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Zooplankton in the Schelde estuary, Belgium and The Netherlands. Spatial and temporal patterns

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The zooplankton fauna of the Zeeschelde estuary (Belgium) was investigated over 10 months by means of monthly sampling. Canonical Correspondence Analysis (CCA) was used to relate the species distribution to environmental factors. The variation in the species data was significantly ($P < 0.05$) related to a set of 10 environmental variables (chlorinity, NH_4^+, temperature, PO_4-P^-, DW, Chl a and Chl b, NO_2-N, NO_3-N and pH). The main spatial and seasonal gradients were associated with chlorinity and temperature respectively. The brackish water zone was dominated by the calanoid Eurytemora affinis in spring, succeeded by Acartia tonsa and mysid species during summer. In the freshwater transect, cyclopoids dominated, together with several cladoceran species. Thermophilic cyclopoid species (Thermocyclops oithonoides, Th. crassus and Mesocyclops leuckarti) occurred during periods of maximal temperature. The cyclopoids Acanthocyclops robustus, Paracyclops poppei and Cyclops vicinus, the cladocerans Daphnia longispina, Chydorus sphaericus and Bosmina longirostris together with the numerically dominant rotifers, oligochaetes, nematodes and juvenile copepods seemed little affected by environmental gradients.

INTRODUCTION

The Schelde estuary, which extends from Gent to Vlissingen, is dominated on the one hand by tides which enter the estuary from the North Sea and on the other hand by the upper drainage which is influencing the system from land site. The Schelde estuary is one of the few remaining estuaries with extensive salt-, brackish- and freshwater tidal river systems in Europe. In particular the freshwater tidal area is a rare habitat in Europe (Meire *et al.*, 1997). The Dutch part of the Scheldt estuary is called the Westerschelde, the Belgian part is called the Zeeschelde (Figure 1).

The Schelde is, as many other estuaries, strongly influenced by human activity and characterized by a high load of organic matter as well as toxic substances (Baeyens *et al.*, 1998). Since the seventies, when water quality was extremely bad, measures were taken. The increase in oxygen concentrations, which started in the eighties, has accelerated since 1995 (Van Damme *et al.*, 1995) and unpublished results clearly indicate an improvement in the water quality.

Most recent investigations on zooplankton in the Zeeschelde date back to samples collected in 1969 (De Pauw, 1973, 1975; Bakker and De Pauw, 1975). In the present study we analyse the temporal and spatial patterns of the zooplankton species composition and abundance over a period of 10 months between 1995 and 1996.

METHOD

Study site

The Zeeschelde is the part of the Schelde estuary situated between the Dutch–Belgium border (57.5 km upstream from Vlissingen) and Gent (160 km upstream from Vlissingen) (Figure 1). The tidal amplitude varies between 5.2 m

