



## Zooplankton community changes in a restoring estuary

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### ABSTRACT

This study describes crustacean (copepods and cladocerans) and rotifer abundance, diversity and community composition in the Scheldt estuary, during 1996–2022. Besides the ongoing environmental change, increased wastewater treatment, followed by the alteration of the main Scheldt channel, have characterized this period. A combination of clustering, correlative and decision tree analysis was used to investigate relationships between zooplankton and the major environmental parameter changes. Since 1996, both crustacean and rotifer communities showed a clear chlorinity-induced separation between the brackish (LSS) and the freshwater (USS) estuary. The effects of water de-eutrophication are visible in both crustacean and rotifer communities, particularly in the freshwater reach. Recently, marks of marinization and intensification of dredging activities, partly linked to changes in phytoplankton are detected on the zooplankton.

### 1. Introduction

Situated at the final reach of drainage basins, estuaries are particularly subjected to combinations of natural and anthropogenic factors, and influenced by what happens in their drainage basins. While still subjected to several anthropogenic impacts, many estuaries worldwide have profited from water quality and biologic communities restoration efforts in the last decades. Long term monitoring results, providing the means to evaluate the ecosystem changes following management decisions are becoming available for several estuaries (Borja et al., 2010; Gee et al., 2010; Ruhl and Rybicki, 2010; Zhang et al., 2021). Biodiversity restoration is an inherent part of these programs, either directly or indirectly, as ecosystem quality and biodiversity are intrinsically related (Beheshti et al., 2021; Xu et al., 2021).

This paper focuses on the estuarine reach of the freshwater and partly the brackish Scheldt estuary, situated in Belgium. The Scheldt estuary is subdivided in the Upper Sea Scheldt (USS) and the lower Sea Scheldt (LSS), which roughly correspond to the tidal fresh and brackish water reach on Belgian territory respectively (Fig. 1).

Besides inorganic and organic pollution arriving from its 21863 km<sup>2</sup> catchment (Brion et al., 2015; Meire et al., 2005; Van Damme et al., 2005), the Scheldt has been impacted by several human actions among

which historic rectifications of its course, loss of intertidal areas and deepening of the main channel to facilitate accessibility to the Antwerp port. The latest deepening took place during 2008–2011 and, associated to regular maintenance dredging, assures a fairway depth of 14.5 m from Vlissingen to Antwerp (Meire et al., 2015).

Since 2005 the SIGMA plan (<https://www.sigmaplan.be/en>) changed its focus from safety to a combination of flood protection with restoration of the natural potential of the Scheldt estuarine system (Van Damme et al., 2005). Measures aim to reduce nutrient and organic waste loads on the drainage basin (Brion et al., 2006) as well as reflooding of wetlands and construction of Controlled Reduced Tidal systems (CRTs) (Cox et al., 2006; Maris et al., 2007; Beauchard et al., 2011). The OMES program (<https://www.omes-monitoring.be/en>) monitors the Scheldt estuary on the Belgian territory in order to evaluate the results of the SIGMA plan.

After a few years of slow improvement of water quality, major changes (i.e. decrease in nutrient concentrations, increase in O<sub>2</sub> concentration) were detected from 2003 onwards in the USS and since 2007 in the LSS (Cox et al., 2009; Mialet, 2010; Chambord et al., 2016). From 2008 onwards, significant spatial changes in the major turbidity zones (MTZ) and marine penetration in the USS took place in parallel with climate fluctuations and intensified dredging activities (Cox et al., 2019;

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De Neve et al., 2020; Amadei Martínez et al., 2023).

This article focuses on the evolution of zooplankton communities, which occupy a central position in the estuarine pelagic food web (Maes et al., 2005), in parallel to the evolution of the Scheldt environment.

The general tendencies observed in the zooplankton community up till 2014 have been well documented (Appeltans et al., 2003; Tackx et al., 2004, 2005; Azémar et al., 2010; Mialet, 2010; Mialet et al., 2011; Chambord et al., 2016). To resume, the first studies carried out since the 1990s describe a general increase in the abundance of zooplankton (crustaceans and rotifers) in the USS, but little changes in the LSS. Rotifers were numerically dominating the zooplankton in both reaches and present higher abundance and taxonomic richness in the USS than in the LSS. Downstream of Antwerp (Fig. 1), the crustacean community was, before 2007, dominated by the calanoid copepod *Eurytemora affinis*. The USS was populated by cyclopoid copepod and cladoceran communities. Since 2007, *E. affinis* started developing in the USS, where it became dominant. Cyclopoid copepods in this reach decreased in abundance, while cladocerans stayed more or less constant in abundance.

The aims of this article are to discern more precisely the response of crustaceans and rotifers by studying species assemblages along the Scheldt estuary, and to update previous studies to 2022. It is assumed that improved water quality may have a major impact on the assemblages at species level, and that recent changes may be related to salinity and MTZ transitions. No assumptions were made about the spatial distribution of communities between LSS and USS due to possible spatial migration of species with marinization. Already several other communities, such as phytoplankton (Amadei Martínez et al., 2023), benthos (Beauchard et al., 2013a; De Neve et al., 2020), fish (Breine et al., 2021), macro-flora (Jacobs et al., 2009) and avifauna (Beauchard et al., 2013b)

have undergone changes in and along the Scheldt estuary during last two decades. Changes in zooplankton top-down controls linked to the evolution of these communities are not included in the variables tested.

## 2. Material and methods

### 2.1. Study site

The Scheldt originates in France, in the region of Saint-Quentin (Aisne) and flows through Belgium to discharge in the North Sea at Vlissingen (The Netherlands). It is a typical rain fed lowland-river, 355 km long, its source being at only 100 m altitude, current velocities surpass  $1 \text{ ms}^{-1}$  only during some flood phases (Meire et al., 2005; Van den Bergh et al., 2005; Brion et al., 2015). The tidal action extends to around 160 km upstream, being stopped at the city of Ghent and as such the Scheldt estuary includes a large reach of tidal fresh water (Meire et al., 2005). The average depth at the Mouth in Vlissingen is 25 m, 20 m at the Dutch-Belgian boarder and 15 m at Melle, the end station of the estuarine monitoring. The average tidal range is 3,8m at Vlissingen, 4, 85 m at at the Dutch- Belgian boarder and 1,96 m at Melle (Meire et al., 2005). The Scheldt estuary is characterized by vertically well-mixed water flows (Baeyens et al., 1998; Van den Bergh et al., 2005), generally showing no salinity or current stratification (Heip, 1988).

The OMES monitoring samples the Sea Scheldt and its main tributaries since 1996 at 29 stations between the Dutch-Belgian border (Grens, "G", Fig. 1) and Melle, near the city of Ghent (Maris et al., 2024). To provide an overview of the spatial and temporal evolution of the zooplankton, data from 4 stations along the brackish water-fresh water gradient were selected for this paper: Grens (G), Antwerp (A), Melle (M), and Dendermonde (D).

