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Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*

M.C. van Riel[1,4], G. van der Velde[1,4,*], S. Rajagopal[1,4], S. Marguillier[2], F. Dehairs[2] & A. bij de Vaate[3,4]

[1]*Department of Animal Ecology and Ecophysiology, Section Aquatic Animal Ecology, Institute for Wetland and Water Research, Radboud University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands*
[2]*Department of Analytical Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium*
[3]*Ministry of Transport, Public Works and Water Management, Institute for Inland Water Management and Waste Water Treatment, P.O. Box 17, 8200 AA Lelystad, The Netherlands*
[4]*Member of Netherlands Centre for River Studies, P.O. Box 177, 2600 MH Delft, The Netherlands*
*(*Author for correspondence: E-mail: G.vandervelde@science.ru.nl)*

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Abstract

The Rhine ecosystem is highly influenced by anthropogenic stresses from pollution, intensive shipping and increased connectivity with other large European rivers. Canalization of the Rhine resulted in a reduction of heterogeneity to two main biotopes: sandy streambeds and riverbanks consisting of groyne stones. Both biotopes are heavily subjected to biological invasions, affecting the rivers food web structure. The Ponto-Caspian amphipods, *Chelicorophium curvispinum* and *Dikerogammarus villosus*, have exerted the highest impact on this food web. The filterfeeding *C. curvispinum* dominated the Rhine food web on the stones in 1998, swamping the stone substrata with mud. However, in 2001 it decreased in numbers, most likely due to top-down regulation caused by increased parasitic and predatory pressure of other more recently invaded Ponto-Caspian species. *D. villosus* showed a fast population increase after its invasion and particularly influenced the macroinvertebrate community on the stones by predaceous omnivory. This species seemed to have maintained its predatory level after its population established. Effects of these mass invaders on the macroinvertebrate community of sandy streambeds in the Rhine are unclear. Here, low densities of macroinvertebrates were observed with the Asiatic clam, *Corbicula fluminea*, as most abundant species. Stable isotope values of food webs from the stones and sand in 2001 were similar. Aquatic macrophytes are nearly absent and the food web is fuelled by phytoplankton and particulate organic matter, originating from riparian vegetation as indicated by similar $\delta^{13}C$ values. Omnivores, filter-, deposit-, and detritus-feeders are the primary and secondary macroinvertebrate consumers and function as keystone species in transferring energy to higher trophic levels. Invaders comprise 90% of the macroinvertebrate numbers, and can be considered ecosystem engineers determining the functional diversity and food web structure of the Rhine by either bottom-up or top-down regulation.

Introduction

The Rhine is an example of a river ecosystem under anthropogenic stress. Dikes reduce its floodplain and the main channel is canalized, reducing its heterogeneity to two main biotopes. The first biotope harbours the river bottom habitats, which are dominated by wave-exposed sand and gravel. The navigation channel itself is disturbed by intensive shipping whirling up the sand sediment, making it unsuitable for zoobenthos. The second biotope is stony substrate found at the riverbanks as riprap or basalt stone groynes (Admiraal et al., 1993). Discharges from potassium mines in France and brown coal mining in Germany salinated the water. Till the 1970s, pollution from municipal and industrial wastewater such as organic loads, heavy metals and pesticides caused a loss of native macrozoobenthos and fish (Van der Velde et al., 1990; Admiraal et al., 1993; Bij de Vaate et al., 2006). When water quality improved by sanitation, recolonization of native macroinvertebrate species and appearance of exotic species was observed in the main channel until the chemical spill of Sandoz in 1986 killed nearly all species living in the Rhine over a stretch of hundreds of kilometres (Den Hartog et al., 1992). Lifting biogeographical barriers by creating canals between large European rivers increased connectivity between the biota of river catchments (Bij de Vaate et al., 2002) and intensified shipping, enabling invasive species to enter the Rhine and to occupy its empty niches. These biological invasions led to interactions between species that had never met before and may have a profound effect on the food web structure of the invaded ecosystem (Dick et al., 1993; Nichols & Hopkins, 1993; Fahnenstiel et al., 1995). Currently, the macroinvertebrate communities of the River Rhine are numerically dominated by exotic species (Van der Velde et al., 2002). Aquatic macrophytes are nearly absent in the Rhine; so particulate organic matter (POM) originating from riparian vegetation and phytoplankton can be considered fuel for the Rhine food web in which macroinvertebrates play a key role transferring energy to higher levels within the food web.

Marguillier et al. (1998) described the Rhine food web between 1996–1998, which harboured successful invaders like the zebra mussel *Dreissena polymorpha* (first observed in 1826), the East-European pikeperch *Sander lucioperca* (1888), the Southern-European freshwater shrimp *Atyaephyra desmarestii* (1915), the Chinese mittencrab *Eriocheir sinensis* (1929), the American crayfish *Orconectes limosus* (1975), the North-American gammarid *Gammarus tigrinus* (1982), the Asiatic clams *Corbicula fluminea* (1988) and *Corbicula fluminalis* (1988), the Ponto-Caspian hydroid *Cordylophora caspia* (1874), and the Ponto-Caspian amphipods *Chelicorophium curvispinum* (1987), *Echinogammarus ischnus* (1989) and *Dikerogammarus villosus* (1995) (Nijssen & De Groot, 1987; Van der Velde et al., 2000, 2002). *C. curvispinum* dominated the Rhine food web, increasing exponentially in numbers since its first colonization in 1987 to densities of hundreds of thousands of specimens per square metre in 1990 (Van den Brink et al., 1991; Rajagopal et al., 1999). This filter-feeder builds housing tubes of mud, changing the stone substrata in the River Rhine to a muddy environment making settling on these substrates difficult for other lithophylic macroinvertebrates. *D. polymorpha* used to be the most dominant species in the Rhine, but was negatively affected by the high densities of *C. curvispinum* (Van den Brink et al., 1991; Jantz, 1996; Van der Velde et al., 1998).

When the Ponto-Caspian invader *D. villosus* entered the Rhine food web in 1995 (Bij de Vaate & Klink, 1995), *C. curvispinum* decreased in numbers (Van der Velde et al., 2000), resulting in a reduction of mud on the stone substrate, leading to an increased diversity of other macroinvertebrates (Haas et al., 2002; Van der Velde et al., 2002). *D. villosus* manifested in the food web as a predator (Van der Velde et al., 2000; Dick et al., 2002), and increased in densities up to 10,000 individuals per square metre of stone substrate. Currently, *D. villosus* and *C. curvispinum* dominate the Rhine food web on this substrate, whereas *C. fluminea* dominates the sandy riverbed in number and biomass.

Since Marguillier et al. (1998) studied the Rhine food web several new invasive Ponto-Caspian species entered the Rhine, such as the triclad flatworm *Dendrocoelum romanodanubiale* (1999), the leech *Caspiobdella fadejewi* (1999), the halacarid *Caspihalacarus hyrcanus* (2000), the isopod *Jaera istri* (1997), the mysids *Hemimysis anomala* (1997) and *Limnomysis benedeni* (1997), and the tubenose goby *Proterorhinus semilunaris* (2002); all of which entered through the Main-Danube Canal opened in 1992 (Bij de Vaate & Swarte, 2001; Van der Velde et al. 2002; Bij de Vaate, 2003). *D. villosus* is assumed to have had the strongest influence on the Rhine food web since its successful invasion. At the time of Marguilliers study, *D. villosus* had just recently invaded the Rhine and possible effects of *D. villosus* had just been noticed in the food web, which was strongly dominated by *C. curvispinum*.

This paper compares the food web of the main channel of the Rhine 1–3 years after the invasion (1996–1998) of *D. villosus*, (Marguillier, 1998) to the food web 6–8 years after its invasion (2001–2003), using macroinvertebrate monitoring data from stone substrates and stable isotope values ($\delta^{13}C$, $\delta^{15}N$). Isotope signatures of organisms are based on actual food assimilation, providing information about the long-term diet of the species (Tieszen et al., 1983; Hobson et al., 1996, 1997) and reveal the direct relationship between the carbon and nitrogen ratios of animals and those of their diets (DeNiro & Epstein, 1978, 1981; Peterson & Fry, 1987). Within a food chain, animals are usually enriched by 1–5‰ (3.4‰ on average) in $\delta^{15}N$ compared to their diet and would allow the determination of trophic position (TP) of the species, whereas $\delta^{13}C$ (1‰ enrichment on average) provides information about the original source of organic matter to the food web (Hobson & Welch, 1992).

