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9 Current velocity shapes co-existence patterns among invasive

10 *Dikerogammarus* species

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24

25 **SUMMARY**

26

27 1. Facilitative interactions among co-evolved representatives of the endemic Ponto-  
28 Caspian fauna are regarded as a major factor of their invasion success. Nevertheless,  
29 the most renowned examples represent interactions between different trophic levels or  
30 functional groups, while ecologically similar species can be expected to show  
31 competition-based niche partitioning.

32 2. Here, we test for differences in the realised niche of three invasive *Dikerogammarus*  
33 species (Crustacea, Gammaridae) in their co-occurring range. We sampled multiple  
34 habitats within sites distributed along the River Danube to test whether some  
35 environmental variables could reveal spatial niche differentiation among the three  
36 species of *Dikerogammarus*, and if so, to test a predictive model outside the zone of  
37 co-occurrence.

38 3. Spatial niche differentiation was present among the species, primarily determined by  
39 current velocity (and associated substrate preference), likely reflecting a stress  
40 tolerance-competitive ability trade-off. Suspended matter concentration was also  
41 relevant, suggesting food resources (through filter feeding) might represent another  
42 important niche axis, somewhat loosening the terms of co-existence between *D.*  
43 *haemobaphes* and the other two species.

44 4. Environmental variables could effectively explain the absence of *D. bispinosus* in the  
45 Lower Danube, implying that the co-existence of the three species is possible only  
46 along a sufficiently wide current velocity gradient, and the observed turnovers are the  
47 result of niche expansion in the absence of the stronger competitor.

48 5. Hence, differences in invasion success may be attributed to a stress tolerance –  
49 competitive ability trade-off. Our results suggest the advantage of *D. villosus* is

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

56

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

59

60   Running title

61   Co-existence patterns among *Dikerogammarus* species

62

### 63   **Introduction**

64

65   As the number of invasive species is increasing in almost all ecosystems, interactions among  
66   them are becoming a pressing conservation issue (Jackson 2015; Kuebbing & Nuñez 2015).

67   Two competing hypotheses predict that either invasion success decreases in time as invaders  
68   accumulate ('biotic resistance'; Elton 1958), or previous colonists can promote the

69   establishment of further species through facilitative interactions, resulting in an 'invasional

70   meltdown' (Simberloff & Von Holle 1999). The endemic Ponto-Caspian species pool is one

71   of the most important sources of aquatic invaders throughout the North-Atlantic region

72   (Ricciardi & MacIsaac 2000; Bij de Vaate *et al.* 2002; Leppäkoski *et al.* 2002). Facilitative

73   interactions among co-evolved representatives of the group are regarded as a major factor of

74   their invasion success (Ricciardi 2001; Gallardo & Aldridge 2015). The most renowned

75 examples are dreissenid mussels providing shelter and food for gammarids (commensalism),  
76 and gobies benefiting from the presence of both invertebrate groups through predation,  
77 presumably at a negligible cost for prey species (Ricciardi 2001; Gallardo & Aldridge 2015).  
78 Nevertheless, these examples represent interactions between different trophic levels or  
79 functional groups, whereas ecologically similar species can be expected to show competition-  
80 based niche partitioning (Chase & Leibold 2003), which might be accompanied by intraguild  
81 predation in certain cases (e.g., gobies and gammarids; Borza, Erős & Oertel 2009; Kinzler *et*  
82 *al.* 2009), adding further complexity to the issue (Polis & Holt 1992). Accordingly, studies on  
83 the well-known invasive *Dreissena* species (Dreissenidae) indicate that quagga mussel (*D.*  
84 *bugensis*) can competitively exclude zebra mussel (*D. polymorpha*) due to its higher  
85 assimilation efficiency, lower respiration rates, and larger body size (Baldwin *et al.* 2002;  
86 Stoeckmann 2003); however, their different physiological tolerances to environmental factors  
87 allow them to co-exist under certain conditions (Jones & Ricciardi 2005; Peyer, McCarthy &  
88 Lee 2009; Karatayev *et al.* 2011; Karatayev, Burlakova & Padilla 2015).

89 The three invasive *Dikerogammarus* species, *D. bispinosus*, *D. haemobaphes*, and *D. villosus*  
90 are also ecologically similar and closely related (Müller, Schramm & Seitz 2002).  
91 *Dikerogammarus villosus* and *D. haemobaphes* have invaded several rivers and canals in  
92 recent decades in Central and Western Europe including the British Isles using both the  
93 Danube-Rhine and the Dnieper-Vistula corridors (Bij de Vaate *et al.* 2002; Grabowski,  
94 Jazdzewski & Konopacka 2007; Labat, Piscart & Fontan 2011; Gallardo & Aldridge 2015;  
95 Rewicz *et al.* 2015). *D. villosus* is however more successful at colonizing lakes and has a  
96 more severe impact on native and other invasive species (reviewed by Rewicz *et al.* 2014). By  
97 contrast, *D. bispinosus* has expanded its range only along the Danube-Rhine corridor, and its  
98 non-native distribution has remained restricted to these two catchments so far (Labat *et al.*  
99 2011). Although the three species frequently co-occur and are apparently able to co-exist in

100 the middle part of the Danube, temporal turnovers have previously been observed at other  
101 sites (see historical context in Methods).

