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

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A B S T R A C T

Data obtained from 14 years of monthly samplings (1996–2009) were used to investigate the response of the crustacean zooplankton community to improving water quality in the Scheldt estuary. A strong reduction of poor water quality indicators, such as NH_4^+ and BOD_5, as well as an increase in oxygen and in chlorophyll *a* concentrations were observed during the study period. During the study period, important changes were observed in the zooplankton community composition and spatial distribution. From 2007 onwards, most of the calanoid population, previously mainly found in the brackish water reach of the estuary, moved to the freshwater, where they reached higher abundances than previously observed. Simultaneously, cyclopoids populations strongly decreased in freshwater while cladocerans did not change their abundance, except during years with high chlorophyll *a* concentrations. Redundancy analyses (RDA) showed that the variability within the calanoid population can be explained by the improvement in water quality. Variability within the cyclopoids and cladoceran community is mainly explained by chlorinity and chlorophyll *a* concentrations. Their presence in the most polluted upstream area until 2007 suggests they are more tolerant to poor water quality than calanoids. Several hypotheses to explain the disappearance of cyclopoids after the move of calanoids to the freshwater are presented and discussed.

1. Introduction

Recent decades have seen an increasing realisation of the need for maintenance of functional and diverse ecosystems in turn leading to restoration efforts on both terrestrial and aquatic systems. The response of organisms to restoration is important for management and several types of organisms have been long used to assess water quality. However, to date, in contrast to the effects of degradation, the biotic response to restoration is less well-known and poorly predictable (Hering et al., 2010). Because of their natural "funnel" function between land and ocean and intensive human use, estuaries are among the most polluted systems worldwide (Dame et al., 2000; Borja et al., 2010; Meng and Liu, 2010). Rates of ecosystem recovery are dependant on the type of stress applied and most estuaries suffer from multi-stress degradation. Restoration efforts in estuaries are relatively recent, indeed several recent papers on estuaries report ongoing pollution rather than restoration, for example the Neuse River and Skidaway River estuaries (Paerl et al., 2004; Verity and Borkman, 2010). At present there is little information on the link between (types of) restoration efforts and responses of various estuarine organisms (Borja et al., 2010). Biotic water quality indicators traditionally involve the benthos, phytoplankton and macrophytes, and zooplankton is generally less considered when studying biological responses to restoration. The labour intensity of studying zooplankton in turbid systems such as estuaries has further reduced studies following response of estuarine zooplankton to restoration. For European systems, the fact that zooplankton is not included in the Water Framework Directive (WFD) has contributed to this paucity despite the response of zooplankton to water quality degradation such as eutrophication being known for estuaries (Verity and Borkman, 2010) suggesting that in reverse, zooplankton is likely to respond to water quality improvement. In freshwater systems, the response and potential pivotal role of zooplankton to the outcome of restoration scenarios has been amply documented

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by both field and modelling studies (e.g. Mazumder, 1994; Benndorf, 1995; Zhao et al., 2008; Chen et al., 2009; Peretyatko et al., 2009; Tadonleke et al., 2009).

The present paper indicates the response of the crustacean zooplankton (copepods and cladocerans) to improving water quality in a temperate estuary. The Scheldt estuary (Belgium/The Netherlands) is one of the few European estuaries that still have an extensive freshwater tidal zone (salinity < 0.5) in its upper reaches (Meire et al., 2005). Having a drainage basin which is heavily impacted by anthropogenic activity, the Scheldt was considered as one of the most polluted systems in Europe during the second half of the 20th century, especially in its freshwater part (Heip, 1988; Baeyens et al., 1998). As a result of substantial emission reduction efforts throughout the watershed, water quality of the Scheldt estuary has substantially improved in the last 2 decades (Van Damme et al., 2005; Cox et al., 2009). Since 1996, this restoration process has been monitored by the multi-disciplinary programme "OMES". The aim is to detect physico-chemical changes occurring in the Scheldt estuary and to relate them to management efforts and future management issues, with a focus on their interaction with biotic communities. In this context, we hypothesise whether zooplankton (crustaceans and rotifers) can detect if the ongoing restoration process influences their abundance, community composition and spatio-temporal distribution pattern. Such changes may be important to the intra- and interspecific competition within the zooplankton community and to the trophic web structure of the estuary. This is also of relevance to the bioindicator potential of the zooplankton community or of certain taxa within this community.

This paper considers abundance data at phylum level for copepods (cyclopoids, calanoids and harpacticoids) and at genera level for cladocerans during the 1996–2009 period. The spatio-temporal evolution of this community is related to the changes in the environmental conditions occurring during this period in the Scheldt estuary. The dominant calanoid copepod species, *Eurytemora affinis*, is considered separately, in view of its recently reported response to improving water quality between 1996 and 2007 in the Scheldt estuary (Mialet et al., 2010).

2. Material and methods

2.1. Study site and data on zooplankton

The Scheldt estuary originates in northern France and runs through Belgium to join the North Sea at Vlissingen in the Netherlands (Fig. 1). In contrast to most other temperate estuaries, the Scheldt estuary is characterized by a vertically well-mixed water column (Baeyens et al., 1998), showing little salinity or current stratification (Heip, 1988). Within the framework of the OMES project, samples have been taken monthly at 16 stations (Fig. 1) from 1996 until present, with an interruption between 2000 and 2002. All stations are situated in the brackish (salinity > 0.5 and 12) and freshwater part (salinity < 0.5) of the estuary (Fig. 1).

2.2. Sampling and analysis

At each station, surface water samples were collected in the middle of the estuary throughout the entire studied period, using bucket hauls from the vessel. A set of environmental variables were measured: 5-day biochemical oxygen demand (BOD_5) was estimated using a WTW OXI 96 oxymeter; pH and temperature were measured using a CONSORT C832 electrode and dissolved oxygen concentration (O_2) a WTW OXI 325, equipped with Clark electrode. Samples were taken for the determination of the concentrations of chlorine (Cl^-), ammonium (NH_4^+-N), nitrates (NO_3^--N), nitrites (NO_2^--N), and orthophosphates (PO_4-P) within 24 h after sampling. They were stored at 4 °C, and analyzed colorimetrically using a SKALAR SA 5100 segmented flow analyzer. Suspended particular matter (SPM) samples were filtered on pre-combusted Whatman GF/C filters. From 1996 to 2001, Chlorophyll *a* (Chl *a*) samples were filtered on 45 μm Sartorius filters, extracted in 90% acetone and analyzed using reversed phase HPLC. Van Damme et al. (1997, 2005) give further details on the methodologies used. From 2002 to present, Chlorophyll *a* (Chl *a*) samples were filtered over a 25 mm diameter Whatman GF/F glass fibre filter.

