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8 How to coexist with the 'killer shrimp' *Dikerogammarus villosus*? Lessons from other
9 invasive Ponto-Caspian peracarids

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24

25 **Abstract**

26

- 27 1. Studying the interactions among coevolved invaders might help us to understand,
28 predict, and perhaps even mitigate their impact on the native biota. We investigated
29 the factors of spatial niche differentiation among invasive Ponto-Caspian peracarids
30 with the aim of revealing how coevolved species can coexist with the 'killer shrimp'
31 *Dikerogammarus villosus*, an invasive gammarid replacing non-Ponto-Caspian species
32 throughout Europe.
- 33 2. Multi-habitat samples from the 3rd Joint Danube Survey were analyzed by partitioning
34 the variation in species density data between environmental and spatial explanatory
35 variable sets. Relevant predictors were identified by forward selection and their role
36 was interpreted based on the RDA triplot. The effect of substrate types was further
37 analyzed in certain species using generalized linear models.
- 38 3. Our analysis revealed characteristic differences in habitat preference (i.e. spatial niche
39 differentiation) among the species allowing coexistence with *D. villosus* at different
40 spatial scales. The relatively small and lean body of *Chaetogammarus ischnus* and
41 *Jaera sarsi* might allow the avoidance of interference with large *Dikerogammarus*
42 specimens by using narrow interstices among pebbles and stones (microhabitat-scale
43 differentiation). The remaining Ponto-Caspian species included in the analysis showed
44 affinity to substrate types (*Obesogammarus obesus*) or current velocity intervals (*D.*
45 *bispinosus*) different from those preferred by *D. villosus* (mesohabitat-scale
46 differentiation), presumably in connection with feeding preferences in some cases (*D.*
47 *haemobaphes*, *Trichogammarus trichiatus*).
- 48 4. Our results provide a framework for a preliminary risk assessment concerning the still
49 high range expansion potential of *D. villosus*; i.e. the identification of the most
50 vulnerable species in the presently not invaded but potentially colonizable regions of

51 the world based on their habitat preference and morphology. The lessons learned from
52 Ponto-Caspian peracarids can be applied to the whole macroinvertebrate fauna, since
53 the same principles (i.e. the avoidance of interference) can be expected to determine
54 their coexistence with *D. villosus*.

55

56 Keywords: alien species, benthos, competition, environmental impact assessment,
57 invertebrates, river

58

59 **1 Introduction**

60

61 The majority of non-indigenous species in any given region originate in a few climatically
62 matching areas strongly connected to the recipient area by anthropogenic transport
63 mechanisms (Hulme, 2009), implying that invader-invader interactions are often determined
64 by coevolution in the native range. Accordingly, coevolved interactions among invaders are a
65 major determinant of invasion impact – in many cases for the worse. Invasive species often
66 promote the establishment of further colonists originating in the same region through
67 facilitative interactions ('invasional meltdown'; Simberloff & Von Holle, 1999) and even if
68 the interaction is essentially competitive (i.e. if the species belong to the same guild), invaders
69 with shared evolutionary history can be expected to show adaptations which allow their stable
70 coexistence (Chase & Leibold, 2003). On the other hand, studying these interactions might
71 help us understand, predict, and perhaps even mitigate the impact of the invaders on the native
72 biota.

73 The recent range expansion of several endemic Ponto-Caspian faunal elements provides a
74 perfect example for the invasion success of coevolved species (Gallardo & Aldridge, 2015;
75 Ricciardi, 2001). Facilitation can be observed among different functional groups, e.g.

76 dreissenid mussels provide food and shelter for gammarids (Gergs & Rothhaupt, 2008; Kobak
77 & Żytkowicz, 2007; Stewart, Miner, & Lowe, 1998), and both groups contribute to the food
78 supply of gobies (Borza, Erős, & Oertel, 2009; Grabowska & Grabowski, 2005; Lederer,
79 Massart, & Janssen, 2006). Although species belonging to the same guild compete for the
80 shared resources, sometimes even resulting in turnovers, e.g. between the two invasive
81 *Dreissena* species (Marescaux et al., 2015; Ricciardi & Whoriskey, 2004), their different
82 tolerances to certain factors allow their long-term coexistence in sufficiently heterogeneous
83 environments (Jones & Ricciardi, 2005; Karatayev et al., 2014; Peyer, McCarthy, & Lee,
84 2009).

85 The gammarid amphipod *Dikerogammarus villosus* (Sowinsky, 1894) is one of the most
86 successful Ponto-Caspian invaders with considerable impact on the biota. Several different
87 macroinvertebrate groups are negatively affected by the appearance of the species (Gergs,
88 Koester, Schulz, & Schulz, 2014; Van Riel et al., 2006); however, the impact is the most
89 dramatic on ecologically similar but competitively weaker gammarids and isopods, which are
90 often driven to local extinction (Dick & Platvoet, 2000). Laboratory experiments suggested
91 that the voracious predatory feeding of the species might be responsible for the declines;
92 however, field evidence is equivocal in this question (Bacela-Spychalska & Van der Velde,
93 2013; Hellmann et al., 2015; Koester, Bayer, & Gergs, 2016; Koester & Gergs, 2014; Van
94 Riel et al., 2006). As *D. villosus* is capable of utilizing several different food sources
95 (Platvoet, Van der Velde, Dick, & Li, 2009), the role of predation in its diet might be context-
96 dependent (Hellmann et al., 2015). Therefore, the primary mechanism of species exclusions
97 might be interference competition, where *D. villosus* forces the weaker competitors to leave
98 their shelter, thereby exposing them to increased predation by fish (Beggel, Brandner,
99 Cerwenka, & Geist, 2016; De Gelder et al., 2016; Kobak, Rachalewski, & Bacela-Spychalska,
100 2016; Van Riel, Healy, Van der Velde, & Bij de Vaate, 2007).

101 The species locally eliminated by *D. villosus* are all native to Europe (e.g. *Gammarus* spp.,
102 *Asellus aquaticus* (Linnaeus, 1758); Borza et al., 2015; Dick & Platvoet, 2000) or North-
103 American invaders in Europe (e.g. *Gammarus tigrinus* Sexton, 1939; Dick & Platvoet, 2000;
104 Leuven et al., 2009); nevertheless, some species were able to persist in the invaded waters by
105 switching habitats (Hesselschwerdt, Necker, & Wantzen, 2008; Platvoet, Dick, MacNeil, Van
106 Riel, & Van der Velde, 2009). On the contrary, Ponto-Caspian peracarids can usually coexist
107 with *D. villosus* within the same waterbody despite the population declines in some cases,
108 which can be ascribed to the extraordinarily high densities before the appearance of the
109 stronger competitor/predator (i.e. niche extension or enemy release; Borza, Huber, Leitner,
110 Remund, & Graf, 2017a; Van Riel et al., 2006). As *D. villosus* could displace all studied
111 species from its preferred habitat (i.e. crevices among stones; Devin, Piscart, Beisel, &
112 Moreteau, 2003; Kobak, Jermacz, & Dzierżyńska-Białończyk, 2015) in aquarium experiments
113 (Kobak et al., 2016; Van Riel et al., 2007), those capable of coexisting with it can be expected
114 to show spatial niche differentiation. Differences in habitat use are obvious in some cases, e.g.
115 several Ponto-Caspian amphipods are psammo-pelophilous (Borza, Huber, Leitner, Remund,
116 & Graf, 2017b) and mysids are epibenthic or semi-pelagic; however, the factors of niche
117 differentiation among lithophilous Ponto-Caspian amphipods are only partially known (Borza
118 et al., 2017a).

