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### Rotifers in the Schelde estuary (Belgium): a test of taxonomic relevance



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Keywords:	Rotifera, Schelde estuary, taxonomic sufficiency, spatio-temporal distribution



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4 1 **Rotifers in the Schelde estuary (Belgium): a test of taxonomic relevance**  
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35 14 **Keywords:** Rotifera; Schelde estuary; taxonomic sufficiency; spatio-temporal distribution  
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4 1 **Abstract**

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6 2 To investigate the reaction of the zooplankton community to improving water quality in  
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8 3 the Schelde estuary, we studied the relationship between rotifer species distribution and  
9  
10 4 environmental factors, and the feasibility of using a coarser level of taxonomic resolution.

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12 5 52 taxa, belonging to 26 genera, were identified, including 22 taxa new for the Schelde.  
13  
14 6 *Brachionus calyciflorus*, *Keratella cochlearis* and *B. angularis* were the most abundant species. The  
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16 7 highest diversity and abundances were observed in the freshwater reach.

17  
18 8 Redundancy analyses (RDA) showed that the main environmental factors explaining  
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20 9 rotifer distribution were chlorinity and seasonal factors (discharge levels, cyclopoid abundance).  
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22 10 Analysis carried out with data at the species and the genus level gave similar ordination plots, but  
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24 11 the positioning of a genus relative to environmental factors did not always adequately represent the  
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26 12 associations between the various species within the genus and environmental factors. Similar  
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28 13 patterns in space and time were observed using taxonomic richness and diversity indices for  
29  
30 14 analyses at species or genus level. Thus, in the context of the restoration of the Schelde estuary, the  
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32 15 identification of rotifer species is very informative, but not essential for detecting important  
33  
34 16 ecological associations.

## 1 Introduction

The worldwide concern to maintain the ecological quality and biodiversity of ecosystems calls for understanding of how various taxa respond to environmental conditions. On the one hand, this requires a solid knowledge of the environmental factors that influence the various taxa within a community, and the interplay between them. On the other hand, it requires the ability to distinguish the various taxa at an ecologically-relevant level. The former is essentially obtained through multivariate correlation analysis, intended to identify as precisely as possible the environmental variables with which the various taxa present in a community are associated. The appropriate level of taxonomic resolution and the associated strength in representing the ecological quality and response of interest is currently subject to considerable debate. The topic is generally considered from a cost/benefit angle, where cost consists of the time, skills and resources needed for species level identification (Quijón and Snelgrove, 2006), and benefit is the ability of the dataset obtained to answer the questions posed. These questions often concern the community response to anthropogenic stress, or evaluating the diversity of ecosystems in a conservation context (Fleishman *et al.*, 2005). Using 'coarser' taxonomic levels, such as genera or families (Somerfield and Clarke, 1995), is one of the strategies proposed to optimize ecological research and survey strategies. In the aquatic environment, benthic organisms are usually used for quality-assessment studies. The feasibility of using coarser taxonomic levels for benthic communities has been investigated in freshwater habitats (Bowman and Bailey, 1997; Marshall *et al.*, 2006), rocky shores (Pagola-Carte and Saiz-Salinas, 2001), gravel and sandy beaches (Schoch and Dethier, 2001; Defeo and Lercari, 2004), lagoons (Mistri and Rossi, 2001), coastal zones (e.g. Gray *et al.*, 1988; James *et al.*, 1995; Somerfield and Clarke, 1995) and deep-sea sedimentary communities (Narayanaswasny *et al.*, 2003; Quijón and Snelgrove, 2006). However, little consideration of this type has so far been applied to pelagic organisms. In view of the ecological importance of rotifers in estuaries, and the difficulty of routinely determining them, it seemed to be worth considering this aspect of the rotifer population of the Schelde estuary.

Within this context, our paper focuses on the planktonic rotifers of the Schelde estuary (Belgium/The Netherlands). This macrotidal estuary is one of the few European estuaries that still has an extensive freshwater tidal zone (< 0.5) in its upper reaches (Meire *et al.*, 2005). The Schelde estuary, and especially its freshwater stretch, was known to be one of the most polluted estuaries in Europe during the 1970s and 1980s (Soetaert and Herman, 1995). Since the 1990s, management efforts, including controlling pollutant levels and wastewater treatment, have resulted in an

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3 1 improvement of the water quality. Over the past ten years, several environmental factors have  
4 2 changed substantially, and these changes have been most marked in the freshwater stretch. Indeed,  
5 3 in the upstream part of the Schelde estuary, a considerable increase in the oxygen concentration  
6 4 and chlorophyll *a*, and decreases in the  $\text{NH}_4^+$  concentration and in BOD5 values have been  
7 5 observed (Cox *et al.*, 2009). Associated with the rise in oxygen concentration, there has been an  
8 6 increase in  $\text{NO}_3^-$  concentration as a result of more intensive nitrification. Furthermore, in the  
9 7 summer silica concentrations ( $\text{SiO}_2$  or DSi (Dissolved Silica)) now drop below limiting values more  
10 8 often than ten years ago (Cox *et al.*, in prep). Runoff, which is an important factor in structuring  
11 9 estuarine spatio-temporal conditions, has varied irregularly over the past decade. While runoff is  
12 10 basically regulated by precipitation and climatic conditions in general, it is also controlled to some  
13 11 extent by human action (e.g., sluice management), and hence is highly relevant to water  
14 12 management. A more extensive overview of the ecological changes that have occurred in the  
15 13 Schelde estuary and current management perspectives is reported by Van Damme *et al.*, 2005,  
16 14 Meire *et al.*, 2005 and Soetaert *et al.*, 2006.

17 15  
18 16 At present, the Schelde can be considered to be an estuary on the way to restoration. To  
19 17 provide managers of this estuary with appropriate advice, the Government of Flanders sponsored a  
20 18 multi-disciplinary monitoring program, 'OMES'. The general aim of the OMES study, which began  
21 19 in 1996, is to describe the changes in the estuarine community during its restoration, and to  
22 20 understand which environmental conditions (or changes in these conditions) have led to this pattern.  
23 21 This information is intended for incorporation in models used to predict impact of management  
24 22 measures on various compartments of the system (Meire *et al.*, 2005).

25 23  
26 24 Because of their key role in the trophic functioning of the estuary (Tackx *et al.*, 2003;  
27 25 Maes *et al.*, 2005), as well as their potential role as bioindicators (Appeltans *et al.*, 2003),  
28 26 zooplankton are an important compartment in the OMES research. This present paper reports a  
29 27 study of the rotifer species composition in the brackish – freshwater reach of the Schelde estuary, as  
30 28 well as its spatio-temporal distribution and diversity during 2002. Rotifers are omnipresent in  
31 29 aquatic systems (Pourriot, 1977; De Ridder and Segers, 1997; Park and Marshall, 2000; Wallace *et al.*,  
32 30 2006; Segers, 2008). They are found mainly in freshwater: a total of 1800 to 2000 species have  
33 31 been reported world-wide, with less than 100 strictly marine species. Estuaries present a particular  
34 32 interesting setting to look at affinities of species and environmental variables. In a highly  
35 33 heterotrophic system such as the Schelde estuary (Soetaert and Herman, 1995), rotifers are likely to  
36 34 form an important link between the microbial web and higher trophic levels (Havens, 1991;  
37 35 Gasparini and Castel, 1997; Griffin and Rippingale, 2001; Froneman, 2002). Unfortunately, there

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3 1 are few species–level datasets on rotifer communities in the literature. This is due to a combination  
4 2 of three factors. Their small size usually requires a compound microscope for identification;  
5 3 illoricate species are more easily identified from living material; few competent taxonomists are  
6 4 currently trained in rotifer taxonomy. Moreover, in estuaries, high concentrations of suspended  
7 5 particulate matter hamper microscopic observation. To the best of our knowledge, the only such  
8 6 report available for a European Atlantic estuary is that for the Elbe estuary (Holst *et al.*, 1998).

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10 7 Our study presents the taxonomic composition (at species level in as far as possible) and  
11 8 the spatio-temporal distribution of the rotifer community in the Schelde estuary. Its relationship to  
12 9 environmental variables is studied, paying special attention to those variables which have changed  
13 10 in the past, and are expected to go on changing as a consequence of restoration.

14 11 To test the feasibility of monitoring the rotifer community at the genus rather than species  
15 12 level, the analysis was carried out at both species and genus level. The results were compared to  
16 13 evaluate whether we obtain the same information about the association between taxa and  
17 14 environmental factors when considering the data at the genus level or at the species level. Diversity  
18 15 indices calculated from species- and genus-level assessments were also examined in relation to the  
19 16 environmental factors.

## 20 17 **Method**

### 21 18 **Study site**

22 19 The Schelde River arises in France and runs through Belgium and The Netherlands, where  
23 20 it enters the North Sea at Vlissingen (Fig. 1). The Schelde estuary, which extends inland as far as  
24 21 the city of Gent, comprises the final 160 km of the river, which extends inland as far as Gent. Three  
25 22 successive salinity zones can be recognized in this stretch: a marine zone (> 15 PSU Practical  
26 23 Salinity Units), a brackish zone 5 (0.5 to 15 PSU) and a freshwater zone (< 0.5 PSU). The tidal  
27 24 amplitude varies between 5.2 m near Antwerp (km 78.5) and 2 m near Gent (km 160).

28 25 Depending on the tidal cycle and the amount of freshwater input, the river has low salinity  
29 26 upstream from roughly Rupelmonde (km 85) to Vlassenbroek (km 118). Downstream, the brackish  
30 27 zone extends to around the Dutch/Belgian border (km 57.5).