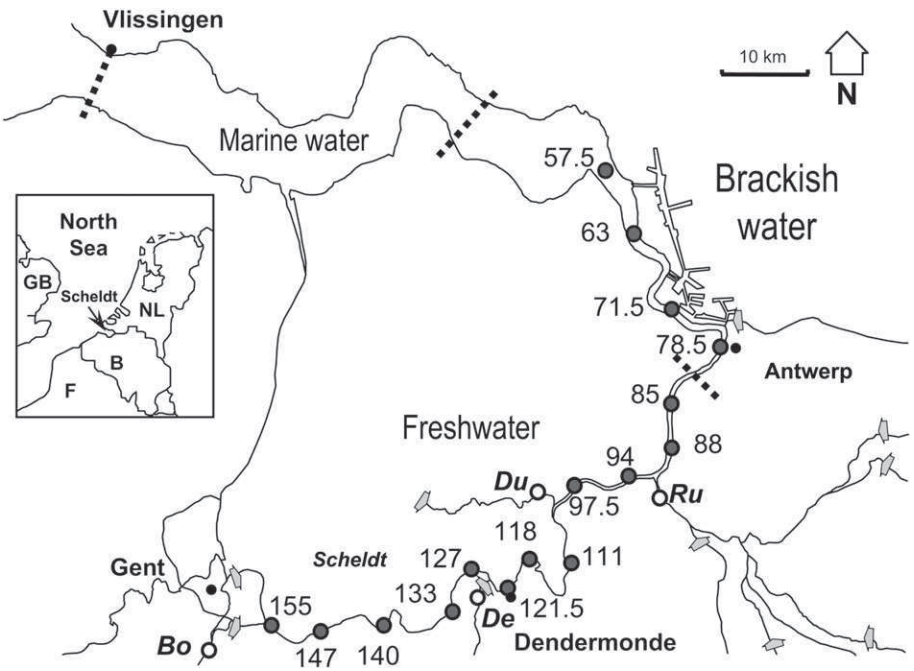


Fig. 1. Map of the Scheldt estuary with OMES sampling stations, designated by their distance, in km, upstream from Vlissingen (mouth). Dotted lines indicate limits between marine water, brackish water and freshwater reaches. Grey arrows indicate the end of tidal influence in the Scheldt and tributaries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

Pigments were then extracted and analyzed by HPLC according to the method of Wright and Jeffrey (1997). More details on the methodologies used are presented in Lionard et al. (2008).

The Flemisch Administration for Waterways and Maritime Affairs provides daily discharge measures (Q) of the BovenScheldt, the Dender and the Rupel. The upstream discharge data at these stations were used to estimate downstream discharge, taking into account all the physical and geomorphological characteristics of the Scheldt estuary (depth, width and relative percentage of each affluent in the total discharge). Daily average discharges for 68 km at the sampling dates are used in our data set. This station is located downstream in the study area and integrates discharge values of upstream stations.

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

2.3. Data analysis

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

The CANOCO software package, version 4.5 (ter Braak, 1987, 1994) was used. The modality of the environmental factors was first analyzed by a Detrended Correspondence Analysis (DCA), using detrending by segments and downweighting for rare species. As the total inertia observed was less than 2.6, a predominance of linear species response curves could be expected; therefore a Principal Components Analysis (PCA) was used to investigate the relationships among environmental factors (ter Braak and Smilauer, 2002). Then the same DCA for the taxa distribution was performed. As for the environmental factors, the total inertia observed was less than 2.6, and so a redundancy analysis (RDA) was used to investigate the relationships between environmental factors and taxa composition. Forward selection of variables was used to select those most closely associated with the spatio-temporal structure of the zooplankton taxa, and to quantify their relative importance. The statistical significance was tested with Monte Carlo permutation tests (499 unrestricted permutations) ($p < 0.05$) and a Bonferroni correction for multiple test was applied. The minimum model so obtained explains the distribution without colinear extra fitting. For each analysis, the variance explanation of each environmental variable (marginal effect), and their additional variance explained when included in the model (conditional effect) (ter Braak and Smilauer, 2002) were also shown. More specific details about this method are given in Borcard et al. (1992).

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

3. Results

3.1. Changes in water quality during the studied period

The evolution of the environmental variables is shown for the brackish and the freshwater areas separately, using the salinity of 0.5 to distinguish the zones (Fig. 2, Table 1). Annual mean BOD_5, NH_4^+-N and PO_4-P (proxy for organic pollution), as well as NO_2^--N, showed a substantial decrease, especially in the freshwater part. NO_3^--N tended to decrease in brackish water while it increased in freshwater. Chl *a* concentration showed no clear tendency with time but increased in freshwater from 2000 to 2004, reaching a maximum annual mean value of 147 $\mu g\,l^{-1}$, then decreased regularly to an annual mean value of 74 $\mu g\,l^{-1}$ in 2009. O_2 concentration increased in both reaches. SPM showed no gradient in the brackish water reach but increased in the freshwater reach (Fig. 2, Table 1). Along the studied period, for most factors associated with water quality (BOD_5, NH_4^--N, NO_2^--N, PO_4-P, O_2), the values observed in the freshwater reach approached the range observed in the brackish reach.

In both zones, considered over the entire 1996–2009 period, Q and T showed no tendency with time, but Q increased strongly during the first half of the study period and decreased after that. Cl^-, as correlated with Q ($p < 0.001$, Spearman rank test between annual mean values) showed the opposite trend with time.

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

The evolution of the abundance of planktonic taxa considered (Figs. 3 and 4) shows that few crustaceans were observed in the brackish water zone, except calanoids. Between 1996 and 2006, *Eurytemora affinis* was observed mainly downstream of 98.5 km and during spring, reaching a maximal monthly mean abundance of 3250 ind m^{-3} during April 2004, considering the entire transect (Fig. 3). *E. affinis* was occasionally found in freshwater, in relative low abundances. Its freshwater abundance increased significantly from 1996 to 2009, especially from 2007 onwards (Fig. 4; Table 1), reaching a monthly mean abundance of 27 900 ind m^{-3} during March 2009 (Fig. 3). This increase was associated with an upstream spreading of populations up to 155 km. Moreover, since 2007 *E. affinis* was also present during summer in the freshwater zone, but not in the brackish water zone.

Other calanoids essentially followed the same evolution as *Eurytemora affinis* (Fig. 3). Between 1996 and 2006, they were found mainly in freshwater water during winter (*Eudiaptomus gracilis*) and in brackish water during summer (*Acartia tonsa*) in relatively low abundance (Figs. 3 and 4). From 2007 to 2008, an important increase in abundance (Fig. 4; Table 1) and a spreading toward the upstream reach (Fig. 3) were observed. However, they were found in very low numbers in the study area during 2009 (Fig. 4).