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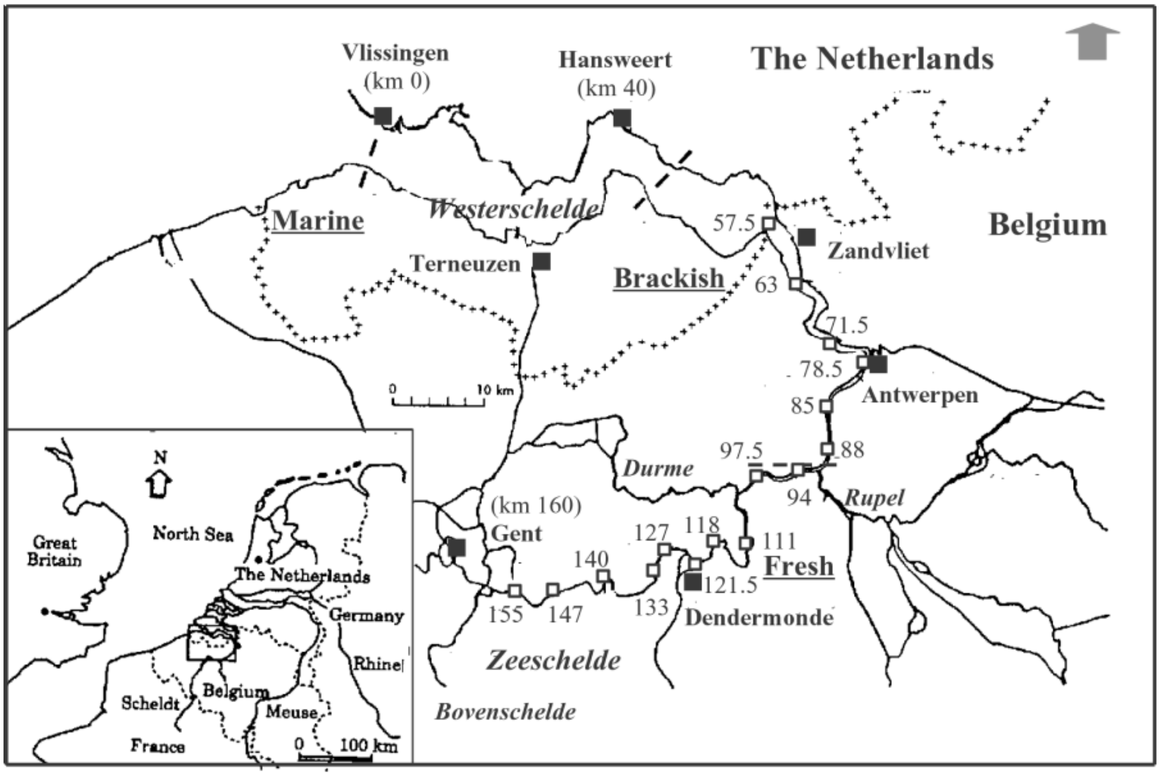

Fig. 1. The Schelde estuary. Sampling stations (white squares) are denoted by their distance in km from the mouth at Vlissingen.

near Antwerpen (km 78.5) to 2 m near Gent (km 160). Vertical salinity stratification is absent but there is a pronounced horizontal salinity gradient, which is subject to tidal and seasonal variation (Middelburg *et al.*, 1995; Baeyens *et al.*, 1998). Between roughly the Dutch–Belgian border (km 57.5) and Rupelmonde (km 85) the water is brackish (0.7–10 p.s.u.). The freshwater (< 0.7 p.s.u.) tidal zone extends, depending on river discharge, between km 100 and km 160, approximately.

Sampling and sample processing

Sampling was carried out in December 1995 and monthly from January till September 1996 at 16 stations (Figure 1). In the following, all stations will be designated by their distance in km upstream from Vlissingen.

Environmental factors were measured at each sampling station. Water sampling was done below surface with a 15 L Niskin bottle. At each station the following environmental variables were measured: pH and temperature using a CONSORT C832 electrode, chlorinity (as a proxy for salinity) and dissolved oxygen concentration (O_2) (WTW OXI 325, equipped with Clark electrode). Water samples were taken for determination of ammonia-(NH_4), nitrate-(NO_3) and nitrite-(NO_2) concentration (Van Damme *et al.*, 1997).

For determination of Suspended Particulate Matter dry weight (DW), Particulate Total Carbon concentration (PTC), Particulate Inorganic Carbon (PIC) concentration, and Chlorophyll *a* (Chl *a*) and *b* (Chl *b*) concentration, 50–100 ml water samples (depending on particulate matter concentration) were filtered on GFC filters, wrapped in Al-foil and stored in deep freeze till analysis. For DW determination, the filters used had been pre-dried and weighed following the procedure described below for the samples. The filters for DW determination were dried at 60°C for 24 h, cooled to room temperature in a dessicator for a few minutes and weighed on a Mettler balance. PTC and PIC concentration were measured by Coulomat and POC concentration was calculated as PTC – PIC. For Chl *a* and Chl *b* analysis, filters were brought into 5 ml of 90% acetone and placed in the refrigerator overnight. Chl *a* and Chl *b* in the extracts were quantified by HPLC using a Waters C_{18} 3.9 × 150 mm column, a Waters Model 440 UV absorbance detector and a Waters 470 scanning fluorescence detector. The solvent mixture used was: 75% methanol, 22% acetone, 3% water. Calibration was done using commercial standards.