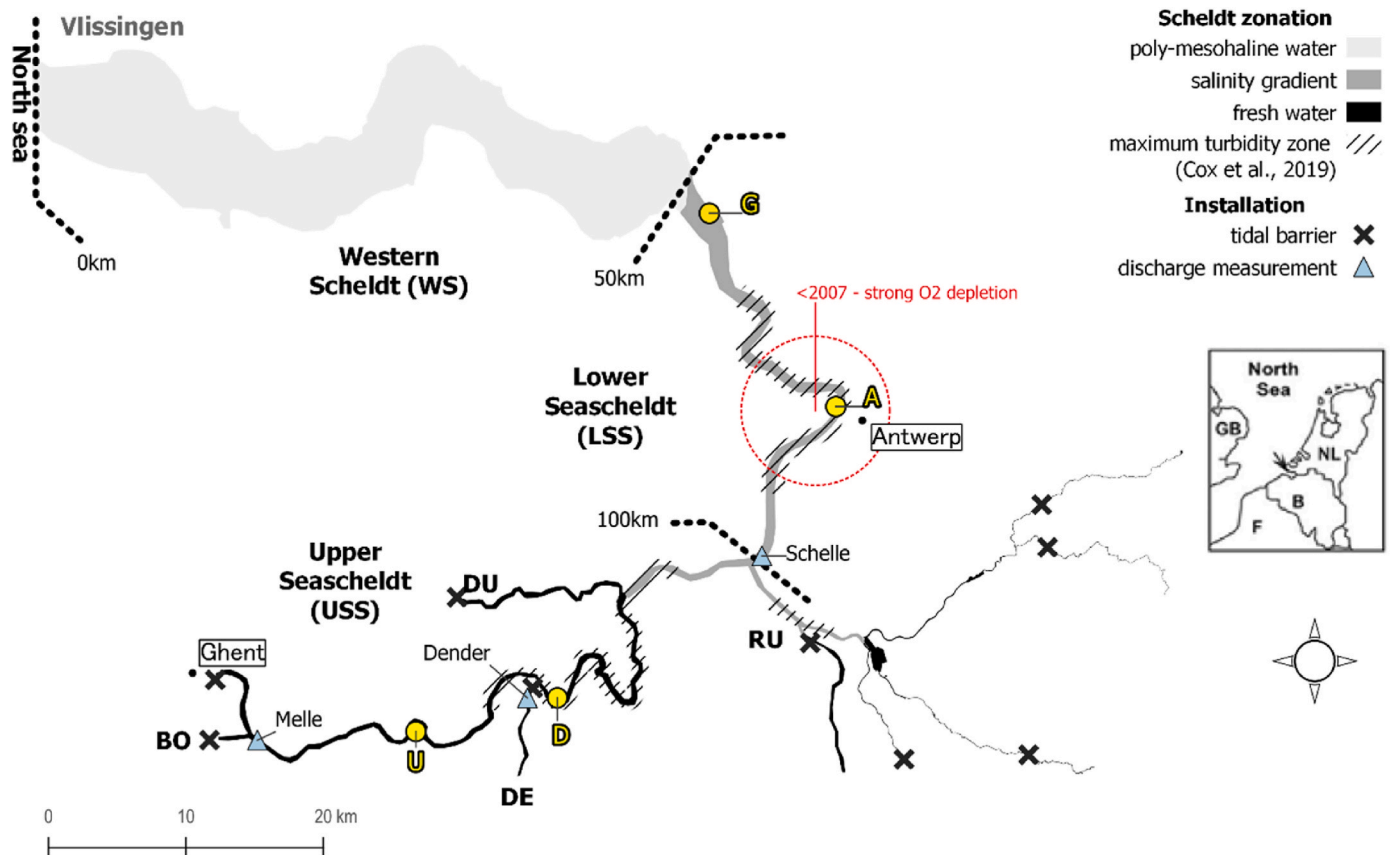


Fig. 1. Localization of the stations studied in the Scheldt estuary (yellow dots, U: Uitbergen, D: Dendermonde, A: Antwerp, G: Grens) with details of the salinity zones borders (grey scale) and the maximum turbidity zones (thin hatchets). Thick broken lines separate the WS from the LSS and the USS. The main tributaries are BO: Bovenschedt, DE: Dender, DU: Durme, RU: Rupel. Inset shows the Scheldt estuary position in Europe. Source: <https://vliz.carto.com/me>. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Dendermonde (D) and Uitbergen (U) (Fig. 1). All are influenced by the tide. The downstream stations G and A, are situated in the strong salinity change reach. At the two other stations, in the USS, water is always fresh with a longer residence time at D than at U.

## 2.2. Sampling and laboratory analysis

For OMES, water quality, phytoplankton and zooplankton communities were monitored. The sampling frequency was monthly or biweekly (March–October between 2009 and 2020). Samplings were tide independent. Water quality parameters were measured from bucket samples taken below the surface and determined using standard methods. Readers are referred to Maris et al. (2024) and Mouth et al. (2024) for the protocols used. Environmental data were downloaded from the Flanders Marine Institute (VLIZ). Discharge data were obtained from the Flanders Hydraulics Research Institute (HIC, <https://waterinfo.vlaanderen.be/>) with measurements at Melle used for U, at Melle + Dender for D, and with calculated discharge at Schelle based on discharges of all Rupel tributaries for A and G (Fig. 1).

For zooplankton sampling, 50 L of below surface water was collected in a bucket and filtered through a 50  $\mu\text{m}$  mesh. Carbonated water was added to the sample to narcotize the zooplankters before conserving them in 4 % formalin. Since 2021, samples were preserved in 70 °C ethanol.

For this article, uneven month samples of the years 1996, 2002, 2009, 2016 and 2022 were analyzed to the lowest possible taxonomic level for both crustaceans (copepods, cladocerans) and rotifers. 1996 is missing for rotifers. For months with two sampling campaigns, only the second one was used. Crustaceans and rotifers were counted, under a stereomicroscope (magnification 12.5X) and a light microscope (magnification 100X) respectively. A minimum of 3 sub-samples and 200 individuals/sample were counted. No zooplankton data are available for December 1996 and January 2002 for all stations, and for January 1996 at station U and July 2002 at station D.

## 2.3. Data analysis

All data analysis was performed using R version 4.1.1 (R Core Team, 2021) completed with the packages “ggplot2” (Wickham, 2016), “vegan” (Oksanen et al., 2022), “FactoMineR” (Husson et al., 2008), “clustsig” (Whitaker and Christman, 2014), “labdsv” (Roberts, 2013), “trend” (Pohlert, 2023) and “rpart” (Therneau and Atkinson, 2022). The alpha threshold was set at 0.05.

To retrace the evolution of the Scheldt pelagic environment, data on discharge, water temperature (TEMP), concentrations of oxygen ( $\text{O}_2$ ), suspended particulate matter (SPM), chloride (Cl), chlorophyll *a* (Chl<sub>a</sub>) and dissolved silica (SOLS) were used. The yearly mean value of each parameter was calculated based on monthly samples or monthly means in the case of two samplings per month. Yearly mean discharge was calculated from continuous measurements (5-min frequency). Missing environmental data were  $\leq 5$  % of total data per parameter and considered NA values in the calculation of the yearly mean.

Each parameter was described during the 1996–2022 study period. The non-parametric Mann-Kendall test was used to detect significant temporal trends (Kendall, 1975; Mann, 1945), with the positive or negative statistic tau indicating the direction of the trend. Pettitt’s non-parametric test was then used to search for significant rupture in the global temporal trend (Pettitt, 1979; Pohlert, 2023). If present, Mann-Kendall’s test was applied independently to detect temporal trends before and after the year of change. Difference between groups of values were tested by a year-paired Wilcoxon test with Bonferroni correction. The ordinal association between each environmental parameter and the discharge was measured with Kendall’s  $\tau$  coefficient.

For each station and each year considered, the mean yearly abundance per zooplankton taxa was calculated. Missing data are considered as NA values. All were summed to give the total annual zooplankton

abundance, using all taxa. Alpha diversity is the number of taxa observed over each full year at a given station. Gamma diversity is the number of taxa considering all 4 stations over each full year. The evenness within the community was calculated on the yearly taxa abundances using Pielou’s index of species evenness (Pielou, 1966). A complete list of taxa observed is given in additional information (table A4 and 5). Trends in data and comparison of group values was done and is reported as for environmental parameters.

Major taxa (with annual relative abundance at least once  $\geq 5$  % of the total abundance) were isolated to increase the statistical power of the following analysis (David, 2017). As such 11 taxa out of 60 for crustaceans and 17 out of 63 for rotifers were used. To quantify the similarity between pairs of communities a Bray-Curtis dissimilarity matrix was applied on log-transformed ( $x+1$ ) abundances of major taxa. The data matrix was projected with an Un-Weighted Pair-Group Method using Arithmetic Averages (UPGMA) clustering dendrogram. A Similarity Profile Analysis (SIM-PROF) determined the number of significant clusters produced (Clarke et al., 2008). Each defined cluster was then considered a typical community. Significant specific indicator taxa for each cluster were detected using the Species Indicator Values (IndVal) (Dufrene and Legendre, 1997).

A multiple factor analysis (MFA) was carried out to correlate the data described previously: environmental parameters, biodiversity values (total abundance, number of taxa and Pielou index) and yearly abundances of the major taxa, without presupposing any causal relationship between the 3 data sets (Escofier and Pagès, 1994). Individuals constituting each typical community are enhanced in the biplots. Discharge was not included in the analysis, because of its strong influence on other parameters such as chlorinity at station scale. Each of the 3 data sets was previously standardized using a z-score by subtracting their mean and dividing by their standard deviation. Crustaceans and rotifers were analyzed separately.

A decision tree was used to test whether environmental factors could segregate community clusters. A continuous regression model (algorithm CART) (Breiman et al., 1984) was built using a two-stage procedure represented by a binary tree (Therneau and Atkinson, 2022). In a first step, a single environmental variable, which best splits the community clusters into two groups, was obtained. Then this process was applied separately to each sub-group, and again. The objective was to find, for each node, the variable and its threshold value that minimizes inertia within the cluster of communities or maximizes inertia between them. The “minsplit” parameter (i.e. the minimum number of observations that must exist in a node for a split to be attempted) was fixed at 2. As some clusters only consist of a single sample, the “minbucket” parameter was set to 1 (i.e. the minimum number of observations in any terminal leaf node). The model with the lowest risk estimate (xerror) was chosen as optimal (David, 2017). It was validated with 2 samples from different clusters (composed of at least 2 samples) which were not included in the first learning: G 1996 and D 2022 for crustaceans, U 2002 and U 2016 for rotifers.

## 3. Results

### 3.1. Environmental data

Since 1995, the Scheldt yearly mean discharge has shown important inter-annual fluctuations, with a minimum average of 72  $\text{m}^3 \text{s}^{-1}$  in 2019 and a maximum of 191  $\text{m}^3 \text{s}^{-1}$  in 2001 at Schelle (Fig. 2). A slightly significant negative linear trend without temporal rupture was detected between 1996 and 2023. Discharges at the 4 stations were significantly positively correlated (Spearman test,  $\rho \geq 0.94$ ,  $p < 0.05$ , data not shown).

No significant temporal trend was detected in the yearly mean water temperature (TEMP) during the entire period studied (1996–2022). Temperatures were not significantly different between the 4 stations and fluctuated around  $13.7 \pm 0.7$  °C across all years and stations (Fig. 3a). No correlation with the yearly discharge was detected for the entire

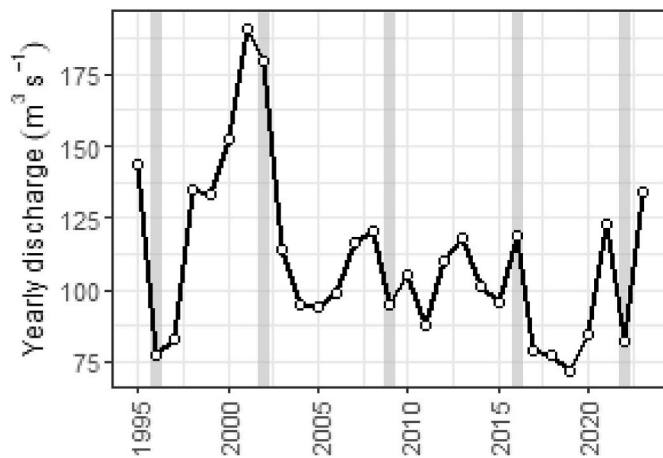


Fig. 2. Yearly discharge at Schelle between 1995 and 2023. The years studied are indicated by the 5 vertical grey stripes.

period studied.