### 31 28 **Sampling and physico-chemical analyses**

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34 29 From February 2002 to December 2002, 16 stations situated along the brackish and  
35 30 freshwater reaches of the estuary were sampled monthly in the middle of the stream (Fig. 1). Water  
36 31 sampling was done just below the surface using a 15-liter Niskin bottle at each sampling station,

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3 1 and the following environmental variables were measured: pH and temperature using a CONSORT  
4 2 C832 electrode and dissolved oxygen concentration (O<sub>2</sub>) (WTW OXI 325, equipped with Clark  
5 3 electrode).  
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9 4 Samples were taken for the determination of the concentrations of chlorine (Cl<sup>-</sup>),  
10 5 ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), orthophosphate (PO<sub>4</sub>-P) and silica (SiO<sub>2</sub>) within  
11 6 24h after sampling. They were stored at 4°C, and analyzed colorimetrically using a SKALAR SA  
12 7 5100 segmented flow analyzer, except for silica (SiO<sub>2</sub>), which was analyzed by ICP-OES (Iris®).  
13 8 Samples for the determination of dissolved organic carbon (DOC) were filtered on Gelman  
14 9 fiberglass filters of 0.45-µm porosity. For DOC determination, a preliminary treatment was  
15 10 performed consisting of H<sub>2</sub>SO<sub>4</sub> acidification, and flushing with nitrogen gas to remove background  
16 11 CO<sub>2</sub>. The DOC was then released by further oxidation to CO<sub>2</sub>, and analyzed using a SKALAR  
17 12 (phenolphthalein, detection at 550 nm). The 5-day biochemical oxygen demand (BOD<sub>5</sub>) was  
18 13 estimated using a WTW OXI 96 oxymeter. Suspended particulate matter (SPM) was determined  
19 14 gravimetrically after filtering on pre-combusted Whatman GF/F 0.7 µm filters which were dried at  
20 15 60°C. Water samples were filtered on 0.45 µm porosity filters and frozen for chlorophyll *a* (Chl *a*)  
21 16 determination. The Chl *a* was extracted by adding N,N-dimethylformamide, and then quantified  
22 17 colorimetrically at 647 and 664 nm (Van Damme *et al.*, 2005). Discharge data were obtained from  
23 18 the Flemish administration for waterways and maritime affairs (AWZ). For zooplankton sampling,  
24 19 50 liters of the surface water were collected in a bucket, and filtered through a 50 µm mesh.  
25 20 Carbonated water was added to the sample to narcotize the rotifers before fixing them with formalin  
26 21 at a final concentration of 4% (Siegfried *et al.*, 1989; Joaquim-Justo *et al.*, 2004).  
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#### 42 22 **Analyses of zooplankton samples**

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44 23 In the laboratory, samples were stained with 3–5 drops of erythrosine, prepared at 0.8 mg  
45 24 per 100 ml of water, to make it easier to detect the organisms in the detritus rich samples. After at  
46 25 least 12 hours, the samples were mixed thoroughly. A subsample was taken using a wide-bore  
47 26 syringe, and screened in a counting wheel using a Leica MZ 9.5 stereomicroscope (9x–90x).  
48 27 Specimens were counted and identified to species whenever possible. When necessary, they were  
49 28 mounted on a slide in glycerin and further observed with a microscope Nikon Optiphot-2 (50x–  
50 29 600x) using Differential Interference Contrast (DIC).  
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#### 58 30 **Data analysis**

59 31 Spatio-temporal trends in the rotifer community, and their relationships to some  
60 32 environmental variables (5-day Biological Oxygen Demand, chlorophyll *a*, chlorinity, ammonium,

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3 1 nitrite, nitrate, dissolved oxygen, pH, orthophosphates, dissolved silica, suspended particulate  
4 matter, temperature, dissolved organic carbon, discharge) were analyzed using multivariate  
5 2 statistics. The environmental factors used were those that had previously been shown to be  
6 3 important in structuring the Schelde zooplankton community (Tackx et al., 2004), most of which  
7 4 are known to have changed in recent years. Abundances of cladocerans, calanoids and cyclopoids  
8 5 were added as environmental factors, because of their possible negative (predation or competition)  
9 6 or positive (association) effect on rotifer distribution. The final dataset for these analyses, which  
10 7 included only the results of sampling for which there were no missing values, contained 154  
11 8 samples, 52 rotifer taxa and 17 environmental variables. The CANOCO software package, version  
12 9 4.5 (ter Braack, 1987; ter Braack, 1994) was used. Taxa abundance data were  $\log(x+1)$  transformed  
13 10 prior to the analysis to obtain a normal distribution. The modality of the taxa distribution was first  
14 11 analyzed by a Detrended Correspondence Analysis (DCA), using detrending by segments. As the  
15 12 total inertia observed was less than 2.6, a predominance of linear species response curves could be  
16 13 expected, and so we used redundancy analysis (RDA), a technique in which the ordination axes are  
17 14 constrained to be linear combinations of provided environmental variables to investigate the  
18 15 relationships between environmental factors and taxa composition. Data were centered and  
19 16 standardized by species prior to analysis. Forward selection of variables was used to select those  
20 17 most closely associated with the spatio-temporal structure of the rotifer community, and to quantify  
21 18 their relative importance. The statistical significance was tested with Monte Carlo permutation tests  
22 19 (499 unrestricted permutations) ( $p < 0.05$ ) and a Bonferroni correction for multiple test was applied.  
23 20 The minimum model so obtained explains the distribution without co-linear extra fitting. RDA  
24 21 biplots are shown using all environmental variables in order to show relations between the most  
25 22 important variables (minimum model) and the others. Moreover, the variation partitioning of the  
26 23 ordination was estimated as described in Borcard *et al.*, 1992 and Borcard and Legendre, 1994,  
27 24 using only the variables selected in the minimum model. Due to the curve shape of the estuary and  
28 25 for a better consideration of the connectivity between the stations in the specific case of an estuary,  
29 26 the distance to the mouth (km) was used as the spatial variable, considering a second degree  
30 27 polynomial, rather than the geographical coordinates.  
31 28

32 29 To test the relevance of the taxonomic level (species versus genus) in detecting the  
33 30 association between taxa and environmental factors, a data reduction to the genus level was made.  
34 31 This new dataset was then analyzed as described above, and the results were compared to those  
35 32 obtained at species level.  
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## Results

### *Taxonomic composition and abundance*

52 rotifer taxa, belonging to 26 genera, were identified. Most of the taxa belonged to the Monogononta (Table 1). About 42% (22 taxa) were new reports for the Schelde estuary. With the exception of *Keratella cruciformis* (Thompson, 1892), all taxa were detected in the freshwater reach, whereas only 28 taxa were found in the brackish water.

### *Spatio-temporal distribution*

As shown in Fig. 2, rotifer abundance was low during winter, and then increased up to its peak in May, reaching 2500 rotifers per liter. The summer abundances were lower, with a maximum of about 500 rotifers per liter between July and October. Considering the year as a whole, the most abundant species were *Brachionus calyciflorus* Pallas, 1766, *Keratella cochlearis* (Gosse, 1851) and *Brachionus angularis* Gosse, 1851, all three of which were found every month. *K. cochlearis* occurred mainly in July and August, when the two *Brachionus* species were less numerous.

From April to October, the abundances were much higher in the fresh water (stations at km 85 to 155) than in the brackish water (stations at km 57.5 to 78), whereas they were similar in these two segments in March and November (Fig. 2). In December, the same trend was observed but only 5 stations were sampled in freshwater. Unfortunately, data were lacking for some stations through the winter months.

### *Relationship with environmental variables: analysis at the species level*

For the rotifer community analysis at the species level, 52 taxa were considered in 154 samples. All environmental factors considered in the analysis contributed significantly ( $p < 0.05$ ) to explaining variability in the distribution of rotifers. Considering the Bonferroni correction for multiple test, 8 environmental factors remained significant: chlorinity, discharge, chlorophyll *a*, temperature, SiO<sub>2</sub>, cyclopoid abundance, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>. The marginal and conditional effects for each variable are shown in Table 2. When considering the importance of the variables themselves, without co-variability of other ones, as estimated by their conditional effects, chlorinity, discharge and cyclopoid abundance are the main factors influencing rotifer distribution (Table 2). The sum of all eigenvalues is 0.494 for analysis with all environmental variables or 0.435 with the minimum model. The first and second RDA axis had eigenvalues of 0.182 and 0.174 respectively, using all environmental variables; 0.179 and 0.169 respectively, in the minimum model. The sum of all axes

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3 1 accounted for 85.8% of the species variation using all environmental variables, and 93.6% when  
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5 2 using only the variables selected for the minimum model.

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7 3 This order corresponds to both the salinity and seasonal variations. The spatial partitioning  
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9 4 of the salinity (chlorinity) in our study zone becomes obvious when we plot the samples grouped by  
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11 5 station which show the brackish stations (km 58, 63, 71 and 78) spread out on the right hand side  
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13 6 and the remaining freshwater ones (km 85 to 151) grouped on the left hand side of the plot (Fig.  
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15 7 3a). The seasonal trend is clearly illustrated by the distribution of the samples in terms of the date  
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17 8 collected (Fig. 3b). In this graph we can clearly see the change along the temperature axis from the  
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19 9 winter months in the bottom right corner to the summer months in the top left corner (Fig. 4a).

