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Contribution of alien peracarid crustaceans to the biocontamination of benthic macroinvertebrate assemblages in Croatian large rivers

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ABSTRACT

Contribution of alien peracarid crustaceans to the biocontamination of benthic macroinvertebrate assemblages in Croatian large rivers

Peracarid crustaceans (orders Amphipoda, Isopoda and Mysida) are one of the most important groups of invaders in fresh and brackish waters. Although large rivers in Croatia have been heavily invaded by peracarid crustaceans, little is known about their impact on local benthic macroinvertebrate assemblages. The main aim of this study was to investigate the contribution of alien Peracarida to the biocontamination of macroinvertebrate assemblages in Croatian large rivers as a proxy measure of their impact. Quantitative sampling was conducted twice (2015 and 2016/2017), at 48 sites on four large rivers (Danube-4, Sava-21, Drava-20, Mura-3). Physicochemical parameters were measured eight times during 2015 and 2016. In total, 16 species were recorded, five native and 11 alien species. The highest number (10 alien species, most from genus *Dikerogammarus* and *Chelicorophium*) were found in the Danube River, while subsets of alien species were found in the Sava (5 spp.) and Drava Rivers (6 spp.), and no alien species were found in the Mura River. The most upstream reaches of the Mura, Drava, and Sava Rivers in Croatia have not yet been colonized by invasive peracarids and the native peracarids are still abundant there. In the Drava River, alien Peracarida had the highest densities. Significant negative correlations were established between the number of native taxa and alien Peracarida species. Proportions of alien peracarids in the total density of benthic macroinvertebrates were spatially variable, the highest average values in the Drava River (24.4 %), and lower in the Danube (20 %) and the Sava (17.7 %) Rivers. Alien Peracarida had the most important contribution to the richness and abundance contamination of macroinvertebrate assemblages. The upstream spread of invasive peracarids will increase biocontamination and could cause a decline of native species richness and abundance in the most upstream reaches of the Sava and Drava Rivers. To prevent further upstream spread of alien Peracarida, "check, clean and dry" protocols should be included in the national regulations.

Key words: invasive species, Crustacea, distribution, densities, biocontamination, macroinvertebrate assemblages, large rivers

RESUMEN

Contribución de crustáceos peracáridos exóticos a la biocontaminación de la comunidad de macroinvertebrados bentónicos en grandes ríos croatas

Los crustáceos peracáridos (órdenes Amphipoda, Isopoda y Mysida) son uno de los grupos invasores más importantes en aguas dulces y salobres. Aunque los grandes ríos de Croacia han sido fuertemente invadidos por crustáceos peracáridos, se sabe poco sobre su impacto en la comunidad de macroinvertebrados bentónicos. El objetivo principal de este estudio fue investigar la contribución de peracáridos exóticos a la biocontaminación de la comunidad de macroinvertebrados en los grandes ríos croatas, como una medida indirecta de su impacto. El muestreo cuantitativo se realizó dos veces (2015 y 2016/2017) en 48 puntos de cuatro grandes ríos (Danubio-4 puntos, Sava-21, Drava-20, Mura-3). Los parámetros fisicoquímicos se midieron ocho veces durante el período de estudio. En total, se registraron 16 especies, cinco nativas y 11 exóticas. El mayor número

(10 especies exóticas la mayoría del género Dikerogammarus *and* Chelicorophium*) se encontró en el río Danubio, seguido de los ríos Drava (6 spp.) y Sava (5 spp.). No se encontraron especies exóticas en el río Mura. Los tramos de cabecera de los ríos Mura, Drava y Sava en Croacia aún no han sido colonizados por peracáridos invasores y los nativos todavía son abundantes. Se establecieron correlaciones negativas significativas entre el número de taxones nativos y especies exóticas de peracáridos. Las proporciones de peracáridos exóticos en la densidad total de macroinvertebrados bentónicos fueron espacialmente variables, siendo los valores promedio más altos en el río Drava (24.4 %) y más bajos en los ríos Danubio (20 %) y Sava (17.7 %). La biocontaminación más importante en la riqueza y abundancia de macroinvertebrados se atribuyó a los peracáridos invasores. La propagación río arriba de estas especies invasoras podría causar una disminución de la riqueza y abundancia de especies nativas en estos tramos, especialmente en los ríos Sava y Drava. Para evitarlo, se debería incluir protocolos de "verificación, limpieza y secado" en las reglamentaciones nacionales.*