Cyclopoids were typically observed in the freshwater zone upstream from 78.5 km (Fig. 3), mainly during late spring and summer. They occurred in varying abundance between 1996 and 2006 (Figs. 3 and 4) but their freshwater abundance decreased significantly from 1996 to 2009 (Fig. 4; Table 1), especially from 2007 onwards. No spatial shift was observed for these taxa (Fig. 3).

As with the cyclopoids, cladocerans also essentially inhabited the freshwater reach, upstream of 98.5 km, mainly during late spring and summer (Fig. 3). Along the study period, their freshwater abundance varied most. However, a marked period of high abundance occurred in the freshwater zone between 2003 and 2005 (Fig. 4), reaching maximum abundance in 2004.

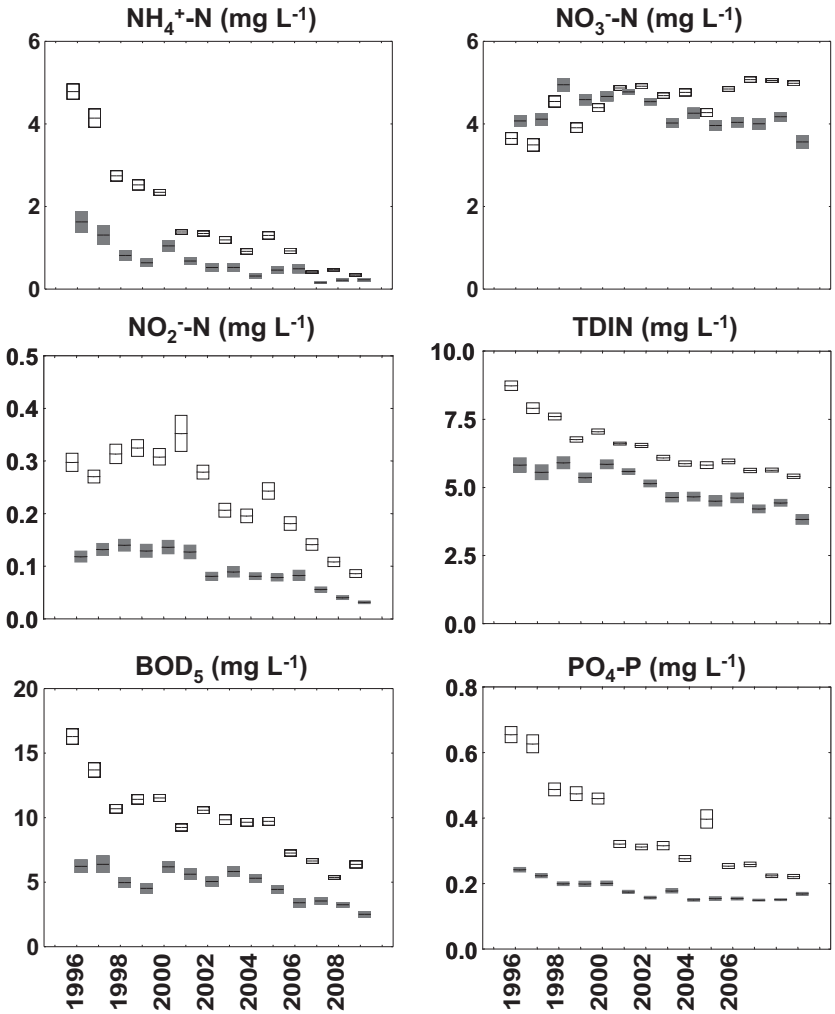


Fig. 2. Evolution of environmental factors in the Scheldt estuary from 1996 to 2009, showing annual mean values (lines) and standard errors (whiskers), in the brackish water part (salinity > 0.5, grey boxes) and in the freshwater part (salinity < 0.5, blank boxes). When present, right Y-axis are for brackish water values.

3.3. Interactions between zooplankton community and water quality

The first RDA, using all environmental factors, explained 27.5% of the total variance in the taxa distribution (Fig. 5). The taxa ordination was first explained by Cl^- (Table 2a) then by factors representing low water quality (NO_2^--N, PO_4-P, NH_4^--N, BOD_5). Other factors appeared to play a minor role. The biplot shows a typical estuarine setting, with *Eurytemora affinis* and other calanoids placed in the left hand side, positively correlated with Cl^- and O_2 concentrations and negatively correlated with factors representing low water quality (see above). NO_2^--N, PO_4-P, NH_4^--N, BOD_5, found in the right-hand side of the biplot, are typical markers for the freshwater area, where water quality is worse than in the brackish part of the estuary and Chl *a* concentrations are higher (Fig. 2). Cyclopoids are placed on this right-hand zone, negatively correlated with Cl^- and positively correlated with Chl *a* concentration. Total cladocerans showed the same correlations than cyclopoids, but genera showed various affinities, with some taxa more correlated with *T* (*Illyocryptus*, *Macrothrix*), than others. Cladocerans were also associated with temperature, typically at maximum values during summer months. Harpacticoid adults seem correlated with Cl^-. As they were observed in low numbers, essentially in the brackish water part, we have chosen not to show results of an RDA based on this taxa (11.4% of the variance explained). Rare cladocerans genera and harpacticoid copepodits are located in the centre of the diagram, with no particular affinities with environmental variables.

Two additional RDAs have been specifically performed for brackish water and freshwater groups (Table 2b, c). Their graphic representations are not shown as they gave essentially the same associations between taxa and environmental factors. The first one (Table 2b) explained 27.1 percent of the total variance of all calanoid adults and copepodits. Their distribution is almost exclusively negatively correlated to factors associated with water quality (Fig. 5, Table 2b). Other factors, including Cl^-, do not appear to explain their distribution (see conditional effects). The second analysis (Table 2c) explained 35.5% of the variance of cladocerans, cyclopoid adults and copepodits. The distribution of these freshwater species was mainly explained by Cl^- then by Chl *a* (Table 2c) with a positive correlation with Chl *a* (Fig. 5).

As can be seen from Figs. 2 and 5, during the study period, the abundance of cladocerans in freshwater followed the same pattern as chlorophyll *a* concentration. Fig. 6 confirms an increase in mean annual cladoceran abundance with mean annual chlorophyll *a* concentration (spearman rank test $p < 0.001$). This increase was particularly strong from 100 μg Chl *a* l^{-1} onwards.