At each station, 50 L of surface water were filtered through a 50 μm net and the collected zooplankton was

fixed in a 4% formaldehyde solution. Samples were analyzed by binocular microscope (4 × 10 magnification) for zooplankton species composition and abundance. Subsamples were taken for counts of small zooplankters (e.g. copepod nauplii, rotifera, nematoda).

Community analysis

The relationship between species distribution and environmental factors was investigated by means of the Canonical Correspondence Analysis (CCA) (Jongman *et al.*, 1995; Ter Braak and Smilauer, 1998), using the CANOCO 4 package. Zooplankton data were used as abundance and ln + 1 transformed. For copepods, all copepodite and adult stages were considered together. For cladocerans, a distinction was made between juvenile stages (all species together) and adults. Down weighting for rare species was performed. A Monte Carlo test using 999 unrestricted permutations was performed to test the significance of the correlations between the environmental factors (mentioned above) and the species distribution.

RESULTS

Zooplankton species composition and numerical abundance

35 zooplankton taxa were identified, most of them down to species or genus level, some at higher levels. The list of taxa is given in Table I.

Total zooplankton abundance averaged over all stations varied from <10 × 10^3 ind m^{-3} in winter, to 183 × 10^3 ind m^{-3} in spring and 267 × 10^3 ind m^{-3} in August (Figure 2).

Rotifera were strongly dominant over the entire salinity range, throughout the year, and were the main contributors to the above mentioned abundance peaks. Dominant genera were *Brachionus*, *Filinia*, *Keratella* and *Rotaria*.

Next to rotifers, copepods were most abundant during the major part of the year. Dominant copepod species were the calanoids *Eurytemora affinis* and *Acartia tonsa* in the brackish zone. In the freshwater area, the dominant copepod was the cyclopoid *Acanthocyclops robustus*. Other species that were regularly occurring, but in low numbers, were *Cyclops* species and *Thermocyclops crassus*. Other copepod species occurred only sporadically (see Table I). Other occurring taxa included Cladocera, Polychaeta, Oligochaeta and Mysidacea. The Cladocera were present mostly in the freshwater part and the dominant species were *Bosmina longirostris*, *Moina brachiata* and three *Daphnia* species: *D. longispina*, *D. magna* and *D. pulex*. Nematoda were most abundant during winter months.

Community analysis

The bi-plots for sample scores and species and environmental variables as a result of the CCA analysis on the total zooplankton dataset are shown in Figure 3a,b. Eigenvalues, percentage of explained variance and correlation coefficients with environmental factors for the first four axes are given in Table II. 74.7% of the variance in the species data was explained by the first three axes. Because of the strong correlation between the seasonal structuring variable 'temperature' and axis 3, Figure 3a,b show biplots of axis 1 and 3. Of the tested environmental variables, Monte Carlo permutations showed, in descending order, chlorinity, NH_4-N, temperature, PO_4,-P, DW, Chl *a* and Chl *b*, NO_2-N, NO_3-N and pH to be significant in explaining the ordination. As can be seen from Table II and the biplot of stations (Figure 3a) and environmental variables, the main spatial and seasonal gradients are associated with axis 1 (chlorinity) and axis 3 (temperature).

