

Research Article

No evidence for intraguild predation of *Dikerogammarus villosus* (Sowinsky, 1894) at an invasion front in the Untere Lorze, Switzerland

Meike Koester* and René Gergs

Institute for Environmental Sciences, University of Koblenz-Landau, Fortstr. 7, 76829 Landau, Germany

E-mail: koester@uni-landau.de(MK), gergs@uni-landau.de (RG)

*Corresponding author

Received: 11 March 2014 / Accepted: 15 July 2014 / Published online: 1 September 2014

Handling editor: Michal Grabowski

Abstract

The establishment of non-indigenous aquatic invertebrates may strongly affect community and food web structures in invaded systems. The invasion of the Ponto-Caspian amphipod *Dikerogammarus villosus* in European waterways, has led to the displacement of other macroinvertebrates, especially other amphipod species. The key factor for the decline of both native and invasive amphipods is assumed to be strong intraguild predation (IGP) by *D. villosus*, as observed in laboratory studies. However, the importance of IGP under field conditions is nearly unknown. Here, we investigated the trophic annidation of the invasive *D. villosus* in a benthic food web in one season in 2011, focusing especially on the relevance of IGP at an invasion front in Switzerland. We combined stable isotope analyses with molecular analyses of *D. villosus*' gut content using a group-specific primer for gammarid amphipods. Analyses of the standard ellipse area showed a strong overlap of the isotopic niches of the amphipod taxa. Niche width analysed by Stable Isotope Bayesian Ellipses in R (SIBER) revealed no significant differences between *D. villosus* and the native gammarid species. Furthermore, we did not detect DNA of native gammarid species in any of the 62 analysed gut contents of *D. villosus* as direct evidence for predation. However, diet of amphipods may vary over the year and we cannot exclude completely the hypothesis that *D. villosus* does predate upon native gammarids. Nevertheless our results indicated that IGP is not the key factor in the displacement of native amphipods and does not seem to play an important role in invasion of the investigated Swiss water body. Hence, factors other than IGP seem to be the driving forces for the observed displacement of native amphipods.

Key words: amphipods, invasion process, stable isotope analyses, SIBER, trophic niche width, genetic gut content analyses

Introduction

The invasion of species, especially in aquatic ecosystems, has become a global concern and represents an increasing threat for native biodiversity (Sala et al. 2000; Chandra and Gerhardt 2008). From the beginning of the 20th century until 1990, the number of invading species in the River Rhine increased six-fold (Kinzelbach 1995), with macroinvertebrate invaders being mostly mussels and crustaceans (Kureck 1992; Chandra and Gerhardt 2008). These invasive species had an impact on the community structure and food web by changing habitat conditions and availability of food resources (e.g. Vander Zanden et al. 1999; Ricciardi and MacIsaac 2000; Gergs et al. 2009).

One of the 100 worst invasive alien species in Europe is the Ponto-Caspian *Dikerogammarus villosus* (Sowinsky, 1894) (DAISIE 2009). This

amphipod species invaded western Europe via the Main-Danube channel and spread rapidly throughout Europe (e.g. Bij de Vaate and Klink 1995; Bij de Vaate et al. 2002). The high reproductive potential of *D. villosus* (Grabowski et al. 2007; Pöckl 2007), its ability to colonize diverse types of substratum (Devin et al. 2003) and its high tolerance of various environmental conditions (e.g. Gabel et al. 2011; Maazouzi et al. 2011; Piscart et al. 2011) are potential reasons for its invasion success. With the establishment of *D. villosus*, the density of many other macroinvertebrate taxa, especially of other amphipod species, in invaded habitats have decreased (e.g. Dick et al. 2002; Bacela-Spychalska and Van Der Velde 2013; Gergs and Rothhaupt 2014). Previous laboratory studies have revealed the strong potential predatory impact of *D. villosus* on other macroinvertebrate species, including other amphipods, and this is assumed to be the key driver of the

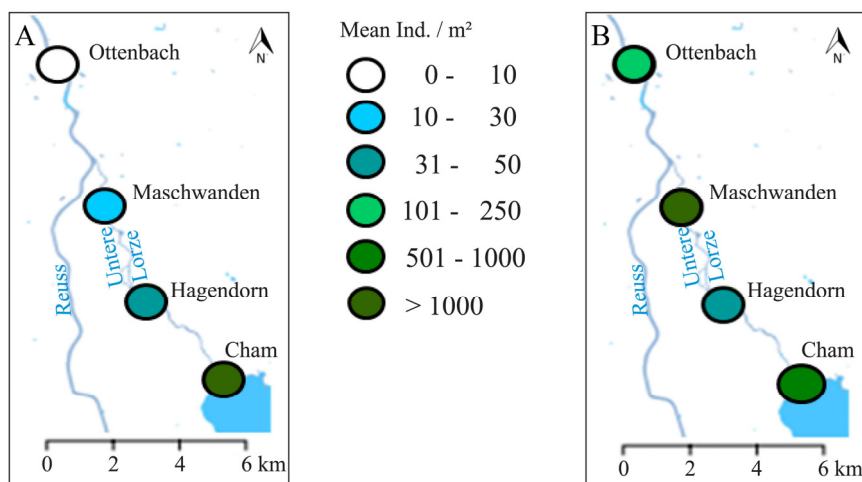


Figure 1. Density and distribution map of *Dikerogammarus villosus* (A) and *Gammarus pulex*/*Gammarus fossarum* (B) (modified after Ortlepp and Mürle 2012, map-source Federal Office of Topography swisstopo) at three sites in the River Untere Lorze. Densities are mean numbers of individuals m^{-2} ($n = 3$ surber-samples per site).

observed displacement (e.g. Dick and Platvoet 2000; Kinzler et al. 2009). However, little is known about the role of intraguild predation (IGP; i.e. predatory interactions between members of the same taxonomic guild, Polis et al. 1989) in the field because natural conditions are much more complex than conditions simulated in laboratory experiments (Kinzler et al. 2009). Therefore, to what extent IGP contributes to the displacement of native amphipod species in the field during an ongoing invasion of *D. villosus* is unknown.

