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# Current velocity shapes co-existence patterns among invasive *Dikerogammarus* species

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## SUMMARY

1. Facilitative interactions among co-evolved representatives of the endemic Ponto-Caspian fauna are regarded as a major factor of their invasion success. Nevertheless, the most renowned examples represent interactions between different trophic levels or functional groups, while ecologically similar species can be expected to show competition-based niche partitioning.
2. Here, we test for differences in the realised niche of three invasive *Dikerogammarus* species (Crustacea, Gammaridae) in their co-occurring range. We sampled multiple habitats within sites distributed along the River Danube to test whether some environmental variables could reveal spatial niche differentiation among the three species of *Dikerogammarus*, and if so, to test a predictive model outside the zone of co-occurrence.
3. Spatial niche differentiation was present among the species, primarily determined by current velocity (and associated substrate preference), likely reflecting a stress tolerance-competitive ability trade-off. Suspended matter concentration was also relevant, suggesting food resources (through filter feeding) might represent another important niche axis, somewhat loosening the terms of co-existence between *D. haemobaphes* and the other two species.
4. Environmental variables could effectively explain the absence of *D. bispinosus* in the Lower Danube, implying that the co-existence of the three species is possible only along a sufficiently wide current velocity gradient, and the observed turnovers are the result of niche expansion in the absence of the stronger competitor.
5. Hence, differences in invasion success may be attributed to a stress tolerance – competitive ability trade-off. Our results suggest the advantage of *D. villosus* is

attributable to its competitive dominance, allowing it to monopolize lentic and/or structured habitats, which represents a fortunate preadaptation to anthropogenic alterations of aquatic ecosystems. The presence of *D. villosus* does not considerably affect the expansion of *D. haemobaphes*; however, the exclusion of *D. bispinosus* from lentic habitats by *D. villosus* probably strongly limits its potential to spread by active dispersal.

Keywords: invasive species, killer shrimp, niche expansion, Ponto-Caspian, tolerance-competition trade-off

Running title

Co-existence patterns among *Dikerogammarus* species

## Introduction

As the number of invasive species is increasing in almost all ecosystems, interactions among them are becoming a pressing conservation issue (Jackson 2015; Kuebbing & Nuñez 2015). Two competing hypotheses predict that either invasion success decreases in time as invaders accumulate (‘biotic resistance’; Elton 1958), or previous colonists can promote the establishment of further species through facilitative interactions, resulting in an ‘invasional meltdown’ (Simberloff & Von Holle 1999). The endemic Ponto-Caspian species pool is one of the most important sources of aquatic invaders throughout the North-Atlantic region (Ricciardi & MacIsaac 2000; Bij de Vaate *et al.* 2002; Leppäkoski *et al.* 2002). Facilitative interactions among co-evolved representatives of the group are regarded as a major factor of their invasion success (Ricciardi 2001; Gallardo & Aldridge 2015). The most renowned examples are dreissenid mussels providing shelter and food for gammarids (commensalism), and gobies benefiting from the presence of both invertebrate groups through predation, presumably at a negligible cost for prey species (Ricciardi 2001; Gallardo & Aldridge 2015). Nevertheless, these examples represent interactions between different trophic levels or functional groups, whereas ecologically similar species can be expected to show competition-based niche partitioning (Chase & Leibold 2003), which might be accompanied by intraguild predation in certain cases (e.g., gobies and gammarids; Borza, Erős & Oertel 2009; Kinzler *et al.* 2009), adding further complexity to the issue (Polis & Holt 1992). Accordingly, studies on the well-known invasive *Dreissena* species (Dreissenidae) indicate that quagga mussel (*D. bugensis*) can competitively exclude zebra mussel (*D. polymorpha*) due to its higher assimilation efficiency, lower respiration rates, and larger body size (Baldwin *et al.* 2002; Stoeckmann 2003); however, their different physiological tolerances to environmental factors allow them to co-exist under certain conditions (Jones & Ricciardi 2005; Peyer, McCarthy & Lee 2009; Karatayev *et al.* 2011; Karatayev, Burlakova & Padilla 2015).

The three invasive *Dikerogammarus* species, *D. bispinosus*, *D. haemobaphes*, and *D. villosus* are also ecologically similar and closely related (Müller, Schramm & Seitz 2002). *Dikerogammarus villosus* and *D. haemobaphes* have invaded several rivers and canals in recent decades in Central and Western Europe including the British Isles using both the Danube-Rhine and the Dnieper-Vistula corridors (Bij de Vaate *et al.* 2002; Grabowski, Jażdżewski & Konopacka 2007; Labat, Piscart & Fontan 2011; Gallardo & Aldridge 2015; Rewicz *et al.* 2015). *D. villosus* is however more successful at colonizing lakes and has a more severe impact on native and other invasive species (reviewed by Rewicz *et al.* 2014). By contrast, *D. bispinosus* has expanded its range only along the Danube-Rhine corridor, and its non-native distribution has remained restricted to these two catchments so far (Labat *et al.* 2011). Although the three species frequently co-occur and are apparently able to co-exist in the middle part of the Danube, temporal turnovers have previously been observed at other sites (see historical context in Methods).

In recent years, considerable effort has been devoted to studying the ecology of *Dikerogammarus* species, especially the most successful *D. villosus*. The interactions among the species have received relatively little attention so far, leaving the terms of their co-existence unidentified. Kley & Maier (2005), investigating a single site in the Upper Danube, could not detect spatial niche segregation between *D. villosus* and *D. bispinosus* along the depth gradient. Kinzler *et al.* (2009) hypothesized that asymmetry in intraguild predation might account for the apparent superiority of *D. villosus* over its relatives. Their experiments did not reveal notable differences in this regard however, and they concluded that factors other than predation might be involved. Kobak, Rachalewski & Bącela-Spychalska (2016) provided experimental evidence of competition between *D. villosus* and *D. haemobaphes*, the latter showing increased dispersal in the presence of its relative. While this mechanism might be involved the observed temporal species turnovers, it cannot account for the stable co-existence of the species in certain river sections.

Co-existence of ecologically similar species can be achieved by several different mechanisms; nevertheless, in spatially heterogeneous environments the species can be expected to show spatial segregation (Chesson 2000; Amarasekare 2003; Chase & Leibold 2003). The multi-habitat samples collected from the River Danube during the latest international longitudinal survey (Joint Danube Survey 3, 2013) allowed us to examine the habitat utilization of the species along wide environmental gradients, which is necessary for detecting niche patterns (Chase 2014; Heino *et al.* 2015). Accordingly, we aimed (i) to test whether there is spatial niche differentiation among the three invasive *Dikerogammarus* species; (ii) to identify the most important niche axes; (iii) to test whether environmental variables can explain the absence of *D. bispinosus* in the lower part of the Danube where the other two species co-exist.

