankton blooms, nutrient concentrations involve a

substantial risk (Comité de Bassin Artois Picardie, 2013). Eutrophication is a widespread type of aquatic pollution (Schindler, 1971, 1974, 2006; Vitousek et al., 1997; Carpenter et al., 1998; Biggs, 2000; Smith, 2003; Smith et al., 2006) which can lead to excessive vegetal development, and have many consequences on water quality, biogeochemical functions and hence ecosystems services (Smith et al., 1999; Garnier et al., 2005).

The low flow displayed by the rivers of the upstream Scheldt watershed also allows zooplankton development: substantial abundances have been found, mainly constituted by small organisms, since the zooplankton community was dominated by rotifers, and crustaceans were mainly copepod nauplii (Le Coz et al, sub.). The present study aims to quantify the predation impact of the zooplankton community (50–250 µm fraction) on the phytoplankton stock and to detect potential selectivity in grazing activity in a number of watercourses situated in the upstream freshwater non-tidal part of the Scheldt watershed.

4.2.3 Material & Methods

4.2.3.1 Study area

The Scheldt takes its source in Northern France, crosses Belgium and flows into the North Sea in Netherlands. Experiments were conducted in 6 sites spread on the upstream watercourses of the Scheldt watershed, at the border between Northern France and Belgium (Fig 1): Nivelle (NIV: 50°28’12.6 ’’N ; 003°27’57.8’’E), Férin (FER: 50°19’20.7’’N ; 003°04’20.0’’E), Brebières (BRE: 50°20’08.4 ’’N ; 003°01’17.9’’E), Don (DON: 50°32’47.4 ’’N ; 002°55’14.2’’E), Erquinghem-Lys (ERQ : 50°40’37.7''N ; 002°50’06.6’’E) and Aire sur la Lys (ASL : 50°38’45.1’’N ; 002°24’36.5’’E). These watercourses are all channelized. Experiments were conducted on 4 occasions: April 2014 (experiments NIV1, FER1, DON1); September 2014 (NIV2, BRE2, DON2); April 2015 (NIV3, FER3, DON3, ASL3, ERQ3); June 2015 (NIV4, DON4, FER4).

4.2.3.2 Zooplankton sampling and environmental conditions

Zooplankton was collected by filtering 50 liters of sub-surface water through a 50µm mesh plankton net using a pump. The collected zooplankton was narcotized with carbonated water, fixed with formaldehyde (4 % final concentration) and stained with erythrosine in the laboratory. Organisms of subsamples were then identified and counted in a counting wheel under a binocular microscope (90 × magnification; minimum 100 individuals per sample), to obtain zooplankton abundances at each site. Most of rotifers and cladocerans were identified to genius, (at the species level for some of them), and for copepods, the distinction was done between nauplii and cyclopoids and calanoids adults and copepodite stages.

To determine Suspended Particulate Matter (SPM) concentrations, 251 to 590 mL sub-surface water was filtered onto pre-weighted Whatman GF/C filters (porosity: 1.2µm). Filters were dried (45 °C, 24h), briefly cooled in a desiccator and weighed to obtain SPM concentrations. Then the filters were burned at 500 °C for 5 hours, and re-weighed to obtain Ash concentration. Organic Matter (OM) concentrations were calculated as the difference between SPM and Ash concentrations.

100 mL samples of sub-surface water was filtered through 0.2 µm cellulose nitrate filters in order to remove bacterial activity, stored at 4 °C and analyzed by Ion Chromatography (Dionex Ics-5000+, Dionex Corp., Sunnyvale, CA, USA) for determination of NO_3^--N and HPO_4^{2-}-P concentrations.

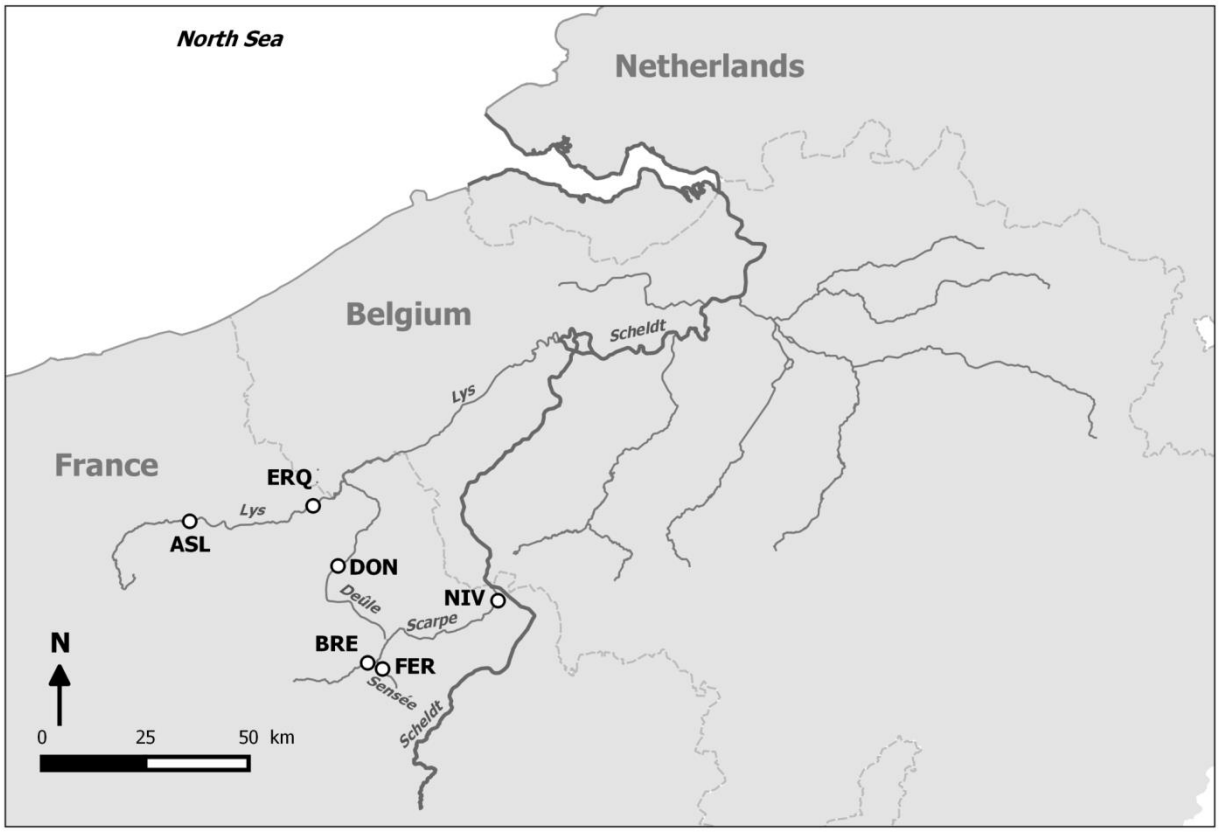

Fig.1: Distribution of the experiment sites on the Scheldt watershed

4.2.3.3 -incubation experiments:

Subsurface natural water was collected and used as represented in Fig 2. Half of the water was filtered through 50 µm sieve, excluding all bigger organisms (containing only organisms which size is below 50 µm), constituting the control water. The remaining water was filtered through a 250 µm sieve, and thus also contained the 50 - 250µm fraction of zooplankton (hereafter called zooplankton or zooplankton community) for which the grazing activity was measured (hereafter

named “grazing” water). Twelve 1L polycarbonate bottles were filled with control water, and 12 other with grazing water. In order to avoid anoxia in the bottles, they were all filled at 900 mL. Six bottles of each treatment were analyzed at the beginning of the experiment, and the 12 remaining bottles (6 control and 6 grazing) were then gathered in a net which was immerged directly in the water course during 24 hours, in order to approach natural conditions of light and temperature.

At the beginning of the experiment (t_0) and at the end of incubation time (t_f), 190 to 620 mL of water, depending on suspended matter concentration, was collected in each bottle and filtered onto Whatman GF/C glass fiber filters. Filters were then stored in liquid nitrogen for further HPLC analyses of phytoplankton pigments.

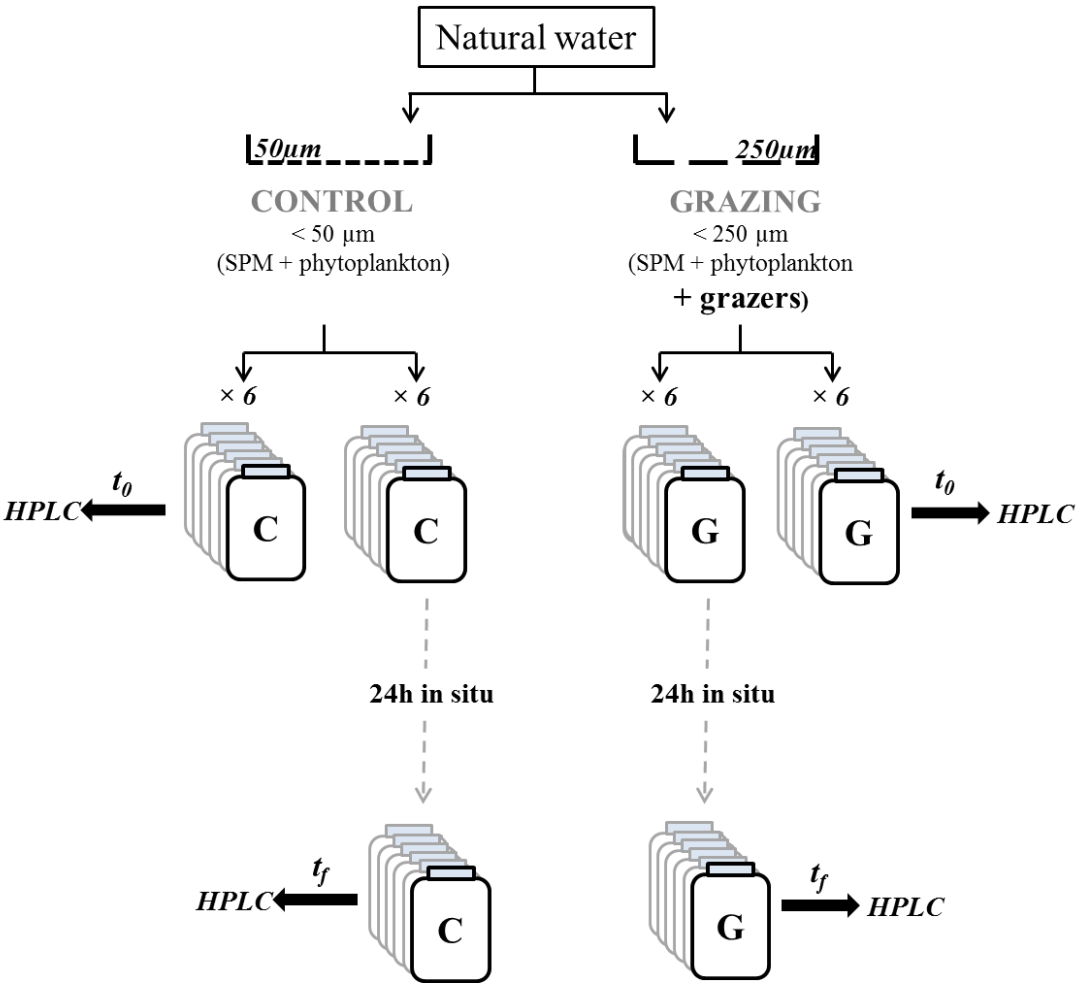

Fig.2: Experimental design of incubation experiments.

4.2.3.4 Pigment extraction, identification and quantification:

GF/C filters were extracted two times (15 min at -20 °C), in a total of 10 mL (5 mL and 5 mL) 98 % cold-buffered methanol - 2 % 1M ammonium acetate solution (following Majdi et al., 2011).

An ultrasonication bath (60 seconds, Branson Ultrasonic bath model 5810) was used to favor algal pigment release. One mL of the solution thus obtained was filtered on 0.2 µm PTFE syringe filter and analyzed using a high-performance liquid chromatograph (HPLC) 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, and at 665 nm to detect chlorophylls and phaeopigments (Wright, 1991).

Pigments were identified by comparing their retention time and absorption spectra with those of pure standards pigments (DHI LAB products, Hørsholm, Denmark) using ChemStation software (version A.10.02, Agilent Technologies inc.). Each pigment concentration was calculated by relating its chromatogram's peak area with the corresponding area of calibrated standard.

Chlorophyll *a* (Chl*a*) was used as a proxy of phytoplankton biomass, while marker pigments detected by HPLC were used as markers of different phytoplankton groups (Jeffrey et al. 1997, Roy et al. 2011). Pigment concentrations were expressed in µg L^{-1}.

4.2.3.5 Estimation of grazing parameters

Differences in mean pigment concentration at the end of the experiment (t_f) between grazing (C_z) and control (C_t) bottles were tested for each pigment in each experiment using Mann-Whitney tests ($p<0.05$).

The variability in replicates (coefficient of variation) of pigments concentrations for which a significant difference was found was then compared to those for which no difference was found, using a Mann-Whitney ($p<0.05$), in order to ensure that the variability of samples was not the reason to detect significant difference between pigments in C_t and C_z.

The following grazing parameters were calculated for each pigment for which a significant concentration difference between C_t and C_z was detected.

The coefficients of mean phytoplankton growth ($\overline{k}$) and of zooplankton grazing (g) were calculated as:

$$\overline{k} = \frac{1}{t} \ln \frac{\overline{C_t}}{\overline{C_0}} \quad (\mathrm{d}^{-1}),$$

with t being the incubation time (in days), $\overline{C_0}$ the mean pigment concentration in control bottles before incubation and $\overline{C_t}$ being the mean pigment concentration in control bottles at the end of the experiments, and

$$g = \frac{1}{t} \ln \frac{\overline{C_t}}{C_{zt}} \quad (\mathrm{d}^{-1}),$$

with C_{zt} being the pigment concentration in each grazing bottle at the end of the experiment. The mean g value for each experiment was calculated as the average of all replicate grazing bottles.

The g/k ratio was used as an estimation of the percentage of growth removed by grazing.

Also, differences in mean pigment concentration between control and grazing bottles at time t_0 were tested; when significant differences were found, theoretical pigment concentrations in control samples at the end of incubation C_{t-theo} were used for calculation of g coefficients, which were calculated as:

$$C_{t-theo} = C_{z0} \times e^{kt}$$

The community clearance rate (Fc), was calculated for the whole zooplankton community contained in 1l of natural water, and is thus given as:

$$Fc = \frac{V \times g}{v} \ (\mathrm{mL\ L^{-1} d^{-1}})$$

Where V is the volume (mL) incubated and v is 900 mL (volume of water incubated).

Community clearance rates (Fc) correspond to the volume of water from which a pigment is removed per unit of time by the grazers contained in 1l of natural water, and represent the predation pressure exerted on the phytoplankton biomass for which the pigment is a marker. In the case of Chl*a*, Fc–Chl*a* represents the community grazing pressure on total phytoplankton biomass.

As a verification of the grazing impact on different algal taxa, the same calculations were also done for each algal group, considering the sum of its marker pigment concentrations (chl *c* + diadinoxanthin + fucoxanthin for diatoms, chl b + violaxanthin + lutein for chlorophytes, alloxanthin for cryptohytes, and zeaxanthin for cyanophytes).

4.2.4 Results

4.2.4.1 Conditions of incubation:

SPM concentrations were on average 17.74 mg L^{-1}, varying between 5.32 and 48.22 mg L^{-1}, and OM concentration was on average 8.00 mg L^{-1} varying between 1.46 and 15.90 mg L^{-1} (Fig. 3). OM accounted for 11 to 97 % of SPM concentration. Chl*a* concentration, ranged from 1.81 to 103.59 µg L^{-1}, with an average concentration of 22.17 µg L^{-1}. NO_3^- and PO_4^{2-} concentrations varied between 166 and 678 µM and between 0.65 and 9.36 µM respectively (Table 1).

