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Food consumption of the invasive amphipod *Dikerogammarus villosus* in field mesocosms and its effects on leaf decomposition and periphyton

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Abstract

Invasive species can affect native communities by replacing competitors, overexploiting prey species or altering ecosystem structure. One example is the Ponto-Caspian amphipod *Dikerogammarus villosus* which has established large populations in European rivers and is widely considered as a main cause for the decline of native benthic invertebrates. This effect has been mainly associated with direct predation, whereas the indirect effects via competition for primary resources are poorly understood and possibly underestimated. To assess the probability of those indirect effects, we performed five outdoor flow-through mesocosm experiments in three European rivers, manipulating the density of *D. villosus.* We quantified its in-situ food consumption during three 24-h gut content surveys in the mesocosms. Gut evacuation rates for correction were measured in the laboratory for different food sources and under continuous feeding. We analysed the invader's effects on primary resources by quantifying periphyton biomass and community leaf litter decomposition in the mesocosms at different *D. villosus* densities. The observed remarkably high food consumption rates (0.38-1.27 mg mg^{-1} d^{-1}, in dry mass/dry body mass) of *D. villosus* can be attributed mainly to its high gut evacuation rates. The leaf litter decomposition rates indicate that *D. villosus* is an efficient shredder; however, there was no effect on the periphyton biomass. Our results indicate that *D. villosus* may be a strong competitor with primary consumers in benthic food webs of invaded rivers, with not only direct but also indirect negative effects on benthic communities. High consumption rates together with an opportunistic feeding behaviour probably promote the invasion success of this amphipod.

Keywords: biofilm grazing, CPOM, daily ration, feeding activity, gut evacuation, river food web, shredder

Introduction

The spread of invasive alien species is a global phenomenon associated with a decline of native biodiversity and changes in ecosystem structure and function (Hooper *et al.*, 2005, Strayer & Dudgeon, 2010). In European and North American river systems, particularly severe effects have been caused by various invasive species from the Ponto-Caspian region (Leuven *et al.*, 2009), among them several amphipods (Bollache *et al.*, 2004, Josens *et al.*, 2005, Palmer & Ricciardi, 2005, van Riel *et al.*, 2006). A prominent example for an invasive amphipod affecting Central European river communities is *Dikerogammarus villosus* SOVINSKY. This species invaded the Rhine System in 1995 (Haas *et al.*, 2002, Leuven *et al.*, 2009) and the Elbe system via the Mittellandkanal in 1998 (Bij de Vaate *et al.*, 2002) and, more recently, spread to Southern and Western Europe (e.g. Italy in 2006, Casellato *et al.*, 2006, United Kingdom in 2010, MacNeil *et al.*, 2010). In the invaded systems, it rapidly establishes high densities and often displaces both native and previously arrived alien species (Haas *et al.*, 2002, Jazdzewski *et al.*, 2004, Koop *et al.*, 2008, Hellmann *et al.*, 2016). Aside from its large body size (maximum 30 mm), other traits such as a high fecundity probably make *D. villosus* a successful invader (Devin *et al.*, 2004, Kley & Maier, 2006, Poeckl, 2009). Its success may be also enhanced by broad tolerance towards environmental factors and/or anthropogenic stressors such as salinity and temperature (Bruijs *et al.*, 2001, Grabowski *et al.*, 2007, Bacela-Spychalska *et al.*, 2013), some pesticides (Bundschuh *et al.*, 2013) and eutrophication (Brauns *et al.*, 2007). Although being generally broad, its tolerances are not always broader than those of other amphipods (Maazouzi *et al.*, 2011, Gergs *et al.*, 2013, Poznanska *et al.*, 2013). However, *D. villosus* is often able to compensate for this by different types of behaviour, such as hiding, low locomotor activity or dominance in competition for refuges (Gabel *et al.*, 2011, Becker *et al.*, 2016, Borza *et al.*, 2017). In addition, the not only omnivorous but highly flexible and opportunistic feeding behaviour of this species can support compensation (e.g. Platvoet *et al.*, 2009b, Dodd *et al.*, 2014). The potentially negative predatory impacts of *D. villosus* were the focus of numerous studies because the species displays very aggressive behaviour against other invertebrates (Dick & Platvoet, 2000, Dick *et al.*, 2002, MacNeil & Platvoet, 2005, Boets *et al.*, 2010). Predation on fish eggs was also observed in laboratory experiments (Casellato *et al.*, 2007, Taylor & Dunn, 2017). Therefore, *D. villosus* is often regarded as a predator with direct negative effects on other benthic species, especially on amphipods in invaded habitats (MacNeil *et al.*, 2011). This may result in negative effects of the *D. villosus* invasion on the ecosystem functions maintained by these prey taxa, such as coarse particular organic matter (CPOM) decomposition. Because *D. villosus* has displayed low shredding efficiencies in several laboratory studies, it is regarded as unable to compensate the loss of other shredders (e.g. Piscart *et al.*, 2011, Boeker & Geist, 2015, Jourdan *et al.*, 2016). On the other hand, some studies suggest this species may have similar shredding capabilities to *Gammarus roeselii* or *G. pulex* (Gergs & Rothhaupt, 2008, Bundschuh *et al.*, 2013, Truhlar *et al.*, 2014). Moreover, effects of *D. villosus* on overall leaf shredding rates depend on abiotic factors, e.g. decrease with increasing flow velocity (Felten *et al.*, 2008) or conductivity (Truhlar *et al.*, 2014). At high temperatures particularly, *D. villosus* seems to be a more efficient shredder than native gammarids (Truhlar *et al.*, 2014, Kenna *et al.*, 2017), although the invader's predation rate also increases with temperature (Van der Velde *et al.*, 2009). With one exception (Felten *et al.*, 2008), all the above mentioned studies comparing *D. villosus* with native amphipods were conducted under laboratory conditions.

D. villosus seems to be an opportunistic feeder with a very broad diet outside the laboratory. The fatty acid composition of *D. villosus* in a French reservoir suggests that decaying terrestrial plant material (including microorganisms) constituted a significant proportion of its diet (Maazouzi *et al.*, 2007). In the River Rhine and the River Elbe, *D. villosus* has a relatively low trophic position, as indicated by its stable isotope signature, and seems to consume plant-based resources in comparable amounts to animal prey (Hellmann *et al.*, 2015). In addition, genetic diet analysis indicates that *D. villosus* does not consume other invertebrates regularly in the River Rhine (Koester *et al.*, 2016). This is in accordance with the morphology of its mouthparts which are not specialized for a predatory life style (Mayer *et al.*, 2008) but are suited for various feeding techniques. Therefore, the omnivorous *D. villosus* might act as a predator but also as a competing primary consumer in a benthic community.

The exceptionally high growth rates and high reproduction potential of *D. villosus* (Devin *et al.*, 2004) suggest that it has high consumption rates, high assimilation efficiency or substantial energy allocation into somatic growth (Gergs & Rothhaupt, 2008, Becker *et al.*, 2016). Either way, the food consumption by extremely dense *D. villosus* populations observed in the field (Haas *et al.*, 2002, Koop *et al.*, 2008, Hellmann *et al.*, 2016) can be expected to have significant effects on resources. However, to date, food consumption rates of *D. villosus* have been only estimated in small-scale laboratory settings (e.g. Gergs & Rothhaupt, 2008, Truhlar *et al.*, 2014, Boeker & Geist, 2015, Jourdan *et al.*, 2016) rather than in the field where more realistic impacts effects on the invaded community can be directly assessed.