## Methods

### *Historical context*

In the Upper Danube, the decline of *D. haemobaphes* (present there since the 1970s; Tittizer *et al.* 2000) could be detected in the 1990-2000s concurrently with the spread of *D. villosus* (Weinzierl, Potel & Banning 1996; Kley & Maier 2006); nevertheless, *D. haemobaphes* is still present in this part of the river (Borza *et al.* 2015). In Lake Balaton, *D. haemobaphes* and *D. bispinosus* were accidentally introduced in 1950 (Ponyi 1956). Most subsequent publications mention *D. villosus* instead of *D. bispinosus* (previously regarded as a subspecies of *D. villosus*; Muskó 1992); however, it was not until the early 2000s that the presence of *D. villosus* (s. str.) was proved (Muskó & Leitold 2003). In 1997, *D. bispinosus* was still abundant and *D. villosus* was not found in the lake (Müller *et al.* 2002), whereas in 2003-2004, *D. villosus* was the dominant species in the nearshore zone, *D. haemobaphes* occurred in relatively low densities mostly in the deeper parts, and *D. bispinosus* was present only sporadically (Muskó *et al.* 2007; Muskó pers. comm.). Similar changes have taken place in recent decades in the lower section of the Danube (< ~1200 river km), as well. According to Cărăuşu, Dobreanu & Manolache (1955), *D. villosus* was present only in the delta of the river even in the middle of the 20th century (not considering the erroneous mention of the species in the Hungarian section; discussed in Borza *et al.* 2015); therefore, it colonized the Lower Danube probably only after the 1960-70s (Popescu-Marinescu *et al.* 2001). On the contrary, although formerly it was relatively common (Popescu-Marinescu *et al.* 2001), recent surveys indicate the almost complete disappearance of *D. bispinosus* in this section of the river (Borza *et al.* 2015).

### *Sample collection and processing*

The samples analyzed in the present study were taken during the 3rd Joint Danube Survey (13 August-26 September 2013) at 55 sites of the river (Fig. 1) between Ulm (river km 2581) and the Delta (km 18, Kiliya branch) by the 'multi-habitat' approach based on, but not strictly following the AQEM protocol (Hering *et al.* 2004). At each site, all available habitat types (four to seven per site) were sampled (altogether 251). Five pooled units covering 25 x 25 cm bottom area were collected for each habitat in the littoral zone by hand net (aperture: 25 x 25 cm, mesh size: 500 µm). All samples were preserved in 4% formaldehyde solution in the field, and stored in 70% ethanol after sorting. Sorting was facilitated by fractioning the material on a set of sieves (mesh sizes: 0.5, 2, 5, 10, 20 mm). In some cases, 2 to 64-fold subsampling of the smallest one or two fractions was necessary due to the extremely high number of juvenile animals in the samples. *Dikerogammarus* specimens (altogether 18 905 individuals) could be identified to species level above ~2 mm body length (15 156 ind.); unidentifiable juveniles were omitted.

### *Data analyses*

We tested for spatial niche differentiation by variance partitioning between environmental and spatial explanatory variables based on redundancy analysis (RDA), using the 'varpart' function in the 'vegan' package (Oksanen *et al.* 2016) in R 3.2.5 (R Core Team 2016) within the overlapping range of the three species (between river km 2258 and 1252, Fig.1) represented by 88 samples from 23 sites (19 of the 120 samples taken in this river section did not contain *Dikerogammarus* spp., and 13 additional samples were omitted due to missing data). We used log(x+1) and Hellinger-transformed (Legendre & Gallagher 2001) count data (individuals per sample) in the analysis, but we show ind./m$^2$ values in the results and in figures for the sake of comparability. We used substrate type (Table 1) and several physicochemical parameters (Table 2) as environmental explanatory variables. We modelled the spatial structure of the study using the asymmetric eigenvector map (AEM) method

(Blanchet, Legendre & Borcard 2008b; Blanchet *et al.* 2011) allowing the consideration of directional spatial processes, induced by the currents in our case. Since the locations of the samples within sites were not recorded, we used a one-dimensional representation of the study design including only sites, and replicated the values of the generated spatial variables (AEM eigenfunctions) for all samples within each site. We constructed several weighting vectors for between-site distances ($d_{ij}$) using the functions $f_1 = 1-d_{ij}/\max(d_{ij})^{\alpha}$ and $f_2 = 1/d_{ij}^{\alpha}$ with different values of α from 1 to 3. Corrected Akaike information criterion values (AICc; Dray, Legendre & Peres-Neto 2006) calculated by the 'ortho.AIC' function in the 'spacemakeR' package (Dray 2013) indicated weighting function $f_2$ with $\alpha = 1$ as optimal (AICc = -168.82) for generating the AEM eigenfunctions. Since the eigenfunctions with positive and negative Moran's *I* values (modelling positive and negative spatial autocorrelation, respectively) both explained a considerable share of the variation (positive: 30.6%, df = 9, F = 5.26, $P < 0.001$; negative: 10.7%, df = 13, F = 1.80, $P = 0.019$), we used both subsets in the analysis, which was possible due to the fact that we only had 22 (number of sites minus one) AEM eigenfunctions for 88 samples. We performed variance partitioning after forward selection (Blanchet, Legendre & Borcard 2008a) on the environmental as well as the spatial explanatory variable sets (with $P = 0.05$) using the 'ordiR2step' function in the 'vegan' package, and tested variance partitions of interest by ANOVA with 9999 permutations. We interpret the importance of environmental variables based on their biplot scores in the model including both environmental and spatial variables.

To provide an insight into the structure of spatial autocorrelation across multiple spatial scales, we constructed Mantel correlograms (Borcard & Legendre 2012) using the 'mantel.correlog' function in the 'vegan' package about (1) the response variables representing both environmentally explainable spatial autocorrelation ('induced spatial dependence') and environmentally not explainable ('true') spatial autocorrelation; Legendre

& Legendre 2012), (2) the residuals of the environmental model (representing true spatial autocorrelation and unexplained induced spatial dependence), and (3) the residuals of the environmental and spatial model (expected to be zero for all spatial scales, if the spatial structure is properly represented in the model). The first distance class in the correlograms represents within-site distances, whereas the subsequent classes were delimited according to the Sturges equation (13 classes with equal widths of 77.4 river km; the last seven are not shown). *P*-values of the Mantel correlation coefficients were calculated with Holm-correction. We mapped the only spatially explainable portion of variance by plotting the fitted site scores (= linear constraints; 'LC' scores) of the first canonical axis in the corresponding partial RDA (featuring AEM eigenfunctions as explanatory variables and environmental variables as covariables) against river km (after Legendre & Legendre 2012).