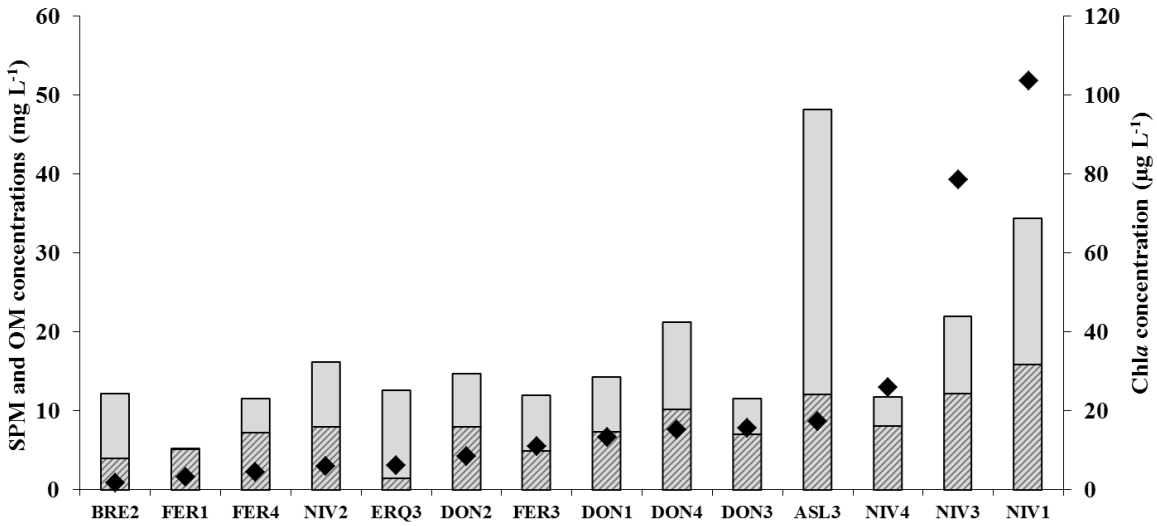

Fig.3: SPM, OM and Chla concentrations for each experiment. Experiments are classified along the x-axis according to increasing Chla concentration

Besides Chl*a*, the following pigments were identified in the natural water: fucoxanthin, diadinoxanthin, diatoxanthin, chlorophyll *c* (markers of diatoms since 19'-butanoyloxyfucoxanthin and 19'-hexanoyloxyfucoxanthin, markers for chrysophytes and prymnesiophytes, were not detected), alloxanthin (marker of cryptophytes), zeaxanthin (marker of cyanophytes), lutein, violaxanthin and chlorophyll *b*, (markers of chlorophytes) (Jeffrey et al., 1997). Beta-carotene (which is not a taxonomic marker, and phaeopigments (pheophytin *a*, pheophorbide *a*) were also detected.

Table 1: Nitrate and phosphate concentrations of natural water used for each experiment.

	µMol /L	
experiment	**NO_3 (µM)**	**PO_4 (µM)**
BRE2	678.24	3.23
FER1	409.80	0.97
FER4	299.14	n.d.
NIV2	237.74	9.36
ERQ3	382.67	4.52
DON2	382.67	6.46
FER3	434.08	0.65
DON1	522.61	6.13
DON4	440.50	7.10
DON3	535.46	4.52
ASL3	605.42	3.87
NIV4	166.35	6.78
NIV3	174.92	1.94
NIV1	184.20	4.52

The fraction of zooplankton (50–250 µm) used in our experiments was constituted mainly by rotifers (23 to 97% of abundance), copepod nauplii (2 to 58%) and adults (0.14 to 25%), and cladocerans (0.05 to 23%). Their abundance, ranging between 22 × 10^3 and 1160 × 10^3 ind.m^{-3}, and their composition were highly variable between incubation experiments (Figs 4 and 5).

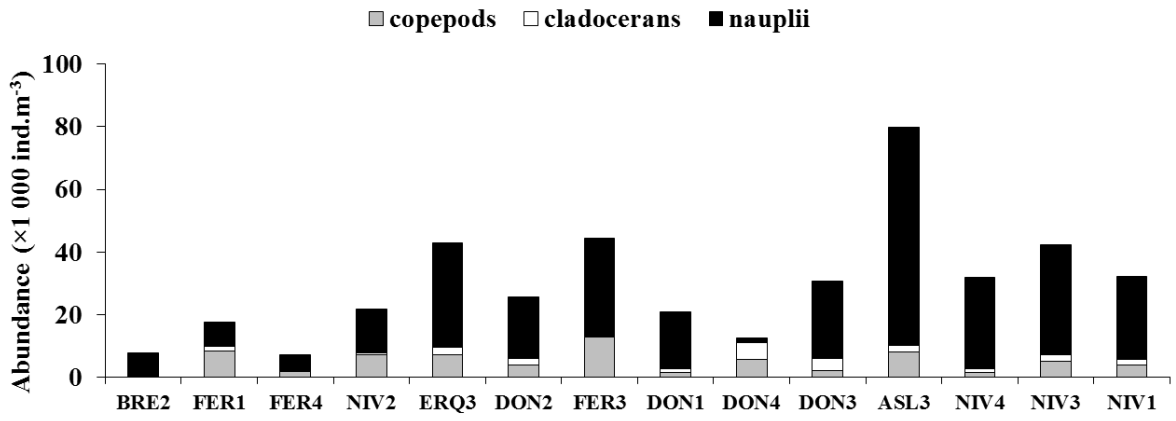

Fig.4. Crustacean abundance at experiment sites and occasions.

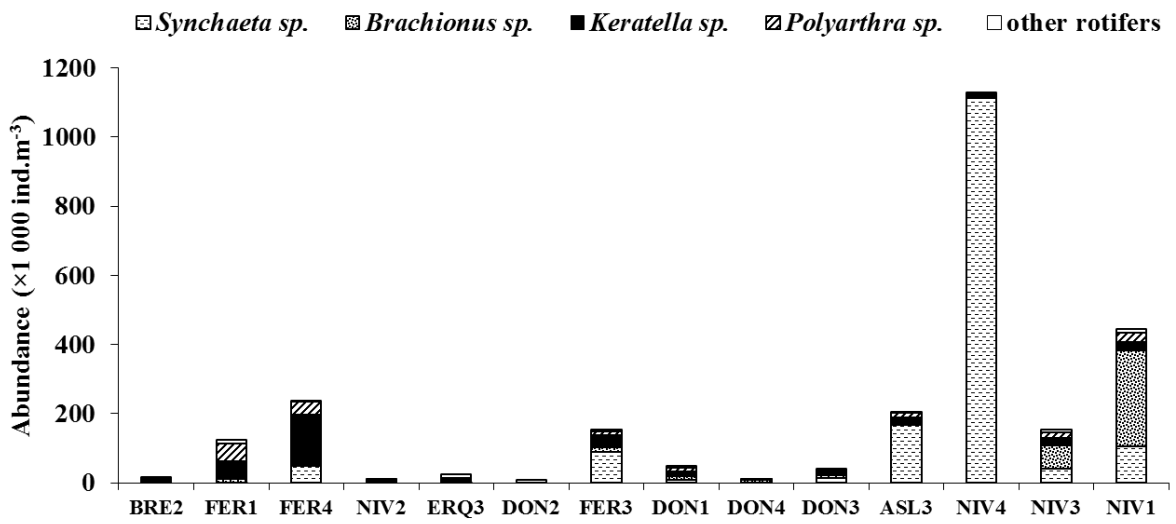

Fig.5 Rotifer abundance at experiment sites and occasions.

Rotifers were represented by a few dominating genera (accounting together to 19 to 97% of the zooplankton assemblage): *Synchaeta* sp., *Brachionus* sp., *Keratella* sp., *Polyarthra* sp.. Other taxa of rotifers represented on average 2% of total rotifer abundance.

4.2.4.2 Community grazing pressure

Final concentrations of Chl*a* were significantly different between grazing and control samples in 5 out of the 14 experiments. Fc values on Chl*a* (Fc – Chl*a*) were varying substantially between experiments (ranging from -362 to 2281 mL L^{-1} d^{-1}; Fig. 6a). Positive Fc – Chl*a* values were found in DON2, ERQ3, and NIV3, indicating a predation on phytoplankton. Negative Fc – Chl*a* values were observed in ASL3 and FER4, reflecting a more important phytoplankton biomass in bottles with zooplankton than in control bottles at the end of the experiment.

Except for ASL3, in which negative growth coefficient (k) for Chl*a* were found, indicating a decrease of phytoplankton biomass in control bottles during the time of the experiment, growth was positive in all experiments. Considering only experiments in which grazing was positive (3 experiments / 14), the 50 – 250 µm zooplankton community removed between 23 and 228% of the Chl*a* stock per day and between 35 and 83% of growth (when it was also positive) (Table 2).

Fc calculated for the different marker pigments and algal groups were also either positive or negative (Fig 6b,c), except for diatoxanthin (maker of diatoms), and chlorophyll *b* (marker of chlorophytes) which never showed significant difference of concentration between grazing and control bottles at the end of the experiments.

NIV1 and DON4 were the only experiments for which no effect of zooplankton could be detected for individual pigments (Fig 6b). The other experiments presented at least one marker pigment

with significant different concentration between treatments at the end of the experiment. Positive Fc values were however observed in only 8/14 experiments, and in 4/14 other experiments only negative Fc values were found. Overall, no pigment was systematically grazed. The most frequently grazed pigments (in 3 experiments for each) were alloxanthin (marker of cryptophytes) and lutein (marker of chlorophytes).

Considering each algal group (using the sum of marker pigments concentrations), no significant difference was detected between final grazing and control bottles for FER1, NIV2 and DON4. However, the results show positive Fc-values for Chlorophytes for 5 experiments, ranging from 190 to 1100 mL $L^{-1}h^{-1}$. Positive Fc values were also found for diatoms in 4 experiments, cryptophytes in 3 experiments, and for cyanophytes at NIV3. Negative values were also found for each group.

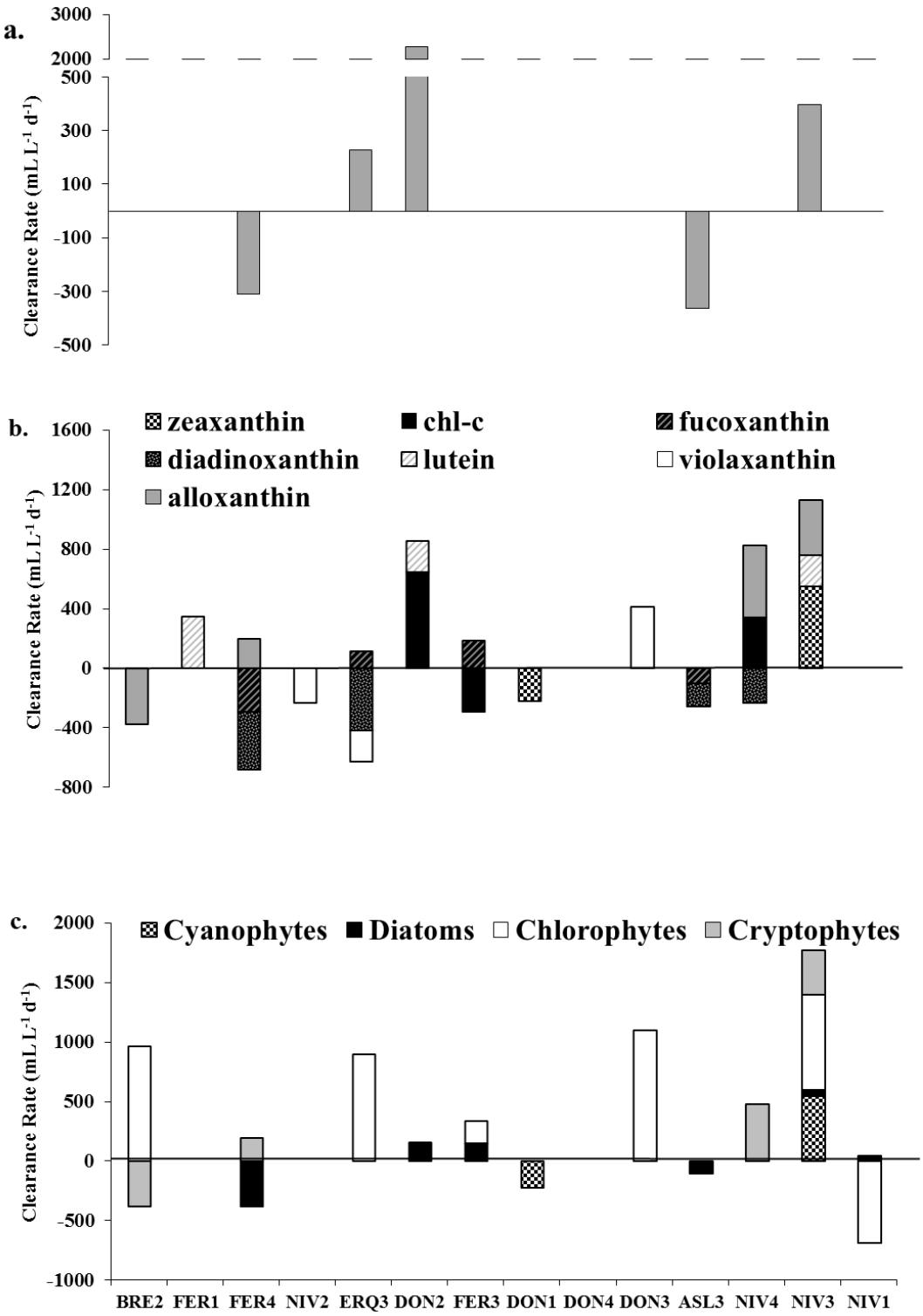

Fig.6 Mean Clearance Rates (N=6) of the 50–250µm zooplankton community on, a) Chla, representing the total phytoplankton biomass, b) on marker pigments, c) on the sum of pigments of each phytoplankton group marker pigments (zeaxanthin for cyanophytes, Chl-c, fucoxanthin and diadinoxanthin for diatoms, lutein and violaxanthin for chlorophytes, and alloxanthin for cryptophytes).

The relation between phytoplankton mortality by zooplankton grazing (g) and phytoplankton growth (or production) (k) coefficients are presented in Fig 7. Within the 14 experiments, most pigments (68%) displayed higher growth than grazing. Negative k values occurred on 3 occasions (17% of cases) and were found for Chl*a*, violaxanthin and alloxanthin.

Table 2: Mean percentage of grazing pressure (±SD) on each detected pigment and on each algal group (calculated from the sum of their marker pigments, see Fig.6 for more details), relatively to the initial stock and to the phytoplankton growth. Average values obtained for each pigment considering all 14 experiments.