Palabras clave: *especies invasoras, crustáceos, distribución, densidades, biocontaminación, comunidad de macroinvertebrados, grandes ríos*

INTRODUCTION

The biodiversity of freshwater ecosystems is highly threatened, primarily due to pollution, habitat loss, and invasive species (Dudgeon et al., 2006; Strayer, 2010). The number of alien and invasive species in European freshwaters, and their intake associated with human activity, is rising (Nunes et al., 2015). Changes in inland waters due to pollution from settlements and industry, other activities such as aquaculture, irrigation system and tourism, canal construction, and the resulting alterations in hydromorphology have all facilitated the spread of alien species (Leuven et al., 2009; Rewicz et al., 2014). Alien macroinvertebrate species (AMS) have replaced native macroinvertebrate fauna in most parts of Europe's large rivers (Van den Brink et al., 1990; Haas et al., 2002; Jazdzewski et al., 2004), while Peracarid crustaceans (Amphipoda, Isopoda, and Mysida) and invasive crayfish (Decapoda) comprise the most important group of AMS in European fresh and brackish waters (Holdich & Pöckl, 2007). Most alien peracarids in European waters originate from the Ponto-Caspian region, spreading upstream through large rivers (Danube, Volga, Vistula Rivers) on ship hulls or in ballast waters (Bij de Vaate et al., 2002; Piscart et al., 2011; Ricciardi & MacIsaac, 2000; Santagata et al., 2009). Others were intentionally introduced in reservoirs to facilitate fish production (Arbačiauskas et al., 2010). Most peracarid crustacean species from the Ponto-Caspian region were introduced in the Middle and Upper Danube River during the 20th century (Borza et al., 2017). Ponto-Caspian amphipods are considered the most important and most successful invasive peracarid crustaceans because of their significant role in the homogenization of the benthic macroinvertebrate community (Rewicz et al., 2014; Van den Brink et al., 1993; Van der Velde et al., 1998). Native amphipods like *Gammarus fossarum*, *G. roeseli* and isopod *Asellus aquaticus* have decreased in abundance or completely disappeared in the presence of invasive amphipods from the Ponto-Caspian region (e. g. Borza et al., 2015; Žganec et al., 2018).

Due to their frequent ecological and economic impacts, the intake of alien species can also be perceived as biological pollution (Elliott, 2003). The negative effects of alien species are difficult to assess and quantify, and require extensive research and great effort, thus a more practical approach is needed to determine the impact of alien species on the ecological status of water bodies (Arbačiauskas et al., 2008). The presence of alien species, regardless of their influence, causes a shift from the natural conditions. As such, they can be seen as biological contaminants that can be quantified using indices based on the proportion of their taxonomic and abundance composition in invaded macroinvertebrate assemblages (Arbačiauskas et al., 2008). Biocontamination indices can serve as an additional element in water quality monitoring, accompanying the existing biological and chemical methods, to obtain a more complete ecological status of a water body, since AMS often change and dominate the invaded communities (MacNeil et al., 2010).

Although alien peracarids are widespread, studies of their relative abundance and proportion in macroinvertebrate communities are scarce. Most studies mention a high abundance of alien peracarids (Josens et al., 2005; Van den Brink et al., 1993; Van Riel et al., 2006a; Wawrzyniak-Wydrowska & Gruszka, 2005), but surprisingly few studies have listed the proportion of alien peracarids in macroinvertebrate assemblages (Gergs & Rothhaupt, 2014; Ricciardi et al., 1997) or studied their contribution to benthic macroinvertebrate assemblages as a proxy of their impact. Therefore, this study aimed to investigate the distribution, density and assemblages of native and alien Peracarida and their relationship with physicochemical parameters in Croatian large rivers. The contribution of alien peracarid crustaceans to the biocontamination of benthic macroinvertebrate assemblages and the relationship between the abundance of alien Peracarida and taxa richness or diversity of whole macroinvertebrate assemblages were assessed. We expect that further upstream spread of invasive species in the Sava, Drava and Mura Rivers could cause decline of the native freshwater biodiversity in Croatia and neighbouring countries.

