This manuscript is textually identical with the published paper:

Borza P, Huber T, Leitner P, Remund N, Graf W (2018): How to coexist with the 'killer shrimp' *Dikerogammarus villosus*? Lessons from other invasive Ponto-Caspian peracarids. Aquatic Conservation: Marine and Freshwater Ecosystems 28(6): 1441-1450.

The original publication is available at:

https://onlinelibrary.wiley.com/doi/10.1002/aqc.2985

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

Péter Borza[1,2], Thomas Huber[3], Patrick Leitner[3], Nadine Remund[4], Wolfram Graf[3]

[1]GINOP Sustainable Ecosystems Group, MTA Centre for Ecological Research, Tihany, Hungary
[2]Danube Research Institute, MTA Centre for Ecological Research, Budapest, Hungary
[3]Department of Water, Atmosphere & Environment, Institute for Hydrobiology & Water Management, BOKU - University of Natural Resources and Applied Life Sciences, Vienna, Austria
[4]UNA - Atelier für Naturschutz und Umweltfragen, Bern, Switzerland

Correspondence: Péter Borza, GINOP Sustainable Ecosystems Group, MTA Centre for Ecological Research, Klebelsberg Kuno utca 3, H-8237 Tihany, Hungary. E-mail: borza.peter@okologia.mta.hu

Abstract

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

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

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

1 Introduction

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

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

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

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

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

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

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

2 Methods

2.1 Sample collection and processing

The macroinvertebrate 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 (Figure 1) between Ulm (river km 2581) and the Delta (river km 18, Kiliya branch) by the ‘multi-habitat’ approach based on the AQEM protocol (Hering, Moog, Sandin, & Verdonschot, 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 specimens in the samples.

2.2 Data analysis

Only free-living, benthic Ponto-Caspian invasive peracarid species were included in the analysis; six gammarids (*Chaetogammarus* (formerly *Echinogammarus*) *ischnus* (Stebbing, 1899), *Dikerogammarus bispinosus* Martynov, 1925, *D. haemobaphes* (Eichwald, 1841), *D. villosus*, *Obesogammarus obesus* (G.O. Sars, 1894), and *Trichogammarus* (formerly *Echinogammarus*) *trichiatus* (Martynov, 1932)), and the isopod *Jaera sarsi* Valkanov, 1936. The niche differentiation among the three invasive *Dikerogammarus* species was analyzed in detail by Borza et al. (2017a) based on the same survey. Nevertheless, *D. bispinosus* and *D. haemobaphes* were included in the present study to allow the comparison of their habitat preferences with that of the other species. Mysids were excluded, since their habitat use is markedly different from *D. villosus* (epibenthic or semi-pelagic). In addition, they reach high abundance mainly in semi-enclosed inlets and slow-flowing sidearms, so they were found only sporadically during the survey (Borza et al., 2015). The filter feeding, tube-dwelling corophiids were excluded, too, since the data suggested that their abundance is primarily determined by the quality and quantity of suspended matter, not habitat characteristics (Borza, Huber, Leitner, Remund, & Graf, 2018). Nevertheless, we share our remarks on the possible mechanisms of their co-existence with *D. villosus* in the Discussion.

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

To provide an insight into the structure of spatial autocorrelation (SA henceforth) across multiple spatial scales, Mantel correlograms (Borcard & Legendre, 2012) were constructed using the 'mantel.correlog' function in the 'vegan' package about (1) the response variables representing both environmentally explainable SA ('induced spatial dependence') and environmentally not explainable ('true') SA (Legendre & Legendre, 2012), (2) the residuals of the environmental model (representing 'true' SA 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 146 river km; the last seven are not shown). *P*-values of the Mantel correlation coefficients were calculated with Holm-correction.

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

3 Results

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

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

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

All seven constrained axes of the RDA explained a significant proportion of the variance (Table 6); nevertheless, the first three axes (cumulative proportion explained: 40.10 %) provide a sufficient basis for the interpretation of the results (Figure 3). Current velocity and TSS – the most important factors of niche differentiation among the three *Dikerogammarus* species (Borza et al., 2017a) – were considerably correlated with all three canonical axes; therefore, the separation of the three *Dikerogammarus* species in the present analysis was observable in three dimensions. *D. villosus* separated from *D. haemobaphes* and *D. bispinosus* along the first and second axes (Figure 3a), whereas the latter two species differentiated primarily along the third axis (Figure 3b). The position of *C. ischnus* and *J. sarsi* was close to *D. haemobaphes* on the first and second axes (Figure 3a), reflecting their preference for gravel (especially micro- and mesolithal). However, the two species separated considerably along the third axis (Figure 3b), owing to the higher affinity of *J. sarsi* to ripraps. *O. obesus* differentiated markedly from all the other species along the second axis (Figure 3), reflecting its association with akal and argyllal. Due to its rarity, the position of *T. trichiatus* was close to the origin of the ordination space (Figure 3). Its only massive occurrence (> 4 000 ind./m^2) was recorded on xylal (Figure 4).

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

4 Discussion

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

The preference of *C. ischnus* for gravel proved to be an effective way to avoid *D. villosus*; however, it resulted in a strong overlap with *D. haemobaphes*, a species capable of similarly aggressive predation as its notorious relative (Bacela-Spychalska & Van der Velde, 2013).