The subtraction method (e.g. Naylor *et al.*, 1989, Gergs & Rothhaupt, 2008) is predominately used in laboratory consumption estimations, because of its simple and time-efficient applicability under standardized conditions. The method is based on the amount of the remaining food after a (most often 24-h) feeding experiment on pre-defined and pre-weighed food sources. In contrast, the *in-situ* method (Bajkov, 1935, Elliott & Persson, 1978) is based on the temporal course of the consumer's gut fullness during 24 hours and is therefore applicable also under field conditions. Consequently, gut content analysis paints a more realistic picture of the actual food consumption under natural conditions – which can differ from laboratory trials. Moreover, the diel feeding activity patterns are easily observed in the field, providing more detailed insights into the predatory and competitive impacts on other benthic invertebrates.

Here, we used the *in-situ* method to estimate the daily food consumption of *D. villosus* in field mesocosms across different conspecific densities. Because of the opportunistic feeding behaviour of *D. villosus*, its food consumption potentially includes CPOM (e.g. leaf litter) and biofilms (e.g. periphyton). This might make the invader an efficient exploitative competitor for benthic shredders and grazers. Therefore, we evaluated the effects of different *D. villosus* biomasses on leaf litter and periphyton in the mesocosms, i.e. under natural conditions. We tested the hypothesis that *D. villosus* would have a positive effect on the community leaf decomposition rate and a negative effect on periphyton biomass. We studied the effects in three lotic ecosystems with a different invasion history and dominance of *D. villosus*: the River Rhine (invaded 1995, low native biodiversity) and the River Elbe (invaded 2001, higher native biodiversity) in Germany, and the River Bure in the Norfolk Broads, U.K. (invaded 2012, higher native biodiversity) (MacNeil *et al.*, 2013, Hellmann *et al.*, 2016).

Methods

Field mesocosm experiments

Five mesocosm experiments were conducted in total, two in the middle section of the River Rhine (km 660, near Sankt Goar, Germany, 50.16987 N, 7.66981 E), two in the upper River Elbe (km 66, near Dresden, Germany, 51.09415 N, 13.65110 E) and one in River Bure (near Wroxham, U.K., 52.714604 N, 1.405625 E). The experiments lasted 4–5 weeks. The Rhine experiments were performed in autumn 2013 (starting Oct 17) and spring 2014 (starting May 9). In River Elbe, the experiments were performed in autumn 2012 (starting Sep 9) and spring 2015 (starting May 7). The experiment in River Bure was performed in spring 2016 (starting

April 26). Before each experiment, high-grade steel mesh baskets (20 mm mesh size, Fig. 1) were filled with natural substratum (from coarse gravel to fist-sized stones, about 20–120 mm grain size). The base area of a basket was 0.1 m^2 and substrate depth was approximately 0.15-0.2 m. Because this depth can be colonized by *D. villosus* in similar coarse substrates of the river bed (L. Richter, personal observations), the used substrate is comparable to field habitat conditions. The baskets were exposed to the river bed, allowing for colonization by site-specific invertebrate communities for 4–6 weeks (for community composition in the baskets, see Table S2 in the appendix). After colonization, the baskets were carefully transferred to the mesocosms (Figure 1), i.e. set into flumes which were mounted on three floating pontoons and closed on both sides with 2-mm steel mesh (except the Elbe experiment in autumn 2012: 16 mm at the upstream end). There were three flumes on each pontoon and the experiments started with eight baskets per flume; baskets were sampled without replacement. The density of *D. villosus* in the flumes was manipulated at the start of the experiment in order to obtain three density treatments (Fig. 1): natural density (reached in the baskets after colonization on the river bed), high density (twice the natural density), and low density (as near zero as possible). This was achieved by the following procedure: all baskets of each flume were very carefully emptied and re-filled (to ensure equal amount of handling) but, as far as possible, all *D. villosus* individuals from the low density treatment flume were transferred to the high density flume. During the experiments, the mesh closing the flumes was cleaned 1-3 times per week. On these occasions, environmental factors were measured, including water temperature (°C), oxygen concentration (mg L^{-1}; multiprobe HQ40d, Hach, USA), current velocity (m s^{-1}; Mini-Air 2, Schiltknecht, Switzerland) and light intensity (except River Bure, due to technical problems) (mmol m^{-2}; portable quantum photometer, LI-COR, USA). Mean values and mean daily ranges between all flumes are given in Table S1 in the appendix.

Monitoring of the benthic community in the mesocosms

Benthic invertebrate density and biomass in the mesocosm flumes were estimated one day after the manipulation (initial sample) and 4 weeks later by emptying one or two baskets from each flume and collecting all invertebrates. The benthic samples were rinsed over a 500 µm sieve and stored in 80 % ethanol. Invertebrates were identified to the lowest possible taxonomic level (Elliot & Mann, 1998, Eggers & Martens, 2001, Glöer & Meier-Brook, 2003, Eiseler, 2005, Eiseler, 2010, Waringer & Graf, 2011), enumerated and total length excluding antennae or appendices was measured to the nearest 0.1 mm using a stage micrometer under a stereo microscope. The individual biomass for each benthic specimen (mg dry mass) in the substrate baskets was calculated from mean length using length-weight relationships (Meyer, 1989, Burgherr & Meyer, 1997, Benke *et al.*, 1999, Hellmann *et al.*, 2013, Hellmann *et al.*, 2015). However, if more than 50 individuals of a single taxon occurred in a basket, only 50 randomly chosen specimens were measured and the mean individual biomass of those specimens was assigned to the remaining specimens of this taxon. The benthic biomass of each taxon (mg basket^{-1}) was calculated as the sum of the individual biomasses.

D. villosus was separated into two separate size classes (adult ≥ 8 mm and juvenile < 8 mm) to account for possible differences in feeding behaviour. Low densities of the morphologically similar *D. haemobaphes* were found in River Elbe. An accurate discrimination from *D. villosus* was possible from 2.5 – 3 mm TL for the experienced researcher, based on the shape, length and spines of the uropods. Only reliably identified *D. villosus* individuals were included in gut content analyses. For an evaluation of competition with other potential grazers and shredders in the benthic community, feeding types were assigned to all taxa according to Tachet (2002) and the www.freshwaterecology.info Database (version 7.0, Schmidt-Kloiber & Hering, 2015). Both databases use relative affinities for the single feeding types which add up to 100% for each taxon, thus facilitating the use of mixed feeding types (Chevenet *et al.*, 1994). Each taxon with an affinity ≥ 10% for the feeding type ‘grazer’ or ‘shredder’ in the literature was assigned to that feeding type, otherwise to the feeding type ‘others’. Taxa with affinities ≥ 10% for both ‘grazer’ and ‘shredder’ were classified according the feeding type with the higher affinity value. The purpose of this procedure was to mirror rather the feeding potential of the invertebrates than their realized feeding behaviour because the actual diet composition is often very variable.