	% stock d^{-1}	% growth
chlorophyll-a	**23 - 228**	**35 - 83**
fucoxanthin	12 - 19	21 - 79
diatoxanthin	no significative Fc	
diadinoxanthin	no positive Fc	
chlorophyll-*c*	35 - 65	48 - 595
diatoms	**5 - 16**	**13 - 50**
violaxanthin	42 - 42	100 - 100
lutein	21 - 35	42 - 417
chlorophyll-*b*	no significative Fc	
chlorophytes	**19 - 111**	**58 - 391**
cyanophytes	**56 - 56**	**73 - 73**
cryptophytes	**20 - 49**	**85 - 97**

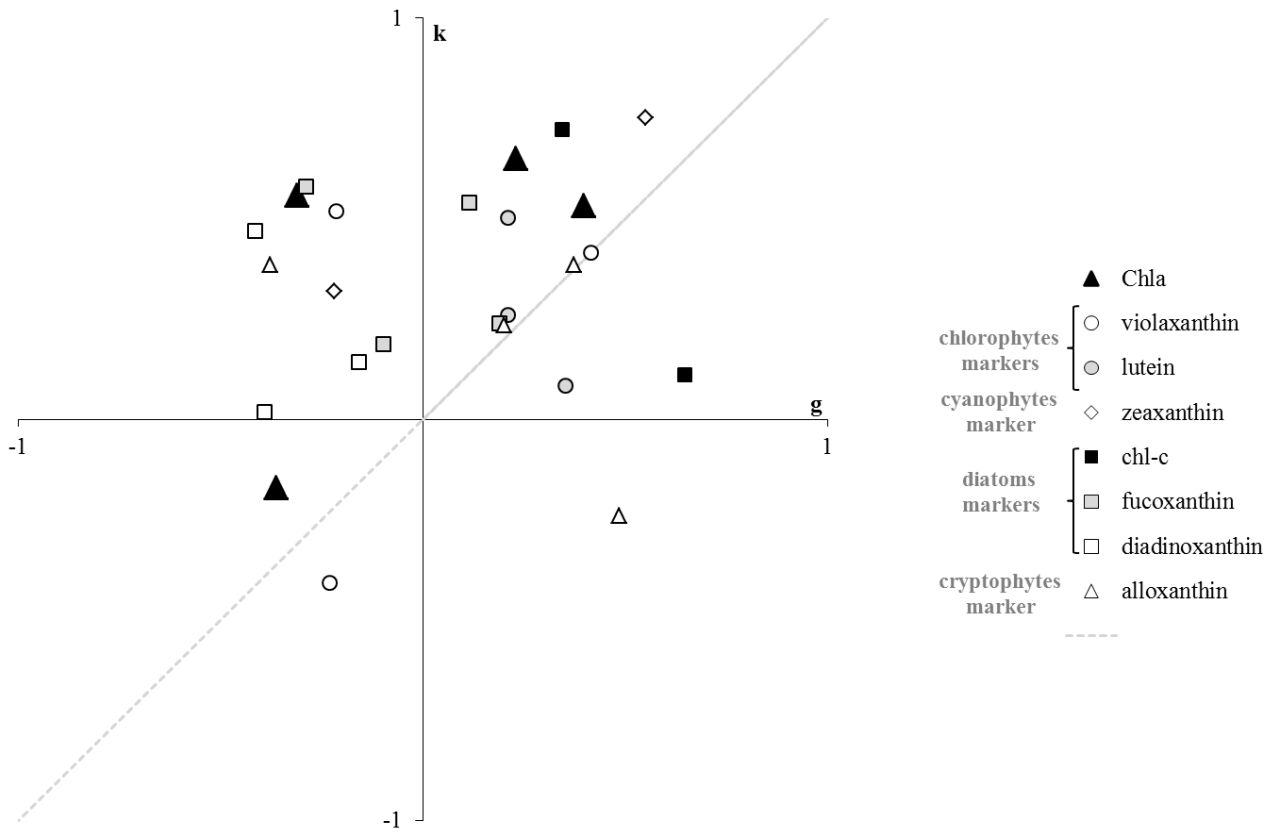

Fig.7 Phytoplankton growth coefficient (k) as a function of phytoplankton mortality due to zooplankton (50–250µm fraction) grazing (g), for each pigments and all incubation experiments. The grey dotted line is the bissector above which growth is higher than grazing and below which grazing is higher than growth

4.2.5 Discussion

The present study investigated the grazing impact of the whole 50–250µm fraction of the zooplankton community, mainly constituted by rotifers and copepod nauplii, on phytoplankton in the upstream non tidal rivers of the Scheldt drainage basin. Zooplankton community grazing was found to remove 23 to 228% of the Chl*a* stock daily, and 35 to 83% of its growth.

In the estuarine reach of the Scheldt, studies of the impact of zooplankton grazing on phytoplankton have provided different results in the marine / brackish waters dominated by calanoid copepods and cirriped nauplii (6–16% d^{-1} of phytoplankton stock; 11–18% of production; Tackx et al., 1990) and the tidal freshwater dominated by rotifers (33–84% d^{-1} of phytoplankton stock; Lionard et al., 2005). The present study shows results rather comparable

to those of the freshwater tidal estuary. However the complexity of planktonic interactions resulted in variable responses between experiments and depending on pigments.

Using Chl*a* to investigate zooplankton grazing focusses on overall phytoplankton biomass variations. However, there is an important diversity of phytoplankton taxa, which are more or less represented in natural water, and in the grazers' prey range. Zooplankton feeding activity on phytoplankton taxa depends in part on the phytoplankton community composition. Phytoplankton species can for example be inedible or unselected by certain zooplankton taxa owing to their quality, size, morphology, palatability, toxicity, or their relative biomass within the phytoplankton community (Paffenhöfer & Van Sant, 1985; Paffenhöfer & Lewis, 1989; Sarnelle et al., 2010; Colina et al., 2015). The investigations on selectivity patterns are all the more important as the grazing activity of zooplankton on specific taxa has structuring effects on phytoplankton communities, and is thus closely related to both predators and prey community dynamics. As such, predator selectivity can influence seasonal successions of phytoplankton species, and also the formation of (excessive) blooms (Gosselain et al., 1998b, Sailley et al., 2015). The use of HPLC technique coupled to grazing experiments allowed to investigate feeding activity on different algal groups, represented by a set of photosynthetic pigments, and permitted to obtain information on zooplankton grazing pressure on specific phytoplankton taxonomic groups: in other words, the selectivity patterns displayed by grazers, in addition to grazing activity on total phytoplankton as quantified using Chl*a* (Burkill et al., 1987; Strom & Welschmeyer, 1991; Obayashi & Tanoue, 2002).

In the present study, Fc-values (i.e. Clearance Rates) calculated for different algal groups suggested a more important grazing activity on chlorophytes. Cryptophytes and diatoms were also grazed, but to a lower extent. Significant zooplankton impact on individual phytoplankton marker pigments was detected in 11 out of 14 experiments, contrarily to what was suggested by results on Chl*a* alone, including 52% positive Fc-values and 48% negative Fc-values (considering all significative couples experiments × pigments). The number of positive Fc-values has also probably been underestimated, because of the impossibility to detect pigments for which concentration was below the limit of quantification (LQ) of the HPLC. This is for example the case for Chl*b*, which in 3 experiments (NIV3, DON3, ERQ3) was detected in the final control bottles while not in the grazing bottles (below LQ), making impossible the Fc calculation but indicating an grazing activity. In addition, Chl*b* decays much faster than lutein (Bianchi et al. 1993), inducing its faster degradation by grazing.

Investigations on individual pigments illustrated the complexity of zooplankton–phytoplankton trophic interactions. For example, fucoxanthin and Chl*c* (which are light harvesting pigments)

can be considered as better markers of diatom biomass than diadinoxanthin (a photoprotective pigment involved in the xanthophyll cycle) (Grant & Louda 2010). However, opposite trends between Chl*c* and fucoxanthin were observed in the FER3 experiment. This could traduce selective feeding among diatoms taxa presenting different fucoxanthin / Chl*c* ratios. Although Chl*c* is frequently used as a marker of diatoms, it can also be found in other algal groups, such as cryptophytes, which could also explain this result.

A taxonomic focus could be useful to clarify the question of grazing impact on cyanophytes: zeaxanthin is frequently used as a marker pigment of cyanophytes but could also have been produced by chlorophytes (Jeffrey et al., 1997), which were present in the water and grazed by zooplankton, according to lutein and Chl*b* results. Cyanophyte blooms have already been reported in the upstream Scheldt watershed, but they are irregular (Prygiel & Leitao, 1994; Noppe et al., 1999). In the study area, where several sites are considered as presenting an eutrophication risk (Comité de Bassin Artois Picardie, 2013), zooplankton grazing activity on cyanophytes could limit phytoplankton development in case of a bloom initiation, if the present results are confirmed.

Rotifers, which constituted the large majority of the grazers considered in the present study, are rather non-selective, but generally preferentially feed on small algae (Rothhaupt, 1990; Hansen et al., 1994; Walz, 1997; Gosselain et al., 1998b). In the studied rivers, as in most temperate freshwater rivers, (Garnier et al., 1995; Gosselain et al., 1998b; Descy et al., 2012), phytoplankton communities are dominated by chlorophytes (mainly with an Equivalent Spheric Diameter <20µm) and diatoms, with occasional development of cryptophytes and cyanophytes (Noppe et al., 1999, Agence de l'Eau Artois-Picardie, com.pers.). An avoidance of diatoms by rotifers could occur, since diatoms can be inedible because of their size, or because of a colonial form, or because their silica frustules are difficult to digest for rotifers (Mialet et al., 2013). This hypothesis should be explored by a more accurate taxonomic investigation using microscopic analysis.

The occurrence of negative Fc-values means that more pigment was present at the end of the experiment in presence of grazers (50–250 µm) than in control bottles (<50µm). Negative grazing rates have already been reported in many studies on zooplankton grazing (Roman & Rublee, 1980; Tackx & Polk, 1986; Nejstgaard et al., 1994, 1997, 2001; Calbet & Landry, 2004; Leising et al., 2005; Stoecker et al., 2015). Several hypotheses are possible to explain negative grazing rates.

The first one relies on the trophic cascade. The potential grazers represented in grazing bottles were constituted by rotifers, copepods, cladocerans, and smaller "micrograzers" (heterotrophic

flagellates, bacteria or ciliates for example). Since the latter are too small to be effectively retained by the 50 µm mesh sieve, they were also present in the control bottles, where they probably feed—at least partially—on phytoplankton (Arndt, 1993; Lionard et al., 2005; Azémar et al., 2007). In the 50–250 µm fraction, copepods and rotifers, which have been shown to feed also on ciliates (Kiørboe, 1998; Nejstgaard et al., 2001; Azémar et al., 2007), could thus decrease phytoplankton grazing by eating "micrograzers". The negative growth (k) found in the present experiments reached -0.49 d^{-1}, which is relatively important. It could also be in part related to a decrease of phytoplankton by microconsumers grazing activity in these bottles. Both negative grazing and growth coefficients could therefore be explained by a grazing activity of bacteria, ciliates and heterotrophic nanoflagellates, impeded by their predators (rotifers and copepods) in the >50 µm fraction.

The second possibility is that excretion by the studied fraction of zooplankton could stimulate phytoplankton growth in the grazing bottles. Zooplankton, and in particular rotifers regeneration of nutrients in forms directly available for phytoplankton (PO_4-P and NH_4-N for example), can stimulate phytoplankton growth (Lehman, 1980; Den Oude & Gulati, 1988; Gulati et al., 1992). The quantification of this stimulation is difficult to separate from the effect of a suppression of micro-organisms grazing and regeneration of nutrients (Elser, 1992; Nejstgaard et al., 2001), however, in the present study, the concentrations of nutrients, exceeding most of times largely the concentrations obtained after adding nutrients in dilution experiments (for example 1.5 to 15.5 µM Nitrate and 0.2 to 1 µM Phosphate in: Nejstgaard et al, 2001), were probably not limiting for phytoplankton growth (except for FER4).

Finally, selectivity of grazers on particular algal taxa can also influence phytoplankton dynamics by changing the competition among species. In particular, by removing a phytoplankton species, grazing can favor the development of other species in competition with the first (Sterner, 1989; Hillebrand et al., 2007; Lewandowska et al., 2014). In this configuration, an increase of phytoplankton biomass is possible in the presence of grazers.

Whereas Chl*a* concentration is generally considered as a good proxy for phytoplankton biomass, its variations can also be related to photoadaptative processes of phytoplankton (McManus, 1995). The light-dependent variations occurring in phytoplankton cellular pigment content oppose light harvesting pigments (such as chlorophylls or fucoxanthin which decrease with light) to photoprotective pigments (such as beta-carotene or zeaxanthin, which increase with light). Cellular content of both types of pigments can vary independently of biomass and carbon content (Dubinsky & Stambler, 2009). The variations of pigment concentrations could also be

related to photoadaptative processes, however, grazing and control bottles were incubated all together, in situ, and submitted to the same light conditions, so this hypothesis is unlikely.

If the hypothesis of the trophic cascade is true, the 50–250 µm zooplankton plays a double role for structuring phytoplankton communities: a direct regulation by grazing activity and a preservation role by limiting predation. This second role may be very important to limit phytoplankton depletion.

Conclusion

Apart from the Meuse, the Moselle (Gosselain et al., 1998a, 1998b) and the Rhine (De Ruyter van Steveninck et al., 1992) rivers have been somewhat neglected for studies on zooplankton–phytoplankton trophic interactions. Yet, this study showed that zooplankton communities play complex and important trophic role in these systems. The variability of responses between the 14 experiments of the present study illustrates the complexity of planktonic interactions. Direct effects of zooplankton grazing must be interpreted together with their indirect effect by interactions with the organisms of the microbial loop, which are not only predators of phytoplankton, but can also compete with phytoplankton for nutrients, or on the contrary regenerate them in forms available for phytoplankton (Currie & Kalff, 1984; Jansson et al., 1996; Hitchcock & Mitrovic, 2013).

Despite the complexity in the results, the present study demonstrated grazing pressures exerted by zooplankton on the phytoplankton community, and in particular on chlorophytes and cryptophytes in several of the watercourses studied. Despite an important proportion of the stock removed daily by grazers in some cases, phytoplankton easily compensated its stock by its growth rates (0.24 to 2.64 d^{-1}). While exerting substantial grazing pressure, the 50 -250 µm zooplankton fraction of the studied systems did not seem to overexploit the natural phytoplankton communities. The occurrence of negative grazing coefficients, suggesting a limitation of micro-organisms predation or competition, could even reveal that the 50 -250µm zooplankton fraction prevents phytoplankton depletion. The dynamics of both communities being closely related, joint studies on phytoplankton-zooplankton dynamics are indispensable, especially since in this type of riverine systems displaying low flow and turbidity, the plankton dynamics may be less influenced by light and hydrology than in more lotic systems (Sterner et al., 1996; Miquelis et al., 1997), and the role of biotic interactions may be more pronounced, as it is the case in lakes.

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DISCUSSION GENERALE

Alors que les précédents travaux décrivant le zooplancton de l'Escaut ont porté jusqu'alors principalement sur la partie estuarienne du fleuve (De Pauw, 1973, 1975 ; Tackx et al., 1995, 2004, 2005 ; Appeltans et al., 2003 ; Azémar et al., 2007 ; Mialet et al., 2010, 2011 ; Chambord et al., 2016), cette thèse a permis de caractériser les propriétés structurelles et fonctionnelles des communautés zooplanctoniques de l'amont du bassin versant. Les résultats obtenus apportent des informations sur l'abondance, la composition, la diversité, la variabilité spatio-temporelle et le rôle trophique de ces communautés, en lien avec les conditions diversifiées auxquelles celles-ci sont exposées dans leur habitat.

○ *Distribution du zooplancton dans le bassin versant de l'Escaut*

La description de la distribution des communautés à l'échelle du fleuve entier (**chapitre 1**) a permis de dégager une vue d'ensemble de la répartition du zooplancton le long de l'Escaut. À cette échelle, le gradient de salinité et l'influence des courants de marée ont été identifiés comme étant les facteurs majeurs de différenciation spatiale des communautés zooplanctoniques (Fig.1). Plusieurs grandes zones fonctionnelles successives ont ainsi pu être identifiées de la source à l'embouchure : la zone non estuarienne d'eau douce, la zone estuarienne d'eau douce, la zone estuarienne d'eau saumâtre et la zone estuarienne marine. Dans ces différents tronçons, les communautés sont soumises à des conditions de vie très différentes et se distinguent nettement.

Avec des abondances situées entre 2×10^4 et 460×10^4 ind. m^{-3} en avril 2013 et entre 0.7×10^4 et 170×10^4 ind. m^{-3} en juin 2015, les communautés zooplanctoniques étaient présentes en abondance dans l'ensemble des tronçons, mais avec une variabilité spatiale importante. La diversité semble minimale dans la zone saumâtre, corroborant l'hypothèse de Remane (1934). Cependant la résolution taxonomique employée reste limitée et sous-estime probablement les valeurs de diversité obtenues. En termes de biomasse, la zone estuarienne d'eau douce semble la plus productive probablement en réponse à un ratio profondeur de mélange/zone photique caractéristique de cette zone et favorable au développement phytoplanctonique (Muylaert et al., 2005).