Size-dependent microhabitat choice is a widely reported phenomenon among gammarids (Devin et al., 2003; Hacker & Steneck, 1990; Jermacz, Dzierżyńska, Poznańska, & Kobak, 2015; Platvoet, Dick, et al., 2009); therefore, we assume that the relatively small-sized and strongly flattened *C. ischnus* (Figure S1) can utilize the deep, narrow interstices among coarse gravel. As only smaller specimens of the more robust *Dikerogammarus* species (Figure S1) can enter the crevices of a given width, *C. ischnus* can avoid direct interference with larger, potentially dangerous individuals. Accordingly, the mesohabitat-preference shown by our results might in fact reflect differences in microhabitat use, since interstices of the preferred width might be most abundant in micro- and mesolithal.

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

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

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

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

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

Not only Ponto-Caspian gammarids are able to partition habitats with *D. villosus*, as demonstrated by the example of *G. tigrinus*, which – contrarily to its decline in rivers – was able to coexist with the stronger competitor by switching to sandy habitats in Lake IJselmeer (Platvoet, Dick, et al., 2009). Similarly, *G. roeselii* was able to persist in Lake Constance in macrophyte stands after the invasion of *D. villosus* (Hesselschwerdt et al., 2008). Most non-Ponto-Caspian peracarids apparently cannot persist in waters where *D. villosus* is present; however, they still inhabit smaller rivers and streams of the invaded regions, implying that they can coexist with it in the same catchment (i.e. macrohabitat scale). Nevertheless, there is no guarantee that all species presently not confronted with *D. villosus* will be able to do so. Although the mechanisms of coexistence suggested by our results and summarized above cannot be regarded as a full list of possibilities for coexistence with *D. villosus*, they provide a framework for a preliminary risk assessment in the presently not invaded but potentially colonizable regions of the world. Morphological and habitat preference data of native species could be compiled and used for identifying the most vulnerable ones (i.e. species with body length/width similar to *D. villosus* and a strict preference for stony substrates and lentic conditions), allowing the elaboration of specific management plans. The lessons learned from Ponto-Caspian peracarids could be applied to other macroinvertebrate groups as well, since the same principles (i.e. the avoidance of physical contact) can be expected to determine their coexistence with *D. villosus*.

Acknowledgements

Joint Danube Survey 3 was organized by the International Commission for the Protection of the Danube River (ICPDR). We would like to thank everyone involved in the organization, field work, and evaluation of the survey for their effort. This work was supported by the MARS project (Managing Aquatic ecosystems and water Resources under multiple Stress) funded by the European Union under the 7th Framework Programme, grant agreement no: 603378, and the GINOP 2.3.2-15-2016-00019 grant. Péter Borza was supported by the Scholarship of the Scholarship Foundation of the Republic of Austria for Post-docs from October 2013 until March 2014 (funding organization: OeAD-GmbH on behalf of and financed by the Scholarship Foundation of the Republic of Austria). We would like to thank two anonymous referees for their useful comments on an earlier version of the manuscript.

References

Bacela-Spychalska, K., Grabowski, M., Rewicz, T., Konopacka, A., & Wattier, R. (2013). The “killer shrimp” *Dikerogammarus villosus* (Crustacea, Amphipoda) invading Alpine lakes: Overland transport by recreational boats and scuba-diving gear as potential entry vectors? *Aquatic Conservation: Marine and Freshwater Ecosystems*, 23, 606–618.

Bacela-Spychalska, K., & Van der Velde, G. (2013). There is more than one “killer shrimp”: Trophic positions and predatory abilities of invasive amphipods of Ponto-Caspian origin. *Freshwater Biology*, 58, 730–741.

Beggel, S., Brandner, J., Cerwenka, A. F., & Geist, J. (2016). Synergistic impacts by an invasive amphipod and an invasive fish explain native gammarid extinction. *BMC Ecology*, 16, 32.

Blanchet, F. G., Legendre, P., & Borcard, D. (2008a). Modelling directional spatial processes in ecological data. *Ecological Modelling*, 215, 325–336.

Blanchet, F. G., Legendre, P., & Borcard, D. (2008b). Forward selection of explanatory variables. *Ecology*, 89, 2623–2632.

Blanchet, F. G., Legendre, P., Maranger, R., Monti, D., & Pepin, P. (2011). Modelling the effect of directional spatial ecological processes at different scales. *Oecologia*, 166, 357–368.

Borcard, D., & Legendre, P. (2012). Is the Mantel correlogram powerful enough to be useful in ecological analysis? A simulation study. *Ecology*, 93, 1473–1481.

Borza, P. (2009). First record of the Ponto-Caspian amphipod *Echinogammarus trichiatus* (Martynov, 1932) (= *Chaetogammarus trichiatus*) (Crustacea: Amphipoda) for the Middle-Danube (Slovakia and Hungary). *Aquatic Invasions*, 4, 693–696.

Borza, P., Csányi, B., Huber, T., Leitner, P., Paunović, M., Remund, N., … Graf, W. (2015). Longitudinal distributional patterns of Peracarida (Crustacea, Malacostraca) in the River Danube. *Fundamental and Applied Limnology*, 187, 113–126.

Borza, P., Erős, T., & Oertel, N. (2009). Food resource partitioning between two invasive gobiid species (Pisces, Gobiidae) in the littoral zone of the River Danube, Hungary. *International Review of Hydrobiology*, 94, 609–621.

Borza, P., Huber, T., Leitner, P., Remund, N., & Graf, W. (2017a). Current velocity shapes co-existence patterns among invasive *Dikerogammarus* species. *Freshwater Biology*, 62, 317–328.