Estimation of the food consumption of *D. villosus*

The estimation of daily food consumption of *D. villosus* was possible in three mesocosm experiments: in the River Rhine, in spring and autumn, and the River Elbe, in spring (not in all five experiments due to logistical and experimental constraints). The daily food rations were estimated during 24-h field samplings, approximately three weeks after the start of the experiment, in the baskets of the natural-density and high-density treatment flumes. In each flume, at least five individuals were collected every 4 hrs, frozen in liquid nitrogen and transported to the laboratory, where they were stored at -18°C until further processing. The contents of pharynx and gut (hereby referred to as gut contents) were separated from the body under a dissecting microscope. Gut contents and body tissue (the latter including the empty gut and pharynx) were placed on separate pre-weighted small glass microfiber filter cuts, freeze-dried for 20-24 hrs at -57°C and weighed to the nearest 0.001 mg. The number of collected individuals (n = 5 to 36 per time point) differed according to the total *D. villosus* abundance in the baskets. If sample size was ≤ 5, each individual was weighed onto a separate filter cut and its gut content on another. If a larger number of individuals per sample was available, 2-3 individuals were pooled and weighed onto one filter cut (and their pooled gut contents on another), in order to save filter material, space and time. In the calculation of the gut fullness index, the contents were related to ‘empty’ mass m_e; here *D. villosus* dry body mass minus dry mass of pharynx contents and gut contents. For pooled individuals, the same was done with the pooled body tissue mass and the pooled gut content mass.

The *in-situ* daily ration of *D. villosus* was estimated from the gut fullness according to Elliott and Persson (1978), as the sum of the consumption during the 4-h sampling intervals. For each interval, the samples from natural and high density flumes were pooled because *D. villosus* biomasses of the treatments did not always differ significantly due to migration effects (C. Winkelmann, unpublished data). The gut fullness indices observed at the intervals were corrected with an exponential evacuation rate, which was estimated in laboratory experiments (Heroux & Magnan, 1996). Two such experiments were conducted, for two experimental food sources (A and B) at 14 ± 1°C (see also Richter *et al.*, in press): Individuals were collected in River Elbe and acclimatized in cages in an indoor flume with stones as refuges. They were fed with willow leaves (*Salix* sp., pre-conditioned for 2 weeks in aerated river water) and live or frozen chironomid larvae. The experimental food sources A (pre-conditioned willow leaves) and B (live chironomid larvae) were provided prior to the actual experiments after a 24-h (food source A) or 12-h starvation phase (food source B). During the evacuation experiments, the individuals were removed from their experimental food source, kept in groups of 5 (A) or 3 (B) and allowed to feed continuously on a well distinguishable second food source (post-A and post-B) for each experimental food. Food source post-A were paper colour-coding dots soaked for 12 h in river water and food source post-B were pre-conditioned willow leaves. Gut content samples were taken at 7 time points (0, 1, 3, 5, 9, 16 and 24 h) starting at the time of switching from food source (A to post-A and B to post-B. The experimental conditions were kept as similar as possible to those in the mesocosms and the river, by providing a near-natural habitat structure with refuges, a slight water movement due to the aeration, a season-specific light-dark cycle of 16:8 h, and keeping the animals in groups. The paper coding dots were used because they were eaten readily and could easily be separated from the leaves during gut analysis. Their digestibility was tested in preliminary experiments over 7 days (Richter et al., in press). Although the paper dots were evacuated more slowly than willow leaves which might have resulted in a slight underestimation of the willow leaf evacuation rate, the animals were not affected negatively. The gut evacuation rate was estimated by fitting an exponential regression to the gut content data over time for each experimental food source. The mean of the negative slopes of the two regressions (0.195 ± 0.039, mean ± se, for chironomid larvae and 0.245 ± 0.048 for willow leaves), 0.22, was used as evacuation rate (expressed in mg mg^{-1} h^{-1}) in the calculation of C_d (Elliott & Persson, 1978).

In order to account for the temperature dependence of food consumption, the *in-situ* daily ration was corrected for the difference between actual mean water temperature during each in-situ consumption experiment (Table 1) and the temperature during the evacuation rate experiments (14°C) by applying Van't Hoff's equation after solving it for the in-situ daily ration (Vant Hoff, 1896). We used a mean Q_{10} value of 1.74 for this correction (Becker *et al.*, 2016). The cumulative daily consumption C_{cum} was calculated in mg dry mass m^{-2} basket base area (mg m^{-2} d^{-1}), for the period between the initial and second benthic sample of each mesocosm experiment, i.e. roughly 4 weeks. Temperature correction with a Q_{10} of 1.74 was applied, using the differences between the actual mean temperature during the consumption experiments and the mean daily temperatures during the whole period.

Evaluation of effects on primary resources

CPOM decomposition was measured directly as leaf decomposition rate during each mesocosm experiment in all rivers (except River Elbe in autumn). We used leaf litter bags filled with 2.5 g pre-conditioned and dried willow leaves (*Salix* sp. from local riparian vegetation), with a mesh size of 1.5 mm and an ample window of 15.0 mm mesh on the upper side to allow invertebrate shredders to access the leaves. The bags were exposed approximately one week after the start of a mesocosm experiment on the substrate surface of every basket. They were sampled weekly for 3-4 weeks by randomly collecting and carefully emptying two bags and weighing the contents after removing all animals and drying at 50°C. Each sampled bag was marked (to avoid double sampling) and re-exposed with about 2.0 g of replacement leaves until the end of the mesocosm experiment in order to avoid affecting decomposition rate by a change in resource availability. Additionally, in two experiments (River Rhine spring; River Elbe spring), 0.2 mm mesh bags excluding macroinvertebrates were exposed and sampled in parallel to get an estimation of the microbial leaf decomposition rate. The leaf decomposition rate was calculated from the decrease of leaf dry mass over time by fitting a linearized negative exponential decay model (Benfield, 2006), for each flume separately.

As an indirect measure of community grazing in the baskets, the periphyton biomass was quantified 4 weeks after the start of each experiment (expressed in mg chlorophyll-a per cm^2 stone surface area). Periphyton was sampled from 2-3 stones out of the uppermost substrate layer in each sampled basket by brushing off the light-exposed surface (total sampled area 165 ± 91 cm^2, mean ± SD) with tap water. The samples were frozen in liquid nitrogen and stored in the dark at -80°C until analysis. The chlorophyll-a content was measured in a defined subsample volume after freeze-drying, homogenization and subsequent ethanol extraction (Wetzel & Likens, 2000) using a luminescence spectrometer (LS 50B, Perkin-Elmer, Rodgau, Germany) at 667 nm emission wavelength. The sampled surface area of the stones was measured for all biofilm samples by carefully wrapping in aluminium foil and weighing the foil cuts afterwards (in relation to a reference cut of 10 cm^2 area).