To test whether environmental factors can account for the broad-scale distributional patterns of *D. bispinosus* (i.e., its absence in the lower section of the river), we used the environmental RDA model to make predictions on the abundance of the species in the area of its absence (93 samples from 25 sites downstream of river km 1252; samples of the substrate type 'macrophytes' were excluded, since they were not represented in the range of co-occurrence). We classified the predicted values as presence or absence based on the predictions on the model input samples using maximum likelihood classification (i.e., the values were assigned to the group where their likelihood assuming Gaussian distribution was higher; we considered priors, i.e., the presence/absence ratio in the modelled data as not informative for the predictions). We repeated the classification using thresholds of 10, 100, and 450 ind./m$^2$ to account for biases arising from sporadic occurrences, which do not necessarily represent the environmental preferences of the species.

Presently available data on the phylogenetic relationships of *Dikerogammarus* spp. (Müller *et al.* 2002) do not provide a stable basis for the discussion of the phylogenetic/evolutionary context of our results (Michał Grabowski *et al.*, University of Łódź, unpublished data).

## Results

*Dikerogammarus villosus* proved to be the most common of the three species in the material; it occurred at every site (altogether 213 samples) with an average (±SD) density of 484±851 ind./m$^2$ (whenever present), and a maximum of 8346 ind./m$^2$. *D. haemobaphes* was present at 36 sites (84 samples) between river km 2415 and 18 (average density: 124±338 ind./m$^2$, max.: 2221 ind./m$^2$), whereas *D. bispinosus* was entirely missing in the lower section of the river, occurring only at 20 sites (54 samples) between river km 2258 and 1252 (average density: 170±376 ind./m$^2$, max.: 1866 ind./m$^2$).

The forward selection procedure on the environmental variables selected current velocity, suspended matter content, substrate type, pH, and depth, while nine spatial variables (four with positive and five with negative Moran's $I$) were retained. Since the minor effect pH (0.2% of the total variance explained alone) could not be interpreted biologically (it ranged only between 7.89 and 8.43), we regarded it as a statistical artefact. Similarly, depth had small explanatory power (0% of the total variance explained alone); its effect could be attributed mainly to its relatively strong correlation with current velocity (Spearman's rank correlation: 0.50); therefore, we excluded these two variables from further analyses.

In the variance partitioning (Fig. 2), the three remaining environmental variables explained 37.9% (df = 11, F = 5.82, $P < 0.001$) of the total variation, whereas spatial variables accounted for 41.4% (df = 9, F = 7.83, $P < 0.001$). There was a considerable overlap between the two groups (23.2%, not testable); 14.7% of the variance (df = 11, F = 3.37, $P < 0.001$) could be explained by environmental factors only, while 18.2% (df = 9, F = 4.50, $P < 0.001$)

was explained exclusively by spatial variables. The variables included in the analysis together accounted for 56.1% (df = 20, F = 6.55, $P < 0.001$) of the total variance.

The Mantel correlogram of the response variables (Fig. 3) indicated significant positive spatial autocorrelation at fine spatial scales (within sites and at 0-77.4 river km), which transitioned into significant negative correlation in the 154.8-232.2 and 232.2-309.5 river km distance classes, whereas in the largest distance classes autocorrelation was not significant. The resulting wave-like shape of the correlogram reflected periodicity in the response of the species along the investigated river section (Legendre & Legendre 2012). The residuals of the environmental model (Fig. 3) showed significant positive spatial autocorrelation within sites as well as in the 77.4-154.8 and 154.8-232.2 river km distance classes, reflecting a similar periodic structure in the only spatially explainable part of variance with a somewhat shorter wave length, which could be associated with dams and major riparian cities (Fig. 4). Significant spatial autocorrelation could not be detected in the residuals of the environmental and spatial model in either of the distance classes (Fig. 3), indicating that the inclusion of AEM eigenfunctions properly accounted for the spatial structure of the study.

As indicated by the RDA triplot (Fig. 5), the main gradient in the community data separated *D. villosus* from *D. bispinosus*, whereas *D. haemobaphes* took an intermediate position on the first ordination axis ($R^2 = 0.461$, df = 1, F = 114.4, $P < 0.001$), and separated from the other two species mainly on the second axis ($R^2 = 0.192$, df = 1, F = 47.7, $P < 0.001$). The third canonical axis was not significant ($R^2 = 0.009$, df = 1, F = 2.29, $P = 0.103$). Current velocity was strongly associated with the main gradient (biplot scores: RDA1: 0.597, RDA2: 0.262), indicating *D. bispinosus* as the most and *D. villosus* as the least rheotolerant among the three species (Appendix 1), whereas the separation of *D. haemobaphes* from the other two species could be explained mostly by suspended matter concentration (biplot scores: RDA1: -0.361, RDA2: 0.366; Appendix 1). *D. villosus* showed affinity to xylal, riprap, argyllal, psammopelal, and pelal, while natural stony substrates (akal, micro-, meso-, macrolithal) and psammal were approximately equally used by the species. Nevertheless, we note that the ordination plot shows only relative substrate usage patterns; in absolute terms all three species avoided fine substrates (Appendix 2).

The environmental RDA model predicted the presence of *D. bispinosus* for 25 samples out of 93 (26.9%) from the river section where it was absent (Fig. 6). Fourteen of these samples were micro- or mesolithal (current velocity: 0-0.22 m/s) and 11 were pelal/psammopelal/psammal (current velocity: 0.09-0.23 m/s). Densities above 10, 100, and 450 ind./$m^2$ were predicted for 19, 10, and 4 samples, respectively.

## Discussion

The density of all three invasive *Dikerogammarus* sp. varied by four orders of magnitude in the material, suggesting that the survey was successful at capturing long environmental gradients allowing conclusions to be made on the niche partitioning of the species. Indeed, environmental variables explained a large portion of the variance, reflecting spatial niche differentiation. The overlap between environmental and spatial variables was strong, indicating that environmental predictors showed definite spatial structure. The also notable part of variance explained only by spatial variables concentrated at two distinct spatial scales; within sites and around 200 river km. The broad-scale autocorrelation can be ascribed to unconsidered environmental or historical factors likely related to major anthropogenic disturbances (large cities and/or impoundments), as suggested by the mapping of this component of variation along the investigated river section (Fig. 4). The within-site autocorrelation also might be attributed to local environmental or historical factors not included in the study; however, it might also reflect mass effect (Leibold *et al.* 2004); i.e., a certain degree of homogenization among the samples within sites through the dispersal of individuals.