Borza, P., Huber, T., Leitner, P., Remund, N., & Graf, W. (2017b). Success factors and future prospects of Ponto–Caspian peracarid (Crustacea: Malacostraca) invasions: Is "the worst over"? *Biological Invasions*, 19, 1517–1532.

Borza, P., Huber, T., Leitner, P., Remund, N., & Graf, W. (2018). Niche differentiation among invasive Ponto-Caspian *Chelicorophium* species (Crustacea, Amphipoda, Corophiidae) by food particle size. *Aquatic Ecology*, DOI: 10.1007/s10452-018-9653-8.

Chase, J. M., & Leibold, M. A. (2003). *Ecological niches: Linking classical and contemporary approaches*. Chicago and London: University of Chicago Press.

Csardi, G., & Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal, Complex Systems*, 1695.

De Gelder, S., Van der Velde, G., Platvoet, D., Leung, N., Dorenbosch, M., Hendriks, H. W. M., & Leuven, R. (2016). Competition for shelter sites: Testing a possible mechanism for gammarid species displacements. *Basic and Applied Ecology*, 17, 455–462.

Devin, S., Piscart, C., Beisel, J. N., & Moreteau, J. C. (2003). Ecological traits of the amphipod invader *Dikerogammarus villosus* on a mesohabitat scale. *Archiv Für Hydrobiologie*, 158, 43–56.

Dick, J. T., & Platvoet, D. (2000). Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267, 977–983.

Gallardo, B., & Aldridge, D. C. (2013). Priority setting for invasive species management: Risk assessment of Ponto-Caspian invasive species into Great Britain. *Ecological Applications*, 23, 352–364.

Gallardo, B., & Aldridge, D. C. (2015). Is Great Britain heading for a Ponto–Caspian invasional meltdown? *Journal of Applied Ecology*, 52, 41–49.

Gergs, R., Koester, M., Schulz, R. S., & Schulz, R. (2014). Potential alteration of cross-ecosystem resource subsidies by an invasive aquatic macroinvertebrate: Implications for the terrestrial food web. *Freshwater Biology*, 59, 2645–2655.

Gergs, R., & Rothhaupt, K.-O. (2008). Effects of zebra mussels on a native amphipod and the invasive *Dikerogammarus villosus*: The influence of biodeposition and structural complexity. *Journal of the North American Benthological Society*, 27, 541–548.

Grabowska, J., & Grabowski, M. (2005). Diel-feeding activity in early summer of racer goby *Neogobius gymnotrachelus* (Gobiidae): A new invader in the Baltic basin. *Journal of Applied Ichthyology*, 21, 282–286.

Hacker, S. D., & Steneck, R. S. (1990). Habitat Architecture and the Abundance and Body-Size-Dependent Habitat Selection of a Phytal Amphipod. *Ecology*, 71, 2269–2285.

Hellmann, C., Worischka, S., Mehler, E., Becker, J., Gergs, R., & Winkelmann, C. (2015). The trophic function of *Dikerogammarus villosus* (Sowinsky, 1894) in invaded rivers: A case study in the Elbe and Rhine. *Aquatic Invasions*, 10, 385–397.

Hering, D., Moog, O., Sandin, L., & Verdonschot, P. F. (2004). Overview and application of the AQEM assessment system. *Hydrobiologia*, 516, 1–20.

Hesselschwerdt, J., Necker, J., & Wantzen, K. M. (2008). Gammarids in Lake Constance: Habitat segregation between the invasive *Dikerogammarus villosus* and the indigenous *Gammarus roeselii*. *Fundamental and Applied Limnology/Archiv Für Hydrobiologie*, 173, 177–186.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, 50, 346–363.

Hulme, P. E. (2009). Trade, transport and trouble: Managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, 46, 10–18.

Jermacz, Ł., Dzierżyńska, A., Poznańska, M., & Kobak, J. (2015). Experimental evaluation of preferences of an invasive Ponto-Caspian gammarid *Pontogammarus robustoides* (Amphipoda, Gammaroidea) for mineral and plant substrata. *Hydrobiologia*, 746, 209–221.

Jones, L. A., & Ricciardi, A. (2005). Influence of physicochemical factors on the distribution and biomass of invasive mussels (*Dreissena polymorpha* and *Dreissena bugensis*) in the St. Lawrence River. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 1953–1962.

Jourdan, J., Westerwald, B., Kiechle, A., Chen, W., Streit, B., Klaus, S., … Plath, M. (2016). Pronounced species turnover, but no functional equivalence in leaf consumption of invasive amphipods in the river Rhine. *Biological Invasions*, 18, 763–774.

Karatayev, A. Y., Burlakova, L. E., Pennuto, C., Ciborowski, J., Karatayev, V. A., Juette, P., & Clapsadl, M. (2014). Twenty five years of changes in *Dreissena* spp. populations in Lake Erie. *Journal of Great Lakes Research*, 40, 550–559.

Kneitel, J. M., & Chase, J. M. (2004). Trade-offs in community ecology: Linking spatial scales and species coexistence. *Ecology Letters*, 7, 69–80.

Kobak, J., Jermacz, Ł., & Dzierżyńska-Białończyk, A. (2015). Substratum preferences of the invasive killer shrimp *Dikerogammarus villosus*. *Journal of Zoology*, 297, 66–76.

Kobak, J., Rachalewski, M., & Bącela-Spychalska, K. (2016). Conquerors or exiles? Impact of interference competition among invasive Ponto-Caspian gammarideans on their dispersal rates. *Biological Invasions*, 18, 1953–1965.