119 According to all indications, *D. villosus* has not reached the borders of its potential range; its
120 further expansion can be reasonably expected. The species has recently established in the
121 British Isles, where climatic factors allow its continued spread even presently (Gallardo &
122 Aldridge, 2013); however, climate change might push the potential distributional limit of the
123 species even farther north (as well as elsewhere in Europe). The species also has the potential
124 to expand its range in the Mediterranean and in the Alpine region, where the transport of
125 recreational ships has already allowed it to colonize relatively small, isolated water bodies

126 (Bacela-Spychalska, Grabowski, Rewicz, Konopacka, & Wattier, 2013; Rewicz et al., 2017;
127 Tricarico et al., 2010). Apparently, ballast water treatment measures have proved successful at
128 halting the influx of Ponto-Caspian species into North America; nevertheless, the appearance
129 of *D. villosus* in the Great Lakes is still considered as a realistic threat (Pagnucco et al., 2015).
130 As *D. villosus* might get into contact with several additional species in the potentially
131 colonizable waters, it is important to understand how it is possible to coexist with this invader.
132 Accordingly, our goal in the present study was to reveal the mechanisms of spatial niche
133 differentiation allowing invasive Ponto-Caspian peracarids to coexist with *D. villosus*. We
134 interpret the results taking the marked morphological differences among the species
135 (Supplementary Information; Figure S1, Table S1-S2) presumably affecting their habitat use
136 into account (Koehl, 1996). We summarize our conclusions as well as previous results on the
137 coexistence mechanisms in a systematic framework, providing a conceptual basis for a
138 preliminary risk assessment related to the potential further range expansion of *D. villosus*.

139

140 **2 Methods**

141

142 *2.1 Sample collection and processing*

143

144 The macroinvertebrate samples analyzed in the present study were taken during the 3rd Joint
145 Danube Survey (13 August-26 September 2013) at 55 sites of the river (Figure 1) between
146 Ulm (river km 2581) and the Delta (river km 18, Kiliya branch) by the ‘multi-habitat’
147 approach based on the AQEM protocol (Hering, Moog, Sandin, & Verdonschot, 2004). At
148 each site, all available habitat types (four to seven per site) were sampled (altogether 251).
149 Five pooled units covering 25 x 25 cm bottom area were collected for each habitat in the
150 littoral zone by hand net (aperture: 25 x 25 cm, mesh size: 500 µm). All samples were

151 preserved in 4% formaldehyde solution in the field, and stored in 70% ethanol after sorting.
152 Sorting was facilitated by fractioning the material on a set of sieves (mesh sizes: 0.5, 2, 5, 10,
153 20 mm). In some cases, 2 to 64-fold subsampling of the smallest one or two fractions was
154 necessary due to the extremely high number of juvenile specimens in the samples.

155

156 2.2 Data analysis

157

158 Only free-living, benthic Ponto-Caspian invasive peracarid species were included in the
159 analysis; six gammarids (*Chaetogammarus* (formerly *Echinogammarus*) *ischnus* (Stebbing,
160 1899), *Dikerogammarus bispinosus* Martynov, 1925, *D. haemobaphes* (Eichwald, 1841), *D.*
161 *villosus*, *Obesogammarus obesus* (G.O. Sars, 1894), and *Trichogammarus* (formerly
162 *Echinogammarus*) *trichiatus* (Martynov, 1932)), and the isopod *Jaera sarsi* Valkanov, 1936.

163 The niche differentiation among the three invasive *Dikerogammarus* species was analyzed in
164 detail by Borza et al. (2017a) based on the same survey. Nevertheless, *D. bispinosus* and *D.*
165 *haemobaphes* were included in the present study to allow the comparison of their habitat
166 preferences with that of the other species. Mysids were excluded, since their habitat use is
167 markedly different from *D. villosus* (epibenthic or semi-pelagic). In addition, they reach high
168 abundance mainly in semi-enclosed inlets and slow-flowing sidearms, so they were found
169 only sporadically during the survey (Borza et al., 2015). The filter feeding, tube-dwelling
170 corophiids were excluded, too, since the data suggested that their abundance is primarily
171 determined by the quality and quantity of suspended matter, not habitat characteristics (Borza,
172 Huber, Leitner, Remund, & Graf, 2018). Nevertheless, we share our remarks on the possible
173 mechanisms of their co-existence with *D. villosus* in the Discussion.

174 Spatial niche differentiation among the species was tested by variance partitioning between
175 environmental and spatial explanatory variables based on redundancy analysis (RDA), using

176 the 'varpart' function in the 'vegan' package (Oksanen et al., 2017) in R 3.2.5 (R Core Team,
177 2016). Ln(x+1) and Hellinger-transformed (Legendre & Gallagher, 2001) count data
178 (individuals per sample) were used in the analysis, but individuals per squaremeter (ind./m²)
179 values are shown in the results and in figures for comparability reasons. Substrate types
180 (Table 1) and several physicochemical parameters (Table 2) were used as environmental
181 explanatory variables. The spatial structure of the study was modelled using the asymmetric
182 eigenvector map (AEM) method (Blanchet, Legendre, & Borcard, 2008a; Blanchet, Legendre,
183 Maranger, Monti, & Pepin, 2011) allowing the consideration of directional spatial processes,
184 induced by water flow in the present case. Two sites (eight samples) were excluded in the two
185 minor arms of the Danube delta (Sulina and Sf. Gheorghe) allowing the one-dimensional
186 representation of the study design. The studied species were not present in 24 samples, and 41
187 additional samples were omitted due to missing values in the explanatory variables, hence 186
188 samples from 47 sites were involved in the analysis. Since the locations of the samples within
189 the sites were not recorded, the values of the generated spatial variables (AEM
190 eigenfunctions) were replicated for all samples within each site. The eigenfunctions both with
191 positive and negative Moran's *I* values (modelling positive and negative spatial
192 autocorrelation, respectively) were used in the analysis, which was possible due to the fact
193 that we only had 46 (number of sites minus one) AEM eigenfunctions for 186 samples.
194 Forward selection was performed (Blanchet, Legendre, & Borcard, 2008b) on the
195 environmental as well as the spatial explanatory variable sets using the 'ordiR2step' function
196 in the 'vegan' package. In each step of the process, the gain in explained variance (adjusted
197 R^2) is tested for all variables one-by-one, and the variable with the highest gain is added to the
198 model until the gain is significantly higher than zero ($P < 0.05$). The two resulting variable
199 sets were included in a variance partitioning ('varpart' function in the 'vegan' package) and
200 variance portions were tested by ANOVA with 9999 permutations. The differentiation among

201 the species and the importance of the environmental variables are interpreted based on the
202 triplot of the model including both environmental and spatial variables.

203 To provide an insight into the structure of spatial autocorrelation (SA henceforth) across
204 multiple spatial scales, Mantel correlograms (Borcard & Legendre, 2012) were constructed
205 using the ‘mantel.correlog’ function in the ‘vegan’ package about (1) the response variables
206 representing both environmentally explainable SA (‘induced spatial dependence’) and
207 environmentally not explainable (‘true’) SA (Legendre & Legendre, 2012), (2) the residuals
208 of the environmental model (representing ‘true’ SA and unexplained induced spatial
209 dependence), and (3) the residuals of the environmental and spatial model (expected to be
210 zero for all spatial scales, if the spatial structure is properly represented in the model). The
211 first distance class in the correlograms represents within-site distances, whereas the
212 subsequent classes were delimited according to the Sturges equation (13 classes with equal
213 widths of 146 river km; the last seven are not shown). *P*-values of the Mantel correlation
214 coefficients were calculated with Holm-correction.