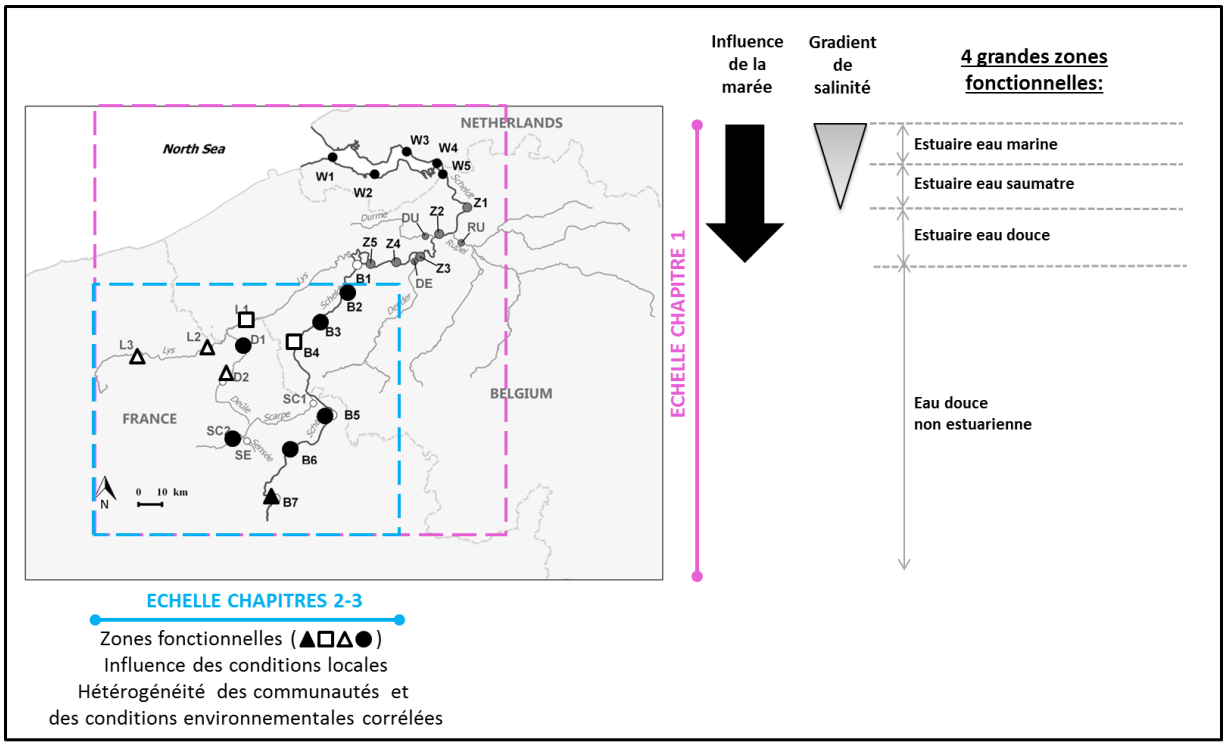

Figure 11. Profils de distribution obtenus et zones fonctionnelles identifiées pour le zooplancton aux échelles du fleuve entier (échelle 1) et du bassin versant non estuarien (échelle 2, exemple de juin 2015).

À l'échelle de la partie amont du bassin (tronçons non estuariens, **chapitres 2 et 3**), les communautés arborent des abondances variables, mais importantes (en moyenne $4.33\times10^5 \pm 9.84\times10^5$ ind. m^{-3} en considérant les 5 campagnes et les 18 stations), principalement dominées par les rotifères, et plus particulièrement par quelques espèces dominant nettement l'assemblage par rapport aux crustacés moins nombreux (eux-mêmes majoritairement représentés par des nauplii de copépodes). La distribution des communautés est corrélée à celle des facteurs environnementaux, bien que ceux-ci ne se soient pas montrés suffisamment homogènes au sein des différentes zones fonctionnelles définies pour établir une influence claire. En adéquation avec le concept de Riverine Ecosystem Synthesis (RES — Thorp et al., 2006), il ressort que les communautés hydrologiquement connectées ne s'avèrent pas systématiquement plus similaires entre elles qu'avec des communautés plus éloignées et non connectées, mais présentant des conditions environnementales plus proches (en termes de facteurs généraux). Cette observation suggère un rôle réduit de l'hydrologie dans la structuration du zooplancton dans les cours d'eau du bassin versant amont de l'Escaut.

Malgré une hydrologie probablement peu influente en amont où les débits sont relativement faibles, la contribution des tributaires à l'abondance et à la composition du zooplancton dans le

cours principal reste difficile à estimer sans davantage de données hydrologiques (**chapitre 1**). La contribution des différents tributaires est probablement variable dans l'Escaut estuarien où les débits sont en partie contrôlés par l'Homme à des fins de navigation et de sécurité (Meire et al., 2005). Que ce soit directement par les apports d'organismes, ou par la modification des conditions hydrologiques, physico-chimiques et trophiques, la présence de confluences le long de l'Escaut semble modifier les communautés, mais leur influence n'a pas pu être quantifiée dans le cadre de la thèse.

○ *Quels sont les facteurs explicatifs de la structure du zooplancton dans la zone non estuarienne ?*

- *Influence de la qualité de l'eau (Etat Ecologique – DCE) ?*

Aucun lien n'a pu être établi entre la distribution des communautés zooplanctoniques et l'état écologique des stations (au sens de la Directive Cadre sur l'Eau), supposé traduire « l'expression de la qualité de la structure et du fonctionnement des écosystèmes aquatiques ».

Puisque le zooplancton est connu pour répondre rapidement aux changements environnementaux (Gannon & Stemberger, 1978), il est probable que les communautés étudiées reflètent les conditions « instantanées » (ou du moins à court terme) du milieu. L'état écologique étant calculé sur 2 années glissantes (au moment où il a été utilisé dans le cadre de ces travaux), il ne traduit pas forcément les conditions au moment de l'échantillonnage (ou pendant la période correspondant au temps de développement du zooplancton dans le tronçon concerné). Le couplage de nos données de zooplancton avec d'une part les données physico-chimiques générales (hors–contaminants) et d'autre part les concentrations des principaux contaminants est cependant supposé mieux refléter les pressions auxquelles sont soumises les communautés.

- *« Des communautés similaires dans des conditions similaires ? »*

Cette question centrale d'écologie, qui concerne l'influence des conditions environnementales dans la structure des communautés (McCune & Allen, 1985 ; Jenkins & Buikema, 1998), a été traitée plus spécifiquement dans les **chapitres 2 et 3**. Elle relève de la prédictibilité de la structure des communautés en relation avec les conditions de l'habitat (une notion particulièrement importante dans un contexte de gestion ou de surveillance des écosystèmes). L'objectif est de définir des zones où les conditions sont homogènes et où le zooplancton reflète cette homogénéité environnementale dans sa composition.

La question en soulève donc une deuxième sous-jacente : comment définir la notion de conditions similaires pour les communautés zooplanctoniques ? En d'autres termes, cette question revient à tester le concept de River Ecosystem Synthesis (Thorp, Thoms & Delong, 2006), et à tenter de définir les caractéristiques environnementales qui caractérisent les potentielles zones fonctionnelles.

Les **paramètres généraux** (hors contaminants) semblent influencer la distribution du zooplancton : les communautés similaires étaient en effet plutôt réparties dans des stations présentant des conditions similaires dans le bassin pour les périodes printanières d'avril 2014, septembre 2014 et avril 2015 (**chapitre 2**). Cependant, ces facteurs ne suffisaient pas seuls à expliquer de manière significative la composition des communautés. Le **chapitre 3** a par ailleurs permis de démontrer que la notion de « conditions similaires » impliquait de nombreuses possibilités et une grande complexité, et que la contamination ne permettait pas de discerner des conditions similaires ou dissimilaires permettant d'expliquer la distribution du zooplancton.

- *Réponses des communautés aux contaminants étudiés et considérations méthodologiques*

Le **chapitre 3** révèle que les **contaminants** considérés dans le cadre de la thèse expliquent une part mineure (environ 7 %) de la variabilité des communautés par rapport aux facteurs généraux eux-mêmes peu influents (environ 14 %).

Il n'est pas exclu que les communautés actuellement en place dans ces cours d'eau- où la pollution est qualifiée d'« historique » - soient constituées d'organismes relativement tolérants aux contaminants présents dans le milieu. En effet, des communautés exposées à une pollution particulière pendant de longues durées peuvent développer une certaine tolérance, la pression de sélection éliminant les espèces les plus sensibles au profit des plus tolérantes (concept de tolérance des communautés induite par la pollution ou : PICT pour « pollution-induced community tolerance » Blanck et al., 1988).

Les effets indirects de la pollution ont été rapportés à plusieurs reprises comme favorisant les rotifères (Fleeger et al., 2003). Il a été démontré, par exemple pour les pesticides, que certains rotifères habituellement peu compétitifs s'avéraient plus tolérants à la contamination (Hanazato, 1998). Plusieurs études rapportent en particulier une augmentation des abondances des genres *Brachionus*, *Keratella*, ou *Polyarthra* après une exposition aux métaux ou aux pesticides, profitant de la diminution des abondances de cladocères, avec qui ils sont en compétition trophique (Brock et al., 1992 ; Jak et al., 1996 ; Sanderson et al., 2002).

La dominance de quelques rotifères, et en particulier de Brachionidés comme *Brachionus* sp. ou *Keratella* sp., dans les cours d'eau étudiés ne s'explique pas nécessairement par le concept de PICT, puisque ce type de communauté est généralement observée dans les rivières (non estuariennes) du monde entier (Lair, 2006). Dans le cadre de la présente étude, le rapport entre les abondances des rotifères et celles des crustacés est plutôt élevé, mais reste comparable avec ceux observés dans les autres cours d'eau (Fig. 2). Cependant, la composition des communautés combinée à leur historique de contamination leur procure potentiellement un niveau de tolérance et de compétitivité expliquant en partie la faible réponse aux contaminations du milieu.

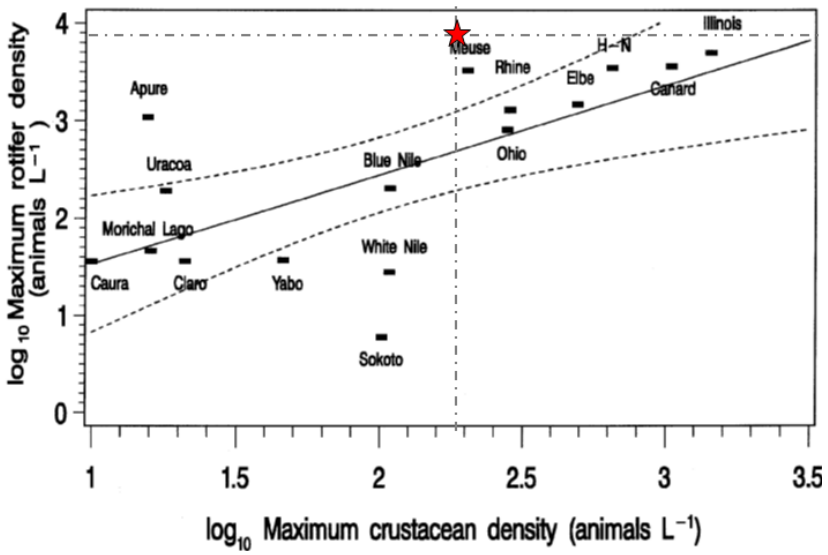

Figure 12. relation entre le log10 de la densité maximale de rotifères et le log10 de la densité maximale de crustacés dans différentes rivières du monde (modifié d'après Kobayashi et al., 1998). Les résultats de la présente étude (bassin versant de l'Escaut non estuarien) sont représentés par l'étoile rouge.

Cette étude s'est concentrée en priorité sur certains contaminants, décrits comme problématiques dans la région (Net et al., 2015a, 2015 b ; Rabodonirina et al., 2015), et liés au contexte historique de pollution d'origine industrielle et urbaine. Un certain nombre de contaminants analysés dans le cadre du projet BIOFOZI, comme les résidus médicamenteux, ou encore les Méthyl-HAP, pourront par la suite être également considérés et venir enrichir les résultats actuels.

Il est aussi important de noter que la méthodologie employée pour tester l'effet des contaminants a été basée sur une approche « top-down », c'est-à-dire en classant les stations à partir de considérations prédéfinies et de manière supervisée. Les seuils du SEQ-Eau (classe d'aptitude à la biologie) ont ici été utilisés pour comparer les réponses des communautés. Les résultats reflètent donc la réponse des communautés zooplanctoniques à la contamination telle qu'elle est définie par le système d'évaluation de qualité des eaux utilisé en France, mais ne correspondent pas nécessairement aux seuils qui peuvent affecter les communautés zooplanctoniques. La définition des classes de « conditions » utilisée dans **le chapitre 3** est donc potentiellement inadaptée aux communautés étudiées. Si d'autres méthodes pourraient être utilisées, celle-ci a toutefois permis de tester ces classes de qualité au regard des réponses du zooplancton, et pourra être comparée éventuellement à d'autres seuils en termes de pertinence pour les communautés.

L'approche utilisée dans le cadre de cette thèse a permis de prendre en compte l'influence des pollutions multiples et les effets directs et indirects sur les communautés, qu'il est possible d'appréhender uniquement en considérant les communautés naturelles dans leur milieu de vie (Fleeger et al., 2003 ; Clements et al., 2016). Les réponses observées étaient faibles et non systématiques, mais sont le reflet de la complexité et de l'hétérogénéité des interactions entre compartiments biotiques et abiotiques dans le milieu naturel.

- *Autres sources de variabilité du zooplancton*

Au terme de ces travaux, une grande part de la variabilité du zooplancton du bassin versant de l'Escaut non estuarien reste inexpliquée (environ 74 %). D'une manière générale, le zooplancton présente une distribution hétérogène, soumise à l'influence de multiples facteurs en interaction, et dépendante de l'échelle d'étude (Pinel-Alloul et al., 1995 ; Jenkins & Buikema, 1998 ; Zhao et al., 2017). À cela s'ajoute l'hétérogénéité importante des conditions de vie inhérente aux réseaux hydrographiques (Biggs et al., 1998 ; Grant et al., 2007). Par ailleurs cette étude n'est pas exhaustive et n'inclut pas un certain nombre de facteurs biotiques et abiotiques potentiellement impliqués dans la structure des communautés (prédation, hydrologie, contaminants non étudiés, etc.). Enfin, il reste difficile d'estimer la part de variabilité d'origine stochastique dans nos données basées sur seulement 5 campagnes d'échantillonnage et 18 stations. Ces premiers résultats pourront cependant être affinés par la suite, en complétant ce jeu de données et en poursuivant les recherches sur différents facteurs explicatifs potentiels non étudiés ici.

○ *Rôle trophique des communautés zooplanctoniques dans la zone non-estuarienne*

La nécessité de comprendre les facteurs expliquant la distribution du zooplancton est d'autant plus fondée qu'il a été démontré une activité trophique importante de ces communautés (**chapitre 4**). En termes d'impact sur le stock total de phytoplancton, le rôle trophique de cette communauté jusqu'alors non documentée de zooplancton, principalement représentée par des petits organismes (rotifères et nauplii de copépodes), équivaut — voire dépasse — la capacité trophique de la communauté estuarienne de la même gamme de taille (Lionard et al., 2005), contribuant ainsi à limiter l'expansion des communautés phytoplanctoniques.

Les résultats mettent aussi en avant l'importance des organismes de la boucle microbienne dans le fonctionnement trophique (et donc biogéochimique) de l'écosystème. L'hypothèse émise à l'issue du **chapitre 4** serait en effet l'existence d'une activité de prédation au sein des communautés d'organismes dont la taille est inférieure à 50 µm, elles-mêmes influencées par la prédation des communautés zooplanctoniques de taille 50-250 µm. Afin de mieux comprendre les interactions qui ont lieu dans cette cascade trophique, des informations plus concrètes sur les organismes qui constituent cette fraction inférieure à 50 µm, comme leur abondance et leur composition taxonomique, pourraient être utiles. Leur pression réelle sur les communautés phytoplanctoniques pourrait également être obtenue en considérant non seulement l'impact de la fraction 50-250 µm dans les expériences d'incubation, mais également celui des fractions inférieures, avec des incubations incluant plusieurs gammes de taille de prédateurs (< 20, 20-50 µm et 50-250 µm par exemple).