Kobak, J., & Żytkowicz, J. (2007). Preferences of invasive Ponto-Caspian and native European gammarids for zebra mussel (*Dreissena polymorpha*, Bivalvia) shell habitat. *Hydrobiologia*, 589, 43–54.

Koehl, M. A. R. (1996). When does morphology matter? *Annual Review of Ecology and Systematics*, 27, 501–542.

Koester, M., Bayer, B., & Gergs, R. (2016). Is *Dikerogammarus villosus* (Crustacea, Gammaridae) a “killer shrimp”in the River Rhine system? *Hydrobiologia*, 768, 299–313.

Koester, M., & Gergs, R. (2014). No evidence for intraguild predation of *Dikerogammarus villosus* (Sowinsky, 1894) at an invasion front in the Untere Lorze, Switzerland. *Aquatic Invasions*, 9, 489–497.

Lederer, A., Massart, J., & Janssen, J. (2006). Impact of round gobies (*Neogobius melanostomus*) on dreissenids (*Dreissena polymorpha* and *Dreissena bugensis*) and the associated macroinvertebrate community across an invasion front. *Journal of Great Lakes Research*, 32, 1–10.

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, 129, 271–280.

Legendre, P., & Legendre, L. F. (2012). *Numerical ecology. 3rd English edition.* (Vol. 24). Amsterdam: Elsevier.

Leuven, R. S., Van der Velde, G., Baijens, I., Snijders, J., Van der Zwart, C., Lenders, H. R., & Bij de Vaate, A. (2009). The river Rhine: A global highway for dispersal of aquatic invasive species. *Biological Invasions*, 11, 1989–2008.

MacNeil, C., Dick, J. T., Platvoet, D., & Briffa, M. (2010). Direct and indirect effects of species displacements: An invading freshwater amphipod can disrupt leaf-litter processing and shredder efficiency. *Journal of the North American Benthological Society*, 30, 38–48.

Marescaux, J., Boets, P., Lorquet, J., Sablon, R., Van Doninck, K., & Beisel, J.-N. (2015). Sympatric *Dreissena* species in the Meuse River : Towards a dominance shift from zebra to quagga mussels. *Aquatic Invasions*, 10, 287–298.

Müller, R., & Eggers, T. O. (2006). Erste Nachweise von *Echinogammarus trichiatus* (Martynov, 1932) in Brandenburg und Berlin (Crustacea: Amphipoda). *Lauterbornia*, 58, 123–126.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., … Wagner, H. (2017). vegan: Community Ecology Package. R package version 2.4-3. http://CRAN.R-project.org/package=vegan.

Pagnucco, K. S., Maynard, G. A., Fera, S. A., Yan, N. D., Nalepa, T. F., & Ricciardi, A. (2015). The future of species invasions in the Great Lakes-St. Lawrence River basin. *Journal of Great Lakes Research*, 41, 96–107.

Peyer, S. M., McCarthy, A. J., & Lee, C. E. (2009). Zebra mussels anchor byssal threads faster and tighter than quagga mussels in flow. *Journal of Experimental Biology*, 212, 2027–2036.

Piscart, C., Mermillod-Blondin, F., Maazouzi, C., Merigoux, S., & Marmonier, P. (2011). Potential impact of invasive amphipods on leaf litter recycling in aquatic ecosystems. *Biological Invasions*, 13, 2861–2868.

Platvoet, D., Dick, J. T., MacNeil, C., van Riel, M. C., & van der Velde, G. (2009). Invader–invader interactions in relation to environmental heterogeneity leads to zonation of two invasive amphipods, *Dikerogammarus villosus* (Sowinsky) and *Gammarus tigrinus* Sexton: amphipod pilot species project (AMPIS) report 6. *Biological Invasions*, 11, 2085–2093.

Platvoet, D., Van Der Velde, G., Dick, J. T., & Li, S. (2009). Flexible omnivory in *Dikerogammarus villosus* (Sowinsky, 1894)(Amphipoda)—Amphipod Pilot Species Project (AMPIS) Report 5. *Crustaceana*, 82, 703–720.

R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rewicz, T., Wattier, R., Rigaud, T., Grabowski, M., Mamos, T., & Bącela-Spychalska, K. (2017). The killer shrimp, *Dikerogammarus villosus*, invading European Alpine Lakes: A single main source but independent founder events with an overall loss of genetic diversity. *Freshwater Biology*, 62, 1036–1051.

Ricciardi, A. (2001). Facilitative interactions among aquatic invaders: Is an“ invasional meltdown” occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 2513–2525.

Ricciardi, A., & Whoriskey, F. G. (2004). Exotic species replacement: Shifting dominance of dreissenid mussels in the Soulanges Canal, upper St. Lawrence River, Canada. *Journal of the North American Benthological Society*, 23, 507–514.

Simberloff, D., & Von Holle, B. (1999). Positive interactions of nonindigenous species: Invasional meltdown? *Biological Invasions*, 1, 21–32.

Stewart, T. W., Miner, J. G., & Lowe, R. L. (1998). Macroinvertebrate communities on hard substrates in western Lake Erie: structuring effects of *Dreissena*. *Journal of Great Lakes Research*, 24, 868–879.

Tricarico, E., Mazza, G., Orioli, G., Rossano, C., Scapini, F., & Gherardi, F. (2010). The killer shrimp, *Dikerogammarus villosus* (Sowinsky, 1894), is spreading in Italy. *Aquatic Invasions*, 5, 211–214.

