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

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To investigate the reaction of the zooplankton community to improving water quality in the Schelde estuary, we studied the relationship between rotifer species distribution and environmental factors, and the feasibility of using a coarser level of taxonomic resolution. Fifty-two taxa, belonging to 26 genera, were identified, including 22 taxa new for the Schelde. *Brachionus calyciflorus*, *Keratella cochlearis* and *B. angularis* were the most abundant species. The highest diversity and abundances were observed in the freshwater reach. Redundancy analyses (RDA) showed that the main environmental factors explaining rotifer distribution were chlorinity and seasonal factors (discharge levels, cyclopoid abundance). Analysis carried out with data at the species and the genus level gave similar ordination plots, but the positioning of a genus relative to environmental factors did not always adequately represent the associations between the various species within the genus and environmental factors. Similar patterns in space and time were observed using taxonomic richness and diversity indices for analyses at species or genus level. Thus, in the context of the restoration of the Schelde estuary, the identification of rotifer species is very informative, but not essential for detecting important ecological associations.

KEYWORDS: Rotifera; Schelde estuary; taxonomic sufficiency; spatio-temporal distribution

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 data set 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 have an extensive freshwater tidal zone (<0.5 PSU) 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 improvement of the water quality. Over the past 10 years, several environmental factors have changed substantially, and these changes have been most marked in the freshwater stretch. Indeed, in the upstream part of the Schelde estuary, a considerable increase in the oxygen concentration and chlorophyll *a*, and decreases in the NH_4^+ concentration and in BOD₅ values have been observed (Cox *et al.*, 2009). Associated with the rise in oxygen concentration, there has been an increase in NO_3^- concentration as a result of more intensive nitrification. Furthermore, in the summer silica concentrations (SiO_2 or DSi (Dissolved Silica)) now drop below limiting values more often than 10 years ago (Cox *et al.*, in preparation). Runoff, which is an important factor in structuring estuarine spatio-temporal conditions, has varied irregularly over the past decade. While runoff is basically regulated by precipitation and climatic conditions in general, it is also controlled to some extent by human action (e.g. sluice management), and hence is

highly relevant to water management. A more extensive overview of the ecological changes that have occurred in the Schelde estuary and current management perspectives is reported by Van Damme *et al.* (Van Damme *et al.*, 2005), Meire *et al.* (Meire *et al.*, 2005) and Soetaert *et al.* (Soetaert *et al.*, 2006).

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

Because of their key role in the trophic functioning of the estuary (Tackx *et al.*, 2003; Maes *et al.*, 2005), as well as their potential role as bioindicators (Appeltans *et al.*, 2003), zooplankton are an important compartment in the OMES research. This present paper reports a study of the rotifer species composition in the brackish–freshwater reach of the Schelde estuary, as well as its spatio-temporal distribution and diversity during 2002. Rotifers are omnipresent in aquatic systems (Pourriot, 1977; De Ridder and Segers, 1997; Park and Marshall, 2000; Wallace *et al.*, 2006; Segers, 2008). They are found mainly in freshwater: a total of 1800–2000 species have been reported world-wide, with less than 100 strictly marine species. Estuaries present a particular interesting setting to look at affinities of species and environmental variables. In a highly heterotrophic system such as the Schelde estuary (Soetaert and Herman, 1995), rotifers are likely to form an important link between the microbial web and higher trophic levels (Havens, 1991; Gasparini and Castel, 1997; Griffin and Rippingale, 2001; Froneman, 2002). Unfortunately, there are few species-level data sets on rotifer communities in the literature. This is due to a combination of three factors. Their small size usually requires a compound microscope for identification; illoricate species are more easily identified from living material; few competent taxonomists are currently trained in rotifer taxonomy. Moreover, in estuaries, high concentrations of suspended particulate matter (SPM) hamper microscopic observation. To the best of our knowledge, the only such report available for a European Atlantic estuary is that for the Elbe estuary (Holst *et al.*, 1998).

Our study presents the taxonomic composition (at species level in as far as possible) and the spatio-temporal distribution of the rotifer community in

the Schelde estuary. Its relationship to environmental variables is studied, paying special attention to those variables which have changed in the past, and are expected to go on changing as a consequence of restoration.

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

METHOD

Study site

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

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

Sampling and physico-chemical analyses

From February 2002 to December 2002, 16 stations situated along the brackish and freshwater reaches of the estuary were sampled monthly in the middle of the stream (Fig. 1). Water sampling was done just below the surface using a 15 L Niskin bottle at each sampling station, and the following environmental variables were measured: pH and temperature using a CONSORT C832 electrode and dissolved oxygen concentration (O_2) (WTW OXI 325, equipped with Clark electrode).

Samples were taken for the determination of the concentrations of chlorine (Cl^-), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate (PO_4-P) and silica (SiO_2) within 24 h after sampling. They were stored at 4°C, and analyzed colorimetrically using a SKALAR SA 5100 segmented flow analyzer, except for silica (SiO_2), which was analyzed by ICP-OES (Iris®). Samples for the determination of dissolved organic carbon (DOC) were filtered on Gelman fiberglass filters of 0.45 μm porosity. For DOC determination, a preliminary treatment was performed consisting of H_2SO_4 acidification, and flushing with nitrogen gas to remove

