

Research Article

The trophic function of *Dikerogammarus villosus* (Sowinsky, 1894) in invaded rivers: a case study in the Elbe and Rhine

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Abstract

The invasive amphipod *Dikerogammarus villosus* has become increasingly dominant in German river ecosystems since the River Rhine invasion in the mid-1990s. Because it is preying on other invertebrate taxa, its appearance is often assumed to be associated with a drastic decrease in species diversity and changes in natural benthic communities. Despite this, the trophic function of *D. villosus* and its predation potential are rarely studied in natural river communities. Here, we assess the trophic function of *D. villosus* in two invaded systems, the Elbe River and the River Rhine, using stable carbon and nitrogen isotope analyses. In the two studied river food webs, *D. villosus* had a generally low trophic position (TP), indicating a likely function as primary consumer or at most as an omnivore. The significantly higher TP in the River Rhine (TP = 2.6) than in the Elbe River (TP = 1.9) suggested a partial use of animal prey in the River Rhine. This was supported by the results of the isotope mixing model SIAR which predicted in addition to a high importance of herbivory in both rivers a likely feeding on other invasive amphipod species in the Rhine community. We conclude that *D. villosus* has a variable but mostly low predacious behaviour, probably depending on the benthic community structure. To allow more realistic trophic analyses for *D. villosus* in the field, we determined the trophic enrichment factors (TEFs) for nitrogen and carbon isotopes of two diet types in the tissue of *D. villosus* in an eight-week laboratory experiment. The carbon isotope enrichment ($\Delta\delta^{13}\text{C}$) from the chironomid diet was negative in the consumer (-1.75‰), whereas the carbon from leaf litter was enriched positively in the tissue (3.27‰). The TEFs for nitrogen ($\Delta\delta^{15}\text{N}$) were more similar for plant and animal diet (leaf litter: 3.56‰ , chironomids: 2.29‰). Because of the high specificity of the enrichment factors with respect to species and food source, we recommend their use in further trophic analyses.

Key words: trophic enrichment factor, stable isotope analysis, trophic position, diet, isotope mixing model, SIAR

Introduction

Invasion of non-indigenous species is assumed to be one of the factors endangering freshwater biodiversity (Sala et al. 2000; Didham et al. 2005). Consequently, the significance of aquatic invaders for the structure and function of river ecosystems seems high, and has become a major concern in the last decades (Bampfyld et al. 2010; Strayer 2010). The very successful Ponto-Caspian invader *Dikerogammarus villosus* (Sowinsky, 1894) has a number of traits enabling it to outcompete other indigenous invertebrates (Grabowski et al. 2007; Rewicz et al. 2014). It has established high

population densities in European rivers such as the Danube River, Moselle River, River Rhine or, more recently, in the Elbe River (Tittizer et al. 2000; Bij de Vaate et al. 2002; Jazdzewski et al. 2002; Devin et al. 2005) and is predicted to invade further freshwater systems (Ricciardi and MacIsaac 2000; MacNeil et al. 2012; Boets et al. 2014; Rewicz et al. 2014).

The predatory behaviour of *D. villosus* towards other benthic invertebrates is often assumed to be one of the most important traits for its invasion success. This has been shown by a number of laboratory experiments concerning the feeding preferences of *D. villosus* (Dick et al. 2002; Krisp

and Maier 2005; Kinzler et al. 2009; MacNeil et al. 2013; Dodd et al. 2014). With their special mouthparts (Mayer et al. 2008), large body sizes (Devin et al. 2003) and high feeding rates (Krisp and Maier 2005; Gergs and Rothhaupt 2008), large adults of *D. villosus* can be potentially stronger predators than other amphipods. Additionally, it has been reported that the decrease of aquatic invertebrate biodiversity is related to the increase of *D. villosus* densities (Dick et al. 2002; Krisp and Maier 2005; Van Riel et al. 2006; MacNeil et al. 2013; Gergs and Rothhaupt 2014). Nevertheless, *D. villosus* might act as an omnivore in aquatic food webs by also using plant-based resources such as leaf litter, algae, detritus and macrophytes (Platvoet et al. 2006; Gergs and Rothhaupt 2008; Maazouzi et al. 2009; Piscart et al. 2011). Due to its wide range in potential food sources, *D. villosus* can be expected to adapt its feeding behavior and its diet composition according to the environmental conditions (Kley and Maier 2005; Kley et al. 2009; Maazouzi et al. 2009; Pellán et al. 2015). This could lead to varying trophic functions under field conditions and the assumed high proportion of predatory feeding might thus not always be achieved in benthic communities of European Rivers. This is supported by the observation that the trophic niche based on stable isotope analyses (SIA) overlapped with other amphipods and that genetic gut content analyses provided no evidence for the consumption of amphipods (Koester and Gergs 2014). The possibility of the predominant use of non-animal food in summer and a high feeding flexibility depending on resource availability was also indicated by fatty acid analyses (Maazouzi et al. 2009). On the other hand, there are field studies about *D. villosus* reporting a high trophic position (TP = 3.7) in the Vistula basin in Poland (Bacela-Spychalska and Van der Velde 2013), and TP values of 2.7 – 3 in the lower reaches of the River Rhine (Van Riel et al. 2006), both showing some of the highest $\delta^{15}\text{N}$ of all amphipods at the site. Both studies therefore stressed the important predatory function of the invader within the benthic community. However, a very high intraspecific variability of resource use has been indicated by a wide range of isotope signatures within the same population of *D. villosus* (Koester and Gergs 2014). These contrasting results concerning the feeding behaviour of *D. villosus* from field and laboratory studies raise the question, whether its trophic function in natural aquatic communities is in fact largely that of a predator or whether its

omnivorous feeding behavior results in a dominant function of a shredder or collector.