Significant temporal trends of the yearly mean SPM were detected in the LSS, but none in the USS (Fig. 3b.). Temporal ruptures in the trends were observed in 2005 at Grens (G) and in 2011 at Antwerp (A), with stable trends before and after this rupture at both stations. In the downstream station G, the average SPM was significantly higher before 2005 ( $105 \pm 53 \text{ mg L}^{-1}$ ) than after ( $52 \pm 12 \text{ mg L}^{-1}$ ). The trend was inverse at A with significantly lower average SPM before 2011 ( $75 \pm 22 \text{ mg L}^{-1}$ ) than after ( $117 \pm 23 \text{ mg L}^{-1}$ ). This maximal SPM concentration at A was comparable to that at G before 2005. In the USS stations Dendermonde (D) and Uitbergen (U), yearly mean SPM concentrations were stable between 1996 and 2022 and significantly higher at D ( $118 \pm 33 \text{ mg L}^{-1}$ ) than at U ( $89 \pm 18 \text{ mg L}^{-1}$ ). SPM at D and at A, after 2011, were not significantly different. At D only, SPM fluctuated inversely to the discharge during the entire period studied ( $\tau = -0.6$ , not significant for the other stations).

In 1996, yearly mean  $\text{O}_2$  concentrations  $<3 \text{ mg L}^{-1}$  were measured at A, D and U, and  $6.3 \text{ mg L}^{-1}$  at G.  $\text{O}_2$  then increased significantly at all four stations until 2009 (Fig. 3c.). Thereafter,  $\text{O}_2$  concentration continued to rise at A and stabilized elsewhere. The yearly mean  $\text{O}_2$  concentration was  $9.1 \pm 0.4 \text{ mg L}^{-1}$ , at G and U (concentrations not significantly different), and stayed significantly higher than at D ( $8.4 \pm 1 \text{ mg L}^{-1}$ ) and at A ( $7.4 \pm 0.4 \text{ mg L}^{-1}$ ). At G only,  $\text{O}_2$  fluctuated inversely with the discharge during the entire period studied ( $\tau = -0.3$ , not significant for the other stations).

The yearly mean concentrations in Chla were significantly higher in the USS than in the LSS and had different temporal trends between stations (Fig. 3d.). In the LSS, Chla increased significantly at G from  $3.5 \mu\text{g L}^{-1}$  in 1996 to more than  $10 \mu\text{g L}^{-1}$  in 2014 (a maximum of  $50.9 \mu\text{g L}^{-1}$  was measured in 2012), before decreasing to  $7.8 \mu\text{g L}^{-1}$  in 2022. At A, concentrations were stable at around  $25.9 \pm 15 \mu\text{g L}^{-1}$  until 2010, before declining to the same level as at G. At A only, Chla correlated positively with the discharge during the entire period studied ( $\tau = 0.3$ , not significant for the other stations). In the USS, a significant temporal rupture was detected in 2011 at D, with stable periods on either side. Chla was significantly higher before 2011 ( $84.4 \pm 50 \mu\text{g L}^{-1}$ ) than after ( $39.4 \pm 15 \mu\text{g L}^{-1}$ ). At U, Chla concentrations stayed stable during the entire study period, fluctuating around  $85.3 \pm 45.0 \mu\text{g L}^{-1}$ , and were significantly higher than at D.

Dissolved silica concentrations (SOLSIS) remained stable from 1996 to 2015–2017, when significant breaks were detected at all four stations (Fig. 3e). In the first period, the yearly mean SOLSIS was stable at all four stations and significantly lower at the downstream G ( $3.1 \pm 0.5 \text{ mg L}^{-1}$ ) than at the upstream U ( $5.2 \pm 0.6 \text{ mg L}^{-1}$ ). Then SOLSIS increased continuously in the LSS up to  $7.0$  and  $9.3 \text{ mg L}^{-1}$  at G and A respectively in 2022. In the USS the concentrations fluctuated around stable averages

significantly higher after 2017 than before and not significantly different between the 2 stations ( $8.8 \pm 1.5 \text{ mg L}^{-1}$ ). No significant correlation with the yearly discharge was detected during the entire period studied.

Yearly mean chlorinity was significantly higher downstream than upstream and significantly negatively correlated with the discharge at station scale over the entire period studied ( $\tau \leq -0.5$ ) (Fig. 3f and g). At station G,  $\text{Cl}^-$  concentration has risen continuously from 1996 to 2022 within the mesohaline range (around  $6 \text{ g L}^{-1}$ ), except in the 2000s when it approached the oligo-mesohaline limit of  $3 \text{ g L}^{-1}$  during a high discharge period (Fig. 2). At A,  $\text{Cl}^-$  concentrations fluctuated over the entire oligohaline range ( $0.3\text{--}3 \text{ g L}^{-1}$ ) and were significantly higher after the temporal rupture detected in 2011 ( $2.0 \pm 0.7 \text{ g L}^{-1}$ ) than before ( $1.2 \pm 0.4 \text{ g L}^{-1}$ ). The water was always fresh in the USS (chlorinity  $<0.3 \text{ g L}^{-1}$ ) with no significant time trends or breaks. Although chlorinity was significantly higher at D than at U during the entire period studied, it should be noted that this difference was amplified in 2009, 2011 and particularly after 2015 (Fig. 3g).

### 3.2. Global zooplankton abundance, diversity and composition

Yearly mean crustacean total abundance varied mostly between  $1 \times 10^3$  and  $10 \times 10^3 \text{ ind. m}^{-3}$ , with a maximum of  $30 \times 10^3$  and  $19 \times 10^3 \text{ ind. m}^{-3}$  at D in 2009 and U in 2022 respectively (Fig. 4a). Temporal variations in crustacean abundance were not correlated between stations (Table 1).

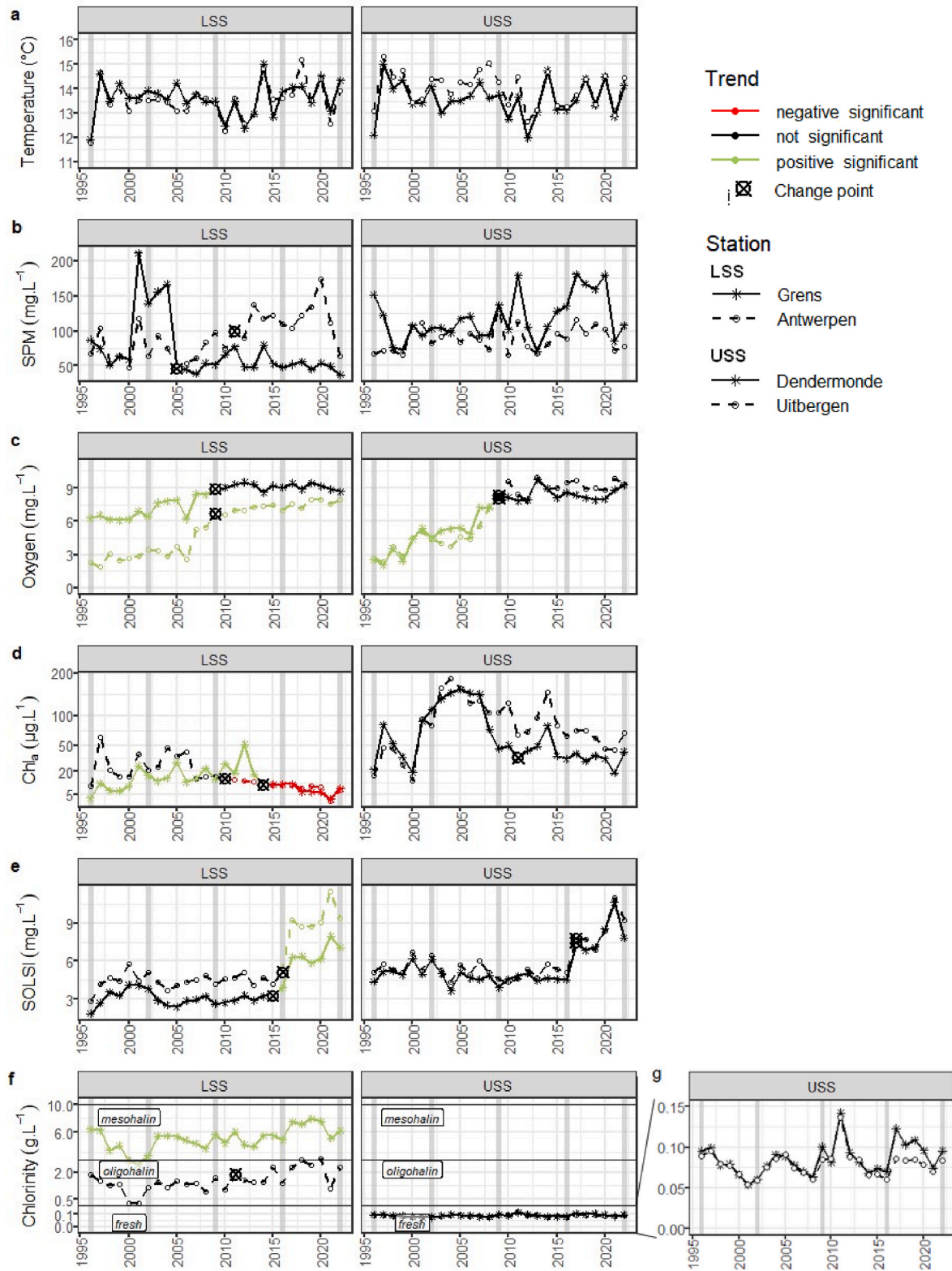
Yearly mean rotifer total abundance varied mostly between  $3 \times 10^3$  and  $150 \times 10^3 \text{ ind. m}^{-3}$ , with a maximum of  $540 \times 10^3$  and  $369 \times 10^3 \text{ ind. m}^{-3}$  observed in 2002 at D and U (Fig. 4b). Particularly in 2022, rotifer abundance was low in the LSS with only 70 and 580 individuals  $\text{m}^{-3}$  at G and A respectively. Except with station D, rotifer abundance fluctuations showed a high rank correlation between stations ( $\rho = 1$ , Table 1), although the p-value is at 0.08.