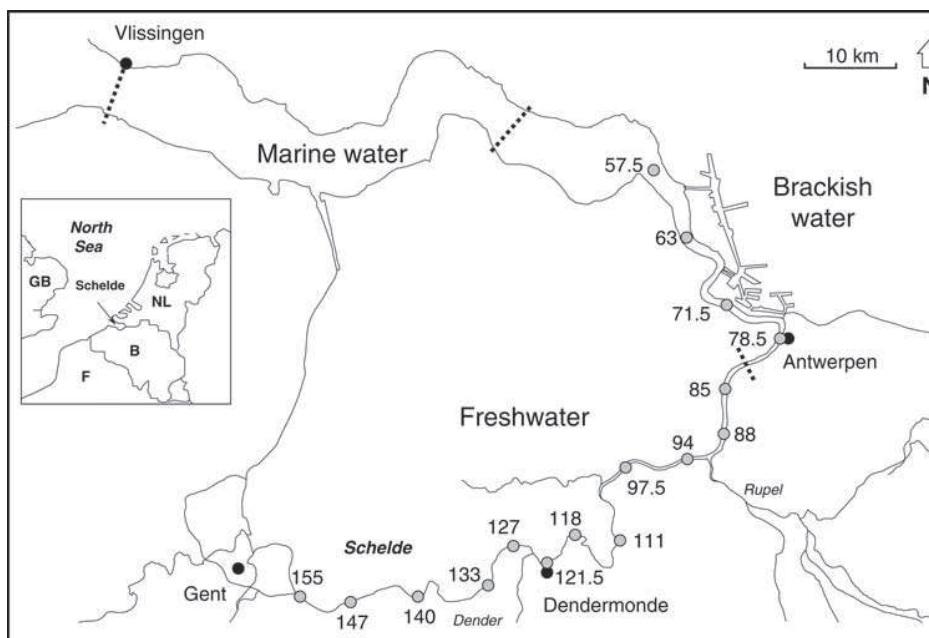


Fig. 1. Map of the Schelde estuary indicating the marine, brackish and freshwater stretches, and the positions of the sampling stations (white circles). Stations are denoted according to their distance from the mouth at Vlissingen in km.

background CO₂. The DOC was then released by further oxidation to CO₂, and analyzed using a SKALAR (phenolphthalein, detection at 550 nm). The 5-day biochemical oxygen demand (BOD₅) was estimated using a WTW OXI 96 oxymeter. SPM was determined gravimetrically after filtering on pre-combusted Whatman GF/F 0.7 µm filters which were dried at 60°C. Water samples were filtered on 0.45 µm porosity filters and frozen for chlorophyll *a* (Chl *a*) determination. The Chl *a* was extracted by adding N,N-dimethylformamide, and then quantified colorimetrically at 647 and 664 nm (Van Damme *et al.*, 2005). Discharge data were obtained from the Flemish administration for waterways and maritime affairs (AWZ). For zooplankton sampling, 50 L of the surface water was collected in a bucket, and filtered through a 50 µm mesh. Carbonated water was added to the sample to narcotize the rotifers before fixing them with formalin at a final concentration of 4% (Siegfried *et al.*, 1989; Joaquin-Justo *et al.*, 2004).

Analyses of zooplankton samples

In the laboratory, samples were stained with three to five drops of erythrosine, prepared at 0.8 mg per 100 mL of water, to make it easier to detect the organisms in the detritus rich samples. After at least 12 h, the samples were mixed thoroughly. A subsample was taken using a wide-bore syringe, and screened in a counting wheel using a Leica MZ 9.5 stereomicroscope (9×–90×). Specimens were counted and identified to species whenever possible. When necessary, they were mounted on a slide in glycerin and further observed with a microscope Nikon Optiphot-2 (50×–600×) using differential interference contrast.

Data analysis

Spatio-temporal trends in the rotifer community, and their relationships to some environmental variables (5-day biological oxygen demand, chlorophyll *a*, chlorinity, ammonium, nitrite, nitrate, dissolved oxygen, pH, orthophosphates, dissolved silica, SPM, temperature, DOC, discharge) were analyzed using multivariate statistics. The environmental factors used were those that had previously been shown to be important in structuring the Schelde zooplankton community (Tackx *et al.*, 2004), most of which are known to have changed in recent years. Abundances of cladocerans, calanoids and cyclopoids were added as environmental factors, because of their possible negative (predation or competition) or positive (association) effect on rotifer distribution. The final data set for these analyses, which included only the results of sampling for which there were no missing values,

contained 154 samples, 52 rotifer taxa and 17 environmental variables. The CANOCO software package, version 4.5 (ter Braack, 1987, 1994) was used. Taxa abundance data were $\log(x + 1)$ transformed prior to the analysis to obtain a normal distribution. The modality of the taxa distribution was first analyzed by a detrended correspondence analysis, using detrending by segments. As the total inertia observed was less than 2.6, a predominance of linear species response curves could be expected, and so we used redundancy analysis (RDA), a technique in which the ordination axes are constrained to be linear combinations of provided environmental variables to investigate the relationships between environmental factors and taxa composition. Data were centered and standardized by species prior to analysis. Forward selection of variables was used to select those most closely associated with the spatio-temporal structure of the rotifer community, and to quantify their relative importance. The statistical significance was tested with Monte Carlo permutation tests (499 unrestricted permutations) ($P < 0.05$) and a Bonferroni correction for multiple test was applied. The minimum model so obtained explains the distribution without co-linear extra fitting. RDA biplots are shown using all environmental variables in order to show relations between the most important variables (minimum model) and the others. Moreover, the variation partitioning of the ordination was estimated as described in Borcard *et al.* (Borcard *et al.*, 1992) and Borcard and Legendre (Borcard and Legendre, 1994), using only the variables selected in the minimum model. Due to the curve shape of the estuary and for a better consideration of the connectivity between the stations in the specific case of an estuary, the distance to the mouth (km) was used as the spatial variable, considering a second degree polynomial, rather than the geographical coordinates.

To test the relevance of the taxonomic level (species versus genus) in detecting the association between taxa and environmental factors, a data reduction to the genus level was made. This new data set was then analyzed as described above, and the results were compared to those obtained at species level.

RESULTS

Taxonomic composition and abundance

Fifty-two rotifer taxa, belonging to 26 genera, were identified. Most of the taxa belonged to the Monogononta (Table I). 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.

Table I: Taxonomic composition of the rotifer fauna of the Schelde estuary

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

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.

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. *Keratella 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 85–155 km) than in the brackish water (stations at 57.5–78 km), whereas they were similar in these two segments in March and November (Fig. 2). In December, the same trend was observed but only five 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, eight environmental factors remained significant: chlorinity, discharge, chlorophyll *a*, temperature, SiO₂, cyclopid abundance, NH₄⁺ and NO₃⁻. The marginal and conditional effects for each variable are shown in Table II. When considering the importance of the variables themselves, without co-variability of other ones, as estimated by their conditional effects, chlorinity, discharge and cyclopid abundance are the main factors influencing rotifer distribution (Table II). 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 axes 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 accounted for 85.8% of the species variation using all environmental variables, and 93.6% when using only the variables selected for the minimum model.