During a monitoring of the benthic community in running waters in the River Untere Lorze, Switzerland in 2011, an invasion front of *D. villosus* was discovered (Figure 1, Ortlepp and Mürle 2012). We hypothesized that IGP by *D. villosus* is an important factor in the displacement of native amphipods at the invasion front in the Untere Lorze. To test this, we analysed the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures of various macro-invertebrate taxa of stored samples collected during the monitoring and genetically analysed the gut content of *D. villosus* using a group-specific primer for gammarid amphipods to detect direct predation on native gammarids.

Material and methods

Samples and sampling sites

Samples originate from the monitoring study of the HYDRA institute (on behalf of the Kanton

Aargau, Luzern, Zug and Zurich) conducted by Ortlepp and Mürle (2012). Samples were collected from three sites in the River Untere Lorze and from one site further downstream in the confluence the Untere Lorze and the River Mittelland-Reuss in March 2011 (Table 1). In the River Untere Lorze, *D. villosus* was found with densities decreasing in the downstream direction, while it was not found in the River Mittelland-Reuss (Figure 1). We used the pre-determined samples made available by the HYDRA institute, which were preserved in 98 % ethanol from this biological monitoring.

The four sampling sites are located in the pre-alpine territory and belong to the catchment area of the River Rhine. The Untere Lorze is a section of the River Lorze that pours out of Lake Zug at Cham, reaching the confluence with the Mittelland-Reuss 10 km downstream, with a drainage basin of approximately 60 km^2 . The mean discharge of the Untere Lorze is 7.3 m^3/s . The Mittelland-Reuss is the lower stretch of the River Reuss that stretches from the outflow of Lake Lucern to the confluence with the River Aare, 72 km downstream, and has a drainage basin of approximately 920 km^2 . The mean discharges of this water body are 110 m^3/s at the Lake Lucern outflow and 140 m^3/s at the confluence with the Aare. The main specific characteristics and exact location of the four sampling sites are given in Table 1. For a detailed description of the sites and the sampling method, see Ortlepp and Mürle (2012).

Table 1. Main characteristics of the two waterbodies Untere Lorze and Mittelland-Reuss and main biotic and abiotic environmental characteristics of the four sampling sites Cham, Hagendorn, Maschwanden and Ottenbach included into this study (after Ortlepp and Mürle 2012).

	Cham	Hagendorn	Maschwanden	Ottenbach
x-coordinate	677 500	675 155	674 090	672 440
y-coordinate	225 990	228 550	231 615	236 640
altitude above sea level [m]	410	395	385	382
T [°C]	4.8	5.7	4.3	6.2
O ₂ [mg/l]	15.7	13.7	13.5	15.2
O ₂ [%]	128	113	108	128
pH	8.1	8.4	8.5	8.7
Conductivity [µS/cm]	293	415	371	321
current [cm/s]	50	100	100	30
Width [m]	12	12	13	62
dominant substratum	empty mussel shells	stones, gravel	gravel	sand, gravel
number of taxa	39	29	69	67
dominant benthic taxa	<i>Dreissena polymorpha</i> (>80 %), <i>Potamopyrgus antipodarum</i> (>5 %), <i>Dikerogammarus villosus</i> (>5 %)	Chironomidae (>50 %), Oligochaeta (12%), Trichoptera (12%)	Chironomidae (38 %), Oligochaeta (30 %), Trichoptera (8 %), Crustacea (8 %)	Chironomidae (>33 %), Oligochaeta (>33 %), Ephemeroptera (10 %), Trichoptera (10 %)

Stable isotope analyses

Preservation in ethanol can lead to an accumulation in $\delta^{15}\text{N}$ of 1 ‰ and in $\delta^{13}\text{C}$ of 2 ‰ (Feuchtmayr and Grey 2003; Syväranta et al. 2011). Since all samples were treated in the same way, the error for all samples was comparable. Only individual adult amphipods (mean size of native amphipods 14.2 ± 4.2 mm, mean size of *D. villosus* 17.8 ± 3.7 mm) were analysed. The gastrointestinal tract of each amphipod was dissected and preserved in ≥ 99.8 % ethanol. We analysed the stable isotope signatures of *D. villosus* from sampling sites Maschwanden (n = 17), Hagendorn (n = 16) and Cham (n = 20) and those of *Gammarus pulex* (Lineaus, 1758)/*Gammarus fossarum* (Panzer, 1835) (as coexisting species) from sampling sites Ottenbach (n = 20), Maschwanden (n = 17) and Hagendorn (n = 13). Since the two species *G. pulex* and *G. fossarum* are hard to distinguish by morphology in some cases, individuals clearly assigned to one of the two species were not available from all sampling sites. For the Ottenbach and Maschwanden sampling sites, for which at least some individuals were distinguishable between the two species, we tested for significant difference in stable isotope signatures ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) between the two *Gammarus* species using a Wilcoxon-Mann-Whitney rank sum test at each sampling site. The test was performed to check if both species occupy a comparable isotopic niche and therefore can be treated as one group. For the Masch-

wanden and Hagendorn sites, we additionally analysed *Rhyacophila* sp. (as a known predator), Simuliidae, Orthoclaadiinae, Diamesinae and *Ancylus fluviatilis* (individuals, n = 5, exceptions are given in the figure legends) to obtain additional information about the food web.