20 10 The species – environmental factors biplot is shown in Fig. 4. Chlorinity, the most  
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22 11 important factor influencing the distribution of rotifer community, is located close to the first axis in  
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24 12 the upper right quadrant. Calanoid copepods, SPM and pH are situated in the right-hand, chlorine–  
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26 13 associated part of the plot. Discharge levels and oxygen concentration are found in the lower right  
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28 14 quadrant, opposite to the cyclopoid abundance and the temperature. Cyclopoid abundance,  
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30 15 chlorophyll *a* and temperature are all associated with the upper left of the ordination, together with  
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32 16 the cladoceran abundance and PO<sub>4</sub>-P concentration. To a lesser extent (non significant after the  
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34 17 Bonferroni correction), NO<sub>2</sub><sup>-</sup> concentration and DBO5 (lower left of the ordination) are all  
35  
36 18 negatively associated with chlorinity. The SiO<sub>2</sub>, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentration vectors are found  
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38 19 almost parallel to the second axis (lower half of the biplot).

39 20 When considering the species distribution in this biplot, the most abundant species are  
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41 21 mainly situated away from the center of the biplot (Fig. 4a), whereas the rare species are all  
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43 22 clustered near the center. Two haline rotifer species, *Synchaeta bicornis* Smith, 1904 and *Keratella*  
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45 23 *cruciformis*, are positively correlated with chlorinity. All the other taxa show greater affinity for  
46  
47 24 freshwater conditions. *Brachionus quadridentatus* Hermann 1783, *B. rubens* Ehrenberg. 1838,  
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49 25 *Pompholyx sulcata* Hudson, 1885, *Keratella tropica* (Apstein, 1907) and *Asplanchna brightwellii*  
50  
51 26 Gosse, 1850 are all positively correlated with high temperatures and chlorophyll *a* concentrations,  
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53 27 and negatively correlated to discharge and oxygen concentration, corresponding to summer  
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55 28 conditions in the top left corner of the biplot. In this quadrant we also find cyclopoid copepods and  
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57 29 cladocerans. The abundant species *B. calyciflorus*, *B. angularis* and *K. cochlearis*, and also *Filinia*  
58  
59 30 *longiseta* (Ehrenberg, 1834) and *K. quadrata* (Müller, 1786) are situated close to the NO<sub>2</sub><sup>-</sup> and  
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31 BOD5 vectors. *B. leydigii* Cohn, 1862, *A. priodonta* Gosse 1850, *Lecane* sp. and *Synchaeta* sp. are  
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33 correlated to high SiO<sub>2</sub>, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations, whereas *Euchlanis dilatata* Ehrenberg, 1832,

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3 1 *Notholca acuminata* (Ehrenberg, 1832) and *Epiphanes* sp. are positively correlated with the  
4 discharge levels.  
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7 3 The biplot of axes 1,3 completes the picture of the association of rotifer species with  
8 environmental factors (Fig. 4b). It essentially confirms the relationship shown in the axis 1,2 biplot  
9 and accentuates the importance of the cyclopid abundance which is, in this graph, the second most  
10 important vector. In this biplot discharge and oxygen concentration, as well as  $\text{SiO}_2$  and  $\text{NO}_3^-$  are  
11 located at the opposite side to  $\text{NH}_4^+$ .  
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17 8 The variation partitioning showed that the environmental variables used in the minimum  
18 model explain 43.8% of the variation in the species matrix, with 33.3% and 10.5% due to non  
19 spatial environmental and spatial environmental factors respectively (Fig. 5). Of the spatial species  
20 variation, 1.7% is not shared by the environmental variables. 54.5% of the variation is unexplained.  
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### 26 13 ***Comparison with analysis at the genus level***

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29 14 The RDA biplot of genus vs. environmental factors is shown in Fig. 6. All environmental  
30 factors tested were found to be significant at  $p < 0.05$  but only 6 remained significant considering the  
31 Bonferroni correction for multiple test. The sum of all eigenvalues is 0.474 for analysis with all  
32 environmental variables or 0.403 with the minimum model. This result is comparable to those  
33 obtained in the species level analysis (0.494 and 0.435 using all environmental variables or the  
34 minimum model). The sum of all the axes accounts for 86.8% of the genus variation using all  
35 environmental variables, and 97.1% when using only the variables selected for the minimum model.  
36 18 The main factors organizing distribution of rotifers considered at genus level are chlorinity and the  
37 discharge (Table 2), as was seen in the species data analysis. Other contributing factors are BOD5,  
38 19 temperature, silica and cyclopid abundance. These results are comparable to those found in the  
39 species level RDA, especially when considering the conditional effects of variables (Table 2).  
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49 25 Some examples illustrated in Fig. 4a,b and Fig. 6a,b allow us to compare results obtained in the  
50 species and genus level analyses. Genus *Asplanchna* is represented by two species. *A. brightwellii*, a  
51 26 summer species, is located in the top left corner of the axis 1,2 biplot (Fig. 4a, ringed). This species  
52 27 is positively associated with high temperatures and Chl *a* concentrations, and negatively with  
53 28 discharge and oxygen concentration. *A. priodonta* is situated at the opposite position to  
54 29 *A. brightwellii* (Fig. 4a, ringed), and is associated with  $\text{SiO}_2$ ,  $\text{NO}_3^-$ , DOC and  $\text{NH}_4^+$ . In the 1,3 biplot  
55 30 *A. priodonta*, which is located rather close to the origin on the first axis, shows little association  
56 31 with environmental factors. On the other hand, the positive correlation between *A. brightwellii* and  
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3 1 temperature, and its negative correlation with discharge levels and oxygen are confirmed. Both  
4 2 species reach similar maximum abundances, but *A. priodonta* was found more frequently, occurring  
5 3 practically throughout the entire period that *A. brightwellii* was absent (Fig. 2). When this analysis  
6 4 was performed using data reduced to the level of genus, *Asplanchna* occurred at a position  
7 5 comparable to that of *A. priodonta* in the 1,2 biplot, and at one comparable to that of *A. brightwellii*  
8 6 in the 1,3 biplot (Fig. 6a,b ringed).

9 7 The eight species of *Brachionus* display different distribution patterns. *B. rubens* is  
10 8 associated with cladoceran abundance, *B. quadridentatus* with high temperature and chlorophyll *a*  
11 9 concentration. *B. calyciflorus* is associated with  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and BOD5. *B. angularis* is positively  
12 10 correlated with BOD5 and negatively with chlorinity. On the other hand, *B. leydigii* shows affinities  
13 11 with  $\text{SiO}_2$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ; *B. urceolaris* Müller, 1773 with  $\text{NH}_4^+$ . *B. variabilis* Hempel, 1896 and  
14 12 *B. diversicornis* (Daday, 1883) are situated among the rare species quite close to the center, and do  
15 13 not seem to be greatly influenced by the factors being considered here. When we performed our  
16 14 analysis at the level of genus for *Brachionus* (Fig. 6, open squares) we found that the genus plot was  
17 15 situated at the extreme left of the second axis, positively correlated with BOD5 and  $\text{NO}_2^-$ , and  
18 16 negatively correlated with chlorinity. This position corresponds to the character of the most  
19 17 abundant *Brachionus* species we found (i.e. *B. calyciflorus* and *B. angularis*), but the associations  
20 18 between the less abundant species and other parameters are not clear.

21 19 Six species of *Keratella* occur in the estuary (Fig. 4, open triangles). *K. cruciformis*, a haline  
22 20 species, is located at the top right hand corner of the plot, and is positively associated with  
23 21 chlorinity. *K. valga* (Erhenberg, 1834), situated quite close to the origin, next to the second axis  
24 22 (upper half), does not show any trend relative to the environmental factors considered. *K. testudo*  
25 23 (Erhenberg, 1832) is situated in the bottom left quadrant on the  $\text{NH}_4^+$  vector, *K. quadrata* and  
26 24 *K. cochlearis* are associated with high BOD5 values and  $\text{NO}_2^-$  concentration, and *K. tropica* with  
27 25 cyclopid abundance and temperature. When we performed our analysis at the level of genus,  
28 26 *Keratella* (Fig. 6, open triangles) was found on the left side of the ordination plot, strongly  
29 27 associated with  $\text{NO}_2^-$  concentration and BOD5. This position corresponds fairly closely to the  
30 28 'mean' for the most abundant species (*K. cochlearis*, *K. quadrata* and *K. tropica*), but does not  
31 29 reflect that of the haline species *K. cruciformis*. Also *K. testudo*, a species which is not associated  
32 30 with the typical left side conditions of high  $\text{NO}_2^-$ , and BOD5, Chl *a* and temperature values, is not  
33 31 well represented by this position of the genus.

32 32 Concerning the variance partitioning, the environmental variables considered in the  
33 33 minimum model explain 40.4% of the variation in the species matrix, with 31.1% and 9.3% due to

1 non spatial environmental and spatial environmental factors respectively (Fig. 5). Spatial genus  
2 variation that is not shared by the environmental variables is negligible with only 0.2%. As  
3 observed with the species level analysis, a large part of the variation (59.4%) is unexplained.

#### *Diversity at the species level*

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The rotifer taxonomic richness,  $R$ , was generally much lower in the brackish zone (0 – 8 taxa) than in freshwater zone (8 – 16 taxa), except during the winter, when the value of  $R$  was similar (8 – 10 taxa) throughout the study area (Fig. 7a). Rotifer diversity, according to the Shannon diversity index,  $H'$ , (Fig. 7b) and its evenness,  $E_{H'}$ , (Fig. 7c), was low in the brackish zone from spring to autumn. The highest values of  $H'$  and  $E_{H'}$  both occurred in winter at all the stations and from early summer till winter just upstream from the brackish zone (km 78 to 120).

