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New occurrence of *Lecane decipiens* (Murray, 1913) and some other alien rotifers in the Schelde estuary (Belgium)

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ABSTRACT. Three alien rotifers were found in the Schelde estuary in spring and summer 2002-2003. *Lecane decipiens* and *Brachionus variabilis*, with a maximum of respectively 6 and 4 ind L⁻¹, were rare and quite localized in the freshwater tidal reach, corresponding to the stage III (non invasive species) in Colautti and Mac Isaac's invasion terminology. This record of *L. decipiens* is the first confirmed one in Europe. *Keratella tropica* was generally present in the freshwater reach but, more tolerant to salinity, it was also found in brackish water. With its maximum abundance of 18 ind L⁻¹, *K. tropica* appeared to be invasive, with a stage IVa (widespread but rare) at the scale of the Schelde estuary. A description with illustrations of these introduced species is given with some information about their ecology.

KEY WORDS : *Lecane decipiens*; *Keratella tropica*; *Brachionus variabilis*; introduced species; Schelde estuary.

INTRODUCTION

As non indigenous species (NIS) entering an ecosystem can induce major changes in community structure and ecosystem functioning, and potentially destroy local species by predation or competition, biological invasions are one of today's concerns in both terrestrial and aquatic systems (PERRINGS et al., 2002; KENNISH, 2002; LEE, 2002; LEE et al., 2003; TABACCHI et al., 2005). For aquatic organisms, shipping activity, with its associated ballast water discharge and hull fouling, is known to be an important mean of transport both overseas and locally (WASSON et al., 2001). Consequently, harbour management strategies take this problem into account in order to prevent biological invasions (HORAN et al., 2002; BATABYAL & BELADI, 2004; BATABYAL et al., 2005).

As the Schelde basin (Belgium and the Netherlands, Fig. 1) is one of the most intensively used shipping ways in Europe, it is a particularly favourable area to the introduction and spreading of non indigenous species. After Rotterdam, the Belgian harbour of Antwerpen, located inland, between km 68 and 89 from the mouth (Vlissingen), is the second European port and the eighth in the world. Navigable for most of its length, the Schelde is connected with a dense canal network giving access to other major ports in the vicinity, as Gent, Brussel, Zeebrugge, Oostend, Liège, and also to other basins, as the Rhine and Meuse basins. The Schelde is also one of the few remaining estuaries in Europe with an extensive freshwater tidal reach (MEIRE et al., 2005; VAN DAMME et al., 2005) and as such offers a large variety of environments to the introduced specimens. Not surprisingly, numerous exotic species, both terrestrial and aquatic have been reported from the area. The Chinese mitten crab, *Eriocheir sinensis* (Milne-Edwards, 1854), is perhaps the best known invader in the Schelde estuary (ANGER, 1991), and the mollusc *Dreissena polymorpha* (Pallas, 1771) has been reported on sluice doors. As some

other examples we cite : the Atlantic croaker, *Micropogonias undulatus* (L.) (STEVENS et al., 2004); the North-American amphipods *Melita nitida* Smith, 1873 and *Inci-socalliope aestuarius* (Watling and Maurer, 1973) reported by FAASSE & VAN MOORSEL (2003).

The zooplankton community is also concerned as a potential invader. The small size of most of these organisms, combined with the production of resting eggs offers them advantages for propagation. *Acartia tonsa* (Dana, 1848), for example, is a North-American native calanoid well established in all European estuaries. *Daphnia ambigua* Scourfield 1947 and *D. parvula* Fordyce, 1901 are alien cladocerans commonly found in Belgium (MAIER, 1996) as well as *Moina weissmanni* Ishikawa, 1896 reported in Flanders by DE MEESTER et al. (2002).

But not all introduced specimens can succeed in establishing themselves. The important variety of situations has given rise to an equally diverse vocabulary, using terms as : alien, exotic, imported, colonizing, invasive... COLAUTTI & MAC ISAAC (2004) have clarified the confused "invasive" species terminology by suggesting a classification based on the "propagule pressure concept" (WILLIAMSON & FITTER, 1996) with different stages in an invading process. Five stages were identified : stage 0 being the potential invading propagules in their donor region; stage I, the propagules are taken into a transport vector (ballast water for example); stage II, the propagules survive the transport and are released in a new environment; stage III, they establish (survive and reproduce) in the recipient region but stay localized and rare; stage IVa, propagules become widespread but rare; stage IVb, propagules are localized but dominant; finally, at stage V they are widespread and dominant. The stages III to V only concern established species in a new environment. Stages IVa and V are considered as "invasive" opposite to stages III and IVb which are "non invasive". This classification was used in the present work.