To assess the trophic function of *D. villosus* in large European rivers, we analysed two benthic communities in the rivers Elbe and Rhine, which differed in invasion history but supported high *D. villosus* densities. The River Rhine was invaded by *D. villosus* in 1995 (Tittizer et al. 2000) and the community is characterized by a high number of invasive taxa (Tittizer 1997). In the Elbe River, the invasion process started some years later (Grabow et al. 1998; Tittizer et al. 2000), and near the sampling site of our study high densities of *D. villosus* were documented for the first time in 2001 (Krieg 2002). Until now, the number of indigenous species in the Elbe River remains relatively high (Schöll and Balzer 1998; Nehring 2006). We compared the trophic function of *D. villosus* between these two ecosystems because it is assumed that predation on native species, which are not adapted to *D. villosus*, is more intense than predation on species dominating ecosystems, which have been invaded a longer time ago (Koester and Gergs 2014). Because of the differences in the invasion histories and present community composition between these two rivers, we hypothesized that *D. villosus* shows a higher proportion of predation in a community with more indigenous species (e.g. in the Upper Elbe) than in an established invasive community (e.g. in the Middle Rhine).

One powerful method to identify trophic functions and feeding pathways is the analysis of carbon and nitrogen isotopes (DeNiro and Epstein 1978, 1981; Post 2002; McCutchan et al. 2003). However, identifying food web interactions by SIA implicitly requires knowledge of the enrichment or depletion of heavy isotopes of nitrogen (^{15}N) and carbon (^{13}C) in a consumer related to its diet, which results from the metabolic fractionation of the assimilated nutrients (Vander Zanden and Rasmussen 2001). Although general values of the trophic enrichment factors (TEFs) are broadly accepted and used for trophic analyses (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001), the actual TEFs vary strongly between or even within consumer species (Tieszen et al. 1983; Vander Zanden and Rasmussen 2001; McCutchan et al. 2003; Caut et al. 2009). Large differences in fractionation in the consumers are, for example, expected to arise from different metabolic pathways due to biochemical compositions of plant and animal sources (Macko et al. 1986; Fantle et al. 1999; Jardine et al. 2005). Nevertheless, mean values

from a large range of animal groups have been used so far in trophic studies of *D. villosus* because published TEFs for amphipods are rare (Vander Zanden and Rasmussen 2001; McCutchan et al. 2003), or even missing for European species. We therefore determined the consumer-specific TEFs for two diet types (one plant source, one animal source) in the laboratory and implemented them into the calculation of trophic position and diet use of *D. villosus* in the field.

Methods

Trophic enrichment laboratory experiment

An 8-week laboratory experiment was conducted with *D. villosus* from February to April 2014. Animals were collected from stones in the Moselle River in Koblenz (Germany; 50.361987°N, 7.563725°E). To allow for acclimation to laboratory conditions and to empty their intestinal tracts, the individuals were kept in aerated river water for three days. In total, 96 individuals were thereafter inserted separately into 100 ml transparent, gravel-filled plastic boxes (bottom with gauze, 1mm mesh size), which were exposed in a laboratory flume (1000 l) filled with a 2:1 mixture of river water and tap water (total ~200 l, current velocity 0.1 m s⁻¹, temperature 15 °C). The water was permanently aerated and filtered, and about half of the water was exchanged at least once a week. The light/dark rhythm was adjusted to 12h/12h.

One half of the animals were fed daily with commercial frozen red chironomid larvae *ad libitum* (chironomid treatment); the other half was fed with willow leaves (*Salix* sp.), which were preconditioned in ventilated river water for at least two weeks prior to the experiment, and exchanged weekly during the experiment (leaf litter treatment). Dead amphipods were eliminated from the boxes daily. For SIA, 10 individuals with a mean body size of 11.2 ± 0.9 mm (± SD) were sampled at the start day of the experiment. Later, three to 11 animals were sampled as available at the days 28, 42 and 56. All individuals were immediately frozen in liquid nitrogen; the food sources were sampled weekly, starting in the second week. All samples were stored at -20°C until further processing.

The physical and chemical water conditions were measured in the laboratory flume at least two times a week (Multi 3430 SET F, WTW, Weilheim, Germany; Appendix 1.1). In addition,

the ammonium concentration in the water was monitored each week and after each water exchange using a photometric test kit (No. 14559, WTW, Weilheim, Germany) to allow quick action in case of water quality problems. For exact determination of ammonium amount during the experiment, the concentrations were measured from frozen water samples using a method based on the Berthelot reaction (modified based on Hansen and Koroleff 1999; Appendix 1.1). The cumulative mortality at the end of the experiment was 47.9 % in the chironomid treatment and 75.0 % in the leaf litter treatment with no significant differences in mortality between the treatments during the experimental course (paired Wilcoxon rank test, $V = 156.5$, $p = 0.14$, $n = 25$).

Field study

The study sites were located in the Upper Elbe near the city Dresden (river km 66, 51.09478°N, 13.64950°E) and in the Middle Rhine near the city Koblenz (river km 560.5, 50.16996°N, 7.66847°E). Both study sites are characterized by sandy and stony substrates with different anthropogenic structures such as groynes and rockfill, while semi-natural riverbanks still remained.

The benthic community was sampled in September 2012 (Elbe) and September 2013 (Rhine) using three substrate baskets filled with the predominant substrate from the study sites (from coarse gravel to hand-sized stones), whereas one basket represented one sample unit. Baskets were exposed on the riverbed near to the riverbank for four to five weeks before sampling to allow colonisation. The baskets (50 × 20 × 20 cm) were made of high-grade steel mesh (20 mm), which allowed an exchange between the river community and the basket community. For sampling, the baskets were lifted up by a crane ship and immediately placed in water-filled plastic barrels. The stones were scrubbed thoroughly with a soft hand brush and organisms were washed out of the gravel. The stone volume of each basket was measured by water displacement to account for different filling quantity.