Three environmental variables; current velocity, substrate type, and suspended matter concentration proved to be important in explaining the niche differentiation of the invasive *Dikerogammarus* species. Since current velocity showed the strongest correlation with the first ordination axis, the effect of this variable appears to be the most prominent. Previous observations on the flow preferences of the species are scarce, especially for *D. haemobaphes* and *D. bispinosus*; nevertheless, the primary role of current velocity is in concert with the longitudinal distribution of the species in the River Dniester, where the abundance of *D. bispinosus* decreased downstream*, D. haemobaphes* was the most widespread, and *D. villosus* was found only in the lagoon, where *D. bispinosus* was missing (Jażdżewski & Konopacka 1988). Field studies involving *D. villosus* reflect strong context-dependency, their conclusions including preference for lentic conditions (Boets *et al.* 2010), relative indifference (Devin *et al.* 2003), and affinity to flow-exposed, but strongly structured habitats (MacNeil & Platvoet 2013); whereas the only flume experiment involving the species demonstrated that it avoids strong currents by using flow-refuges (Felten, Dolédec & Statzner 2008). Our results confirm that the species is primarily rheophobic, but it can utilize lentic microhabitats offered by obstacles in the water (i.e., stones, wood, and macrophytes) even in relatively fast-flowing sections of rivers.

Flow conditions are recognized as a primary factor of community organization in rivers and streams, affecting organisms primarily through drag forces and food provision (Biggs, Nikora & Snelder 2005). Since both of the more rheotolerant species (*D. bispinosus* and *D. haemobaphes*) are able to persist in still water (e.g., in Lake Balaton), we assume that the disturbance effect of currents is more pronounced in this case, implying a stress tolerance vs. competitive ability trade-off among the species (Grime 1977; Chase & Leibold 2003; Kneitel

& Chase 2004). Although the importance of this mechanism has been tested mostly on terrestrial plants (e.g., Liancourt, Callaway & Michalet 2005; Peterson, Rice & Sexton 2013), it has been demonstrated that hydrodynamic stress can generate such co-existence patterns; e.g., among sessile mussels, where a stronger byssal thread attachment comes at a cost of growth rate and reproductive output (Zardi *et al.* 2006). Remarkably, this mechanism is involved in the niche partitioning of the two invasive *Dreissena* species, as well (Peyer *et al.* 2009). Regarding the invasive *Dikerogammarus* species, the components of the trade-off are yet to be determined; nevertheless, the high abundance of *D. villosus* in drift samples (Van Riel, Van der Velde & Bij de Vaate 2011) might be an indication that currents represent an important mortality factor for the species, whereas the competitive dominance of *D. villosus* might involve behavioural aspects (Kobak *et al.* 2016) as well as differences resource utilization, as suggested by its higher functional response compared to *D. haemobaphes* (Bovy *et al.* 2015).

The role of substrate preference in relation to the niche differentiation of *Dikerogammarus* species is less univocal. There was a considerable overlap among the species, since they all avoided soft substrates and reached high densities on gravel of various particle size; however, *D. villosus* was also abundant on riprap, macrophytes, and wood, in accordance with previous observations (e.g., Devin *et al.* 2003; Boets *et al.* 2010). Considering that the substrate types *D. villosus* preferred and the other two species avoided are the ones which offer the most protection against currents (Davis & Barmuta 1989; Green 2005), this pattern might also be explained by the ability of *D. villosus* to monopolize lentic microhabitats, implying that the role of substrate preference cannot be separated from the effect of current velocity within the confines of our study.

Since all three species are omnivorous and capable of filter feeding (Ponyi 1956; Platvoet *et al.* 2006), the role of suspended matter might be related to their feeding preferences;

nevertheless, an indirect connection (e.g., through other filter feeding organisms) is also possible. Although the feeding ecology of *D. villosus* has been studied extensively in recent years (reviewed by Rewicz *et al.* 2014), comparative studies among the invasive *Dikerogammarus* species are relatively rare and far from providing a comprehensive picture (Ponyi 1956; Kinzler *et al.* 2009; Bacela-Spychalska & Van der Velde 2013; Bovy *et al.* 2015). We assume that food might represent another important niche axis further decreasing interspecific competition among the three species. However, to prove and fully understand its role, further studies taking all relevant environmental variables into account would be necessary.

The environmental RDA model predicted the presence of *D. bispinosus* in around a quarter of the samples from the area of its absence; nevertheless, the majority of habitats were predicted to support low densities of the species. Many of the samples classified as suitable (even two of the best four) represented soft substrates (with relatively strong currents), which were avoided by the species within its present range. In addition, the substrates characterized as micro- or mesolithal in the Lower Danube consisted mainly of mollusc shells (not gravel, as in the preferred habitats within the upper and middle river sections), providing more protection from currents than gravel (most of these samples were dominated by *D. haemobaphes*). Even if there are suitable habitat patches for the species in this section of the river, they might be too small and isolated to support viable populations. In summary, we conclude that environmental variables, namely the general lack of current-exposed gravel deposits can effectively explain the absence of *D. bispinosus* in the Lower Danube. Accordingly, its former abundance in this river section as well as in Lake Balaton can be regarded as niche expansion, reflecting its ability to capitalize on the absence of *D. villosus* by using lentic habitats. However, the competitively superior *D. villosus* may dislodge *D.*

*bispinosus*, resulting in increased fish predation (de Gelder *et al.* 2016); which implies that the co-existence of the species is possible only along a sufficiently wide current velocity gradient.

The same mechanism could be responsible for the decline of *D. haemobaphes* in the Upper Danube and in Lake Balaton after the appearance of *D. villosus*, although the conflict between these two species appears to be less pronounced. The fact that it did not completely disappear indicates that – although in a subordinate position – it can coexist with the stronger competitor both in lacustrine and fluvial environments. Similarly, although minor niche expansion may reasonably be expected, there is no indication of a strong negative interaction between *D. haemobaphes* and *D. bispinosus*. Since *D. haemobaphes* occupies the middle of the current velocity gradient, it faces a direct interference with both species. Therefore, selection might have led to differentiation (i.e., character displacement; Schluter 2000) on another niche axis, possibly food resources, allowing it to mitigate interspecific competition. *Dikerogammarus villosus* and *D. bispinosus* interfere less under normal circumstances due to their more effective differentiation along the current velocity gradient; therefore, their overlap on this supposed second niche axis might be higher, resulting in a more severe competition if lentic habitats are first occupied by *D. bispinosus*.