215 The effect of substrate types was further analysed in a univariate context using generalized
216 linear models (GLM) on count data of *C. ischnus*, *J. sarsi*, and *O. obesus* (*T. trichiatus* was
217 excluded from this analysis due to its rarity in the material, and *Dikerogammarus* species
218 were excluded since factors other than substrate type have strong influence on their habitat
219 preferences; Borza et al. 2017a). The negative binomial family with log link function was
220 used (‘glm.nb’ function in the ‘MASS’ package; Venables & Ripley, 2002) since it provided a
221 better fit than Poisson and quasi-Poisson models based on the distribution of the deviance
222 residuals (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Pairwise comparisons among the
223 parameter estimates of substrate types were made using the ‘glht’ function in the ‘multcomp’
224 package (Hothorn, Bretz, & Westfall, 2008) in with Tukey correction. As *J. sarsi* did not

225 occur at all on psammopelal, this substrate type was not included in the model and it was
226 substituted with zeros in the pairwise comparisons.

227

228 **3 Results**

229

230 All target species were present in almost the entire studied section of the Danube, except for
231 *D. bispinosus* (Table 3; Borza et al., 2017a). *D. villosus* proved to be the most widespread and
232 – on average – most abundant during the survey, followed by *C. ischnus* and *O. obesus*, which
233 in turn reached a maximal density even higher than *D. villosus* (Table 3). *J. sarsi* was still
234 more abundant than the two remaining *Dikerogammarus* species, while *T. trichiatus* was
235 rather rare (Table 3).

236 The forward selection procedure on the environmental variables selected substrate types, total
237 suspended solids (TSS), dissolved oxygen concentration, total nitrogen concentration, current
238 velocity, and total phosphorus concentration (Table 4), explaining 25.75% of the total
239 variation (Table 5). The forward selection on the spatial variables selected 19 AEM
240 eigenvectors explaining 29.17%; nevertheless, the overlap between the two variable sets was
241 considerable (together they accounted for 38.53 %; Table 5).

242 The Mantel correlogram of the response variables indicated significant positive SA in the
243 smallest three distance classes (0-292 river km), significant negative SA at intermediate
244 distances (292-876 river km), whereas in the largest distance classes SA was not significant
245 (Figure 2). The inclusion of environmental predictors in the model decreased SA
246 considerably; however, it remained significantly positive between 0 and 146 river km
247 distances (Figure 2). SA was not significant among the residuals of the model including
248 environmental and spatial predictor variables in any of the distance classes (Figure 2).

249 All seven constrained axes of the RDA explained a significant proportion of the variance
250 (Table 6); nevertheless, the first three axes (cumulative proportion explained: 40.10 %)
251 provide a sufficient basis for the interpretation of the results (Figure 3). Current velocity and
252 TSS – the most important factors of niche differentiation among the three *Dikerogammarus*
253 species (Borza et al., 2017a) – were considerably correlated with all three canonical axes;
254 therefore, the separation of the three *Dikerogammarus* species in the present analysis was
255 observable in three dimensions. *D. villosus* separated from *D. haemobaphes* and *D. bispinosus*
256 along the first and second axes (Figure 3a), whereas the latter two species differentiated
257 primarily along the third axis (Figure 3b). The position of *C. ischnus* and *J. sarsi* was close to
258 *D. haemobaphes* on the first and second axes (Figure 3a), reflecting their preference for
259 gravel (especially micro- and mesolithal). However, the two species separated considerably
260 along the third axis (Figure 3b), owing to the higher affinity of *J. sarsi* to riprap. *O. obesus*
261 differentiated markedly from all the other species along the second axis (Figure 3), reflecting
262 its association with akal and argyllal. Due to its rarity, the position of *T. trichiatus* was close
263 to the origin of the ordination space (Figure 3). Its only massive occurrence ($> 4\ 000$ ind./m²)
264 was recorded on xylal (Figure 4).

265 The GLMs confirmed the results of the RDA regarding the substrate preference of the three
266 species included in this analysis. *C. ischnus* and *J. sarsi* showed a marked affinity to different
267 sizes of gravel and xylal, while the latter also preferred riprap (Figures 4, 5a-b, Tables S3,
268 S4). *O. obesus* preferred argyllal and smaller sizes of gravel (akal and microlithal; Figures 4,
269 5c, Table S5). The relatively few significant comparisons with akal and macrolithal are in part
270 attributable to the low number of samples with these substrate types, reflecting their rarity in
271 the studied river section.

272

273 **4 Discussion**

274

275 Our analysis revealed characteristic differences in habitat preference among the species,
276 indicating spatial niche differentiation primarily determined by substrate types. The remaining
277 five significant variables accounted for only minor portions of the variance. The effect of
278 current velocity and TSS is attributable mainly to their importance in the niche differentiation
279 among the three *Dikerogammarus* species (Borza et al., 2017a). The role of total phosphorus
280 concentration was similar to TSS due to their relatively strong correlation (Spearman's rank
281 correlation: 0.364), whereas total nitrogen and dissolved oxygen concentration did not show
282 clear association with any of the species, so their effect is individually not interpretable.

283 The preference of *C. ischnus* for gravel proved to be an effective way to avoid *D. villosus*;
284 however, it resulted in a strong overlap with *D. haemobaphes*, a species capable of similarly
285 aggressive predation as its notorious relative (Bacela-Spychalska & Van der Velde, 2013).
286 Size-dependent microhabitat choice is a widely reported phenomenon among gammarids
287 (Devin et al., 2003; Hacker & Steneck, 1990; Jermacz, Dzierżyńska, Poznańska, & Kobak,
288 2015; Platvoet, Dick, et al., 2009); therefore, we assume that the relatively small-sized and
289 strongly flattened *C. ischnus* (Figure S1) can utilize the deep, narrow interstices among coarse
290 gravel. As only smaller specimens of the more robust *Dikerogammarus* species (Figure S1)
291 can enter the crevices of a given width, *C. ischnus* can avoid direct interference with larger,
292 potentially dangerous individuals. Accordingly, the mesohabitat-preference shown by our
293 results might in fact reflect differences in microhabitat use, since interstices of the preferred
294 width might be most abundant in micro- and mesolithal.

295 We assume that the same mechanism might explain the similar substrate-preference of *J.*
296 *sarsi*, a species even smaller and more flattened than *C. ischnus*. The fact that it was even
297 more abundant on riprap than *C. ischnus* might indicate that its co-existence with *D. villosus*
298 is even less problematic.

299 Morphological and behavioural adaptations might account for the habitat preference of *O.*
300 *obesus*, as well. This species can burrow itself into fine sediments (P. Borza, pers. obs.). It can
301 form holes in clay which might serve as shelter, explaining the high observed density of the
302 species on this substrate type. In sand, however, the animal gets entirely buried under the
303 particles, which might be an effective predator escape mechanism, but not a sustainable
304 lifestyle. Nevertheless, other factors – such as food availability or substrate stability – also
305 might be attributable for the low density of *O. obesus* on sand. The peculiar body shape of the
306 species might have another advantage; when bent, the narrow anterior and posterior tips along
307 with the wide central body part form a wedge, allowing the animal to fit into the relatively
308 shallow and wide gaps among the particles of fine gravel. The ability to utilize this substrate
309 type is an effective way to avoid large *Dikerogammarus* specimens (Devin et al., 2003), and it
310 also might account for the higher invasion potential of the species as compared to psammo-
311 pelophilous Ponto-Caspian amphipods (Borza et al., 2017b).