Statistical analyses

The effect of *D. villosus* biomass on leaf decomposition rate and periphyton biomass was analysed by fitting linear mixed-effects models (Pinheiro, 2000, Bates *et al.*, 2015a) using the R package lme4 (Bates *et al.*, 2015b). This allowed the combined statistical analysis of all experiments. Thus, not only (small) differences in abiotic environmental factors between the flumes within an experiment are accounted for, but also the larger seasonal and river-related differences. *D. villosus* biomass values were square root-transformed to approximate normal distribution. For CPOM decomposition rate as response variable, *D. villosus* biomass (both size classes together, mean of the initial and 4 weeks samplings) was included in the models as fixed effects and experiment and pontoon as random effects, pontoon being nested within experiment. Two sets of models were fitted: one with a common slope of the *D. villosus* effect for all experiments (a-models) and one with a random slope, i.e. the slope was potentially influenced by the experiment (b-models). The effects on periphyton biomass (chl-a as response) were modelled separately for total *D. villosus* biomass and juveniles only (< 8 mm). Here, *D. villosus* biomass (4 weeks after start) and season were included as fixed effects because of the suspected strong seasonality of periphyton growth. Similarly, we fitted two sets of models, one with fixed and one with random slope, for the *D. villosus*-season interaction effect on periphyton. We compared all the models, including the null models without fixed effects, using Akaike’s Information Criterion, AIC (Johnson & Omland, 2004) to find the optimal models. The daily rations of juvenile and adult *D. villosus* in the respective mesocosm experiments were compared using permutation tests, stratified by sampling time. All statistical analyses and graphical procedures were carried out using R (version 3.3.3, R

Core Team, 2017).

Results

In most mesocosm experiments, *D. villosus* constituted a substantial proportion of the total benthic biomass (Fig. 2, Table S2) both in the high-density and natural-density treatments. We observed comparatively low *D. villosus* biomass only in the River Rhine in autumn and in the recently invaded River Bure. In our experimental units, non-native taxa dominated the benthic communities in the rivers Rhine (87.3 – 97.7% biomass) and Elbe (74.0 – 94.5%), in contrast to River Bure (4.6 – 15.7%). Potential grazers aside from *D. villosus*, were important in the River Elbe (in autumn, mainly the invasive isopod *Jaera sarsi*) and the River Bure (native and invasive snails) but occurred in low biomasses in the River Rhine. Potential shredders other than *D. villosus* included the invasive amphipod *Echinogammarus ischnus* (syn. *Chaetogammarus ischnus*) in the River Rhine and, in low numbers, *D. haemobaphes* in the River Elbe in spring, both species showing omnivorous feeding, which includes leaf-shredding. In the River Bure, the main native shredder was the caddis larva *Halesus radiatus*.

D. villosus had high *in-situ* consumption rates in all mesocosms, consuming average daily rations of 38 – 127% of the body weight (Table 1). The gut fullness index (Fig. 3) of adults and juveniles was highest in the River Rhine, spring, and lowest in the River Elbe, spring, but indicated no distinct diel pattern of feeding activity in any of the experiments. At almost all sampling times, juveniles had a slightly higher mean gut fullness index than adults, with the largest differences occurring in the evening and night hours. Applying the same evacuation rate to both size classes, the daily ration of juveniles was always higher than that of adults in the respective mesocosm experiment (permutation tests, stratified by sampling time, n = 41 – 176, $p < 0.01$ for all experiments).

Considerable amounts of food were probably consumed by *D. villosus* in the baskets of the mesocosms, with maximum estimates of 11.8 g m^{-2} basket base area. The consumption by the adults constituted the major proportion of the total daily food consumption (C_{cum}) of *D. villosus* in the mesocosms in the two experiments in River Rhine (Table 1). This was due to the high proportion of adult biomass in the mesocosms (87% of the total *D. villosus* biomass in autumn and 85 % in spring). In contrast, in River Elbe, the biomass of adults and juveniles was similar (57 % adults of biomass) and the proportions in consumption nearly equal for both size classes (adults: 49% of C_{cum}).

Leaf decomposition rate showed an overall increase with higher *D. villosus* biomass in the four analysed mesocosm experiments (Fig. 4). The best model (based on the lowest AIC with $|\Delta AIC| \geq 2.0$ to the second best one), included *D. villosus* biomass as fixed effect and pontoon and experiment as random effects (m1a, Table 2). This indicates a significant effect of *D. villosus* biomass on CPOM decomposition in the mesocosms. The model with a variable slope for the single experiments did not describe the data more accurately than that with a uniform slope, which suggests that the underlying mechanisms of the increasing CPOM decomposition rates were similar in the experiments despite different rivers and seasons. The CPOM decomposition rate in the fine mesh bags in the natural density treatment flumes was generally lower than in the coarse mesh bags of the same flumes. This indicates that macroinvertebrates accounted for a part of CPOM decomposition in the mesocosms, although their importance seemed to differ between the experiments. In River Elbe, spring, at a high density of potential shredders, the CPOM decomposition in the fine mesh bags was much lower (0.019 g d^{-1} compared to 0.052 ± 0.011 g d^{-1} mean ± sd). In River Bure, at a low shredder density, it was only slightly lower in the fine mesh bags (0.011 g d^{-1} compared with 0.016 ± 0.011 g d^{-1}). The periphyton (chl-a) showed no clear relationship to either total or juvenile *D. villosus* biomass (Fig. 5). None of the models was better than the respective null model; in fact, all models were very similar according to AIC (Table3). Although periphyton biomass was mostly higher in spring than in autumn, the effect of season was also not significant according to the model selection.

Discussion

The impact of invasive species on the trophic structure and function of communities is often negative but seems to be context-dependent (Kratina *et al.*, 2014, Jackson *et al.*, 2017). This might apply also to the omnivorous *D. villosus*, as in our field mesocosm study, we observed that the invasive *D. villosus* is a remarkably strong consumer in the Central European Rivers Elbe and Rhine. Its ability to ingest more food than its own body weight per day in field mesocosms exceeded expectations from laboratory-based experiments (MacNeil *et al.*, 2011, own unpublished data, Maier *et al.*, 2011, Truhlar *et al.*, 2014). Although *D. villosus* does use periphyton and leaf litter in the field to considerable proportions (Hellmann *et al.*, 2016, Koester *et al.*, 2016), we found no effect on periphyton biomass in any of the five experiments. However, our hypothesis postulating positive effects of *D. villosus* on leaf litter decomposition was supported by the data from four mesocosm experiments in three rivers with different benthic communities. This might be explained by the fact that *D. villosus* was an important or even the dominant shredder in terms of biomass in River Elbe. Even when other invertebrate shredders are present (in the River Rhine, previously invaded species) *D. villosus* can be an efficient shredder in river ecosystems, enhancing the community leaf litter recycling. In the systems studied here, there were nearly no native gammarids and low densities of other native shredders. This precluded a test of the common assumption that the invasion of *D. villosus* negatively affects ecosystem functioning (i.e. leaf litter decomposition) due to the replacement of (more efficient) native shredders (e.g. MacNeil *et al.*, 2011, Jourdan *et al.*, 2016). Our findings indicate that there may be exceptions from this assumption, considering the high feeding potential of *D. villosus* and depending on the community and other environmental factors. For instance, when comparing leaf shredding rates of *D. villosus* and native amphipods, the larger body size of *D. villosus* (Kenna *et al.*, 2017) and its higher feeding efficiency at higher temperatures (Truhlar *et al.*, 2014) should be taken into account. Furthermore, it seems that intraguild predation is not always as important in the field as indicated by laboratory observations (reviewed in Jackson *et al.*, 2017). The assumption of *D. villosus* feeding substantially on CPOM was supported also by our observation that many individuals were found in and on the leaf bags, particularly in the coarse mesh area on the upper side that was obviously not suitable as a refuge because it was exposed to light. Some bags had extremely high *D. villosus* densities, which might have even dampened the biomass effects on leaf decomposition rate due to spatial interference competition in the high-density treatments. *D. villosus* is able to shred leaves due to the morphology of its mouthparts (Mayer *et al.*, 2008) and CPOM is a valuable enough food source, in particular in combination with the adhering biofilm of fungi and bacteria containing essential fatty acids (Maazouzi *et al.*, 2007, Maazouzi *et al.*, 2009). Even if animal prey can be expected to be assimilated more easily,, it is conceivable that the opportunistic and flexible feeder *D. villosus* used the easily available CPOM. Selecting the most abundant or consistently available food resource, even if it is not the energetically most profitable resource (per weight unit), can be a successful foraging strategy for some consumers (Real, 1990, Worischka *et al.*, 2015). Therefore, the relative impact of *D. villosus* is likely to depend on the community structure as well as the availability of different food sources.