This order corresponds to both the salinity and seasonal variations. The spatial partitioning of the salinity (chlorinity) in our study zone becomes obvious when we plot the samples grouped by station

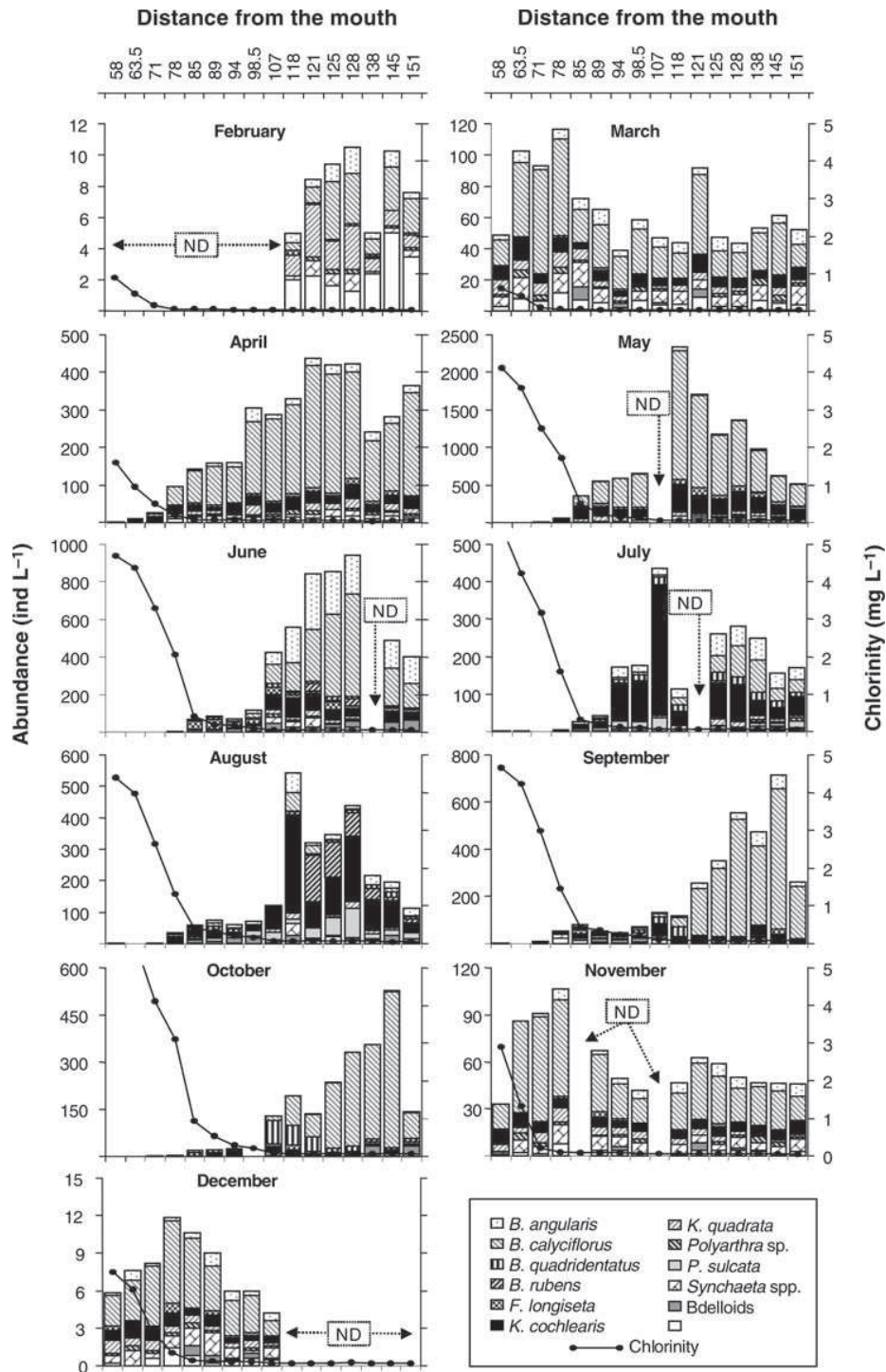


Fig. 2. Spatio-temporal distribution of rotifers in the Schelde estuary. The abscissa shows the distance from the mouth of the estuary (in km). The left ordinate indicates the rotifer abundance (bars); the right ordinate indicates the chlorinity (line). ND, no data available.

which show the brackish stations (58, 63, 71 and 78 km) spread out on the right hand side and the remaining freshwater ones (85–151 km) grouped on the left hand side of the plot (Fig. 3A).

The seasonal trend is clearly illustrated by the distribution of the samples in terms of the date collected (Fig. 3B). In this graph, we can clearly see the change along the temperature axis from the winter months in the

Table II: Variance explained by the environmental variables tested with the rotifer data set at the species and the genus level

All variables							
Species				Genus			
Marginal effects		Conditional effects		Marginal effects		Conditional 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
O ₂	0.11	Cyclo.	0.06	DBO5	0.10	Cyclo.	0.05
Chl <i>a</i>	0.11	SiO ₂	0.03	NO ₂	0.10	SiO ₂	0.03
T	0.10	NH ₄	0.02	O ₂	0.10	DBO5	0.02
BOD5	0.10	Chl <i>a</i>	0.02	T	0.09	T	0.01
NO ₂	0.10	T	0.01	Chl <i>a</i>	0.09	Chl <i>a</i>	0.01
SiO ₂	0.10	NO ₃	0.01	NH ₄	0.08	NO ₂	0.01
PO ₄	0.09	NO ₂	0.01	SiO ₂	0.08	NH ₄	0.01
Cyclo.	0.08	PO ₄	0.01	Cycl.	0.08	O ₂	0.01
NH ₄	0.07	DOC	0.01	PO ₄	0.07	NO ₃	0.00
NO ₃	0.05	O ₂	0.01	DOC	0.05	DOC	0.01
Clado.	0.04	pH	0.00	NO ₃	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	PO ₄	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

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 <i>a</i>	0.11	Cyclo.	0.06	BOD5	0.10	Cyclo.	0.05
T	0.10	SiO ₂	0.03	T	0.09	SiO ₂	0.03
SiO ₂	0.10	NH ₄	0.02	SiO ₂	0.08	BOD5	0.02
Cyclo.	0.08	Chl <i>a</i>	0.02	Cyclo.	0.08	T	0.01
NH ₄	0.07	T	0.01				
NO ₃	0.05	NO ₃	0.01				

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.

bottom right corner to the summer months in the top left corner (Fig. 4A).