For the SIA, individuals of all obviously and sufficiently abundant taxa and samples of basic resources (such as macrophytes, moss, and leaf litter) were picked out from each basket. In addition, biofilm was scrubbed from the stone surfaces and particulate organic material (POM) was pipetted from the excess water in the barrel. Adult individuals of *D. villosus* (>9 mm, three females and three males) were collected separately

per basket. For the analyses of all other invertebrates, three to 20 individuals (depending on their body size) were pooled per basket. All basic resources were cooled for further transport and animals were immediately frozen in liquid nitrogen. For the analysis of the benthic species composition, all remaining organisms were washed out from the basket substrate and stored in 96 % ethanol.

Sample processing

To determine the species composition and densities in the substrate baskets, animals were identified to the lowest feasible taxonomic level and enumerated. The body length or head width of the individuals were measured (± 0.1 mm) to estimate a mean biomass of the populations using size-mass-relationships (Meyer 1989; Benke et al. 1999; Baumgärtner and Rothhaupt 2003; Hellmann et al. 2013). In case of amphipods, all individuals from a subsample representing a quarter or an eighth were measured in their natural curved posture. To establish specific size-mass relationships for amphipods, individuals with different sizes were measured, separately dried at 60°C for 24 h, and weighted with a micro balance (± 0.01 mg). A power function was fitted to the measured data of body sizes (x) and dry weight (y) for *D. villosus* ($y=0.0058x^{2.92}$; $r^2=0.94$, $n=98$), *Echinogammarus ischnus* ($y = 0.0224x^{2.04}$; $r^2 = 0.86$, $n = 99$) and *Chelicorophium* spp. ($y=0.0534x^{1.51}$; $r^2=0.70$, $n=96$). In order to include the different stone volumes per basket into the calculation of benthic density, a correction factor ($1/\text{sampled stone volume}$) was used.

For SIA, the basic resources POM and biofilm were pelletized by centrifugation (4 °C, 15,000 RCF, 5–10 min). For most of the species, guts were removed, while very small primary consumers were processed without removing the guts. All samples were dried at 60°C up to 48 hours. The individuals of *D. villosus* from the laboratory experiment were also prepared for analyses by removing the guts. All laboratory samples were freeze-dried at –51°C for 24 h (Christ Alpha, Osterode, Germany). After drying, all SIA samples were grounded and about 0.2–1 mg of the animal samples and 3–5 mg of the non-animal samples was packed into tin capsules (5 × 9 mm, IVA Analysentechnik e. K., Germany).

Stable isotope analyses and calculations

The ratios of nitrogen and carbon isotopes of all samples were analysed with a Delta Advantage

isotope ratio mass spectrometer connected to a Flash HT elemental analyser (*Thermo Finnigan*, Bremen, Germany). The values of the stable isotopes are expressed as δ notation relative to the Vienna Pee Dee Belemnite (VPDB) international standards for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$ in per mille units (‰). The precision for stable isotope values (SD) was 0.12 ‰ for carbon and 0.05 ‰ for nitrogen. The proportions of C and N were determined by calibrating with an internal standard in the Flash elemental analyser during the SIA. The molar C:N ratio was calculated from these proportions for the different diets from the laboratory experiment.

All statistical and model calculations were performed with the software R (version 3.1.0, R Development Core Team 2014). The TEFs for nitrogen and carbon were calculated from the differences between the mean isotope signatures of *D. villosus* and the sources in the trophic enrichment experiment. For that purpose, all isotope signatures of the sources over the entire experimental time were averaged. For identifying the values for the calculation of the mean *D. villosus* signatures, the signatures were compared between the sampling points (t_0 , t_{28} , t_{42} , t_{56}) in each treatment by a multiple comparison test (Tukey's [HSD] with Bonferroni correction). Finally, only those values were included, which showed no significant change to the next sampling points, assuming the metabolic turnover of the diet in the consumer tissue to be terminated. After testing the normal distribution and variance homogeneity, a transformation by x^{-6} was necessary for $\delta^{13}\text{C}$ of *D. villosus* in the chironomid treatment. The means and standard deviations of the TEFs were estimated by choosing randomized consumer-diet differences from the specific dataset with a randomization simulation (10,000 iterations).

The trophic position of the consumer *D. villosus* and the necessary baseline correction were estimated after the equations of Vander Zanden and Rasmussen (1999). The $\Delta\delta^{15}\text{N}$ is represented by the mean of diet-typical TEFs for nitrogen measured in our laboratory experiment ($\Delta\delta^{15}\text{N} = 2.93$ ‰). The river-specific trophic baseline was derived from the primary consumers at each river site (scrapers, gatherers and particle feeders) and was calculated separately for each food web in the Elbe River and River Rhine.

The relative contribution of the different resources to *D. villosus* diet (adults >9 mm) was assessed using a Bayesian isotopic mixing model in R (SIAR; Parnell et al. 2010). The model incorporates the uncertainty in the isotope signatures

of the consumers, resources and trophic enrichment factors and allows the evaluation of the possible sources (Smith et al. 2013; Appendix 2). The means and standard deviations of the diet-specific TEFs measured in the laboratory for *D. villosus* were used in the model calculation (see Table 1). Because the predictive power of the mixing model is higher with a lower number of resources (Phillips et al. 2014), the number of resources was reduced as much as possible by averaging their signatures, whenever they were not significantly different (verified by Welch two sample test). However, care was taken to prevent eliminating important information. Therefore, only resources from the same diet type were averaged. For the River Elbe, the plant sources (leaf litter and macrophytes) were combined, also grazing animals (*Ancylus fluviatilis* and *Baetis fuscatus*) and particle feeders (*Hydropsyche* spp. and *Eiseniella tetraedra*). For the River Rhine, the organic matter sources (POM and biofilm), the plant sources (leaf litter and macrophytes) and particle feeding animals (*Chelicorophium* spp. and Simuliidae) were combined. We are aware that the discriminatory power might be reduced above six or seven resources and the interpretation will be more uncertain (Phillips et al. 2014). In spite of this, we included all available resources into the model calculations because a high number of potential resources was reported for *D. villosus* (Rewicz et al. 2014) and previously observed (R. Gergs, M. Koester, unpubl. data). One of our main objectives was to assess which resources were probably used and which were of minor interests. In the light of the expected highly variable feeding, we felt that an *a priori* exclusion of certain resources would reduce the information gaining from the model, which has also been acknowledged before (Phillips et al. 2014).