In conclusion, our study confirmed that similarly to *Dreissena* species, the interaction among the invasive *Dikerogammarus* species can be characterised by competitive niche partitioning, implying the species do not facilitate each other's establishment, although *D. villosus* might facilitate the spread of *D. haemobaphes*, as suggested by Kobak *et al.* (2016). Nevertheless, these results do not necessarily contradict the invasional meltdown hypothesis, if we assume that the aforementioned facilitative interactions act as a stronger community assembly factor, since they operate among different trophic levels or functional groups, whereas negative interactions only determine which functionally more-or-less redundant species represent the guilds. We have identified current velocity as the most decisive factor in determining the co-existence patters of *Dikerogammarus* species, which might lead to a better understanding of their invasion characteristics, and could be used for refining predictions of their potential distributions (Gallardo & Aldridge 2013). Our results suggest that the apparently different invasion success of the species corresponds to their position on the stress tolerance-competitive ability axis, implying that the advantage of *D. villosus* is attributable to its competitive dominance, allowing it to monopolize lentic and/or structured habitats, which represents a fortunate preadaptation to anthropogenic alterations of waters; i.e., ripraps, impoundments, and fish passes (MacNeil & Platvoet 2013). The presence of *D. villosus* does not considerably impede the expansion of *D. haemobaphes*; however, it can be expected to reach high densities only in rivers and only if the food supply is favourable. On the contrary, the exclusion of *D. bispinosus* from lentic habitats by *D. villosus* probably strongly limits its potential to spread by active dispersal; therefore, it can be expected to continue its expansion only if it can reach other gravel-bed rivers by jump dispersal.

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Captions

**Fig. 1** Sampling sites along the River Danube. White squares: *D. villosus*, grey circles: *D. haemobaphes*, black triangles: *D. bispinosus*. The dashed ellipse encompasses the river section where the three species co-occurred during the survey (river km 2258-1252); the dark shaded area corresponds to the River Danube basin. Codes of the riparian countries: DE: Germany, AT: Austria, SK: Slovakia, HU: Hungary, HR: Croatia, RS: Serbia, RO: Romania, BG: Bulgaria, MD: Moldova, UA: Ukraine.

**Fig. 2** The partitions of variance explained by the two sets of explanatory variables (Env.: environmental variables, AEM: spatial variables). The size of the circles is not proportional to the explained variance.

**Fig. 3** Mantel correlograms of the response variables (squares/solid line), the residuals of the environmental model (circles/dashed line), and the residuals of the environmental and spatial model (triangles/dotted line). The distance class at 0 river km corresponds to within-site distances. Solid symbols indicate significant correlations (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). Numbers on the top of the graph indicate the number of pairs involved in the calculation of correlations for each distance class. Symbols are connected only to visualize the trends.

**Fig. 4** The distribution of the only spatially explainable partition of variance along the investigated river section (fitted site scores of the first canonical axis in the partial RDA featuring AEM eigenfunctions as explanatory variables and environmental variables as covariables). Solid line: smoothed spline with a smoothing parameter of 0.5, upward arrows: major riparian cities, downward arrows: sampling sites in impounded river sections.

**Fig. 5** Triplot showing the results of the RDA including both environmental and spatial explanatory variables (WA scores, species scaling). Empty circles represent samples. Dvill:

*D. villosus*, Dhae: *D. haemobaphes*, Dbis: *D. bispinosus*. Substrate types: AKA: akal, ARG: argyllal, MAL: macrolithal, MEL: mesolithal, MIL: microlithal, PEL: pelal, PPE: psammopelal, PSA: psammal, RIP: rip-rap, XYL: xylal. Thick arrows: continuous environmental variables (cur: current velocity, sus: suspended matter), thin arrows: AEM eigenfunctions (spatial variables).

**Fig. 6** Kernel density of predicted values of the environmental RDA model for *D. bispinosus*. Grey area: samples in the area of its absence (downstream of rkm 1252), solid line: absences in its range of occurrence (between river km 2258 and 1252), dashed line: presences in its range of occurrence. The increasingly dark sections indicate samples classified as > 0, > 10, > 100, and > 450 ind./m$^2$, respectively.

**Table 1** Definitions of substrate types used in the study.

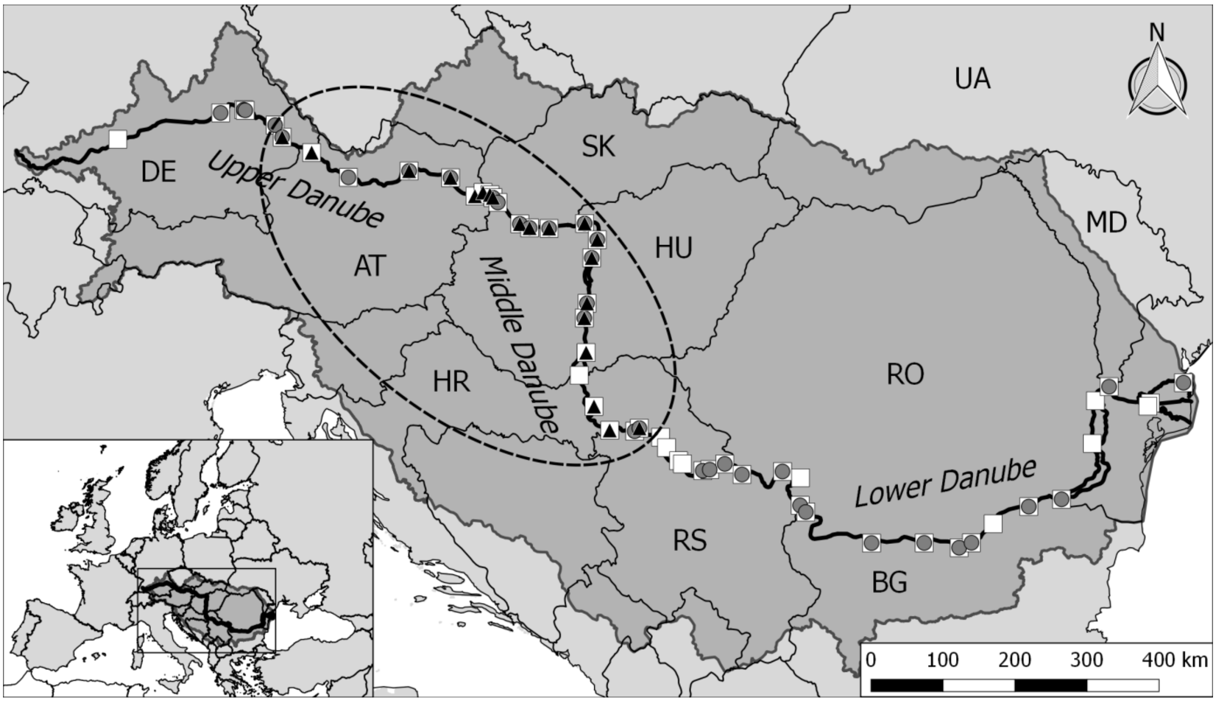
**Table 2** Physicochemical parameters used as environmental explanatory variables in the study. The parameters were measured A: for all samples (averaged over the five sampling units), B: at two points per site near the river banks, or C: at one point per site in the middle of the channel.

**Appendix 1** Log(x+1) transformed density of *Dikerogammarus* spp. as a function of current velocity and suspended matter content within their respective range of occurrence during the survey.