MATERIAL AND METHODS

Field sampling and laboratory analyses

Sampling sites were located along four major

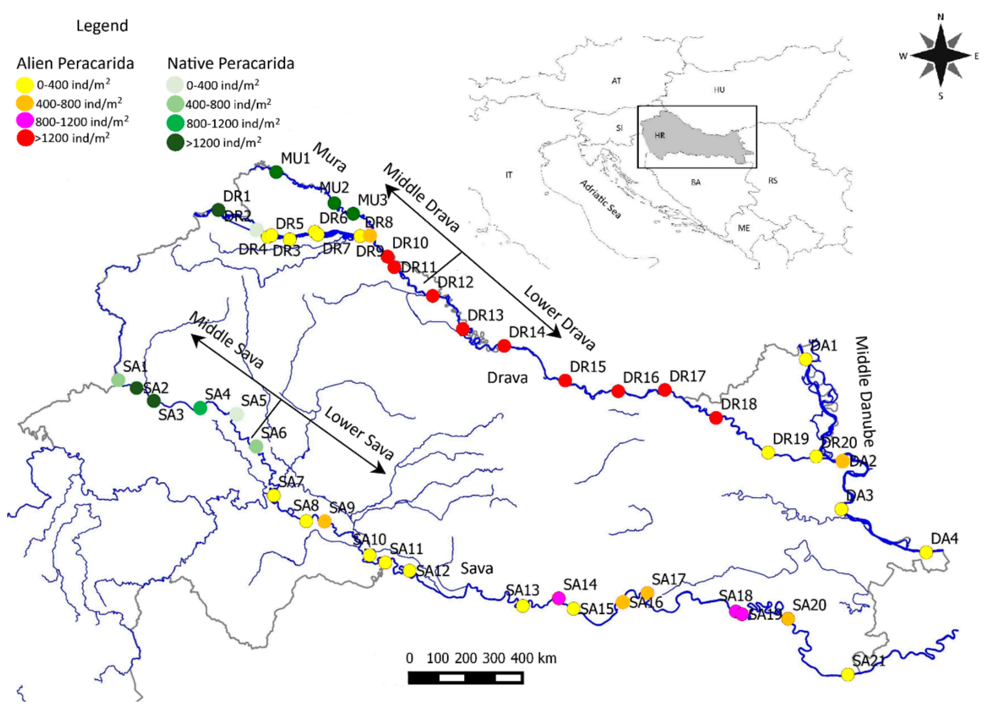

Figure 1. Sampling sites (48) along the four major large rivers in Croatia: the Danube-DA (4 sites), Sava-SA (21), Drava-DR (20), and Mura-MU (3) Rivers. At all sites, four categories of average densities are shown for alien and native peracarid crustaceans by colour. The inset map shows the position of the study area in Croatia (Pannonian lowland ecoregion in grey) and Europe, with country codes (HR: Croatia, AT: Austria, BA: Bosnia and Herzegovina, HU: Hungary, IT: Italy, ME: Montenegro, SI: Slovenia, RS: Serbia). *Puntos de muestreo (48) a lo largo de los cuatro grandes ríos principales de Croacia: los ríos Danubio-DA (4 puntos), Sava-SA (21), Drava-DR (20) y Mura-MU (3). En todos los puntos, se muestran cuatro categorías de densidades promedio para crustáceos peracáridos exóticos y nativos por color. El mapa insertado muestra la posición del área de estudio en Croacia (ecorregión de tierras bajas de Panonia en gris) y Europa, con códigos de país (HR: Croacia, AT: Austria, BA: Bosnia y Herzegovina, HU: Hungría, IT: Italia, ME: Montenegro, SI: Eslovenia, RS: Serbia).*

rivers in Croatia: Mura (3 sites), Drava (20), Sava (21), and Danube (4) Rivers (Fig. 1). In total, samples were taken at 48 sites: in the main course of the river (44 sites) and at two reservoirs on the Drava River (Čakovec and Dubrava Reservoirs; four sites, two in the littoral and two in the deeper parts of each reservoir, Fig. 1). All sampling sites are located in the Pannonian (Hungarian) lowland ecoregion (ER11) (Illies, 1978).