The daily food consumption by the total *D. villosus* population reached maximum values of more than 10 g dry mass m^{-2} basket area in the field mesocosms due to the high feeding rate of the juveniles and the high biomass of the adults. With a dependency on any single food source and even at a mixed diet, this consumption is enormous and shows the considerable potential of this invader as a predator or exploitative competitor. Although these values are coarse estimates, the results are quite transferrable to ‘real’ field conditions because *D. villosus* reaches densities of more than 3000 ind m^{-2} in Central European rivers (Haas *et al.*, 2002) and can dominate macroinvertebrate communities in terms of biomass (Hellmann *et al.*, 2015). The combination of the high consumption rates and high benthic densities of this invader suggests the existence of drastic effects on resources (basal resources and/or potential prey) under natural conditions. It is therefore possible that aside from the often observed strong direct effects of *D. villosus* on other species, such as predation (e.g. Dick & Platvoet, 2000, MacNeil *et al.*, 2011) or displacement from microhabitats (e.g. Casellato *et al.*, 2008 , Borza *et al.*, 2017), indirect effects by exploitation competition may also contribute to the negative consequences for invaded communities.

The large difference between our consumption estimates and the values found in other studies can be attributed mainly to methodology, i.e. the experimental conditions as well as the estimation method itself. Most estimations of the feeding rate of *D. villosus* were performed in the laboratory under highly artificial conditions, such as small experimental tanks and providing a modicum of refuge (e.g. Truhlar *et al.*, 2014, Boeker & Geist, 2015). Also the use of single individuals (Gergs & Rothhaupt, 2008, Piscart *et al.*, 2011, Jourdan *et al.*, 2016) might affect the feeding rate. A combination of more semi-natural conditions in laboratory feeding experiments, such as larger tanks with abundant refuge availability and the keeping of the animals in groups, with realistic estimations of gut evacuation rates, can result in much higher feeding rates (0.54-0.89 mg mg^{-1} d^{-1}, dry mass/ dry body mass, Richter *et al.*, in press) compared to the above-mentioned studies (all less than 0.4 mg mg^{-1} d$^{-1)}$. Therefore, the higher feeding rates observed in our field mesocosms are plausible. Assuming that field experiments mirror the complex situation in river ecosystems better than laboratory assays, we suggest that our findings improve the estimate of the potential impact of *D. villosus* in invaded European rivers.

The periodicity of feeding activity of *D. villosus* was weak in the mesocosms with average gut fullness being relatively constant but showing high between-individual variation. This is in accordance with behavioural observations from other studies, such as a high between- and within-individual variability in swimming activity (Bierbach *et al.*, 2016) and a strong affinity for refuges such as stones or pebbles (Platvoet *et al.*, 2009a, Kobak *et al.*, 2015). The latter behaviour enables the animals to feed even in the presence of predators, e.g. fish. An important assumption of the gut content method is the strong mathematical dependence of consumption rate on gut evacuation rate (Elliott & Persson, 1978, Worischka & Mehner, 1998) which, physiologically, may in turn depend on the ingestion rate (Eggers, 1977). This was observed for *Daphnia* sp. (Gillis *et al.*, 2005) but is likely to occur in many other invertebrates. Thus, the amplitude of gut fullness over time might be dampened by the fact that ingested food is evacuated more slowly when no fresh food is following. The dependence was accounted for in the consumption estimation by using an evacuation rate determined under continuous feeding. Although it is not possible to eliminate its influence on in-situ gut content, we assume periodicity of feeding activity to be of minor importance, because *D. villosus* has been observed to have no distinct diurnal activity rhythm in previous behavioural experiments (Richter *et al.* , in press; P. Lommatzsch, unpublished data). Continuous feeding of *D. villosus* over the whole day, especially of the more predatory adults, would have consequences for all potential prey animals, reducing the possibilities for predator avoidance to merely spatial segregation.

Because *D. villosus* is at least able to feed on periphyton (Platvoet *et al.*, 2009b), we analysed also potential grazing effects. However, the periphyton quantity was not influenced by *D.*

villosus biomass in the mesocosms. The first possible explanation is the presence of more efficient grazers in some experiments, such as snails (especially *Viviparus contectus* Millet, 1813*,* Table S2) in River Bure. They might have masked any *D. villosus* effects simply due to much higher biomasses and higher grazing rate. Another reason for the lack of *D. villosus* effects on periphyton could be the dominance of bottom-up effects on autotrophic periphyton (Keldsen, 1996, Sturt *et al.*, 2011) in the only slightly shaded flumes. This is supported by our observation of strong algal periphyton growth during the three spring experiments with a temporary dominance of filamentous algae especially in River Elbe. A third explanation is that *D. villosus*, especially the adults, probably used other resources such as CPOM. Juveniles, which can be assumed to have a higher proportion of algae in their diet (Platvoet *et al.*, 2006, and own, non-quantitative observations during the gut content analyses), most likely accounted for a minor part of the total consumption except in River Elbe in spring.

In conclusion, *D. villosus* is probably not only a predator but also a competitor for some basal resources in many benthic food webs and has the potential to positively affect the ecosystem function of leaf litter decomposition. The combination of high consumption rates, and omnivorous and opportunistic feeding behaviours probably contributes to the population persistence of this invader (Kratina *et al.*, 2012) and its strong potential to alter the structure and dynamics of native benthic communities.

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Tables

Table 1 Daily food consumption of *D. villosus* in the mesocosm experiments. C_d and $C_{d, Q10}$ = daily ration in dry mass per dry body mass without and with temperature correction, TL = mean total length of the analysed individuals, n = total number of samples (all samples of a 24-h survey, number in brackets = total number of individuals if they were pooled for part of the samples), T_w = water temperature during 24-h survey, C_{cum} = cumulative daily consumption based on mean *D. villosus* biomass, Prop. C_{cum} = proportion of size class in total cumulative daily consumption, aut = autumn, spr = spring. The standard error of C_d was calculated using a bootstrap procedure (Efron, 1979).