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

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

125

## 126 **Methods**

### 127 *Historical context*

128 In the Upper Danube, the decline of *D. haemobaphes* (present there since the 1970s; Tittizer  
129 *et al.* 2000) could be detected in the 1990-2000s concurrently with the spread of *D. villosus*  
130 (Weinzierl, Potel & Banning 1996; Kley & Maier 2006); nevertheless, *D. haemobaphes* is  
131 still present in this part of the river (Borza *et al.* 2015). In Lake Balaton, *D. haemobaphes* and  
132 *D. bispinosus* were accidentally introduced in 1950 (Ponyi 1956). Most subsequent  
133 publications mention *D. villosus* instead of *D. bispinosus* (previously regarded as a subspecies  
134 of *D. villosus*; Muskó 1992); however, it was not until the early 2000s that the presence of *D.*  
135 *villosus* (s. str.) was proved (Muskó & Leitold 2003). In 1997, *D. bispinosus* was still  
136 abundant and *D. villosus* was not found in the lake (Müller *et al.* 2002), whereas in 2003-  
137 2004, *D. villosus* was the dominant species in the nearshore zone, *D. haemobaphes* occurred  
138 in relatively low densities mostly in the deeper parts, and *D. bispinosus* was present only  
139 sporadically (Muskó *et al.* 2007; Muskó pers. comm.). Similar changes have taken place in  
140 recent decades in the lower section of the Danube (< ~1200 river km), as well. According to  
141 Cărauşu, Dobreanu & Manolache (1955), *D. villosus* was present only in the delta of the river  
142 even in the middle of the 20<sup>th</sup> century (not considering the erroneous mention of the species in  
143 the Hungarian section; discussed in Borza *et al.* 2015); therefore, it colonized the Lower  
144 Danube probably only after the 1960-70s (Popescu-Marinescu *et al.* 2001). On the contrary,  
145 although formerly it was relatively common (Popescu-Marinescu *et al.* 2001), recent surveys  
146 indicate the almost complete disappearance of *D. bispinosus* in this section of the river (Borza  
147 *et al.* 2015).

### 148 *Sample collection and processing*

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

#### 162 *Data analyses*

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

174 (Blanchet, Legendre & Borcard 2008b; Blanchet *et al.* 2011) allowing the consideration of  
175 directional spatial processes, induced by the currents in our case. Since the locations of the  
176 samples within sites were not recorded, we used a one-dimensional representation of the study  
177 design including only sites, and replicated the values of the generated spatial variables (AEM  
178 eigenfunctions) for all samples within each site. We constructed several weighting vectors for  
179 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  
180 different values of  $\alpha$  from 1 to 3. Corrected Akaike information criterion values (AICc; Dray,  
181 Legendre & Peres-Neto 2006) calculated by the ‘ortho.AIC’ function in the ‘spacemaker’  
182 package (Dray 2013) indicated weighting function  $f_2$  with  $\alpha = 1$  as optimal (AICc = -168.82)  
183 for generating the AEM eigenfunctions. Since the eigenfunctions with positive and negative  
184 Moran’s  $I$  values (modelling positive and negative spatial autocorrelation, respectively) both  
185 explained a considerable share of the variation (positive: 30.6%,  $df = 9$ ,  $F = 5.26$ ,  $P < 0.001$ ;  
186 negative: 10.7%,  $df = 13$ ,  $F = 1.80$ ,  $P = 0.019$ ), we used both subsets in the analysis, which  
187 was possible due to the fact that we only had 22 (number of sites minus one) AEM  
188 eigenfunctions for 88 samples. We performed variance partitioning after forward selection  
189 (Blanchet, Legendre & Borcard 2008a) on the environmental as well as the spatial  
190 explanatory variable sets (with  $P = 0.05$ ) using the ‘ordiR2step’ function in the ‘vegan’  
191 package, and tested variance partitions of interest by ANOVA with 9999 permutations. We  
192 interpret the importance of environmental variables based on their biplot scores in the model  
193 including both environmental and spatial variables.

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



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

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

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

## 225 **Results**

226 *Dikerogammarus villosus* proved to be the most common of the three species in the material;  
227 it occurred at every site (altogether 213 samples) with an average ( $\pm$ SD) density of  $484\pm 851$   
228 ind./m<sup>2</sup> (whenever present), and a maximum of 8346 ind./m<sup>2</sup>. *D. haemobaphes* was present at  
229 36 sites (84 samples) between river km 2415 and 18 (average density:  $124\pm 338$  ind./m<sup>2</sup>, max.:  
230 2221 ind./m<sup>2</sup>), whereas *D. bispinosus* was entirely missing in the lower section of the river,  
231 occurring only at 20 sites (54 samples) between river km 2258 and 1252 (average density:  
232  $170\pm 376$  ind./m<sup>2</sup>, max.: 1866 ind./m<sup>2</sup>).