By comparing the Rhine food web at different stages of invasion by Ponto-Caspian species we may explain; (1) whether macroinvertebrate communities in the Rhine are changed by invasions (2) the position of successful invaders in the food web, (3) source fuels for the food web and (4) changes to the major trophic pattern of the food web after invasion.

Material and methods

Sampling sites

Food web items were collected from the main distributaries of the Rhine in the Netherlands (river Waal; 5° 48′ E; 51° 51′ N) in the vicinity of Nijmegen during July–September 1996, August 1997 and August 1998. Samples were taken from the main channel of the river Waal from the groyne stone riverbanks, from the sandy streambeds and from cooling water intake sieves of an adjacent electric power plant. Sampling of food web items was repeated in the summers between April 2001 and April 2003. Macroinvertebrate species on the stone substrate and the sandy river bottom were sampled and surveyed monthly at the same site during June–September 1998 and June–September 2001.

Field collection and analysis of macroinvertebrate communities

Groyne stones were sampled at a water depth of 2 m by means of a polyp-grab operated with a hydraulic crane from a ship. Six stones were sampled monthly during June–September 1998 and June–September 2001. Macroinvertebrates were carefully brushed off the stones, collected and preserved in 70% ethanol, sorted by species, and counted in the laboratory using a stereomicroscope. The total surface area of the stones was measured in order to quantify the densities of macroinvertebrate species per square metre substrate.

Macroinvertebrates inhabiting the sandy bottom in between the groynes were sampled using a hydraulic grab (0.475×0.335 m spread, 0.15 m height), which sampled a surface area of 0.159 m^2. Large macroinvertebrates were first sieved out using a coarse sieve (5 mm mesh), and the smaller macroinvertebrates present in the sand samples were collected by subsequently whirling up the sand in water and sieving out the macroinvertebrates. Five sand bottom samples were taken each month.

A microcosm experiment was carried out to investigate the predatory impact of *D. villosus* on *C. curvispinum*. Fifty plastic cups were filled with tap water, provided with five individuals of *C. curvispinum* each and placed in a climate room; 25 cups at 15 °C and 25 cups at 25 °C with a 9/15 h dark/light regime. To 15 cups of each series one individual of *D. villosus* was added. The survival of *C. curvispinum* in absence and presence of *D. villosus* was observed every day. Dead *C. curvispinum* present in the cups with *D. villosus* were replaced. Consumed *C. curvispinum* were counted.

Field collection and laboratory treatments of food web items

Food web items were collected from the shore, water layer, stone substrate and sandy bottom of the river Waal. Samples were sorted by species, washed with distilled water and dried for 48 h at 70 °C after which specimens were ground to a fine powder using a mortar with pestle and liquid nitrogen. Due to low individual weight of some macroinvertebrate species and juvenile fish, a composite tissue sample was prepared by pooling a number of whole specimens. Shells of molluscs were removed before grinding. For adult fish and larger crustaceans, muscle tissue was used because of its slow turnover rate and would provide a history of food assimilation over periods of months and exclude short-term variability (Gearing, 1991).

Samples measured during the summers of 1996 and 1997 were treated as follows (see Marguillier, 1998). For dissolved inorganic carbon ($\text{DIC} = \Sigma\,\text{CO}_2 = [\text{CO}_{2(\text{aq})}] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]$), 50 ml of water was collected in serum vials kept airtight after 1 ml of saturated HgCl_2 solution was added to stop bacterial activity. DIC extraction followed procedures by Kroopnick (1974). POM was collected by filtering 1 l of water using Whatman GF/C filters (1.2 μm pore size), dried at 60 °C for 24 h and stored in clean petri dishes until isotopic ratio analysis. The latter was preceded by a hydrochloric acid vapour treatment under vacuum in order to remove calcium carbonate. Zooplankton was collected using a light trap. Silt material fixed on groyne stones of the breakwater was scraped off and macroinvertebrates present in the silt were collected. The δ^{13}C value for phytoplankton was derived from the δ^{13}C values of POM and DIC because isolation of phytoplankton from other suspended material was not possible and precluded direct measurement. According to Mook & Tan (1991), the average carbon isotope fractionation value of POM derived from primary production is −23‰ relative to DIC. Addition of this value with the DIC value obtained in this study (−9.65‰) was considered to give a reasonable idea of phytoplankton δ^{13}C value (−32‰). The δ^{15}N value (10.56‰) for phytoplankton was estimated by subtracting the value of 3.4‰, set as the difference between two successive trophic levels (Minagawa & Wada, 1984), from the mean δ^{15}N of all filter-, deposit- and detritus-feeders (13.96‰).

Isotope analysis

Carbon and nitrogen stable isotope compositions were measured with a Carlo Erba NA 1500 elemental analyser coupled online *via* a Finnigan Conflo III interface with a ThermoFinnigan DeltaPlus mass-spectrometer. Carbon and nitrogen isotope ratios are expressed in delta notation (δ^{13}C, δ^{15}N) relative to Vienna PDB and atmospheric nitrogen.

For comparing macroinvertebrate isotope compositions between the food web between 1996 and 1998 and that between 2001 and 2003, baseline variation was corrected for (Cabana & Rasmussen, 1996) by adding 4.39‰ (i.e. the mean decrease in δ^{15}N values of the primary consumers *D. polymorpha*, *C. fluminea* and *C. fluminalis*) to the mean δ^{15}N value measured in 2001–2003 for each species.

Trophic levels were estimated according to the method of Vander Zanden & Rasmussen (1999) based on the following overall δ^{15}N–δ^{13}C relationship:

$$\delta^{15}\text{N}_{e,\text{primaryconsumer}} = \frac{6.34}{1 + \exp[9.67 + (0.356 * \delta^{13}\text{C}_{m,\,\text{primaryconsumer}})]} \tag{1}$$

In which $\delta^{15}\text{N}_e$ is the estimated δ^{15}N value for a species, calculated from the measured δ^{13}C value ($\delta^{13}\text{C}_m$) for that species in the food web studied using the overall δ^{15}N–δ^{13}C relationship (1). The food web specific deviation from the general baseline curve was calculated for every primary consumer in the Rhine food web as follows:

$$\text{residual} = \delta^{15}\text{N}_{m,\text{primaryconsumer}} - \delta^{15}\text{N}_{e,\,\text{primaryconsumer}} \tag{2}$$

In which $\delta^{15}\text{N}_m$ is the δ^{15}N value measured for the species in the food web studied. The residual value can be calculated for every primary consumer in the food web studied (2). Subsequently, the mean residual value (U_{residual}) of all primary consumers from the food web can be calculated. The TP of each consumer in the food web can be estimated by combining the general baseline curve (1) with the mean residual value of the Rhine food web:

$$\delta^{15}\mathrm{N}_{\mathrm{corrected}} = \frac{6.34}{1 + \exp[9.67 + (0.356 * \delta^{13}\mathrm{C}_{\mathrm{consumerX}})]} + U_{\mathrm{residual}} \quad (3)$$

where $\delta^{15}\mathrm{N}_{\mathrm{corrected}}$ is the food web-corrected baseline δ^{15}N value. Equation (3) produces an appropriate baseline δ^{15}N value for each species based on the δ^{13}C signature of the species, the general δ^{15}N–δ^{13}C relationship and the food web specific deviation (U_{residual}). Finally, the TP of the species in the food web (consumer X) is estimated as follows:

$$\mathrm{TP}_{\mathrm{consumer\,x}} = ((\delta^{15}\mathrm{N}_{\mathrm{consumer\,x}} - \delta^{15}\mathrm{N}_{\mathrm{corrected}})/3.4) + 2 \quad (4)$$

where $3.4 =$ one trophic level increment (mean enrichment) in δ^{15}N.

Cluster analysis and statistical analysis

Cluster analysis was performed on the stable isotope values for food web items in 1996–1998 (Fig. 4) and 2001–2003 (Fig. 5), using euclidean distances and complete linkage (Statistica 4.5) and was used for interpretation of the food web, not for statistical proof. Differences in δ^{15}N between different life stages and sexes of *D. villosus* (Fig. 6), and differences in densities of macroinvertebrate species and taxa (Fig. 2) between June–September 1998 and June–September 2001 were tested for each species using a *t*-test (SPSS 11.5) for independent samples.