Experiment	**size class**	**C_d ± se (g g^{-1} d^{-1})**	**$C_{d,Q10}$**	**TL ± sd (mm)**	**n**	**T_w ± sd (°C)**	**C_{cum} ± sd (g m^{-2} d^{-1})**	**Prop. C_{cum} (%)**
Rhine, aut	adult	0.43±0.02	0.38	10.92±1.61	110	11.8±0.1	1.0±0.7	81.5
	juvenile	0.64±0.06	0.56	6.12±1.00	54			18.5
Rhine, spr	adult	0.71±0.03	0.95	11.48±1.52	42 (126)	19.1±0.8	11.8±5.0	84.0
	juvenile	0.96±0.07	1.27	4.87±0.86	85			16.0
Elbe, spr	adult	0.36±0.03	0.46	9.15±1.20	122 (168)	18.6±1.1	8.5±5.1	55.5
	juvenile	0.51±0.03	0.65	5.39±1.76	185 (265)			44.5

Table 2 Model selection of linear mixed models with CPOM decomposition rate and periphyton chl-a, respectively, as dependent variables. For periphyton chl-a, one set of models was built for total *D. villosus* biomass and one for only juvenile *D. villosus* biomass as fixed effect. Dvill = *D. villosus* biomass (all size classes), Dvill$_{juv}$ = juvenile *D. villosus* biomass (< 8 mm), pont = pontoon, exper = experiment (pontoon always nested within experiment), Df = degrees of freedom of the model, AIC = Akaike's information criterion, LogLik = logarithm of maximum likelihood.

Model	**Parameters**		**Df**	**AIC**	**LogLik**
	fixed effects	**random effects**			
response: CPOM decay rate					
m1b	Dvill	pont, exper (random slope)	9	-72.8*	45.4
m0b	-	pont, exper (random slope)	8	-69.0	42.5
m1a	Dvill	pont, exper	5	-80.3*	45.1
m0a	-	pont, exper	4	-74.4	41.2
response: Periphyton (chl-a)					
m3b	Dvill, season (interaction)	pont, exper (random slope)	11	198.4	-88.2
m3a	Dvill, season (interaction)	pont, exper	7	197.4	-91.7
m2a	Dvill, season	pont, exper	6	198.3	-93.1
m1ad	Dvill	pont, exper	5	196.8	-93.4
m1as	season	pont, exper	5	199.3	-94.6
m0a	-	pont, exper	4	198.5	-95.3
m3b	Dvill$_{juv}$, season (interaction)	pont, exper (random slope)	11	210.2	-94.1
m3a	Dvill$_{juv}$, season (interaction)	pont, exper	7	202.2	-94.1
m2a	Dvill$_{juv}$, season	pont, exper	6	201.2	-94.6
m1ad	Dvill$_{juv}$	pont, exper	5	200.5	-95.2
m1as	season	pont, exper	5	199.3	-94.6
m0a	-	pont, exper	4	198.5	-95.3

* Likelihood ratio test: $p < 0.05$, df = 1, Chi^2 = 4.64 resp. 4.01

Figures

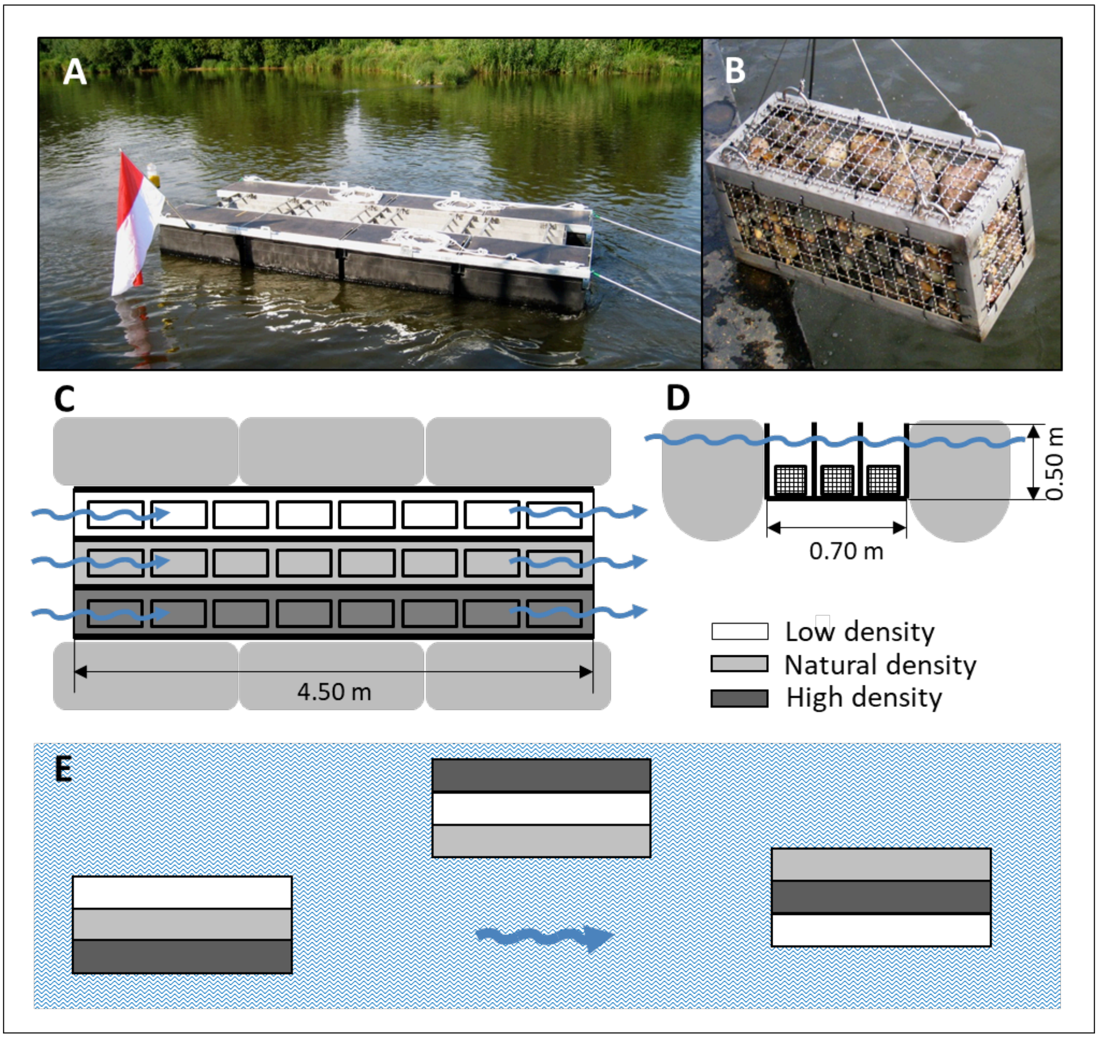

Figure 1 (A) Mesocosm in the river, (B) substrate basket before exposure on the river bed for colonization, (C) schematic drawing (top view) of a mesocosm with three flumes containing eight colonized substrate baskets each. The baskets were open at the top during the experiments. The three density treatments were achieved by manipulating the *D. villosus* density. Grey arrows indicate the flow of water through the flumes which were closed with 2-mm steel mesh at the prow and stern ends. (D) Schematic drawing (cross section) of a mesocosm. (E) Position of the mesocosms in the river (100-200 m apart from each other) and distribution of the treatments in each mesocosm during experiments. Drawings are not to scale.