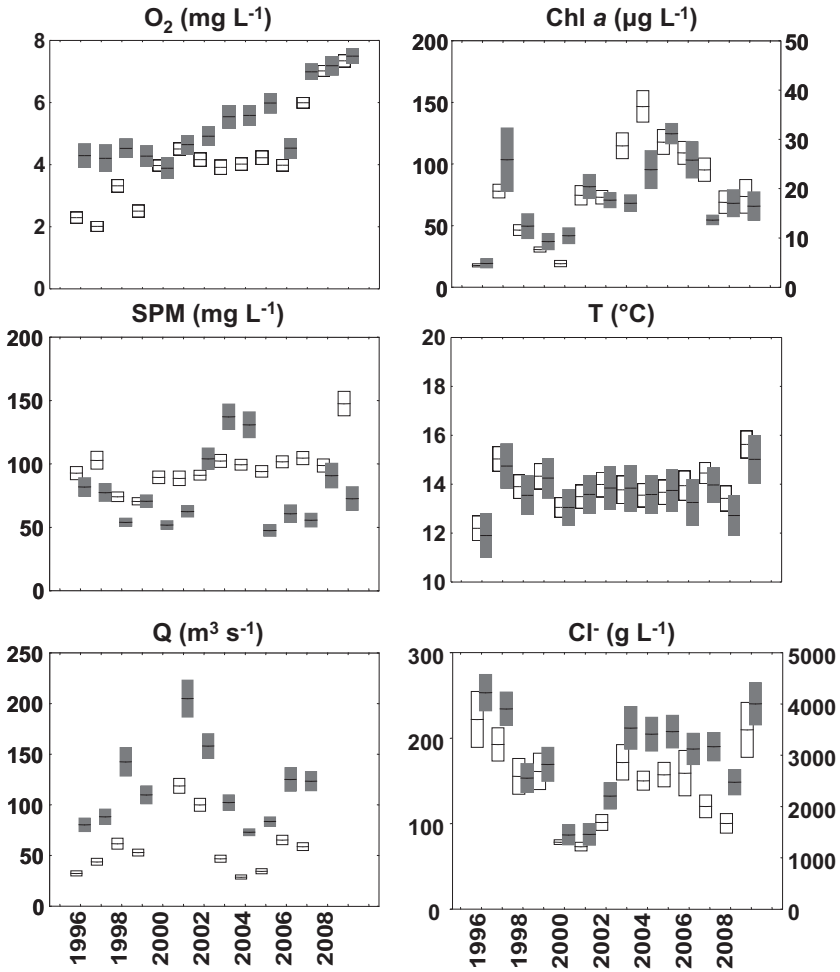


Fig. 2. (*continued*).

4. Discussion

4.1. Changes in water quality during the studied period

Since the mid-nineties, the water quality of the Scheldt estuary had shown very important changes. Major tendencies observed in this study confirm those previously observed (Van Damme et al., 1995; Cox et al., 2009). BOD_5, NH_4^+-N, NO_2^--N and PO_4-P concentrations continue to decrease particularly in the freshwater reach (Fig. 2), where water quality has always been worse than in the brackish part (Van Damme et al., 1995). O_2 increased regularly in both the brackish and in the freshwater reaches. The slight increase of NO_3^--N in the freshwater part can be explained by the fact that since the second half of the seventies, the zone with high nitrification rates moved upstream due to the increase of oxygen concentrations and caused a substantial impact on the N-load (Soetaert and Herman, 1995).

Cox et al. (2009), using data collected between 1967 and 2008, report a concomitant nutrient concentration decrease and Chl *a* increase for the freshwater 118–155 km stretch and explain this evolution as a switch between two steady states. From a high nutrient — hypereutrophic state — which inhibited phytoplankton primary production — , the Scheldt has recently evolved to a lower nutrient — high phytoplankton production — eutrophic equilibrium, because NH_4^+ concentrations have fell below the primary production inhibiting level. Our study generally confirms this tendency, considering annual mean values between 1996 and 2009 over the entire 57.5–155 stretch. Apart from runoff, Cl^-, SPM and Chl *a* concentrations, all environmental variables considered followed a gradual, unidirectional change during the entire study period, either from a certain year onwards in both in the fresh and brackish water stretch. The pattern observed for Cl^- may be

Table 1
Resulting *R* values of a Spearman rank test for tendencies with time of mean annual values of environmental factors and of abundance of zooplankton groups The variables considered are presented in Figs. 2 and 4 for both zones (*: $p < 0.05$; ***: $p < 0.001$; ns: non significant).

Variable	Freshwater	Brackish water
NH_4-N	−0.97***	−0.94***
NO_3	0.79***	−0.58*
NO_2-N	−0.83***	−0.87***
TDIN	−0.98***	−0.91***
PO_4	−0.95***	−0.84***
BOD_5	−0.93***	−0.82***
Chl *a*	ns	ns
O_2	0.87***	0.87***
SPM	0.57*	ns
CL	ns	ns
Q	ns	ns
TEMP	ns	ns
E. affinis	0.86***	ns
Calanoids except *E. affinis*	ns	ns
Cyclopoids	−0.63*	ns
Cladocerans	ns	ns