233 The forward selection procedure on the environmental variables selected current velocity,  
234 suspended matter content, substrate type, pH, and depth, while nine spatial variables (four  
235 with positive and five with negative Moran's *I*) were retained. Since the minor effect pH  
236 (0.2% of the total variance explained alone) could not be interpreted biologically (it ranged  
237 only between 7.89 and 8.43), we regarded it as a statistical artefact. Similarly, depth had small  
238 explanatory power (0% of the total variance explained alone); its effect could be attributed  
239 mainly to its relatively strong correlation with current velocity (Spearman's rank correlation:  
240 0.50); therefore, we excluded these two variables from further analyses.

241 In the variance partitioning (Fig. 2), the three remaining environmental variables explained  
242 37.9% (df = 11, F = 5.82,  $P < 0.001$ ) of the total variation, whereas spatial variables  
243 accounted for 41.4% (df = 9, F = 7.83,  $P < 0.001$ ). There was a considerable overlap between  
244 the two groups (23.2%, not testable); 14.7% of the variance (df = 11, F = 3.37,  $P < 0.001$ )  
245 could be explained by environmental factors only, while 18.2% (df = 9, F = 4.50,  $P < 0.001$ )

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

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

261 As indicated by the RDA triplot (Fig. 5), the main gradient in the community data separated  
262 *D. villosus* from *D. bispinosus*, whereas *D. haemobaphes* took an intermediate position on the  
263 first ordination axis ( $R^2 = 0.461$ ,  $df = 1$ ,  $F = 114.4$ ,  $P < 0.001$ ), and separated from the other  
264 two species mainly on the second axis ( $R^2 = 0.192$ ,  $df = 1$ ,  $F = 47.7$ ,  $P < 0.001$ ). The third  
265 canonical axis was not significant ( $R^2 = 0.009$ ,  $df = 1$ ,  $F = 2.29$ ,  $P = 0.103$ ). Current velocity  
266 was strongly associated with the main gradient (biplot scores: RDA1: 0.597, RDA2: 0.262),  
267 indicating *D. bispinosus* as the most and *D. villosus* as the least rheotolerant among the three  
268 species (Appendix 1), whereas the separation of *D. haemobaphes* from the other two species  
269 could be explained mostly by suspended matter concentration (biplot scores: RDA1: -0.361,  
270 RDA2: 0.366; Appendix 1). *D. villosus* showed affinity to xylal, riprap, argyllal,

271 psammopelal, and pelal, while natural stony substrates (akal, micro-, meso-, macrolithal) and  
272 psammal were approximately equally used by the species. Nevertheless, we note that the  
273 ordination plot shows only relative substrate usage patterns; in absolute terms all three species  
274 avoided fine substrates (Appendix 2).

275 The environmental RDA model predicted the presence of *D. bispinosus* for 25 samples out of  
276 93 (26.9%) from the river section where it was absent (Fig. 6). Fourteen of these samples  
277 were micro- or mesolithal (current velocity: 0-0.22 m/s) and 11 were  
278 pelal/psammopelal/psammal (current velocity: 0.09-0.23 m/s). Densities above 10, 100, and  
279 450 ind./m<sup>2</sup> were predicted for 19, 10, and 4 samples, respectively.

## 280 **Discussion**

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

294 certain degree of homogenization among the samples within sites through the dispersal of  
295 individuals.

296 Three environmental variables; current velocity, substrate type, and suspended matter  
297 concentration proved to be important in explaining the niche differentiation of the invasive  
298 *Dikerogammarus* species. Since current velocity showed the strongest correlation with the  
299 first ordination axis, the effect of this variable appears to be the most prominent. Previous  
300 observations on the flow preferences of the species are scarce, especially for *D. haemobaphes*  
301 and *D. bispinosus*; nevertheless, the primary role of current velocity is in concert with the  
302 longitudinal distribution of the species in the River Dniester, where the abundance of *D.*  
303 *bispinosus* decreased downstream, *D. haemobaphes* was the most widespread, and *D. villosus*  
304 was found only in the lagoon, where *D. bispinosus* was missing (Jażdżewski & Konopacka  
305 1988). Field studies involving *D. villosus* reflect strong context-dependency, their conclusions  
306 including preference for lentic conditions (Boets *et al.* 2010), relative indifference (Devin *et*  
307 *al.* 2003), and affinity to flow-exposed, but strongly structured habitats (MacNeil & Platvoet  
308 2013); whereas the only flume experiment involving the species demonstrated that it avoids  
309 strong currents by using flow-refuges (Felten, Dolédec & Statzner 2008). Our results confirm  
310 that the species is primarily rheophobic, but it can utilize lentic microhabitats offered by  
311 obstacles in the water (i.e., stones, wood, and macrophytes) even in relatively fast-flowing  
312 sections of rivers.