Le couplage des expériences d'incubation avec l'analyse HPLC des différents pigments phytoplanctoniques détectés, développé dans les travaux de thèse de Chambord (2016) portant sur le tronçon estuarien d'eau douce de l'Escaut, a permis de quantifier l'impact de la pression de prédation du zooplancton sur les différents groupes algaux. Les résultats ont montré une sélection trophique des organismes zooplanctoniques 50-250 µm sur les chlorophytes, et dans une moindre mesure sur les cryptophytes et les diatomées. Bien qu'il soit également suggéré, l'impact sur les cyanophycées reste encore à confirmer.

Les cyanophycées sont des procaryotes (bactéries) photosynthétiques particulièrement problématiques en eau douce et peuvent être à l'origine d'efflorescences parfois toxiques (Paerl et al., 2001). Des concentrations élevées en nutriments, associées à un temps de résidence des eaux suffisamment important pour permettre la croissance, et combinées à une prédation faible, représentent des conditions optimales pour le développement d'efflorescences (Paerl et al., 2001). Dans les cours d'eau canalisés et riches en nutriments qui constituent le bassin versant

amont de l'Escaut, certains taxons de cyanobactéries ont tendance à se développer en période estivale (par exemple les genres *Aphanocapsa*, *Planktothrix, Phormidium, Pseudanabaena* — comm pers. AEAP). Des cas d'efflorescences ont également été rapportés dans la région (Prygiel & Leitao, 1994), et l'état des lieux réalisé en 2013 dans le cadre de la DCE décrit un pourcentage important de cours d'eau dans les bassins de la Lys et de la Deûle comme étant « potentiellement eutrophes » (Comité de Bassin Artois Picardie, 2013). Ces cours d'eau présentent des concentrations en nutriments propices à l'eutrophisation, mais les efflorescences ne se produisent pas. Dans ce contexte, le rôle des consommateurs primaires est donc un aspect primordial à prendre en compte. Certaines études ont mis en évidence la capacité du zooplancton, et notamment des rotifères, à se nourrir de certaines cyanophycées (Starkweather & Kellar, 1983; Weithoff & Walz, 1995; Work & Havens, 2003). Des investigations plus poussées doivent donc être menées pour compléter nos résultats.

La détermination par observation microscopique des groupes phytoplanctoniques présents dans les expériences d'incubation présentées en **chapitre 4** pourra venir compléter ces résultats et apporter des précisions sur l'impact du broutage zooplanctonique sur les cyanophycées. Des premières observations microscopiques ont permis d'ores et déjà de mettre en évidence une diminution significative des cyanobactéries du genre *Pseudanabaena* sp. durant les expériences d'incubation en présence de la fraction 50-250 µm à Aire sur la Lys en avril 2015 (Nguyen, 2016).

Outre les observations microscopiques, les résultats obtenus dans le **chapitre 4** pourraient être affinés en améliorant les connaissances sur la composition pigmentaire des différents groupes algaux (chemotaxonomie). Le logiciel CHEMTAX (Mackey et al., 1996), fréquemment utilisé pour traduire les compositions pigmentaires en contribution des groupes algaux et des cyanobactéries à la biomasse phytoplanctonique, pourrait être utilisé à cet effet. Bien que la résolution taxonomique de ce type de technique soit limitée, le traitement des données pigmentaires issues d'analyses HPLC par CHEMTAX évite l'omission de certaines algues de très petite taille difficiles à détecter par microscopie. Son utilisation nécessite néanmoins de connaitre les ratios [pigment marqueur]/[chlorophylle *a*] pour les différents groupes algaux et cyanobactéries, or ce ratio est fortement variable en fonction des conditions environnementales (Geider, 1987 ; Descy et al., 2009). En eau douce, ces ratios sont disponibles en majorité pour les lacs, et les ratios utilisés en eau douce de l'estuaire de l'Escaut par Lionard et al. (2005, 2008) sont inadaptés et montrent des résultats incohérents avec nos données (d'après les premiers essais, en comparaison avec quelques données taxonomiques issues des premières observations microscopiques et des comptages de l'Agence de l'eau). Ces ratios pourraient être obtenus par analyse des pigments de cultures algales. Dans un premier temps, des échantillons de

cyanobactéries ont été récoltés en période d'efflorescence dans la zone d'étude et seront analysés par HPLC et microscopie afin d'affiner la méthode HPLC pour caractériser la composition taxonomique des cyanobactéries problématiques de la région.

○ *Implications dans le fonctionnement biogéochimique de l'écosystème*

Les différentes études de cette thèse mettent en évidence d'une part l'existence de communautés zooplanctoniques hétérogènes en amont du bassin versant de l'Escaut, soumises à l'influence de multiples facteurs dont une grande partie reste encore non-identifiée, et d'autre part un rôle trophique important de ces communautés zooplanctoniques. Par conséquent, et compte tenu des liens étroits entre structure et fonction des écosystèmes (Cummins, 1974), on peut supposer que des facteurs intervenant dans la structure des communautés zooplanctoniques peuvent influencer l'impact trophique des communautés et engendrer d'importantes répercussions sur la dynamique du phytoplancton. En matière de gestion, la maitrise de paramètres influençant la structure des communautés et donc contribuant au contrôle du phytoplancton pourrait donc s'avérer être un moyen de lutter contre les efflorescences nuisibles.

Afin de mieux comprendre les liens existant entre les communautés zooplanctoniques et phytoplanctoniques, des suivis temporels avec une fréquence plus importante seraient aussi envisageables. En effet, les résultats des **chapitres 1 et 2** suggèrent des variations saisonnières à la fois des communautés de zooplancton et de phytoplancton, probablement inter-corrélées. Les successions saisonnières observées dans les lacs (notamment décrites par Sommer et al., 1986) peuvent probablement être — au moins partiellement — transposées aux cours d'eau présentant des conditions hydrologiques aussi favorables au développement planctonique que celles de notre zone d'étude (Lair, 2006). Dans les travaux présentés ici, seules 5 périodes ont été considérées, couvrant les mois d'avril 2013, avril 2014, septembre 2014, avril 2015 et juin 2015. La présente thèse ne visait pas à décrire la dynamique temporelle des communautés, mais révèle que celle-ci est probablement d'une importance capitale pour mieux comprendre les mécanismes impliqués dans la structure des communautés zooplanctoniques. Elle a permis de mettre en évidence une probable répétitivité des patrons de distribution des communautés entre les mois d'avril, mais suggère une différentiation saisonnière avec les mois d'été (juin et septembre).

Plusieurs modèles ont été construits pour décrire le fonctionnement biogéochimique des cours d'eau à l'échelle du bassin versant. Le modèle RIVERSTRAHLER, qui a été appliqué entre autres à l'Escaut pour simuler les transferts de nutriments dans le bassin (Billen et al., 2005, 2009 ; Thieu

et al., 2009, 2010a, 2010 b ; Gypens et al., 2013), implémente bien le zooplancton comme compartiment. Cependant, seule la température est prise en considération comme variable influençant sa dynamique, et l'impact du zooplancton sur le phytoplancton est considéré de manière globale, sans intégrer les profils de sélectivité trophique mis en évidence dans le cadre de cette thèse et d'autres travaux (Tackx et al., 1989, 2003 ; DeMott, 1995 ; Gasparini et al., 1999 ; Azémar et al., 2007). L'importante variabilité structurelle et fonctionnelle du zooplancton pourrait être mieux prise en compte pour venir enrichir ce type de modèles utiles à la compréhension du fonctionnement biogéochimique à l'échelle de l'écosystème.

○ *Potentiel indicateur du zooplancton ?*

Si le zooplancton permet de fournir une vision synthétique de la qualité de l'eau, reflétant les caractéristiques fonctionnelles du milieu, à ce stade, il est encore nécessaire d'affiner la compréhension des interactions entre les communautés et leur environnement dans les cours d'eau du bassin de l'Escaut. Notamment, un certain nombre de contaminants analysés dans le cadre du projet BIOFOZI pourront être combinés à ceux qui ont déjà été étudiés dans le **chapitre 3**. Néanmoins, la part de déterminisme dans la structure des communautés doit être suffisante pour pouvoir utiliser ce compartiment en tant qu'indicateur. Les systèmes lacustres où le zooplancton a été recommandé comme élément de qualité biologique pour la DCE (Moss, 2007 ; Nõges et al., 2009 ; Jeppesen et al., 2011 ; Haberman & Haldna, 2014), sont des milieux relativement stables et homogènes (Lampert, 1997), mais il en est autrement dans les systèmes plus complexes que forment les cours d'eau. En effet, les résultats obtenus dans les **chapitres 1 à 3** illustrent bien la difficulté d'appréhender les mécanismes qui interviennent dans la distribution et la structure des communautés zooplanctoniques du bassin versant amont de l'Escaut. Bien que la distribution du zooplancton soit corrélée à celle des conditions environnementales (paramètres généraux hors contaminants), la variabilité spatiale et temporelle des réponses conduit à une faible prédictibilité des profils de distribution, et il n'est pas encore possible de caractériser des conditions « homogènes » pour le zooplancton. Or cette prédictibilité est une condition importante pour la mise en place de bio-indication. La réponse des communautés aux contaminations est quant à elle probablement trop dépendante du « contexte » : variabilité de la composition des communautés, historique de contamination de ces communautés influençant leur tolérance à certains contaminants, superposition des contaminations multiples (Clements et al., 2012, 2016).

Dans l'estuaire, le suivi temporel du zooplancton a mis en évidence des changements avec l'amélioration de la qualité de l'eau. Ces travaux ont montré que le copépode calanoïde

Eurytemora affinis a colonisé la partie eau douce de l'Estuaire, en parallèle à l'amélioration de la qualité de l'eau se traduisant par une augmentation des concentrations en oxygène et une diminution des concentrations en ammonium (Appeltans et al., 2003 ; Mialet et al., 2010, 2011 ; Chambord, 2016). Néanmoins la mise en évidence de la présence d'*E. affinis* dans le bassin versant (amont) est une nouvelle donnée intéressante qui pourrait témoigner d'une certaine accessibilité des habitats étudiés à ce taxon très bien étudié dans les milieux estuariens. L'absence de suivis réguliers du zooplancton ni de données historiques détaillées dans le bassin versant ne permettent pas d'identifier la période (date) d'apparition d'*E. affinis*. Seuls des suivis futurs pourraient mettre l'accent sur l'importance ou pas de la présence de cette espèce dans le bassin versant de l'Escaut.

E. affinis est un complexe d'espèces cryptiques connu pour sa forte capacité adaptative vis-à-vis de la salinité permettant à certaines population de coloniser rapidement les milieux d'eau douce (Lee, 1999 ; Lee & Bell, 1999 ; Lee & Petersen, 2003 ; Winkler et al., 2008). Dans nos cours d'eau, où les concentrations en oxygène (en moyenne 9.9 mg L^{-1}) et en ammonium (en moyenne 0.54 mg L^{-1}) sont favorables au développement d'*E. affinis* (si l'on considère les seuils : [O2]>4 mg L^{-1} et [N-NH_4^+]<2 mg L^{-1} pour que le milieu soit accessible à *E. affinis* décrits dans Chambord et al, 2016), ce sont donc d'autres paramètres que la concentration en O_2 et NH_4^+ qui limitent le développement de ce taxon dans la partie non estuarienne du bassin versant. Néanmoins, les abondances d'*E. affinis* restent faibles et très sporadiques dans le bassin non estuarien, hormis pour quelques stations de la Lys où elles sont plus importantes (Aire sur la Lys, Erquinghem-Lys et Wervicq notamment), et positivement corrélées à la température, qui varie entre 14 et 20 °C dans les eaux où l'espèce a été observée. Dans l'estuaire d'eau douce, *E. affinis* se développe également à des températures plus élevées qu'en eau saumâtre, et plus particulièrement lorsque celles-ci sont supérieures à 15 °C (Chambord et al., 2016).

Des suivis temporels à plus long terme, comme effectués dans la partie estuarienne de l'Escaut, pourraient également s'avérer pertinents dans le bassin en amont. Le fait que les communautés zooplanctoniques répondaient faiblement aux diverses sources de contamination (**chapitre 3**) est potentiellement lié à une certaine tolérance de ces taxons à la pollution. Si tel est le cas, des changements pourraient être observés en cas de modification des conditions, et en particulier de la qualité de l'eau, comme décrit précédemment dans l'estuaire.

La compréhension du fonctionnement des écosystèmes est une question centrale en écologie, et est devenue d'autant plus nécessaire avec le développement des activités humaines et les perturbations qui en ont découlé. Le potentiel du zooplancton en tant que bio-indicateur de la qualité de l'eau dans les cours d'eau du bassin amont de l'Escaut reste donc pour l'heure limité

par la variabilité importante des réponses des communautés zooplanctoniques aux conditions de contamination, et la faible prédictibilité des relations entre les communautés et les conditions du milieu. Cependant, un suivi régulier et à fréquence temporelle adaptée des communautés zooplanctoniques (par exemple synchrone avec les suivis phytoplanctoniques) pourrait permettre la détection de changements de conditions environnementales, et être utilisé dans le cadre de suivis des restaurations de ces milieux.

Les travaux de cette thèse ont également permis de démontrer le rôle trophique important de communautés zooplanctoniques jusqu'alors non-décrites. La probable capacité de contrôle des communautés phytoplanctoniques a été mise en évidence, impliquant également les organismes de la boucle microbienne. En particulier, l'impact trophique du zooplancton sur les cyanobactéries a été suggéré et mérite une attention particulière dans les recherches futures.

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ANNEXE

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Simultaneous Detection of 13 Endocrine Disrupting Chemicals in Water by a Combination of SPE-BSTFA Derivatization and GC-MS in Transboundary Rivers (France-Belgium)

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Abstract The occurrence of endocrine-disrupting chemicals (EDCs) in the aquatic environment has brought increasing concern due to their potential adverse impacts on ecosystems and humans. These compounds are generally present in complex water matrices, such as surface waters at trace levels (ng L^{-1}) making their analysis difficult. In this work, an analytical method for the simultaneous determination of 13 EDCs, including 5 steroid estrogens, 1 progestogen, 1 androgen, and 6 endocrine-disrupting phenols in water, was developed using solid phase extraction (SPE), derivatization, and gas chromatography-mass spectrometry (GC-MS). The method was validated by spiking the 13 EDCs to the interest matrix. The recovery was in the range of 52–71% with an average of 62%. The limits of quantification were 1 and 5–10 ng L^{-1} for phenolic compounds and hormones, respectively. The validated method was applied to assess the contamination level of the targeted EDCs in 15 sites collected from six rivers located at the cross-border area of Northern France and Belgium. The majority of the considered compounds were detected in the sampling sites and among them, bisphenol A (BPA) was found at the highest level which can be up to 286 ng L^{-1}. However, NP was the most frequently detected, followed by BPA and PG.

Keywords Endocrine-disrupting compounds · Surface waters · Derivatization · GC-MS

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1 Introduction

Endocrine-disrupting chemicals (EDCs) such as steroid estrogens, both natural and synthetic, and phenolic chemicals are widely used as surfactants, plasticizer, preservatives, disinfectants, and antiseptics (Silva et al. 2002; Jobling et al. 2004; Roepke et al. 2005). They have attracted a large worldwide scientific attention due to their widespread distribution into ecosystems and aquatic organisms, and potentially adverse health effects

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(Jobling and Tyler 2006; Fernandez et al. 2007; Lee et al. 2010; Sim et al. 2010; Gomez et al. 2012). EDCs have been linked to infertility, feminization, premature puberty, developmental problems, attention-deficit hyperactivity disorder, obesity, diabetes, impaired immune function, endocrine cancers (prostate, ovarian and breast), birth deformities, metabolic syndrome, and other diseases (Jobling et al. 2004; Roepke et al. 2005; De Coster and van Larebeke 2012; Rogers et al. 2013). Due to the adverse physiological effects of EDCs for human and wildlife, it is therefore critical to develop a reliable method for their reliable quantification.