Truhlar, A. M., Dodd, J. A., & Aldridge, D. C. (2014). Differential leaf-litter processing by native (*Gammarus pulex*) and invasive (*Dikerogammarus villosus*) freshwater crustaceans under environmental extremes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 24, 56–65.

Van Riel, M. C., Healy, E. P., Van der Velde, G., & Bij de Vaate, A. (2007). Interference competition among native and invader amphipods. *Acta Oecologica*, 31, 282–289.

Van Riel, M. C., Van der Velde, G., Rajagopal, S., Marguillier, S., Dehairs, F., & Bij de Vaate, A. (2006). Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. *Hydrobiologia*, 565, 39–58.

Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S. Fourth Edition.* New York: Springer.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York: Springer Science and Business Media.

Tables

TABLE 1 Definitions of substrate types used in the study.

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
Chaetogammarus ischnus	18 - 2415	47	114	25.6	6.4 - 154.4	12816.0
Dikerogammarus bispinosus	1252 - 2258	20	54	27.2	9.6 - 115.2	1865.6
Dikerogammarus haemobaphes	18 - 2415	36	84	17.6	6.4 - 64.0	2220.8
Dikerogammarus villosus	18 - 2581	54	213	169.6	41.6 - 566.4	8345.6
Jaera sarsi	18 - 2415	36	106	94.4	35.2 - 234.4	4652.8
Obesogammarus obesus	18 - 2362	46	140	25.6	6.4 - 129.6	10688.0
Trichogammarus trichiatus	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 step	Added variable	Cumulative var. explained	*df*	*F*	*P*
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	%	*df*	*F*	*P*
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 avaliable for axes).

Canonical axis	*df*	Variance %	*F*	*P*
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%