Figure 3a shows the brackish water stations (km 57.5–km 97) to be situated at the right side of the plot, associated with high oxygen concentrations, especially in winter (October–February). With few exceptions, the freshwater stations occupy the left side of the plot and the vicinity of axis 2. Most winter observations are situated below axis 1, at the opposite side of the temperature vector. Besides chlorinity, the brackish water stations are characterized by higher oxygen concentrations than the freshwater stations, especially during winter. Nutrient concentrations are maximal in the freshwater zone, situated at the left of the biplot. DW and POC arrows point to the lower left quadrant of the plot, being highest in winter in the freshwater zone, while Chl *a* and Chl *b* are situated in the left upper quadrant, associated with high temperatures in the freshwater part of the estuary.

The species biplot is shown in Figure 3b. The calanoid copepods *Eurytemora affinis* and *Acartia tonsa*, Polychaetes, Mysids and the harpacticoids *Microarthridion littorale* and *Pseudobradya* sp. typically inhabit the brackish water zone situated at the right side of the plot. The harpacticoids and *E. affinis*, situated in the lower right corner, are associated with high O_2 concentrations, while *A. tonsa*, polychaetes and mysids are associated with higher temperatures. Cyclopoids and Cladocerans are characteristic for the freshwater zone, at the left side of axis 3. The Cyclopoid copepods *Thermocyclops crassus*, *Thermocyclops oithonoides* and *Mesocyclops leuckarti*, as well as the cladoceran *Ilyocryptus agilis* are observed in the upper part of the plot, associated with high temperatures.

Table I: Zooplankton taxa observed

Phylum: Aschelminthes
Class: Nematoda
Phylum: Rotifera
Class: Eurotatoria
Order: Transversiramida
Family: Brachionidae
Brachionus spec.
Keratella spec.
Order: Protoramida
Family: Filinidae
Filinia spec.
Order: Septiramida
Polyarthra spec.
Class: Digononta
Order: Bdelloidea
Family: Habrotrochida
Rotaria spec.
Phylum: Annelida
Class: Polychaeta
Class: Oligochaeta
Phylum: Arthropoda
Sub Phylum: Crustacea
Class: Copepoda Edwards 1840
Order: Calanoida Sars 1903
Family: Acartiidae Sars 1903
Acartia tonsa Dana 1848
Family: Temoridae Giesbrecht 1892
Eurytemora affinis (Poppe 1880)
Temora longicornis (Muller 1792)
Order: Cyclopoida Burmeister 1834
Family: Cyclopidae Sars 1913
Acanthocyclops robustus (Sars 1863)
Cyclops strenuus strenuus Fischer 1851
Cyclops vicinus vicinus Ulianine 1875
Diacyclops bicuspidatus (Claus 1857)
Eucyclops serrulatus (Fischer 1851)
Mesocyclops leuckarti (Claus 1857)
Metacyclops gracilis (Lilljeborg 1853)
Metacyclops problematicus Dumont 1973
Paracyclops poppei (Rehberg, 1880)
Thermocyclops crassus (Fischer 1857)
Thermocyclops oithonoides (Sars 1863)
Order: Harpacticoida Sars 1903
Family: Canthocamptidae Lang 1948
Canthocamptus staphylinus (Jurine 1820)
Family: Tachidiidae Lang 1948
Microarthridion littorale (Poppe 1881)
Family: Ectinosomatidae Sars 1903
Pseudobradya spec.
Family: Ameiridae Lang 1936
Nitocra lacustris (Schmankevitch 1875)
Family: Macrothricidae Norman and Brady, 1867
Ilyocryptus agilis Kurz (1878)
Class: Malacostraca Latreille 1803
Order: Mysidacea Boas 1883
Mesopodopsis slabberi (Van Beneden 1861)
Class: Branchiopoda Latreille 1817
Family: Bosminidae
Bosmina longirostris (Müller 1785)
Family: Daphniidae
Ceriodaphnia quadrangula (Müller 1785)
Ceriodaphnia reticulata (Jurine 1820)
Daphnia longispina Müller 1785
Daphnia magna Straus 1920
Daphnia pulex Leydig 1860
Moina brachiata (Jurine 1820)
Family: Chydoridae
Chydorus sphaericus (Müller 1785)
Leydigia acanthocercoides (Fischer 1854)