The aims of this study were (1) to report the presence of some introduced rotifers (*Keratella tropica*, *Lecane decipiens* and *Brachionus variabilis*) in the Schelde estuary, (2) to present morphological characteristics of these

species in the Schelde focussing on their diagnosis, with new illustrations and (3) to discuss their ecology and their present invasive stage in the Schelde.

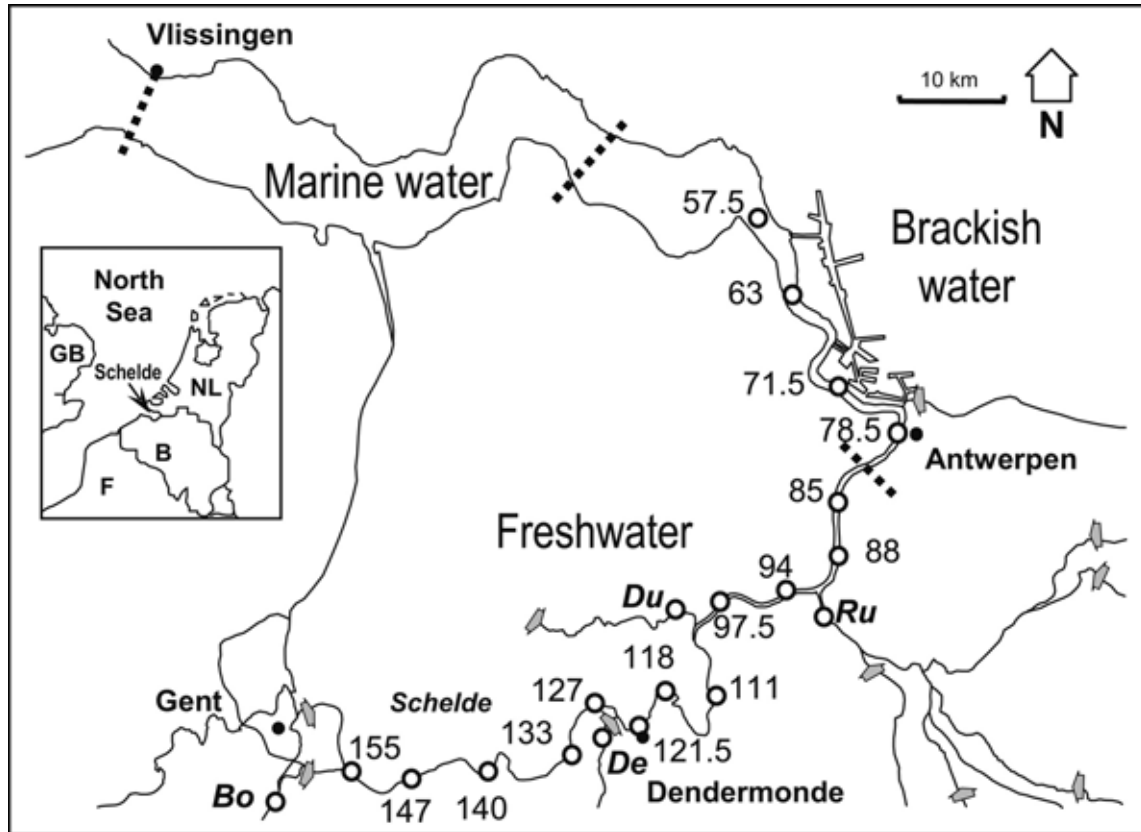


Fig. 1. – Map of the Schelde estuary indicating the marine, brackish and freshwater stretches and the position of the sampling stations (white circles). Stations in the Schelde are denoted according to their distance to the mouth, at Vlissingen. Stations in the tributaries were called “Ru” for Rupel, “Du” for Durme, “De” for Dender and “Bo” for Boven Schelde. Gray arrows indicate position of upper limit of tidal influence.

MATERIALS AND METHODS

The material examined (Table 1) was obtained in 2002-2003 from campaigns in the brackish and freshwater reaches of the Schelde estuary during the multi disciplinary monitoring of the OMES program (Flemish government research program), including investigations about the zooplankton diversity and distribution. 16 stations, identified by their distance (in km) to the mouth of the estuary in Vlissingen and 4 tributaries (Rupel, Durme, Dender and Boven Schelde), were monthly sampled (Fig. 1).

50 litres of the surface water were collected in the middle of the stream with a bucket from a ship and filtered over a 50µm mesh size. Carbonated water was added to the sample in order to narcotize rotifers before fixing them with formalin at a final concentration of 4%. The tributaries were sampled from the shore, at about one km from their mouth.

In 2003, some specimens were also collected from the shore in Antwerpen, Dendermonde and Gent.