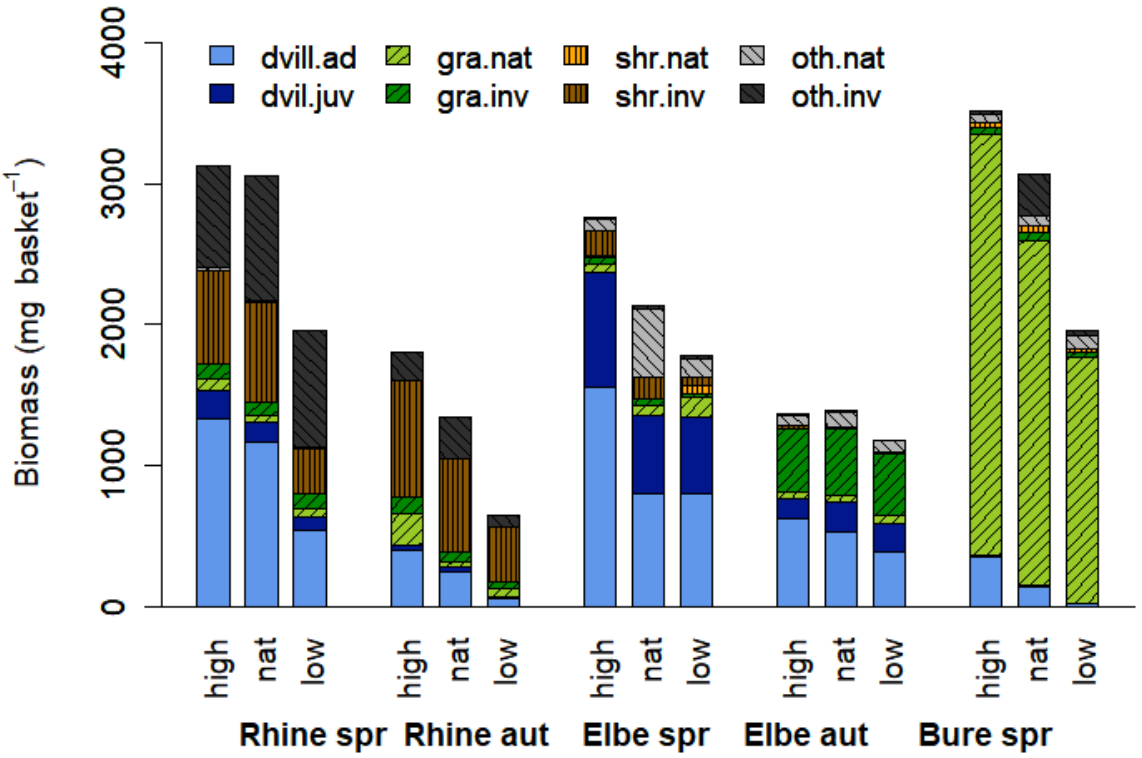

Figure 2 Biomass of benthic invertebrates, grouped by feeding type, in the mesocosm flumes with the three *D. villosus* density treatments (mean of 0- and 4-week sampling except Elbe, autumn: only 4-week sampling). Dvill = *D. villosus* (not included in any of the three feeding types but regarded separately), ad ≥ 8 mm, juv < 8 mm, gra =grazer, shr = shredder, oth = others. inv = invasive or non-native taxa. aut = autumn, spr = spring. For detailed community composition see Table S2 in the appendix.

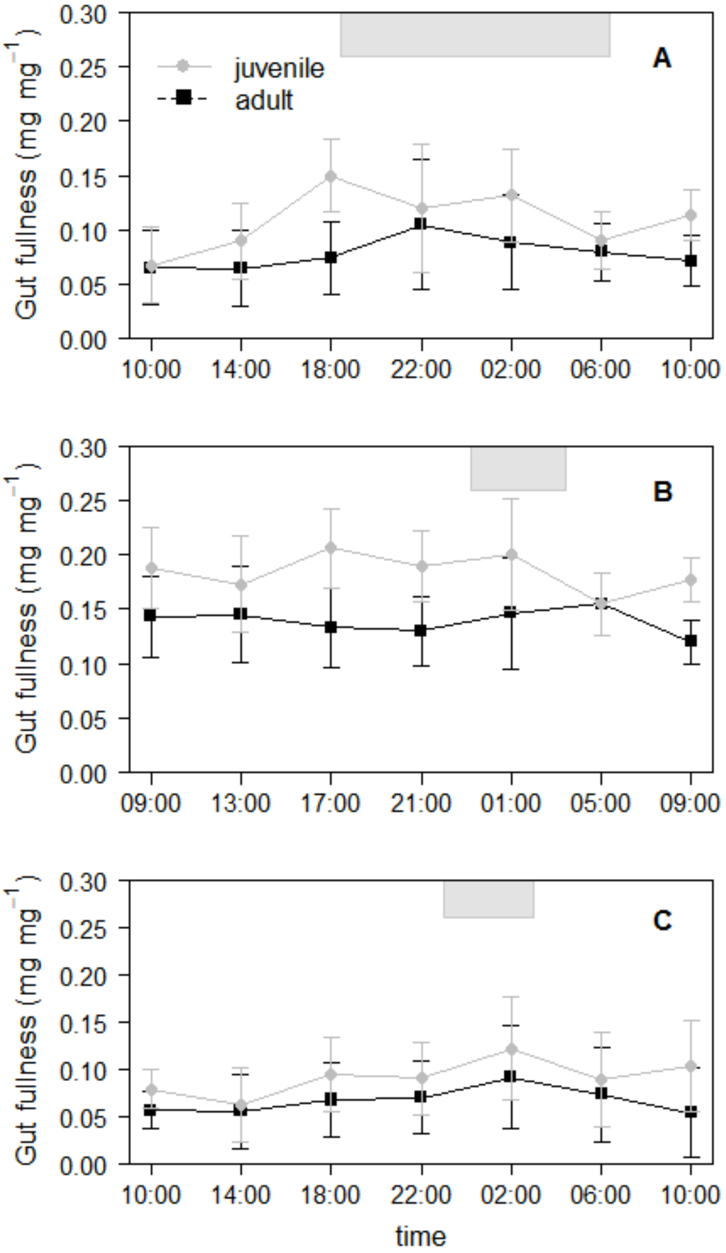

Figure 3 Gut fullness index of *D. villosus* in the mesocosms, measured in mg gut contents mg^{-1} empty body mass (adult, > 8 mm, black squares, and juveniles, < 8 mm, grey circles): (A) River Rhine, autumn 2013 at 11.8 ± 0.1°C water temperature, n = 5 – 27 per time point, (B) River Rhine, spring 2014 at 19.1 ± 0.8°C, n = 6 – 12 per time point, (C) River Elbe, spring 2015 at 18.6 ± 1.1°C, n = 7 – 35 per time point. All values in dry mass per dry body mass. Time corresponds to CET in (a) and to CEST in (b) and (c), grey areas mark the dark periods between sunset and sunrise.