The species–environmental factors biplot is shown in Fig. 4. Chlorinity, the most important factor influencing the distribution of rotifer community, is located close to the first axis in the upper right quadrant. Calanoid copepods, SPM and pH are situated in the right-hand, chlorine-associated part of the plot. Discharge levels and oxygen concentration are found in the lower right quadrant, opposite to the cyclopid abundance and the temperature. Cyclopid abundance, chlorophyll *a* and temperature are all associated with the upper left of the ordination, together with the cladoceran abundance and PO₄-P concentration. To a lesser extent (non-significant after the Bonferroni correction), NO₂⁻ concentration and DBO5 (lower left of the ordination) are all negatively associated with chlorinity. The SiO₂,

NO₃⁻ and NO₃⁻ concentration vectors are found almost parallel to the second axis (lower half of the biplot).

When considering the species distribution in this biplot, the most abundant species are mainly situated away from the center of the biplot (Fig. 4A), whereas the rare species are all clustered near the center. Two haline rotifer species, *Synchaeta bicornis* Smith, 1904 and *Keratella cruciformis*, are positively correlated with chlorinity. All the other taxa show greater affinity for freshwater conditions. *Brachionus quadridentatus* Hermann 1783, *B. rubens* Ehrenberg, 1838, *Pompholyx sulcata* Hudson, 1885, *Keratella tropica* (Apstein, 1907) and *Asplanchna brightwellii* Gosse, 1850 are all positively correlated with high temperatures and chlorophyll *a* concentrations, and negatively correlated to discharge and oxygen concentration, corresponding to summer conditions in the top left corner of the biplot. In this

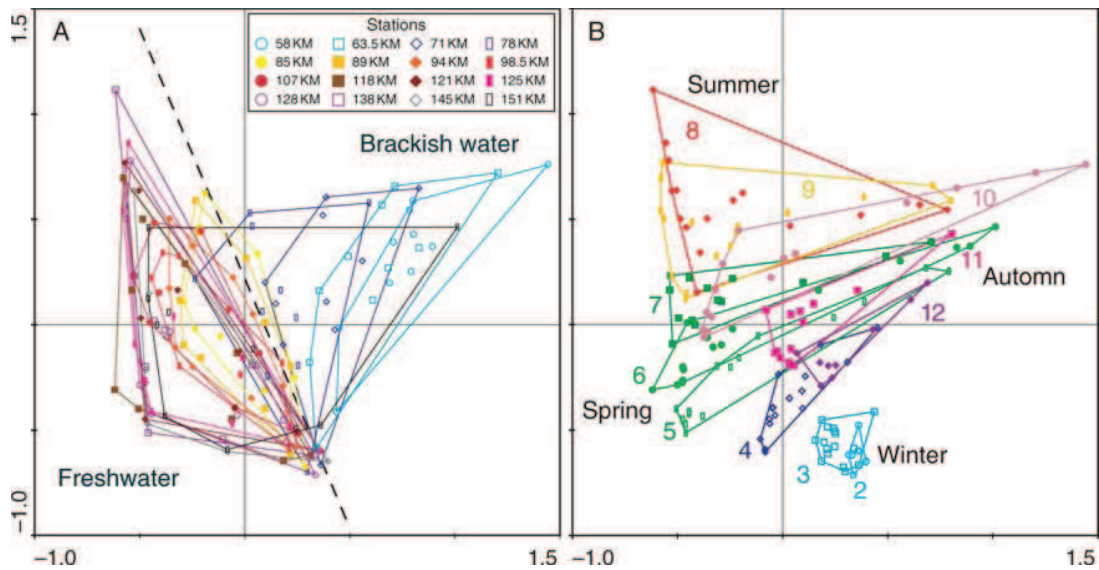


Fig. 3. RDA biplot, axis 1,2, showing the distribution of the samples grouped by station location (A) and by date (B), the number indicating the month. See Fig. 4A for the position of the environmental factors vectors.

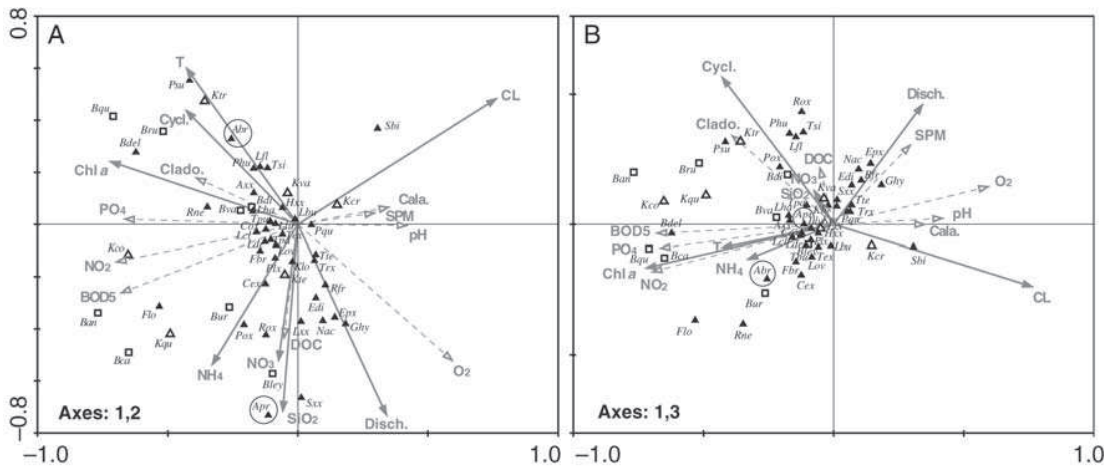


Fig. 4. RDA biplot (A) axis 1,2; (B) axis 1,3 showing the distribution of the rotifers at species level in the Schelde estuary as a function of the environmental parameters. Most significant environmental variables (minimum model) are shown as full arrows. Other variables are shown as dotted arrows. See Table I for nomenclature of species, and Table III for the abbreviations of the environmental factors.

quadrant, we also find cyclopoid copepods and cladocerans. The abundant species *B. calyciflorus*, *B. angularis* and *K. cochlearis*, and also *Filinia longiseta* (Ehrenberg, 1834) and *K. quadrata* (Müller, 1786) are situated close to the NO₂⁻ and BOD₅ vectors. *Brachionus leydigii* Cohn, 1862, *A. priodonta* Gosse 1850, *Lecane* sp. and *Synchaeta* sp. are correlated to high SiO₂, NO₃⁻ and NH₄⁺ concentrations, whereas *Euchlanis dilatata* Ehrenberg, 1832, *Notholca acuminata* (Ehrenberg, 1832) and *Epiphanes* sp. are positively correlated with the discharge levels.