According to the Croatian national river typology, sampling sites were distributed within three river types. The most upstream reaches of the Drava (sites DR1-DR12) (Middle Drava) and Sava Rivers (SA1-SA5) (Middle Sava) and the whole Croatian part of the Mura River (MU1-MU3) belong to the category of "Very large lowland rivers – lower course of the Mura River and middle course of the Sava and Drava Rivers" (HR-R_5B). The downstream reaches of the Drava (DR13-DR18) (Lower Drava) and Sava River (SA6-SA21) (Lower Sava) belong to the category "Very large lowland rivers – lower course of the Sava and Drava Rivers" (HR-R_5C). The Croatian reach of the Middle Danube River belongs to the category "Very large lowland rivers – the Danube" (HR-R_5D) (Regulation on Water Quality Standard; Official Gazette 73/13, 151/14, 78/15, 61/16, 80/18).

Sampling was conducted on two occasions (first sampling occurred in 2015 and the second one in 2016 or 2017) with a hand net (25x25 cm, mesh size 500 µm) in a shallow bank area of rivers at 44 locations or in the littoral zone of reservoirs (2 sites). Sampling occurred during stable and low water levels, following the AQEM sampling protocol (AQEM, 2002). Replicate quantitative samples (20x0.0625 m^2) were collected at each site on all microhabitats covering more than 5 %. In the two sampling sites of deeper parts of the two Drava River reservoirs (DR4 and DR7), three replicate samples were taken using an Ekman grab (3x0.0225 m^2) only in 2015. Samples were preserved in 96 % ethanol in the field and subsequently stored in 70 % ethanol in the laboratory. Using a binocular stereomicroscope (Olympus SZX10) in the laboratory, macroinvertebrates were isolated from sediment and organic detritus and stored in 70 % ethanol for later identification. Determination keys used for identifying peracarid crustaceans were: Amphipoda - Cărăusu et al. (1955), Karaman & Pinkster (1977a, b), Pinkster (1993), Eggers & Martens (2001); Isopoda - Argano (1979), Veuille (1979), Mysida - Dobson (2012), Wittmann et al. (2016). Other taxa were identified to the lowest possible taxonomic level for other studies (Ćuk et al., 2019; in press).

Water samples were collected on 4 sampling dates in 2015 (March, July, October, December) and 4 sampling dates in 2016 (March, May, August, December) at 46 sites. In the deep sections of reservoirs (sites DR4 and DR7), measurements were not taken from the bottom of the reservoirs, and these two sites were excluded from the statistical analyses. The following 27 physico-chemical parameters were analysed according to standard analytical methods for assessment of surface water quality (ISO norms): water temperature (°C), pH, conductivity (µS/cm), total suspended solids (mg/L), alkalinity m-value (mg $CaCO_3$/L), total hardness (mg $CaCO_3$/L), dissolved oxygen (mg O_2/L), oxygen saturation (%), biological oxygen demand (BOD_5) (mg O_2/L), chemical oxygen demand (COD-Mn) (mg O_2/L), ammonia (NH^{4+}) (mg N/L), nitrites (NO^{2-}) (mg N/L), nitrates (NO^{3-}) (mg N/L), total nitrogen (mg N/L), inorganic nitrogen (mg N/L), organic nitrogen (mg N/L), orthophosphates (PO_4^{3-}) (mg P/L), total phosphorus (mg P/L), total organic carbon (TOC) (mg/L), dissolved organic carbon (DOC) (mg/L), calcium (mg/L), magnesium (mg/L), sodium (mg/L), potassium (mg/L), chlorides (mg/L), fluorides (mg/L) and sulphates (mg/L). Substrate composition was assessed using the AQEM protocol (AQEM, 2002) at 46 sites. For analyses, three aggregated fractions were used: hard substrate (mega-, macro-, microlithal and akal), soft substrate (psammal, psammopelal and argylal), and phytal. The subbasin size (calculated using GIS tools), which is positively correlated with discharge, was used for each site instead of discharge data since actual discharge data were not available for each sampling site. The effect of geographic location was examined by including geographic coordinates (X, Y Gauss – Krueger, zone 5), distance from the Danube (with four Danube sites having the value 0), and altitude into the analyses.