Salinity and temperature were measured *in situ* using an YSI 650 MDS multimeter with an YSI 600 R sensor.

TABLE 1

List of material examined, with the number of specimens and sampling station. All stations were in the Schelde estuary except Gent *, in the Boven Schelde. D indicates the distance from the mouth at Vlissingen.

	Number	Station	D (km)	Date
<i>L. decipiens</i>	1	Vlassenbroek	118	05/2002
	2	Dendermonde	121.5	05/2002
	2	Dendermonde	121.5	03/2003
	2	Sint Onolfs	127	06/2002
	2	Wetteren	147	06/2002
	2	Melle	155	06/2002
	4	Gent *	–	06/2002
<i>B. variabilis</i>	2	Temse	97.5	07/2002
	1	Mariekerke	111	06/2002
	3	Mariekerke	111	07/2002
	1	Vlassenbroek	118	07/2002
	3	Wetteren	147	07/2002
<i>K. tropica</i>	2	Kruibeke	85	07/2002
	2	Mariekerke	111	07/2002
	2	Dendermonde	121.5	08/2002
	3	Sint Onolfs	127	08/2002
	2	Appels Veer	133	08/2002
	2	Uitbergen	140	07/2002
	2	Wetteren	147	07/2002

In the laboratory, samples were stained with 4-5 drops of erythrosine, prepared at 0.8mg per 100mL of water, to facilitate detection of the organisms in the detritus rich samples. The samples were screened with a stereomicroscope Leica MZ 9.5 (9x – 90x) and specimens of interest mounted on a slide in glycerine. These were further observed with a microscope Nikon Optiphot-2 (50x – 600x) fit with a DIC process and a camera lucida for drawings and measurements.

RESULTS AND DISCUSSIONS

The following species have been diagnosed as alien species in the Schelde estuary :

Lecane decipiens (Murray, 1913)

Description : *Lecane* with a smooth ovate lorica being about four-fifths as wide as long (Fig. 2). Dorsal plate anteriorly narrower than ventral, without lateral extensions but sharp triangular projections at the antero-lateral corners. Deep lateral sulci between the dorsal and ventral plates. The lateral margins of the dorsal plate do not reach the head aperture margin. This aperture having coincident margins, strongly concave, with sinuses very deep, V-shaped and rounded posteriorly. Toe single and long, parallel sided for half and tapering gradually to point, without claw.

Diagnosis : This species can be mistaken with the common *L. hamata* (Stokes, 1896) and with *L. serrata* (Hauer, 1933). It differs from the first by the lateral margin of the dorsal plate which does not reach the head aperture, and from the second by its lorica without any ornamentation.

Measurements : The toe (length), the dorsal and ventral plates (width and length) were measured on 10 specimens from the Schelde. The mean size with standard deviation are given in Table 2.

TABLE 2

Mean size and standard deviation of *Lecane decipiens* Dorsal plate (DP), ventral plate (VP) and toe. 10 specimens were measured.

	DP length	DP width	VP length	VP width	Toe length
Mean size (µm)	76.5	62.9	80.8	54.8	37.2
Standard deviation	3.7	5.3	3.1	3.2	0.5

Distribution and abundance : *L. decipiens* was found in spring (May and June 2002) in 5 stations of the upper reaches of the estuary and in the Boven-Schelde (Fig. 3). The water temperature measured was between 19.4°C, in May, and 25.1°C, in June. *L. decipiens* also appeared in winter (March 2003) in one station (Dendermonde, km 121.5) at a water temperature of 10.0°C. The area inhabited by this *Lecane* covered around 40km, from Vlassenbroek (km 118) to the Boven-Schelde station, upstream from Gent. The salinity ranged from 0.09 to 0.11g L⁻¹. The maximum abundance was 6.1 ind L⁻¹, in June.