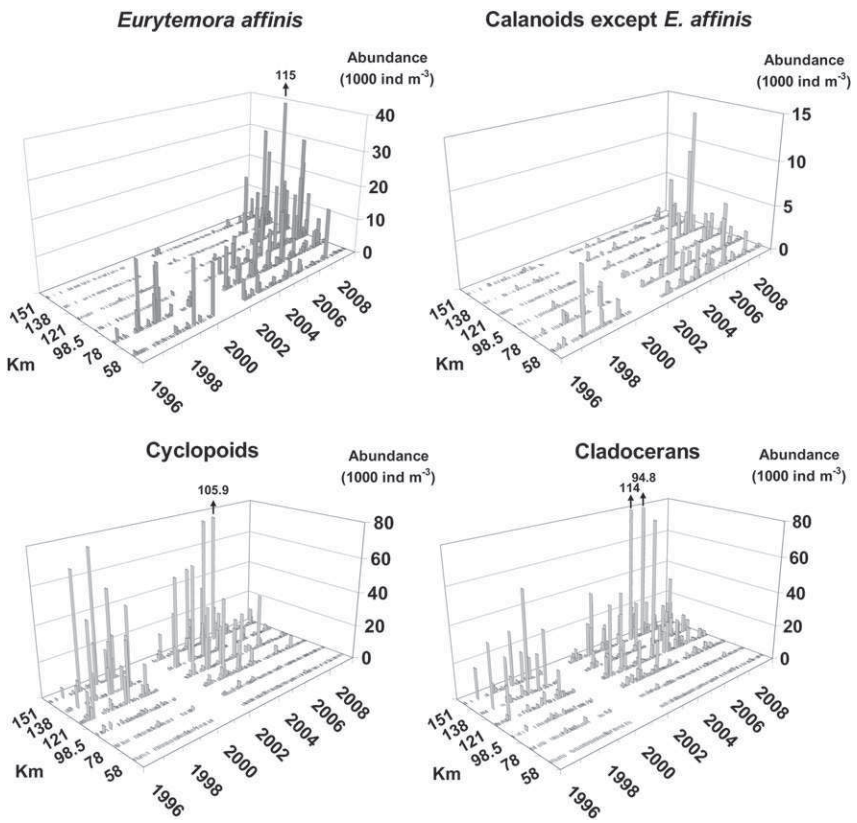


Fig. 3. Evolution of spatial and temporal distribution of abundance (monthly values for each station) of planktonic crustaceans in the Scheldt estuary, from 1996 to 2009: *Eurytemora affinis* adults, other calanoid adults, cyclopoid adults and cladoceran adults.

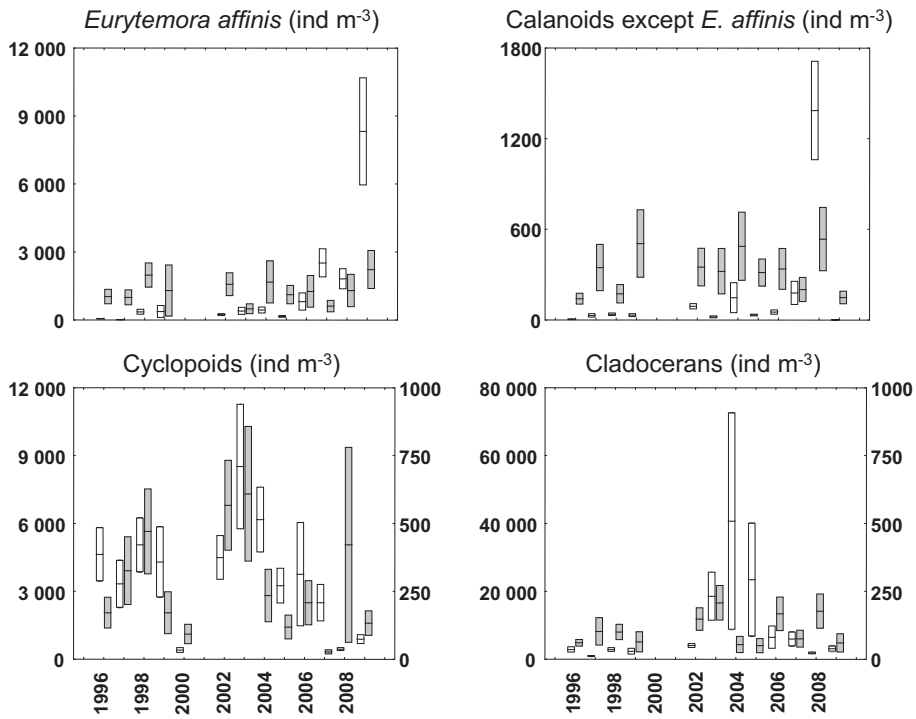
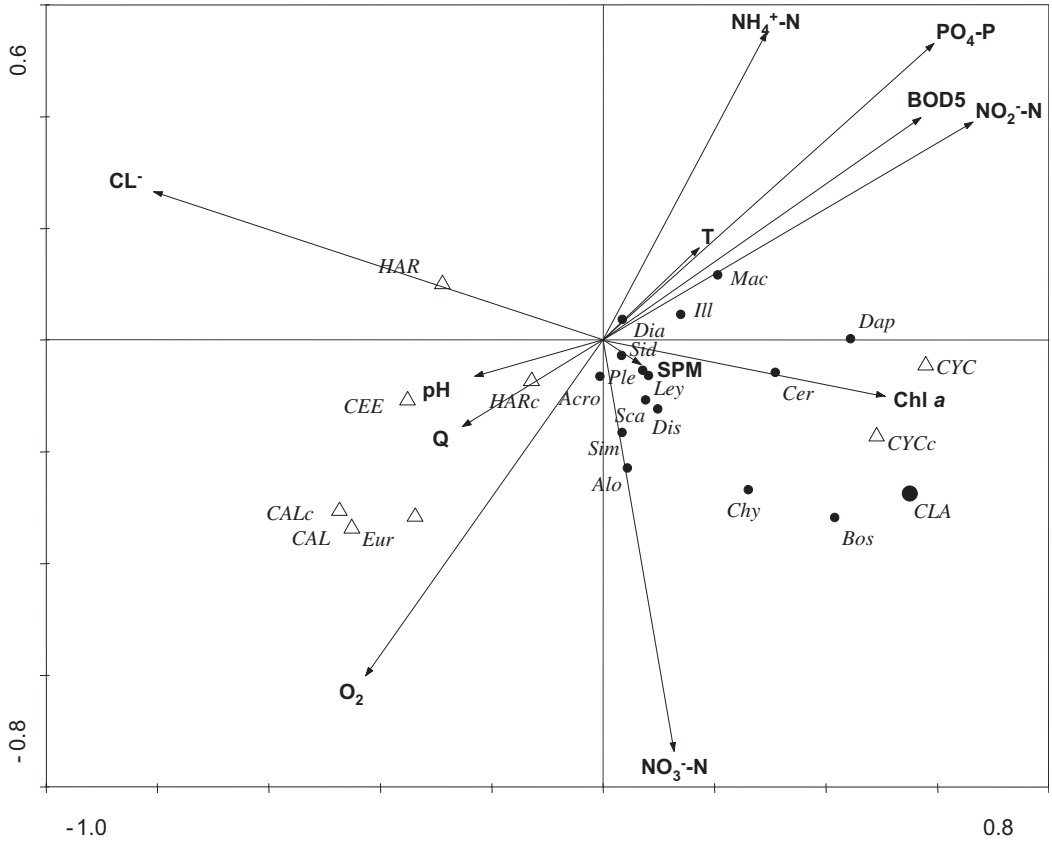


Fig. 4. Evolution of the abundance of planktonic crustaceans in the Scheldt estuary, showing annual mean values (lines) and standard errors (whiskers), in the brackish water part (salinity > 0.5, grey boxes) and in the freshwater part (salinity < 0.5, blank boxes). When present, right Y-axis are for brackish water values.