**Appendix 2** Log(x+1) transformed density of *Dikerogammarus* spp. on different substrates within their respective range of occurrence during the survey. Horizontal line: median, box: interquartile range, whiskers: 1.5 times the interquartile range distance from the upper or lower quartile, or the minimum/maximum, if that falls out of the range. Abbreviations as in Fig. 3, plus MPH: macrophytes. Numbers on the top of the graphs indicate the number of samples/sites of the given substrate types in the range of occurrence of the species.

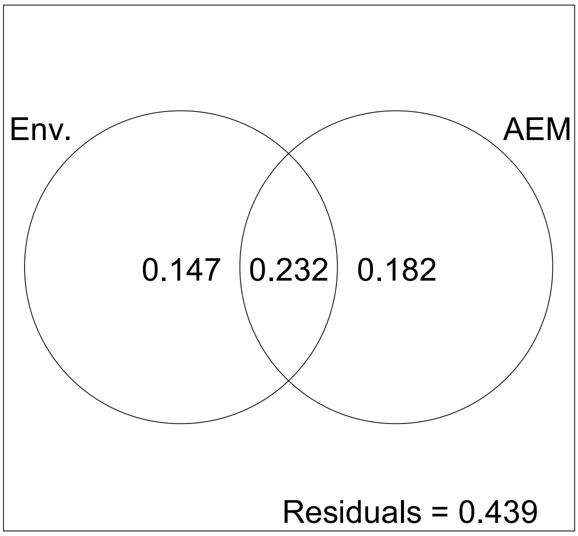
651 Figures

652 Fig. 1



653

654 Fig. 2



655

656 Fig. 3

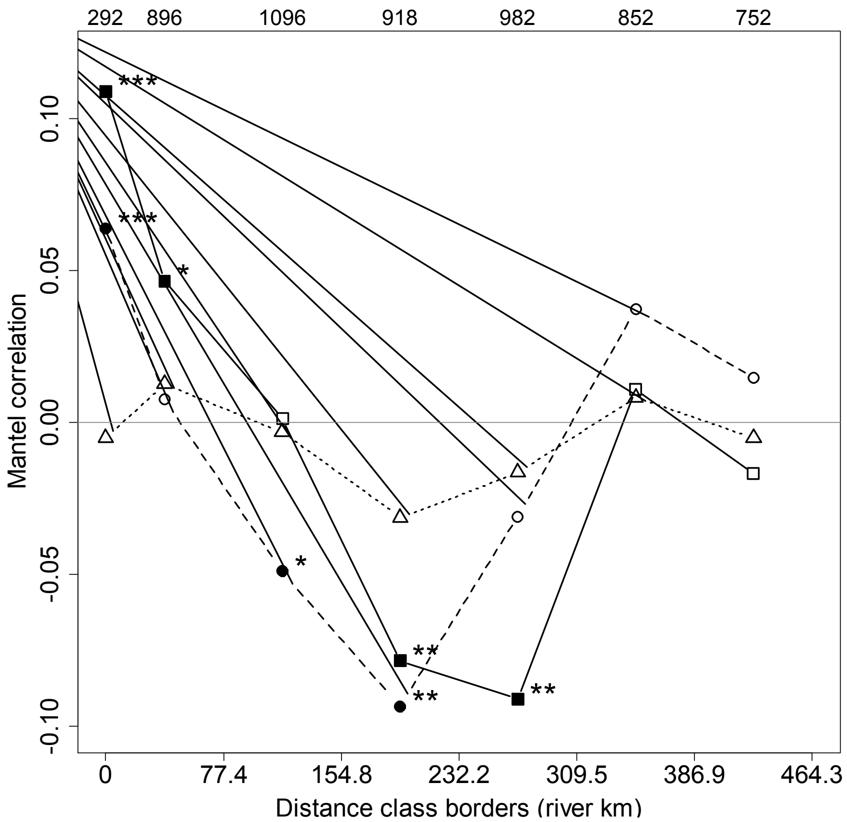

292
896
1096
918
982
852
752
Mantel correlation
0.10
0.05
0.00
-0.05
-0.10
***
*
***
*
**
**
**
0
77.4
154.8
232.2
309.5
386.9
464.3
Distance class borders (river km)