Results

Trophic enrichment factors and diet turnover in D. villosus

The signatures of the nitrogen and carbon isotopes of *D. villosus* in the leaf litter treatment, as the means of all samplings when no further change was recorded, were higher than the mean diet signatures, resulting in a positive trophic enrichment of both isotopes (Table 1). In the chironomid treatment, the mean consumer nitrogen signature was higher whereas the carbon signature was lower than the mean diet signatures, resulting in a positive trophic enrichment factor for nitrogen

and a negative enrichment factor (i.e. depletion) for carbon (Table 1).

In the chironomid treatment, *D. villosus* completed the turnover of the diet in the tissue within four weeks because $\delta^{15}\text{N}$ did not differ from each other after day 28 ($p > 0.05$, Tukey's [HSD] test). The $\delta^{13}\text{C}$ did not differ from each other after day 42 ($p > 0.05$), which indicated a turnover time of six weeks. For both isotopes, significant differences of the consumer signatures were observed between the values of the start (t_0) and all other sampling points ($p < 0.001$). The half-life turnover of tissue isotopes was estimated to be 1.2 days for nitrogen and 13.9 days for carbon.

In the leaf litter treatment, $\delta^{13}\text{C}$ did not change significantly between day 28 and the other sampling dates ($p > 0.05$, Tukey's [HSD] test), thus a large part of the diet turnover was accomplished within four weeks. This is also indicated by a half-life time of about 35 days for carbon and the large differences between start values and all other values ($p < 0.001$). The $\delta^{15}\text{N}$ of *D. villosus* did not change significantly between day 42 and 56 ($p > 0.05$), thus the diet turnover seemed to be largely completed within six weeks. The first significant change in the consumer signature was observed between the start value and day 42 ($p < 0.001$). Due to the almost linear decrease of $\delta^{15}\text{N}$ in *D. villosus* during the experiment, no asymptotic exponential function could be fitted to these data and consequently no half-life time could be calculated (see Appendix 1.2). Further results of the experiment are given in Appendix 1.2.

The benthic communities at the river sites

During the field study in autumn 2012 and 2013, 16 native and five invasive taxa (genus or species level) were found in the Elbe River, whereas nine native and eight invasive taxa were found in the River Rhine (Table 2). The mean biomass and density of *D. villosus* at both river sites were high (Table 2) and did not differ significantly (Student's t-tests, density: $p = 0.09$, $t = 2.26$; biomass: $p = 0.44$, $t = 0.87$, $n = 3$). In addition, the relative proportions of juveniles (<6 mm) on the *D. villosus* density were similar in both rivers (Elbe: 0.64 ± 0.12 , Rhine: 0.47 ± 0.08 , means $\pm$ SE; Student's t-test, $p = 0.27$, $t = 1.27$, $n = 3$), indicating no general differences in population dynamics.

In the Elbe River, *D. villosus* contributed a biomass proportion of 64% to the benthic community, forming therefore the most important taxon by far because no other taxa reached similar high biomasses (Table 2). Considering the benthic densities, the

Table 1. Signatures of stable isotopes $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ (means $\pm$ SD) of the diet given in the laboratory experiment (all measures, $n = 23$ leave samples, $n = 21$ chironomid samples) and of *D. villosus* when the diet turnover in its tissue was completed (included time points t_i and n given in brackets). The trophic enrichment factor (TEF) $\Delta \delta^{13}\text{C}$ ‰ or $\Delta \delta^{15}\text{N}$ ‰ is the mean increase between the signature of the diet and consumer tissue (mean $\pm$ SD).

Treatment	δ	Diet signature	<i>D. villosus</i> signature		TEF
Leaf litter	^{13}C	-29.11 ± 0.64	-25.84 ± 0.83	$(t_{28}, t_{42}, t_{56}, 12)$	3.27 ± 1.29
	^{15}N	6.62 ± 1.86	10.18 ± 0.77	$(t_{42}, t_{56}, 7)$	3.56 ± 1.97
Chironomid	^{13}C	-19.75 ± 0.69	-21.48 ± 1.01	$(t_{42}, t_{56}, 16)$	-1.75 ± 1.18
	^{15}N	8.81 ± 0.29	11.11 ± 0.41	$(t_{28}, t_{42}, t_{56}, 21)$	2.29 ± 0.49

Table 2. Densities (individuals/l) and biomasses (mg dry weight/l) of each taxon in the rivers Elbe and Rhine, found at the sampling sites after the four-week exposure (August to September) of the substrate baskets (means $\pm$ SE, $n = 3$ samples); the native (nat) or invasive (inv) origin of each taxon is given in brackets. The taxon *Chelicorophium* spp. includes the species *C. curvispinum* and *C. robustum*, *Hydropsyche* spp. means the total amount of all identified and non-identified species.