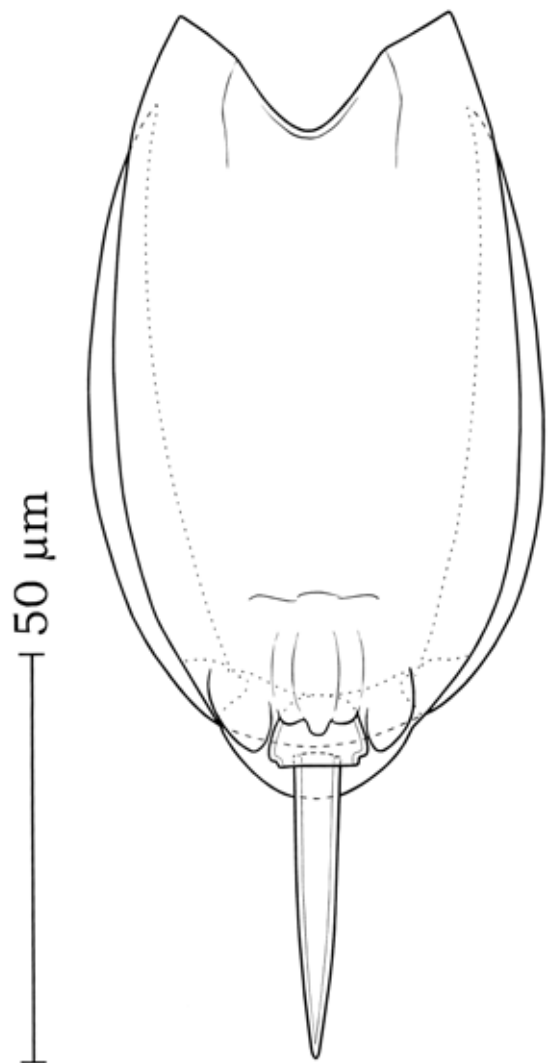


Fig. 2. – Ventral view of a female of *Lecane decipiens*.

Discussion : The measurements showed that individuals from the Schelde estuary were rather small but in accordance with the data previously reported on the species (KOSTE, 1978; SHARMA, 1980; SEGERS, 1995).

This *Lecane* originating from South and Central America was cited in Europe although all of these occurrences were uncertain or misidentified *L. hamata* (PEJLER, 1962; BRUNHES et al., 1982; SEGERS et al., 1993; SEGERS, 1995; SEGERS, 1996). The problem of reliability of the non-illustrated records as well as for some illustrated ones is well known in rotifer studies (DUMONT, 1983; KOSTE & SHIEL, 1989; SEGERS, 1998; SEGERS, 2001). The presence of *L. decipiens* in the Schelde basin in various locations (6 stations) and time (2002-2003) indicates, despite its quite low abundance in our plankton samples, the possible establishment of a rather abundant population in its typical benthic-periphytic environment. This is the first confirmed occurrence in Europe. Its presence in March 2003 associated with low temperatures indicates a large tolerance to this factor whereas the salinity range in which *L. decipiens* occurred was low.