Numerous analytical methods have been proposed to monitor the EDCs in water samples (Kuster et al. 2004; Petrovic et al. 2004; Jiménez-Díaz et al. 2015; Guedes-Alonso et al. 2014; Huang et al. 2011; Scognamiglio et al. 2016). Among them, gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography-liquid chromatography tandem-mass spectrometry (LC-MS/MS) are the most currently used for the simultaneous analysis of EDCs. However, GC-MS offers good separation, identification capabilities and exhibit high sensitivity for lower experiment cost (Zhang and Zuo 2005). But, in order to enhance detection sensibility and best separation resolution in GC-MS analysis, silylation is required to derivatize hydroxyl groups of EDCs, and thus reduce polarity, increase volatility and thermal stability of the analytes. The most common derivatization reagent is the N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) (Liu et al. 2004; Lei et al. 2009; Zhang et al. 2006). Indeed, the GC-MS analytical technique linked with BSTFA as a derivatization agent allows identification of a large range of organic compounds which contain –OH and/or –COOH moieties (Net 2010). BSTFA is usually used in combination with a small proportion of catalyst trimethylchlorosilane (TMCS) to enhance derivatization efficiency (Duong et al. 2010). However, optimal conditions such as temperature, heating time of derivatization and equilibration time vary strongly from one compound to another.

In this work, firstly, the optimal conditions for the simultaneous quantification of 13 EDCs have been determined. Secondly, the method was applied to assess the concentration of 13 EDCs in dissolved phase in water collected from fifteen sites in six rivers located in the cross-border area Northern France-Belgium and the upstream Scheldt watershed.

2 Materials and Methods

2.1 Chemicals

EDCs standards: progesterone, testosterone, 17α-estradiol, 17α-ethynylestradiol, 17β-estradiol, estriol, estrone,4-nonylphenol, bisphenol A, bisphenol F, bisphenol E, bisphenol C and bisphenol G, were purchased from Sigma-Aldrich (Saint-Louis, USA) with a purity of 99%. The silylation derivatization reagent N,O-bis [trimethylsilyltrifluoroacetamide] w/1% trimethylchlorosilane (BSTFA 1% TMCS) were obtained from Restek (Bellefonte, USA). 17 β-estradiol-d2, progesterone-C13 and bisphenolA-d16 with a purity of 99% were used as internal standards for steroid estrogens and phenolic chemicals. Supel-Select HLB SPE cartridges (200 mg/6 mL) were purchased from Sigma-Aldrich (Saint-Louis, USA). HPLC grade ethyl acetate, dichloromethane (DCM), methanol, acetonitrile and hexane were purchased from Dislab (Lens, France). Ultrapure water (Milli-Q) was produced by a Millipore apparatus (18.2 MΩ cm^{-1} resistivity). Sodium sulfate, silica and diatomaceous earth were calcinated at 450 °C overnight to eliminate the potential contaminants.

2.2 Study Sites and Sampling

The studied area is part of the watershed of the Scheldt, which presents high anthropogenic activities and high population density. Moreover, its historical industrialization was among the highest in Europe (Boughriet et al. 2007; Net et al. 2014a, b; 2015; Rabodonirina et al. 2015). These sites have been known to be among the most contaminated areas by trace metallic elements and organic pollutants. However, there is lack of data available in the literature concerning EDCs residues for the study area.

The sampling campaign was conducted in early spring 2015, from 07/04 to 10/04/2015 in 15 sites in six rivers located at the cross-border area Northern France-Belgium (Fig. 1). Six sites were located along the Scheldt river (Fresnes, Neuville, Crevecoeur, Warcoing, Berchem, Zingem), three sites along the Lys River (Aire sur-la-Lys, Erquinghem-Lys, Wervicq), two on the Deûle river (Don and Wambrechies), two on the Scarpe River (Brebières and Nivelle), one on the Sensée River (Férin), and one on the Sambre River (Jeumont). Water samplings were performed using pre-cleaned amber glass 2.5 L bottles that were immediately capped

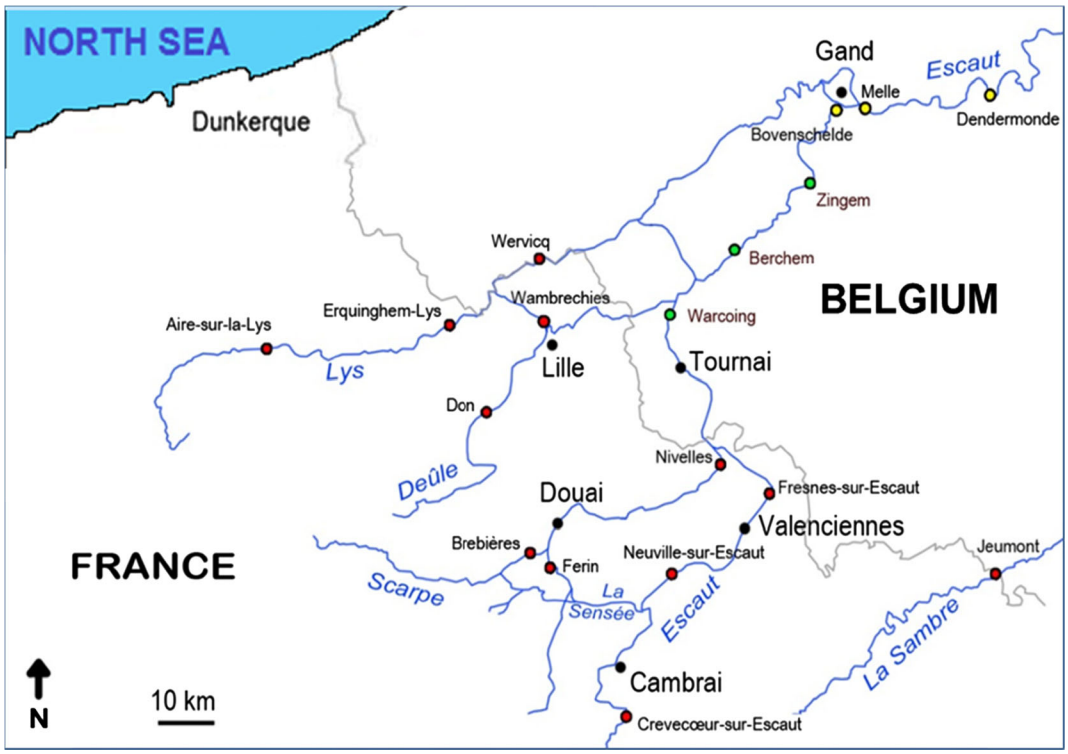


Fig. 1 Studied sites at the cross-border area Northern France-Belgium and the watershed upstream of the Scheldt. Sampling sites are indicated by *red* and *green* cycles respectively for the studied areas located in France and in Belgium

with Teflon-lined lid. Water samples were directly filtered using 0.45 μm Whatman glass microfiber filters to separate the dissolved phase from suspended solid matter (SSM). Targeted EDCs residues in filtered water were extracted using solid phase extraction (SPE).

During the sampling, classical parameters such as pH, temperature (T), dissolved oxygen (O_2), turbidity, and potential (E) have been measured directly in the field.

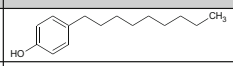
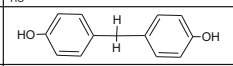
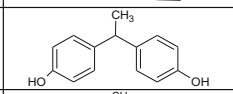
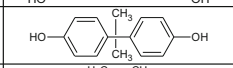
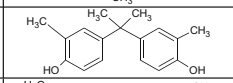
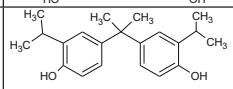
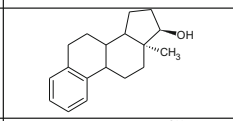
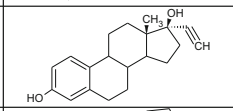
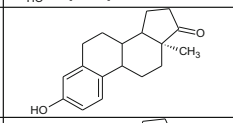
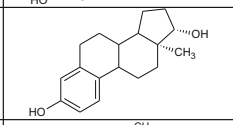
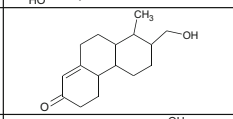
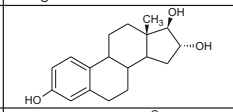
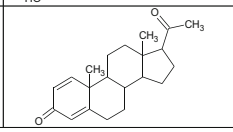
2.3 Targeted Analytes

In this work, 13 endocrine disrupting chemicals were analyzed. (i) Hormones: Progesterone (PG), Testosterone (TST) and five estrogens namely 17α-estradiol (αE1), 17α-ethynylestradiol (αEE2), 17β-estradiol (βE2), estriol (E3) and estrone (E1). (ii) Phenolic contaminants (PCs) including 5 bisphenols (BPs): 4-nonylphenol (NP), bisphenol A (BPA), bisphenol F (BPF), bisphenol E (BPE), bisphenol C (BPC) and bisphenol G (BPG). Their structures, acronyms, chemical formula, molecular mass, molecular mass of derivative compound, retention time (RT), quantifier ions, recoveries and limit of quantification (LOQ) are presented in Table 1.

2.4 Solid Phase Extraction (SPE)

Filtered water samples (500 mL) were spiked with internal standards (17β-estradiol-d2, progesterone-$C^{13}$and bisphenol A-d16). The SPE cartridges, containing hydrophilic-lipophilic-balanced (HLB) copolymer were chosen for the extraction/pre-concentration of EDCs. SPE cartridges were placed on 12-port Visiprep vacuum manifold and conditioned sequentially with 3 mL of ethyl acetate/methanol (1/1, *v/v*), 3 mL of methanol and 3 mL of ultrapure Millipore-Q water (pH 2). Then, filtered water was extracted at a flow rate ~5 mL/min. The cartridges were washed with 3 mL of methanol-water (2/3, *v/v*) then dried under vacuum for 1 h. The analytes were eluted from the sorbents with 9 mL of ethyl acetate/acetone (1/1, *v/v*) at a flow rate of 1–2 mL/min. The eluate was dried under a gentle stream of nitrogen and transferred into GC injection vial by solubilizing in 50 μL of acetonitrile. Finally, each extract was subjected to derivatization with BSTFA.

Table 1 Trivial name, acronyms, elemental composition, chemical structure, molecular weight (Mw), molecular weight after silylation (Mw-TMS), retention time (RT), characteristic ions (m/z), correlation coefficient (R^2), method quantification limits (LOQ), and method detection limits (LOD) of 13 EDCs

Trivial name and acronyms	Chemical formula	Chemical structure	Mw	Mw-TMS	RT (min)	Ions (m/z)	Recovery (%; $n \geq 3$)	R^2	LOQ (ng L^{-1})	LOD (ng L^{-1})
4-Nonylphenol NP	$C_{15}H_{24}O$		220	292	7.61	179; 292	63±1.4	0.997	1	0.33
Bisphenol F BPF	$C_{13}H_{12}O_2$		200	344	8.92	344; 343	68±2.2	0.997	1	0.33
Bisphenol E BPE	$C_{14}H_{14}O_2$		214	394	9.06	369	70±2.3	0.997	1	0.33
Bisphenol A BPA	$C_{15}H_{16}O_2$		228	372	9.27	357; 358	67±1.6	0.997	1	0.33
Bisphenol C BPC	$C_{17}H_{20}O_2$		256	400	9.68	386; 400	71±2.2	0.997	1	0.33
Bisphenol G BPG	$C_{21}H_{28}O_2$		312	456	9.7	441; 456	70±2.1	0.997	1	0.33
17α-estradiol αE2	$C_{18}H_{24}O_2$		272	416	12.33	282; 342; 416	53±1.7	0.998	10	3.33
17α-ethynylestradiol αEE2	$C_{20}H_{24}O_2$		296	440	12.41	425; 285	52±2.3	0.998	10	3.33
Estrone E1	$C_{18}H_{22}O_2$		270	342	12.59	257; 342; 343	58±1.9	0.996	10	3.33
17β-estradiol E2	$C_{18}H_{24}O_2$		272	416	12.7	342; 416	54±1.3	0.989	10	3.33
Testosterone TST	$C_{19}H_{28}O_2$		288	360	13.24	129; 226; 345	62±2.1	0.985	5	1.66
Estriol E3	$C_{18}H_{24}O_3$		288	504	14.3	311; 386; 504	54±1.8	0.998	10	3.33
Progesterone PG	$C_{21}H_{30}O_2$		314	–	14.64	124; 299; 314	68±2.2	0.989	5	1.66

2.5 Derivatization

Each extract was derivatized with 50 μL of derivatization reagent (BSTFA containing 1% TMCS) and heated at 65 °C. Indeed, the temperature and the duration of derivatization were studied and the optimal condition was obtained with 65 °C and 2 h of heating. For the calibration curve, the linearity range between 0.1 and 10 μg/mL was appropriate for every compound and has been used in this study. For the calibration curve, 50 μL of standard mixture solution (10 mg L^{-1}) were spiked with 10 μL internal standard (0.01 g L^{-1}) then were

derivatized by adding 50 μL derivatization reagent and heat at 65 °C for 1 h as for the extract. After the derivatization reaction, the derivatives were kept at room temperature during15 min prior to GC-MS analysis.

2.6 GC-MS Analysis

The extracts were analyzed using a Varian 3900 gas chromatograph (GC) equipped with a deactivated fused-silica guard column (5 m, 0.25 mm i.d.) and a fused-silica capillary Phenomenex XLB (60 m length, 0.25 mm i.d., 0.25 μm film thickness) and coupled with a Varian Ion Trap Saturn 2000 Mass Spectrometer (MS). The carrier gas was helium, held at a constant flow rate of 1 mL min^{-1}. One microliter of each sample was injected in the splitless mode at 280 °C and the injector was purged with helium after 1 min. The temperature of the GC was programmed as follows: initial temperature 100 °C, held for 2 min, 5 °C/min ramped to 250 °C then 3 °C/min ramped to 300 °C and held for 2.33 min. The transfer line and the ion trap were respectively held at 280 and 220 °C. Each targeted compound was identified based on the retention time (RT) and the mass spectrum (m/z) from chromatogram of standard solutions acquired in full scan (FS) mode. Quantification was then performed in the single ion storage (SIS), MS/MS or multiple reaction monitoring (MRM) modes for better selectivity. Response factors were determined relative to the internal standards response and to standard mixtures.

3 Results and Discussions

3.1 Method Development

3.1.1 Initial Temperature Effect Study

The initial temperature plays an important role in the analysis of the endocrine disrupting chemicals (EDCs) with GC-MS. It was set to allow fast removal of the solvent without losses of the analytes. To study the injector temperature effect, three experiments were carried out in FS mode by using a 5 μg/mL of standard solution EDCs: 50, 80, and 100 °C. Figure 2 shows the chromatograms obtained with the initial temperature 50, 80, and 100 °C. Best detection was obtained with initial injector temperature set at 100 °C. Accordingly, the temperature of the GC was programmed as follows: initial temperature 100 °C, held for 2 min, 5 °C/min ramped to 250 °C then 3 °C/min ramped to 300 °C and held for 2.33 min.

3.1.2 Derivatization Optimization

The E1,αE2, TST, and NP contain one hydroxyl group in their structure, while EE2, E2, BPA, BPC, BPF, BPG, and BPE contain two hydroxyl groups and the E3 contains three hydroxyl groups. Direct analysis of these compounds by using GC-MS could not allow good efficiency. To enhance the detection sensitivity and separation resolution for the analysis of target EDCs by GC-MS, derivatization is an essential step to increase their volatility and thermal stability. The common derivatization reagents which proved their efficiency for the EDCs are silylation reagents, such as BSTFA or N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) (Thurman et al. 2013). These reagents silylate the hydroxyl groups of the EDCs to get trimethylsilyl (TMS) derivative (Fig. 3). However, this stepwise derivatization method has significant problems such as incomplete derivatization and the derivate instability.