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

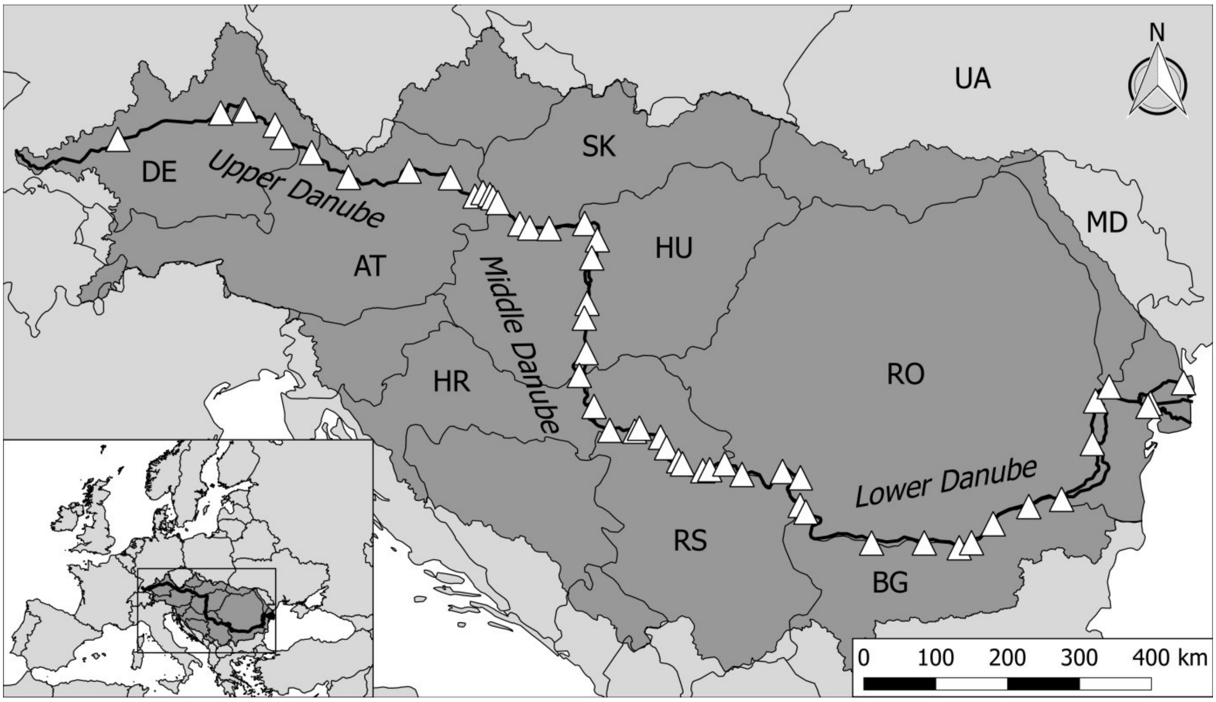


FIGURE 2

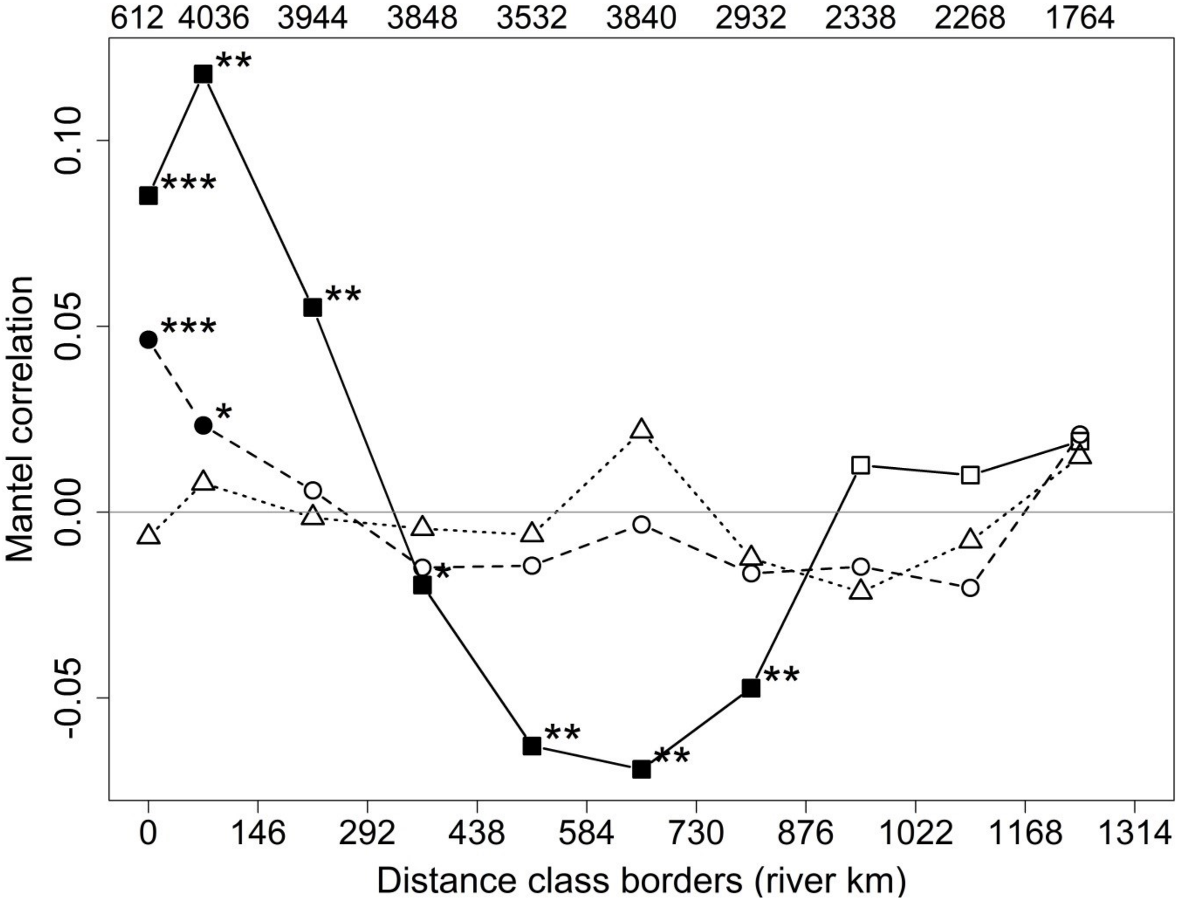