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

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

Axes	1	2	3	4
Eigenvalues	0.189	0.059	0.012	0.005

Sum of all canonical eigenvalues: 0.275

Fig. 5. RDA biplots axis 1 & 2, showing the distribution of planktonic crustaceans (copepods: blank triangles; cladocerans: black circles) in the Scheldt estuary, as a function of the environmental factors. See text for the abbreviations of the environmental factors. Taxa CAL and CLA, were added as supplementary data after the analysis, as they already include several taxa.

explained by variations in runoff, as mean annual values are significantly correlated with mean annual Q values (Spearman rank, $p = 0.02$). As there is no trend in runoff during the study period, there is no obvious explanation for the increase in SPM concentration in the freshwater zone. Contrarily to our observations, Cox et al. (2009) observe a quasi continuous increase in annual mean Chl *a* concentration in the freshwater 118–155 km stretch between 2001 and 2008. As our study area is considerably more extended (57.5–155 km), this illustrates the interest of considering detailed spatial trends when monitoring restoration results. This consideration is however beyond the scope of this paper.

4.2. Changes in zooplankton distribution during the 1996–2009 period and potential effect of water quality improvement

The spatio-temporal distribution of the zooplankton in the Scheldt estuary has been relatively little studied prior to the OMES project. A first inventory of the spatio-temporal distribution of both phytoplankton and zooplankton over its entire marine–freshwater transect (0–155 km) for 1967–1969 was given by De Pauw (1975, unpublished PhD thesis). Published studies concern only the copepod *Eurytemora affinis* (De Pauw, 1973) or the zooplankton community in the brackish water part during short periods (Bakker and De Pauw, 1975; Bakker et al., 1977). Soetaert and Van Rijswijk

Table 2
Variance in the zooplankton abundance explained by the environmental factors in different RDAs.

Marginal effects		Conditional effects	
Variable	Lambda1	Variable	LambdaA
a) RDA with all taxa			
Cl^-	0.13	Cl^-	0.13
NO_2^--N	0.09	PO_4-P	0.05
PO_4-P	0.08	NO_2^--N	0.03
BOD_5	0.07	NH_4^+-N	0.02
O_2	0.06	O_2	0.01
Chl *a*	0.05	Chl *a*	0.01
NH_4^+-N	0.04	pH	0.01
NO_3^--N	0.04	NO_3^--N	0.01
Q	0.02	SPM	0.01
T	0.02	*T*	0
pH	0.01	BOD_5	0
SPM	0	*Q*	0
Sum of all canonical eigenvalues: 0.275			
b) RDA with only taxa EUR, CEE, CALc			
PO_4-P	0.15	PO_4-P	0.15
NO_2^--N	0.14	O_2	0.04
BOD_5	0.12	Cl^-	0.03
O2	0.11	NO_2^--N	0.02
NH_4^+-N	0.09	*T*	0.01
Cl^-	0.09	Chl *a*	0.01
Chl *a*	0.03	*Q*	0.01
pH	0.02	BOD_5	0
NO_3^--N	0.02	pH	0
Q	0.02	NO_3^--N	0
T	0.01	NH_4^+-N	0
SPM	0	SPM	0
Sum of all canonical eigenvalues: 0.271			
c) RDA with only taxa CLA, CYC, CYCc			
Cl^-	0.22	Cl^-	0.22
Chl *a*	0.1	Chl *a*	0.05
NO_2^--N	0.09	NH_4^+-N	0.02
BOD_5	0.06	PO_4-P	0.02
PO_4-P	0.05	NO_2^--N	0.01
NO_3^--N	0.04	O_2	0.01
O_2	0.03	NO_3^--N	0.01
Q	0.02	*T*	0.01
T	0.02	*Q*	0
NH_4^+-N	0.01	SPM	0
SPM	0.01	pH	0
pH	0	BOD_5	0
Sum of all canonical eigenvalues: 0.355			

(1993) sampled zooplankton from 0 to 78.5 km over two annual cycles (1989–1991). Since 1996, the OMES monitoring is the first consistent, long term study on zooplankton in the Scheldt estuary over the brackish–freshwater transect (57.5–155 km). The use of a 14 year monitoring database allows us to analyse the association of the earlier mentioned improvement of water quality to the evolution of the zooplankton community. Even with a Bonferroni correction, all the environmental factors considered contributed significantly to explain the distribution of crustaceans in the Scheldt. The data set shows the colinearity of environmental factors, typical for an estuarine gradient situation. This is well shown by differences between marginal and conditional effects (Table 2): after taking into account the variance explained by the first factors (Cl^- for Table 2a and c; PO_4-P for Table 2b), the variance explained by following factors is considerably reduced in the conditional effect.

In the present study, after the salinity, environmental factors associated with water quality (NO_2^--N, PO_4-P, NH_4^+-N, BOD_5 and O_2) are the dominant factors which explain the ordination of crustacean taxa (Table 2a). Contrary to the strong seasonal

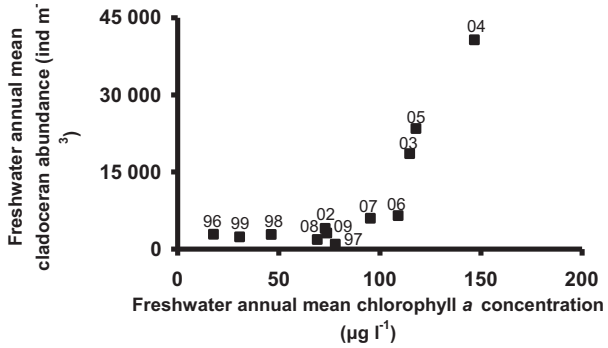


Fig. 6. Relation between annual mean cladoceran abundance and chlorophyll *a* concentration in the freshwater part of the Scheldt estuary.

influence reported by Soetaert and Van Rijswijk (1993) and Tackx et al. (2004) within annual cycles, in this long term study, factors such as *T* and Chl *a*, as seasonal and food availability proxies, appear less important than water quality (Table 2a). No obvious strong influence of the water quality on the taxa distribution was mentioned in above-cited studies. However, these studies occurred before the major physico-chemical changes which occurred in the estuary (Van Damme et al., 2005). Therefore, in the long term, the influence of water quality improvement dominates over seasonal changes in environmental conditions in determining environmental conditions for zooplankton in the Scheldt estuary.

In considering the RDAs on calanoids, cyclopoids and cladocerans separately (Table 2b, c), the marginal effect of the environmental factors showed that the distribution of calanoids is most strongly affected by water quality. The distribution of cyclopoids and cladocerans is conversely mainly explained by Chl *a* in addition to Cl^- and not by factors associated with water quality. Their highest abundances were found in the same years (2003–2005) as the strongest phytoplankton blooms (Figs. 2 and 4). The fact that cladoceran mean annual abundance increases with Chl *a* concentration, especially from 100 $\mu g\,l^{-1}$ onwards (Fig. 6), suggests a potential threshold effect of the phytoplankton availability on the cladoceran abundance. In Chilean lakes, De los Rios and Soto (2007) have also shown a strong response of daphnid abundance to eutrophication.