#### *Comparison with analysis at the genus level*

It can be seen that taxonomic richness follows a very similar course over time and space, whether calculated at species or at genus level (Fig. 8). Similar trends are observed for  $H'$  and  $E_{H'}$  indices (not shown). Pearson's correlation coefficients between  $R$ ,  $H'$  and  $E_{H'}$  indices calculated from species and genus level data were 0.88, 0.91, and 0.92 respectively ( $p < 0.01$ ).

#### *Correlation between the diversity indices and environmental factors*

The correlation between diversity indices obtained from both datasets is given in Table 3. 28 significant correlations were observed at the species level, versus 31 at the genus level. Most, but not all, of the correlations revealed (70%) concern the same indices and factors. The species level, for example, shows a correlation between  $R$  and  $E_{H'}$  and SPM, which is not detected at genus level. On the other hand, more significant correlations are detected with temperature, DOC and discharge when using the genus-level instead of the species-level dataset.

## **Discussion**

### *Taxonomic composition*

The first aim of this study was to inventory the rotifer taxonomic composition of the Schelde estuary. The taxonomic list for the Schelde estuary has increased since previous studies (De

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3 1 Pauw, 1975; Tackx *et al.*, 2004), with 22 new taxa being reported. The main reason for this increase  
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5 2 was probably the taxonomic effort made in this study, although introduced species are commonly  
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7 3 observed in areas with heavy shipping activity such as the Schelde estuary (Carlton, 1996; Johnson  
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9 4 and Padilla, 1996; Ruiz *et al.*, 2000; Wasson *et al.*, 2001).

10  
11 5 In the Schelde estuary as in other estuaries (Holst *et al.*, 1998; Park and Marshall, 2000;  
12  
13 6 Rougier *et al.*, 2005; Lam-Hoai *et al.*, 2006), rotifers originating from freshwater are dominant. The  
14  
15 7 rare *Synchaeta bicornis* and *Keratella cruciformis* are the only typically brackish rotifers (Koste,  
16  
17 8 1978; Holst *et al.*, 1998; Segers, 2007) found in our samples.

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19 9 The rotifer abundance observed in the Schelde is about the same as that observed in the  
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21 10 Elbe estuary (Holst *et al.*, 1998), the nearest estuary for which rotifer data are available. Moreover,  
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23 11 most species are found in both these estuaries, exhibiting similar temporal patterns. The most  
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25 12 abundant species occurring in the Schelde estuary (*B. calyciflorus*, *B. angularis* and *K. cochlearis*)  
26  
27 13 are cosmopolitan planktonic species (Pontin, 1978; De Ridder and Segers, 1997). *K. cochlearis* and  
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29 14 *K. quadrata* are considered to be generalist rotifers, feeding on bacteria, detritus and flagellates  
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31 15 (Pourriot, 1977; Starkweather and Bogdan, 1980; Arndt, 1993), and as a consequence are well  
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33 16 adapted to high concentrations of suspended particulate matter (SPM). The genera *Cephalodella*,  
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35 17 *Colurella*, *Lecane*, *Lepadella*, are generally benthic or periphytic (Pontin, 1978; Nogrady *et al.*,  
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37 18 1995; Segers, 1995; Duggan, 2001). As expected, they were rare in our surface samples. *Keratella*  
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39 19 *tropica*, *Brachionus variabilis* and *Lecane decipens* (Murray, 1913) are the only exotic rotifers  
40  
41 20 observed in the Schelde. Their occurrence and ecology has been detailed in Azémar *et al.* (2007).  
42

#### 43 22 ***Relationship between rotifer species distribution and environmental variables***

44  
45 23 Earlier analyses of the distribution of the Schelde zooplankton community have shown  
46  
47 24 salinity and temperature to be the main structuring factors for the total zooplankton community  
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49 25 (Soetaert and Van Rijswijk, 1993; Tackx *et al.*, 2004). Both these reports considered rotifers as a  
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51 26 group. Our study shows that, also for the rotifer community, salinity (chlorinity) is the main  
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53 27 structuring (spatial) factor (Fig. 4, Fig. 5, Fig. 6). Temperature is less important in explaining rotifer  
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55 28 community structure and is preceded by other typically seasonal factors such as discharge and Chl  
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57 29 *a*. Moreover, considering the Bonferroni correction, temperature is not significant while cyclopoids,  
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59 30 mainly present during warm season, are the third environmental parameter structuring the rotifer  
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31 distribution. This might be explained by the fact that, while the mean temperature over the entire

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3 1 transect varies between 4.2 and 23.6°C in time, it varies maximally 2.6°C between stations within  
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5 2 each sampling campaign.

6  
7 3 Besides the seasonal aspect, the effect of the discharge level can also be explained through  
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9 4 the positioning of the salinity gradient. In winter, from November till March, when the freshwater  
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11 5 flow is greatest, the rotifer population seems to be displaced downstream (Fig. 2). No difference in  
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13 6 rotifer abundance was observed between the so called freshwater and brackish water reaches during  
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15 7 this period, in contrast to the differences reported in other temperate estuaries or in tropical estuaries  
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17 8 during the rainy season (Holst *et al.*, 1998; Park and Marshall, 2000; Rougier *et al.*, 2005; Lam-  
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19 9 Hoai *et al.*, 2006). However, during high discharge periods, the entire transect sampled in this study  
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21 10 consisted of freshwater (< 0.5 PSU). Unfortunately, rotifers were not studied further downstream, in  
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23 11 the Dutch part of the estuary. More complete data are required before we can attempt to  
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25 12 characterize the rotifer community and its geographical distribution during winter.

26  
27 13 The importance of Chl *a* and SiO<sub>2</sub> as structuring variables of the rotifer community can be  
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29 14 explained by their higher concentrations in the upstream part of the transect than the downstream  
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31 15 part, as well as their seasonal variations. (Fig. 2; Fig. 4). Species such as *B. quadridentatus* and  
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33 16 *K. cochlearis*, which are associated with high Chl *a* concentrations (Fig. 4), are known to be  
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35 17 herbivorous, and hence are likely to benefit from high phytoplankton concentrations (Reynolds,  
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37 18 1984; Hlawa and Heerkloss, 1994; Heerkloss and Hlawa, 1995). Effect of temperature on rotifer  
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39 19 species composition and abundance, whether directly through its physiological effect or indirectly  
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41 20 through its association with the phytoplankton growth season, is clear from Fig. 2.

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43 21 The fact that cladoceran and cyclopoid copepod populations also peak during summer  
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45 22 (Azémar, unpublished data) explains their association with temperature-related rotifer taxa,  
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47 23 although populations of some rotifers are probably directly correlated to the crustacean abundance.  
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49 24 For instance, *Brachionus rubens* is known to be an epizoic rotifer associated with cladocerans  
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51 25 (Galliford, 1953; May, 1989; Iyer and Rao, 1993). The introduced *B. variabilis* which is  
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53 26 occasionally found in Belgium (Dumont, 1983; Azémar *et al.*, 2007) also has been reported to be  
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55 27 epizoic but less frequently (May, 1989). Both *Brachionus* species are associated with the cladoceran  
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57 28 abundance vector in our analysis. Some specimens of *B. rubens* were observed fixed on *Daphnia*  
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59 29 spp. bodies during the sample analysis. The small number of such observations is probably due to  
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61 30 the mechanical effect of the filtration process, and the addition of the fixative.

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3 1 All of the environmental factors considered contribute significantly to explaining the  
4 2 spatio-temporal distribution of rotifers in the Schelde, at both the species and the genus levels but  
5 3 the Bonferroni correction limits this significance to respectively 8 and 6 main factors. This is due, in  
6 4 part, to the colinearity between the factors, most of which change gradually across the estuarine  
7 5 chlorinity distribution. This colinearity is well shown by differences between marginal and  
8 6 conditional effects (Table 2): after having taken into account the variance explained by the 2 first  
9 7 variables (Cl<sup>-</sup> and discharge), the variance explained by following variables is considerably reduced  
10 8 in the conditional effect.

11 9 The variables considered here are those that have been chosen for the routine monitoring of  
12 10 the restoring Schelde estuary, because of they are known to represent water quality (Van Damme *et*  
13 11 *al.*, 2005). Moreover, these environmental factors have all changed as a consequence of the ongoing  
14 12 restoration process, and are likely to continue changing in the future. Thus, for the practical purpose  
15 13 of advising those managing the estuary, our findings do make it possible to find out which taxa are,  
16 14 for example, favored by high Chl *a* concentrations, or those that are not hindered by high NH<sub>4</sub><sup>+</sup>  
17 15 concentrations. These factors are, to some extent, manageable.

### 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 17 ***Relationships with environmental variables: comparison of species and genus level***

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35 19 The analyses performed with the dataset reduced to the genus level (Fig. 6) and using all  
36 20 the environmental variables generally showed the same trends as those of the most abundant  
37 21 species. The sum of all eigenvalues (0.474 and 0.494 at the genus and the species level respectively)  
38 22 and the variation explained in the dataset (86.8% and 85.8% at the genus and the species level  
39 23 respectively) were also comparable in both cases. In our data, genera were often represented by one  
40 24 dominant species accompanied by a few other relatively rare ones. As shown by the examples of  
41 25 *Brachionus* and *Keratella*, the genus position in the ordination plot in these cases corresponds  
42 26 closely to that of the most abundant species within the genus considered. However, the position  
43 27 found for the genus does not give an adequate picture of the association between the less abundant  
44 28 species, or those that are only present for a short period, and environmental factors. The example of  
45 29 the genus *Asplanchna*, illustrates this observation. In this case, the two species had similar  
46 30 abundance, but their mean value did not adequately represent either.