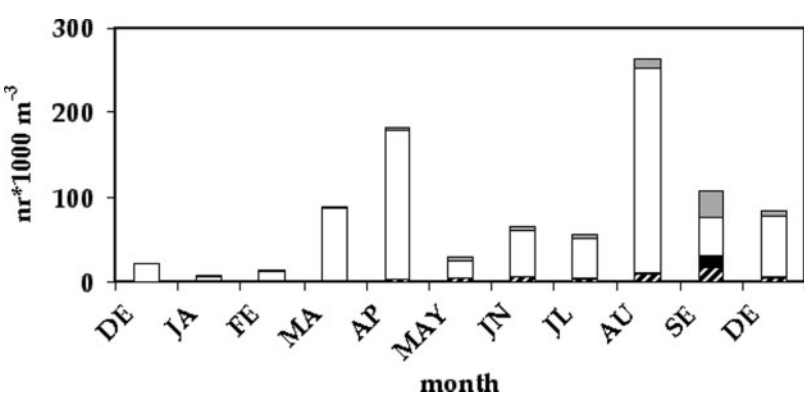

Fig. 2. Numerical abundance of main taxonomic groups (mean of all stations sampled) for each month; Copepoda (striped), Cladocera (black), Rotifera (white), and other mesozooplankton organisms (grey).