Fig. 4

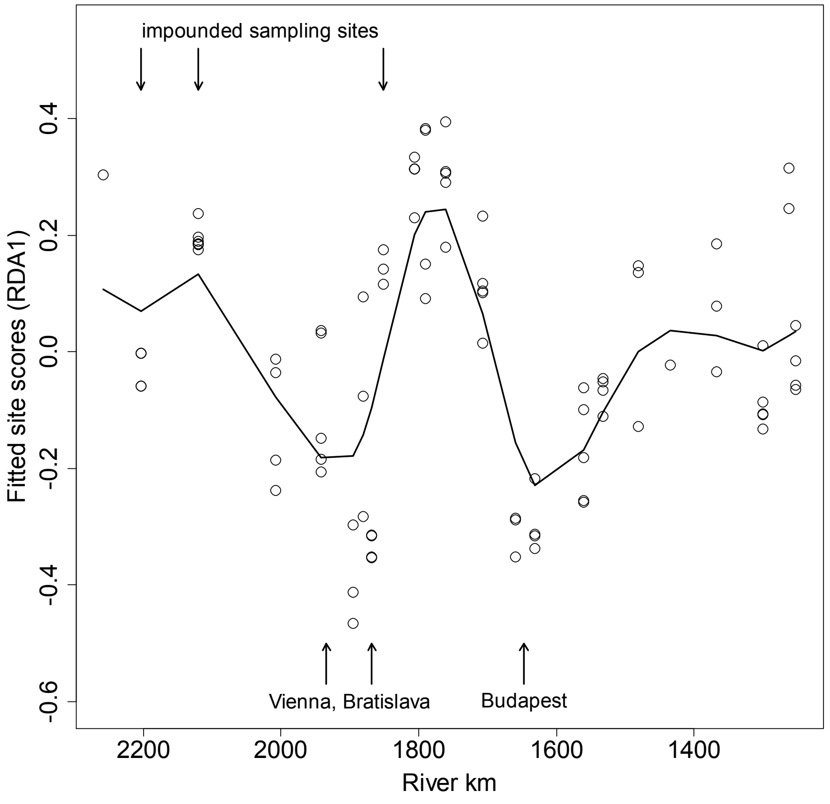
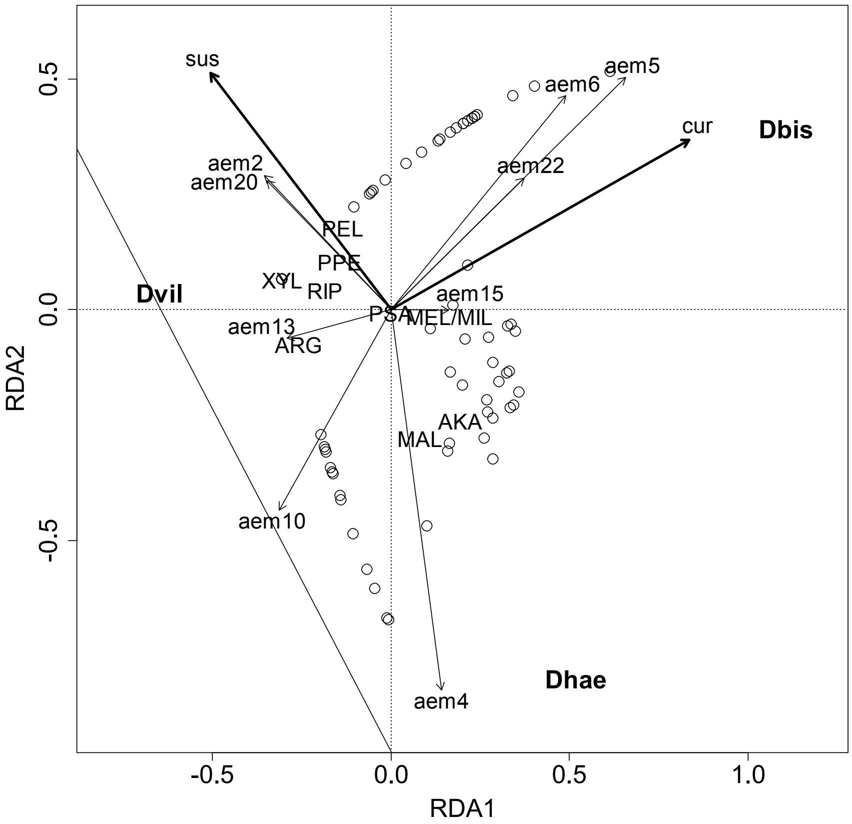
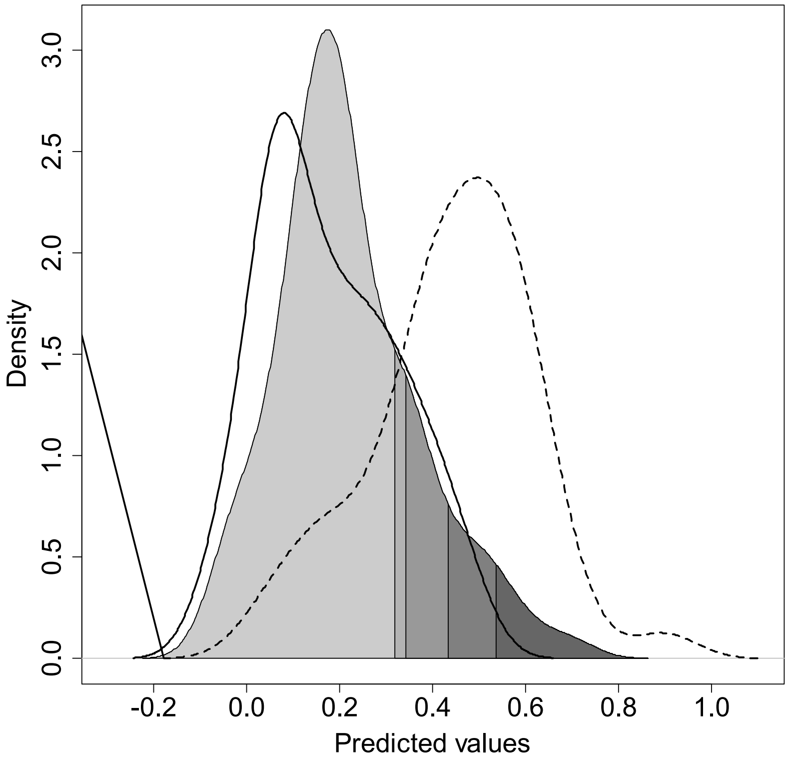

impounded sampling sites
Fitted site scores (RDA1)
0.4
0.2
0.0
-0.2
-0.4
-0.6
Vienna, Bratislava
Budapest
2200
2000
1800
1600
1400
River km

Fig. 5



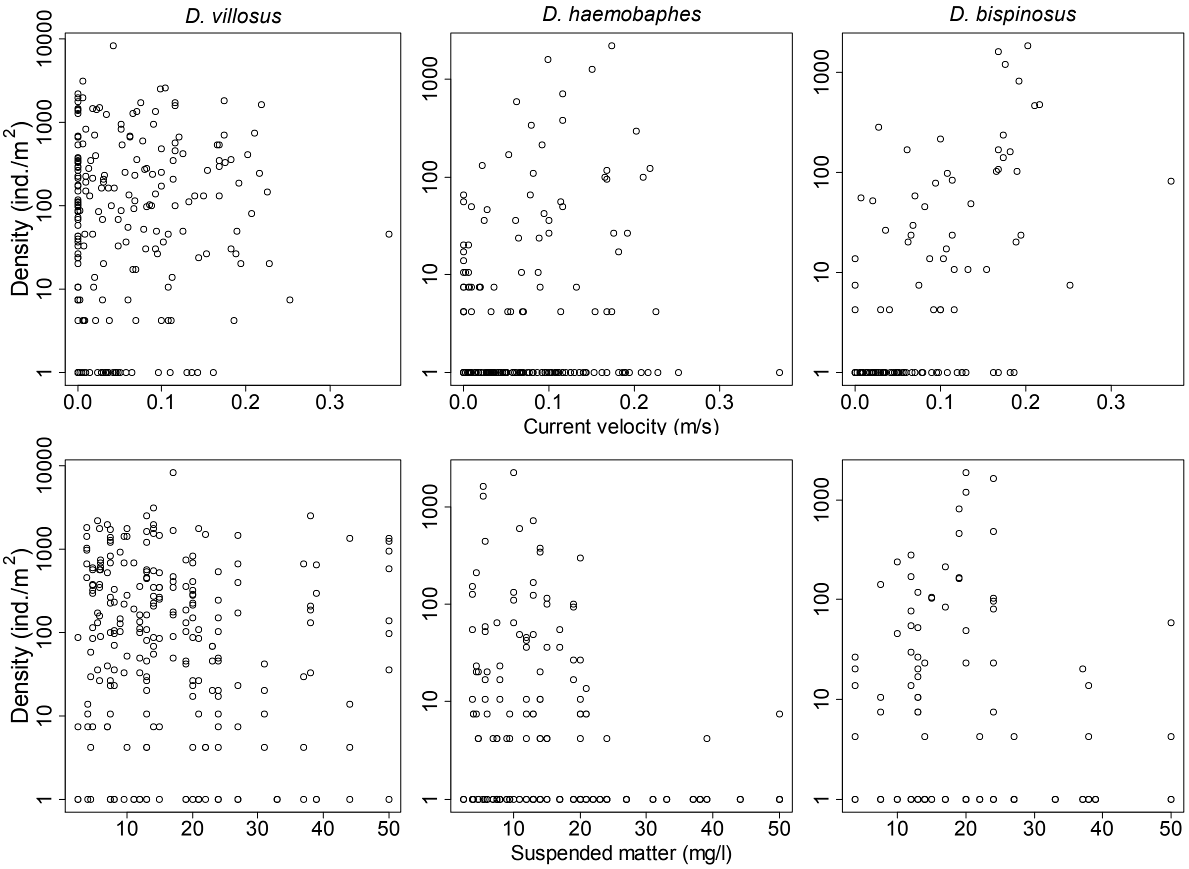
sus
aem5
aem6
cur
Dbis
aem2
aem20
aem22
PEL
PPE
XYL
RIP
Dvil
aem15
PSA
MEL/MIL
aem13
ARG
AKA
MAL
aem10
Dhae
aem4
RDA2
RDA1
0.5
0.0
-0.5
1.0