Results

Changes in the macroinvertebrate community of the Rhine

Macroinvertebrate communities on groyne stones changed during the invasion and population increase of *D. villosus* (Fig. 1). Between June and September 1998, *C. curvispinum* dominated the macroinvertebrate community during the summer with population peaks in June and July (Fig. 1a) and decreased during August and September. Between June and September 2001, population densities of *C. curvispinum* had strongly decreased and peaked in August (Fig. 1b), and other macroinvertebrate species also became abundant. Overall changes in densities of the most dominant macroinvertebrates on stone substrate between both years are shown in Figure 2a. The increase of *D. villosus* ($p = 0.005$) coincides with a significant increase of *D. polymorpha* ($p = 0.005$) and *Ancylus fluviatilis* ($p = 0.02$) and a highly significant decrease of *C. curvispinum* ($p = 0.001$). Populations of *Hypania invalida*, *D. romanodanubiale* ($p = 0.012$) and Gastropoda ($p = 0.003$) other than *A. fluviatilis*, showed a smaller, significant decrease in density. Laboratory experiments confirmed that *D. villosus* could negatively affect populations of *C. curvispinum* (Fig. 8). Survival of *C. curvispinum* (Fig. 8) was higher at a high temperature (25 °C) than at a lower temperature (15 °C). At high temperature survival of *C. curvispinum* was strongly reduced in presence of *D. villosus* due to predation.

On the sandy bottom, *C. fluminea* dominated macroinvertebrate communities during the summer of 1998, with the highest densities observed in July. Oligochaetes and Chironomidae were also relatively abundant, but overall densities were low (Fig. 1c). During 2001, densities of macroinvertebrates were higher than observed in 1998 and biodiversity had increased. *C. fluminea* dominated the community during August and September (Fig. 1d). A comparison of the densities of various taxa in both years shows most taxa had increased in number on the sand bottom (Fig. 2b).

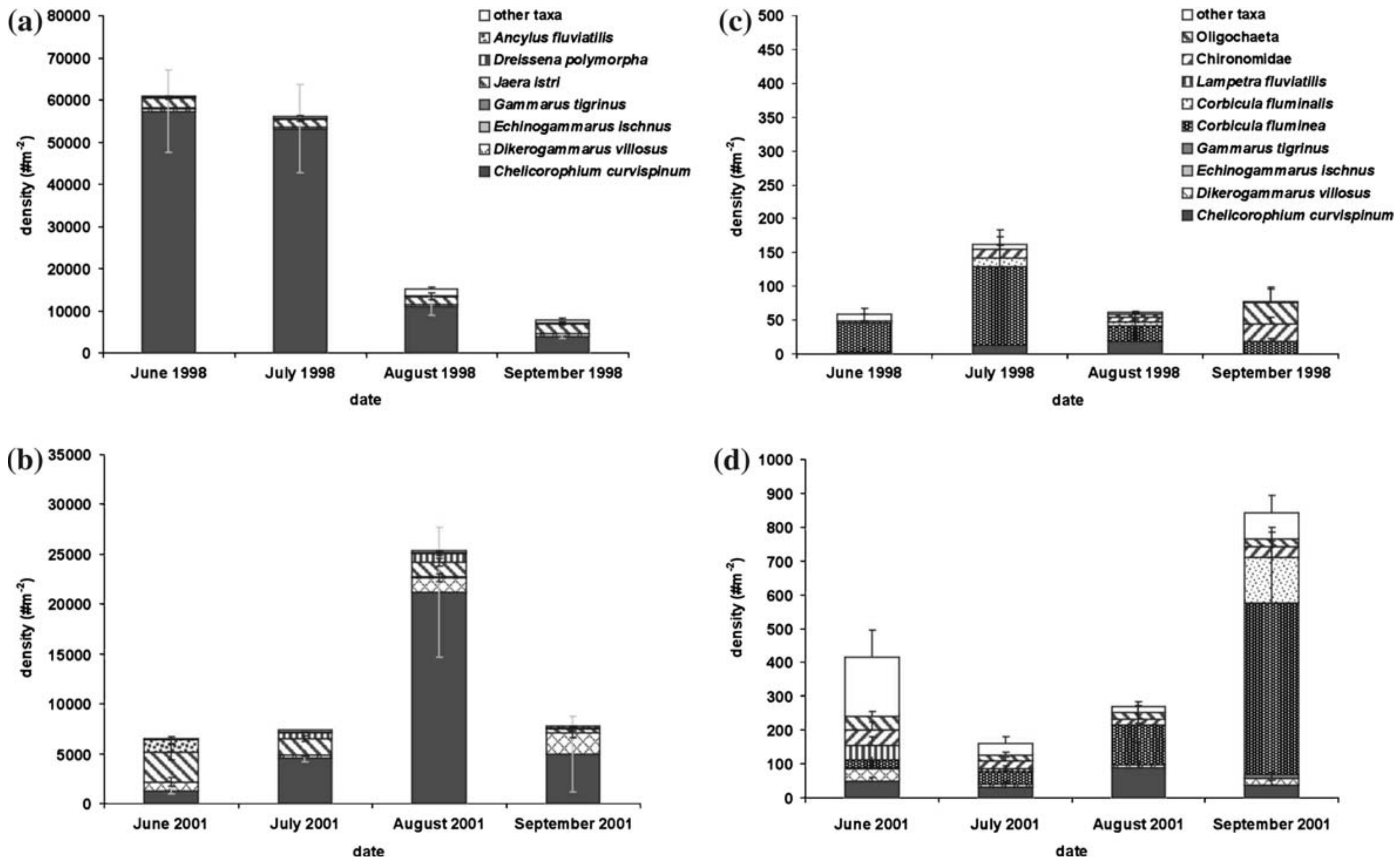

Figure 1. Macroinvertebrate species composition on the stones (a, b) and on the sandy bottom (c, d) in the Waal in June–September 1998 (a, c) and June–September 2001 (b, d).

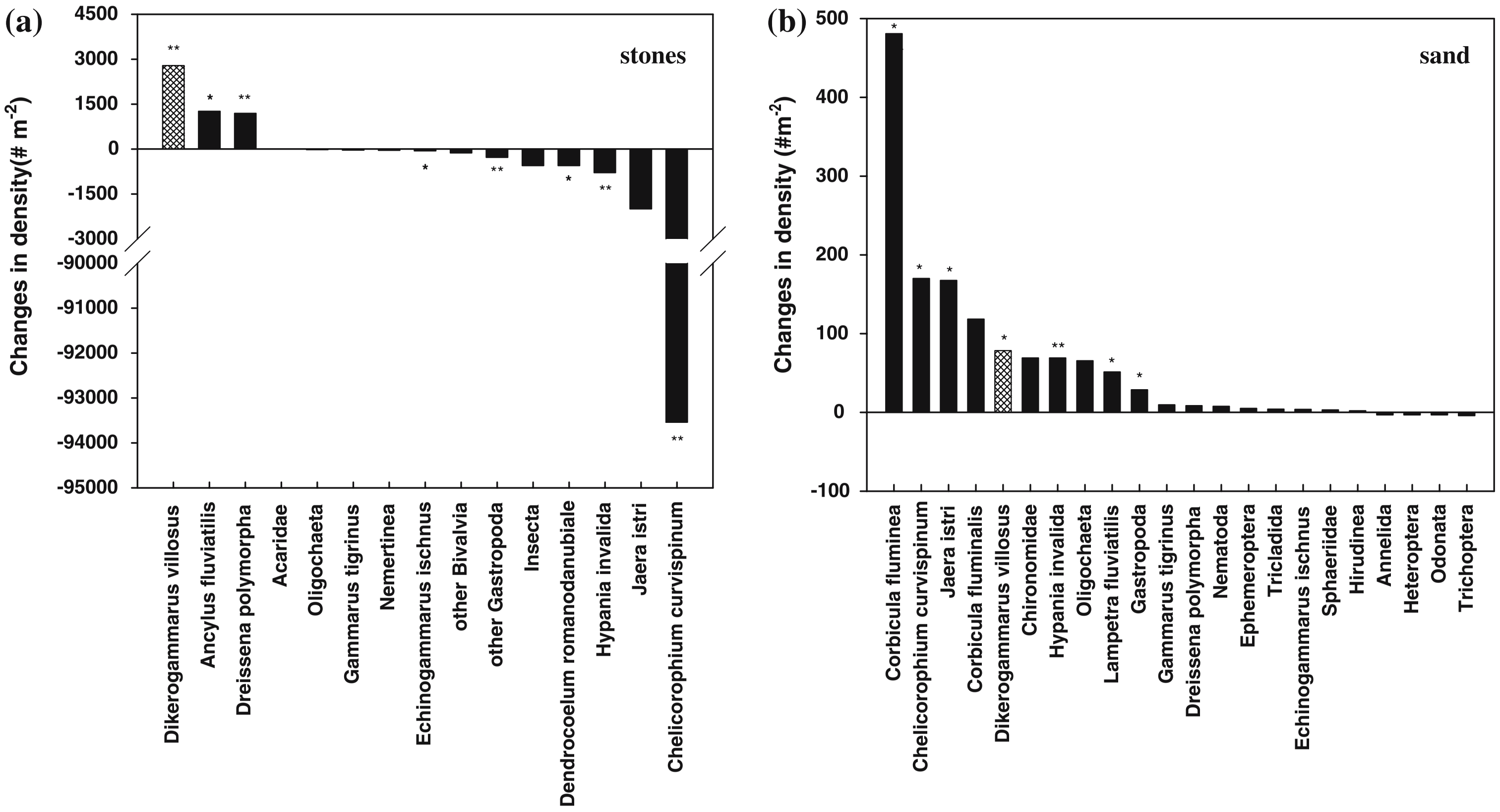

Figure 2. Changes in densities of the total numbers of *D. villosus* (shaded columns) and other macroinvertebrate species and taxa (black columns) on the stone substrate (a) and on the sandy bottom (b) in the Waal in the period 1998–2001. Significance levels: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