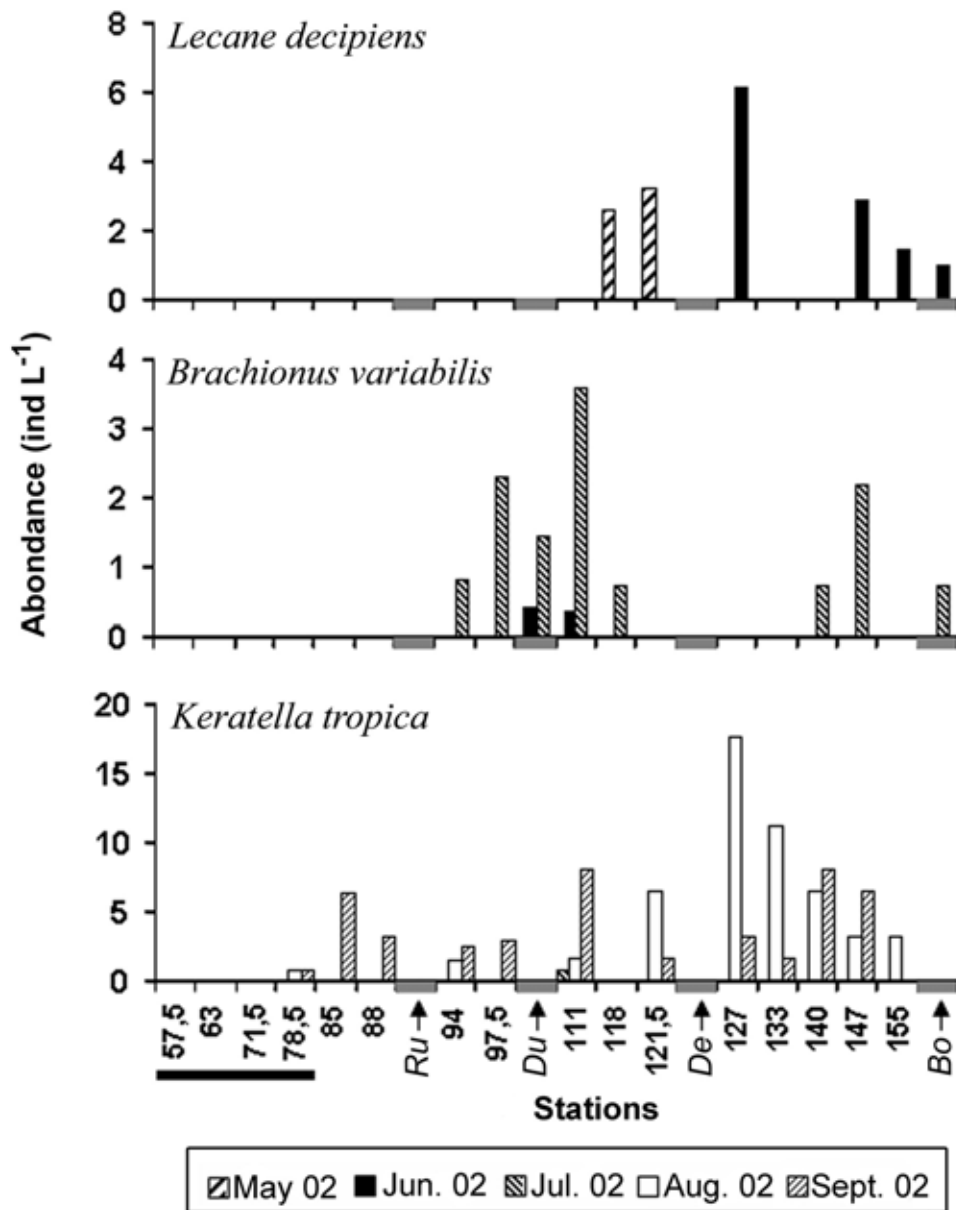


Fig. 3. – Abundance of *L. decipiens*, *B. variabilis* and *K. tropica* in the Schelde estuary in 2002. Stations in the Schelde are denoted according to their distance (in km) to the mouth, at Vlissingen. Stations in the tributaries were called “Ru” for Rupel, “Du” for Durme, “De” for Dender and “Bo” for Boven Schelde. The black line indicates the brackish area of the estuary.

According to the invasive species terminology of COLAUTTI & MAC ISAAC, 2004, this species can be considered as “non invasive” with a stage III (established species, localized and numerically rare). In May, the rotifer population was dominated by *B. calyciflorus* Pallas, 1766,

and by both *B. calyciflorus* and *B. angularis* Gosse, 1851 in June. During these periods, *L. decipiens* never represented more than 0.7% of the total rotifer abundance (Fig. 4). We can assume that the impact of *L. decipiens* on the functioning of the ecosystem is negligible.