All samples were dried at 60 °C, ground, weighed and placed into tin capsules (0.2 – 1.2 mg). Isotope ratios of carbon and nitrogen isotopes were measured using a Delta Advantage Isotope Ratio Mass Spectrometer connected to a Flash HT elemental analyser (Thermo Finnigan). Values of the stable carbon and nitrogen isotopes are presented as δ -values relative to the international reference standards Vienna PeeDee belemnite for carbon and atmospheric N₂ for nitrogen in units of per mille (‰). Repeated analyses of an internal standard resulted in typical accuracy (± 1 SD) of 0.12 and 0.05 ‰ for carbon and nitrogen, respectively.

We statistically analysed the results using the statistical software package R (R Development Core Team 2013). The trophic niche and trophic niche width of the different amphipod species were determined and compared using analyses of the Stable Isotope Bayesian Ellipses in R (SIBER) implemented in the R software package SIAR (Version 4.1.3; Jackson et al. 2011). We used standard ellipse area (SEA) calculated from the variance and covariance of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ amphipod data as a measure of the core isotopic niche (Jackson et al. 2012). According to Jackson et al. (2011) the SEA is less sensitive to small

sample sizes than the six metrics established by Layman et al. (2007) and can be used for data with $n > 10$. Bayesian estimations of the standard ellipses (SEA_B), suggested to tackle the problem of sensitivity to sample size and outliers (Jackson et al. 2011), were calculated to test for significant differences in trophic niche width with Bonferroni correction of the significance level for multiple comparisons. The percentage overlap of the isotopic niche area for coexisting amphipod species was determined using the function *overlap()* and the total niche area (TA) of the respective species.

Genetic analyses of the gut content

The DNA in the gut content of *D. villosus* was identified using PCR with the primer set Gam18S specific for gammarid amphipods (Koester et al. 2013). DNA was extracted with a modified salt extraction protocol (e.g. Gemmer and Gergs 2013) from the dissected gastrointestinal tract of *D. villosus* individuals used for stable isotope analyses (except for the Cham site, where 15 additional individuals were analysed). Each gut content was homogenized in 440 μ l salt extraction buffer (0.4 M NaCl, 10 mM Tris-HCl pH 8.0, and 2 mM EDTA pH 8.0) using a TissueLyser II (Qiagen; 1 min at 15 Hz) and then mixed with 90 μ l of 10 % SDS and 5 μ l of 10 mg/ml proteinase K. The mixtures were incubated for 1 h at 60 °C with shaking at 400 rpm on a thermomixer, with vortexing every 10 min. Then, 350 μ l 5 M NaCl was added, and the mixture was vortexed for 1 min and then centrifuged for 30 min at 13,000 rpm at room temperature in a Hitachi CT 15RE microcentrifuge. The supernatant (approximately 600 μ l) was transferred to a fresh 1.5 ml reaction tube, carefully mixed with 600 μ l isopropanol, and stored at -20 °C overnight. The samples were then centrifuged for 20 min at 13,000 rpm at room temperature in the microcentrifuge, and the supernatant was discarded. Pellets containing DNA were washed with 70% ethanol, dried, and dissolved in 50 μ l sterile ddH₂O. Since DNA of the digested prey is likely to be degraded (King et al. 2008), the DNA extract from each gut content was tested using a universal primer set ('NSF1419/20' ATAACAGGTCTGTGATGCCC, 'NSR1642/16' GACGGGCGGTGTGTRC) targeting the nuclear small subunit (18S) rDNA (Jarman et al. 2006). Since, this universal primer set targets the same rDNA region as the specific primer set Gam18S, the presence and functional efficiency of the used templates for the gut content analyses

with this primer set was assured directly from the tested individuals. Such a control with this universal primer set is important to prove the effectiveness of the PCR detection, because the quality of the template is a crucial step for the detection with a specific primer. DNA extracts for which functional efficiency were not proven with the universal primer set were excluded from further analyses.

The PCR reactions were conducted as described in Koester et al. (2013). DNA extract (1 μ l) was mixed with 9 μ l of a reaction mixture containing 0.5 μ M of each primer (Eurofins MWG Operon), 0.025 mM dNTPs, 1 \times reaction buffer S (10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂; PeqLab Biotechnologie GmbH) and 0.05 U Taq DNA polymerase (PeqLab Biotechnologie GmbH). We used the following PCR profile: 94 °C for 4 min, followed by 25 cycles of 94 °C for 30 s, 67 °C annealing temperature for 30 s, 72 °C for 90 s, plus a final extension step at 72 °C for 10 min. To prove the success of the PCR reaction, we used two controls with pure DNA from *G. pulex* and *G. fossarum* and one negative control with pure DNA from *D. villosus*. Amplified fragments were separated on 1.5 % agarose gels for 35 min at 100 V/cm; a 100-bp DNA ladder was used as size standard (Carl Roth GmbH + Co. KG). DNA was visualized using the gel documentation system Biostep Argus X1 (biostep GmbH, Jahnsdorf, Germany).