Table 1. Mean isotopic values ($\delta^{13}C$, $\delta^{15}N$), for items occurring in the Rhine food web in 1996–1998, with corresponding standard deviations. Arranged according to trophic level

	Code	*n*	*n*[b]	Size range (cm)	Means		SD		Range		Trophic level
					$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	
Sander lucioperca	Sl	77	–	3.4–42.6	17.75	−27.14	1.26	2.73	20.67–14.12	−32.44 to −19.85	3.17
Cottus gobio	Cg	6	–	4.9–7.1	17.75	−26.01	0.76	0.96	19.35–17.12	−27.40 to −24.46	3.17
Gymnocephalus cernuus	Gc	44	–	4.0–12.5	17.43	−27.47	0.93	1.17	19.59–15.73	−30.64 to −25.40	3.07
Gobio gobio	Gg	5	–	8.4–11.2	17.30	−25.82	0.34	0.15	19.15–8.57	−54.14 to −13.90	3.04
Platichthys flesus	Pf	3	–	21.8–25.5	17.22	−26.64	0.75	0.87	17.79–16.37	−27.23 to −25.64	3.01
Aspius aspius	Aas	7	–	5.5–8.3	16.57	−26.99	1.39	1.16	18.49–14.47	−28.85 to −25.61	2.82
Perca fluviatilis	Pf	53	–	4.2–18.0	16.54	−28.59	0.81	1.27	18.35–15.06	−30.64 to −24.80	2.81
Scardinius erythrophthalmus	Se	2	–	16.3	16.43	−26.12	0.36	2.29	16.68–16.17	−27.74 to −24.50	2.78
Blicca bjoerkna	Bb	84	–	3.2–31.5	16.42	−27.69	0.99	1.46	20.28–14.15	−32.47 to −25.37	2.78
Anguilla anguilla	Aa	35	–	10.5–68.2	16.35	−28.18	1.35	1.64	18.51–13.49	−31.16 to −24.34	2.76
Osmerus eperlanus	Oe	2	–	6.1–6.5	16.18	−28.37	0.46	0.54	16.50–15.86	−28.75 to −27.99	2.71
Rutilus rutilus	Rr	53	–	3.3–29.0	16.18	−28.09	1.30	1.57	19.42–12.94	−32.02 to −24.62	2.70
*Eriocheir sinensis**	Es	1	–	4.0–5.0	16.16	−28.26	na	na	na	na	2.70
Cobitis taenia	Ct	1	–	5.0	16.01	27.45	na	na	na	na	2.66
Alburnus alburnus	Aal	20	–	4.6–15.0	15.94	−28.11	1.46	1.00	19.13–14.19	−30.28 to −26.29	2.64
Palaemon longirostris	Pl	1[a]	6	na	15.62	−26.73	na	na	na	na	2.54
Atyaephyra desmarestii	Ad	1[a]	5	na	15.56	−30.34	na	na	na	na	2.52
Gasterosteus aculeatus	Ga	5	–	3.4–5.3	15.56	−29.48	3.19	1.92	19.17–9.54	−32.50 to−27.38	2.52
*Orconectes limosus***	Ol	8	–	0.7–6.0 (carapace)	15.58	−26.69	0.75	0.11	na	na	2.43
Leuciscus cephalus	Lc	3	–	4.0–5.2	15.12	−26.29	1.29	0.85	16.55–14.04	−27.06 to −25.39	2.40
Hydropsyche spec.	Hs	3	–	na	15.04	−29.45	na	na	na	na	2.37
Abramis brama	Ab	12	–	9.0–33.0	14.82	−28.12	0.95	1.20	16.28–13.15	−30.46 to −26.89	2.31
Dikerogammarus villosus	Dv	1[a]	>10	na	14.65	−28.17	1.04	1.67	na	na	2.26

Corbicula fluminea	Cfa	1[a]	>10	na	14.48	−29.60	na	na	na	na	2.21
Chironomidae	C	3[a]	±150	na	14.44	−28.41	na	na	na	na	2.19
Ephydatia fluviatilis	Ef	–	–	na	14.37	−28.28	na	na	na	na	2.17
Gammarus tigrinus	Gt	1[a]	>11	na	14.33	−29.52	3.23	1.81	na	na	2.15
Corbicula fluminalis	Cfs	1[a]	>10	na	14.26	−29.33	na	na	na	na	2.14
Bithynia tentaculata	Bt	1[a]	>10	na	14.24	−27.83	na	na	na	na	2.14
Chondrostoma nasus	Cn	1	–	5.7	14.14	−29.30	na	na	na	na	2.11
Cyprinus carpio	Cca	2	–	9.1–9.2	14.13	−24.82	0.46	2.21	14.46–13.80	−26.38 to −23.25	2.10
Chelicorophium curvispinum	Ccu	1[a]	>10	na	14.09	−28.54	na	na	na	na	2.09
Dreissena polymorpha	Dp	1[a]	>10	na	13.78	−29.51	na	na	na	na	2.00
Cordylophora caspia	Cc	1[a]	>10	na	13.61	−28.79	na	na	na	na	1.95
Copepods	Cp	1[a]	>10	na	13.14	−27.85	na	na	na	na	na
Plumatella repens	Pr	1	>10	na	12.67	−31.14	na	na	na	na	1.67
Mud tubes of *C. curvispinum*	T	5	–	na	12.12	−26.69	0.19	0.36	12.00–10.05	−28.62 to −26.69	na
Silt	S	2	–	na	12.08	−26.67	na	na	na	na	na
Lampetra fluviatilis adult (silver colour)	Lfad	3	–	12.0–34.8	11.36	−25.53	0.36	2.57	11.62–10.95	−27.70 to −22.70	1.29
Cladophora glomerata	Cg	1	–	na	11.13	−22.96	na	na	na	na	na
Phytoplankton	Phyt	1	–	na	10.56	−32.00	na	na	na	na	na
POM	POM	1	–	na	10.25	−24.27	0.21	0.23	na	na	na
Lampetra fluviatilis juvenile (brown colour)	Lfj	3	–	9.0–13.0	9.81	−22.53	0.65	1.04	9.81–8.57	−23.65 to −21.60	0.69
Plumatella fungosa	Pf	1	>10	na	9.08	−28.75	na	na	na	na	0.62
DIC	DIC	1	–	na		−9.65	na	na	na	na	na

Items are sorted by their values of d 15N/14N.

[a] Pooled samples.

[b] Size of pooled samples.

na = Not analysed.