The biplot of axes 1,3 completes the picture of the association of rotifer species with environmental factors (Fig. 4B). It essentially confirms the relationship shown in the axis 1,2 biplot and accentuates the importance of the cyclopoid abundance which is, in this graph, the second most important vector. In this biplot discharge and oxygen concentration, as well as SiO₂ and NO₃⁻ are located at the opposite side to NH₄⁺.

The variation partitioning showed that the environmental variables used in the minimum model explain

43.8% of the variation in the species matrix, with 33.3 and 10.5% due to non-spatial environmental and spatial environmental factors, respectively (Fig. 5). Of the spatial species variation, 1.7% is not shared by the environmental variables; 54.5% of the variation is unexplained.

Comparison with analysis at the genus level

The RDA biplot of genus vs. environmental factors is shown in Fig. 6. All environmental factors tested were found to be significant at $P < 0.05$, but only six remained significant considering the Bonferroni

correction for multiple test. The sum of all eigenvalues is 0.474 for analysis with all environmental variables or 0.403 with the minimum model. This result is comparable to those obtained in the species level analysis (0.494 and 0.435 using all environmental variables or the minimum model). The sum of all the axes accounts for 86.8% of the genus variation using all environmental variables, and 97.1% when using only the variables selected for the minimum model. The main factors organizing distribution of rotifers considered at genus level are chlorinity and the discharge (Table II), as was seen in the species data analysis. Other

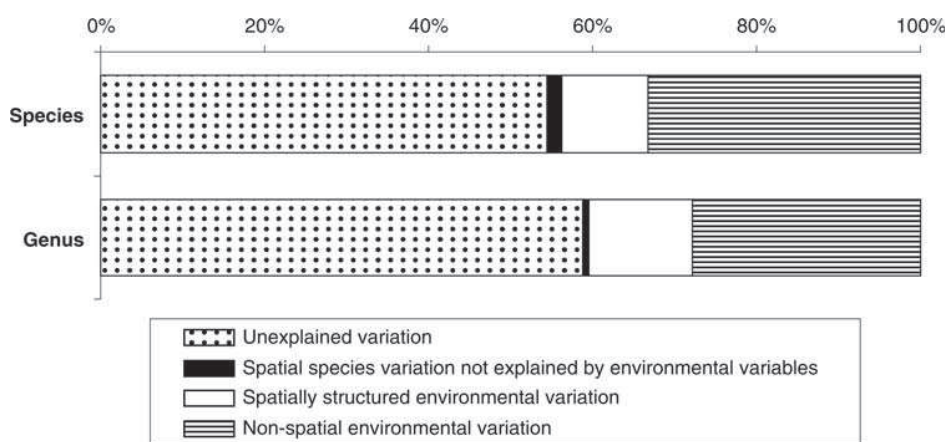


Fig. 5. Variation partitioning of the rotifer data matrix at the species and genus level considering environmental variables selected in the minimum model, using the distance to the mouth as typical spatial factor.

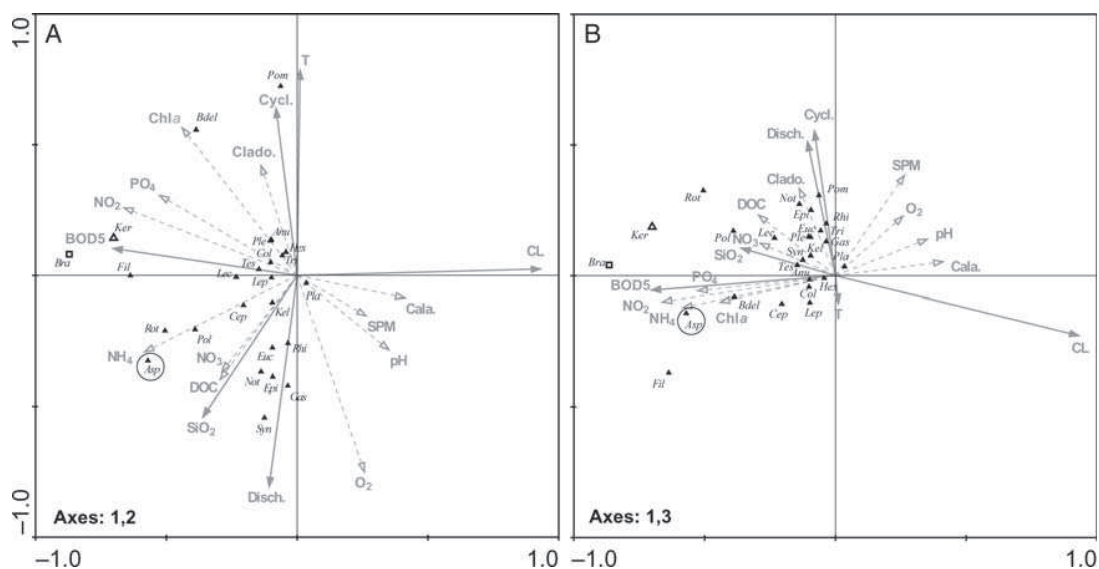


Fig. 6. RDA biplot (A) axis 1,2 and (B) axis 1,3 showing the distribution of the rotifers at genus level in the Schelde estuary as a function of the environmental parameters. Most significant environmental variables (minimum model) are shown as full arrows. Other variables are shown as dotted arrows. See Table I for nomenclature of genera, and Table III for the abbreviations of environmental factors.

contributing factors are BOD5, temperature, silica and cyclopoid abundance. These results are comparable to those found in the species-level RDA, especially when considering the conditional effects of variables (Table II).

Some examples illustrated in Figs 4A and B and 6A and B allow us to compare results obtained in the species and genus-level analyses. Genus *Asplanchna* is represented by two species. *Asplanchna brightwellii*, a summer species, is located in the top left corner of the axis 1,2 biplot (Fig. 4A, ringed). This species is positively associated with high temperatures and Chl *a* concentrations, and negatively with discharge and oxygen concentration. *Asplanchna priodonta* is situated at the opposite position to *A. brightwellii* (Fig. 4A, ringed), and is associated with SiO₂, NO₃⁻, DOC and NH₄⁺. In the 1,3 biplot *A. priodonta*, which is located rather close to the origin on the first axis, shows little association with environmental factors. On the other hand, the positive correlation between *A. brightwellii* and temperature, and its negative correlation with discharge levels and oxygen are confirmed. Both species reach similar maximum abundances, but *A. priodonta* was found more frequently, occurring practically throughout the entire period that *A. brightwellii* was absent (Fig. 2). When this analysis was performed using data reduced to the level of genus, *Asplanchna* occurred at a position comparable to that of *A. priodonta* in the 1,2 biplot, and at one comparable to that of *A. brightwellii* in the 1,3 biplot (Fig. 6A and B, ringed).