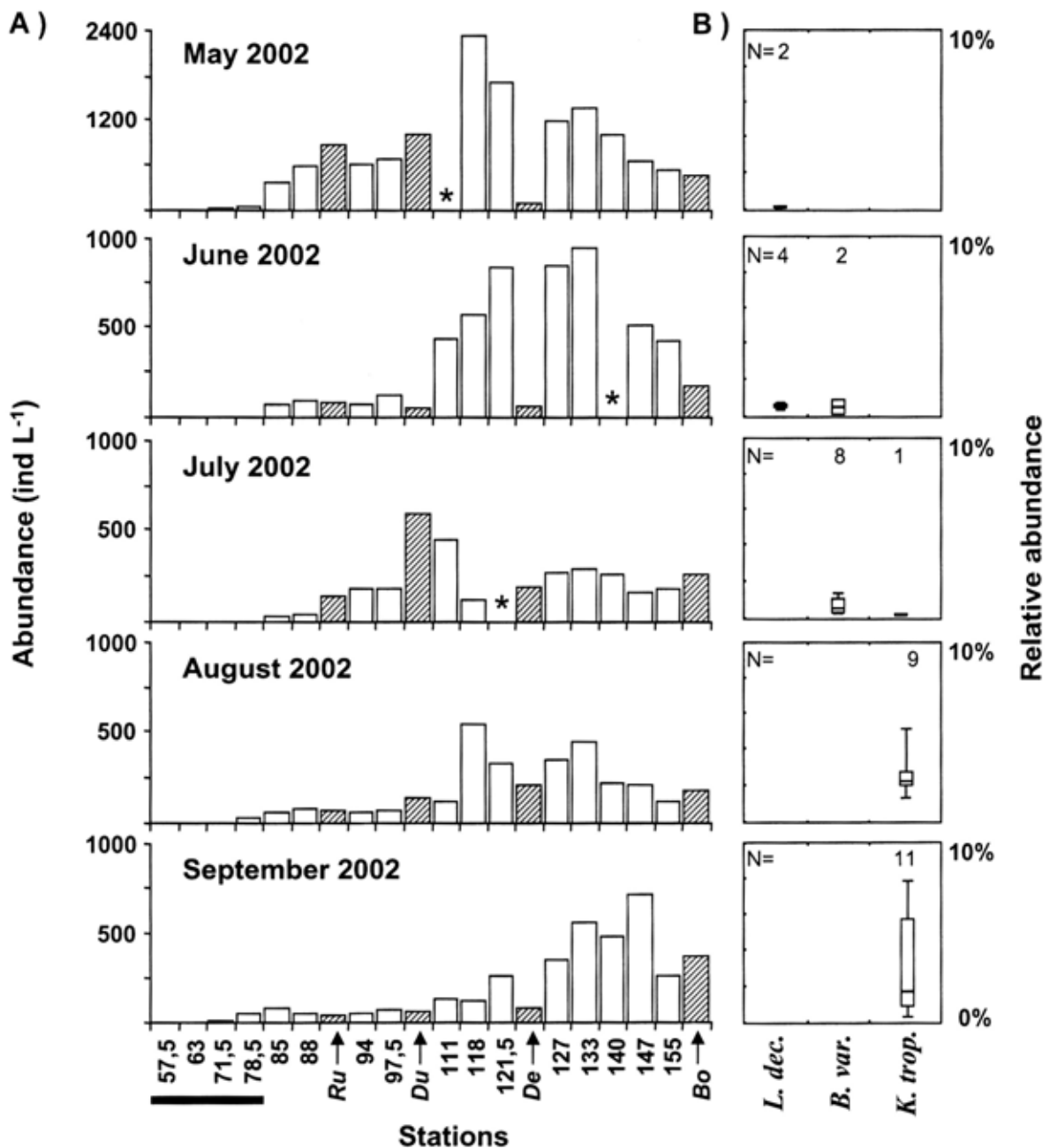


Fig. 4. – Total abundance (ind L⁻¹) of rotifers (A) from may to september 2002 in the Schelde estuary (white bars) and its tributaries (striped bars). Stations are denoted as in Fig. 3. Asterisk indicates an absence of sample. The relative abundance (B) of the introduced rotifers, *L. decipiens* (*L. dec.*), *B. variabilis* (*B. var.*) and *K. tropica* (*K. trop.*) compared to the total rotifer abundance is represented by box-plots with the mean, the minimum and maximum relative abundance at the stations where they occurred. N indicates the number of these stations.

Brachionus variabilis Hempel, 1896

Description and diagnosis : Lorica flattened dorso-ventrally bearing six spines at the antero-dorsal margin; the median ones being the longest; the intermediate and the lateral barely equal (Fig. 5). Antero-ventral margin with two rounded median projections. Two posterior spines, very variable, can appear or not, and reach a considerable length. All specimens found in the Schelde had

long spines. This *Brachionus* presents a dorsal tongue-shaped projection over the foot opening which characterise the species. The similar *B. novae-zelandiae* (Morris, 1913) does not have such projection.

Measurements : The length of the lorica with and without spines was measured on 10 specimens from the Schelde, as well as their width. The mean size with standard deviation is given in Table 3.

Distribution and abundance : *B. variabilis* was only present in the upper reaches of the estuary (Fig. 3) in summer, with quite low abundances. The maximum abundance occurred in July with 4 ind L⁻¹. Compared to the total rotifer population, the relative abundance was quite low, reaching a maximum of 1.4% of the rotifer abundance (Fig. 4). The area inhabited by *B. variabilis* covered around 70km, from Steendorp (km 94) to the Boven-Schelde station, upstream from Gent. The salinity ranged from 0.09 to 0.24 g L⁻¹. Water temperature was 20.2 to 24.1°C.

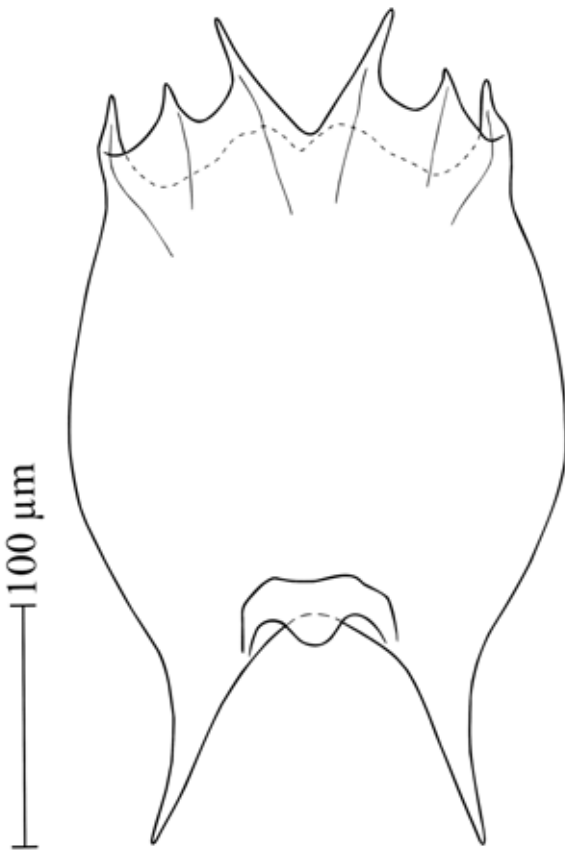


Fig. 5. – Dorsal view of a female of *Brachionus variabilis*.