* carapace width, ** carapace length

Table 2. Mean isotopic values ($\delta^{13}C$, $\delta^{15}N$), for items occurring in the Rhine food web in 2001–2003, with corresponding standard deviations

	Code	*n*	n^b	Size range (cm)	Means		SD		Range		Trophic level
					$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	
Lampetra fluviatilis adults	Lfa	3	–	13.0–22.5	16.27	−20.20	0.10	0.47	16.35–16.20	−20.54 to −19.86	5.08
Sander lucioperca	SI	28	–	9.5–51.0	15.96	−28.49	1.47	1.60	17.85–11.64	−31.77 to −25.67	3.98
Palaemon longirostris	PI	3	–	5.0–5.5	15.22	−26.75	0.89	0.07	16.02–14.26	−26.82 to −26.69	4.05
Eriocheir sinensis	Es	18	–	4.1–7.5 (carapace)	14.55	−27.03	0.49	0.89	15.85–13.91	−29.14 to −25.57	3.81
Abramis brama	Ab	20	–	10.0–30.0	14.52	−27.54	1.35	2.10	16.11–12.79	−33.77 to −24.66	3.71
Blicca bjoerkna	Bb	22	–	9.5–30.5	14.15	−28.58	1.17	1.61	15.96–11.07	−33.84 to −26.75	3.43
Rutilus rutilus	Rr	14	–	7.0–24.5	13.87	−29.10	0.86	1.72	15.41–11.99	−34.85 to −26.82	3.27
Perca fluviatilis	Pf	22	–	7.5–28.0	13.64	−28.62	1.72	1.98	16.03–10.52	−32.85 to −26.15	3.28
Orconectes limosus	OI	4	–	8.5–10.4	13.56	−26.69	0.28	1.40	13.97–13.37	−28.37 to −24.99	3.57
Porifera gemmula	Pogem	6[a]	500	0.1	13.35	−30.19	1.02	1.10	14.94–12.57	−29.59 to −26.56	na
Ancylus fluviatilis	Af	5[a]	35	na	12.32	−28.79	3.03	1.17	15.11–8.16	−30.19 to −27.34	3.16
Pomphorhynchus spec.	Ps	1[a]	6	na	11.60	−27.67	na	na	na	na	2.83
Dikerogammarus villosus adults > 1.2 cm	Dvbigad	5	10	1.3–2.1	11.27	−26.09	1.00	0.37	12.31–10.61	−28.07 to −26.03	3.03
Porifera	Po	6	–	na	11.06	−28.36	0.51	1.68	11.55–10.08	−30.89 to −26.49	2.56
Hydropsyche bulgaromanorum	Hb	1[a]	15	na	10.95	−29.27	0.07	2.76	11.00–10.89	−31.20 to −27.31	2.39
Dikerogammarus villosus adults	Dvad	9[a]	50–100	1.0–1.3	10.93	−26.33	0.97	0.49	12.63–8.96	−27.92 to −25.64	2.86
Jaera istri	Ji	15[a]	300–500	0.1–0.5	10.88	−26.68	0.83	0.48	14.74–10.03	−27.22 to −26.33	2.78
Chelicorophium curvispinum	Ccu	18[a]	±150	na	10.74	−28.08	0.90	1.12	12.03–8.40	−29.05 to −26.49	2.51
Dikerogammarus villosus mean	Dv	33[a]	10–150	0.4–2.1	10.64	−26.48	1.21	0.49	12.31–8.07	−31.20 to −25.64	2.71
Dikerogammarus villosus juveniles	Dvjuv	22[a]	150	0.4–0.8	10.53	−26.54	1.29	0.51	11.72–8.07	−27.72 to −25.69	2.70
Gammarus tigrinus	Gt	1[a]	30	0.8–1.3	10.48	−26.63	0.65	0.43	11.18–9.32	−27.13 to −25.78	2.68
Echinogammarus ischnus	Ei	3[a]	5	na	10.22	−26.04	0.38	0.48	10.52–9.79	−26.44 to −25.50	2.69
Moss	moss	6	–	na	10.01	−30.82	2.23	5.63	11.53–6.06	−35.15 to −23.25	na
Corbicula fluminalis	Cfs	1[a]	20	na	9.86	−30.37	0.00	0.00	na	na	1.92
Corbicula fluminea	Cfa	1[a]	20	na	9.80	−29.50	0.25	1.16	10.08–9.40	−30.68 to −20.03	2.02
Chironomidae	C	6[a]	±150	na	9.78	−30.64	1.99	1.75	11.63–6.39	32.77 to −28.71	1.87

Dreissena polymorpha	Dp	44[a]	±20	1.0–3.0	9.68	−28.81	0.78	1.73	12.70–8.37	−32.17 to −22.65	2.09
Anodonta anatina	Aa	1	–	10.0	9.61	−29.40	na	na	na	na	1.98
Lampetra fluviatilis juveniles, larvae	Lfj	3[a]	30	1.5–8.5	9.61	−28.11	1.67	3.67	11.49–8.30	−31.70 to −24.37	2.17
Mud tubes of *C. curvispinum*	T	9	–	na	9.50	−24.23	1.08	3.07	12.02–8.43	−31.34 to −21.77	na
Hypania invalida	Hi	1[a]	70	na	9.38	−26.4	0.00	0.00	na	na	2.39
Cordylophora caspia	Cc	1	–	na	9.28	−28.84	0.27	0.22	9.47–9.09	−29.00 to −28.68	1.96
Plant matter	Pm	23	–	na	9.22	−22.77	1.10	2.86	11.68–7.64	−28.88 to −16.56	na
Green algae	Algae	1	–	na	9.22	−16.23	na	na	na	na	na
Bryozoa	Bryo	5	–	na	9.03	−26.70	0.30	2.14	9.44–8.72	−29.59 to −24.54	2.24
Carex acuta	Ca	1	–	na	7.70	−28.54	na	na	na	na	na
Mud	mud	3	–	na	5.88	−18.02	3.36	4.62	9.08–2.39	−22.83 to −13.62	na
Potentilla anserina	Pa	1	–	na	5.75	−28.80	na	na	na	na	na
Cladophora glomerata	Cg	1	–	na	5.57	−21.00	na	na	na	na	na
Clinclidotus spec.	Cs	1	–	na	5.26	−33.84	na	na	na	na	na
Equisetum arvense	Ea	1	–	na	4.95	−28.59	na	na	na	na	na
Lythrum salicaria	Ls	1	–	na	4.83	−27.06	na	na	na	na	na
Festuca arundinacea	Fg	1	–	na	4.62	−30.02	na	na	na	na	na
Plantago major	Pm	1	–	na	4.49	−28.80	na	na	na	na	na
Rubus fruticosus	Rf	1	–	na	4.02	−28.88	na	na	na	na	na
Salix alba	Sa	7	–	na	3.89	−27.81	2.73	1.19	7.64–1.55	−29.94 to −26.50	na
Rorippa sylvestris	Rs	1	–	na	3.86	−28.46	na	na	na	na	na
Eryngium campestre	Ec	1	–	na	3.51	−29.70	na	na	na	na	na
Taraxacum officinale	To	1	–	na	2.60	−29.43	na	na	na	na	na
Cirsium arvense	Ca	1	–	na	2.55	−29.56	na	na	na	na	na
Achillea millefolium	Am	1	–	na	2.18	−29.43	na	na	na	na	na
Calamagrostis epigejos	Ce	1	–	na	1.96	−28.14	na	na	na	na	na
detritus	det	13	–	na	1.86	−27.12	0.28	0.06	2.06–1.66	−27.17 to −27.08	na

[a] Pooled samples.

[b] Size of pooled samples.

na = Not analysed.

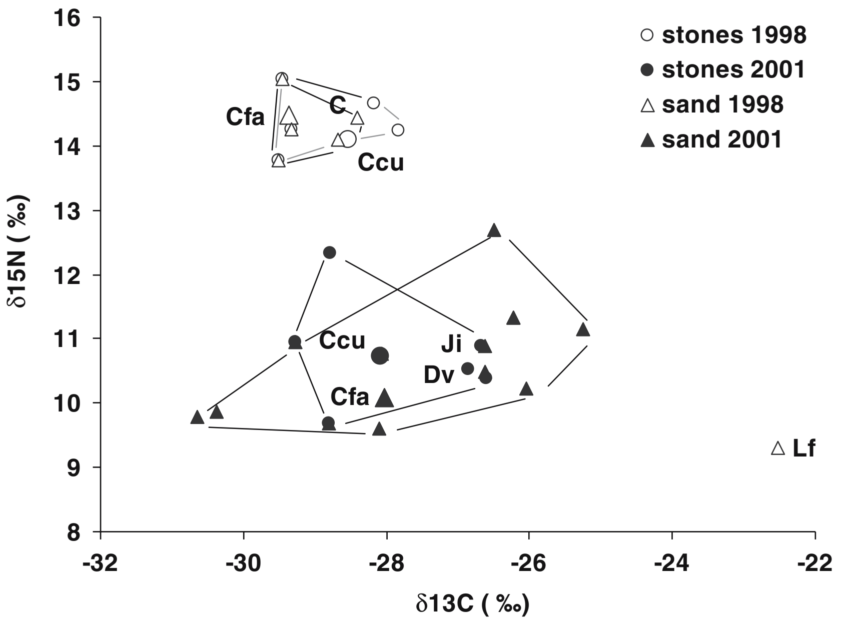

Figure 3. Distributions of $\delta^{13}C$ and $\delta^{15}N$ values in the food web of the two different Rhine biotopes viz. stones and sand in 1998 and 2001. The isotopic values of the dominant species in the biotopes are indicated by enlarged symbols and are coded: Cfa: *Corbicula fluminea*, Ccu: *Chelicorophium curvispinum*, Dv: *Dikerogammarus villosus*, Ji: *Jaera istri* and C: Chironomidae, Lf: *Lampetra fluviatilis* larvae.