In this study, the hydroxyl groups were derivatized by using BSTFA catalyzed with 1% of TMCS (50 μL of BSTFA, 1% TMCS) and heating at 65 °C. The influence of the reaction time (heating time) was carried out to investigate the optimum condition of heating. Heating times of 30 min, 1 h, 1 h 30 min, 2 h, 3 h and 4 h have been performed and the results are shown in Fig. 4a. The equilibrium time after heating can also influence significantly on the detection (Fig. 4b). Indeed, after heating at 65 °C for 2 h, the mixtures were cooled at room temperature during 0 min, 15 min, 1 h, 2 h, 4 h, 6 h, 8 h and 10 h prior to GC-MS analysis. The results showed that the best efficiencies were obtained when heated at 65 °C during 2 h except for EE2 and αE2 of which better detection was obtained when heating during 1 h. However, these two compounds (EE2 and αE2) still presented a high intensity of detection when heating during 2 h. For the equilibration time after heating, the optimum was obtained with 15 min of equilibration at room temperature for all the selected compounds. The best compromise for the simultaneous detection of these hormones and alkylphenols was to heat at 65 °C during 2 h and keep at room temperature for 15 min for equilibration.

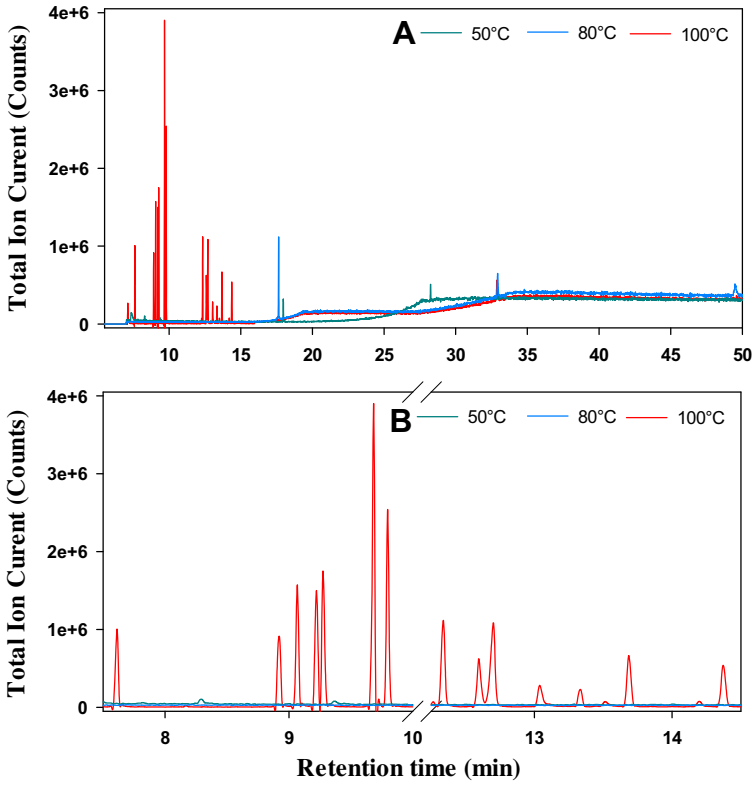


Fig. 2 Chromatograms of the 13 EDCs with injector temperature 50, 80, and 100 °C. **a** Between 5 and 50 min. **b** A zoom between 7.5 and 15 min

3.1.3 Method Validation

The optimum conditions were validated and linearity range, repeatability of these 13 hormones and alkylphenols were studied. For this purpose, a series of injections of the mixture of targeted compounds at the concentration ranging from 0.001 to 10 μg mL^{-1} (0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 μg mL^{-1}) with 1 μg mL^{-1} of internal standards was used to determine the linear concentration range. For each concentration, the analysis was performed in triplicate. Table 1 presents the details on the RT, LOQ, LOD, quantifier ions (m/z), and correlation coefficient (R^2) of each targeted EDC.

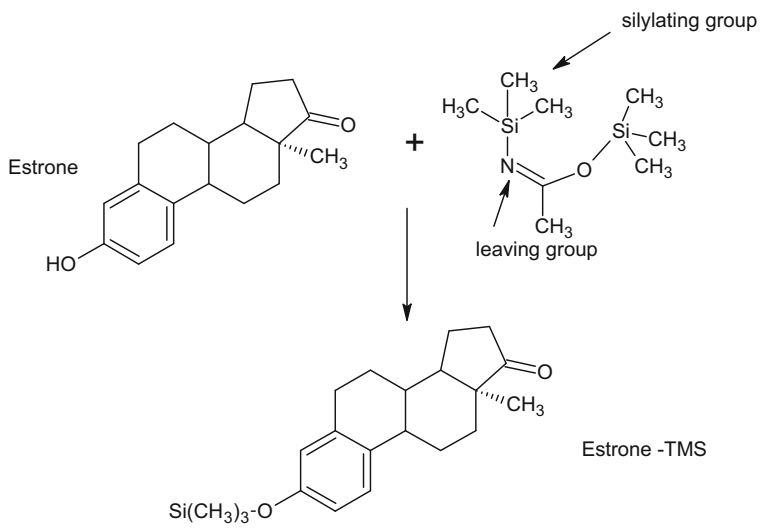


Fig. 3 The silylation reaction of estrone with BSTFA

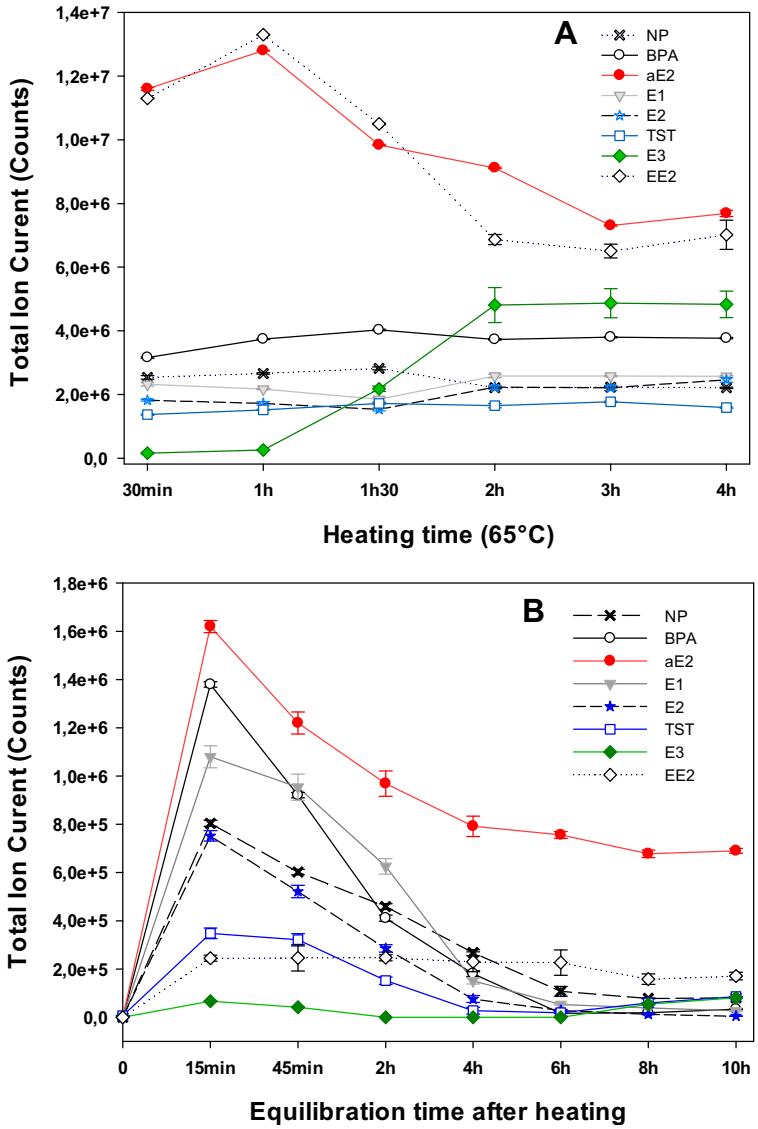


Fig. 4 **a** Effect of heating time on the reaction of the derivatization at 65 °C. **b** Effect of equilibration at room temperature after heating at 65 °C of hormones and alkylphenols ($n = 3$)

In this work, the limits of detection and quantification obtained for the studied compounds were lower than the values reported in previous studies. In a study conducted by Vega-Morales (Vega-Morales et al. 2010) using HPLC-DAD, the method limits of detection (LOD) obtained for the estrogens and progesterone were 600 and 300 ng L^{-1}, respectively. In another study by Queiroz (Queiroz et al. 2014) using HPLC/HRMS, the LOD for estrogens was 9.3-12.4 ng L^{-1} and for BPA was 2.1 ng L^{-1}. In another study, Selvaraj (Selvaraj et al 2014) reported an LOD of 1.5 ng L^{-1} for BPA and 1.1 for estrogens when using GC/MS.

The recoveries obtained with our method of the simultaneous extraction ranged from 52 to 71% (Table 1). In the literature, there is only very few research which focused on the analysis of these EDCs simultaneously. However, many studies were focused on the extraction of these compounds separately for each family. Our recoveries were lower than those obtained when each family of EDCs was extracted separately. Indeed, each family has different physical-chemical properties which makes difficult to the simultaneous extraction. For example, Queiroz et al. (2014) have reported the recoveries ranging from 12 to 87%, and Viglino et al. (2008) obtained the recoveries ranging from 46 to 87% when working on the simultaneous analysis. Compared to these simultaneous analyses, our results were satisfactory since we have no recovery lower than 52%.

Table 2 Individual concentration of the EDCs in the 15 sites

Compounds	Concentration of individual EDC (ng L^{-1})												
	NP	BPF	BPE	BPA	BPC	BPG	αE2	EE2	E1	E2	TST	E3	PG
Aire sur LaLys	14.9 ± 0.4	77.7 ± 0.4	NQ	38.8 ± 0.2	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	6.1 ± 0.1
Berchem	16.9 ± 0.4	NQ	NQ	76.4 ± 0.2	NQ	10.3 ± 0.1	NQ	NQ	70.2 ± 0.1	NQ	NQ	NQ	6.0 ± 0.01
Brebieres	20.1 ± 0.4	91.9 ± 0.4	NQ	286 ± 2.0	24.26 ± 0.4	NQ	NQ	NQ	NQ	NQ	NQ	NQ	10.9 ± 0.5
Crevecoeur	1.9 ± 0.4	NQ	NQ	39.5 ± 0.2	68.22 ± 0.2	NQ	NQ	NQ	116.2 ± 0.4	NQ	NQ	NQ	6.3 ± 0.4
Don	20.3 ± 0.4	NQ	NQ	51.7 ± 0.3	NQ	20.9 ± 0.4	NQ	NQ	NQ	NQ	5.8 ± 0.5	21.0 ± 0.4	6.7 ± 0.1
Erquinghem	8.1 ± 0.5	NQ	NQ	27.7 ± 0.2	NQ	NQ	NQ	NQ	NQ	NQ	6.5 ± 0.4	70.7 ± 0.1	NQ
Ferin	3.6 ± 0.1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	5.4 ± 0.5	NQ	7.3 ± 0.4
Frenes	15.5 ± 0.5	NQ	NQ	11.8 ± 0.3	NQ	NQ	NQ	NQ	NQ	NQ	NQ	47.6 ± 0.4	5.7 ± 0.1
Jeumont	25.9 ± 0.7	15.7 ± 0.6	NQ	42.5 ± 0.1	NQ	NQ	NQ	NQ	NQ	NQ	5.8 ± 0.4	NQ	7.9 ± 0.1
Neuville	7.4 ± 0.7	NQ	NQ	11.9 ± 0.1	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Nivelle	25.7 ± 0.1	NQ	NQ	21.6 ± 0.1	NQ	NQ	NQ	NQ	34.2 ± 0.6	NQ	NQ	77.7 ± 0.2	NQ
Wambrechies	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Warcoing	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Wervicq	24.2 ± 0.5	44.0 ± 0.6	NQ	NQ	NQ	NQ	NQ	NQ	92.0 ± 0.2	NQ	NQ	NQ	NQ
Zingem	9.2 ± 0.1	NQ	NQ	6.8 ± 0.1	NQ	NQ	NQ	NQ	NQ	3.6 ± 0.1	NQ	NQ	6.4 ± 0.1

NQ not quantified (<LOQ)

Table 3 The comparison of EDCs concentrations (ng L^{-1}) detected in aquatic environment in Northern France-Belgium with literature

EDCs	Rivers in northern France-Belgium						Rivers in Europe	Rivers, north America	Rivers, China	Rivers, Japan
	Lys River	Deûle River	Scarp River	Sensée River	The Scheldt	Sambre River				
NP	15.7 ± 0.7	10.2 ± 0.8	22.9 ± 0.7	3.6 ± 0.5	8.5 ± 0.4	25.9 ± 1.3	NQ–644 (83%)	NQ–40 (50%)	36–33 (100%)	51.40–144.10 (100%)
BPF	40.6 ± 0.7	NQ	46.0 ± 0.7	NQ	NQ	15.7 ± 0.6	NQ–180	-	-	-
BPA	22.2 ± 0.4.0	25.9 ± 0.3	153.8 ± 1.0	NQ	24.4 ± 0.6	42.5 ± 0.1	NQ–66	NQ–12 (41%)	6–331 (100%)	16.50–150.2 (100%)
BPE	NQ	NQ	NQ	NQ	NQ	NQ	-	-	-	-
BPC	NQ	NQ	12.0 ± 0.2	NQ	11.4 ± 0.1	NQ	-	-	-	-
BPG	NQ	20.9 ± 0.4	NQ	NQ	10.3 ± 0.1	NQ	-	-	-	-
αE2	NQ	NQ	NQ	NQ	NQ	NQ	<2	NQ–74 (5%)	NQ–2 (22%)	-
EE2	NQ	NQ	NQ	NQ	NQ	NQ	NQ		NQ–1 (5%)	-
E1	30.7 ± 0.3	NQ	17.1 ± 0.2	NQ	31.1 ± 0.2	NQ	<0.40–33	NQ–112 (7%)	NQ–65 (61%)	17.1–44.5 (100%)
E2	NQ	NQ	NQ	NQ	NQ	NQ	<0.10–3.60	NQ–93 (10%)	NQ–2 (11%)	2.6–7.7 (100%)
TST	2.2 ± 0.1	2.9 ± 0.3	NQ	5.4 ± 0.5	NQ	5.8 ± 0.4	1.60–3.40	-	-	-
PG	2.0 ± 0.0	3.3 ± 0.1	5.4 ± 0.1	7.3 ± 0.1	4.1 ± 0.1	7.3 ± 0.1	-	-	-	-
E3	23.6 ± 0.3	10.5 ± 0.5	38.9 ± 0.1	NQ	7.9 ± 0.4	NQ	-	NQ–51 (21%)	NQ–1 (5%)	<0.2
Ref.	This work						Hermann et al., 2002; Labadie and Budzinski 2005; Raycoguesdes-alonso et al., 2014; Raycoguesdes-alonso et al., 2014	Boyd et al., 2004	Peng et al. 2008	Furuichi et al., 2004

The values in the parentheses are the detection frequencies

NQ not quantified (<LOQ), "-" no data

Moreover, the coefficients of variation were less than 10%. This was regarded as satisfactory, considering that the 13 EDCs were extract simultaneously.

3.2 Application for Surface Water

3.2.1 Physicochemical Characteristics of Waters

In the laboratory, SSM was also measured. The values of the field measured water parameters and SSM values parameters are listed in Table 1S. Generally, the water bodies were relatively neutral or slightly basic with pH values of 7.68–8.57 and well oxygenated with dissolved oxygen concentration ranging between 6.5 and 18.44 mg L^{-1}. The temperature was ranging from 11.2 to 16.1 °C. However, SSM along the Scheldt presented a large variation ranging from 2.47 at Brebières to 66.36 mg L^{-1} at Berchem.

3.2.2 Occurrence of EDCs in the Environment

Table 3 presents the concentration of every individual compound detected in each sampling site. The concentration of each compound varied significantly from site to site. NP was the most frequently detected and followed by BPA and PG. Among the selected hormones, PG was the most frequently detected. Its concentration varied from <LOQ to 10.9 ± 0.5 ng L^{-1} (Brebieres) (Table 2). These concentrations were in the same order as those reported by Labadie and Budzinski (2005) in the Jalle d'Eysines River near Bordeaux, France.