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

319 & Chase 2004). Although the importance of this mechanism has been tested mostly on  
320 terrestrial plants (e.g., Liancourt, Callaway & Michalet 2005; Peterson, Rice & Sexton 2013),  
321 it has been demonstrated that hydrodynamic stress can generate such co-existence patterns;  
322 e.g., among sessile mussels, where a stronger byssal thread attachment comes at a cost of  
323 growth rate and reproductive output (Zardi *et al.* 2006). Remarkably, this mechanism is  
324 involved in the niche partitioning of the two invasive *Dreissena* species, as well (Peyer *et al.*  
325 2009). Regarding the invasive *Dikerogammarus* species, the components of the trade-off are  
326 yet to be determined; nevertheless, the high abundance of *D. villosus* in drift samples (Van  
327 Riel, Van der Velde & Bij de Vaate 2011) might be an indication that currents represent an  
328 important mortality factor for the species, whereas the competitive dominance of *D. villosus*  
329 might involve behavioural aspects (Kobak *et al.* 2016) as well as differences resource  
330 utilization, as suggested by its higher functional response compared to *D. haemobaphes*  
331 (Bovy *et al.* 2015).

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

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

344 nevertheless, an indirect connection (e.g., through other filter feeding organisms) is also  
345 possible. Although the feeding ecology of *D. villosus* has been studied extensively in recent  
346 years (reviewed by Rewicz *et al.* 2014), comparative studies among the invasive  
347 *Dikerogammarus* species are relatively rare and far from providing a comprehensive picture  
348 (Ponyi 1956; Kinzler *et al.* 2009; Bacela-Spychalska & Van der Velde 2013; Bovy *et al.*  
349 2015). We assume that food might represent another important niche axis further decreasing  
350 interspecific competition among the three species. However, to prove and fully understand its  
351 role, further studies taking all relevant environmental variables into account would be  
352 necessary.

353 The environmental RDA model predicted the presence of *D. bispinosus* in around a quarter of  
354 the samples from the area of its absence; nevertheless, the majority of habitats were predicted  
355 to support low densities of the species. Many of the samples classified as suitable (even two  
356 of the best four) represented soft substrates (with relatively strong currents), which were  
357 avoided by the species within its present range. In addition, the substrates characterized as  
358 micro- or mesolitoral in the Lower Danube consisted mainly of mollusc shells (not gravel, as  
359 in the preferred habitats within the upper and middle river sections), providing more  
360 protection from currents than gravel (most of these samples were dominated by *D.*  
361 *haemobaphes*). Even if there are suitable habitat patches for the species in this section of the  
362 river, they might be too small and isolated to support viable populations. In summary, we  
363 conclude that environmental variables, namely the general lack of current-exposed gravel  
364 deposits can effectively explain the absence of *D. bispinosus* in the Lower Danube.

365 Accordingly, its former abundance in this river section as well as in Lake Balaton can be  
366 regarded as niche expansion, reflecting its ability to capitalize on the absence of *D. villosus* by  
367 using lentic habitats. However, the competitively superior *D. villosus* may dislodge *D.*

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

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

384 In conclusion, our study confirmed that similarly to *Dreissena* species, the interaction among  
385 the invasive *Dikerogammarus* species can be characterised by competitive niche partitioning,  
386 implying the species do not facilitate each other's establishment, although *D. villosus* might  
387 facilitate the spread of *D. haemobaphes*, as suggested by Kobak *et al.* (2016). Nevertheless,  
388 these results do not necessarily contradict the invasional meltdown hypothesis, if we assume  
389 that the aforementioned facilitative interactions act as a stronger community assembly factor,  
390 since they operate among different trophic levels or functional groups, whereas negative  
391 interactions only determine which functionally more-or-less redundant species represent the  
392 guilds. We have identified current velocity as the most decisive factor in determining the co-



393 existence patters of *Dikerogammarus* species, which might lead to a better understanding of  
394 their invasion characteristics, and could be used for refining predictions of their potential  
395 distributions (Gallardo & Aldridge 2013). Our results suggest that the apparently different  
396 invasion success of the species corresponds to their position on the stress tolerance-  
397 competitive ability axis, implying that the advantage of *D. villosus* is attributable to its  
398 competitive dominance, allowing it to monopolize lentic and/or structured habitats, which  
399 represents a fortunate preadaptation to anthropogenic alterations of waters; i.e., ripraps,  
400 impoundments, and fish passes (MacNeil & Platvoet 2013). The presence of *D. villosus* does  
401 not considerably impede the expansion of *D. haemobaphes*; however, it can be expected to  
402 reach high densities only in rivers and only if the food supply is favourable. On the contrary,  
403 the exclusion of *D. bispinosus* from lentic habitats by *D. villosus* probably strongly limits its  
404 potential to spread by active dispersal; therefore, it can be expected to continue its expansion  
405 only if it can reach other gravel-bed rivers by jump dispersal.

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- 602

603 Captions

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

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

613 **Fig. 3** Mantel correlograms of the response variables (squares/solid line), the residuals of the  
614 environmental model (circles/dashed line), and the residuals of the environmental and spatial  
615 model (triangles/dotted line). The distance class at 0 river km corresponds to within-site  
616 distances. Solid symbols indicate significant correlations (\*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P <$   
617  $0.001$ ). Numbers on the top of the graph indicate the number of pairs involved in the  
618 calculation of correlations for each distance class. Symbols are connected only to visualize the  
619 trends.