FIGURE 3

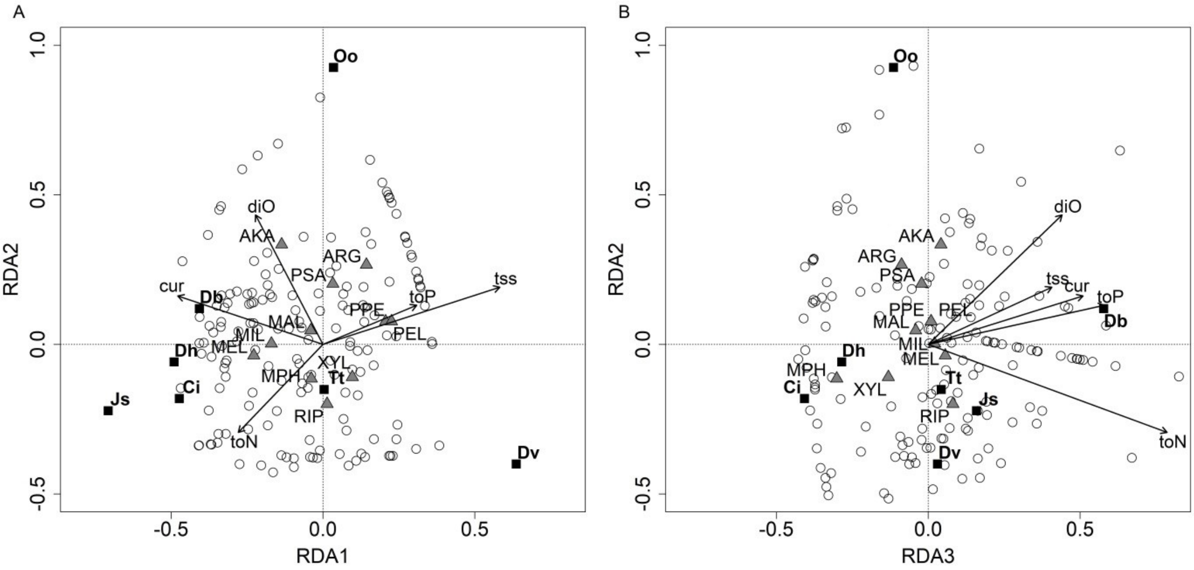


FIGURE 4

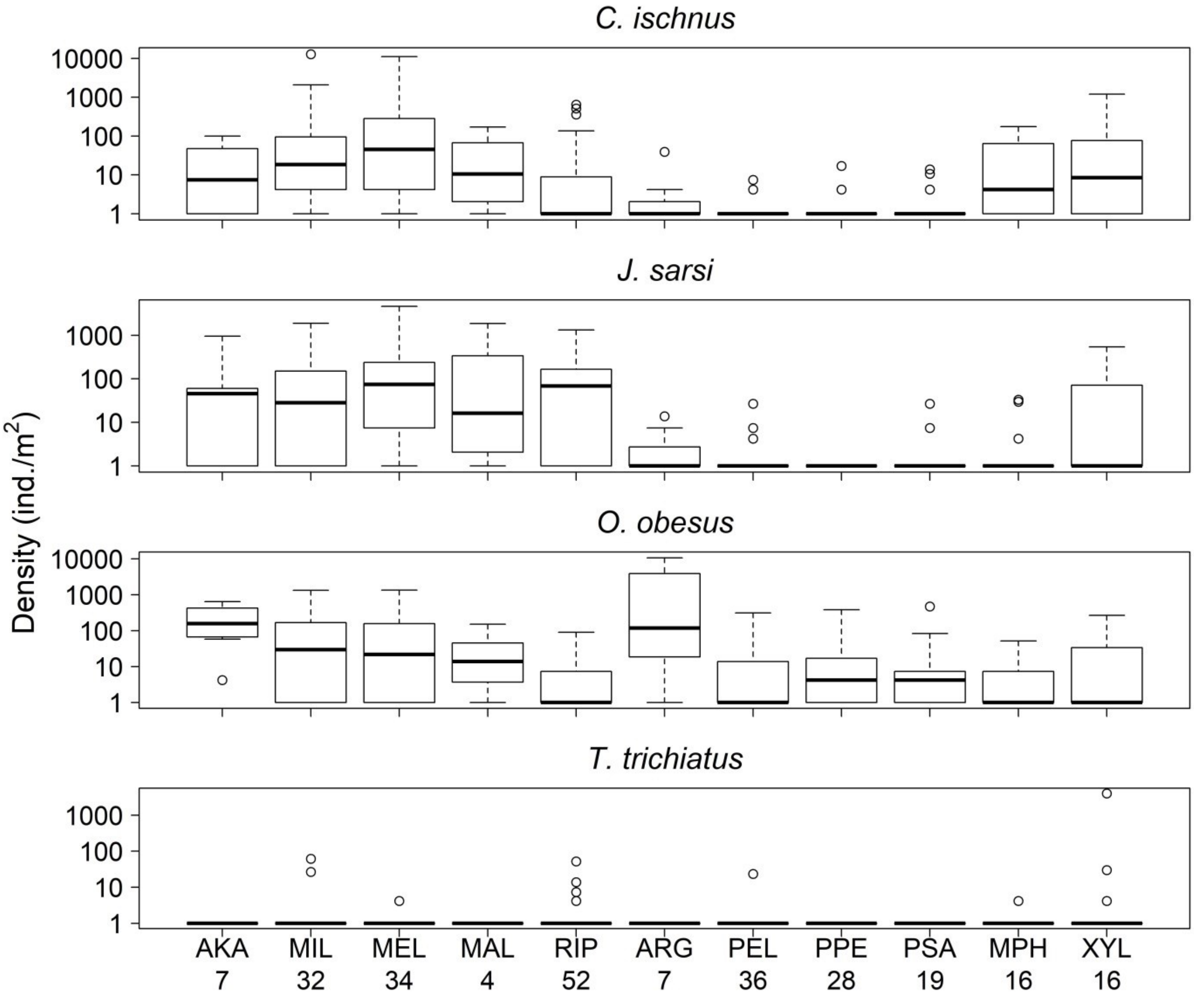
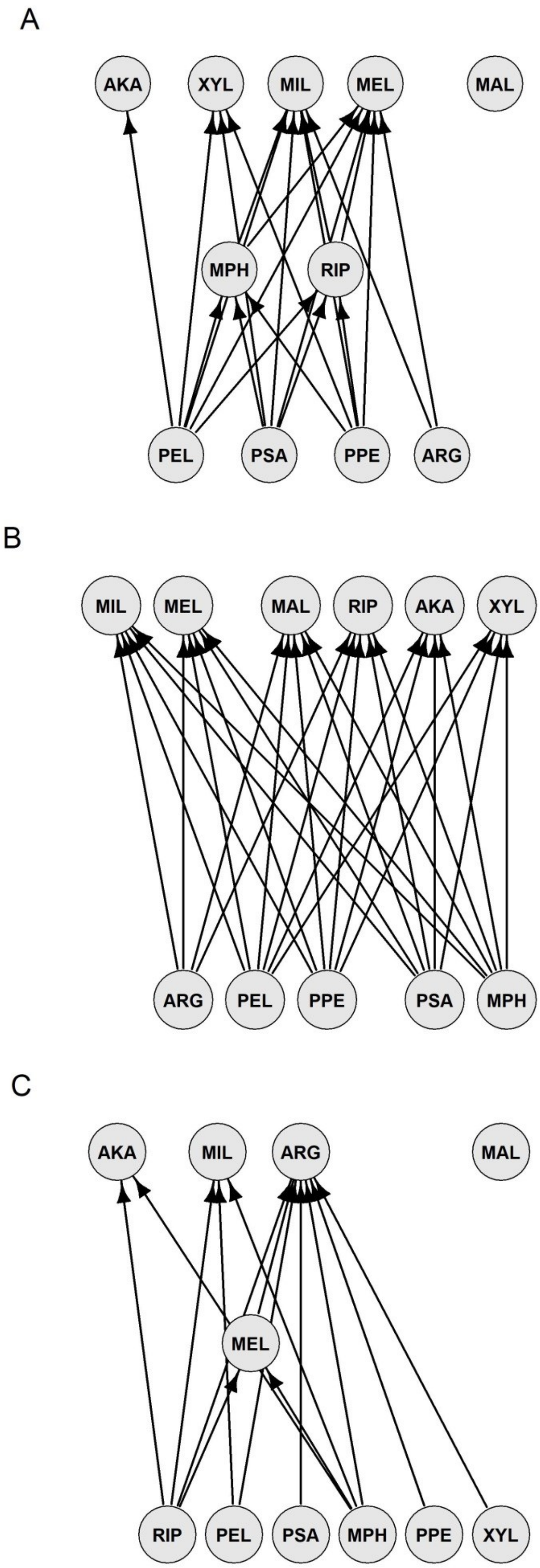