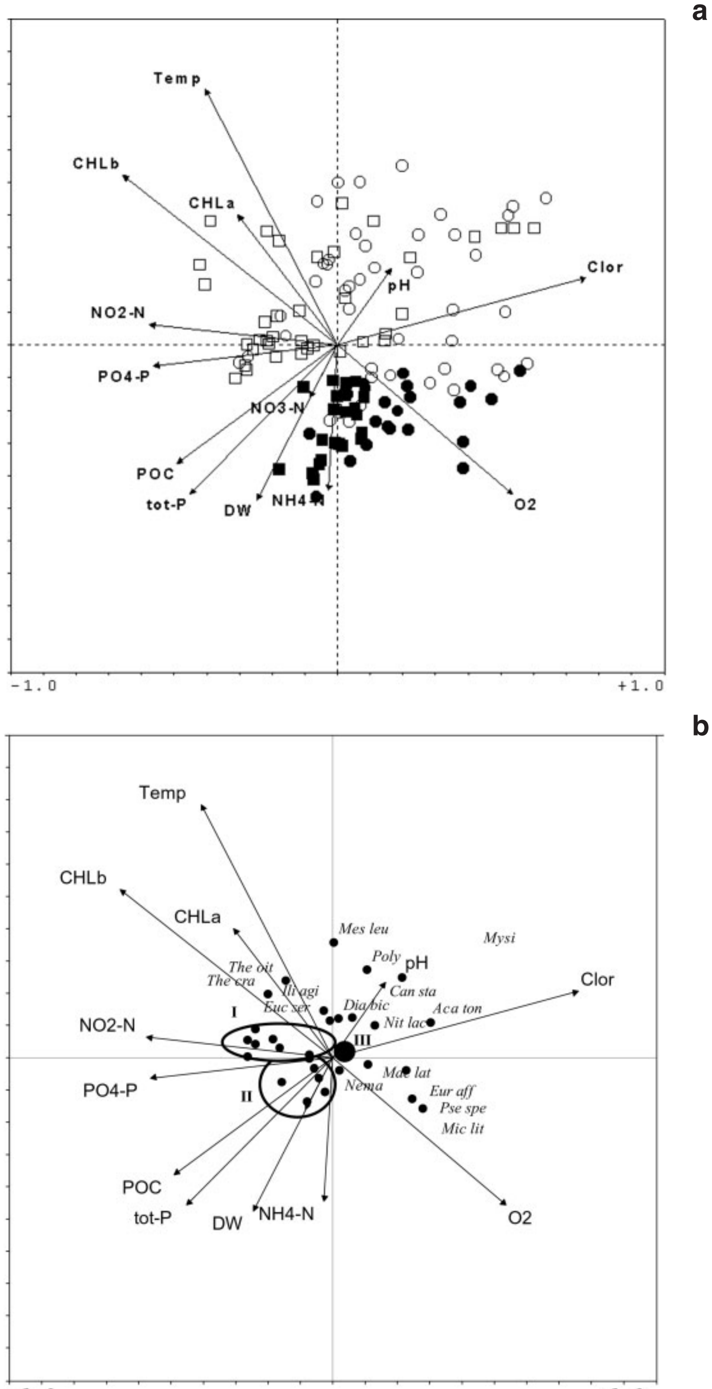

Fig. 3. Results of CCA analysis. Axis 1 and 3 biplot for: (**a**) environmental variables vectors and stations. Brackish water zone (km 57.5–97) in winter (December–February): black circles. Brackish water zone during the remainder of the year: white circles. Freshwater zone (km 111–160) in winter: black squares and during the remainder of the year: white squares. (**b**) environmental variables and species. Species abbreviations outside of lists: (in alphabetical order): *Acartia tonsa* (Ac ton), *Canthocamptus staphylinus* (Can sta), *Diacyclops bicuspidatus* (Dia bic); *Eurytemora affinis* (Eur aff); *Ilyocryptus agilis* (Ili agi), *Eucyclops serratus* (Euc ser), *Mesocyclops leucartii* (Mes leu), *Microarthridion littorale* (Mic lit), polychaeta (Poly), *Thermocyclops oithonoides* (The oit) and *Thermocyclops crassus* (The cra).
List I: *Acanthocyclops robustus*, *Ceriodaphnia quadrangula*, *Ceriodaphnia reticulata*, *Daphnia magna*, *Daphnia pulex*, juvenile Cladocera and *Moina brachiata*.
List II: *Bosmina longirostris*, *Chydorus sphaericus*, *Cyclops strenuus*, *Daphnia longispina*, *Leydigia acanthocercoides*, oligochaeta and *Paracyclops poppei*.
List III: *Cyclops vicinus*, rotifers and small copepodites.

Table II: Results of CCA analysis

Axis	1	2	3	4
Eigenvalues	0.222	0.128	0.070	0.049
Cumulative percentage variance of species–environment relationship	39.5	62.3	74.7	83.5
Correlation coefficient				
Chlorinity	0.691	−0.344	0.151	−0.196
Temperature	−0.370	−0.219	0.575	−0.077

List I, located above axis 3, includes the dominant species *Daphnia pulex* and *Moina brachiata*, as well as *D. magna*, *Ceriodaphnia quadrangula*, and *C. reticulata*. Also included, and located close to the origin are the dominant cyclopoid *Acanthocyclops robustus* and juvenile cladocerans. List II, located below axis 3 at lower temperatures, includes the cladocerans *Daphnia longispina*, *Bosmina longirostris* and *Leydigia acanthocercoides*, the cyclopoids *Cyclops strenuus strenuus* and *Paracyclops poppei* and oligochaetes.

List III consists of *Cyclops vicinus vicinus*, rotifers and small copepodites, which are situated close to the origin of the ordination, as is the case for several other abundant taxa such as *Acanthocyclops robustus*, *Cyclops strenuus*, *Bosmina longirostris* as well as juvenile cladocerans, oligochaete larvae and nematodes.