Both crustacean and rotifer total yearly mean abundance were significantly higher at the USS station D and U as at the LSS stations G and A during the entire period studied. Rotifers composed at least 80 % of all the zooplankton community abundance all along the years studied in the USS (Fig. 5). In the LSS the rotifer abundance fluctuated between 99 and 70 % of total zooplankton abundance. In 2022 crustacean yearly abundances were higher than those of rotifers and composed 83 % of the zooplankton at G and 53 % at A.

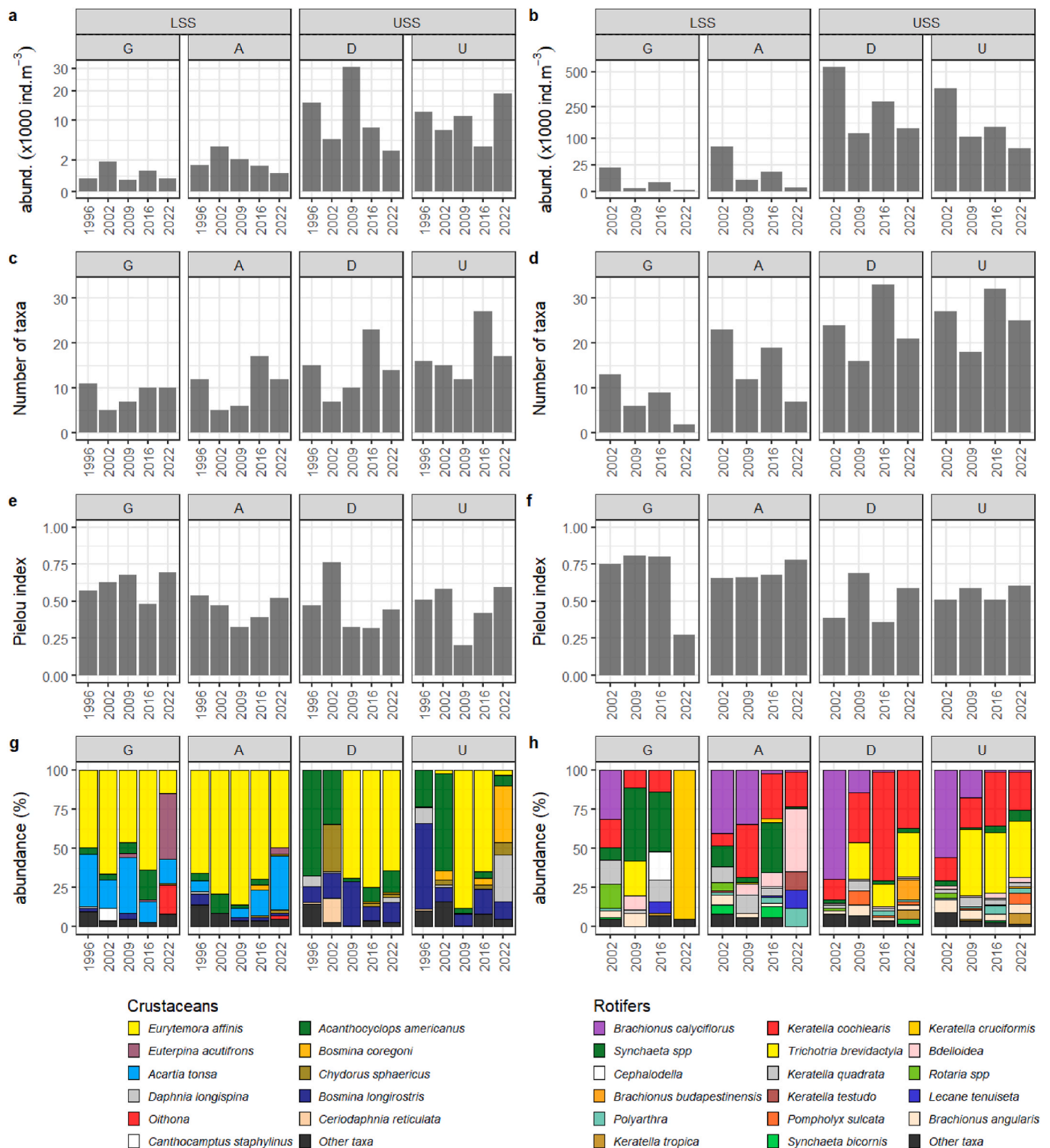
Considering all stations and years, the crustacean community counted a total of 60 taxa (26 copepods and 34 cladocerans) and the rotifer community 63. During the entire period studied, the number of taxa was significantly higher at the USS stations D and U than at the LSS stations G and A for both crustaceans and rotifers. Crustacean  $\alpha$  diversity showed a minimum in 2002 (5–7 taxa), except at U where it stayed slightly higher in 2002 than in 2009 (15 and 11 taxa respectively) and a maximum in 2016 with 10–27 taxa noted by station (Fig. 4c). A decrease was observed in 2022 at all stations, rendering  $\alpha$  diversity close to the 1996 level but higher than in 2002 and 2009. These fluctuations were positively correlated between stations for crustaceans, except between the two extreme stations G and U (Table 1).

In the rotifer community, the number of taxa generally varied between 8 and 25 (Fig. 4d). Minimal  $\alpha$  diversity was noted in 2022 at G and A (2 and 6 taxa respectively) and maximal at D and U in 2016 (33 and 32 taxa respectively). Temporal  $\alpha$  diversity fluctuations between stations of the LSS (G and A) and of the USS (D and U), showed a high rank correlation ( $\rho = 1$ , Table 1), although the p-values are at 0.08.

Pielou index of evenness mostly fluctuated between 0.4 and 0.7 for both communities. For crustaceans, lowest values were observed in 2009 at U (0.2). For rotifers, a significantly higher evenness of the community was detected at the LSS stations G and A ( $0.7 \pm 0.2$ ) than at the USS stations D and U ( $0.5 \pm 0.1$ ) (Fig. 4f, not significant for crustaceans). Low rotifer community Pielou values were observed at D in 2002 and 2016 (0.4), and a minimum was reached in 2022 at G (0.3).



**Fig. 3.** Yearly mean values for the main environmental parameters of the Scheldt, between 1996 and 2022, by station: a) water temperature and concentration of: b) suspended particles, c) oxygen, d) chlorophyll *a*, e) dissolved silica, f) chlorine with a zoom on freshwater values in g). The years studied are indicated by vertical grey stripes. Significant change points in the temporal trends are shown with a crossed circle. Temporal trends on each period (before/after rupture if present) are shown with a color code (red: significantly negative, green: significantly positive, black: stable). Note that in graph d) and f) the y-axis is squared transformed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 4.** Yearly mean values per station for: total abundance of a) crustaceans and b) rotifers (note that y-axes are squared transformed for better visibility), taxonomic richness of c) crustaceans and d) rotifers, Pielou index for e) crustacean and f) rotifer and relative abundance for the major taxa of g) crustacean and h) rotifer communities. Stations are ordered according to the downstream - upstream gradient (G: Grens, A: Antwerp, D: Dendermonde, U: Uitbergen).

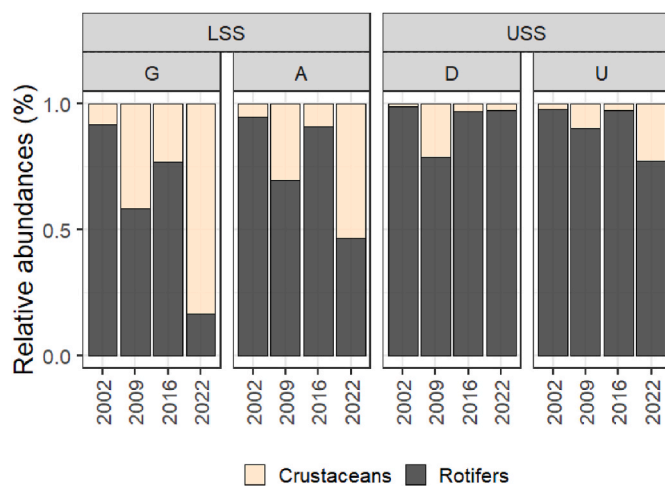
The fluctuation between stations was not correlated for crustaceans nor rotifers (Table 1).