C. ischnus
J. sarsi
O. obesus
T. trichiatus
Density (ind./m^2)
10000
1000
100
10
1
AKA
7
MIL
32
MEL
34
MAL
4
RIP
52
ARG
7
PEL
36
PPE
28
PSA
19
MPH
16
XYL
16

FIGURE 5


A
AKA
XYL
MIL
MEL
MAL
MPH
RIP
PEL
PSA
PPE
ARG
B
MIL
MEL
MAL
RIP
AKA
XYL
ARG
PEL
PPE
PSA
MPH
C
AKA
MIL
ARG
MAL
MEL
RIP
PEL
PSA
MPH
PPE
XYL

Supporting Information

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

Péter Borza, Thomas Huber, Patrick Leitner, Nadine Remund, Wolfram Graf

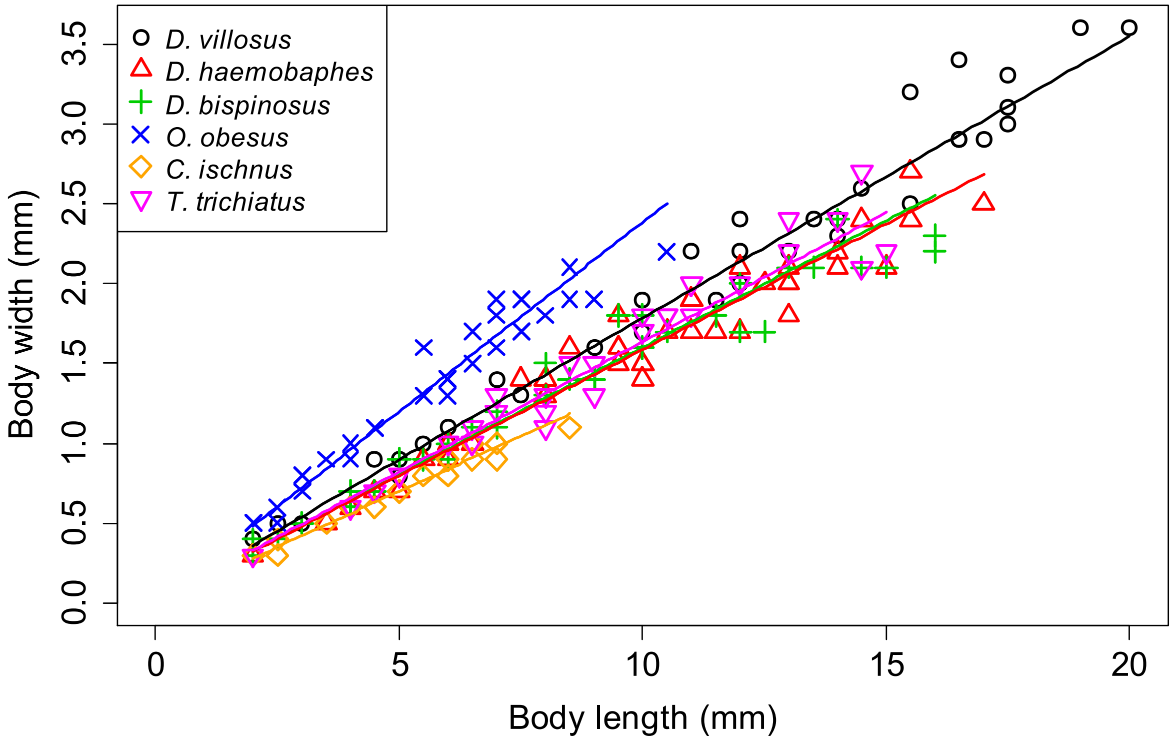


Figure S1 Body length-body width relationships in the studied gammarid species; given only as an illustration of their characteristic morphological differences. The measurements were made by ocular micrometer on specimens collected in several different waters in Hungary (collection of the Danube Research 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. ischus* grow considerably smaller and deviate from the standard body shape in opposing directions. Note: the characteristic body shape of *O. obesus* and *C. ischus* 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 esimate ± SE
Chaetogammarus ischnus	23	2.0-8.5	-1.952 ± 0.025
Dikerogammarus bispinosus	36	2.0-16.0	-1.812 ± 0.027
Dikerogammarus haemobaphes	42	2.0-17.0	-1.822 ± 0.029
Dikerogammarus villosus	38	2.0-20.0	-1.704 ± 0.029
Obesogammarus obesus	32	2.0-10.5	-1.414 ± 0.024
Trichogammarus trichiatus	31	2.0-15.0	-1.791 ± 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	*t*	*P*
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	*z*	*P*
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
```