Taxon	Elbe River		River Rhine	
	Density	Biomass	Density	Biomass
Bivalvia				
<i>Corbicula fluminea/fluminalis</i> (inv)	0.4 ± 0.2	0.56 ± 0.45	5.6 ± 2.3	2.83 ± 1.9
<i>Dreissena bugensis rostriformis</i> (inv)	-	-	2.3 ± 1.5	11.18 ± 8.03
<i>Pisidium</i> spp. (nat)	0.6 ± 0.3	0.07 ± 0.04	-	-
Gastropoda				
<i>Ancylus fluviatilis</i> (nat)	6.2 ± 1.1	2.20 ± 0.3	-	-
<i>Bithynia tentaculata</i> (nat)	-	-	1.0 ± 0.1	2.07 ± 2.0
<i>Theodoxus fluviatilis</i> (inv) ^a	-	-	2.4 ± 1.2	8.49 ± 4.39
Turbellaria				
<i>Dendrocoelum romanodanubiale</i> (inv)	-	-	6.6 ± 3.5	0.36 ± 0.24
Oligochaeta				
<i>Eiseniella tetraedra</i> (nat)	0.5 ± 0.3	106.94 ± 77.91	-	-
Oligochaeta	1.3 ± 0.4	0.08 ± 0.04	4.5 ± 3.3	0.21 ± 0.12
Crustacea				
<i>Chelicorophium</i> spp. (inv)	0.3 ± 0.1	0.08 ± 0.02	2309.1 ± 722.9	391.89 ± 122.05
<i>Dikerogammarus haemobaphes</i> (inv)	7.5 ± 5.2	14.91 ± 11.93	-	-
<i>Dikerogammarus villosus</i> (inv)	254.1 ± 116.7	361.3 ± 240.9	89.8 ± 47.3	240.7 ± 32.6
<i>Echinogammarus ischnus</i> (inv)	-	-	700.6 ± 64.6	274.55 ± 25.57
<i>Jaera sarsi</i> (inv)	581.3 ± 99.1	66.27 ± 11.3	208.9 ± 33.8	23.78 ± 3.85
Ephemeroptera				
<i>Baetis fuscatus</i> (nat)	2.46 ± 1.1	0.30 ± 0.17	-	-
<i>Heptagenia sulphurea</i> (nat)	4.2 ± 0.7	1.28 ± 0.07	-	-
<i>Potamanthus luteus</i> (nat)	0.04 ± 0.04	0.001 ± 0.001	-	-
Trichoptera				
<i>Cheumatopsyche lepida</i> (nat)	7.7 ± 2.5	0.80 ± 0.31	0.3 ± 0.3	0.04 ± 0.04
<i>Ecnomus tenellus</i> (nat)	0.04 ± 0.04	0.001 ± 0.001	8.3 ± 3.2	0.09 ± 0.03
<i>Hydropsyche</i> spp. (total)	10.1 ± 0.9	2.78 ± 0.26	375.2 ± 63.3	62.50 ± 12.48
<i>H. bulgaromanorum</i> (nat)	0.6 ± 0.1	0.44 ± 0.18	6.5 ± 2.6	5.01 ± 2.2
<i>H. contubernalis</i> (nat)	5.4 ± 0.6	2.00 ± 0.21	262.2 ± 39.5	39.92 ± 6.08
<i>H. exocellata</i> (nat)	0.1 ± 0.04	0.05 ± 0.02	38.0 ± 10.7	13.67 ± 3.15
<i>H. incognita/pellucidula</i> (nat)	0.2 ± 0.1	0.18 ± 0.11	3.8 ± 3.2	1.39 ± 1.03
<i>Lepidostoma basale</i> (nat)	0.1 ± 0.1	0.01 ± 0.01	-	-
<i>Psychomyia pusilla</i> (nat)	2.0 ± 0.7	0.10 ± 0.03	-	-
Coleoptera				
<i>Elmis</i> sp. (nat)	0.04 ± 0.04	0.01 ± 0.01	-	-
<i>Oulimnius</i> sp. (nat)	-	-	0.3 ± 0.3	0.62 ± 0.62
Diptera				
Chironomidae	21.1 ± 4.9	0.46 ± 0.13	32.6 ± 8.9	0.30 ± 0.05
<i>Simulium</i> spp. (nat)	86.1 ± 29.6	4.47 ± 1.53	19.8 ± 4.2	0.92 ± 0.24
Total community	986.1 ± 147.7	565.41 ± 223.16	3767.1 ± 920.01	1020.53 ± 179.04

^abased on Gergs et al. (2014)

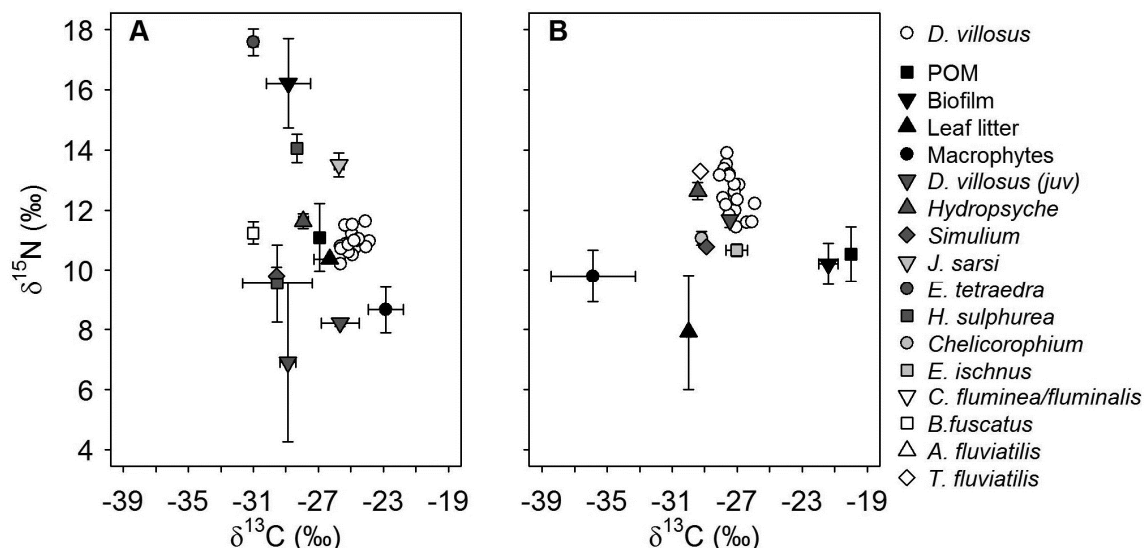


Figure 1. Stable isotope signatures of adult *D. villosus* with a body length >9 mm (white circles, single values) and measured values of its possible diet (means $\pm$ SD, $n = 1 - 4$ samples) in the food webs of the Elbe River (A) and the River Rhine (B). *D. villosus* (juv) were juveniles with a body length <6 mm; *Hydropsyche* included the taxa *H. contubernalis*, *H. bulgaromanorum* and *H. exocellata*; *Chelicorophium* included the taxa *C. robustum* and *C. curvispinum* (signatures of sources are not corrected by TEFs; for complete scientific names see Table 3).