Results

No significant differences in the isotopic signature between *G. pulex* and *G. fossarum* were observed at the tested sampling sites (Wilcoxon-Mann-Whitney test; $\delta^{13}\text{C}$: Ottenbach $p = 0.335$ and Maschwanden $p = 0.052$; $\delta^{15}\text{N}$: Ottenbach $p = 0.189$ and Maschwanden $p = 0.177$). This result suggests that the two species occupy a comparable isotopic niche at our sampling sites. Therefore, stable isotope data of both native amphipod species and of those individuals, which were not possible to distinguish between the two species, were combined. Further statistical analyses were performed for *G. pulex/G. fossarum* together.

Adult individuals of native and invasive amphipods co-occurred at the two sampling sites at the invasion front (Maschwanden and Hagendorn). At both sites, the nitrogen signature of *D. villosus* individuals ($\Delta 3 - 4$ ‰) and *G. pulex/G. fossarum* ($\Delta \sim 2.5$ ‰) showed substantial intraspecific variations (Figure 2).

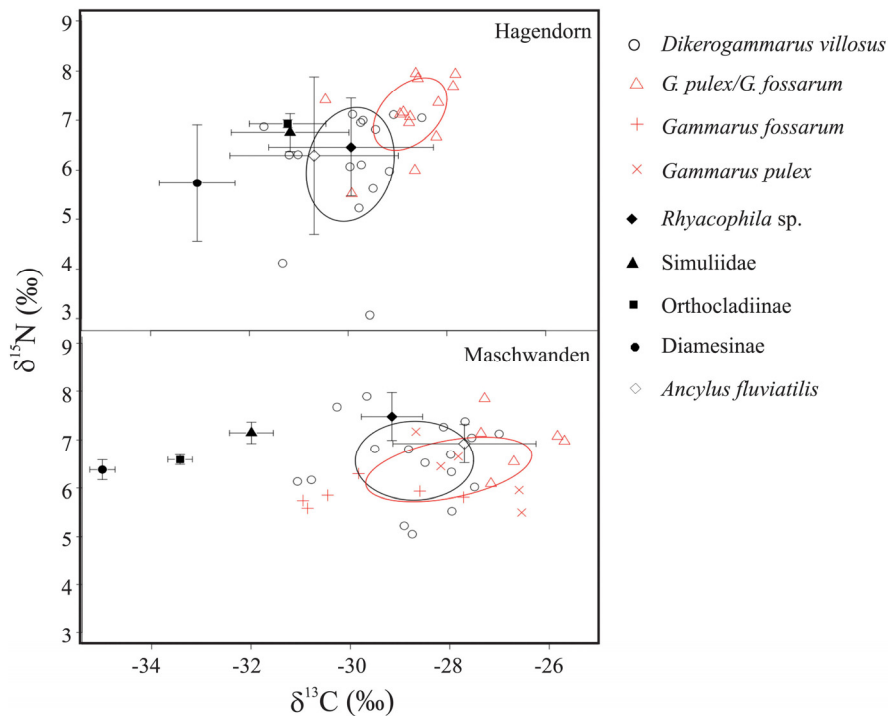


Figure 2. Stable isotope bi-plot illustrating the isotopic niche of *Dikerogammarus villosus* and *Gammarus pulex/Gammarus fossarum*. The standard ellipse area (SEA) for *D. villosus* (black) and *G. pulex/G. fossarum* (red) is shown. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values \pm SD of *Rhyacophila*, Simuliidae, Orthocladiinae ($n = 4$ at Maschwanden), Diamesinae ($n = 3$ at Maschwanden) and *Ancylus fluviatilis* ($n = 3$ at Hagendorn) are given as additional information of the food web.

Most amphipods at the two sampling sites showed comparable or even lower $\delta^{15}\text{N}$ values than *A. fluviatilis* (a well-defined consumer I). At the site at the foremost front of the invasion (Maschwanden), approximately one-third of the *D. villosus* and *G. pulex/G. fossarum* individuals showed $\delta^{15}\text{N}$ values comparable to those of the predatory *Rhyacophila* sp., while the other individuals of both amphipod species showed values up to approximately 2 ‰ lower than those of *Rhyacophila* sp. The nitrogen signature from one-third of the *D. villosus* individuals and one-half of the *G. pulex/G. fossarum* individuals was even slightly lower than that of collector gatherers (Diamesinae). The $\delta^{15}\text{N}$ values of most amphipod individuals at Hagendorn were similar to the values of *Rhyacophila* sp. at the same site. Some *D. villosus* individuals showed lower $\delta^{15}\text{N}$ values (up to 2 ‰), whereas one-third of the *G. pulex/G. fossarum* individuals showed slightly higher $\delta^{15}\text{N}$ values (< 1 ‰).

The carbon signature of *D. villosus* individuals ($\Delta 3.5 - 4$ ‰) and *G. pulex/G. fossarum*

individuals ($\Delta 4 - 5$ ‰) showed strong intraspecific variations at both sites. Overall, all amphipod individuals were enriched in ^{13}C compared to Simuliidae, Orthocladiinae and Diamesinae species. One-half of the *D. villosus* individuals and two-thirds of the *G. pulex/G. fossarum* individuals had higher $\delta^{13}\text{C}$ values than *Rhyacophila* sp. at the Maschwanden sampling site. At the Hagendorn sampling site, the intraspecific variation in the carbon signature of both *D. villosus* and *G. pulex/G. fossarum* was lower, and only some individuals of both amphipod species showed $\delta^{13}\text{C}$ values slightly more enriched than those of *Rhyacophila* sp.