DISCUSSION

The CCA analysis revealed that, besides chlorinity, restraining species like *E. affinis*, *A. tonsa,* mysids and polychaetes downstream of km 97, the main environment gradient was formed by the seasonal temperature changes. These allowed the development of typical thermophilic species such as *Thermocyclos crassus, T. oithonoides* and *Mesocyclops leuckarti* during summer in the freshwater reaches. *T. crassus* exhibits an optimal development around 25°C. At lower temperature, the rate of development decreases and this species is probably outcompeted by cyclopoid copepods which have a higher rate of development at temperatures below 25°C, like *Cyclops vicinus* and *Diacyclops bicuspidatus* (Maier, 1989). *T. crassus* has also been observed in the Donkmeer (Belgium), where it was replacing another thermophilic species, *Thermocyclops oithonoides* (Dumont, 1965). *T. oithonoides* was also observed in the Zeeschelde, but in extremely low abundance. This species develops at lower temperature than *T. crassus* and is much more susceptible to eutrophication (Dumont, 1965).

With the exception of the calanoid *Eurytemora affinis* and the harpacticoids *Microarthridion littorale* and *Pseudobradia* sp., no organisms were associated with increasing O_2 concentrations. Abundant taxa like rotifers, the cyclopoid *Acanthocyclops robustus*, *Cyclops strenuus* and small copepodites of calanoids and cyclopoids, together with the cladocera *Bosmina longirostris*, oligochaete larvae and nematodes were all located close to the origin (Figure 3b). In the case of small copepodites, this can be explained by the fact that both calanoid and cyclopoid copepodites were quantified together, thus encompassing the entire chlorinity gradient and co-occurring variation in environmental conditions. Similarly, for rotifers, oligochaetes and nematodes, the low taxonomic level of resolution could cause this lack of relationship to environmental conditions. For the other species, it is more likely to be a genuine picture of their environmental tolerance.

As in most freshwater tidal estuaries (Heinbokel *et al.*, 1988; Pace *et al.*, 1992; Gosselain *et al.*, 1994, Kobayashi *et al.*, 1996), rotifers dominated the planktonic metazoa in the Zeeschelde. *Brachionus* was the dominant genus. Sladecek (Sladecek, 1983) classifies this genus as characteristic to α-β mesosaprobe water, indicative for moderate to high organic pollution. It had been reported earlier by Verraes (Verraes, 1968) at the mouth of the river Rupel which discharges the unpurified water of Brussels into the Schelde. Also the other genera observed (*Filina*, *Keratella* and *Rotaria*), are generally known as cosmopolitan, eurythermic freshwater with some tolerance for salinity, classified as α to β mesosaprobic genera (Kolkwitz and Marsson, 1909; Remane, 1929; Evens, 1954; De Ridder, 1959, 1963; Sladecek, 1983; De Pauw, 1975).

A. robustus is a widely distributed species, occurring in lakes and pools (Dumont, 1965; Illies, 1967). It has previously been found in the Schelde at Lillo (Leloup and Konietzko, 1956) and near the mouth of the river Rupel (Verraes, 1965). *C. strenuus* is widely distributed in Europe [North American and Asian reports have yet to be confirmed and may not be reliable (Fiers, personal observation)], with a preference for freshwater and low salinity (Rylov, 1935). They are characterized as α-β mesosaprobic (Kolkwitz and Marsson, 1909; Caspers and Schultz, 1964; Sladecek, 1983).

Oligochaetes strongly dominate the benthic community in the freshwater reaches of the Zeeschelde (Seys *et al.*, 1999), and are well known as very tolerant to organic pollution (Brinkhurst, 1980; Giere and Pfannkuche, 1982).

In conclusion, the majority of genera and species observed in the freshwater stretches of the Zeeschelde

are typically tolerant of medium to strongly polluted waters. Beside the seasonal effect of temperature, little spatial segregation in zooplankton composition was observed among the freshwater stations sampled.