47 31 In our study, however, the use of the genus level as surrogate for the species, while  
48 32 inevitably resulting in a loss of ecological information, particularly pertaining to the less abundant

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3 1 or rare species, does not substantially change the general pattern of the spatio-temporal distribution  
4 2 of the rotifer community as a function of environmental factors. Comparison between the variation  
5 3 partitioning of the data considered at the species and the genus level do not show noticeable  
6 4 differences. The spatially linked environmental variation explained is relatively small (10.5% at the  
7 5 species level). This is probably due to the absence of real gradients in the distribution of the  
8 6 environmental factors measured all along the transect. In fact, most variables vary differently inside  
9 7 the small brackish area (3 stations) and the extensive freshwater area (13 stations) (Van Damme *et*  
10 8 *al.*, 2005).

11 9  
12 10 Considering the taxonomic richness,  $R$ , (Fig. 7a), the area investigated can also be divided  
13 11 into two zones, corresponding to the brackish-water and the freshwater zones respectively. Fairly  
14 12 homogeneous values of richness are observed within both zones. Except during winter, the rotifer  
15 13 community in the brackish zone of the Schelde is less diverse than that in the freshwater zone. Few  
16 14 species are able to cope with the wide variations of environmental factors in the brackish zone  
17 15 (Remane and Schlieper, 1958). In the Schelde, the most abundant rotifers occurring in the brackish  
18 16 water zone consist of a few tolerant freshwater species (*B. calyciflorus*, *B. quadridentatus*, *K.*  
19 17 *cochlearis*, *K. quadrata*). As most rotifers currently live in freshwater and simply follow the  
20 18 current, they die as salinity values increase. Within the freshwater zone, in contrast to the  
21 19 homogeneity of the taxonomic richness, both Shannon's diversity index ( $H'$ , Fig. 7b) and evenness  
22 20 ( $E_{H'}$ , Fig. 7c) display some noticeable differences in the relative abundance of taxa between the  
23 21 upper and lower freshwater reaches. The upper reach of the freshwater tidal zone (km 78-105) is  
24 22 dominated by few taxa ( $H'$  generally below 0.6, and  $E_{H'}$  below 0.4). In comparison, the downstream  
25 23 reach of the freshwater zone has about the same richness  $R$  but shows higher values of  $H'$  and  $E_{H'}$   
26 24 (except in spring). Moreover, rotifers are numerically less abundant in the lower reach of this  
27 25 freshwater zone than further upstream (Fig. 2). This reach often corresponds to a maximum  
28 26 turbidity zone (MTZ) (Baeyens *et al.*, 1998; Herman and Heip, 1999; Chen *et al.*, 2005; Meire *et*  
29 27 *al.*, 2005; Van Damme *et al.*, 2005) where the concentration of particulate matter and pollutants  
30 28 generate restricting ecological conditions (Soetaert and Van Rijswijk, 1993; Van Damme *et al.*,  
31 29 2005).

32 30  
33 31 As shown in Fig. 8, richness measured at the genus or species level followed a very similar  
34 32 trend over space and time. This was also the case for  $H'$  and  $E_{H'}$ . In our dataset, 9 out of 27 genera  
35 33 were plurispecific, and 18 monospecific. However, the plurispecific genera contained 64% of the  
36 34 species observed. The monospecific genera include 10 cases in which the species could not be  
37 35 identified, so we cannot be certain that they were indeed monospecific. The co-variation of diversity  
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3 1 measured at species and genus level in our dataset is partly due to the fact that calculating diversity  
4 2 at the species and genus level produced the same result for 36% of the species. It is difficult to say  
5 3 whether this type of rotifer population composition is common in estuaries. The taxonomic  
6 4 resolution used by Holst (Holst *et al.*, 1998) in the study on the Elbe rotifers is different from ours  
7 5 for some genera. These authors used non fixed samples, which enabled them to identify more non-  
8 6 loricate species.

13  
14 7 As diversity at both taxonomic levels varies over both time and space, it is not surprising to  
15 8 find that the indices obtained from both taxonomic levels also generally correlate significantly with  
16 9 the same environmental factors (Table 3). As we have already mentioned, most of the  
17 10 environmental factors considered in Table 3 can be expected to continue to change with the ongoing  
18 11 restoration of the Schelde estuary. Diversity is typically of interest in water management, and so  
19 12 compiling an inventory rotifer diversity offers relevant information related to the evolution of  
20 13 environmental variables. The fact that this can be done at the genus level facilitates including the  
21 14 rotifer community in routine monitoring programs.

28  
29 15 In general, the feasibility of higher taxa as surrogates for species-level patterns appears to  
30 16 depend mainly on the taxonomic composition of the taxonomic group considered. In the case of the  
31 17 marine benthic invertebrates, Maurer (2000) judges that loss of ecological information resulting  
32 18 from the use of Taxonomic Sufficiency (TS) make this method unacceptable even though it does  
33 19 save cost. The exclusion of rare species has serious effects on general ecological observations and  
34 20 theory, and runs counter to current biodiversity assessment and bio indicator research (Maurer,  
35 21 2000). From this point of view, the use of the coarser identification level is not appropriate, or  
36 22 should at least be limited to well explored areas, where the biodiversity has already been well  
37 23 documented (Quijón and Snelgrove, 2006). The time saved by identifying organisms to a coarser  
38 24 level depends on the number of species within each group, on whether the numerically dominant  
39 25 species belong to several taxonomically complicated groups or not, and on the level of taxonomic  
40 26 expertise available (Dauvin *et al.*, 2003). In estuarine environments, the high concentration of  
41 27 suspended particulate matter poses a serious problem for studying plankton samples, and rotifers in  
42 28 particular. Finding small transparent animals in the samples is very difficult, even after staining. In  
43 29 the freshwater part of the Schelde estuary, the SPM consists mainly of organic matter, so that it is  
44 30 not possible to use ludox separation of the organisms from the heavier fraction as applied to  
45 31 zooplankton samples by Soetaert and Van Rijswijk (1993) in the brackish zone of the estuary. On  
46 32 the other hand, once they are found within the suspended matter, an experienced person using a  
47 33 good stereomicroscope can identify most rotifers present up to species level without major

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3 1 problems. Nevertheless, the rotifer classification is largely perfectible: all areas are not equally  
4 2 intensively studied so literature is not consistent everywhere. Identifications are usually based on  
5 3 morphology which is insufficient for numerous group or species as attested by molecular studies  
6 4 (Fontaneto *et al.*, 2007; Fontaneto *et al.*, 2008; Fontaneto *et al.*, 2009; Kaya *et al.*, 2009). Thus, the  
7 5 *Brachionus* and *Keratella* from the Schelde can be identified at the species level at a glance, but one  
8 6 should bare in mind the existence of species complexes, such as, for example, *Brachionus plicatilis*,  
9 7 *Keratella cochlearis*. Most of the rotifers in the estuary are loricated. The surface ornamentation of  
10 8 the lorica is of taxonomic importance (Wallace *et al.*, 2006), because they include traits that are  
11 9 visible even on fixed samples. Soft bodied rotifers, such as *Synchaeta*, *Rotaria* and other Bdelloids,  
12 10 once fixed with formalin often have their corona and foot retracted, making it difficult to identify  
13 11 them even to genus level. Live samples or scanning electron microscopy (SEM) would be required  
14 12 to identify them, which would considerably increase the methodological constraints for monitoring  
15 13 purposes (Hollowday, 2002).

16 14 From this study of the rotifer population in the context of the restoration of the Schelde  
17 15 estuary, we conclude that, in such studies, it is very informative but not essential to start with a  
18 16 species-level inventory. A lack of taxonomic competence for species-level identification should not  
19 17 prevent scientists from monitoring estuarine rotifer populations. At least in the case of the Schelde  
20 18 estuary, most of the ecological information (community structure as a function of spatio-temporal  
21 19 variations in environmental conditions, the relationship between diversity and changes in  
22 20 environmental factors) are also obtained when using the genus level analysis.

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**Table and Figure legends**

Table 1. Taxonomic composition of the rotifer fauna of the Schelde estuary. The designation codes used for the multivariate analyses are shown in bold. Symbols: \* - new taxa for the Schelde; b - taxa present in the brackish water zone; f - taxa present in the freshwater zone.

Table 2. Variance explained by the environmental variables tested with the rotifer dataset at the species and the genus level. Variables were considered using the complete set or only the most significant ones, after a Bonferroni correction (minimum model). Marginal and conditional effects are shown.

Table 3. Significance of Spearman rank correlations obtained between R, H' and E<sub>H'</sub> from both datasets (i.e. species and genus level) with environmental factors (indicated with the abbreviations used for following analyses). \*: significant at p<0.05. \*\*\*: significant at p<0.01. Dark gray: significance detected with both datasets; dark shading: significance detected with the species dataset only. Light gray: significance detected with the genus level dataset only.

Fig. 1. Map of the Schelde estuary indicating the marine, brackish and freshwater stretches, and the positions of the sampling stations (white circles). Stations are denoted according to their distance from the mouth at Vlissingen in km.

Fig. 2. Spatio-temporal distribution of rotifers in the Schelde estuary. The abscissa shows the distance from the mouth of the estuary (in km). The left ordinate indicates the rotifer abundance (bars); the right ordinate indicates the chlorinity (line). ND: no data available.