TABLE 3

Brachionus variabilis length and standard deviation. 10 specimens were measured.

	Total length	Length without spines	Width
Mean size (μm)	348.2	197.5	206.7
Standard deviation	3.9	3.3	4.4

Discussion : The first occurrence of *B. variabilis* in Europe was reported in the boat racing canal in Gent (COUSSEMENT et al., 1976). Its sporadic occurrence in Belgium was noted by DUMONT (1983): “(...) *Brachionus variabilis* (...) suddenly appeared “en masse” in Belgium during the hot summer of 1976. After a short re-occurrence in 1977, it died out (?) again”. At present, this American species, originally described from Havana, Illinois, and recorded from the Palearctic, Neartic, Orien-

tal, Australian and Neotropical regions (DE RIDDER & SEGERS, 1997) is observed in the Schelde. All specimens were found in freshwater. In Belgium, this species could develop in high numbers around Antwerpen (e.g. Blokkesdijk) and in the creek region of the North West, (e.g. Sint Jan in Eremo) according to an anonymous referee. Considering its large distribution, the non-indigenous status of *B. variabilis* in Belgium is arguable. As well as *L. decipiens*, *B. variabilis* can be considered as “non invasive”, stage III (localized and numerically rare). Further data are necessary to precise the status of the Schelde basin population but an invasive stage II would suppose some recurrent introductions and dispersal along the estuary to explain its large distribution in the estuary. Nevertheless this species is not sufficiently abundant to have a significant impact on the system.

***Keratella tropica* Apstein, 1907**

Description : Lorica flattened dorso-ventrally bearing three median plates; a fourth small posterior plate characterise the species; three pairs of marginal plates (Fig. 6). Six spines at the anterior-dorsal margin, with the median ones, long and curved ventral ward. The lateral ones are usually a little longer than the intermediate. Two unequal posterior spines both variable in length but the right one is always longer than the left one which can be absent. The variations of the posterior spines length are illustrated in Fig. 7 with the different patterns found in the Schelde.

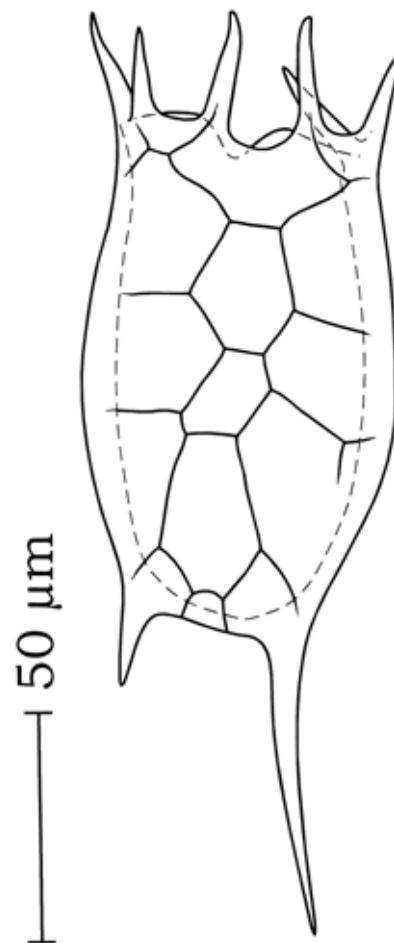


Fig. 6. – Dorsal view of a female of *Keratella tropica*.

Diagnosis : The small hindmost median plate of the lorica easily differentiates *Keratella tropica* from *Keratella valga* (Ehrenberg, 1834).

Measurements : The length of the lorica without spines was measured on 15 specimens from the Schelde, as well as the length of the two posterior spines. The mean size with standard deviation is given in Table 4.

Distribution and abundance : *K. tropica* was mainly present in the upper reaches of the estuary (Fig. 3) in the late summer (July, August and September). Some specimens were also found in Antwerpen (km 78.5) in the brackish water. The salinity ranged from 0.09 to 2.62g L⁻¹. Water temperature ranged between 18.5 and 23.6°C. The maximum abundance, observed in August, was 17.6 ind L⁻¹. The species was also observed the following years (2003-2004) during the same season. The relative

TABLE 4

Mean size and standard deviation of *Keratella tropica* lorica and posterior spines. 15 specimens were measured.