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

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

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

632 **Fig. 6** Kernel density of predicted values of the environmental RDA model for *D. bispinosus*.  
633 Grey area: samples in the area of its absence (downstream of rkm 1252), solid line: absences  
634 in its range of occurrence (between river km 2258 and 1252), dashed line: presences in its  
635 range of occurrence. The increasingly dark sections indicate samples classified as > 0, > 10, >  
636 100, and > 450 ind./m<sup>2</sup>, respectively.

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

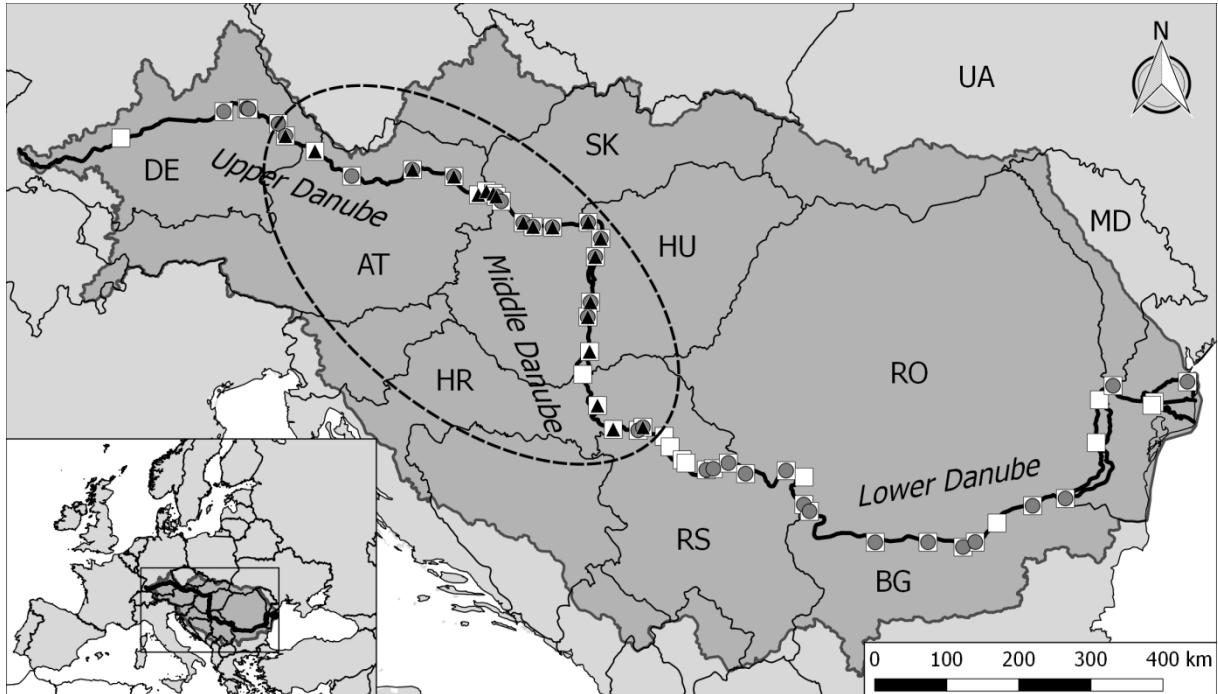
638 **Table 2** Physicochemical parameters used as environmental explanatory variables in the  
639 study. The parameters were measured A: for all samples (averaged over the five sampling  
640 units), B: at two points per site near the river banks, or C: at one point per site in the middle of  
641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