Based on the standard ellipses, *D. villosus* did not occupy a higher trophic position than the native *G. pulex/G. fossarum* at the Maschwanden and Hagendorn sampling sites. At both sampling sites, the standard ellipses showed a strong overlap of the isotopic niches of *D. villosus* and *G. pulex/G. fossarum* (Figure 2), which suggested that they have a similar feeding strategy. The overlap was stronger at Maschwanden (67.8 % of

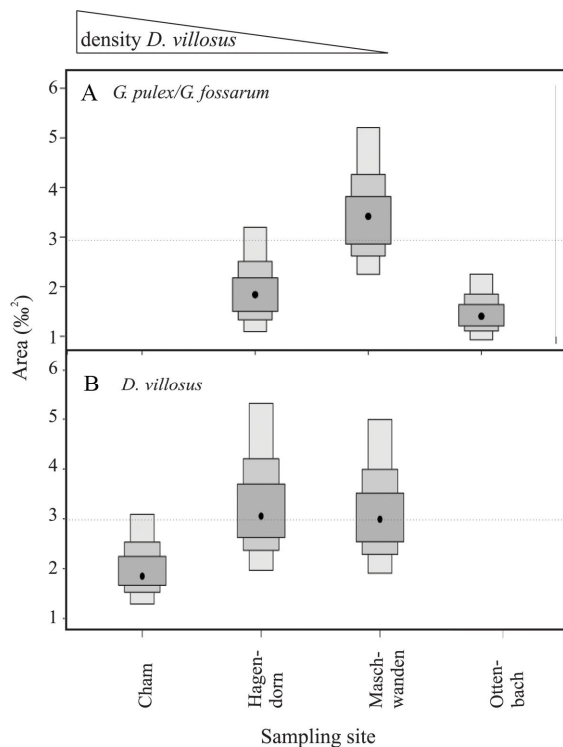


Figure 3. Density plot showing the confidence intervals of the standard ellipse areas for *Gammarus pulex*/*Gammarus fossarum* (A) and *Dikerogammarus villosus* (B) at the four sampling sites. Black points correspond to the mean standard ellipse area. Boxes shaded from dark to light grey represent the 50 %, 75 % and 95 % confidence intervals.

the total niche area of *D. villosus* and 65.3 % of the total niche area of *G. pulex*/*G. fossarum*) than at Hagendorn (7.7 % of the total niche area of *D. villosus* and 15.6 % of the total niche area of *G. pulex*/*G. fossarum*). The Bayesian estimations of the standard ellipses (SEA_B) of co-occurring *D. villosus* and *G. pulex*/*G. fossarum* were not significantly different, i.e. the niche width of the native and invasive species did not differ ($p > 0.05$; Figure 3). The niche widths of *D. villosus* at the three sampling sites in the Untere Lorze did not differ significantly ($p > 0.05$). The niche width of *G. pulex*/*G. fossarum* was significantly lower at Ottenbach than at Maschwanden ($p \leq 0.005$), whereas the isotopic niche width at Hagendorn did not significantly differ from these two sites ($p > 0.05$).

Genetic analyses of 62 DNA extracts from the gut content with the universal primer set for 18S rDNA yielded a fragment of the of 200 – 240

basepairs length according to Jarman et al. (2006), indicating the presence and functional efficiency of the 18S rDNA in the gut content extracts. Analyses with the specific Gam18S primer set did not detect DNA of *G. pulex*/*G. fossarum* in any of the gut content samples of *D. villosus* from the Maschwanden ($n = 17$), Hagendorn ($n = 15$) and Cham ($n = 30$) sampling sites. This was not a result of failed PCR since agarose gel electrophoresis of the PCR product of the controls (DNA extracted from *G. pulex* and *G. fossarum*) yielded a fragment of the appropriate length, (312 basepairs, Koester et al. 2013), and that of the negative control revealed no fragment.

Discussion

In an earlier study upon amphipods in main tributaries of the River Rhine in the Netherlands, Van Riel et al. (2006) found that *D. villosus* showed slightly higher $\delta^{15}N$ value than *Gammarus tigrinus*, *Echinogammarus ischnus* and *Chelicorophium curvispinum*, but differences in the mean values were about 1 ‰, which is lower than one trophic level. In our study, we found similar $\delta^{15}N$ values for the different amphipod species and overlapping isotopic niches, which militates against predation of *D. villosus* on *G. pulex*/*G. fossarum*. This conclusion is also supported by the results of the genetic analyses of the gut content, in which no *G. pulex*/*G. fossarum* DNA was detected in the gut of any of the 62 analysed *D. villosus* individuals, i.e. these individuals of the invasive amphipod did not recently ingest individuals or parts of the native amphipods. Even though we cannot exclude completely that *D. villosus* does predate upon native gammarids, IGP seems to be not the key factor in the displacement of native amphipod species during the *D. villosus* invasion in the Untere Lorze, even though earlier studies have shown that *D. villosus* is a stronger potential intraguild predator than *G. pulex* and *G. fossarum* (Kinzler and Maier 2003; MacNeil and Platvoet 2005; Kinzler et al. 2009). Such a minor importance of IGP in this invasion might be due to the more complex conditions and available food resources in the field, as the predatory strength of amphipods can be influenced by habitat structure and abiotic as well as biotic conditions (Dick and Platvoet 1996; MacNeil et al. 2004). Besides this, the fact that the amphipods do not show higher $\delta^{15}N$ values than a consumer I (*A. fluviatilis*) indicates a less predacious feeding strategy of the amphipods, which also militates against strong importance of IGP. However, van

der Velde et al. (2009) showed that the predatory behaviour of *D. villosus* can change between seasons. Since only samples from March 2011 were available for our study, we can only make a point about the feeding strategy of the amphipods and the role of IGP in this one season.