312 *Trichogammarus trichiatus* was relatively rare in our material; however, since its density
313 varied within a wide range, we felt that it would be useful to publish our data. Its inclusion in
314 the analysis did not change the overall results, since the Hellinger-transformation gives low
315 weight to rare species (Legendre & Gallagher, 2001). Information on the habitat preference of
316 *T. trichiatus* is scarce in the literature apart from invasion reports noting its occurrence on
317 gravel as well as riprap (e.g. Borza, 2009); however, the data of Müller & Eggers (2006)
318 suggest its affinity to plants. Our results support this; the massive occurrence of the species on
319 woody debris suggests a differentiation from *D. villosus* at the mesohabitat scale. As *D.*
320 *villosus* is rather ineffective at detritus decomposition according to most studies (Jourdan et
321 al., 2016; MacNeil, Dick, Platvoet, & Briffa, 2010; Piscart, Mermillod-Blondin, Maazouzi,
322 Merigoux, & Marmonier, 2011; however, Truhlar, Dodd, & Aldridge, 2014 came to a
323 different conclusion), the affinity of *T. trichiatus* to organic materials might indicate a

324 difference in their feeding preferences. Nevertheless, further data are needed to test our
325 observation on the substrate choice of the species, as well as its potential connection to
326 feeding.

327 In summary, co-existence with *D. villosus* can be achieved at different spatial scales (Kneitel
328 & Chase, 2004). Species considerably smaller and/or flatter than *D. villosus* (e.g. *C. ischnus*
329 and *J. sarsi*) might be able to persist in the same mesohabitat by avoiding it at the
330 microhabitat scale. We assume that this mechanism plays a role in the case of corophiids, as
331 well, coupled with the protection of the tube, which might keep *D. villosus* away at least when
332 the animals form dense colonies among/under stones.

333 Most Ponto-Caspian gammarids show a substrate preference different from *D. villosus*, thus
334 avoiding it at the mesohabitat scale. Environmental factors allowing niche differentiation
335 include current velocity (*D. haemobaphes* and especially *D. bispinosus*; Borza et al., 2017a),
336 and sediment grain size (*O. obesus* and all psammo-pelophilous species; Borza et al., 2017b).
337 Differences in feeding preferences also might lead to stable coexistence if the availability of
338 food sources is spatially heterogeneous, leading to spatial differentiation between the
339 competitors. This mechanism might play a role in the coexistence of *D. haemobaphes* with *D.*
340 *villosus* in relation to suspended matter (Borza et al., 2017a), and possibly also in the case of
341 *T. trichiatus*, showing affinity to organic habitats.

342 Not only Ponto-Caspian gammarids are able to partition habitats with *D. villosus*, as
343 demonstrated by the example of *G. tigrinus*, which – contrarily to its decline in rivers – was
344 able to coexist with the stronger competitor by switching to sandy habitats in Lake IJsselmeer
345 (Platvoet, Dick, et al., 2009). Similarly, *G. roeselii* was able to persist in Lake Constance in
346 macrophyte stands after the invasion of *D. villosus* (Hesselschwerdt et al., 2008). Most non-
347 Ponto-Caspian peracarids apparently cannot persist in waters where *D. villosus* is present;
348 however, they still inhabit smaller rivers and streams of the invaded regions, implying that

349 they can coexist with it in the same catchment (i.e. macrohabitat scale). Nevertheless, there is
350 no guarantee that all species presently not confronted with *D. villosus* will be able to do so.
351 Although the mechanisms of coexistence suggested by our results and summarized above
352 cannot be regarded as a full list of possibilities for coexistence with *D. villosus*, they provide a
353 framework for a preliminary risk assessment in the presently not invaded but potentially
354 colonizable regions of the world. Morphological and habitat preference data of native species
355 could be compiled and used for identifying the most vulnerable ones (i.e. species with body
356 length/width similar to *D. villosus* and a strict preference for stony substrates and lentic
357 conditions), allowing the elaboration of specific management plans. The lessons learned from
358 Ponto-Caspian peracarids could be applied to other macroinvertebrate groups as well, since
359 the same principles (i.e. the avoidance of physical contact) can be expected to determine their
360 coexistence with *D. villosus*.

361

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363

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374

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568 **Tables**

569

570 **TABLE 1** Definitions of substrate types used in the study.

571

Substrate type	Abbreviation	Definition
riprap	RIP	rocks of variable size, artificial
macrolithal	MAL	blocks, large cobbles; grain size 20 cm to 40 cm
mesolithal	MEL	cobbles; grain size 6 cm to 20 cm
microlithal	MIL	coarse gravel; grain size 2 cm to 6 cm
akal	AKA	fine to medium-sized gravel; grain size 0.2 cm to 2 cm
psammal	PSA	sand; grain size 0.063-2 mm
psammopelal	PPE	sand and mud
pelal	PEL	mud (organic); grain size < 0.063 mm
argyllal	ARG	silt, loam, clay (inorganic); grain size < 0.063 mm
macrophytes	MPH	submerged macrophytes, including moss and Characeae
xylal	XYL	tree trunks, dead wood, branches, roots

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.

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	9.29-497.90 µS/cm
Dissolved O ₂ concentration (DO)	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	7.77-8.43
Dissolved organic carbon concentration	combustion catalytic oxidation/NDIR [EN 1484:2002]	b	1.59-7.63 mg/L
Total nitrogen concentration (TN)	spectrophotometry [EN ISO 11905]	b	0.52-2.95 mg/L
Total phosphorus concentration (TP)	spectrophotometry [EN ISO 6878]	b	0.02-0.11 mg/L
Total suspended solids (TSS)	gravimetry [EN 872]	c	2.5-50.0 mg/L

TABLE 3 Range, occurrence, and density of the species during the survey (IQR: interquartile range).

Species	Occurrence			Density (ind./m ² , when present)		
	Range (river km)	No. of sites	No. of samples	Median	IQR	Maximum
<i>Chaetogammarus ischnus</i>	18 - 2415	47	114	25.6	6.4 - 154.4	12816.0
<i>Dikerogammarus bispinosus</i>	1252 - 2258	20	54	27.2	9.6 - 115.2	1865.6
<i>Dikerogammarus haemobaphes</i>	18 - 2415	36	84	17.6	6.4 - 64.0	2220.8
<i>Dikerogammarus villosus</i>	18 - 2581	54	213	169.6	41.6 - 566.4	8345.6
<i>Jaera sarsi</i>	18 - 2415	36	106	94.4	35.2 - 234.4	4652.8
<i>Obesogammarus obesus</i>	18 - 2362	46	140	25.6	6.4 - 129.6	10688.0
<i>Trichogammarus trichiatus</i>	18 - 2354	10	14	9.6	3.2 - 28.0	4012.8

TABLE 4 Consecutive steps of the forward selection procedure on the environmental variables. The seventh step is only shown for comparability; the seventh variable (pH) was not included in the model since the *P*-value exceeded 0.05.