Crustacean community composition changed substantially over time, especially in the USS. While in 1996 and 2002, the calanoid copepod *Eurytemora affinis* dominated the crustacean community only at the LSS stations and was quasi absent at the USS, it had become

dominant at all stations in 2009 and remained so in 2016 (Fig. 4d). In 2022 *E. affinis* became scarce at the 2 opposite stations G and U. The cyclopoid *Acanthocyclops americanus*, important to dominant in the USS in 1996 and 2002, constituted less than 10 % of the crustaceans at all stations in 2009, 2016 and 2022. This species was the only cyclopoid observed at all stations until 2016, before disappearing from G and A in

**Table 1**  
Spearman rho correlation and p-value between stations for each crustacean and rotifer demographic parameter, for the five or four years studied.

|                     |                  | Abundance |         | Nb of taxa |         | Pielou index |         |       |
|---------------------|------------------|-----------|---------|------------|---------|--------------|---------|-------|
|                     |                  | rho       | p-value | rho        | p-value | rho          | p-value |       |
| Crustaceans (n = 5) | G-A              | 0.3       | 0.683   | 0.9        | 0.042*  | 0            | 1       |       |
|                     | G-D              | -0.4      | 0.517   | 1          | 0.005** | 0.2          | 0.783   |       |
|                     | G-U              | -0.6      | 0.350   | 0.8        | 0.112   | 0.2          | 0.783   |       |
|                     | A-D              | 0.4       | 0.517   | 1          | 0.014*  | 0.6          | 0.350   |       |
|                     | A-U              | -0.4      | 0.517   | 1          | 0.005** | 0.6          | 0.350   |       |
|                     | D-U              | -0.2      | 0.783   | 0.9        | 0.054*  | 0.8          | 0.133   |       |
|                     | Rotifers (n = 4) | G-A       | 1       | 0.083      | 1       | 0.083        | -0.4    | 0.750 |
|                     |                  | G-D       | 0.8     | 0.333      | 0.6     | 0.417        | -0.4    | 0.750 |
|                     |                  | G-U       | 1       | 0.083      | 0.6     | 0.417        | -0.4    | 0.750 |
|                     |                  | A-D       | 0.8     | 0.333      | 0.6     | 0.417        | -0.4    | 0.750 |
| A-U                 |                  | 1         | 0.083   | 0.6        | 0.417   | 0.4          | 0.750   |       |
| D-U                 |                  | 0.8       | 0.333   | 1          | 0.083   | 0.6          | 0.417   |       |



**Fig. 5.** Relative abundances measured between the yearly mean total abundances of the crustaceans and the rotifers.

2022.

Except in 2002 at A, the calanoid *Acartia tonsa* was observed in all years in the LSS. The species was absent from the two upstream stations. In 2022 the harpacticoid *Euterpina acutifrons* and the cyclopoid *Oithona* spp., represented around 60 % of the community at G and were observed for the first time at A.

The cladoceran *Bosmina longirostris* was absent or scarce at the LSS stations and showed variable importance between the years at the USS stations. It was dominant in 1996 at U and accounted for around 20 % of the total abundance in all other years at both USS stations. *Chydorus sphaericus*, *Bosmina coregoni* and *Daphnia longispina*, rare in the LSS stations, and present in variable low abundances at USS stations until 2016, presented 75 % of the crustacean community in 2022 at the upstream station U, but much less (18 %) at D.

Among the most abundant rotifer species (Fig. 4h), *Brachionus calyciflorus* was dominant at the four stations in 2002 and decreased in

importance since 2009 to represent less than 2 % of the communities in 2016 and 2022. Except in 2022 at G, *Keratella cochlearis* was observed at all stations and years, with a yearly relative abundance between 8 and 69 %.

The genus *Synchaeta* and *S. bicornis* (the only species counted separately within the genus *Synchaeta*), were well represented in the LSS communities (3–47 % of total abundance) in the first three years studied before tending to disappear in 2022. *Trichotria brevidactyla* developed mainly at the 2 upstream stations D and U, representing between 14 up to 43 % of the community in 2016 and 2022. Earlier it was only observed in 2009 at G (22 %).

### 3.3. Detection of homogeneous community clusters and their indicator taxa

Four typical communities were detected for the crustaceans (Fig. 7a). The two major branches had an average Bray-Curtis dissimilarity of 57 %, and divided the LSS (G and A) and the USS (D and U) communities. In the LSS, the communities of all the years were grouped together (cluster 4), characterized by *Acartia tonsa*. For the USS, both D and U were clustered in 1996 with D 2002 (cluster 1) and were characterized by *Ceriodaphnia reticulata* (maximum Indval value of 1). In 2009 and 2016 the two stations were clustered (cluster 3), with D 2022. *Eurytemora affinis* characterized this cluster. Station U in 2002 and 2022 formed cluster 2 with *Bosmina coregoni* as indicator taxon.

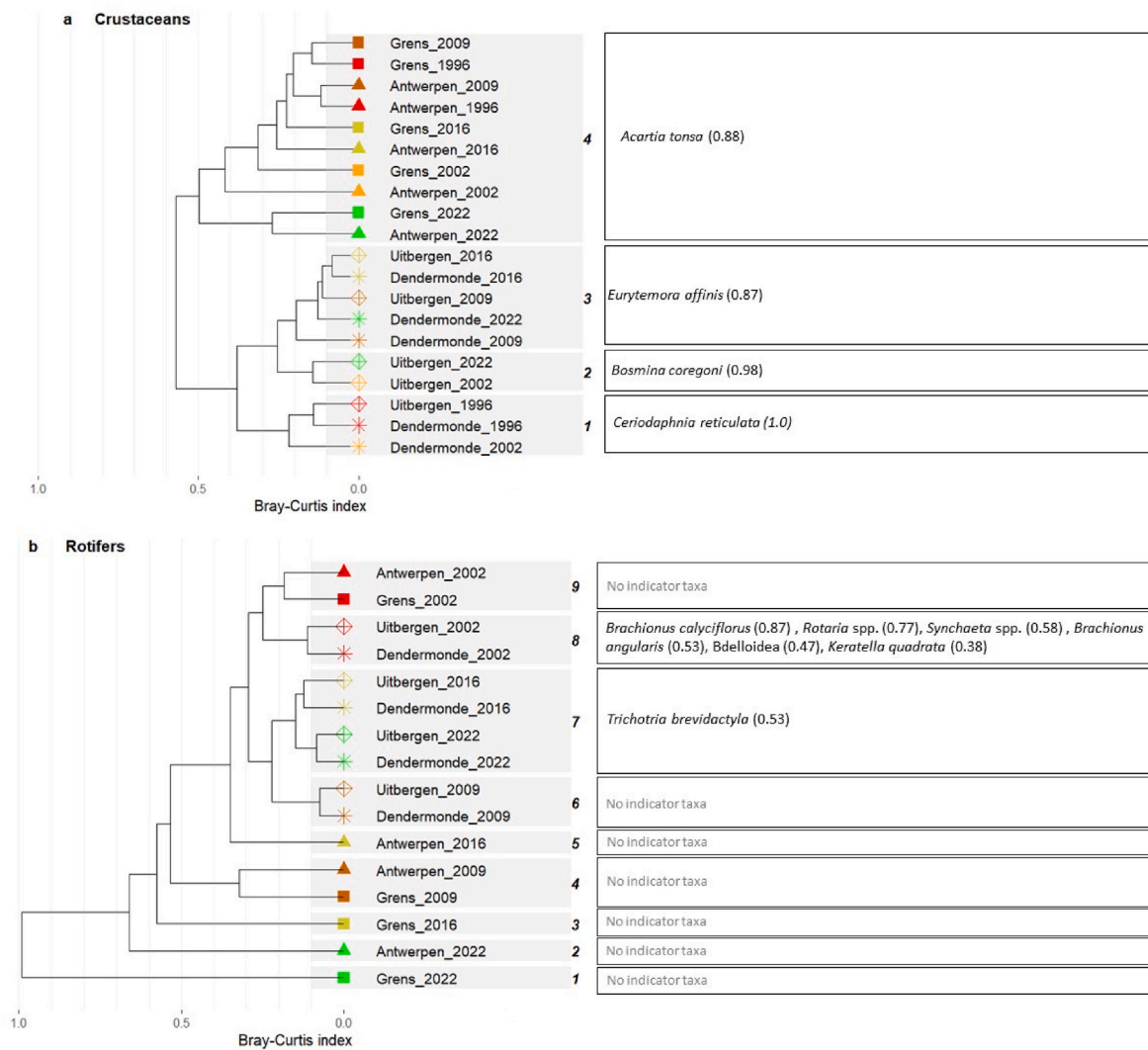
Nine typical communities were detected for rotifers (Fig. 7b). At the highest level (average Bray-Curtis dissimilarity of 99 %), G 2022 was split from the other observations in cluster 1, without typical taxa. Next, G and A stayed separately for the years 2022 (A in cluster 2) and 2016 (clusters 3 and 5 respectively). Both were grouped by year in 2009 (cluster 4) and 2002 (cluster 9). This LSS cluster is the closest of those of the USS in 2002 (U and D in cluster 9) for which *Brachionus calyciflorus*, *Rotaria* spp., *Synchaeta* spp., *Brachionus angularis*, Bdelloidea and *Keratella quadrata* were indicator taxa. Finally, cluster 7 grouped U and D in 2016 and 2022 with *Trichotria brevidactyla* as typical taxa.

### 3.4. Correlations between environment, zooplankton biodiversity and communities

For crustaceans, the first three dimensions of MFA contribute 57.4 % of the data variance. Among the variables with the best quality of representation on the factorial map ( $\cos^2 \geq 0.5$ , Table 2a),  $\text{Cl}^-$  is associated with dim.1 and opposed LSS to USS, the latter related to total crustacean abundance (Fig. 7a and b). This spatial dimension explained 27 % of the data variance.

On dim.2 SOLSI is correlated with the abundance of the cladoceran *Chydorus sphaericus* (Fig. 7a–d). The year 2022 at all stations, and 1996 in the USS, are positively associated with the axis, explaining 17.9 % of the data variance.  $\text{O}_2$ , most defining dim.3, is anticorrelated with *Ceriodaphnia reticulata* abundance and shows a clear temporal gradient explaining 12.8 % of the data variability (Fig. 7c and d).

For rotifers, the first three dimensions of the MFA contribute 65.1 % of the data variance. As for crustaceans, dim.1 synthesises a spatial segregation between LSS and USS stations representing 36.6 % of the data variance (Fig. 7e and f). Among the variables with  $\cos^2 \geq 0.5$  (Table 2b),  $\text{Cl}^-$  is anticorrelated with Chl<sub>a</sub> as well as with total rotifer abundance, number of taxa and abundances of *Keratella quadrata*, Bdelloidea, *Brachionus angularis* and *Synchaeta* spp. (except *S. bicornis*). The abundance of *Trichotria brevidactyla* is well represented on dim.2, and is particularly opposed to U and D in 2002 (cluster 8). SOLSI, most defining dim.3, is correlated with *Keratella tropica* abundance. The year 2022 is, at all stations, positively associated with axis 3 explaining 12.4 % of the data variance, while the earlier years (except U 2009) are found on the negative side of this axis (Fig. 7g and h).