The smaller overlap of the isotopic niche at Hagendorn than at Maschwanden might imply that *G. pulex*/*G. fossarum* shifts to another feeding niche as the density of the invasive competitor *D. villosus* increases. However, the reverse effect or a niche shift based on different resource availability between sites cannot be excluded. Nevertheless, overlapping isotopic niches indicated that *G. pulex*/*G. fossarum* and *D. villosus* occupy a similar trophic level and hence have a similar predatory strength on other organisms. This confirms recent studies showing that *D. villosus* is not only a predator but also an omnivore with flexible feeding strategies (e.g. Mayer et al. 2009; Platvoet et al. 2009). The relatively similar niche widths of co-occurring *D. villosus* and *G. pulex*/*G. fossarum* together with the niche overlap indicates that these amphipods have both a comparable and a flexible feeding strategy. At first glance, the significantly lower niche width of *G. pulex*/*G. fossarum* at Ottenbach, where *D. villosus* was not present, suggests that *G. pulex*/*G. fossarum* is less specialized in the presence of the invasive competitor and uses a larger food spectrum to avoid potential competition. However, the environmental conditions of the two water bodies differ (Mattmann 2005, but see also Table 1), and therefore other explanations for the lower niche width at Ottenbach are more likely. For example, the variability in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ at the base of the food web can vary in different ecosystems and further propagate to higher trophic levels resulting in different isotopic niche widths (Post 2002). In addition, different environmental conditions may affect the assimilation of food items into tissue, which can have an impact on the total isotopic variation of a consumer (Layman et al. 2007).

Besides its feeding behaviour, the high reproductive potential and rapid growth of *D. villosus* (e.g. Kley and Maier 2006; Grabowski et al. 2007; Pöckl 2007) make the species a strong competitor not only for food but also for space. In a substratum choice experiment of each individual species, *D. villosus* preferred microhabitats of gravel with a low percentage of sand and stones, whereas *G. pulex* showed no clear substratum preference (Van Riel et al. 2007). However, in the presence of *D. villosus*, *G. pulex* is found less

frequently in gravel (MacNeil and Platvoet 2005; Boets et al. 2010), independent of the density of *D. villosus* (Van Riel et al. 2007). Such an exclusion from shelter-providing habitats leads to increased swimming activity of *G. pulex* in the water column and increased exposure to predatory fish and might therefore play an important role in the observed displacement of native amphipod species by *D. villosus* (Boets et al. 2010). Microhabitat distribution might be also of importance during an ongoing invasion process. For example, at the Cham sampling site (Figure 1), individuals of native amphipod species were only found in one of the three surber-samples, whereas individuals of *D. villosus* were found in all three surber-samples at that sampling site (see Orllepp and Mürle 2012). Furthermore, at the Maschwanden sampling site, with the lowest density of *D. villosus*, the highest density of *G. pulex* and *G. fossarum* in the River Untere Lorze was recorded. Therefore, there are indications that the native *G. pulex* and *G. fossarum* will become replaced by the invasive *D. villosus* in the River Untere Lorze during this ongoing invasion process.

Based on our findings, we conclude that a combination of different factors is responsible for the observed displacement of other native and invasive amphipod species. The success of *D. villosus* and the replacement of indigenous species might be further due to the high tolerance of *D. villosus* to various environmental conditions (e.g. Gabel et al. 2011; Piscart et al. 2011; Bundschuh et al. 2013). However, our results are from one case study of a recent invasion of *D. villosus*, with a limited number of sites and samples from one season, and generalizations cannot be drawn. *D. villosus* is predicted to further increase its range expansion on a global scale (Devin et al. 2003), thereby causing a potential threat to the diversity of newly invaded systems (Dick and Platvoet 2000). Further studies are therefore needed to understand the importance of both IGP and interspecific competition in the field.

Acknowledgements

We thank the Hydra Institute (especially Uta Mürle and Johannes Orllepp) and AquaPlus for providing samples, species distribution data and information about sampling sites and conditions. We thank Karen A. Brune for linguistic revision of the manuscript and anonymous reviewers and the associated editor for their helpful comments. This study was financed by the German Research Foundation (DFG; project GE 2219/3-1) and the University of Koblenz-Landau.