Fig. 6

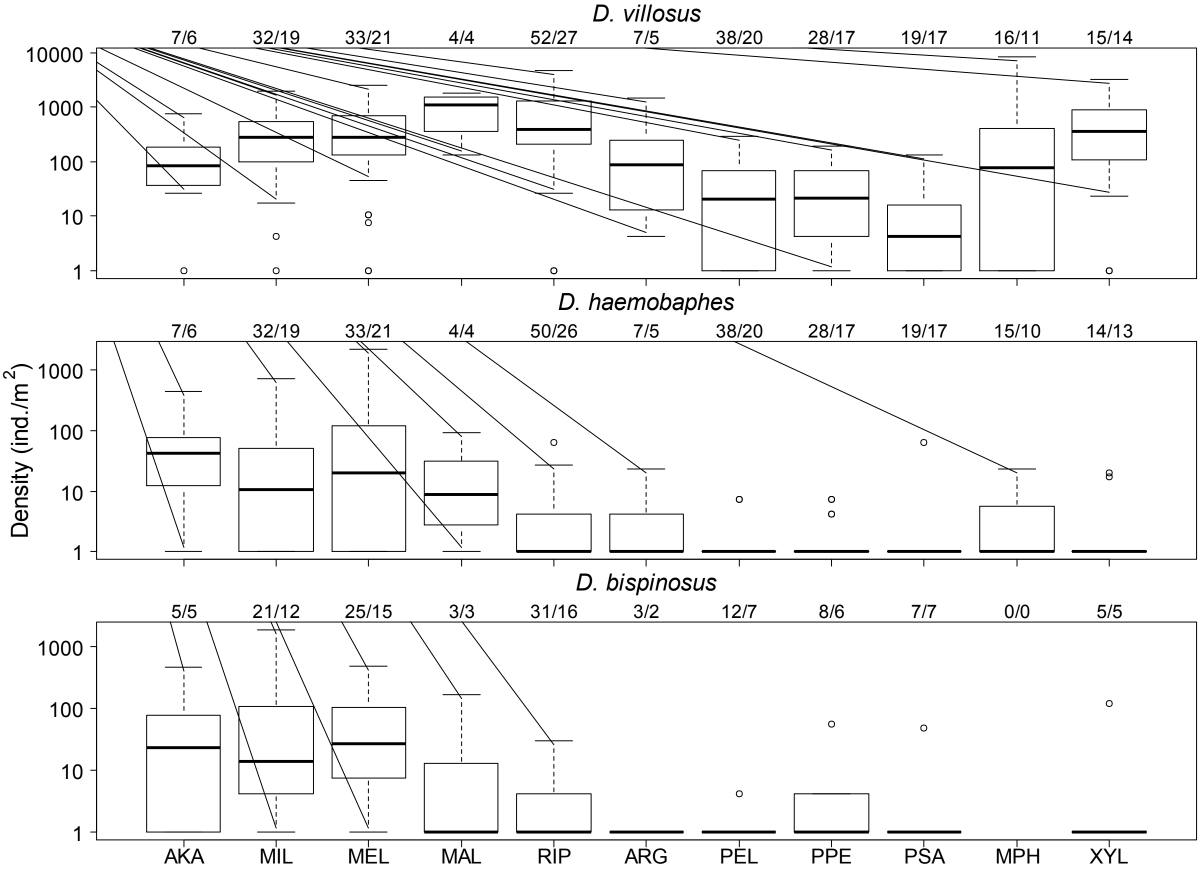


Density
Predicted values
0.0
0.5
1.0
1.5
2.0
2.5
3.0
-0.2
0.2
0.4
0.6
0.8

Appendix 1



Appendix 2



Tables

Table 1

| Substrate type | Definition |
|---|---|
| riprap | rocks of variable size, artificial |
| macrolithal | blocks, large cobbles; grain size 20 cm to 40 cm |
| mesolithal | cobbles; grain size 6 cm to 20 cm |
| microlithal | coarse gravel; grain size 2 cm to 6 cm |
| akal | fine to medium-sized gravel; grain size 0.2 cm to 2 cm |
| psammal | sand; grain size 0.063-2 mm |
| psammopelal | sand and mud |
| pelal | mud (organic); grain size < 0.063 mm |

| | |
|---|---|
| argyllal | silt, loam, clay (inorganic); grain size < 0.063 mm |
| macrophytes | submerged macrophytes, including moss and Characeae |
| xylal | tree trunks, dead wood, branches, roots |

Table 2

| Parameter | Method [standard] | Measurement | Range |
|---|---|---|---|
| Current velocity | Marsh-McBirney Flo-Mate™ Model 2000 portable electromagnetic flow meter approx. 5 cm above the bottom | A | 0-0.37 m/s |
| Depth | measuring stick | A | 0.1-1.2 m |
| Chlorophyll-a concentration | spectrophotometry [DIN 38412] | B | 0.10-18.77 µg/L |
| Conductivity | YSI EXO2 portable multiparameter sonde from motor-boat | B | 9.29-566.00 µS/cm |
| Dissolved $O_2$ concentration | YSI EXO2 portable multiparameter sonde from motor-boat | B | 5.89-10.42 mg/L |
| pH | YSI EXO2 portable multiparameter sonde from motor-boat | B | 7.89-8.43 |
| Dissolved organic carbon concentration | combustion catalytic oxidation/NDIR [EN 1484:2002] | B | 1.59-7.63 mg/L |
| Total nitrogen concentration | spectrophotometry [EN ISO 11905] | B | 0.52-3.37 mg/L |
| Total phosphorus concentration | spectrophotometry [EN ISO 6878] | B | 0.02-0.11 mg/L |
| Suspended matter concentration | gravimetry [EN 872] | C | 2.5-50.0 mg/L |