Forward selection		Cumulative var.			
step	Added variable	explained	<i>df</i>	<i>F</i>	<i>P</i>
Step 1	Substrate types	17.10%	10	4.82	< 0.0001
Step 2	Total suspended solids	20.19%	1	7.78	< 0.0001
Step 3	Dissolved O ₂ conc.	22.09%	1	5.24	0.0004
Step 4	Total N conc.	23.99%	1	5.32	0.0004
Step 5	Current velocity	24.90%	1	3.09	0.0143
Step 6	Total P conc.	25.75%	1	2.96	0.0161
(Step 7)	pH	26.06%	1	1.71	0.1280

TABLE 5 The result of the variance partitioning (A + B + C + D = 1).

Variance fraction	%	<i>df</i>	<i>F</i>	<i>P</i>
Environmental and spatial variables (A+B+C)	38.53%	34	4.41	< 0.0001
Environmental variables (A+B)	25.75%	15	5.28	< 0.0001
Spatial variables (B+C)	29.17%	19	5.01	< 0.0001

Overlap (B)	16.39%	not testable		
Environmental variables alone (A)	9.36%	15	2.69	< 0.0001
Spatial variables alone (C)	12.78%	19	2.86	< 0.0001
Residuals (D)	61.47%	not testable		

TABLE 6 Variance explained by the canonical axes (not comparable with the results of the variance partitioning since adjusted R^2 -values are not available for axes).

Canonical axis	<i>df</i>	Variance %	<i>F</i>	<i>P</i>
RDA1	1	18.67%	66.24	< 0.0001
RDA2	1	13.86%	49.18	< 0.0001
RDA3	1	7.57%	26.84	< 0.0001
RDA4	1	4.31%	15.28	< 0.0001
RDA5	1	2.67%	9.47	< 0.0001
RDA6	1	1.86%	6.61	< 0.0001
RDA7	1	0.89%	3.16	0.0127

Residual	178	50.17%
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Figure legends

FIGURE 1 Macroinvertebrate sampling sites during the 3rd Joint Danube Survey. 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.

FIGURE 2 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.

FIGURE 3 Triplot showing the results of the RDA including six environmental and the 19 spatial explanatory variables ('WA' scores, species scaling). A: RDA1 vs. RDA2, B: RDA3 vs. RDA2. Empty circles represent samples. Ci: *Chaetogammarus ischnus*, Db: *Dikerogammarus bispinosus*, Dh: *Dikerogammarus haemobaphes*, Dv: *Dikerogammarus villosus*, Js: *Jaera sarsi*, Oo: *Obesogammarus obesus*, Tt: *Trichogammarus trichiatus*. Arrows represent continuous environmental variables (cur: current velocity, diO: dissolved oxygen concentration, toN: total nitrogen concentration, toP: total phosphorus concentration, tss: total suspended solids). Substrate type abbreviations as in Table 1. AEM eigenfunctions are not shown for the sake of perspicuity.

FIGURE 4 Density of the species on the different substrate types ($\log(x)+1$ -transformed).

Abbreviations as in Table 1.

FIGURE 5 Network representations of the pairwise comparisons of the parameter estimates of substrate types in the GLMs (created using the ‘igraph’ package; Csardi & Nepusz, 2006).

A: *C. ischnus*, B: *J. sarsi*, C: *O. obesus*. Nodes represent substrate types (abbreviations as in

Table 1), arrows represent significant differences ($P < 0.05$), pointing at the larger value.

Numerical results are shown in Tables S3-5.