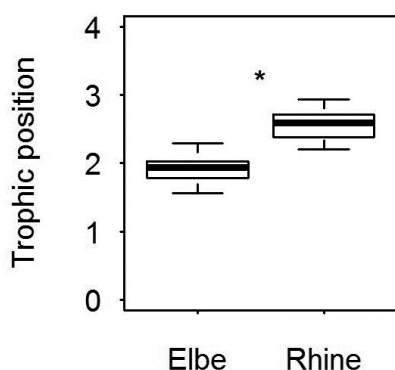


Figure 2. Trophic position of adult *D. villosus* (body length >9 mm) in the Elbe River and the River Rhine (Box-Whisker plots, median, quartiles, 10th and 90th percentiles; *p value < 0.001, Student's t-test).

small-bodied isopods *Jaera sarsi* had the highest proportion (59%) on the total density, followed by *D. villosus* (26%). High densities were also observed for *Simulium* spp. and Chironomidae (both Diptera, proportion ~11%), whereas specimens of all other taxa were represented in low numbers in the samples from the Elbe River (<1% on benthic density, Table 2). In contrast, the biomass proportion of *D. villosus* in the River

Rhine was only one third of that in the Elbe (23.6%). The three important amphipod taxa (*D. villosus*, *Echinogammarus ischnus*, *Chelicorophium* spp.) reached there 89% of the benthic biomass with similar proportions each (Table 2). Regarding the proportions on the benthic density, *D. villosus* reached 2.4% and was out-numbered by *E. ischnus* (18.6%), *Chelicorophium* spp. (61%), *Hydropsyche* spp. (10%), and *J. sarsi* (7%). The total benthic density was higher in the River Rhine than in Elbe River (Student's t-test, $p = 0.041$, t value = -2.98), whereas total biomasses did not differ (Student's t-test, $p = 0.19$, t value = -1.59; Table 2).

Trophic position and food sources of D. villosus

In the Elbe River, the large individuals of *D. villosus* (>9 mm) were found consistently at the lower limit of the $\delta^{15}\text{N}$ -range of all primary consumer taxa in the food web (10.21–11.64 ‰) and the values of the individual animals showed a low variation (SD = 0.36) (Figure 1). In the River Rhine, on the other hand, the adults showed a higher variability in nitrogen signatures (SD = 0.72) with approximately half of the individuals at the upper limit of the $\delta^{15}\text{N}$ range of all primary consumers (11.44–13.9 ‰) (Figure 1). The large difference in the nitrogen signatures was reflected in the significant difference between the trophic positions of *D. villosus* in the two river food webs (Student's t-test, $t = -10.09$, $p < 0.001$, Figure 2).

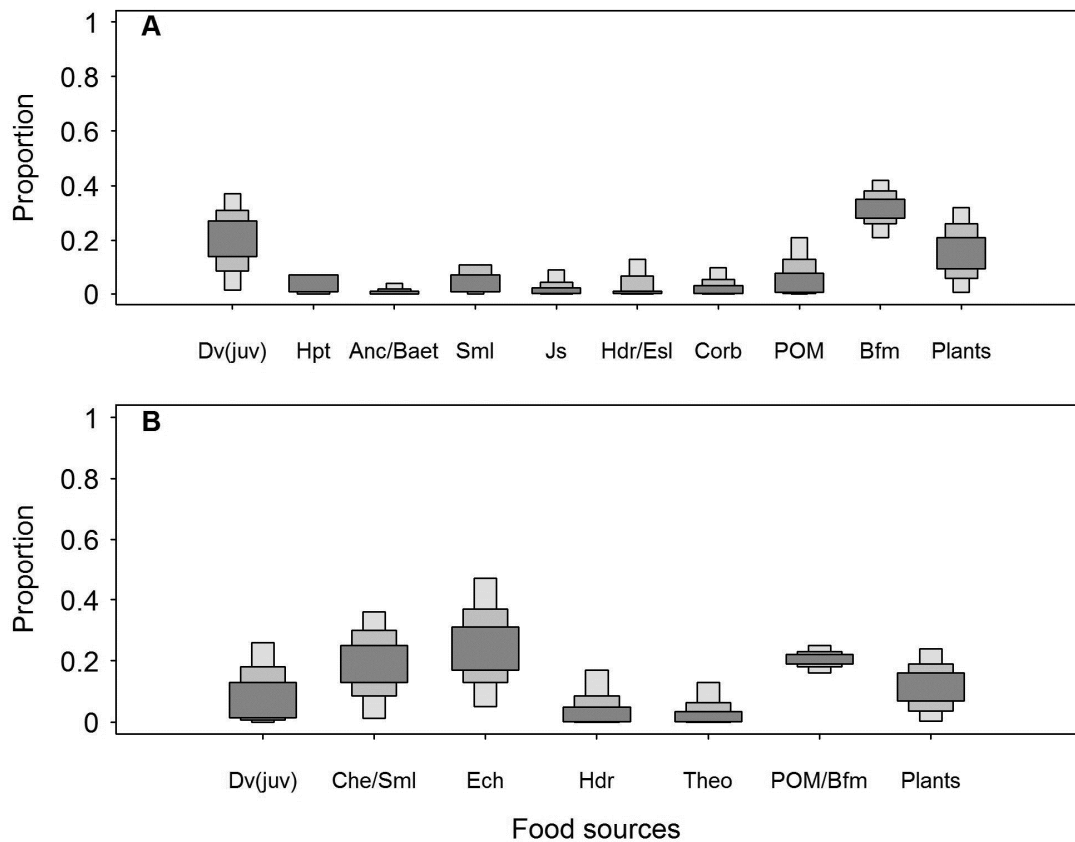


Figure 3. Diet contributions for the consumer *D. villosus* (adults >9 mm) in the Elbe River (A) and River Rhine (B), given as relative proportion of each source. The boxes give the range of 95%, 75% and 50% probability interval (light to dark grey) for the different sources (for source abbreviations see Table 3). Some sources were combined due to overlapping isotope signatures (see Methods and Table 3).