Food web studies by stable isotopes

Macroinvertebrate food webs of the different biotopes; groyne stones and sandy bottom

Mean values of $\delta^{13}C$ of the food web on the stones in 1998 were comparable to those observed in 2001 and ranged from −29.51‰ (*D. polymorpha*) to −27.34 i.s.o. −27.83‰ (*A. fluviatilis*) and from −29.76‰ (Bivalvia) to −26.61‰ (Annelida), respectively (Fig. 3). However, mean values of $\delta^{15}N$ of the food web on the sand bottom as well as the stones differed between 1998 and 2001. The mean $\delta^{15}N$ values of the groyne stone food web ranged from 13.78‰ (*D. polymorpha*) to 15.04‰ (Insecta) in 1998 and from 9.68‰ (*D. polymorpha*) to 12.32‰ (*A. fluviatilis*) in 2001. Mean values of $\delta^{13}C$ of species on the sandy bottom food web in 1998 and 2001 ranged from −29.51‰ (*D. polymorpha*) to −22.53‰ (*Lampetra fluviatilis* larvae) and from −30.64‰ (Chironomidae) to −26.04‰ (*E. ischnus*), respectively (Fig. 3), and are comparable to $\delta^{13}C$ values of the food web on stones. The mean $\delta^{15}N$ values of species in the sand food web ranged from 9.31‰ (*L. fluviatilis* larvae) to 15.04‰ (Insecta) in 1998 and from 9.61‰ (*L. fluviatilis* larvae) to 12.70‰ (*D. romanodanubiale*) in 2001. Mean $\delta^{15}N$ values have decreased since 1998 for most species in the food webs of both biotopes.

Total food web of the Rhine

Three trophic levels can be distinguished in the food web between 1996 and 1998 (Fig. 4). The primary level is based on POM (cluster 1) or suspended organic matter and phytoplankton (cluster 2). The second level harboured most species and consisted of primary and secondary consumers (cluster 3), mainly macroinvertebrates inhabiting the stone substrate. The third level consists of top predators (cluster 4). The mean carbon and nitrogen isotope ratios ($\delta^{13}C$, $\delta^{15}N$) of items occurring in the Rhine food web for the period of 1996–1998 ranged from −31.14‰ (*Plumatella repens*) to −22.53‰ (*Lampetra fluviatilis* larvae) for $\delta^{13}C$ and from 9.08‰ (*Plumatella fungosa*) to 17.75‰ (*Sander lucioperca*) for $\delta^{15}N$ (Fig. 4, Table 1).

Two clusters formed the base of the food web, the first cluster consisting of POM (containing both phytoplankton and organic detrital material (Marguillier, 1998)), *Cladophora glomerata* and *L. fluviatilis* larvae, and the second cluster containing

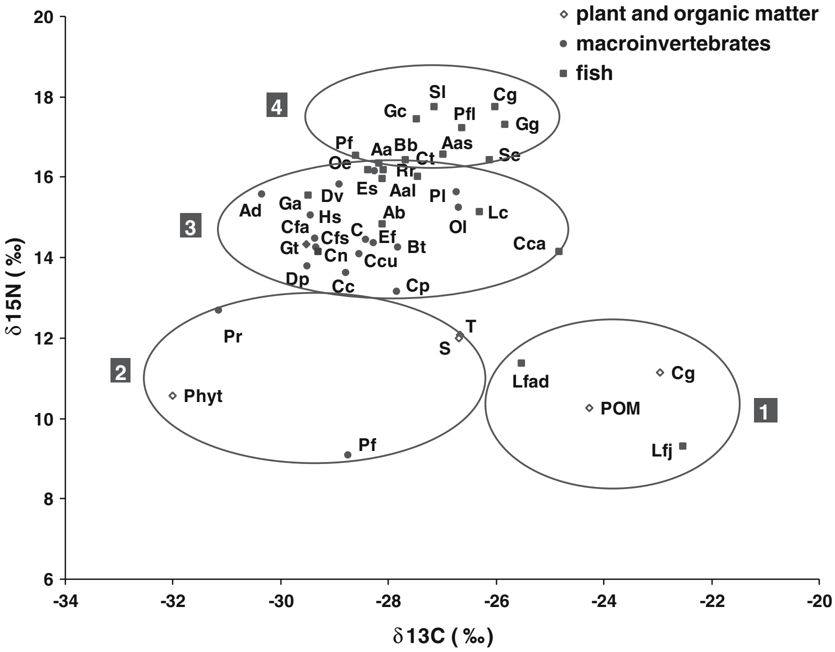

Figure 4. Stable isotopic values of organisms in the River Rhine food web for the period of 1996–1998 according to Marguillier et al. (1998). Mean values are given in the figure, corresponding standard deviations, standard errors of the mean and full names of the abbreviations are shown in Table 1. The circles 1–4 indicate the different clusters of trophic groups (obtained using Statistica 4.5).

phytoplankton, silt, housing tubes of *C. curvispinum* and both *Plumatella* species. The larvae of the river lamprey *L. fluviatilis* showed isotopic values distinct to that of the parasitic adults. Values of δ^{13}C for muddy housing tubes of *C. curvispinum* are similar to that of silt material. Isotopic carbon composition of DIC from the Waal was −9.65‰, which suggests phytoplankton in the river to have a mean δ^{13}C value of −32.00‰.

The second level of the food web consisted of primary consumers, filter, deposit and detritus feeders, with δ^{15}N values ranging from 13.14‰ (copepods) to 14.65‰ (*D. villosus*). The δ^{13}C values from these primary consumers and omnivores ranged from −24.82‰ *C. carpio* to −31.97 (*A. desmaresti*). These values show considerable depletion in ^{13}C relative to the δ^{13}C value of POM. *Palaemon longirostris* and *Orconectes limosus* are on average comparable in isotopic level, indicating a similar feeding preference.

Mean isotopic values of zoobenthivorous and/or zooplankton feeding fish like bream (*Abramis brama*), eel (*A. anguilla*) and roach (*Rutilus rutilus*) are comparable to the group of primary and secondary consumers mentioned and are clustered together with secondary consumers as well as with zoobenthivorous fish, functioning as transitive species from the second to the third trophic level of the Rhine food web. Zoobenthivorous fish as *Blicca bjoerkna*, *Gymnocephalus cernuus* and piscivorous fish *Perca fluviatilis* and *S. lucioperca* represent the highest predatory level in the Waal food web.

In the food web between 2001 and 2003, three trophic levels similar to those of the food web between 1996 and 1998 could be distinguished (Fig. 5) extended by a cluster of riparian plant species. The mean carbon and nitrogen isotope ratios (δ^{13}C, δ^{15}N) of items occurring in the Rhine food web for the period of 2001–2003 ranged between −33.84‰ (the moss *Cinclidotus* spec.) and −16.23‰ (green algae) for δ^{13}C and 1.86‰ (detritus) and 16.27‰ (*L. fluviatilis*, adults) for δ^{15}N (Fig. 5, Table 2).