References

- Bacela-Spychalska K, Van Der Velde G (2013) There is more than one 'killer shrimp': trophic positions and predatory abilities of invasive amphipods of Ponto-Caspian origin. *Freshwater Biology* 58: 730–741, <http://dx.doi.org/10.1111/fwb.12078>
- Bij de Vaate A, Jazdzewski K, Ketelaars H, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1159–1174, <http://dx.doi.org/10.1139/f02-098>
- Bij de Vaate A, Klink A (1995) *Dikerogammarus villosus* Sowinski (Crustacea: Gammaridae) a new immigrant in the Dutch part of the Lower Rhine. *Lauterbornia* 20: 51–54
- Boets P, Lock K, Messiaen M, Goethals PLM (2010) Combining data-driven methods and lab studies to analyse the ecology of *Dikerogammarus villosus*. *Ecological Informatics* 5: 133–139, <http://dx.doi.org/10.1016/j.ecoinf.2009.12.005>
- Bundsschuh M, Gergs R, Schadt S, Schulz R (2013) Do differences in sensitivity between native and invasive amphipods explain their coexistence in Lake Constance? A case study with lambda-cyhalothrin. *Chemosphere* 92: 483–489, <http://dx.doi.org/10.1016/j.chemosphere.2013.01.106>
- Chandra S, Gerhardt A (2008) Invasive species in aquatic ecosystems: issue of global concern. *Aquatic Invasions* 3: 1–2, <http://dx.doi.org/10.3391/ai.2008.3.1.1>
- DAISIE (2009) Handbook of Alien Species in Europe. Springer, Dordrecht, The Netherlands
- Devin S, Piscart C, Beisel J-N, Moreteau JC (2003) Ecological traits of the amphipod invader *Dikerogammarus villosus* on a mesohabitat scale. *Archiv für Hydrobiologie* 158: 43–56, <http://dx.doi.org/10.1127/0003-9136/2003/0158-0043>
- Dick JTA, Platvoet D (1996) Intraguild predation and species exclusion in amphipods: the interaction of behaviour, physiology and environment. *Freshwater Biology* 36: 375–383, <http://dx.doi.org/10.1046/j.1365-2427.1996.00106.x>
- Dick JTA, Platvoet D (2000) Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proceedings of the Royal Society of London, Series B* 267: 977–983, <http://dx.doi.org/10.1098/rspb.2000.1099>
- Dick JTA, Platvoet D, Kelly DW (2002) Predatory impact of the freshwater invader *Dikerogammarus villosus* (Crustacea: Amphipoda). *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1078–1084, <http://dx.doi.org/10.1139/f02-074>
- Feuchtmayr H, Grey J (2003) Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. *Rapid Communications in Mass Spectrometry* 17: 2605–2610, <http://dx.doi.org/10.1002/rcm.1227>
- Gabel F, Pusch MT, Breyer P, Burmester V, Walz N, Garcia X-F (2011) Differential effects of wave stress on the physiology and behaviour of native versus non-native benthic invertebrates. *Biological Invasions* 13: 1843–1853, <http://dx.doi.org/10.1007/s10530-011-0003-1>
- Gemmer I, Gergs R (2013) Characterization of the first twelve microsatellite loci for the amphipod *Gammarus roeselii* (Crustacea: Amphipoda). *Conservation Genetics Resources* 5: 955–957, <http://dx.doi.org/10.1007/s12686-013-9941-5>
- Gergs R, Rinke K, Rothhaupt KO (2009) Zebra mussels mediate benthic-pelagic coupling by biodeposition and changing detrital stoichiometry. *Freshwater Biology* 54: 1379–1391, <http://dx.doi.org/10.1111/j.1365-2427.2009.02188.x>
- Gergs R, Rothhaupt K-O (2014) Invasive species as driving factors for the structure of benthic communities in Lake Constance, Germany. *Hydrobiologia*: 1–10, <http://dx.doi.org/10.1007/s10750-014-1931-4>
- Grabowski M, Bacela K, Konopacka A (2007) How to be an invasive gammarid (Amphipoda: Gammaroidea) – comparison of life history traits. *Hydrobiologia* 590: 75–84, <http://dx.doi.org/10.1007/s10750-007-0759-6>
- Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80: 595–602
- Jackson MC, Donohue I, Jackson AL, Britton JR, Harper DM, Grey J (2012) Population-Level Metrics of Trophic Structure Based on Stable Isotopes and Their Application to Invasion Ecology. *PLoS ONE* 7: e31757, <http://dx.doi.org/10.1371/journal.pone.0031757>
- Jarman SN, Redd KS, Gales NJ (2006) Group-specific primers for amplifying DNA sequences that identify Amphipoda, Cephalopoda, Echinodermata, Gastropoda, Isopoda, Ostracoda and Thoracica. *Molecular Ecology Notes* 6: 268–271, <http://dx.doi.org/10.1111/j.1471-8286.2005.01172.x>
- King RA, Read DS, Traugott M, Symondson WOC (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology* 17: 947–963, <http://dx.doi.org/10.1111/j.1365-294X.2007.03613.x>
- Kinzelbach R (1995) Neozoans in European waters—Exemplifying the worldwide process of invasion and species mixing. *Experientia* 51: 526–538, <http://dx.doi.org/10.1007/BF02143203>
- Kinzler W, Kley A, Mayer G, Waloszek D, Maier G (2009) Mutual predation between and cannibalism within several freshwater gammarids: *Dikerogammarus villosus* versus one native and three invasives. *Aquatic Ecology* 43: 457–464, <http://dx.doi.org/10.1007/s10452-008-9206-7>
- Kinzler W, Maier G (2003) Asymmetry in mutual predation: possible reason for the replacement of native gammarids by invasives. *Archiv für Hydrobiologie* 157: 473–481, <http://dx.doi.org/10.1127/0003-9136/2003/0157-0473>
- Kley A, Maier G (2006) Reproductive characteristics of invasive gammarids in the Rhine-Main-Danube catchment, South Germany. *Limnologica - Ecology and Management of Inland Waters* 36: 79–90
- Koester M, Claßen S, Gergs R (2013) Establishment of group-specific PCR primers for the identification of freshwater macroinvertebrates. *Conservation Genetics Resources* 5: 1091–1093, <http://dx.doi.org/10.1007/s12686-013-9998-1>
- Kureck A (1992) Neue Tiere im Rhein: Die Wiederbesiedlung des Stroms und die Ausbreitung der Neozoen. *Naturwissenschaften* 79: 533–540, <http://dx.doi.org/10.1007/BF01131409>
- Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88: 42–48, [http://dx.doi.org/10.1890/0012-9658\(2007\)88\[42:CSIRPF\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2)
- Maazouzi C, Piscart C, Legier F, Hervant F (2011) Ecophysiological responses to temperature of the "killer shrimp" *Dikerogammarus villosus*: Is the invader really stronger than the native *Gammarus pulex*? *Comparative Biochemistry and Physiology. Part A* 159: 268–274
- MacNeil C, Dick JTA, Johnson MP, Hatcher MJ, Dunn AM (2004) A species invasion mediated through habitat structure, intraguild predation, and parasitism. *Limnology and Oceanography* 49: 1848–1856, <http://dx.doi.org/10.4319/lo.2004.49.5.1848>
- MacNeil C, Platvoet D (2005) The predatory impact of the freshwater invader *Dikerogammarus villosus* on native *Gammarus pulex* (Crustacea: Amphipoda); influences of differential microdistribution and food resources. *Journal of Zoology* 267: 31–38, <http://dx.doi.org/10.1017/S0952836905007351>
- Mattmann B (2005) Untersuchung der Reuss und ihrer Zuflüsse unterhalb des Vierwaldstättersees in den Jahren 1999–2003: Zusammenstellung der Untersuchungen zur Wasserqualität Herausgeber. http://www.uwe.lu.ch/download/afu/ap/9/Reussbericht_99-03.pdf