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

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

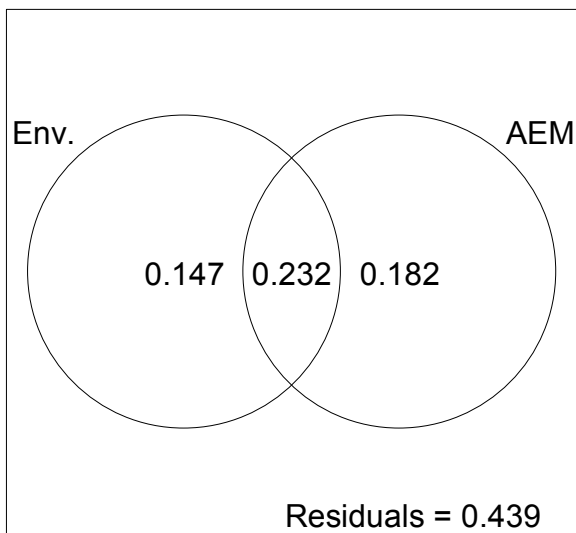
651 Figures

652 Fig. 1



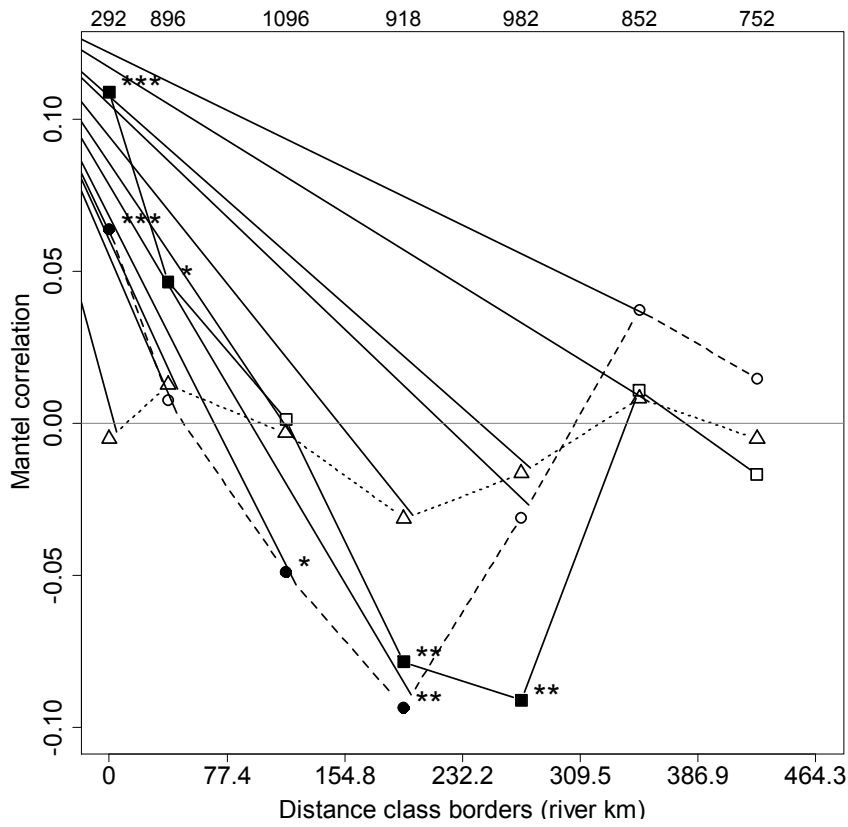
653

654 Fig. 2



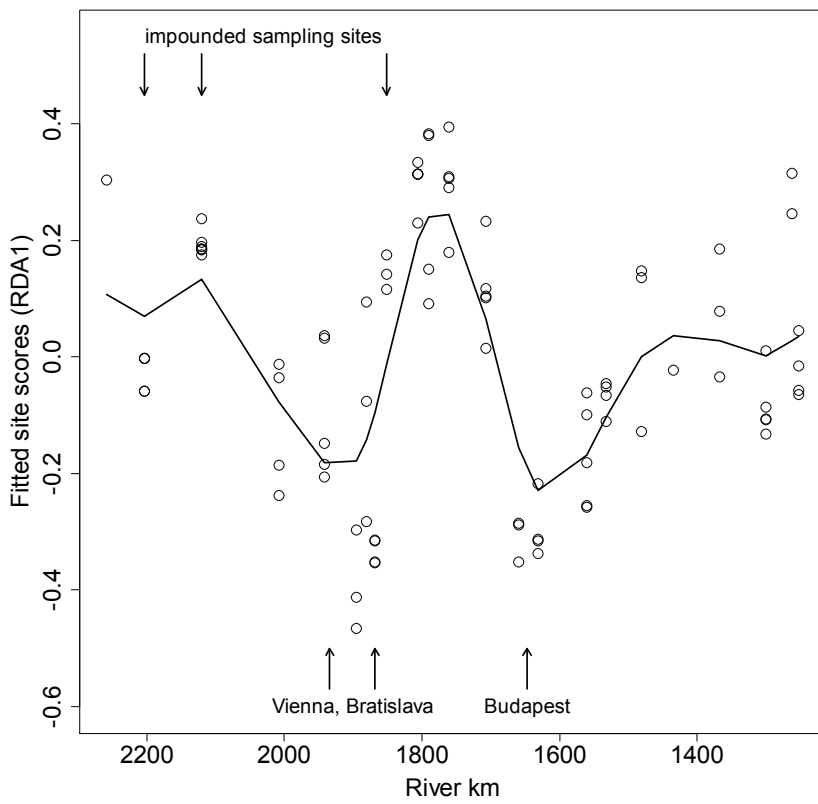