Riparian plants and *Cinclidotus* spec. were clustered together (cluster 1), showing the lowest δ^{15}N values in distance from the aquatic consumer food web. The mean δ^{13}C values of these plants were similar to those of primary and secondary consumers and ranged from −27.06 (*Lythrum*

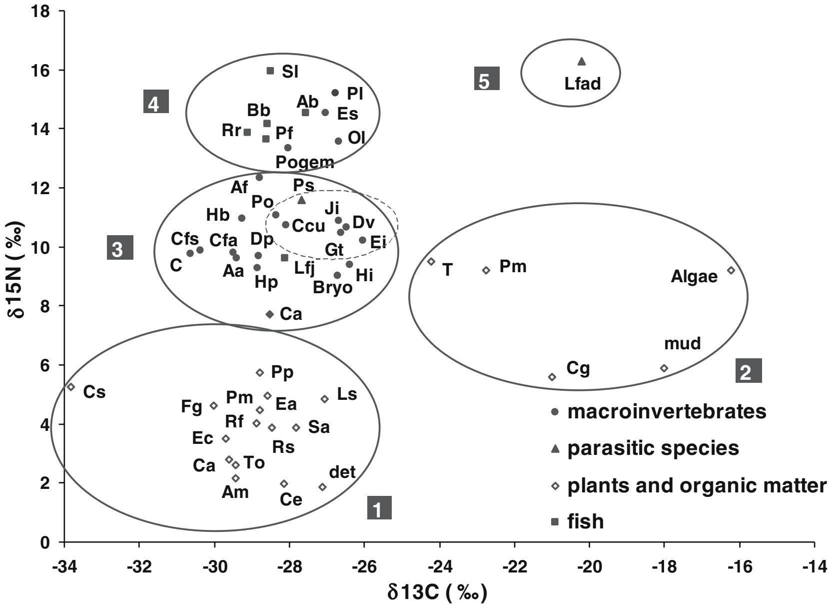

Figure 5. Stable isotopic values of organisms in the River Rhine food web for the period of 2001–2003. Mean values are given in the figure, corresponding standard deviations, standard errors of the mean and full names of the abbreviations are shown in Table 2. The circles 1–5 indicate the different clusters of trophic groups (obtained using Statistica 4.5).

salicaria) to −33.84 (*Cinclidotus* spec.). The second cluster at the first trophic level consisted of green algae, mud, plant matter and housing tubes of *C. curvispinum*. Green algae and decomposing plant matter in the Waal show a scattered pattern of δ^{13}C values with no clear enrichment in δ^{13}C compared to detritus and silt.

The δ^{13}C values from the primary consumers (cluster 3), filter, deposit and detritus feeders and omnivores ranged from −26.04 (*E. ischnus*) to −30.64 (chironomids), showing less depletion in δ^{13}C relative to detrital organic matter (difference −1.08 to −3.52‰) than in the food web between 1996 and 1998. Isotopic values of the Asian clams *C. fluminalis* and *C. fluminea*, the American gammarid *G. tigrinus*, the Ponto-Caspian amphipods *C. curvispinum*, *E. ischnus* and *D. villosus* and the isopod *Jaera istri* are within the range of other filter, deposit and detritus feeders and omnivores. Among these groups, the amphipods *C. curvispinum* and *D. villosus* have the highest δ^{15}N value (10.74 ± 0.90‰ and 10.64 ± 1.21‰).

Fish species (cluster 4) show δ^{13}C values (−29.10 ± 1.72‰ to −24.94 ± 5.05‰) similar to those of most macroinvertebrate species. Their δ^{15}N values, however, indicate a higher trophic level of feeding, similar to the level of *Palaemon longirostris*, and *Eriocheir sinensis*, all clustered with the highest δ^{15}N values, and are the top-predators of the food web. *P. longirostris* and *O. limosus* are less comparable in isotopic level in the food web between 2001 and 2003, indicating a less similar feeding preference during this period. Isotopic nitrogen values of the organisms in the food web between 2001 and 2003 were lower than of those of the food web between 1996 and 1998 for almost all species, whereas the isotopic carbon values were similar (Figs. 3–5). Piscivorous fish (cluster 4 and 5) are the top predators in the Rhine food web.

Comparison of the isotope signatures of the most abundant macroinvertebrate species from both food webs after correction of the differences in baseline δ^{15}N values (Fig. 6) shows comparable isotope values for most macroinvertebrate species for both food webs. Lower mean δ^{13}C values for *D. villosus* and *G. tigrinus* and a differently related position of *C. curvispinum* to *D. villosus* for the different periods are remarkable. During 1996–1998, isotopic values of *D. villosus* related to those of other macroinvertebrates indicated chironomids and *C. curvispinum* as potential prey items (Figs. 6 and 8) whereas these values were less related in 2001–2003. Standard

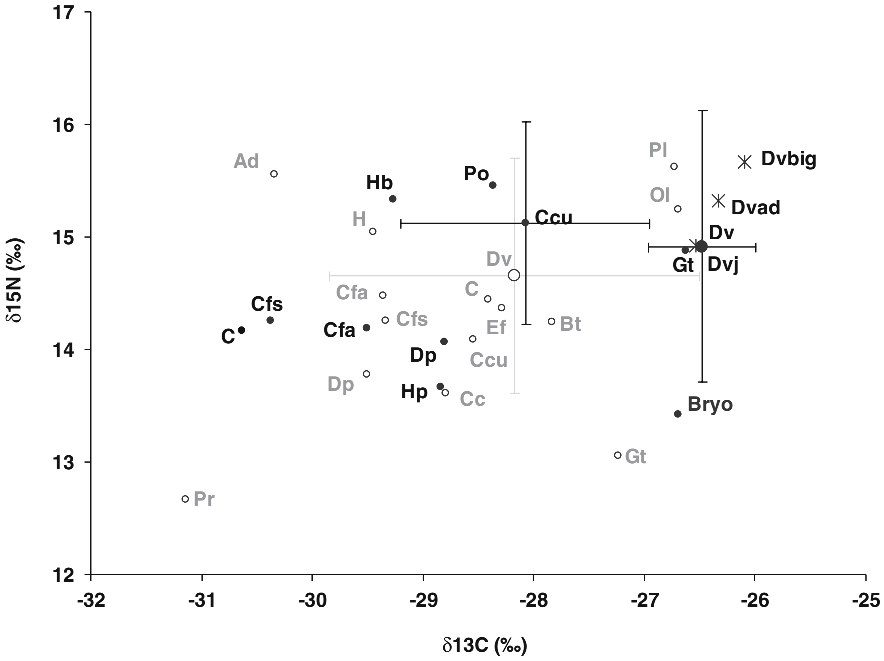

Figure 6. Mean stable isotope values of macroinvertebrate species from both food webs after correction for the nitrogen depletion in the river system after 1998. Standard deviations are shown for the species *C. curvispinum* and *D. villosus*.

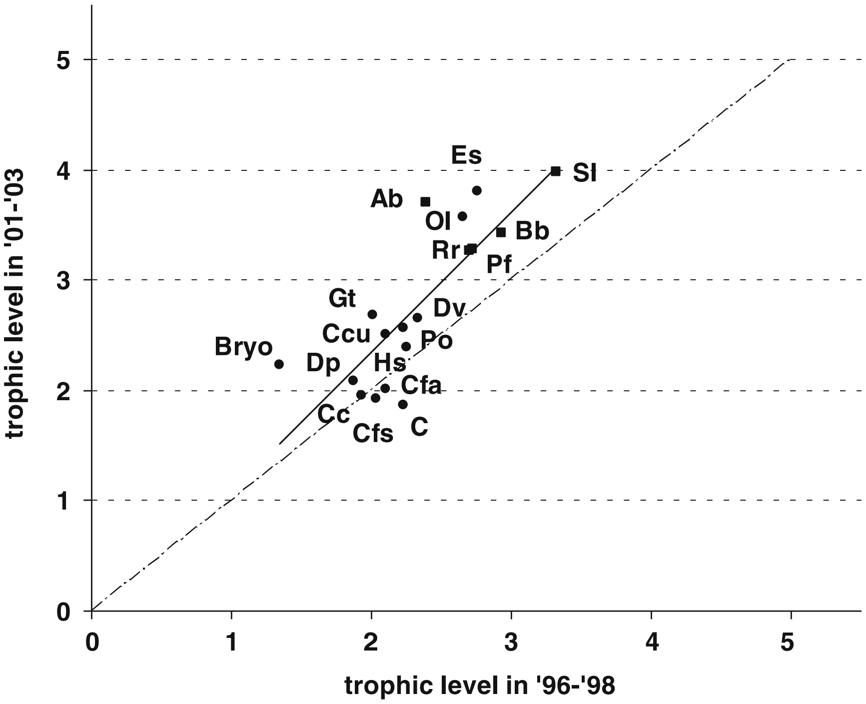

Figure 7. Trophic position of Rhine food web species during invasion and after establishment of *D. villosus*. Codes indicate the species according to the codes given in Table 1 and 2. The trend line compares the TP of the same species between the two different time periods.

deviations of *D. villosus* however still overlap those of *C. curvispinum*, indicating that *D. villosus* with the highest $\delta^{15}N$ values (i.e. bigger adults) would probably still prey on *C. curvispinum*, as is demonstrated in the laboratory experiment (Fig. 8). Differences in $\delta^{15}N$ between different life stages of *D. villosus* were however not significant ($p > 0.05$).