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3 1 Fig. 3. RDA biplot, axis 1,2, showing the distribution of the samples grouped by station location (a)  
4 and by date (b), the number indicating the month. See Fig. 4a for the position of the environmental  
5 2 factors vectors.  
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11 5 Fig. 4. RDA biplot a) axis 1,2; b) axis 1,3 showing the distribution of the rotifers at species level in  
12 the Schelde estuary as a function of the environmental parameters. Most significant environmental  
13 6 variables (minimum model) are shown as full arrows. Other variables are shown as dotted arrows.  
14 7 See Table 1 for nomenclature of species, and Table 3 for the abbreviations of the environmental  
15 8 factors.  
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23 11 Fig. 5. Variation partitioning of the rotifer data matrix at the species and genus level considering  
24 12 environmental variables selected in the minimum model, using the distance to the mouth as typical  
25 13 spatial factor.  
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31 15 Fig. 6. RDA biplot a) axis 1,2 and b) axis 1,3 showing the distribution of the rotifers at genus level  
32 16 in the Schelde estuary as a function of the environmental parameters. Most significant  
33 17 environmental variables (minimum model) are shown as full arrows. Other variables are shown as  
34 18 dotted arrows. See Table 1 for nomenclature of genera, and Table 3 for the abbreviations of  
35 19 environmental factors.  
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42 21 Fig. 7. Distribution of a) the taxonomic richness (R), b) Shannon's diversity index (H') and c) the  
43 22 evenness ( $E_H$ ) in the estuary as a function of the distance from the mouth (at Vlissingen) and of time  
44 23 (in months).  
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51 25 Fig. 8. Species richness, R, calculated from species- (black diamonds) and from genus- (open  
52 26 squares) level data along the transect sampled for each month of the year.  
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1 Table 1

2

<b>Monogononta</b>			
<i>Anuraeopsis</i> sp.	<b>Axx</b> * f	<i>Keratella valga</i> (Ehrenberg, 1834)	<b>Kva</b> f
<i>Asplanchna brightwellii</i> Gosse, 1850	<b>Abr</b> b f	<i>Lecane bulla</i> (Gosse, 1851)	<b>Lbu</b> * b f
<i>Asplanchna priodonta</i> Gosse 1850	<b>Apr</b> b f	<i>Lecane closteroerca</i> (Schmarda, 1859)	<b>Lcl</b> * f
<i>Brachionus angularis</i> Gosse, 1851	<b>Ban</b> b f	<i>Lecane decipiens</i> (Murray, 1913)	<b>Lde</b> * f
<i>Brachionus calyciflorus</i> Pallas, 1766	<b>Bca</b> b f	<i>Lecane flexilis</i> (Gosse, 1886)	<b>Lfl</b> f
<i>Brachionus diversicornis</i> (Daday, 1883)	<b>Bdi</b> * f	<i>Lecane hamata</i> (Stokes, 1896)	<b>Lha</b> * f
<i>Brachionus leydigii</i> Cohn, 1862	<b>Bley</b> * b f	<i>Lecane luna</i> (Müller, 1776)	<b>Llu</b> * f
<i>Brachionus quadridentatus</i> Hermann 1783	<b>Bqu</b> b f	<i>Lecane</i> sp.	<b>Lxx</b> b f
<i>Brachionus rubens</i> Ehrenberg, 1838	<b>Bru</b> f	<i>Lepadella ovalis</i> (Müller, 1786)	<b>Lov</b> f
<i>Brachionus urceolaris</i> Müller, 1773	<b>Bur</b> b f	<i>Notholca acuminata</i> (Ehrenberg, 1832)	<b>Nac</b> b f
<i>Brachionus variabilis</i> Hempel, 1896	<b>Bva</b> f	<i>Platylas quadricornis</i> (Ehrenberg, 1832)	<b>Pqu</b> * b f
<i>Cephalodella</i> sp.	<b>Cex</b> f	<i>Ploesoma</i> sp.	<b>Plx</b> * f
<i>Colurella</i> sp.	<b>Cox</b> f	<i>Ploesoma hudsoni</i> (Imhof, 1891)	<b>Phu</b> * f
<i>Epiphanes</i> sp.	<b>Epx</b> b f	<i>Polyarthra</i> sp.	<b>Pox</b> b f
<i>Euchlanis dilatata</i> Ehrenberg, 1832	<b>Edi</b> b f	<i>Pompholyx sulcata</i> Hudson, 1885	<b>Psu</b> * b f
<i>Filinia brachiata</i> (Rousselet, 1901)	<b>Fbr</b> f	<i>Rhinoglena frontalis</i> Ehrenberg, 1853	<b>Rfr</b> * b f
<i>Filinia longiseta</i> (Ehrenberg, 1834)	<b>Flo</b> b f	<i>Synchaeta</i> sp.	<b>Sxx</b> b f
<i>Gastropus hyptopus</i> (Ehrenberg, 1838)	<b>Ghy</b> * b f	<i>Synchaeta bicornis</i> Smith, 1904	<b>Sbi</b> * b f
<i>Hexarthra</i> sp.	<b>Hxx</b> f	<i>Testudinella</i> sp.	<b>Tex</b> f
<i>Kellicottia longispina</i> (Kellicott, 1879)	<b>Klo</b> * b f	<i>Testudinella patina</i> (Hermann, 1783)	<b>Tpa</b> * f
<i>Keratella cochlearis</i> (Gosse, 1851)	<b>Kco</b> b f	<i>Trichocerca pusilla</i> (Jennings, 1903)	<b>Tpu</b> f
<i>Keratella cruciformis</i> (Thompson, 1892)	<b>Kcr</b> * b	<i>Trichocerca similis</i> (Wiersejski, 1886)	<b>Tsi</b> f
<i>Keratella quadrata</i> (Müller, 1786)	<b>Kqu</b> b f	<i>Trichocerca</i> sp.	<b>Trx</b> f
<i>Keratella testudo</i> (Ehrenberg, 1832)	<b>Kte</b> * b f	<i>Trichotria tetractis</i> (Ehrenberg, 1830)	<b>Tte</b> * f
<i>Keratella tropica</i> (Apstein, 1907)	<b>Ktr</b> b f		
<b>Bdelloidea</b>			
<i>Dissotrocha</i> sp.	<b>Dxx</b> * f	<i>Rotaria neptunia</i> (Ehrenberg, 1832)	<b>Rne</b> f
<i>Rotaria</i> sp.	<b>Rox</b> b f	Other Bdelloids	<b>Bdel</b> b f

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4 1 **Table 2**  
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6 27 **All variables**

SPECIES				SPECIES			
Marginal Effects		Marginal Effects		Marginal Effects		Marginal Effects	
Variable	Variable	Variable	Variable	Variable	Variable	Variable	Variable
CL	0,15	CL	0,15	CL	0,18	CL	0,18
Disch.	0,12	Disch.	0,13	Disch.	0,11	Disch.	0,11
O2	0,11	Cyclo.	0,06	DBO5	0,10	Cyclo.	0,05
Chl a	0,11	SiO2	0,03	NO2	0,10	SiO2	0,03
T	0,10	NH4	0,02	O2	0,10	DBO5	0,02
BOD5	0,10	Chl a	0,02	T	0,09	T	0,01
NO2	0,10	T	0,01	Chl a	0,09	Chl a	0,01
SiO2	0,10	NO3	0,01	NH4	0,08	NO2	0,01
PO4	0,09	NO2	0,01	SiO2	0,08	NH4	0,01
Cyclo.	0,08	PO4	0,01	Cycl.	0,08	O2	0,01
NH4	0,07	DOC	0,01	PO4	0,07	NO3	0,00
NO3	0,05	O2	0,01	DOC	0,05	DOC	0,01
Clado.	0,04	pH	0,00	NO3	0,04	Clado.	0,01
DOC	0,04	Clado.	0,01	pH	0,04	pH	0,00
pH	0,04	BOD5	0,01	Clado.	0,04	PO4	0,01
Cala.	0,03	Cala.	0,00	Cala.	0,04	SPM	0,00
SPM	0,02	SPM	0,00	SPM	0,03	Cala.	0,00