The eight species of *Brachionus* display different distribution patterns. *Brachionus rubens* is associated with cladoceran abundance, *B. quadridentatus* with high temperature and chlorophyll *a* concentration. *Brachionus calyciflorus* is associated with NH₄⁺, NO₂⁻ and BOD5. *Brachionus angularis* is positively correlated with BOD5 and negatively with chlorinity. On the other hand, *B. leydigii* shows affinities with SiO₂, NO₃⁻ and NH₄⁺; *B. uceolaris* Müller, 1773 with NH₄⁺. *Brachionus variabilis* Hempel, 1896 and *B. diversicornis* (Daday, 1883) are situated among the rare species quite close to the center, and do not seem to be greatly influenced by the factors being considered here. When we performed our analysis at the level of genus for *Brachionus* (Fig. 6, open squares), we found that the genus plot was situated at the extreme left of the second axis, positively correlated with BOD5 and NO₂⁻, and negatively correlated with chlorinity. This position corresponds to the character of the most abundant *Brachionus* species we found (i.e. *B. calyciflorus* and *B. angularis*), but the associations between the less abundant species and other parameters are not clear.

Six species of *Keratella* occur in the estuary (Fig. 4, open triangles). *Keratella cruciformis*, a haline species, is located at the top right hand corner of the plot, and is

positively associated with chlorinity. *Keratella valga* (Erhenberg, 1834), situated quite close to the origin, next to the second axis (upper half), does not show any trend relative to the environmental factors considered. *Keratella testudo* (Erhenberg, 1832) is situated in the bottom left quadrant on the NH₄⁺ vector, *K. quadrata* and *K. cochlearis* are associated with high BOD5 values and NO₂⁻ concentration, and *K. tropica* with cyclopoid abundance and temperature. When we performed our analysis at the level of genus, *Keratella* (Fig. 6, open triangles) was found on the left side of the ordination plot, strongly associated with NO₂⁻ concentration and BOD5. This position corresponds fairly closely to the “mean” for the most abundant species (*K. cochlearis*, *K. quadrata* and *K. tropica*), but does not reflect that of the haline species *K. cruciformis*. Also *K. testudo*, a species which is not associated with the typical left side conditions of high NO₂⁻, and BOD5, Chl *a* and temperature values, is not well represented by this position of the genus.

Concerning the variance partitioning, the environmental variables considered in the minimum model explain 40.4% of the variation in the species matrix, with 31.1 and 9.3% due to non-spatial environmental and spatial environmental factors, respectively (Fig. 5). Spatial genus variation that is not shared by the environmental variables is negligible with only 0.2%. As observed with the species-level analysis, a large part of the variation (59.4%) is unexplained.

Diversity at the species level

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 (78–120 km).

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 (data 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).

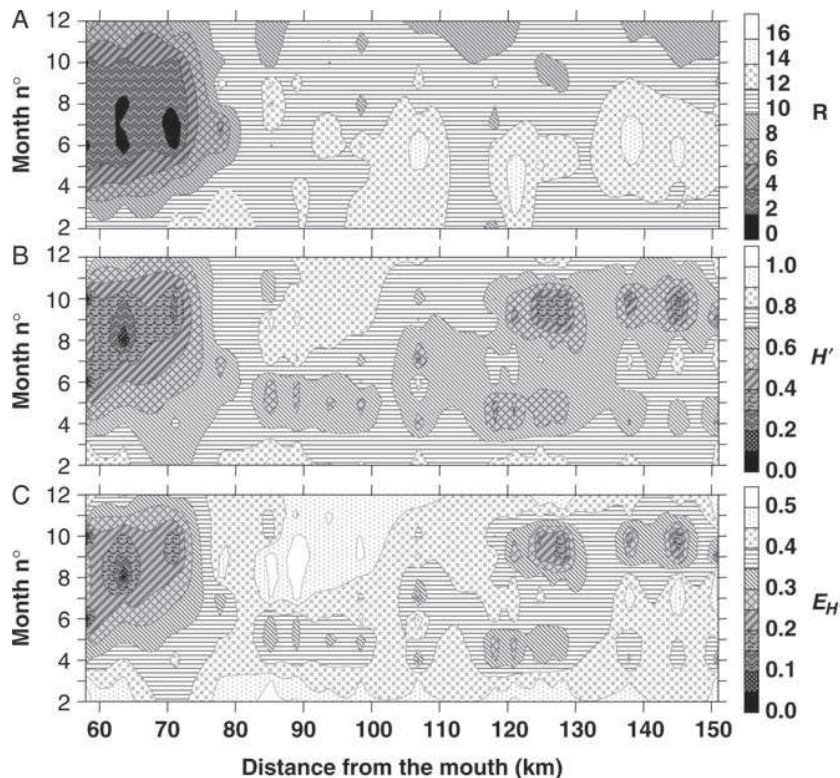


Fig. 7. Distribution of (A) the taxonomic richness (R), (B) Shannon's diversity index (H') and (C) the evenness (E_H) in the estuary as a function of the distance from the mouth (at Vlissingen) and of time (in months).

Correlation between the diversity indices and environmental factors

The correlation between diversity indices obtained from both data sets is given in Table III. Twenty-eight 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 data set.

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 Pauw, 1975; Tackx *et al.*, 2004), with 22 new taxa being reported. The main

reason for this increase was probably the taxonomic effort made in this study, although introduced species are commonly observed in areas with heavy shipping activity such as the Schelde estuary (Carlton, 1996; Johnson and Padilla, 1996; Ruiz *et al.*, 2000; Wasson *et al.*, 2001).