Besides the thermophylic copepods *T. crassus*, *T. oithonoides*, *M. leuckartii* and *M. gracilis*, three cladocerans, (*C. reticulata* and *L. acanthocercoides*, and *M. brachiata*) were observed in this study which were not reported in a previous species list of the zooplankton of the Zeeschelde (De Pauw, 1975). All three cladocerans are tolerant species, adapted to organic pollution, which may not have thrived in the Zeeschelde during the study of De Pauw (De Pauw, 1975), carried out in the years 1967–1969, when environmental conditions were deteriorating but not at their worst level (Van Damme *et al.*, 1995). The occurrence of the thermophiles could be explained by the very high temperatures in August 1995 (up to 25°C), which was the maximum observed during the period 1965–1995 (Van Damme *et al.*, 1995).

Of particular interest is the presence of *Metacyclops problematicus*; this is the second record of this species for Belgium and Europe (Dumont, 1973). *Metacyclops problematicus* was described from the river Samber (Belgium), a heavily polluted river. The animals occurred in its polysaprobic zone and co-occurred with the ubiquitous *Paracyclops fimbriatus* (Fisher, 1853). Current studies (Fiers, personal observation) on the cyclopoid fauna of Belgium revealed that *M. problematicus* is widely distributed and occurs typically in heavily polluted waters where conditions exclude other cyclopine species.

Environmental conditions in the brackish part are generally better than in the freshwater part of the Zeeschelde and this zone harbors some typical estuarine and marine zooplankton species.

Eurytemora affinis is a euryhaline and eurythermic copepod, which is considered as a typical estuarine species of the Northern Hemisphere (De Pauw, 1973; Castel and Feurtet, 1989; Soltonpour-Gargari and Wellershaus, 1987). The species normally has its optimum around 0 p.s.u. In the Schelde, this salinity zone is characterized by very low oxygen concentrations (Soetaert and Van Rijswijk, 1993), which explains why, in the Schelde, *E. affinis* has shifted its peak abundance towards the brackish water area, where oxygen concentrations are higher (Sautour and Castel, 1995; this study). However, in January, February and March 1996, when temperatures were low and oxygen concentration around the 0 p.s.u. zone was >4 mg L^{-1}, the copepod was also found in the freshwater part of the Zeeschelde (up to km 127). During the remainder of the year 1996, *E. affinis* was again restricted to the zone downstream of Antwerp (km 78.5). During the 1999 and 2000 monitoring campaigns, the species was regularly observed in the freshwater reaches (unpublished data). These observations could reflect a possible response of the species to improving oxygen concentrations in the freshwater stretch. This hypothesis is examined in another paper (Appeltans *et al.*, 2004).

A. tonsa succeeded *E. affinis* in the brackish water zone during the summer, as observed in many temperate estuaries, brackish lagoons and coastal areas (Bakker and De Pauw, 1975; Baretta and Malschaert, 1988; Paffenhöfer and Stearns, 1988). Mysids are important components of the hyperbenthos in the entire Westerschelde (Mees *et al.*, 1993).

Water quality in the Schelde is expected to improve further in the future, as a consequence of ongoing and intensified water purification in the Schelde estuary and its tributaries. This evolution will, in itself, provide an interesting setting for inter specific competition among the presently dominant tolerant zooplankton species and some of the more sensitive ones. Water quality improvement will probably also affect feeding conditions for zooplankton (e.g. increase in phytoplankton–detritus ratio). The trophic structure of the ecosystem (Muylaert, 1999), may as such also change in the future.

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EDITOR'S NOTE:

Salinity (S) [Practical Salinity Units 1978] in seawater is currently defined in relationship to chlorinity (Cl) thus:

$$S = 1.80655\ Cl$$

(Unesco, 1981, *Unesco tech. Pap. mar. Sci.*, Nos. 36, 37, 38; Boucher, J., 2000, Salinity, chlorinity and salt in seawater, http://bell.mma.edu/~jbouch/OS212S00F/index.htm, consulted 07 Nov. 2003). In estuaries, however, differences in the relative concentrations of ions in the diluting river water may cause departures from the relationship between chlorinity and salinity, especially at low salinities.

– IRJ