Table 3. Contributions of different food sources (proportion in %) to the consumer *D. villosus* in the Elbe River and the River Rhine given as means and 95% interval. Abbreviations (abbr.) are given for every source as shown in Figures 4. Juvenile *D. villosus* had body sizes <6 mm. Some sources were combined due to overlapping isotope signatures (see Methods).

Sources	Abbr.	Elbe River		River Rhine	
		Mean	95% int.	Mean	95% int.
<i>D. villosus</i> (juvenile)	Dv(juv)	20.2	1.4 – 37	12.1	0 – 26
<i>Heptagenia sulphurea</i>	Hpt	2.3	0 – 6.7	-	-
<i>Ancylus fluviatilis/Baetis fuscatus</i>	Anc/Baet	1.4	0 – 3.9	-	-
<i>Hydropsyche</i> spp.	Hdr	-	-	6.0	0 – 17
<i>Hydropsyche/Eisaniella tetraedra</i>	Hdr/Esl	4.9	0 – 13	-	-
<i>Simulium</i> spp.	Sml	7.5	0 – 17	-	-
<i>Jaera sarsi</i>	Js	3.1	0 – 9.2	-	-
<i>Chelicorophium/Simulium</i>	Che/Sml	-	-	19.4	1.3 – 36
<i>Echinogammarus ischnus</i>	Ech	-	-	25.4	5 – 47
<i>Corbicula fluminea/fluminalis</i>	Corb	3.7	0 – 10	-	-
<i>Theodoxus fluviatilis</i>	Theo	-	-	4.5	0 – 13
Biofilm	Bfm	31.6	21 – 42	-	-
POM	POM	8.6	0 – 21	-	-
POM/biofilm	POM/Bfm	-	-	20.3	16 – 25
Macrophytes/leaf litter	Plants	16.7	0.5 – 32	12.3	0.3 – 24

In the River Rhine, the mean TP of *D. villosus* (2.6 ± 0.2 , $n = 18$, $\pm$ SD) was higher than that of individuals in the Elbe food web (1.9 ± 0.2 , $n = 18$, mean $\pm$ SD; Figure 2), indicating a more predaceous behaviour in the River Rhine. The mean body length of adult *D. villosus* analysed for SIA was 12.1 ± 2.3 mm in Elbe and 12.5 ± 1.7 mm in Rhine (means $\pm$ SD).

The results of the mixing model were consistent with the trophic positions. The main food sources of *D. villosus* in the Elbe River were plants and biofilm (Figure 3A, Table 3). Almost all invertebrate taxa were predicted to be non-relevant resources, because the 95% probability intervals include 0% usage of the respective resource indicating a relatively uncertain estimation using these diets by the model calculation (Table 3). Only juvenile *D. villosus* were predicted to be a relevant resource. In the River Rhine, the diet contribution was more variable than in the Elbe River, indicating a flexible use of the most food sources (Figure 3B, Table 3). The diet in the River Rhine included a higher proportion of invertebrates, especially other amphipod species, than in the Elbe food web but a similar intense use of plant-based resources. In addition, the ranges of animal diet proportions predicted by the model were frequently large and most of them reached values of 0% at the low end of the 95% intervals indicating a high uncertainty in diet use (Table 3). The smaller ranges of plant-based resources, like POM and biofilm, with a low end of 95% interval of at least 16% indicate a certain high use of these diets.

Discussion

Our results showed clearly that *D. villosus* was less predaceous at the two studied field sites than one might expect on the basis of previous studies. Neither in the Elbe River nor in the River Rhine did we observe a predator-like trophic position in the river communities at the respective sampling site and season. In fact, the $\delta^{15}\text{N}$ was similar to or even lower than that of typical primary consumers at the same sampling sites (e.g. snails, mayflies). While strict invertebrate predators typically show a trophic position of about 3 (Benke et al. 2001; Anderson and Cabana 2007; Hellmann et al. 2013), the trophic positions of individual *D. villosus* in our field study ranged between 1.6 and 2.9. While the maximum values indicate some predaceous behavior, the lower values are within the usual range for primary consumers (e.g. grazers, shredders) depending on

the resources (Anderson and Cabana 2007). Mean TP values of 1.9 in Elbe River and 2.6 in River Rhine indicate omnivorous feeding with a probable high proportion of plant-based sources in the diet. Although the trophic function of the invader *D. villosus* in European river ecosystems is still in debate, because of several contradictory observations in the laboratory (e.g. Dick et al. 2002; Boets et al. 2010; Dodd et al. 2014) and in the field (e.g. Van Riel et al. 2006; Koester and Gergs 2014), there are only a few other publications based on the stable isotope method within natural food webs. A recent study reported a similarly high intraspecific variability in nitrogen signatures within *D. villosus* populations and similarly weak evidence for predation (Koester and Gergs 2014). In Lake Constance, *D. villosus* seemed not to be distinctly more predaceous than *Gammarus roeseli* because stable isotope signatures did not differ significantly (Rothhaupt et al. 2014). A study in several German lakes showed similar trophic positions to our study with values lower than 2.5 (Brauns et al. 2011). There are of course other studies, observing relative high trophic positions and nitrogen signatures of the invader compared to other species in the food web (Van Riel et al. 2006; Bacela-Spychalska and Van der Velde 2013). However, considering the high natural feeding variability within one population, a comparison to our results is difficult to find, in some cases because of the very low number of samples (Bacela-Spychalska and Van der Velde 2013) or in others because samples were pooled (Van Riel et al. 2006). Furthermore, a very high temporal and spatial variability in the trophic function of *D. villosus* is indicated by former studies in the Rhine catchment area (Van Riel et al. 2006; Koester and Gergs 2014) and by our results as well. The invader might therefore act as an important predator during invasion and switch afterwards to other food sources; or independently of invasion history may vary in its predominant trophic role between sampling sites or rivers. This might even explain the contradictory findings of previous studies and illustrates that a very flexible feeding behaviour can be considered as a beneficial trait for a successful invader.