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

The rotifer abundance observed in the Schelde is about the same as that observed in the Elbe estuary (Holst *et al.*, 1998), the nearest estuary for which rotifer data are available. Moreover, most species are found in both these estuaries, exhibiting similar temporal patterns. The most abundant species occurring in the Schelde estuary (*B. calyciflorus*, *B. angularis* and *K. cochlearis*) are cosmopolitan planktonic species (Pontin, 1978; De Ridder and Segers, 1997). *Keratella cochlearis* and *K. quadrata* are considered to be generalist rotifers, feeding on bacteria, detritus and flagellates (Pourriot,

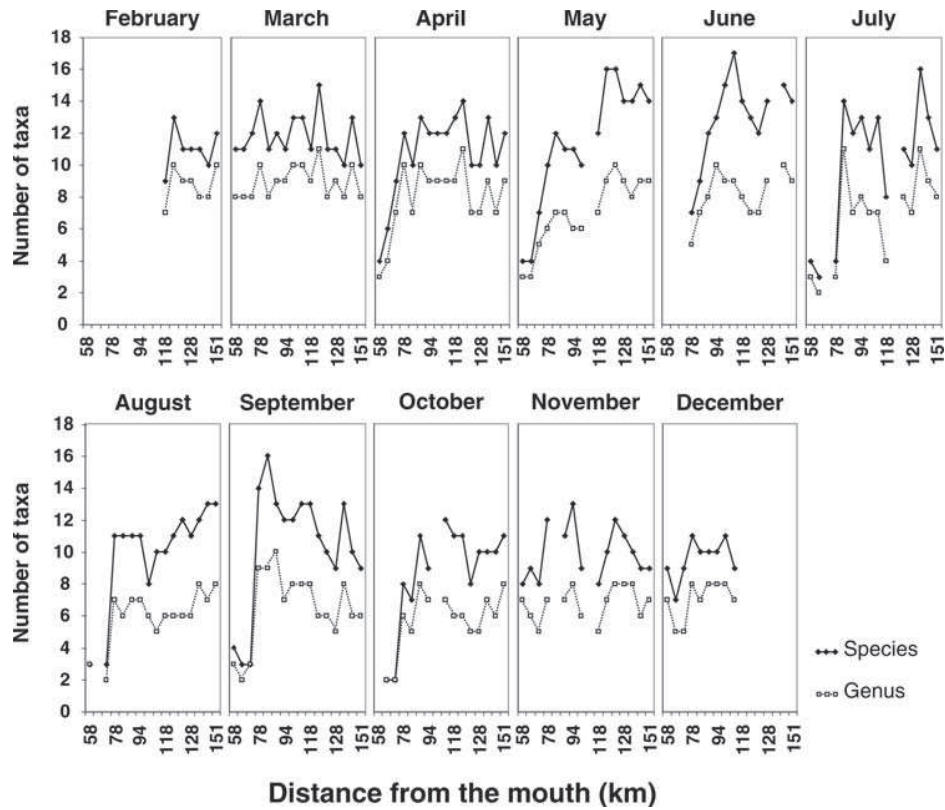


Fig. 8. Species richness, R , calculated from species- (black diamonds) and from genus- (open squares) level data along the transect sampled for each month of the year.

Table III: Significance of Spearman rank correlations obtained between R , H' and E_H from both data sets (i.e. species and genus level) with environmental factors (indicated with the abbreviations used for following analyses)

	SPECIES			GENUS		
	R	H'	E_H	R	H'	E_H
5-day Biological Oxygen Demand (BOD5)	***		***	***		***
Chlorophyll a (Chl a)	***		***		***	***
Chlorinity (CL)	***	***	***	***	***	***
Ammonium (NH_4^+)	***		***	***		***
Nitrite (NO_2^-)	***		***	***		***
Nitrate (NO_3^-)	***		***	***		*
Dissolved Oxygen (O_2)			***		*	***
pH	***	***				
Orthophosphate (PO_4-P)	***		***	***	***	***
Dissolved Silica (SiO_2)	***	***		***	*	
Suspended Particulate Matter (SPM)	*		*			
Temperature (T)		*		***	***	*
Dissolved Organic Carbon (DOC)	***	***		***	***	*
Discharge (Disch.)		***		***	***	***
Cladoceran abundance (Clado.)	***		***	***		***

*Significant at $P < 0.05$. ***Significant at $P < 0.01$. Dark gray, significance detected with both data sets; dark shading, significance detected with the species data set only; light gray, significance detected with the genus level data set only.

1977; Starkweather and Bogdan, 1980; Arndt, 1993), and as a consequence are well adapted to high concentrations of SPM. The genera *Cephalodella*, *Colurella*, *Lecane*, *Lepadella* are generally benthic or periphytic (Pontin, 1978; Nogrady *et al.*, 1995; Segers, 1995; Duggan, 2001). As expected, they were rare in our surface samples. *Keratella tropica*, *Brachionus variabilis* and *Lecane decipens* (Murray, 1913) are the only exotic rotifers observed in the Schelde. Their occurrence and ecology has been detailed in Azémar *et al.* (Azémar *et al.*, 2007).

Relationship between rotifer species distribution and environmental variables

Earlier analyses of the distribution of the Schelde zooplankton community have shown salinity and temperature to be the main structuring factors for the total zooplankton community (Soetaert and Van Rijswijk, 1993; Tackx *et al.*, 2004). Both these reports considered rotifers as a group. Our study shows that, also for the rotifer community, salinity (chlorinity) is the main structuring (spatial) factor (Figs 4–6). Temperature is less important in explaining rotifer community structure and is preceded by other typically seasonal factors such as discharge and Chl *a*. Moreover, considering the Bonferroni correction, temperature is not significant while cyclopoids, mainly present during warm season, are the third environmental parameter structuring the rotifer distribution. This might be explained by the fact that, while the mean temperature over the entire transect varies between 4.2 and 23.6°C in time, it varies maximally 2.6°C between stations within each sampling campaign.

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

The importance of Chl *a* and SiO₂ as structuring variables of the rotifer community can be explained by their higher concentrations in the upstream part of the

transect than the downstream part, as well as their seasonal variations (Figs 2 and 4). Species such as *B. quadridentatus* and *K. cochlearis*, which are associated with high Chl *a* concentrations (Fig. 4), are known to be herbivorous, and hence are likely to benefit from high phytoplankton concentrations (Reynolds, 1984; Hlawa and Heerkloss, 1994; Heerkloss and Hlawa, 1995). Effect of temperature on rotifer species composition and abundance, whether directly through its physiological effect or indirectly through its association with the phytoplankton growth season, is clear from Fig. 2.