Figures

FIGURE 1

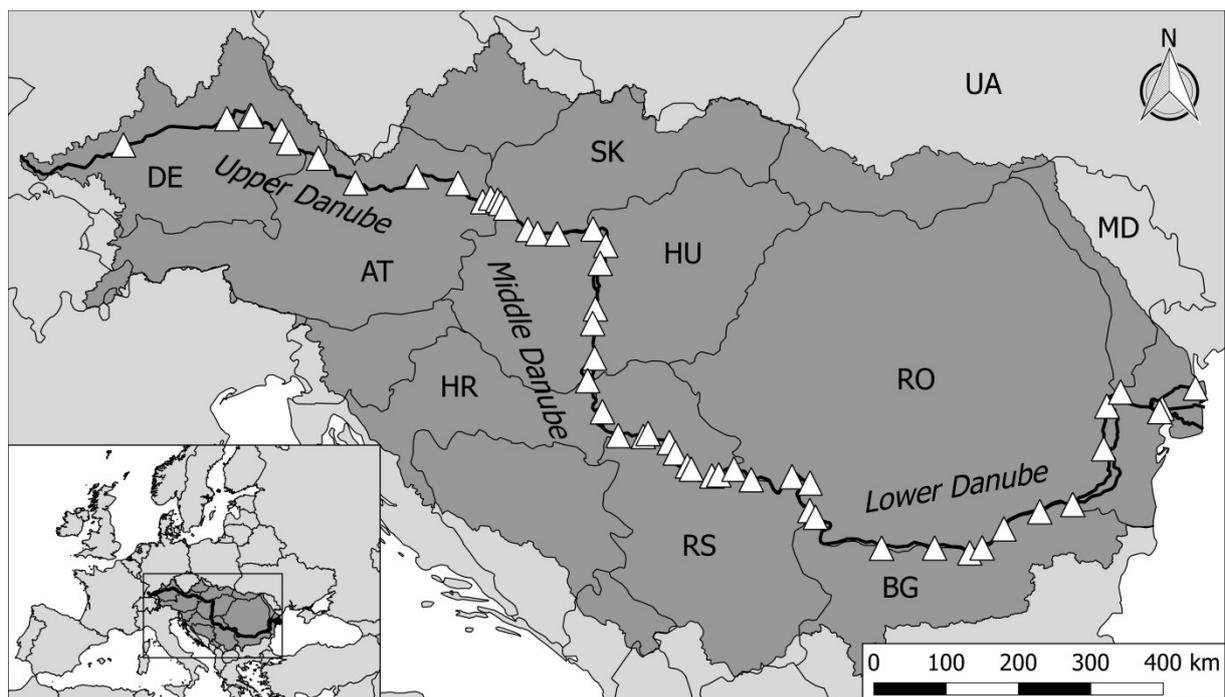


FIGURE 2

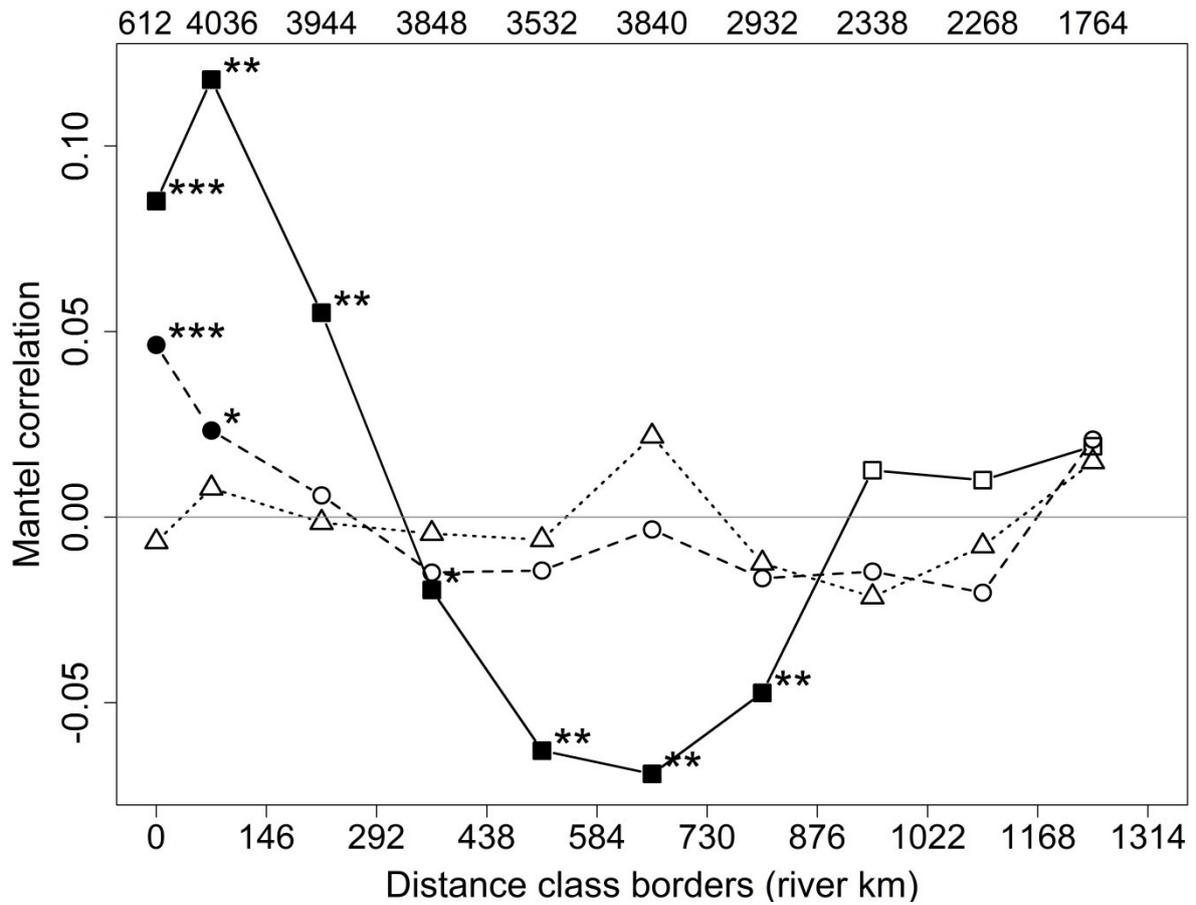


FIGURE 3

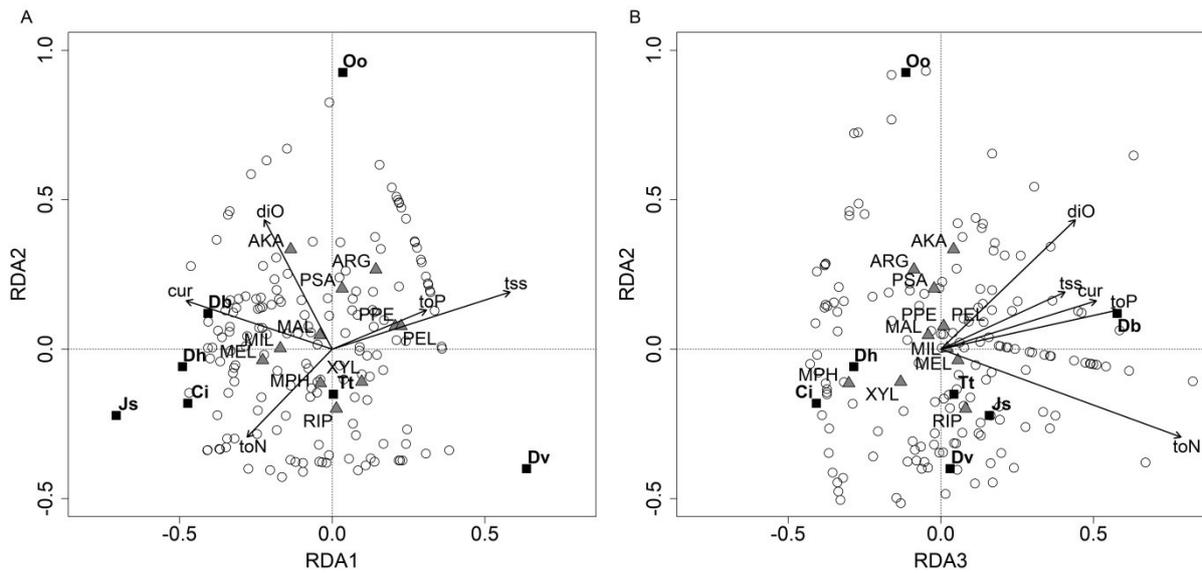


FIGURE 4

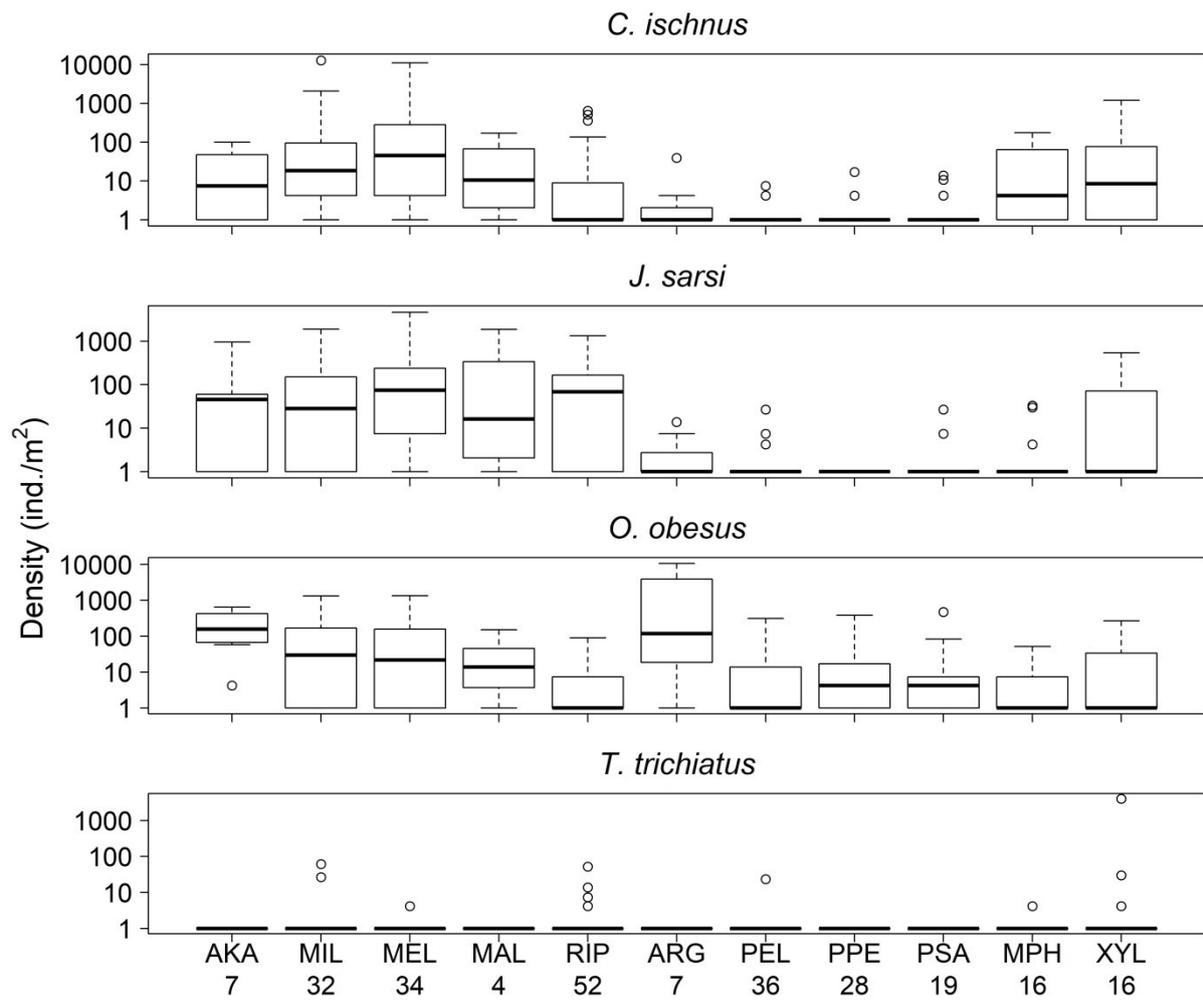
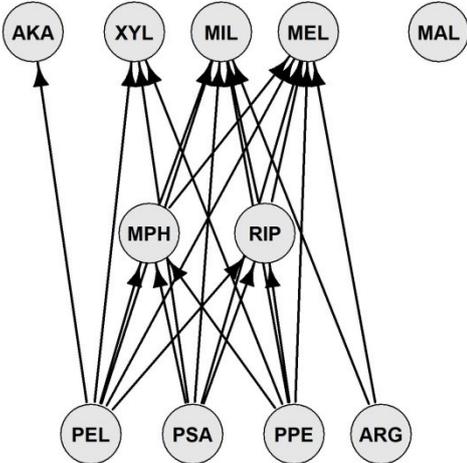
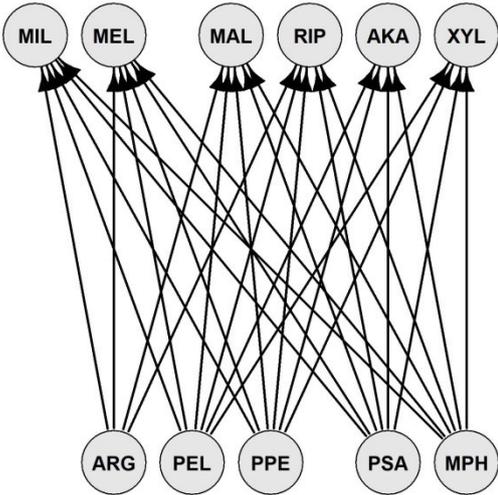