- Mayer G, Maier G, Maas A, Waloszek D (2009) Mouthpart morphology of *Gammarus roeselii* compared to a successful invader, *Dikerogammarus villosus* (Amphipoda). *Journal of Crustacean Biology* 29: 161–174, <http://dx.doi.org/10.1651/08-3056R.1>
- Ortlepp J, Mürle U (2012) Gewässerschutzfachstellen der Kantone Aargau, Zug, Zürich und Luzern (2011): Biologische Untersuchung der Mittelland-Reuss, Kleinen Emme und Unteren Lorze. Fachbericht Makrozoobenthos (Untersuchungen vom Februar / März 2011). Hydra AG, St. Gallen
- Piscart C, Kefford BJ, Beiseld JN (2011) Are salinity tolerances of non-native macroinvertebrates in France an indicator of potential for their translocation in a new area? *Limnologia* 41: 107–112
- Platvoet D, van der Velde G, Dick JTA, Li S (2009) Flexible omnivory in *Dikerogammarus villosus* (Sowinsky, 1894) – Amphipod Pilot Species Project (AMPIS) Report 5. *Crustaceana* 82: 703–720, <http://dx.doi.org/10.1163/156854009X423201>
- Pöckl M (2007) Strategies of a successful new invader in European freshwaters: fecundity and reproductive potential of the Ponto-Caspian amphipod *Dikerogammarus villosus* in Austrian Danube, compared with the indigenous *Gammarus fossarum* and *G. roeseli*. *Freshwater Biology* 52: 50–63, <http://dx.doi.org/10.1111/j.1365-2427.2006.01671.x>
- Polis GA, Myers CA, Holt RD (1989) The Ecology and Evolution of Intraguild Predation: Potential Competitors That Eat Each Other. *Annual Review of Ecology and Systematics* 20: 297–330, <http://dx.doi.org/10.1146/annurev.es.20.110189.001501>
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718, [http://dx.doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)
- R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org>
- Ricciardi A, MacIsaac HJ (2000) Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. *Trends in Ecology & Evolution* 15: 62–65, [http://dx.doi.org/10.1016/S0169-5347\(99\)01745-0](http://dx.doi.org/10.1016/S0169-5347(99)01745-0)
- Sala OE, Chapin FS, III, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* 287: 1770–1774, <http://dx.doi.org/10.1126/science.287.5459.1770>
- Syväranta J, Martino A, Kopp D, Cereghino R, Santoul F (2011) Freezing and chemical preservatives alter the stable isotope values of carbon and nitrogen of the Asiatic clam (*Corbicula fluminea*). *Hydrobiologia* 658: 383–388, <http://dx.doi.org/10.1007/s10750-010-0512-4>
- van der Velde G, Leuven REW, Platvoet D, Bacela K, Huijbregts MJ, Hendriks HM, Kruijt D (2009) Environmental and morphological factors influencing predatory behaviour by invasive non-indigenous gammaridean species. *Biological Invasions* 11: 2043–2054, <http://dx.doi.org/10.1007/s10530-009-9500-x>
- Van Riel MC, Healy EP, Van der Velde G, Bij de Vaate A (2007) Interference competition among native and invader amphipods. *Acta Oecologia* 31: 282–289, <http://dx.doi.org/10.1016/j.actao.2006.12.006>
- Van Riel MC, van der Velde G, Rajagopal S, Marguillier S, Dehairs F, Bij de Vaate A (2006) Trophic relationships in the Rhine food web during and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. *Hydrobiologia* 565: 39–58, <http://dx.doi.org/10.1007/s10750-005-1904-8>
- Vander Zanden MJ, Casselman JM, Rasmussen JB (1999) Stable isotopes evidence for the food web consequences of species invasions in lakes. *Nature* 401: 464–467, <http://dx.doi.org/10.1038/46762>