	Lorica	Left posterior spine	Right posterior spine
Mean size (µm)	109.3	56.2	9.3
Standard deviation	4.3	17.2	8.0

abundance was much higher than that of *L. decipiens* and *B. variabilis* : negligible in July 2002 (0.2% of the rotifer population) but between 1.3 and 5.1% in August 2002, and 0.6 to 7.9% in September 2002 (Fig. 4) according to the station where this species occurred.



Fig. 7. – Diversity of patterns in posterior spines of *Keratella tropica* in the Schelde estuary.

Discussion : This *Keratella* is widely distributed in tropical freshwaters and also in subtropical and temperate areas in summer. Specimens from the Schelde estuary were rather small in size compared to those from other locations and generally they had a short left posterior spine. The spatio-temporal distribution of the different forms of *K. tropica* did not present any tendency : specimens with long and short posterior left spine were both present during summer in all locations. HYNES (1970) reported the existence of a diminutive form of this species from a river (Batuluoya, Ceylon) and attributes it to an adaptation to running waters. According to GREEN (1980), the spine length was correlated with the presence of calanoid copepods. In the Schelde, calanoids were rare during summer but the cyclopoid copepod *Acanthocyclops trajani* Mirabdullayev & Defaye, 2002 (previously identified as *A. robustus*), was dominant in the freshwater tidal and is a potential predator of rotifers.

As in other estuaries, *K. tropica* was mainly found in the freshwater reaches of the estuary (SHARMA & NAIK, 1996; HOLST et al., 1998; SHIEL, 1986) but it shows a tolerance to salinity, being present in the brackish area as well. It appears to be resistant to pollutions and eutrophic conditions (DUGGAN et al., 2002; MISHRA & SAKSENA, 1998), as encountered in the Schelde estuary. Moreover, it's presence in the entire freshwater area of the estuary lead us to consider it as a stage VIa (widespread but

numerically rare) invasive species. In 1980, LEENTVAAR reported its presence in the cooling water of a factory in the Netherlands. Its warm-stenothermy determines its occurrence. *K. tropica* is now a common rotifer in Europe in summer and it is at present impossible to tell whether this is truly non-indigenous (SEGERS, pers. com.). Whatever its invasive status is, with its rather important relative abundance (max 7.9% of the rotifer abundance), *K. tropica* could have a significant role in the trophic web of the estuary.

GENERAL DISCUSSION

The OMES study was the only recent one focusing on rotifer diversity in the Schelde estuary. As such, the three species considered in this paper could have been introduced a long time ago without being observed till 2002. The date of their introduction in the Schelde remains unknown. All have been recorded in Europe before (see DE RIDDER & SEGERS, 1997). Nevertheless, the present report of *L. decipiens* is the first illustrated, hence reliable, record from Europe. All previous illustrated records of the species from Europe are misidentified *L. hamata* (SEGERS, 1996).

Even if the shipping is clearly an important vector of propagation for some species, it cannot be generalized to

all species. Transcontinental transport concentrates in marine and brackish waters so these ships are probably less appropriate for propagation of typically freshwater species as *L. decipiens* and *B. variabilis*. The freshwater reaches of the Schelde however also deal with a lot of more localized transport, which could facilitate dispersion within the adjacent basins of Rhine and Meuse.

For introduced species found in low abundance it is problematic to determine whether they are established (stages III and IVa) or not (stage II) in their new environment. The regular shipping traffic may lead to multiple introductions year by year. Nevertheless, the distribution and frequency of the occurrences are informative to determine the invading stage. *Keratella tropica* is the only rotifer species appearing to be invasive (stage IVa) at the scale of the investigated stretch of the Schelde estuary. *Lecane decipiens* and *Brachionus variabilis* are non invasive (stage III).

In combination with the adaptation capacity of some exotic species, as *K. tropica*, the actual climate change could be favourable to spreading of introduced species. Global change models predict an increase of run-off in North and Western Europe (ARNELL, 1999). MUYLAERT et al. (2001) showed changes in phytoplankton composition of the Schelde following freshets. During the period 1996-2000, the run-off of the Schelde has strongly and continuously increased and STRUYF et al. (2004) have shown a possible effect on the modification on the Schelde estuarine nutrient fluxes. In 2002 the discharge of the Schelde was indeed very high (VAN DAMME et al., 2005). These phenomena have a strong influence on the ecosystem functioning, which increases the difficulty to predict the becoming of the introduced species. Nevertheless, the 3 probably alien rotifers presented here show a development during the warmest periods of the year. Hence, an increase of the water temperature could facilitate their development in this area. However, to our knowledge, no rotifer has up to date been reported to be invasive in any estuary.

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