FIGURE 5

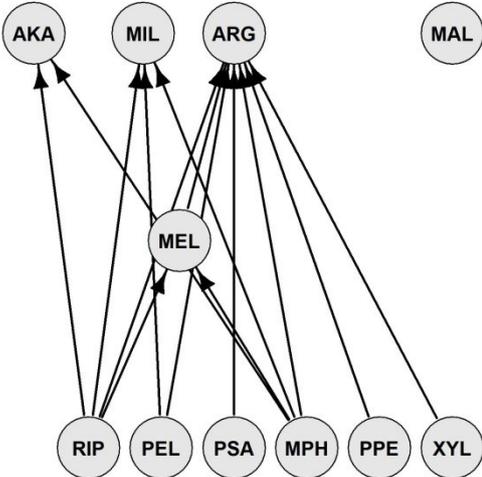
A



B



C



Supporting Information

How to coexist with the 'killer shrimp' *Dikerogammarus villosus*? Lessons from other invasive Ponto-Caspian peracarids

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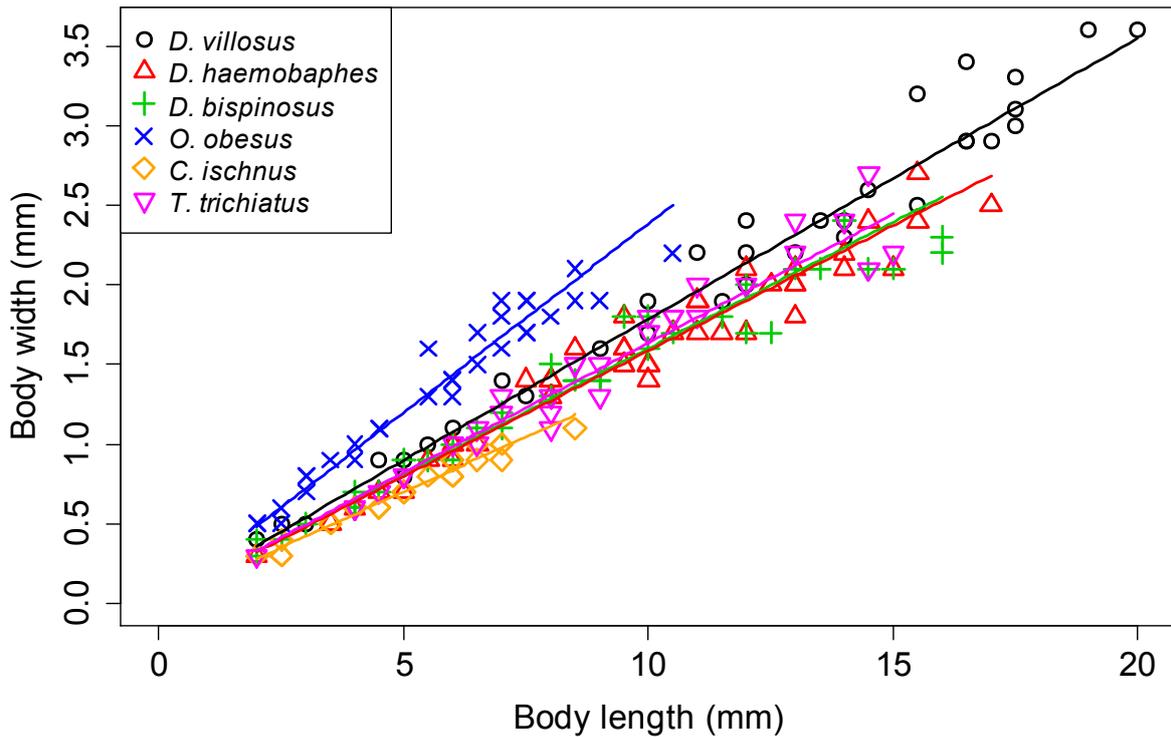


Figure S1 Body length-body width relationships in the studied gammarid species; given only as an illustration of their characteristic morphological differences. The measurements were made by ocular micrometer on specimens collected in several different waters in Hungary (collection of the Danube Research Institute, Budapest, Hungary). The largest specimens measured here do not represent the maximal sizes reported in the literature, but approximate it. While the majority of the included gammarids attain body sizes > 15 mm and differ little in their body proportions, *O. obesus* and *C. ischnus* grow considerably smaller and deviate from the standard body shape in opposing directions. Note: the characteristic body shape of *O. obesus* and *C. ischnus* is also reflected in their scientific names (*obesus*: fat, plump; *ischnus*: thin, lean). The dorsoventrally flattened isopod *Jaera sarsi* attains 2-3 mm body length and ~0.5 mm body height. The line segments represent the fitted linear models (see Table S1 and S2 for details).