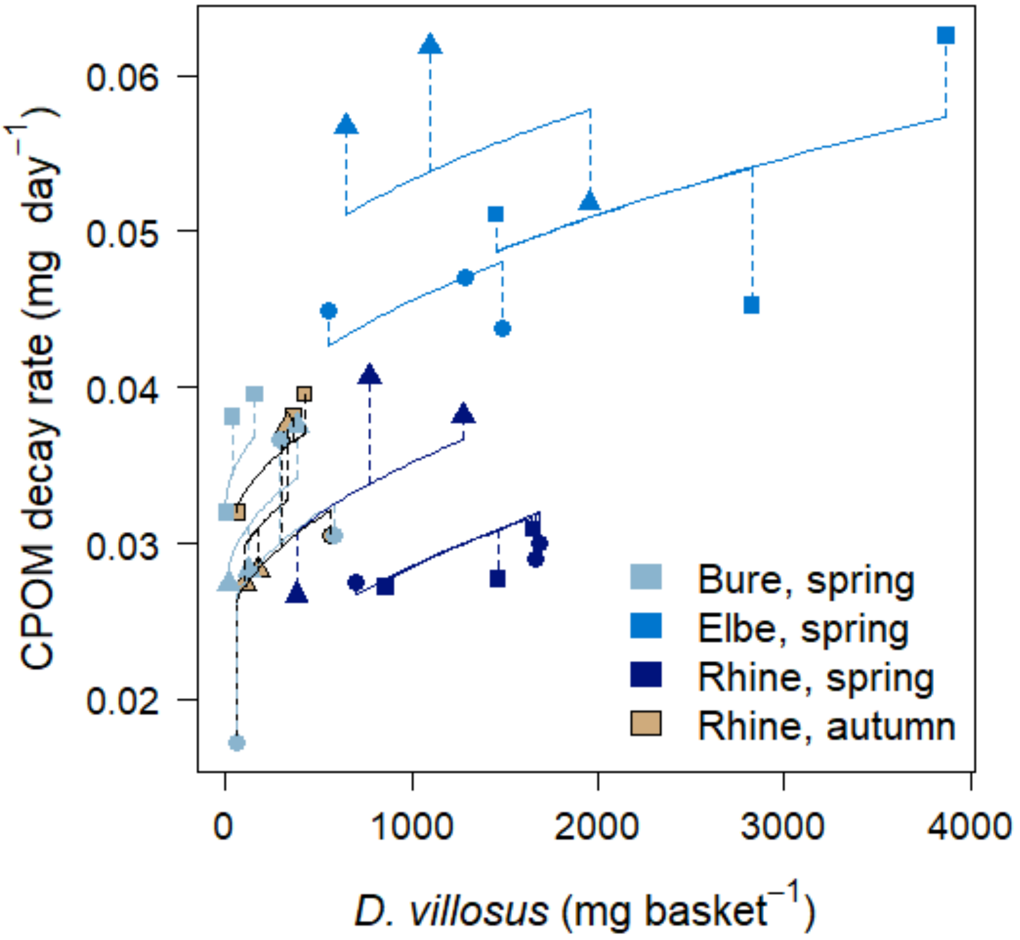

Figure 4 Leaf decomposition rate (mg day^{-1}) and *D. villosus* biomass (mg basket^{-1}; all size classes, dry mass, mean of start and 4-week sample of each mesocosm experiment). Colours indicate the mesocosm experiments and symbols (squares, triangles and circles) indicate the three pontoons used in each experiment, with three mesocosm flumes each. Regression lines: linear mixed-effects model with residuals (dashed). The regression lines are curved to account for the square-root transformation of biomass. For model specifications see text.

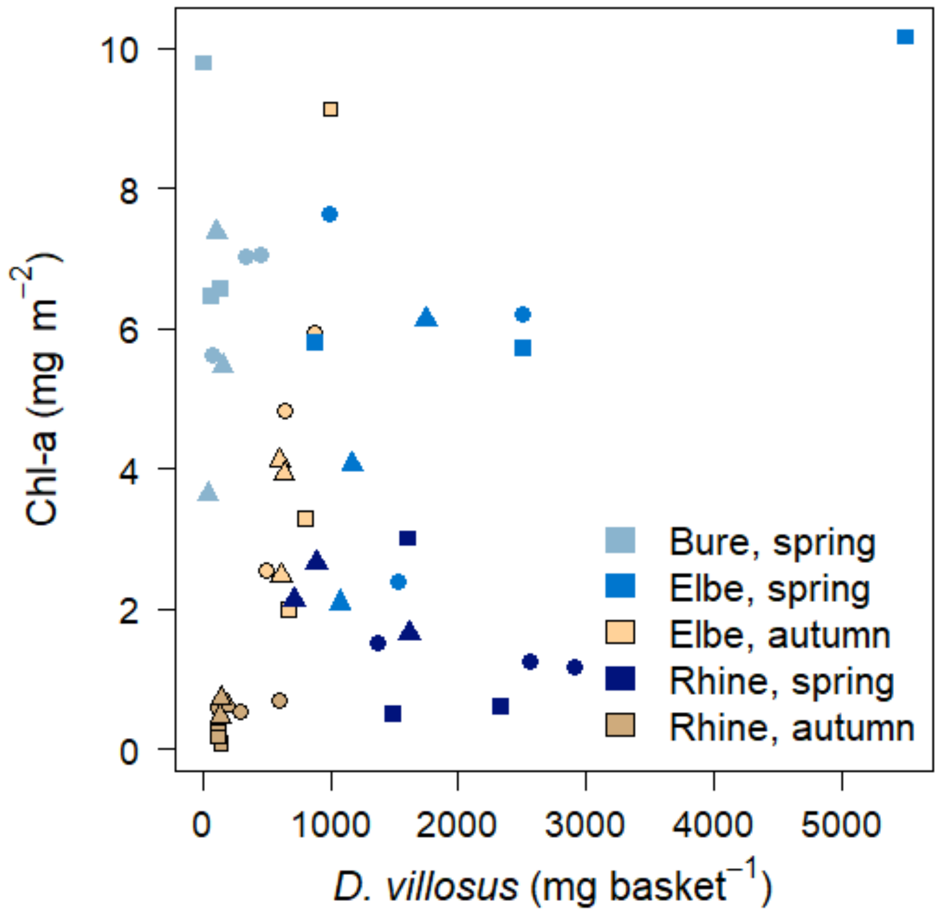

Figure 5 Autotrophic biofilm (chl-a) and *D. villosus* biomass (all size classes, dry mass) in the mesocosms, sampled 4 weeks after start. Colours indicate the mesocosm experiments and symbols (squares, triangles and circles) indicate the three pontoons used in each experiment, with three flumes each. Linear mixed-effects model showed no fixed effects of *D. villosus* biomass or season. For model specifications see text.