**Fig. 6.** Hierarchical trees (UPGMA) based on the Bray-Curtis dissimilarity matrix on log-transformed (x+1) abundances of a) the major crustacean taxa and b) the major rotifer taxa. Homogeneous community clusters (SIMPROF analysis) are grouped in grey rectangles and referenced by number (on the right edge of each box). Taxa which are significantly characteristic for each cluster are listed and the Indval index specified in brackets.

### 3.5. Environmental thresholds separating community clusters

Variables selected by the model in the tree construction for the 4 crustacean community clusters are Cl<sup>-</sup> and O<sub>2</sub> (Fig. 8a). The prediction is good for both verification samples: G 1996 is correctly placed in cluster 4 and D 2022 in cluster 3.

Chlorinity is the first environmental factor dividing crustacean clusters. Cluster 4, grouping all the LSS samples was characterized by yearly mean Cl<sup>-</sup> ≥ 557 mg L<sup>-1</sup>, other clusters by Cl<sup>-</sup> concentrations below this limit. Oxygen concentration then divides the USS samples with a threshold of 6.3 mg L<sup>-1</sup>. Samples from 1996 to 2002 (Cluster 1) were below this limit, while those from 2009 to 2022 were above (Cluster 3). Cluster 2 was divided into two branches: U 2002 in the lower oxygen concentration range and U 2022 in the higher one.

Variables selected in the tree construction for the 9 rotifer community clusters are Cl, O<sub>2</sub>, and Chla (Fig. 8b). The prediction is good for both verification samples: U 2016 is correctly placed in cluster 7 and U 2022 in cluster 8.

As with crustaceans, chlorinity is the primary environmental factor separating clusters of freshwater USS communities from brackish water LSS communities, with a threshold value of 557 mg L<sup>-1</sup>. In freshwater, O<sub>2</sub> was the next node retained. Cluster 7 (2016 and 2022) was split from

the others (2002 and 2009) by O<sub>2</sub> concentration ≥ 8.5 mg L<sup>-1</sup>. In brackish water, a threshold value of 16 µg L<sup>-1</sup> of Chla dissociated cluster 9 (A and G 2002) from all the others with lower concentrations (Clusters 1 to 5 from 2009 to 2022).

## 4. Discussion

The present paper evaluates the effect of changes in environmental conditions co-occurring with the SIGMA plan application in the Scheldt estuary between 1996 and 2022 on both crustacean (mesozooplankton) and rotifer communities. As mentioned in the introduction, several papers have illustrated this period but since the species list of Tackx et al. (2005), essentially the calanoid copepod *Eurytemora affinis*, as dominant mesozooplankton species, has received attention (Appeltans et al., 2003; Mialet, 2010; Mouth et al., 2024). Other mesozooplankton organisms were generally considered at suborder and order levels (Tackx et al., 2005; Mialet et al., 2011; Chambord et al., 2016) and rotifers were analyzed on short time scales (Azémar et al., 2007, 2010). The fact that, in this study, species level analyses was only carried out at 6–7 year intervals may hide some short-term variations in the zooplankton evolution. A comparison between all OMES crustacean and rotifer data and those selected for this paper is given in additional information (A1, 2 and

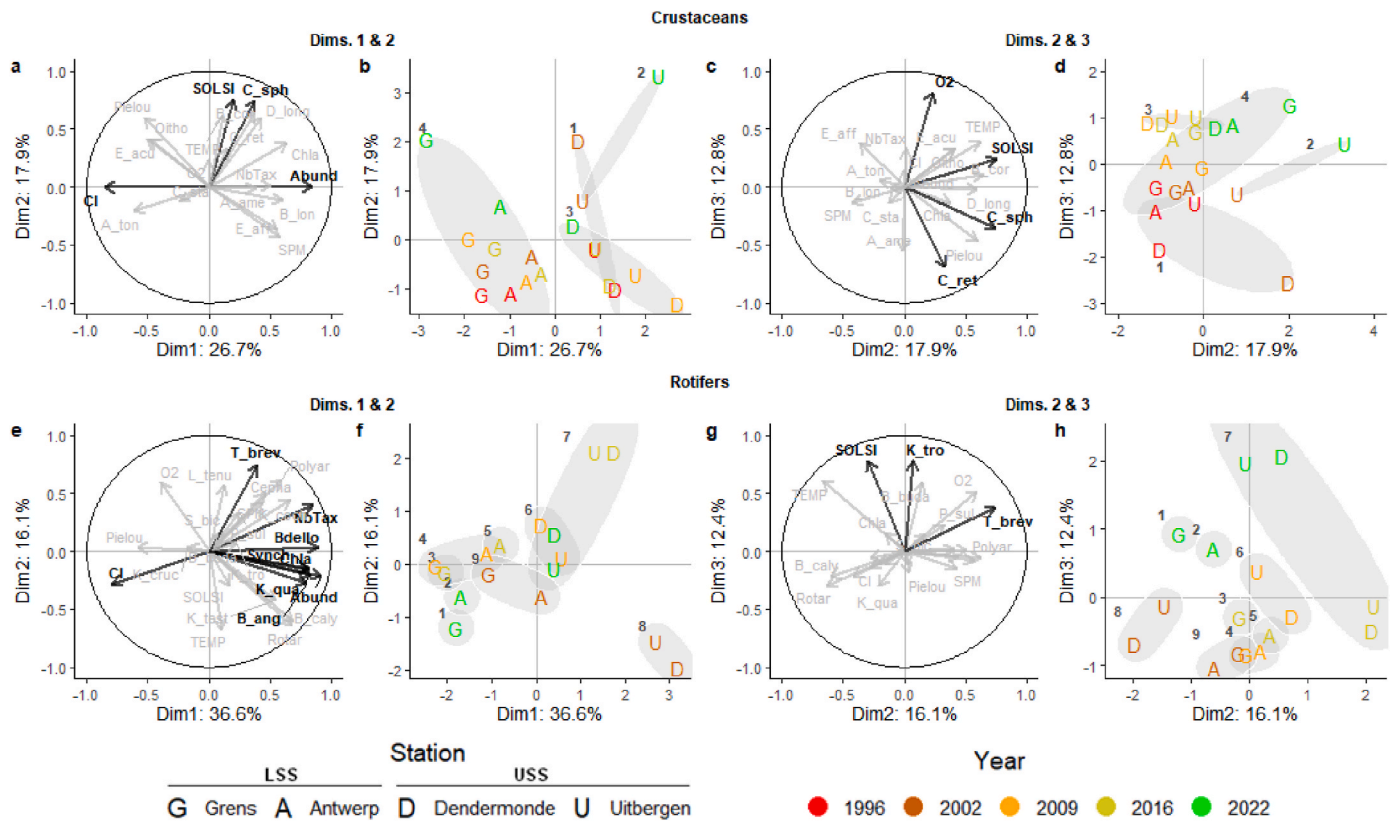


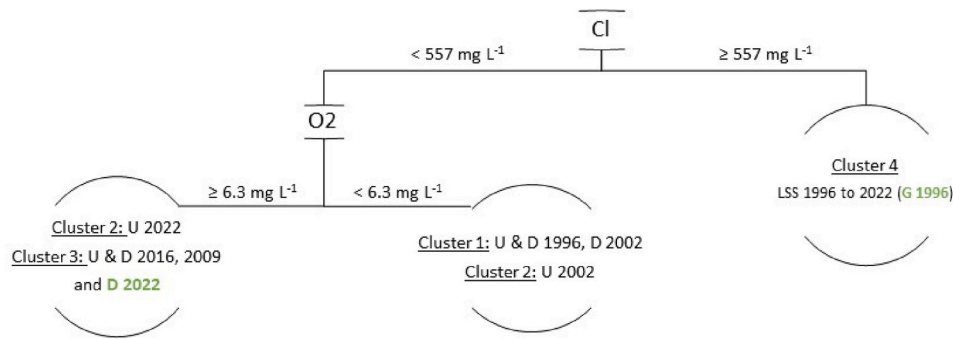
Fig. 7. Multiple factor analysis (MFA) maps for environmental parameters, biodiversity index and abundances of the major taxa applied on a) to d) crustaceans, e) to h) rotifers. Variables sufficiently represented on the dimensions ( $\cos^2 \geq 0.5$ , Table 2) are indicated with a black arrow, others are in grey. Individuals are plotted and typical community clusters indicated by grey ellipses with numbers specified (see Fig. 6). Note that 1996 is missing for rotifers. See Table 2 below for abbreviations.