655

656 Fig. 3



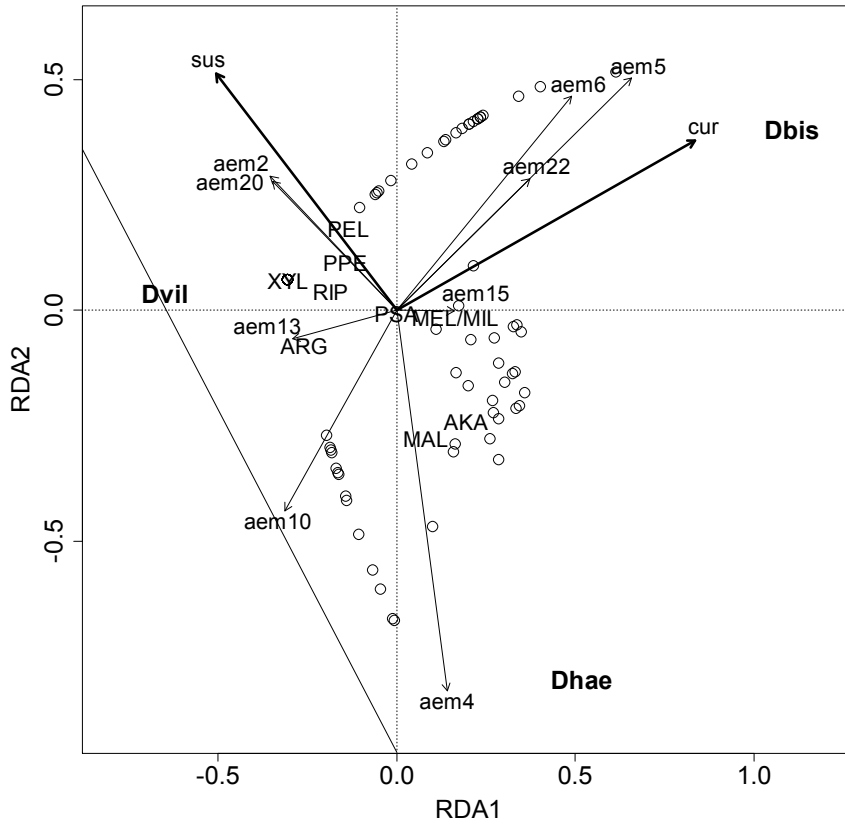
657

658 Fig. 4



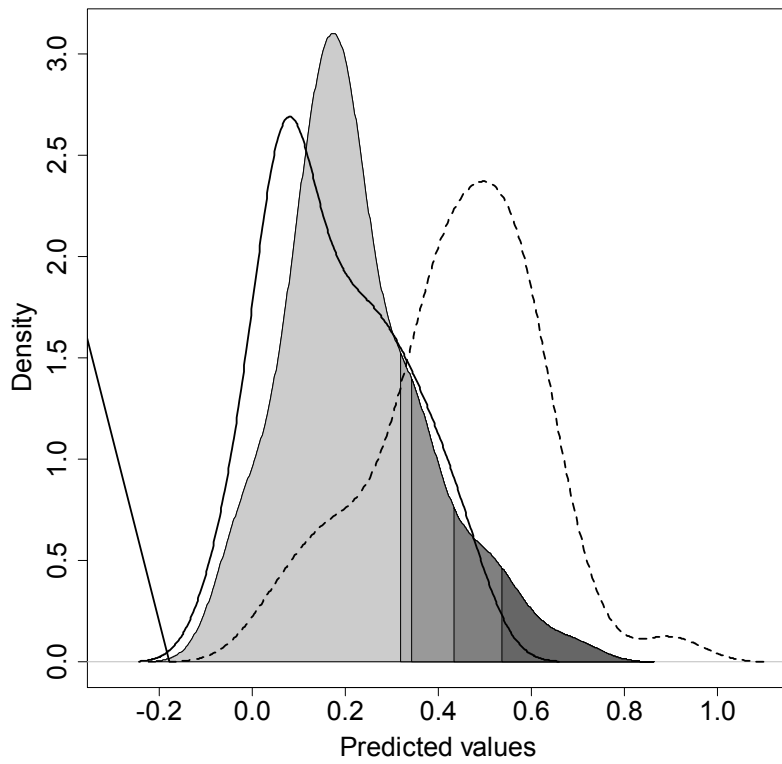
659

660 Fig. 5

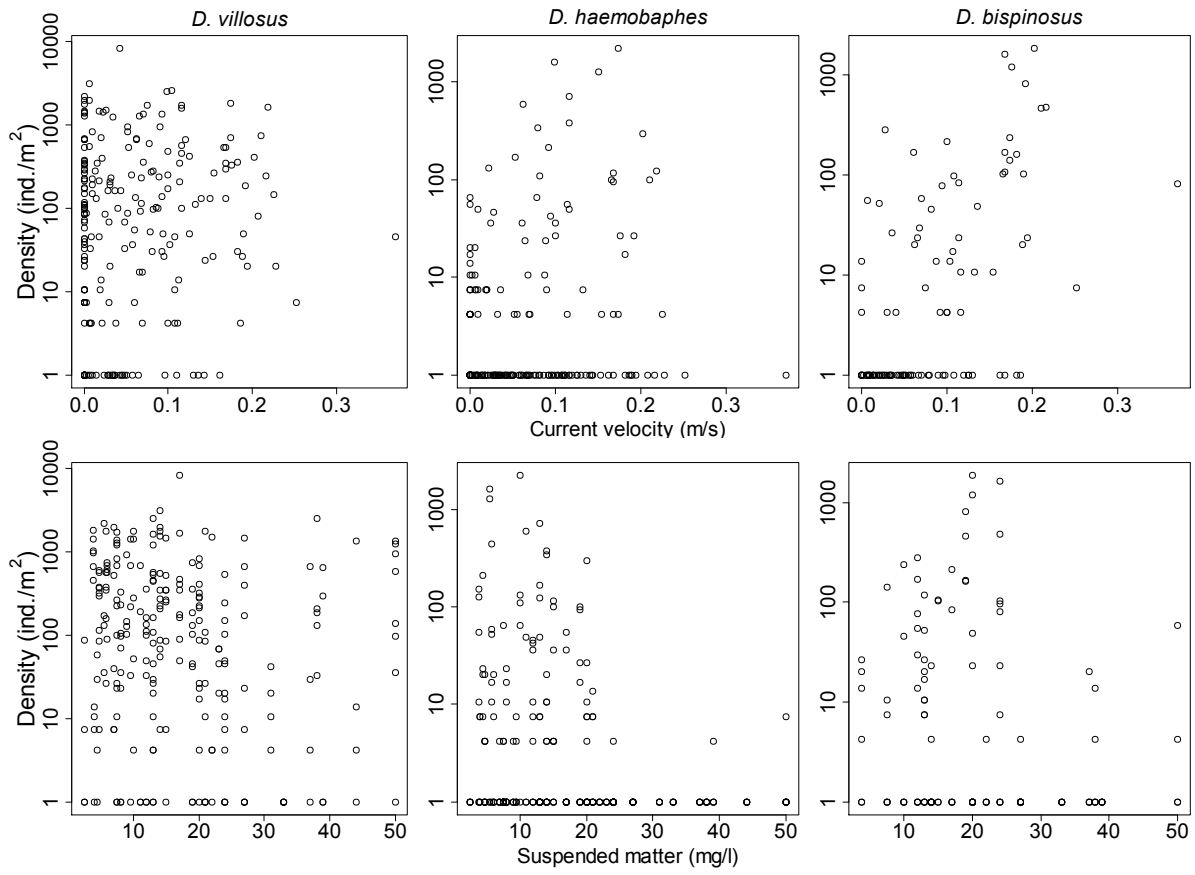


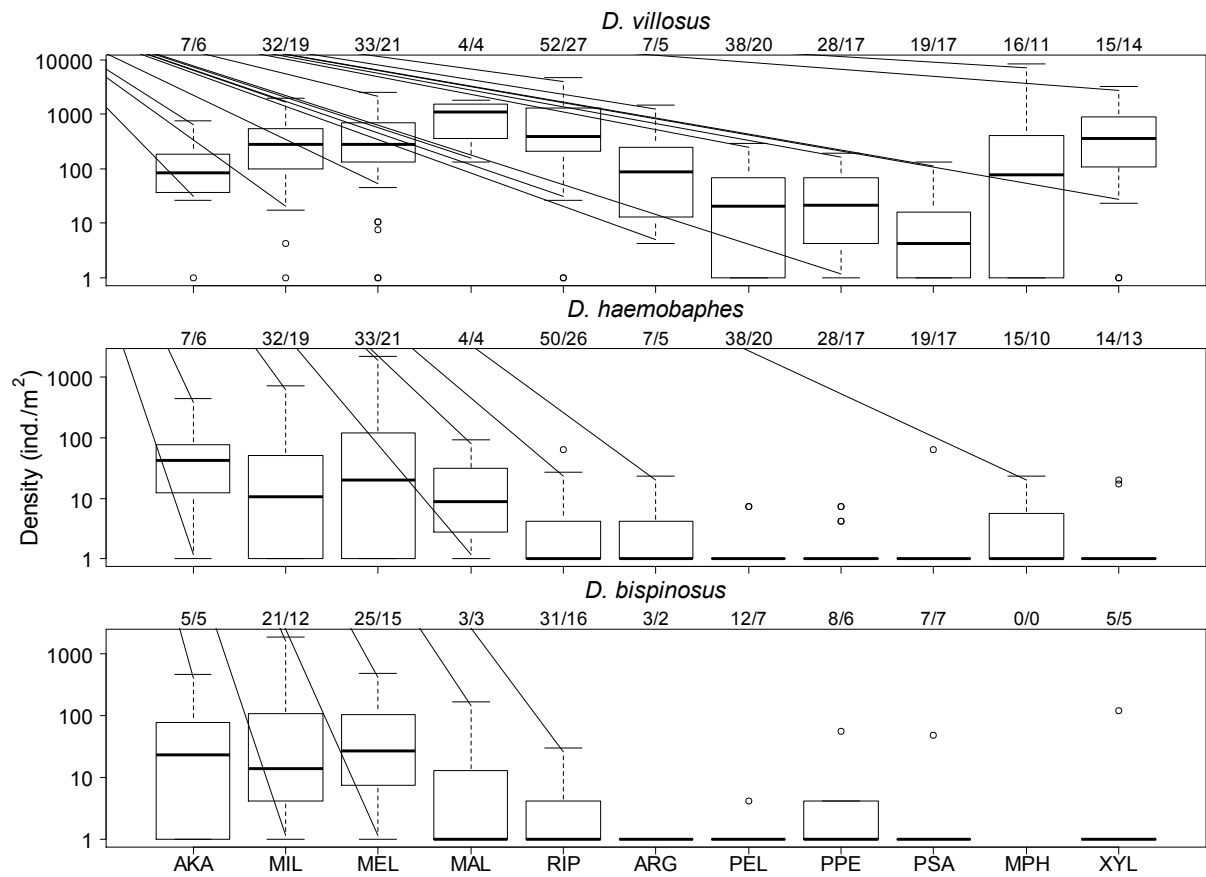
661

662 Fig. 6



663





667

668 Tables

669 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

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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 <sub>2</sub> 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

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