The food web between 2001 and 2003 shows higher trophic levels for most of the primary and secondary consumers (Fig. 7, Tables 1 and 2) than the food web between 1996 and 1998. The TP of *D. villosus* shifted proportionally to changes in the trophic level of most of the species in the food web.

Discussion

The Ponto-Caspian amphipods *C. curvispinum* and *D. villosus* have had a huge impact on the Rhine food web as they represent 80–90% of its macroinvertebrate community in number and biomass. *C. curvispinum* dominated the food web on groyne stones in 1998 and kept its dominance in 2001 next to *D. villosus*, although population densities of *C. curvispinum* had drastically been reduced. Various factors that generate bottom-up or top-down regulation of macroinvertebrate communities could be responsible for this decrease. As abiotic factors on which *C. curvispinum* depends, i.e. temperature and chlorophyll-*a* (Rajagopal et al., 1999), did not inhibit its development as the mean water temperature was somewhat higher in 2001 (13.2 °C in 1998, 13.9 °C in 2001), mean nitrogen concentration decreased from 4.33 g l^{-1} in 1998 to 2.23 g l^{-1} in 2001 and mean chlorophyll-*a* densities increased from 4 μg l^{-1} in 1998 to 10 μg l^{-1} in 2001, its population reduction is not likely caused by low resource availability or by altered abiotic factors. Top-down control by predation by *D. villosus* or by various top-predators (Kelleher et al., 1998) combined with increased predation or parasitic pressure from the triclad *D. romanodanubiale*, the halacarid *Caspihalacarus hyrcanus* and the acanthocephalan *Pomphorhynchus* spec. (Van Riel et al., 2003) could more evidently have caused this decline.

Most of the changes in the higher trophic levels of the Rhine food web between 1998 and 2001 are ascribed to Ponto-Caspian invasions. High numbers of *C. curvispinum* as food item could pave the pathway of invasions of predators and parasites like *E. ischnus*, *D. villosus*, *Jaera istri*, the mentioned predators and parasites as well as the gobies *P. semilunaris* and *Neogobius melanostomus*, causing an invasional meltdown of Ponto-Caspian species in the river Rhine (Ricciardi, 2001; Van der Velde et al., 2002, 2006).

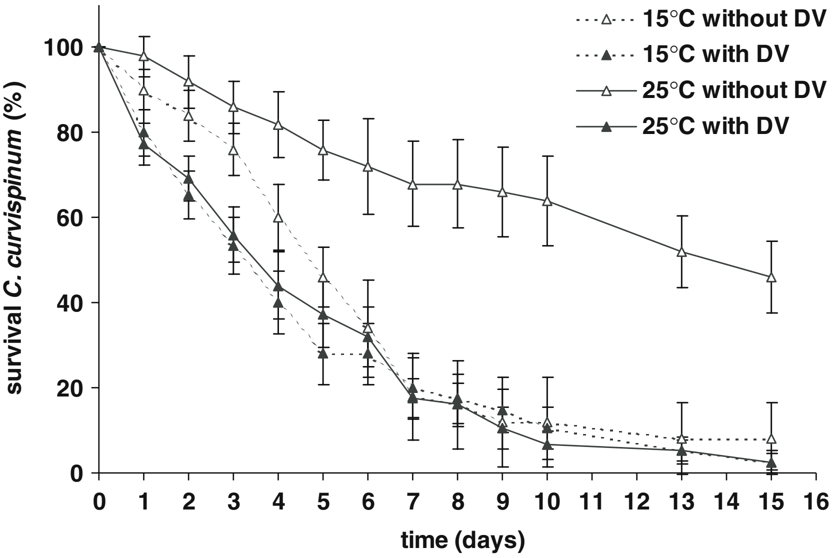

Figure 8. Effect of predation by *D. villosus* (DV) on survival of *C. curvispinum* at two water temperatures in a laboratory experiment. Bars indicate standard error of the mean.

Macroinvertebrate communities on these stone substrata have shown the largest changes in structure due to the subsequent success of various invaders competing for space, refuges and resources. It is assumed that the rapid increase of the omnivorous *D. villosus* that proved to be a strong competitor (Wijnhoven et al., 2003; Van Riel et al., 2004) determined the structure of the macroinvertebrate community on stones in 2001.

On the sand substrate, little has changed since the invasion of *C. curvispinum* and *D. villosus*. The Asiatic clam *C. fluminea* has been dominating the sandy streambeds since 1988 and densities of other macroinvertebrates remained low, probably due to high disturbance of the biotope by waves and shipping whirling up the sediment and to high predation pressure in this open environment. Ponto-Caspian amphipods were present in very low numbers and most individuals found were juveniles. However, the density and diversity of the macroinvertebrate community on the sand substrate increased between 1998 and 2001. Especially, *C. fluminea* and in September also *C. fluminalis* contributed to this increase perhaps related to much higher chlorophyll-*a* values in 2001.

Successful invaders in the Rhine food web are mainly present as primary consumers (e.g. *C. curvispinum, D. polymorpha, J. istri*). The more predaceous omnivorous Ponto-Caspian gammarids such as *D. villosus* are secondary consumers preying on macroinvertebrates as well as scavenging on carcasses and feeding on algae and phytoplankton. *D. villosus* shows high δ^{15}N values in comparison to other amphipod species, indicating a higher predatory level for this gammarid. Primary consumers invading the Waal did not change their food source years after invasion, but predatory, omnivorous invaders seem to be able to shift their diet, which could be affected by changes in prey species availability as densities of *C. curvispinum*, their most abundant presumed prey species in the period 1996–1998, decreased in the period 1998–2001. The δ^{15}N values of individual *D. villosus* did not significantly depend on body length and life stage, but adults with body lengths larger than 1.2 cm exceeded the values of the smaller ones, indicating a slightly higher trophic level.

The composition of the food web between 2001 and 2003 is comparable to that of the food web between 1996 and 1998. Input of terrestrial plant material, phytoplankton, benthic algae and POM fuel both food webs. Terrestrial plants seem to fuel the aquatic web, as they show comparable δ^{13}C values. Input of aquatic macrophytes is of minor influence, as their abundance in the main channel of the Rhine is negligible. The second trophic level is formed by primary consumers, consisting of filter-, detritus- and deposit-feeder and omnivores, of which most are invasive macroinvertebrate species. The most important source of food for this group is probably phytoplankton and suspended organic matter. The zoobenthic-zooplanktivorous and piscivorous fish occupy the highest trophic level of the food web. The isotopic composition of the larvae and adults of *L. fluviatilis* are distinct from other organisms in the Rhine food web and from each other, due to different feeding patterns. Larvae probably feed on benthic algae and microorganisms present in the sand bottom, but as adult become ectoparasitic on fish.

The overall δ^{15}N values of the Rhine food web decreased in isotopic nitrogen ratio indicating a lower anthropogenic nitrogen input (Cabana & Rasmussen, 1996; Hansson et al., 1997; McClelland et al., 1997) into the river Rhine. From 1998 to 2001, the nitrogen content of the water layer at Lobith, The Netherlands, diminished from 4.33 to 2.23 ± 0.21 g l^{-1}. Carbon isotopic values appeared to be unaltered. Trophic levels in both food webs can be compared after correcting for the variation in baseline δ^{15}N (Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen, 1999), according to the overall δ^{15}N–δ^{13}C relationship described by Vander Zanden & Rasmussen (1999). After this correction, the trophic level of most invertebrate species does not seem to have altered much. *D. villosus* continues the high predatory level it showed at the beginning of its invasion, but probably extended its diet as δ^{13}C shifted to more enriched values, likely to be related to changes in prey species availability.

Summarizing the characteristics of the Rhine food web, roughly three trophic levels can be distinguished. The food web is fuelled by POM, mostly originating from riparian plants, and phytoplankton (Admiraal et al., 1994), which makes the Rhine food web comparable to other large river food webs depending on riparian input (Thorp et al., 1998; Huryn et al., 2002). Macroinvertebrate species are keystone species, transferring food from phytoplankton and POM to higher trophic levels and eventually top-predators. As most of these keystone primary consumers in the Rhine are invaders, mostly crustaceans and bivalves, these invaders could be considered ecosystem engineers affecting functional diversity and food web structure of the community at the expense of insects (Vitousek, 1990; Crooks, 2002; Van der Velde et al., 2006). This engineering becomes more evident as numbers of a high impact invader increase and can result in bottom-up regulation, as *C. curvispinum* showed by swamping the ecosystem with muddy tubes, or top-down regulation by means of predation and competition as is observed for *D. villosus*.

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