Table 3 presents the mean values of each compound in each river. High heterogeneity of concentrations was observed between the rivers. The concentrations of E1 detected in the six rivers at Nord-Pas-de-Calais and at the cross-border France-Belgium were similar to the ones measured in the Mississippi River (Zhang et al. 2007), but slightly higher than those measured in

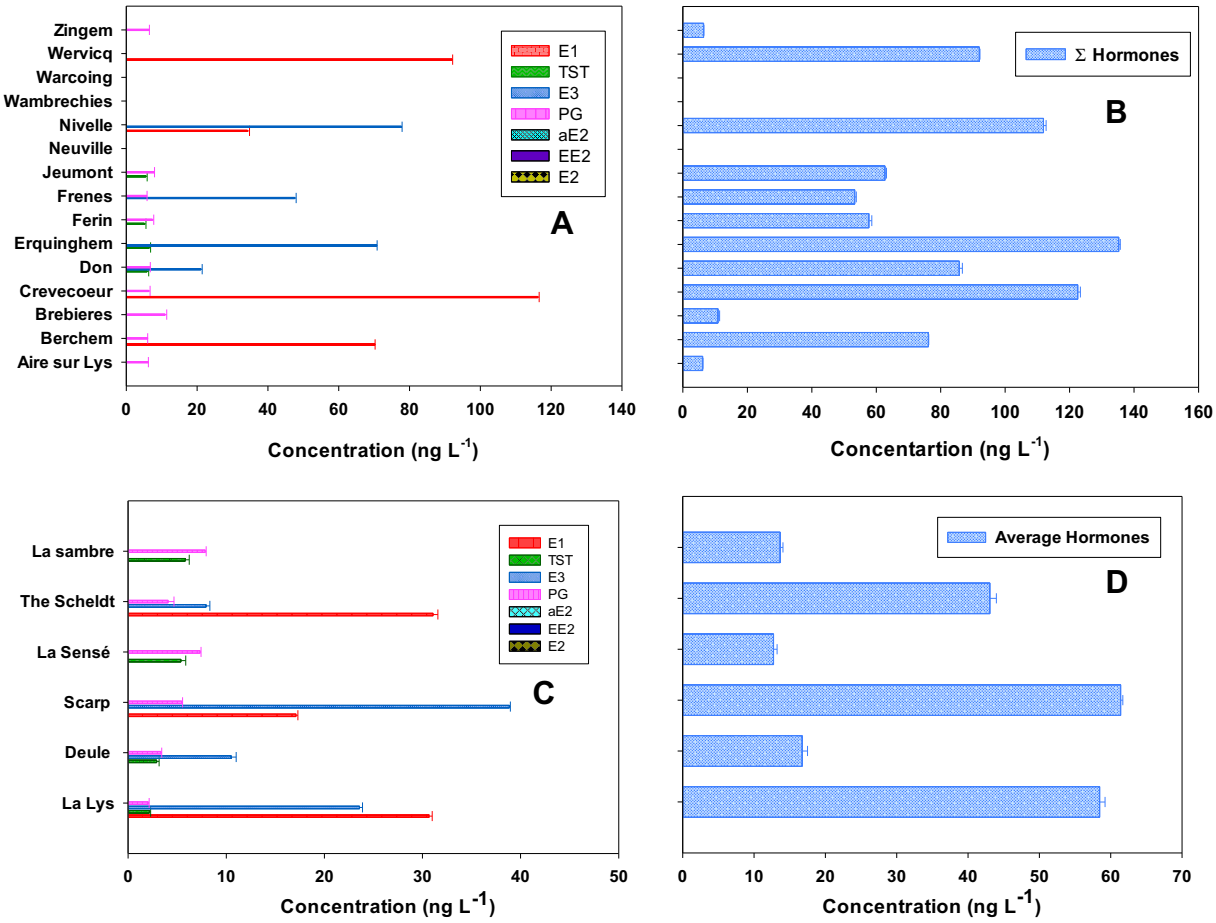


Fig. 5 Concentration of each individual hormone in each site (**a**), in each river (**b**), the ∑Hormones in each site (**c**) and in each river (**d**)

Guangzhou River, China (Peng et al. 2008) (Table 3). The variability of E1 level in the sampling sites may be explained by their local sources located near to the sampling site. It can also be related to the population density and the agricultural or industrial practices. The Scarp River was the most contaminated river (Fig. 4c). This may be due to the fact that it is closely surrounded by big cities (Lille, Douai and Cambrai) where urban and industrial activities are concentrated. For testosterone, the concentration was close to LOQ value. Similar results were reported in Gran Canaria, Spain (Rayco et al. 2013). The low concentrations of testosterone detected in our samplings sites may be due to its instability in natural waters. Indeed, a recent study has reported that testosterone is rapidly transformed in natural water (Vulliet et al. 2010). Above all, in global, high levels of hormones were detected at Crevecoeur and Nivelle.

3.2.3 Hormone Residues

The level of E1 was higher than those of the other steroid hormones with the maximum detected at Crevecoeur (116.2 ± 0.4 ng L^{-1}) (Fig. 5).

E2 and αE2 were detected at trace level (≤2 ng L^{-1}). Similar observations has been reported in the literature (Net et al. 2014). These may be due to the instabilities of E1, E2 and αE2 in surface water. Indeed, E1, E2 and αE2 have been reported unstable in natural environment (Ternes et al. 1999). αEE2 was not detected at any site. This may be because of the low amount consumed for contraception purpose; it

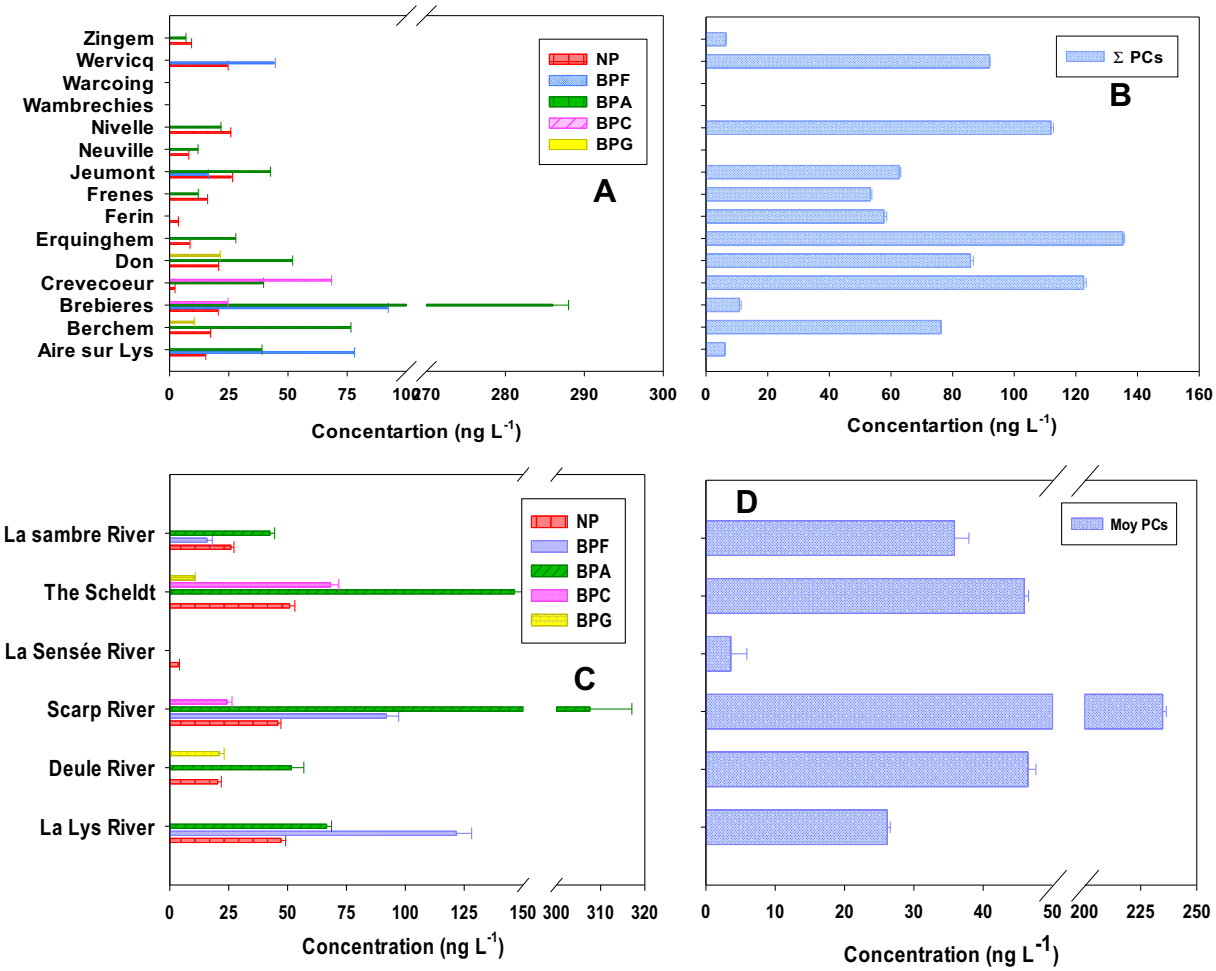


Fig. 6 Concentration of individual phenolic contaminants PCs in the 15 sites (**a**), in each river (**c**), the ΣPCs concentration in each site (**b**) and in each river (**d**)

is used only in doses of approximatively 30 μg of αEE2/woman/day. For comparison, daily production of naturel estrogens is close to 150 μg/woman/day and progesterone production ranges from 3 to 30 mg/woman/day (Labadie and Budzinski 2005). Moreover, E1 is more stable than αEE2 and E2 (E2 > EE2 > E1) (Feng 2005).

3.2.4 Phenolic Contaminants Residues

NP, the metabolite of nonylphenol ethoxylates surfactants, which are widely used in detergents and emulsifiers, was predominant among the five selected phenolic contaminants and was detected in all samples. The contamination level was detected from <LOQ to 25.9 ng L^{-1} (Table 2). These values are in the same order of the one reported by Peng et al. (2008) (Table 3). BPA is primarily used as an intermediate in the production of polycarbonate plastic, epoxy and other specialty resins. BPA is mainly used in the polycarbonate include glazing and sheeting, household equipment, electrical and electronic goods, electronic storage media, including bottles, utensils, and containers. It is also used in the production of phenoplast, phenolic, polyvinylchloride unsaturated, polyester resins, and thermal paper. Arnold et al. (2013) report high concentrations of BPA which can be up to 1900, 2970, and 4230 ng L^{-1} in natural environment detected respectively in North America, Europe and Asia. Our result showed the maximum concentration of BPA at 286 ng L^{-1} detected in Brebières, a site of the Scarpe River (Fig. 6). For the studied area, BPA concetrations recorded in the fifteen sites were similar to those found in North America (Arnold et al. 2013) but higher than those found in river of Guangzhou, China (Peng et al. 2008).

Both the tolerable daily intake (TDI) set by the EU Commission and the reference dose (RfD) established by the United State Environmental Protection Agency (US-EPA) are 0.05 mg BPA/kg body weight/day. Due to this restriction, other bisphenols (BPs) such as BPF, BPE, BPC, and BPG, considered as substitutes for BPA in industrial applications are starting to be used for the production of epoxy resins. These BPs have shown moderate to slight acute toxicity and estrogenic effects similar to those of BPA (Gallart-Ayala et al. 2011). BPF was detectable in 26.7% of the samples; it was detected from <LOQ to 91.9 ng L^{-1}. These concentrations were lower than those reported in North Rhine-Westphalia, Germany (Hermann et al. 2002).

For the other BPs, less information has been published. BPC and BPG were detected in only two sites with the maximum concentration of 68.2 and 20.9 ng L^{-1} (Table 2). BFE was detected at below LOQ for all sites. Figure 6 shows the concentration of individual PCs and the sum of PCs (ΣPCs) in each site and in each river.

4 Conclusion

EDCs are an important class of emerging contaminants. Their reliable quantification can be difficult due to their presence in trace levels in natural environment. In this work, main parameters which influence on the efficiency of detection of 13 EDCs have been optimized using a combination of SPE, BSTFA derivatization and GC/MS analysis. The method has a good recovery and LOQs of the method were ranging from 1 to 10 ng L^{-1}. This method was applied to assess the contamination of the 13 EDCs in 15 sites located at the cross-border area Northern France and Belgium. The results showed significant contamination by the EDCs in the 15 sites located on six rivers (The Scheldt, the Lys, Deûle, the Scarpe, the Sensée and the Sambre Rivers). High concentration of Bisphenol A and Estrone were detected in Brebières and Crevecoeur. Globally, the Scarpe and the Scheldt Rivers were the most contaminated by PCs and hormones, lowest concentration levels of PCs and hormones were detected for the Deûle and the Sensée River, respectively. EDCs in natural environment can be a serious threat to ecosystem functioning. To minimize their impacts on ecosystems and human health, it seems essential to develop cost-effective technology to improve their elimination yield from wastewaters and thus limit the problem from the source and/or to remediate the contaminated natural environment.

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Zooplankton distribution and trophic role in the Scheldt Watershed

ABSTRACT:

Structural and functional characteristics of the Scheldt basin zooplankton have been studied in relation with environmental conditions presented in watercourses.

The Scheldt runs for 350 km from Northern France to the Netherlands, crossing Belgium. Its basin drains an area subject to multiple pollutions originating from industrial, agricultural and domestic activities. As a consequence, ecological states of watercourses are unsatisfactory according to the Water Framework Directive (WFD) evaluation. So far, studies on Scheldt zooplankton have focused on estuarine reaches, and the upstream basin's communities were scarcely documented.

From source to mouth of the Scheldt, 4 functional zones have been identified presenting different habitat conditions for zooplankton, defined mainly by salinity gradient and tidal influence.

At the scale of the non-tidal watershed, spatial and temporal variability of zooplankton communities have been explored, in relation with environmental conditions. Zooplankton distribution showed a strong heterogeneity, inducing little predictability concerning ecological status (WFD), environmental conditions, and contamination patterns of sites (considering a set of persistent organic pollutants and trace metals characteristics of the study area). However, an important trophic role has been highlighted. The zooplankton community between 50-250 µm showed a selective feeding on some algal groups, particularly on chlorophytes, and and the results suggest a grazing activity on cyanobacteria.

Key words : zooplankton, Scheldt watershed, Water Framework Directive, multiple contamination, trophic selectivity.

Distribution et rôle trophique du zooplancton dans le bassin versant de l'Escaut

RESUME :

Les caractéristiques structurelles et fonctionnelles du zooplancton du bassin versant de l'Escaut ont été étudiées en relation avec les conditions environnementales présentées dans les cours d'eau.

L'Escaut est un fleuve long de plus de 350km répartis entre la France, la Belgique et les Pays-Bas. Son bassin versant draine un territoire soumis à de nombreuses pressions d'origine anthropique industrielle, agricole et domestique. Par conséquent, d'après l'évaluation de la Directive Cadre sur l'Eau, l'état écologique des cours d'eau est jugé plutôt insatisfaisant. Les précédentes études sur le zooplancton de l'Escaut ayant porté principalement sur les tronçons estuariens du fleuve, les communautés zooplanctoniques des cours d'eau de l'amont du bassin versant, dans la zone transfrontalière entre la France et la Belgique étaient jusqu'alors non documentées à notre connaissance.

De la source à l'embouchure de l'Escaut, 4 grandes zones fonctionnelles ont pu être identifiées présentant différentes conditions d'habitat pour le zooplancton, définies principalement par le gradient de salinité et l'influence de la marée.

A l'échelle de la partie non estuarienne du bassin versant, la variabilité spatiale et temporelle du zooplancton a été explorée, en relation avec les conditions environnementales. La distribution du zooplancton a montré une forte hétérogénéité, induisant une faible prédictibilité dans au regard de l'état écologique (DCE), des conditions environnementales et des profils de contamination des sites (en considérant une gamme de polluants organiques persistants et métaux traces caractéristiques de la zone d'étude). En revanche, un rôle trophique important a pu être mis en évidence, avec une sélectivité trophique de la fraction zooplanctonique 50 -250 µm envers les groupes algaux, en particulier les Chlorophytes, et un broutage suggéré sur les cyanobactéries.

Mots clés : zooplancton, bassin versant de l'Escaut, Directive Cadre sur l'Eau, contaminations multiples, sélectivité trophique.