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

All of the environmental factors considered contribute significantly to explaining the spatio-temporal distribution of rotifers in the Schelde, at both the species and the genus levels but the Bonferroni correction limits this significance to, respectively, eight and six main factors. This is due, in part, to the colinearity between the factors, most of which change gradually across the estuarine chlorinity distribution. This colinearity is well shown by differences between marginal and conditional effects (Table II): after having taken into account the variance explained by the two first variables (Cl⁻ and discharge), the variance explained by following variables is considerably reduced in the conditional effect.

The variables considered here are those that have been chosen for the routine monitoring of the restoring Schelde estuary, because they are known to represent water quality (Van Damme *et al.*, 2005). Moreover, these environmental factors have all changed as a consequence of the ongoing restoration process, and are likely to continue changing in the future. Thus, for the practical purpose of advising those managing the estuary, our findings do make it possible to find out which taxa are, for example, favored by high Chl *a* concentrations, or those that are not hindered by high

NH_4^+ concentrations. These factors are, to some extent, manageable.

Relationships with environmental variables: comparison of species and genus level

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

In our study, however, the use of the genus level as surrogate for the species, while inevitably resulting in a loss of ecological information, particularly pertaining to the less abundant or rare species, does not substantially change the general pattern of the spatio-temporal distribution of the rotifer community as a function of environmental factors. Comparison between the variation partitioning of the data considered at the species and the genus level do not show noticeable differences. The spatially linked environmental variation explained is relatively small (10.5% at the species level). This is probably due to the absence of real gradients in the distribution of the environmental factors measured all along the transect. In fact, most variables vary differently inside the small brackish area (3 stations) and the extensive freshwater area (13 stations) (Van Damme *et al.*, 2005).

Considering the taxonomic richness, R (Fig. 7A), the area investigated can also be divided into two zones, corresponding to the brackish-water and the freshwater zones, respectively. Fairly homogeneous values of richness are observed within both zones. Except during winter, the rotifer community in the brackish zone of the Schelde is less diverse than that in the freshwater zone. Few species are able to cope with the wide variations of environmental factors in the brackish zone

(Remane and Schlieper, 1958). In the Schelde, the most abundant rotifers occurring in the brackish water zone consist of a few tolerant freshwater species (*B. calyciflorus*, *B. quadridentatus*, *K. cochlearis* and *K. quadrata*). As most rotifers currently live in freshwater and simply follow the current, they die as salinity values increase. Within the freshwater zone, in contrast to the homogeneity of the taxonomic richness, both Shannon's diversity index (H' , Fig. 7B) and evenness ($E_{H'}$, Fig. 7C) display some noticeable differences in the relative abundance of taxa between the upper and lower freshwater reaches. The upper reach of the freshwater tidal zone (78–105 km) is dominated by few taxa (H' generally below 0.6, and $E_{H'}$ below 0.4). In comparison, the downstream reach of the freshwater zone has about the same richness R but shows higher values of H' and $E_{H'}$ (except in spring). Moreover, rotifers are numerically less abundant in the lower reach of this freshwater zone than further upstream (Fig. 2). This reach often corresponds to a maximum turbidity zone (Baeyens *et al.*, 1998; Herman and Heip, 1999; Chen *et al.*, 2005; Meire *et al.*, 2005; Van Damme *et al.*, 2005) where the concentration of particulate matter and pollutants generate restricting ecological conditions (Soetaert and Van Rijswijk, 1993; Van Damme *et al.*, 2005).

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

As diversity at both taxonomic levels varies over both time and space, it is not surprising to find that the indices obtained from both taxonomic levels also generally correlate significantly with the same environmental factors (Table III). As we have already mentioned, most of the environmental factors considered in Table III can be expected to continue to change with the ongoing restoration of the Schelde estuary. Diversity is typically of interest in water management, and so compiling an

inventory rotifer diversity offers relevant information related to the evolution of environmental variables. The fact that this can be done at the genus level facilitates including the rotifer community in routine monitoring programs.

In general, the feasibility of higher taxa as surrogates for species-level patterns appears to depend mainly on the taxonomic composition of the taxonomic group considered. In the case of the marine benthic invertebrates, Maurer (Maurer, 2000) judges that the loss of ecological information resulting from the use of taxonomic sufficiency (TS) makes this method unacceptable even though it does save cost. The exclusion of rare species has serious effects on general ecological observations and theory, and runs counter to current biodiversity assessment and bio indicator research (Maurer, 2000). From this point of view, the use of the coarser identification level is not appropriate, or should at least be limited to well explored areas, where the biodiversity has already been well documented (Quijón and Snelgrove, 2006). The time saved by identifying organisms to a coarser level depends on the number of species within each group, on whether the numerically dominant species belong to several taxonomically complicated groups or not, and on the level of taxonomic expertise available (Dauvin *et al.*, 2003). In estuarine environments, the high concentration of SPM poses a serious problem for studying plankton samples, and rotifers in particular. Finding small transparent animals in the samples is very difficult, even after staining. In the freshwater part of the Schelde estuary, the SPM consists mainly of organic matter, so that it is not possible to use Ludox separation of the organisms from the heavier fraction as applied to zooplankton samples by Soetaert and Van Rijswijk (Soetaert and Van Rijswijk, 1993) in the brackish zone of the estuary. On the other hand, once they are found within the suspended matter, an experienced person using a good stereomicroscope can identify most rotifers present up to species level without major problems. Nevertheless, the rotifer classification is largely perfectible: all areas are not equally intensively studied so literature is not consistent everywhere. Identifications are usually based on morphology which is insufficient for numerous group or species as attested by molecular studies (Fontaneto *et al.*, 2007, 2008, 2009; Kaya *et al.*, 2009). Thus, the *Brachionus* and *Keratella* from the Schelde can be identified at the species level at a glance, but one should bear in mind the existence of species complexes, such as, for example, *Brachionus plicatilis* and *Keratella cochlearis*. Most of the rotifers in the estuary are loricated. The surface ornamentation of the lorica is of taxonomic importance (Wallace *et al.*, 2006), because they include traits that are visible

even on fixed samples. Soft bodied rotifers, such as *Synchaeta*, *Rotaria* and other Bdelloids, once fixed with formalin often have their corona and foot retracted, making it difficult to identify them even to genus level. Live samples or scanning electron microscopy (SEM) would be required to identify them, which would considerably increase the methodological constraints for monitoring purposes (Hollowday, 2002).

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

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