Table S1 Number of specimens, body length range, and model parameters of the species included in the analysis. A linear model without intercept was fitted on ln-ln transformed data (power function, necessary since standard deviation increased with body length) including all species in R 3.2.5 (R Core Team, 2016). As the species-body length interactions could be neglected, the model contains one parameter for ln-transformed body length (estimated as 0.992 ± 0.012 , indicating an approximately linear relationship), and one parameter for each species (included in the table). Adjusted $R^2 = 0.982$.

Species	No. of specimens	Body length range (mm)	Model parameter estimate \pm SE
<i>Chaetogammarus ischnus</i>	23	2.0-8.5	-1.952 \pm 0.025
<i>Dikerogammarus bispinosus</i>	36	2.0-16.0	-1.812 \pm 0.027
<i>Dikerogammarus haemobaphes</i>	42	2.0-17.0	-1.822 \pm 0.029
<i>Dikerogammarus villosus</i>	38	2.0-20.0	-1.704 \pm 0.029
<i>Obesogammarus obesus</i>	32	2.0-10.5	-1.414 \pm 0.024
<i>Trichogammarus trichiatus</i>	31	2.0-15.0	-1.791 \pm 0.029

Table S2 Pairwise comparisons of the species parameters of the model, calculated by the ‘glht’ function in the ‘multcomp’ package (Hothorn et al., 2008) with Tukey correction. Ci: *Chaetogammarus ischnus*, Db: *Dikerogammarus bispinosus*, Dh: *Dikerogammarus haemobaphes*, Dv: *Dikerogammarus villosus*, Oo: *Obesogammarus obesus*, Tt: *Trichogammarus trichiatus*.

Null hypothesis	Estimate	Std. error	<i>t</i>	<i>P</i>
Db - Ci = 0	0.140	0.023	6.169	< 0.001
Dh - Ci = 0	0.131	0.023	5.743	< 0.001
Dv - Ci = 0	0.249	0.023	10.641	< 0.001
Oo - Ci = 0	0.538	0.023	23.766	< 0.001
Tt - Ci = 0	0.161	0.024	6.811	< 0.001
Dh - Db = 0	-0.009	0.019	-0.495	0.996
Dv - Db = 0	0.108	0.019	5.567	< 0.001
Oo - Db = 0	0.398	0.021	19.367	< 0.001
Tt - Db = 0	0.021	0.020	1.024	0.908
Dv - Dh = 0	0.118	0.019	6.347	< 0.001
Oo - Dh = 0	0.407	0.020	19.888	< 0.001
Tt - Dh = 0	0.030	0.020	1.535	0.639
Oo - Dv = 0	0.290	0.021	13.748	< 0.001
Tt - Dv = 0	-0.087	0.020	-4.35	< 0.001
Tt - Oo = 0	-0.377	0.021	17.547	< 0.001

Table S3 Pairwise comparisons of the parameter estimates of substrate types in the GLM for *C. ischnus*. Abbreviations as in Table 1, significance codes: ‘***’: $P < 0.001$; ‘**’: $P < 0.01$; ‘*’: $P < 0.05$; ‘.’: $P < 0.1$.

Hypothesis	Estimate	Std. error	<i>z</i>	<i>P</i>
MIL - AKA = 0	2.94763	1.05662	2.790	0.1454
MEL - AKA = 0	3.31438	1.05107	3.153	0.0543 .
MAL - AKA = 0	0.48206	1.58624	0.304	1.0000
RIP - AKA = 0	0.24202	1.02070	0.237	1.0000
ARG - AKA = 0	-1.63974	1.37811	-1.190	0.9810
PEL - AKA = 0	-3.89639	1.11228	-3.503	0.0179 *
PPE - AKA = 0	-3.02604	1.10519	-2.738	0.1656
PSA - AKA = 0	-3.00600	1.16732	-2.575	0.2395
MPH - AKA = 0	0.16713	1.14868	0.145	1.0000
XYL - AKA = 0	1.37055	1.14700	1.195	0.9803
MEL - MIL = 0	0.36675	0.61969	0.592	0.9999
MAL - MIL = 0	-2.46557	1.33993	-1.840	0.7332
RIP - MIL = 0	-2.70560	0.56663	-4.775	< 0.001 ***
ARG - MIL = 0	-4.58737	1.08556	-4.226	< 0.01 **
PEL - MIL = 0	-6.84402	0.71862	-9.524	< 0.001 ***
PPE - MIL = 0	-5.97366	0.70759	-8.442	< 0.001 ***
PSA - MIL = 0	-5.95362	0.80118	-7.431	< 0.001 ***
MPH - MIL = 0	-2.78050	0.77378	-3.593	0.0127 *
XYL - MIL = 0	-1.57708	0.77127	-2.045	0.5892
MAL - MEL = 0	-2.83232	1.33556	-2.121	0.5337
RIP - MEL = 0	-3.07235	0.55623	-5.524	< 0.001 ***
ARG - MEL = 0	-4.95412	1.08016	-4.586	< 0.001 ***
PEL - MEL = 0	-7.21077	0.71045	10.150	< 0.001 ***
PPE - MEL = 0	-6.34042	0.69929	-9.067	< 0.001 ***
PSA - MEL = 0	-6.32037	0.79385	-7.962	< 0.001 ***
MPH - MEL = 0	-3.14725	0.76619	-4.108	< 0.01 **
XYL - MEL = 0	-1.94383	0.76366	-2.545	0.2553
RIP - MAL = 0	-0.24003	1.31178	-0.183	1.0000
ARG - MAL = 0	-2.12180	1.60566	-1.321	0.9600
PEL - MAL = 0	-4.37845	1.38424	-3.163	0.0516 .
PPE - MAL = 0	-3.50810	1.37855	-2.545	0.2558
PSA - MAL = 0	-3.48805	1.42884	-2.441	0.3141
MPH - MAL = 0	-0.31493	1.41366	-0.223	1.0000
XYL - MAL = 0	0.88849	1.41229	0.629	0.9999
ARG - RIP = 0	-1.88177	1.05063	-1.791	0.7641
PEL - RIP = 0	-4.13841	0.66468	-6.226	< 0.001 ***
PPE - RIP = 0	-3.26806	0.65274	-5.007	< 0.001 ***
PSA - RIP = 0	-3.24802	0.75317	-4.312	< 0.001 ***
MPH - RIP = 0	-0.07490	0.72395	-0.103	1.0000
XYL - RIP = 0	1.12852	0.72128	1.565	0.8840
PEL - ARG = 0	-2.25665	1.13981	-1.980	0.6358

PPE - ARG = 0	-1.38629	1.13289	-1.224	0.9766
PSA - ARG = 0	-1.36625	1.19358	-1.145	0.9857
MPH - ARG = 0	1.80687	1.17536	1.537	0.8955
XYL - ARG = 0	3.01029	1.17372	2.565	0.2451
PPE - PEL = 0	0.87035	0.78830	1.104	0.9892
PSA - PEL = 0	0.89039	0.87328	1.020	0.9942
MPH - PEL = 0	4.06352	0.84820	4.791	< 0.001 ***
XYL - PEL = 0	5.26694	0.84592	6.226	< 0.001 ***
PSA - PPE = 0	0.02004	0.86422	0.023	1.0000
MPH - PPE = 0	3.19316	0.83888	3.806	< 0.01 **
XYL - PPE = 0	4.39658	0.83657	5.255	< 0.001 ***
MPH - PSA = 0	3.17312	0.91920	3.452	0.0211 *
XYL - PSA = 0	4.37654	0.91709	4.772	< 0.001 ***
XYL - MPH = 0	1.20342	0.89325	1.347	0.9543