The strong reduction of cyclopoid abundance in the brackish water is probably due to their decrease in freshwater reaches, which limited their expansion in downstream direction. Indeed, as found during 1996 (Tackx et al., 2004), cyclopoids copepods found in the brackish zone almost always belonged to the dominant freshwater species *Cyclops vicinus vicinus* and *Acanthocyclops trajani* (not shown).

Among the copepods, *Eurytemora affinis* showed the greatest displacement. This calanoid is known to be typical of the brackish–freshwater transition zone in European estuaries (Sautour and Castel, 1995). In the Scheldt, previous studies have noticed the absence of *E. affinis* from the freshwater–brackish water fringe and upstream reaches during the sixties (De Pauw, 1973) and the late eighties (Soetaert and Van Rijswijk, 1993). This was explained by the very bad water quality in this salinity range, situated around the highly polluted zone of Antwerp (78.5 km, Fig. 1). The first observations of the copepod in the Antwerp area occurred in the mid-1990s and this population shift was attributed to the improving oxygen concentrations (Appeltans et al., 2003). Since 1996 (Fig. 3) *E. affinis* has been regularly abundant around Antwerp and as such well settled in the same salinity range as in other European temperate estuaries (Sautour and Castel, 1995). Between 2001 and 2006, *E. affinis* was sporadically observed in the upstream freshwater part of the estuary (Fig. 3). From 2007 onwards, these

occasional occurrences have switched to a consistent high abundance of *E. affinis* in the freshwater area. *E. affinis* now reaches very high abundance with maxima (155 000 ind m^{-3}) comparable with those of freshwater stretch of the Seine estuary (Mouny and Dauvin, 2002). In the Gironde and in the Ems, maximal freshwater abundances are <20 000 ind m^{-3} (Sautour and Castel, 1995). Other calanoids, although much less abundant, have also increased substantially in the freshwater reach in 2007–2009. Since its water quality has improved, the freshwater part of the Scheldt estuary seems to have become a suitable area for calanoids. However, in the last 3 years, the increase in calanoid abundance in the freshwater area has been extremely large and we question whether this is this a sporadic boom or a real trend. Mialet et al. (2010), using Scheldt spring data on *E. affinis* between 1996 and 2007, showed that *E. affinis* can occur in the upstream freshwater stretch (salinity < 0.5) when mean upstream O_2 concentration is >4 mg l^{-1} and the minimum O_2 concentration, which is always observed around the middle of the estuary, is >1.3 mg l^{-1}. A possible explanation for the abrupt invasion of the freshwater reach by *E. affinis* (and other calanoids) since 2007 could be that environmental conditions are steadily above the limit level. Indeed, verifying the 2007–2009 spring data shows that conditions exceed this over the entire study zone for *E. affinis* with an upstream expansion during 91% of the monthly sampling campaigns, while during 1996–2006, this was only the case in 56% of the campaigns. It remains to be investigated if this recent apparent acceleration in the water quality in the Scheldt estuary is related to the gradual commissioning of the purification plant built in Brussels around 2007–2008, reducing organic loads reaching the Scheldt through the Rupel tributary (Fig. 1) (Van Damme et al., 2005) or to the regime shift from a hypertropic to a autrophic system as suggested by Cox et al. (2009).

Since 2007, *Eurytemora affinis* was also observed during summer in the freshwater zone. This could be explained by the fact that salinity is most stressful for *E. affinis* at high temperatures (i.e. 25 °C), as shown Kimmel and Bradley (2001). Bradley (1975, 1991) observed the same phenomenon in the Chesapeake Bay, as *E. affinis* migrates to lower salinities as temperatures increases. *E. affinis* is a euryhaline species. Lee showed that in the past century, *E. affinis* brackish populations invaded freshwater reaches, in European systems as well as in North American and in Asian ones, in a few generations (Lee, 1999; Lee et al., 2003). It is likely that *E. affinis* prefers low salinity habitats because they reduce its energy investment in osmoregulation. In addition to salinity, environmental conditions in the freshwater reach may generally be better suited to increase calanoid than cyclopoid population dynamics. This seems unlikely, as in estuarine systems, calanoids are generally associated with brackish water and cyclopoids with freshwater regions but then zooplankton in estuaries have mainly been studied after the water quality was already degraded. Hence there is the need for further research on restoration processes in estuaries.

Tackx et al. (2003) have shown that *Eurytemora affinis* is capable of selecting phytoplankton among the detritus-dominated suspended matter in the Scheldt estuary, and can satisfy its nutritional demands solely by its phytoplankton ingestion. It is thus unlikely that the higher phytoplankton concentration in the freshwater stretch than in the brackish water (Fig. 2) has encouraged the move by *E. affinis* to the freshwater, or that *E. affinis* really profited from better feeding conditions in this area. Moreover, chlorophyll *a* concentration in the freshwater zone has decreased since 2004 (Fig. 2). Considering the above, the improving water quality (strong reduction of NH_4^+-N, and BOD_5, increase in O_2 concentration) seems the most likely cause of the shift of *E. affinis* from the brackish water to the upstream freshwater Scheldt. Nevertheless, it is not yet possible to categorically be definite about an establishment of an *E. affinis* population in the freshwater zone.

4.3. Potential effect of biotic interactions on zooplankton community

The upstream shift of calanoids is concomitant with a quasi-complete disappearance of cyclopoids from this area. This suggest that cyclopoids, which appear to be less sensitive to poor water quality (Table 2c), were able to live in the upstream freshwater area as long as calanoids were absent. Once water quality in the freshwater stretch became permissive for calanoids, they rapidly became dominant, even during summer, which created an overlap with the cyclopoid spatio-temporal distribution. Hence it is possible to advance several possible explanations for this substantial change in spatial composition of the Scheldt zooplankton community although we are unaware of any literature comparison between calanoids, cyclopoids or cladocerans concerning their tolerance to salinity, pollutant or oxygen ranges.