Table 2

Representation ( $\cos^2$ ) of variables to each MFA dimension for a) crustaceans and b) rotifers. Variables are listed in descending order of total  $\cos^2$ . Variables in bold are considered sufficiently represented to be discussed ( $\cos^2 \geq 0.5$ ). The percentage contribution of each dataset to the dimensions is shown in *italic*.

| a  |             |             |             |       | b  |             |             |             |       |
|--|-------------|-------------|-------------|-------|--|-------------|-------------|-------------|-------|
| CRUSTACEANS (1996–2022)                  | Dim.1       | Dim.2       | Dim.3       | TOTAL | ROTIFERS (2002–2022)                       | Dim.1       | Dim.2       | Dim.3       | TOTAL |
| <b>Environmental parameters</b>          | <i>30 %</i> | <i>39 %</i> | <i>39 %</i> |       | <b>Environmental parameters</b>            | <i>29 %</i> | <i>46 %</i> | <i>68 %</i> |       |
| (Cl) Chlorinity                          | <b>0.73</b> | 0           | 0.02        | 0.75  | (TEMP) Subsurface water temperature        | 0.01        | 0.45        | 0.37        | 0.83  |
| (O2) Oxygen concentration                | 0           | 0.05        | <b>0.67</b> | 0.72  | (O2) Oxygen concentration                  | 0.15        | 0.35        | 0.27        | 0.77  |
| (SOLSI) Dissolved silica                 | 0.04        | <b>0.57</b> | 0.06        | 0.67  | (Cl) Chlorinity                            | <b>0.64</b> | 0.08        | 0.02        | 0.74  |
| (Chla) Chlorophyll <i>a</i>              | 0.40        | 0.14        | 0.02        | 0.56  | (SOLSI) Dissolved silica                   | 0.03        | 0.09        | <b>0.61</b> | 0.73  |
| (SPM) Suspended particulate matter       | 0.34        | 0.19        | 0.02        | 0.55  | (Chla) Chlorophyll <i>a</i>                | <b>0.67</b> | 0.02        | 0.02        | 0.71  |
| (TEMP) Subsurface water temperature      | 0.01        | 0.38        | 0.15        | 0.54  | (SPM) Suspended particulate matter         | 0.19        | 0.18        | 0.02        | 0.39  |
| <b>Biodiversity index</b>                | <i>36 %</i> | <i>15 %</i> | <i>19 %</i> |       | <b>Biodiversity index</b>                  | <i>35 %</i> | <i>9 %</i>  | <i>2 %</i>  |       |
| (Pielou) Pielou index                    | 0.28        | 0.36        | 0.22        | 0.86  | (Abund) Total abundance                    | <b>0.84</b> | 0.05        | 0.01        | 0.9   |
| (Abund) Total abundance                  | <b>0.71</b> | 0           | 0           | 0.71  | (NbTax) Number of taxa                     | <b>0.72</b> | 0.16        | 0           | 0.88  |
| (NbTax) Number of taxa                   | 0.26        | 0           | 0.11        | 0.37  | (Pielou) Pielou index                      | 0.34        | 0           | 0.03        | 0.37  |
| <b>Major taxa (abundances)</b>           | <i>34 %</i> | <i>45 %</i> | <i>42 %</i> |       | <b>Major taxa (abundances)</b>             | <i>35 %</i> | <i>45 %</i> | <i>30 %</i> |       |
| (C_sph) <i>Chydorus sphaericus</i>       | 0.14        | <b>0.56</b> | 0.12        | 0.82  | (Rotar) <i>Rotaria</i> spp.                | 0.45        | 0.41        | 0.09        | 0.95  |
| (C_ret) <i>Ceriodaphnia reticulata</i>   | 0.02        | 0.11        | <b>0.47</b> | 0.60  | (B_caly) <i>Brachionus calyciflorus</i>    | 0.48        | 0.37        | 0.05        | 0.90  |
| (E_aff) <i>Eurytemora affinis</i>        | 0.31        | 0.14        | 0.14        | 0.59  | (T_brev) <i>Trichotria brevidactyla</i>    | 0.16        | <b>0.55</b> | 0.14        | 0.85  |
| (B_cor) <i>Bosmina coregoni</i>          | 0.14        | 0.42        | 0.01        | 0.57  | (K_qua) <i>Keratella quadrata</i>          | <b>0.69</b> | 0.04        | 0.09        | 0.82  |
| (E_acu) <i>Euterpina acutifrons</i>      | 0.25        | 0.17        | 0.11        | 0.53  | (Bdello) <i>Bdelloidea</i>                 | <b>0.79</b> | 0           | 0           | 0.79  |
| (D_long) <i>Daphnia longispina</i>       | 0.18        | 0.35        | 0           | 0.53  | (Polyar) <i>Polyarthra</i> spp.            | 0.35        | 0.38        | 0           | 0.73  |
| (Oitho) <i>Oithona</i> sp.               | 0.23        | 0.17        | 0.11        | 0.51  | (B_ang) <i>Brachionus angularis</i>        | <b>0.64</b> | 0.08        | 0           | 0.72  |
| (A_ame) <i>Acanthocyclops americanus</i> | 0.14        | 0           | 0.3         | 0.44  | (Synch) <i>Synchaeta</i> spp.              | <b>0.67</b> | 0.02        | 0.01        | 0.70  |
| (A_ton) <i>Acartia tonsa</i>             | 0.38        | 0.04        | 0           | 0.42  | (K_tro) <i>Keratella tropica</i>           | 0.05        | 0.01        | <b>0.61</b> | 0.67  |
| (B_lon) <i>Bosmina longirostris</i>      | 0.36        | 0.01        | 0           | 0.37  | (K_coch) <i>Keratella cochlearis</i>       | 0.44        | 0.2         | 0           | 0.64  |
| (C_sta) <i>Canthocamptus staphylinus</i> | 0.06        | 0.01        | 0.02        | 0.09  | (K_test) <i>Keratella testudo</i>          | 0.3         | 0.18        | 0.05        | 0.53  |
|  |             |             |             |       | (Cepha) <i>Cephalodella</i> sp.            | 0.21        | 0.25        | 0           | 0.46  |
|  |             |             |             |       | (B_buda) <i>Brachionus budapestinensis</i> | 0.01        | 0.02        | 0.35        | 0.38  |
|  |             |             |             |       | (L_tenu) <i>Lecane tenuiseta</i>           | 0.02        | 0.32        | 0.01        | 0.35  |
|  |             |             |             |       | (P_sul) <i>Pompholyx sulcata</i>           | 0.06        | 0.11        | 0.05        | 0.22  |
|  |             |             |             |       | (S_bic) <i>Synchaeta bicornis</i>          | 0.01        | 0.03        | 0.02        | 0.06  |
|  |             |             |             |       | (K_cruc) <i>Keratella cruciformis</i>      | 0.03        | 0           | 0.01        | 0.04  |

## a Crustaceans



## b Rotifers

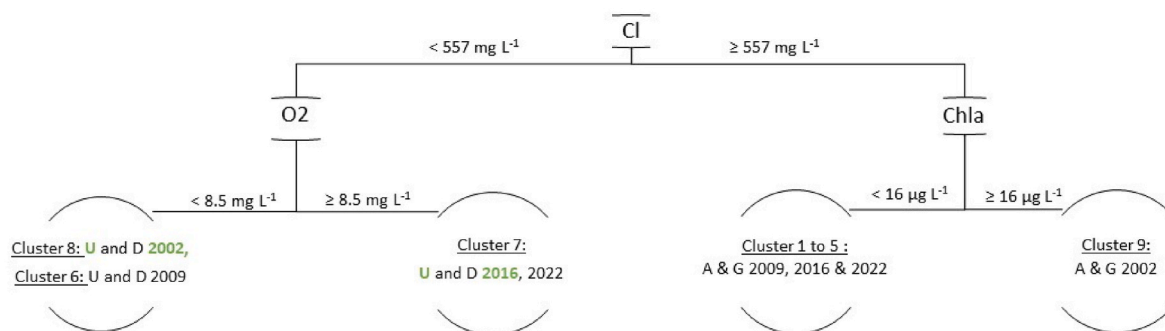


Fig. 8. Optimal decision trees for a) crustacean clusters and b) rotifer clusters.

3) and shows that they generally reveal similar trends in time and space.

This discussion will use community clustering coupled with multivariate analysis and decision tree to try to elucidate the main co-evolutions of environmental factors and zooplankton communities. While little used in ecology, decision tree analysis has proven its usefulness in habitat modeling (Coz-Rakovac et al., 2008; Debeljak and Džeroski, 2011; Itsukushima, 2023). Being aware that the decision tree results are not necessarily the 'sole and exact' environmental reasons for changes in the zooplankton communities, we believe it offers the most logical pathway to discern some major trends.

Over the 4 or 5 years considered, the average crustacean abundances measured per year in the Scheldt estuary (mostly from  $1 \times 10^3$  to  $10 \times 10^3$  ind.  $m^{-3}$ ) are within the large range observed in both fresh and salt lotic systems (Paturej and Gutkowska, 2015; Yermolaeva et al., 2021; Horn et al., 2023). Rotifer yearly mean abundances were at least 10 times higher than those of crustaceans, comparable to observations in non-tidal running freshwaters (Zimmermann-Timm et al., 2007; Burdis and Hoxmeier, 2011). The total number of 123 taxa observed, composed of 60 crustaceans (copepods 21 %, cladocerans 28 % of the total richness) and 63 rotifers (51 %), is in line with reports for other temperate estuaries (Modéran, 2010; Rothenberger et al., 2014; Yermolaeva et al., 2021).

A significant difference in the composition of crustacean and rotifer communities between LSS and USS, which account for most of the data variance, is visible for all selected years since 1996. Both abundances and numbers of taxa of both crustaceans and rotifers were higher in the freshwater USS than in the brackish LSS, suggesting a better development of the zooplankton in freshwater (Fig. 4a and b, 7a and e, Table 2), in line with other ecological studies (Bakker et al., 1994; Callieri et al., 2006; Whitfield and Elliott, 2011; Whitfield et al., 2012).

In the brackish water LSS, copepod species well adapted to salinity, such as the euryhaline species *Acartia tonsa* (Dussart, 1967; Mauchline, 1998; Pedroso et al., 2007), the major marker of LSS stations, and

*Euterpina acutifrons* or *Oithona* sp. (Dussart, 1969) are found (Figs. 4h and 7a). The  $557 \text{ mg Cl}^- \text{ L}^{-1}$  splitting value, within the oligohaline range (i.e.  $300\text{--}3000 \text{ mg L}^{-1}$ ), was nevertheless reached at station A of the LSS between 2000 and 2002, a period of very high discharges (Figs. 2 and 3f). It is worth noting the near-absence of the euryhaline species *A. tonsa* (Dussart, 1967; Mauchline, 1998; Pedroso et al., 2007) (Fig. 4g) either as a response to this lower salinity or because the plankton community was displaced further downstream by the high discharge.