We predicted that predation by *D. villosus* would be more important in a community that was invaded more recently (Upper Elbe), than in a benthic community that had already adapted to the invasive amphipod species (Middle Rhine). However, our results contradicted this assumption because the trophic position of *D. villosus* was

lower in Elbe River even though invasion occurred later than in the River Rhine. Nevertheless, the benthic community of the Elbe River contained distinctly more native species, even though some of them were only observed in low densities. In contrast, very few native taxa with low densities were observed in the Middle Rhine whereas other invasive amphipods outnumbered *D. villosus*. Our observations seem to be representative for the benthic community composition in these river sections (Schöll 2009) and the River Rhine might therefore be characterized as a river of invasives (Leuven et al. 2009). It can therefore be assumed that the predation potential of *D. villosus* is strongly influenced by the site-specific benthic community and the specific prey availability. In the Elbe River, potential prey taxa as possible prey were not easily available, because most of them reached less than 5 % of the amphipod density. It therefore seems possible that the low abundance of large prey species (mainly mayflies, caddisflies, and amphipods), could be responsible for the minor use of animal sources in the Elbe River. On the other hand, although particularly taxa such as dipterans and isopods were a preferred prey in laboratory experiments (Dick et al. 2002; Boets et al. 2010; Dodd et al. 2014), these seemed to have a low relevance at our field sites in spite of their high abundances. It is comprehensible that *D. villosus* is forced to feed on only three of ten present resources, leaf litter/plants, biofilm and juveniles of its own kind, because in late summer in the Elbe River other prey species are less available due to their life cycles. In contrast to this situation, there are high amounts of potential large prey organisms in the Rhine, mostly other invasive amphipods. The higher prey availability might explain the higher trophic position of adult *D. villosus* in the River Rhine and the more flexible feeding with a tendency to more predacious behaviour, indicated by the similar use of five out of seven possible sources.

A critical point, confining the results of the mixing model, is the high number of the included resources because it is proven to reduce the power of the data interpretation (Phillips et al. 2014). However, omnivorous species can be expected to use a higher number of different resources than recommended for the model input, thus their inclusion seemed to be more realistic for the studied invader accepting the lower model certainty. The low importance of most of the resources in the diet of *D. villosus* could only be proven by including all possible resources

into the mixing model. However, care has to be taken not to over-interpret the model results. A food source, of which the lower 95% interval did not exceed a proportion of zero, can hardly be interpreted as a certain diet because there is a probability of 25% that this source is not used at all. The well-defined ranges of most of the sources in both rivers show a relatively clear interpretable picture of the diet utilisation of *D. villosus* and underline the high importance of biofilm, POM and plants for the diet of the invasive amphipod. A further problem might be that sources, laying in the same direction in the isotope biplot, could be substituted by each other and the use of a specific diet could remain doubtful. However, this seemed not to be a present problem in this study because most of the important sources were those with distinct stable isotope signatures, especially plant-based diet.

Although we interpret the difference in the predation potential of *D. villosus* between the two rivers to be a result of the river-specific benthic communities, the question might be asked whether the feeding of *D. villosus* might have caused a change in the community composition. It seems possible that *D. villosus* predation affects the species composition because differences in benthic community have been observed before and after *D. villosus* invasion (Van Riel et al. 2006). However, we do not think this is likely at our sampling sites because most of the native insect species were strongly endangered or even lost before the occurrence of *D. villosus* in the Middle Rhine due to the increasing anthropogenic utilisation associated with organic pollution and structural degradation (Tittizer et al. 1992) and no intense predation on those native insect taxa still present was indicated by our results. In addition, although amphipods were the most probable prey item for *D. villosus* in River Rhine, as observed in other studies (Kinzler et al. 2009; Van Riel et al. 2009; Boets et al. 2010), they seemed not to be negatively affected in terms of density.

Considering the importance of *D. villosus* as an invader in European running waters and its predation potential, the application of the SIA method to quantify its trophic function in natural communities has become an important tool (Van Riel et al. 2006; Brauns et al. 2011; Bacela-Spychalska and Van der Velde 2013; Koester and Gergs 2014; Rothhaupt et al. 2014). The small number of field studies explicitly based on SIA illustrates the large gap of knowledge regarding the trophic variability of *D. villosus* in time and

space. Because *inter alia* the lack of specific enrichment factors constrained the interpretation of isotope studies (Caut et al. 2009), we determined TEFs in laboratory experiments for *D. villosus*, showing a considerable diet-specific variability for one consumer. While the TEFs for nitrogen in this study lay inside the range of published discrimination factors, values for carbon enrichment strongly differ from the values given in meta-analyses (e.g. Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001). Therefore, using generalized mean values seemed not to be well transferable to our study and would have resulted in a considerable overestimation of predatory feeding and underestimation of plant-based diet. Because of the large difference between fractionation of plant-based and animal food sources for this omnivore, we explicitly recommend the inclusion of diet-typical TEFs in trophic analyses. We are convinced that the diet-specific values provided here could also be beneficial for other studies assessing the trophic role of the *D. villosus* in order to evaluate the variability in field studies.

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The following supplementary material is available for this article:

Appendix 1. Supplementary results of the trophic enrichment experiment:

Appendix 1.1. The abiotic conditions during the trophic enrichment experiment in the flume.

Appendix 1.2. Change of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the consumer *D. villosus* because of the tissue fractionation, and change in the diet during the experimental time in leaf litter treatment and chironomid treatment.

Appendix 2. The simulated mixing polygon for the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ -biplot of the single consumer signatures *D. villosus* and the average source signatures including the TEF's for the Elbe food web and the Rhine food web.

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