30  
31 **Minimum model**

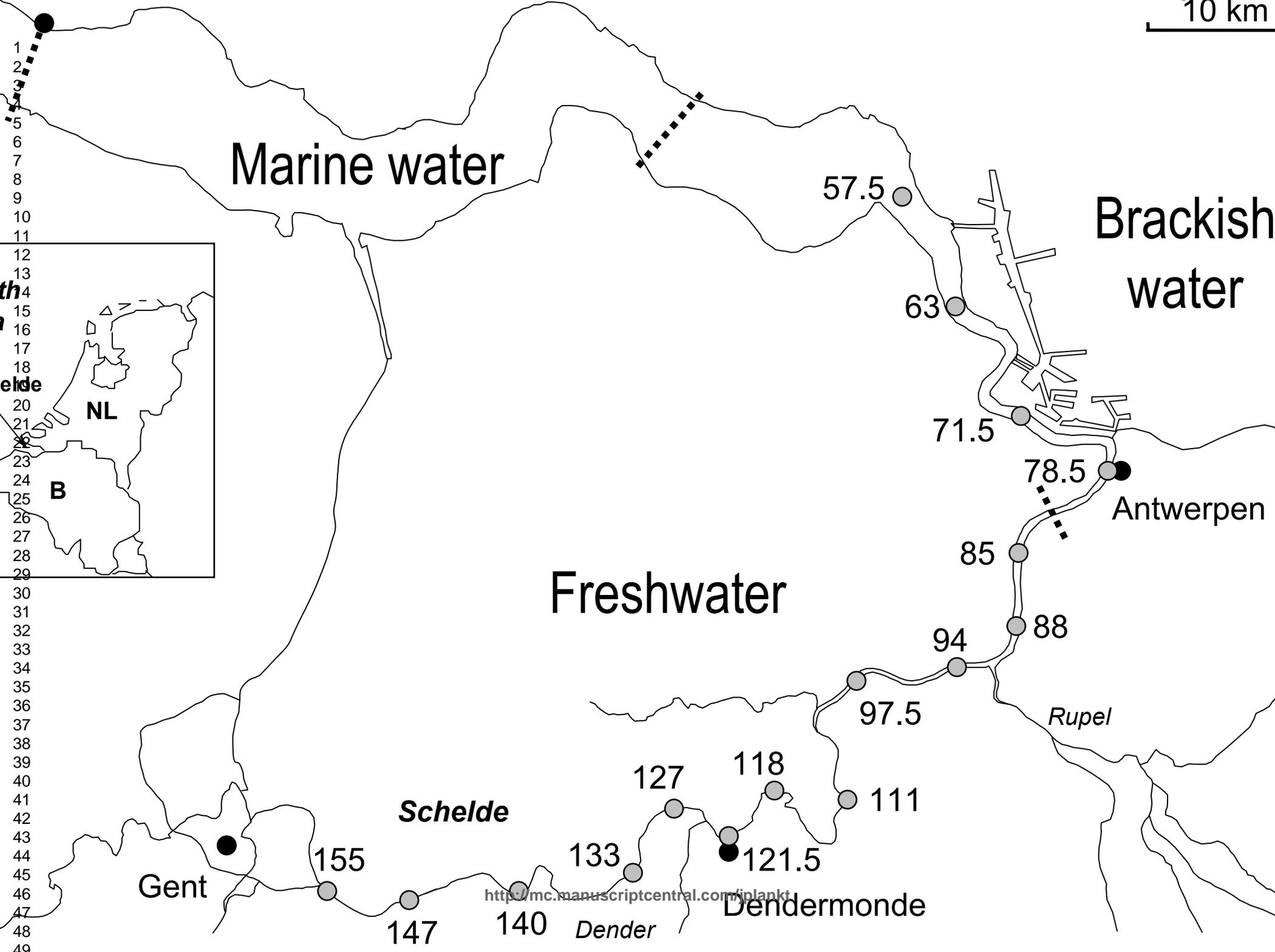
SPECIES				GENUS			
Marginal Effects		Conditional Effects		Marginal Effects		Conditional Effects	
Variable	Lambda1	Variable	LambdaA	Variable	Lambda1	Variable	LambdaA
CL	0,15	CL	0,15	CL	0,18	CL	0,18
Disch.	0,12	Disch.	0,13	Disch.	0,11	Disch.	0,11
Chl a	0,11	Cyclo.	0,06	BOD5	0,10	Cyclo.	0,05
T	0,10	SiO2	0,03	T	0,09	SiO2	0,03
SiO2	0,10	NH4	0,02	SiO2	0,08	BOD5	0,02
Cyclo.	0,08	Chl a	0,02	Cyclo.	0,08	T	0,01
NH4	0,07	T	0,01				
NO3	0,05	NO3	0,01				

1 Table 3

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	SPECIES			GENUS		
	R	H'	E <sub>II</sub>	R	H'	E <sub>II</sub>
5-day Biological Oxygen Demand (BOD5)	***		***	***		***
Chlorophyll <i>a</i> (Chl <i>a</i> )	***		***		***	***
Chlorinity (CL)	***	***	***	***	***	***
Ammonium (NH <sub>4</sub> <sup>+</sup> )	***		***	***		***
Nitrite (NO <sub>2</sub> <sup>-</sup> )	***		***	***		***
Nitrate (NO <sub>3</sub> <sup>-</sup> )	***		***	***		*
Dissolved Oxygen (O <sub>2</sub> )			***		*	***
pH	***	***				
Orthophosphate (PO <sub>4</sub> -P)	***		***	***	***	***
Disolved Silica (SiO <sub>2</sub> )	***	***		***	*	
Suspended Particulate Matter (SPM)	*		*			
Temperature (T)		*		***	***	*
Dissolved Organic Carbon (DOC)	***	***		***	***	*
Discharge (Disch.)		***		***	***	***
Cladoceran abundance (Clado.)	***		***	***		***

3



Marine water

Brackish water

Freshwater

Antwerpen

Gent

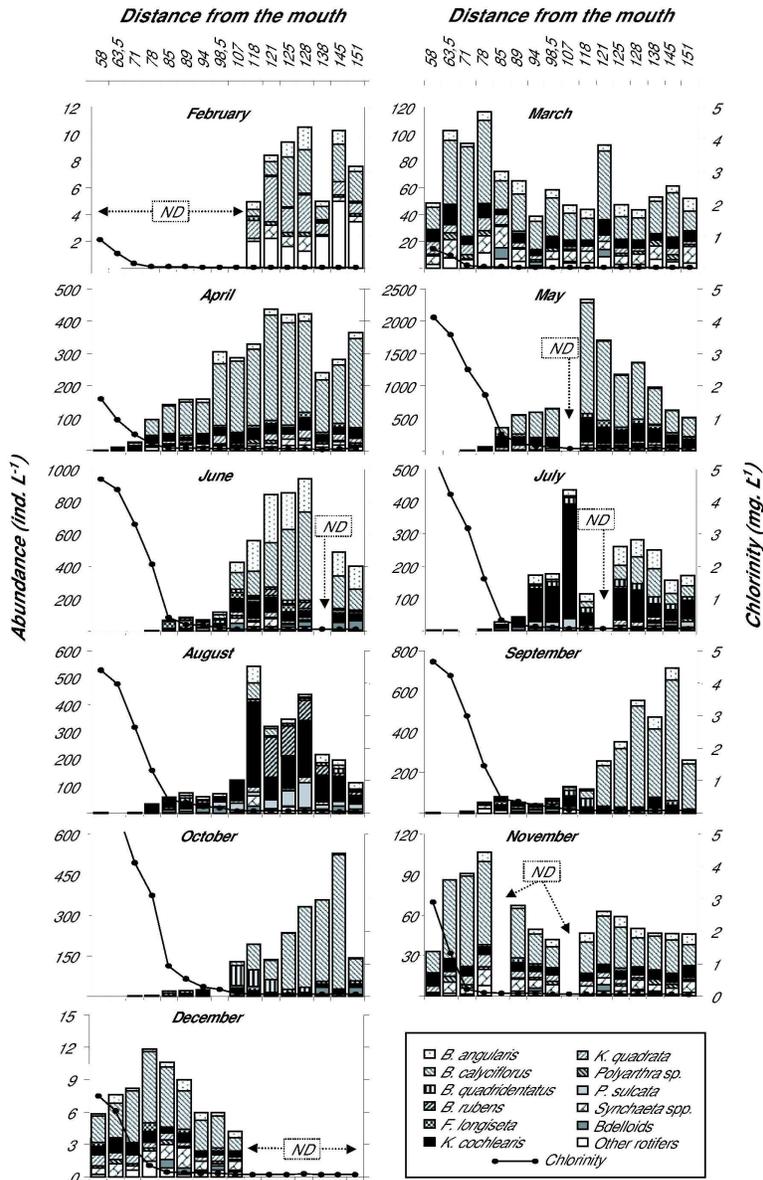
Schelde

Dender

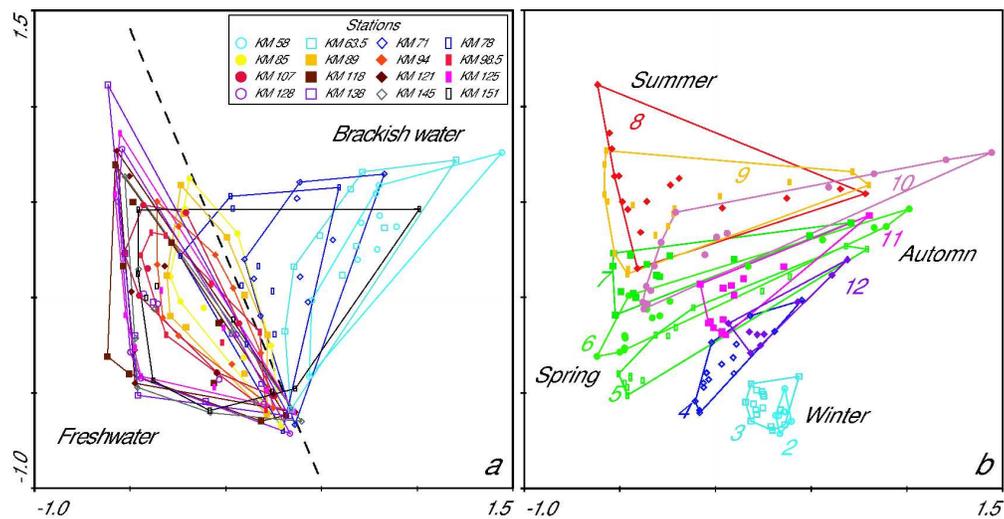
Dendermonde

Rupel

http://mc.manuscriptcentral.com/jplankt



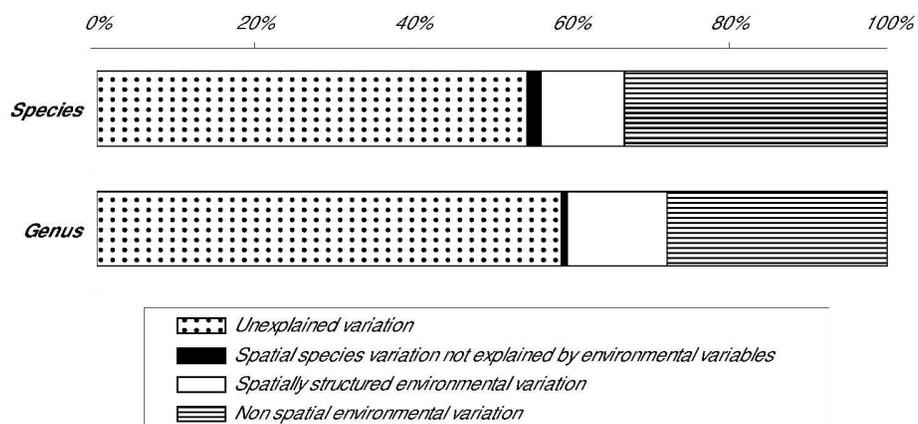
Spatio-temporal distribution of rotifers in the Schelde estuary.  
152x237mm (600 x 600 DPI)