An effect of the recent human-induced marinization appears clearly in the rotifer community, particularly sensitive to salinity (Azémar et al., 2010; Bielańska-Grajner and Cudak, 2014). For the first time, rotifer abundances were indeed lower than crustaceans in 2022 at both LSS stations (Fig. 5). Hall and Lewandowska (2022) carried out a zooplankton community mesocosm study in the mesohaline range and report a shift from a rotifer-dominant to a crustacean-dominant community at salinity above 7.5 PSU (approximately  $420 \text{ mg L}^{-1} \text{ Cl}^-$ , De Pauw, 1975). As crustacean abundance remained quite stable (Fig. 3a), a similar shift could be in progress here. This seems to be particularly true at the most downstream station G, where rotifer community has shifted to a sparse and poorly diverse community dominated by the euryhalin species *Keratella cruciformis* (Godske, 1968; Koste, 1978; Holst et al., 1998; Segers, 2007) (Fig. 4b–d, f and g). Less pronounced changes are visible in the crustacean community (not significant, Fig. 4g and 6). Copepods *Euterpina acutifrons* and *Oithona* sp. were more numerous, the latter also identified as thriving in the Eastern Scheldt (Netherlands), favored not only by marinization but also by the phytoplankton changes induced by the invasion of filter-feeding bivalves (Cloern and Jassby, 2012; Horn et al., 2023).

Restoration efforts of the Scheldt water quality were particularly evidenced by the oxygen concentration (Fig. 3c) (Cox et al., 2009, 2019). Gradual significant changes over time are clearly detected in the fresh water zooplankton community since 1996, particularly in crustaceans marked by the installation of *E. affinis* (cluster 3, Fig. 4g and 6a)

(Chambord et al., 2016), related to the increase in  $O_2$ . Major shifts in the USS community compositions were proposed by the decision tree around a yearly threshold of  $6.3 \text{ mg } O_2 \text{ L}^{-1}$  for crustaceans and  $8.5 \text{ mg } O_2 \text{ L}^{-1}$  for rotifers, separated the pre- from the post-water de-eutrophication period in 2009 (Fig. 8a and b) corresponding to earlier reports for the dominant copepod *E. affinis* (Appeltans et al., 2003; Mialet, 2010; Mialet et al., 2011). The cladoceran *Ceriodaphnia reticulata* and 6 rotifers species characterized conditions prior to wastewater treatment effects (Fig. 6a and b). The first one, whose abundance was opposite to oxygen concentrations (Fig. 7a–d) is a common, often dominant, cladoceran described as inhabiting eutrophic waters (Błędzki and Rybak, 2016). Among the indicator rotifers, *Brachionus calyciflorus*, *B. angularis* and *Keratella quadrata* are known to be able to eat bacteria (Gilbert, 2022; Obertegger and Wallace, 2023), which must have been a dietary advantage before management efforts, as bacteria were then more abundant in the water than afterwards (Dijkman and Kromkamp, 2006; Ouattara et al., 2009; Chambord et al., 2016).

Improvement in oxygenation of the brackish water reach over the study period (Fig. 3c), did not disrupt the crustacean community, showing no significant composition changes in time. In contrast, the rotifer community was highly variable (Fig. 6b) but none of the 6 community-types identified (i.e. clusters) in the LSS are directly related to  $O_2$  (Fig. 7e–h, Fig. 8b). A side effect of improved water quality was the quasi-continuous decrease in phytoplankton biomass since 2009, recently amplified by the marinization process (Amadei Martínez et al., 2023; see also below) (Fig. 3d). Chla is proposed as a control factor for rotifer communities by the decision tree, which can explain the shift from the 2002 community to the more recent ones, below  $16 \mu\text{g L}^{-1}$  (Fig. 8b). The fact that the LSS rotifers, but not the crustacean community were linked to Cha suggests that rotifers are more responsive to phytoplankton biomass than the crustaceans living in brackish water. Because of the anti-correlation between chlorinity and Chla in this downstream part of the estuary (Amadei Martínez et al., 2023), the cumulative effect of these 2 variables on the zooplankton communities should be further explored.

More recently, zooplankton communities are related to an increase in SOLSI, explaining around 15 % of the data variance. As Amadei Martínez et al. (2023) describe a global decrease in phytoplankton biomass since 2002 associated to a recent fall in diatom relative abundance and a rise in cyanobacteria in the Scheldt freshwater reach, which explains a decrease in silica consumption by diatoms. Less diatoms, one of the main food resources for zooplankton in estuary (Tackx et al., 2003; Cabrol et al., 2015) could influence zooplankton development and community composition, depending on the diatom-dependence and selection capacity of the different taxa. The restoration of intertidal marshes following the SIGMA plan, improving the system's capacity to recycle biogenic silica (Jacobs et al., 2008, 2013; Van Damme et al., 2009), also boosts SOLSI concentration in the water. Although no community-type was strictly associated with this parameter, the abundances of the highly ubiquitous cladoceran *Chydorus sphaericus* and the alien rotifer *Keratella tropica*, the only one of the three rotifer species reported in 2002 by Azémar et al. (2007), could reflect ongoing phytoplanktonic transitions in USS.

The main latest significantly change within the USS was the disappearance of *E. affinis* at the most upstream station U (Fig. 4g), clustering the crustacean communities of the years 2002 and 2022 (cluster 2, Fig. 6a), so before and after the de-eutrophication. As,  $NH_4^+$  and  $O_2$ , known to be limiting factors for *E. affinis*, stayed below its tolerance limit (unpublished results) (Chambord et al., 2016; Mouth et al., 2024), an impact of the recent feeding resources shift described above is favored. As Lee et al. (2013) found that high food concentration significantly increases low-salinity tolerance of *E. affinis*, one can hypothesize that recent marinization could aid this species to persist at D, but not at the upstream station U, under declining conditions of consumable phytoplankton. Indeed, Chla, SPM and chlorinity were lower at U than at D (Fig. 3), where considerable marine inflows and maximum turbidity is

measured during low summer discharges since 2008 (Cox et al., 2019) (Fig. 3b and f). Continuous OMES monitoring data presented in additional information confirms the negative trend of the species which began from 2020 onward (supplementary material A 3).

Despite strong environmental changes, no significant shift in the composition of the crustacean community at U is detected between the two years, and the small pelagic indicator cladoceran *Bosmina coregoni* found favorable living conditions in both 2002 and 2022 (Fig. 6a). The presence of *E. affinis* alone would have inhibited the rest of the community, validating its invasive character in freshwater (Lee, 1999; Winkler et al., 2008).

The deepening of the Scheldt fairway, in addition to facilitating marine entrance in the estuary, has complex interactions with water physico-chemistry quality, freshwater runoff and sediment erodibility, which, since around 2008, occasionally lead to higher SPM concentrations (Fig. 3b) (Cox et al., 2019). The attribution of *Trichotria brevidactyla*, a species known to live in the sediment (Koste, 1978; Jersabek and Leitner, 2013), as the indicator of the 2016 and 2022 USS rotifer communities (Fig. 6b and 7e to h) could reflect a significant zooplankton response to sediment remobilizations, explaining 16.1 % of the variance in the data.

## 5. Conclusion

The environmental parameters influencing crustacean and rotifer abundance, diversity and community structure in the Scheldt estuary are interlinked which complicates interpretation of zooplankton observations. The combined clustering, multi-variate and decision tree approach applied in this study however helped to indicate the most likely (combination of) environmental parameters giving rise to various crustacean and rotifer communities: chlorinity, oxygen, phytoplankton composition and biomass (quantified as Chla concentration) and sediment mobilization.

Crustacean and rotifer species composition showed a significantly stable spatial segregation between the downstream brackish (LSS) and the upstream freshwater (USS) reach since 1996, evolving in a different way and also affected abundances and diversities.

Among the measures of the SIGMA plan, the impact of the reduction of nutrient and organic waste loads on USS zooplankton is evidenced by this study from 2009 onwards. Both crustacean and rotifer community showed significant composition shifts related to oxygen increase, as the gradual disappearance of highly eutrophy resistant and bacterial feeding species and the expansion of *Eurytemora affinis*. In brackish water LSS, crustacean community seemed very stable without repons to the major water quality improvement, while rotifer community appeared more sensitive to phytoplankton biomass decrease.

In 2016, the development of psammic rotifer *Trichotria brevidactyla* probably marks a significant impact of the intensification of dredging activities on USS zooplankton. More recently (2022), marinization induced a major shift from a rotifer-dominant to a crustacean-dominant zooplankton community in LSS, strongly affecting communities at G, in the downstream part of the Belgium reach. A minor crustacean species change occurred in this part of the estuary while a major community shift appeared at the upstream station U. An influence of complex interactions between phytoplankton (biomass and composition) and salinity is suspected, and calls for intensive monitoring of ongoing ecological processes.

## CRedit authorship contribution statement

**Anaëlle Bernard:** Writing – original draft, Visualization, Formal analysis, Data curation. **Frédéric Azémar:** Validation, Data curation, Conceptualization. **Tom Maris:** Validation, Funding acquisition, Data curation. **Patrick Meire:** Validation, Project administration. **Luz Amadei Martínez:** Validation. **Céleste Mouth:** Validation, Data curation. **Elisa Bou:** Validation, Data curation. **Michèle Tackx:** Writing – original

draft, Supervision, Project administration, Funding acquisition, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2025.109450>.

### Data availability

Data will be made available on request.

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