As it is also difficult to imagine that improving water quality has a negative effect on cyclopoids, calanoids may have reduced cyclopoid abundance by either competition or predation. Using long term (8 months) enclosure experiments with lake calanoids and cyclopoids, Soto and Hurlbert (1991) showed that calanoids could strongly depress cyclopoid populations. These authors explain this effect by a reduction of phytoplankton or rotifer food for cyclopoid nauplii or by calanoid predation on cyclopoid nauplii. Also, Dodson (1975) has shown that in freshwater ponds, calanoids and cyclopoids can feed on each other's nauplii. However, as the key copepod species of the present study were not the same, extending these conclusions to the Scheldt estuary is difficult. In a more general way, we further refrain from using literature on zooplankton response to restoration available from freshwater (mainly lentic) systems to explain the trends observed in the Scheldt, as zooplankton dynamics in these systems are governed mainly by phenomena such as stratification and clear water–turbid water states, which are not acting in the well-mixed Scheldt estuary (Baeyens et al., 1998).

Using stable isotope signatures to study the lower trophic web in the York river, (Virginia, USA) Hoffman et al. (2008) have shown that *Eurytemora affinis*, the dominant cladoceran *Bosmina freyi* and cyclopoids have similar signatures, and can switch from autochthonous produced phytoplankton to allochthonous matter. In the Scheldt estuary however, *E. affinis* occurs mainly in spring, while *B. freyi* and cyclopoids occur later in the year. Considering the above mentioned capacity for phytoplankton selection of *E. affinis,* it is possible that this dominant calanoid performs better in selecting high quality food than cyclopoids. Moreover, algae species composition during spring and summer in the Scheldt estuary seems to have changed from 1996 (Van Burm, unpublished data). During spring 2007 and 2008, large diatoms (*Actinocyclus normanii*) dominated the community whereas during spring 1993, small species (*Cyclotella meneghiniana*, *Skeletonema costatum*) dominated (Muylaert and Sabbe, 1999). This shift in phytoplankton size composition could also influence the feeding efficiency and development of the various mesozooplankton species, but also of rotifers, which are numerically dominant in the entire estuary (Tackx et al., 2004) and form another potential high quality prey to the mesozooplankton. Indeed, the capacity of both freshwater and marine copepods to feed on rotifers is well known (Williamson, 1987; Conde-Porcuna and Declerck, 1998; Ciros-Perez et al., 2004). Lapesa et al. (2004) report that the saltwater species calanoid *Arctodiaptomus salinus* feeds more efficiently on rotifers that the co-occurring cyclopoid *Diacyclops bicuspidatus*. As for algae, it is possible that *E. affinis* feeds better on rotifers than cyclopoids. Azémar et al. (2010) showed that during 2002, rotifers reached their peak abundance in spring and autumn (up to 2 500 000 ind m^{-3} in the freshwater zone), thus alternating with cladocerans and cyclopoids population peaks. This alternation could reflect a predation of

cyclopoids and/or cladocerans on rotifers. Indeed, the two dominant cyclopoids species in the Scheldt estuary, *Cyclops vicinus vicinus* and *Acanthocyclops trajani*, are able to develop to the adult stage on a pure algal diet (Hansen and Santer, 1995; Hopp and Maier, 2005), but feed essentially on rotifers as adults (Brandl and Fernando, 1978; Brandl, 2005). Rotifer abundance data for the Scheldt are not yet available for the complete period studied so it is difficult to evaluate their potential role in the recent evolution of the zooplankton community in this estuary. Nevertheless, first observations indicate that rotifer mean abundances were particularly low in 2008 (about 4 times less than in 2004 and 2 times less than in 2002). This raises the hypothesis of a recent rotifers predation following *E. affinis*' shift, which could have hampered the development of cyclopoid populations. Also, there are no data available on ciliates. As they can contribute to a relatively important part of the copepod diet in estuarine systems (Calbet and Saiz, 2005), they could explain recent changes in mesozooplankton distribution.

In addition, the top down control on the mesozooplankton could influence the dominance among taxa, for example by selective predation (De Mott, 1995; Lu and Xie, 2001). During the early 1990s, when *Eurytemora affinis* was still very abundant in the brackish water part of the Scheldt, the diet of the mysid *Neomysis integer* in this zone consisted practically solely of *E. affinis* (Fockedey and Mees, 1999). In addition, in the brackish water zone of the Scheldt (78.5 km and downstream), *E. affinis* and various hyperbenthic species form an important food resource for the diet of juveniles of dominant fish species such as sprat and herring (Maes et al., 2005). While water quality improvement has resulted in higher abundance of zooplankton predators in the freshwater area (Verslycke et al., 2004). Within the inventory of fish species reported in the Scheldt estuary (Breine et al., 2010), an increase of species richness and abundance has been observed in the freshwater zone since 2007 (Breine, unpublished PhD thesis data). This could have increased predation pressure on crustaceans, but there is no obvious reason why these predators would feed selectively on cyclopoids rather than on other copepods.

Contrarily to cyclopoids, cladocerans showed no decrease since 2007 and thus are apparently less hampered by biological interactions occurring in the freshwater zone than cyclopoids. While capable of selective feeding (Wong et al., 2006), cladocerans are generally considered as filter feeders which are less selective than copepods (De Mott, 1988). Because of this feeding mode, they may not compete with calanoids and cyclopoids. This can also explain why their abundance followed the same trend as Chl *a* concentration (Figs. 2, 4 and 6).

The fact that changes in some other parameters, not measured by OMES, could have led to the observed changes in the zooplankton community cannot be excluded: in addition to pollutant loads, potential changes in the benthos could interact with the pelagic community. In a long term study over zooplankton community of a German lagoon, Feike et al. (2007) observed important and rapid changes in calanoid (*Eurytemora affinis* and *Acartia tonsa*) and rotifer abundance just after the introduction of an invasive polychaete species. These authors suggest that this benthic species caused the depletion of rotifer resting eggs by grazing or bioturbation. Hence in the upper Scheldt estuary, improving water quality could have affected the benthic community and indirectly changed living conditions for the plankton.

In conclusion, it is apparent that the improving water quality in the Scheldt estuary, and mainly in the freshwater stretch, is paralleled with major shifts in the spatial distribution of the zooplankton taxa: an increase in calanoid copepod and cladoceran abundance, while cyclopoids are strongly decreasing. These changes in the zooplankton community structure raise a number of fundamental questions on tolerance for environmental conditions and trophic competition between the taxa calanoida, cyclopoida and cladocera. Unfortunately, little information on water quality tolerance or biotic interactions among freshwater estuarine crustacean plankton taxa is available. Answering these questions will require mesocosm experiments using estuarine taxa, combined with in situ studies on trophic interactions (stable isotopes, fatty acids, observation of gut contents). Finally, the recent increase in dissolved oxygen concentration in the freshwater part may lead to more intense phytoplanktonic blooms (Cox et al., 2009). The leads to the need to test the hypothesis that, given changed distribution patterns, zooplankton can regulate phytoplankton production.

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