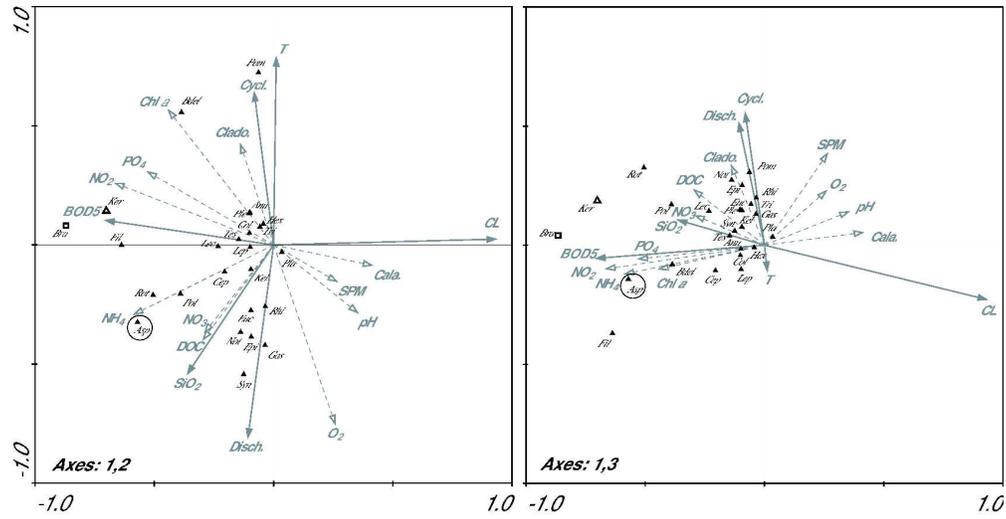
RDA biplot showing the distribution of the samples grouped by station location and by date  
286x147mm (600 x 600 DPI)

Peer Review



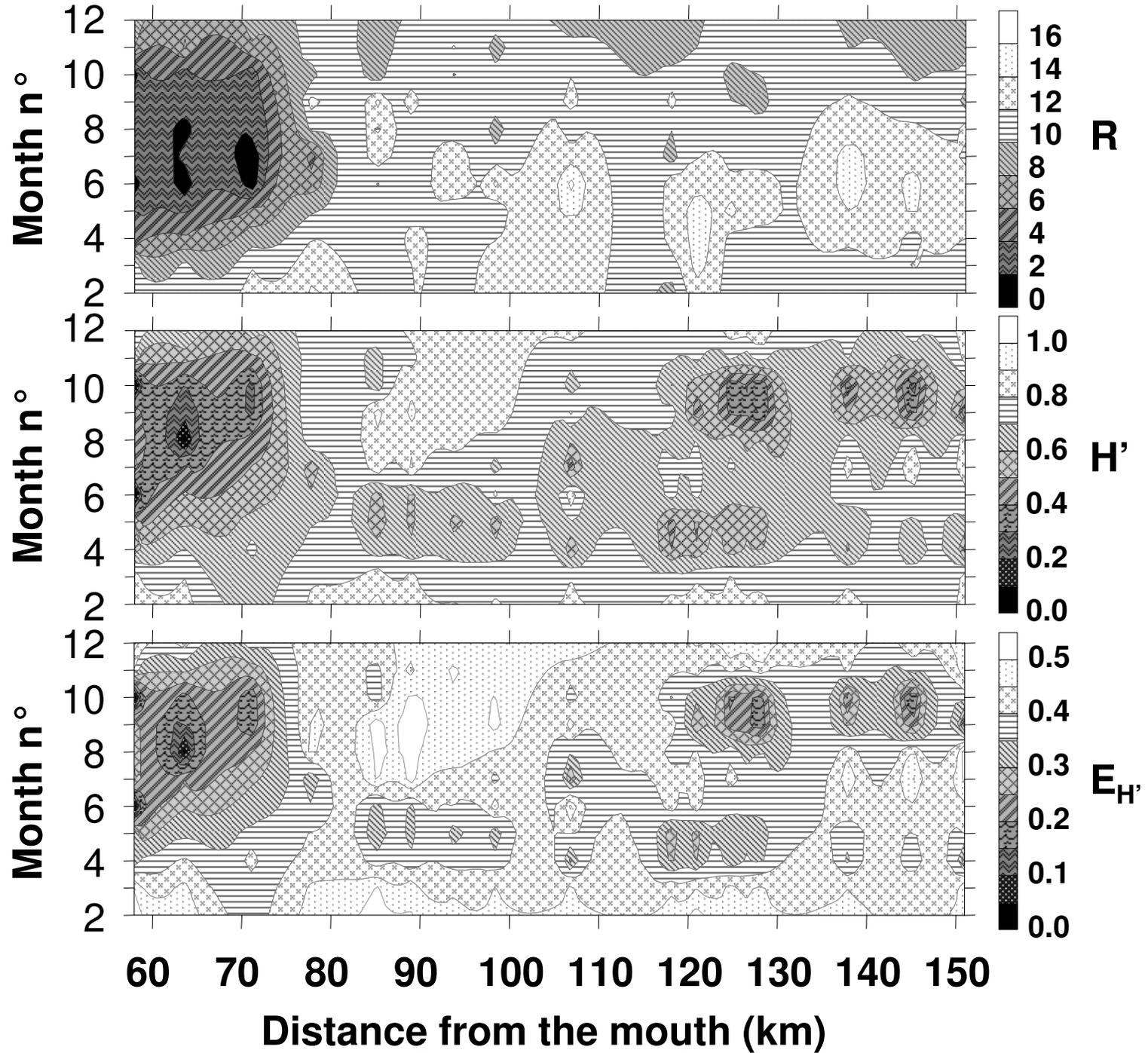


Variation partitioning of the rotifer data matrix at the species and genus level  
202x91mm (600 x 600 DPI)



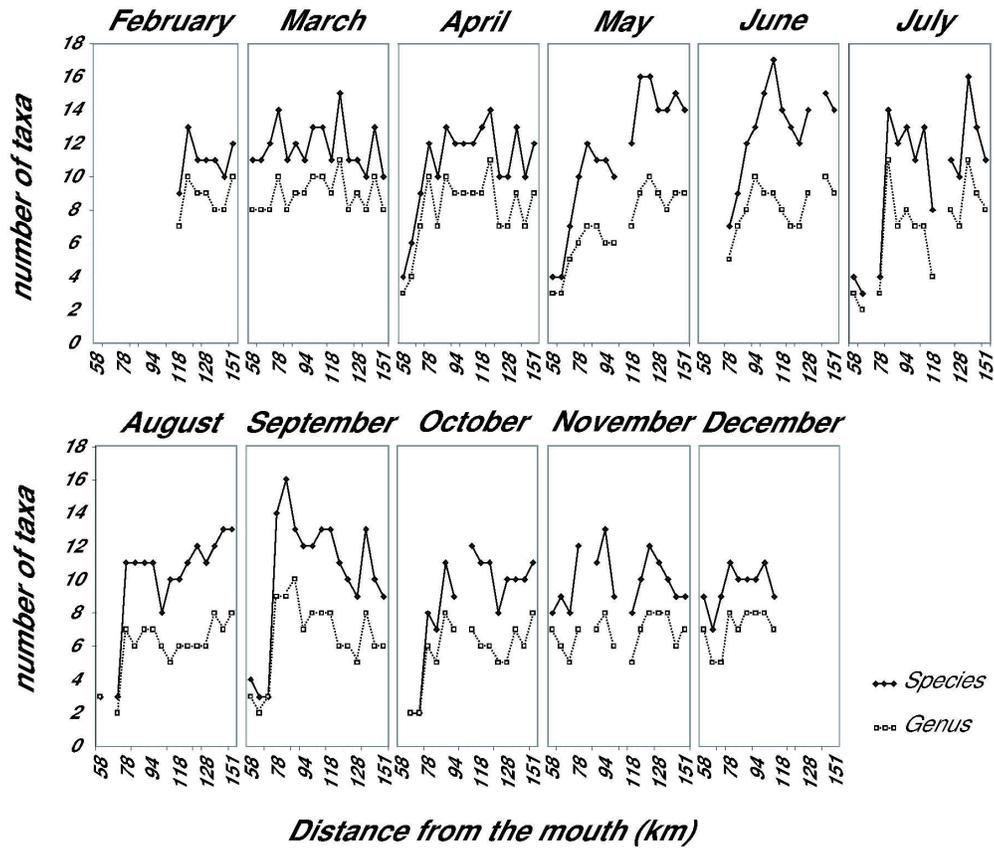
RDA biplot showing the distribution of the rotifers at genus level in the Schelde estuary as a function of the environmental parameters.  
286x147mm (600 x 600 DPI)

er Review



Distance from the mouth (km)

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Species richness, R, calculated from species- (black diamonds) and from genus- (open squares) level data along the transect sampled for each month of the year.  
230x196mm (600 x 600 DPI)