Table 1. Average (in bold), minimum and maximum densities (in parenthesis) in the upper row, and proportions in total abundance of macroinvertebrates in the lower row for each species in all four large rivers and at two river reaches of the Drava and Sava Rivers calculated only for sites where species was found. ˝ indicates that the species was found at only one reach of the Sava or Drava River and that the values for the whole river are the same. The last row shows the number of native and alien species. Sites abbreviations below alien species names represent the most upstream sites where the species was found, while the same abbreviations for native species represent the most downstream sites. In cases without ranges, species were found at only one site. Species in bold are alien species while species in bold and asterisk (*) represent invasive species. *Densidad promedio (en negrita), mínima y máxima (entre paréntesis) en la fila superior y proporciones en abundancia total de macroinvertebrados en la fila inferior para cada especie en los cuatro grandes ríos y en dos tramos de los ríos Drava y Sava se calcularon solo para los puntos donde se encontraron especies. ˝ indica que la especie se encontró en un solo tramo del río Sava o Drava y que los valores para todo el río son los mismos. La última fila muestra el número de especies nativas y exóticas. Las abreviaturas de puntos debajo de los nombres de las especies exóticas representan los puntos más altos donde se encontró la especie, mientras que las mismas abreviaturas para especies nativas representan los sitios más bajos en el río. En los casos sin áreas de distribución, las especies se encontraron en un solo punto. Las especies en negrita son especies exóticas, mientras que las especies en negrita y asterisco (*) representan especies invasoras.*

	Middle Sava (SA1-SA5)	Lower Sava (SA6-SA21)	Sava (SA1-SA21)	Mura	Middle Drava (DR1-DR12)	Lower Drava (DR13-DR20)	Drava (DR1-DR20)	Danube
Amphipoda								
Chelicorophium curvispinum	0	**50.8** (4.8-177.0)	˝	0	**2554.4** (1221.6-4327.2)	**2244.0** (7.6-4771.2)	**2328.6** (7.6-4771.2)	**147.8** (72.9-293.5)
(SA8, DR10)	0	**2.7** (0.2-12.4)	˝	0	**40.9** (25.1-59.8)	**28.3** (1.9-62.0)	**31.7** (1.9-62.0)	**9.56** (5.8-17.8)
Chelicorophium robustum	0	0	0	0	0	0	0	**27.8** (4.5-67.1)
	0	0	0	0	0	0	0	**4.6** (0.3-16.4)
Chelicorophium sowinskyi	0	**217. 6** (0.9-884.5)	˝	0	0	**190.6** (11.9-676.8)	˝	0
(SA8, DR14)	0	**8.7** (0.2-37.7)	˝	0	0	**2.4** (0.2-8.8)	˝	0
Dikerogammarus bisponosus	0	0	0	0	0	0	0	**9.2**
	0	0	0	0	0	0	0	**0.2**
Dikerogammarus haemobaphes	0	**141.0** (1.2-593.2)	˝	0	0	0	0	**5.7** (0.9-10.6)
(SA7)	0	**7.7** (0.1-42.8)	˝	0	0	0	0	**1.3** (2.5-21.4)
Dikerogammarus villosus	0	**36.4** (12.7-69.6)	˝	0	**551.7** (2.4-1734.0)	**329.2** (42.8-1136.4)	**440.4** (2.4-1734.0)	**116.4** (44.0-176.5)
(SA8, DR3)	0	**2.4** (0.5-6.9)	˝	0	**13.0** (0.2-35.6)	**7.0** (1.5-22.9)	**10.0** (0.2-35.6)	**9.5** (2.5-21.4)
Echinogammarus ischnus	0	0	0	0	0	**69.0** (1.6-146.4)	˝	**9.6** (6.0-15.3)
(DR14)	0	0	0	0	0	**1.2** (0.4-3.0)	˝	**0.8** (0.2-1.5)
Obesogammarus obesus	0	0	0	0	0	0	0	**11.3** (0.4-33.0)
	0	0	0	0	0	0	0	**0.9** (0.02-2.0)
Gammarus fossarum	**1183.8** (209.6-2424.7)	**470.8**	**1065.0** (209.6-2424.7)	**4068.8** (3727.2-4336.8)	**44.3**	0	˝	0
(SA6, DR12)	**19.5** (1.3-38.9)	**11.5**	**18.2** (1.3-38.9)	**74.1** (69.4-80.2)	**0.6**	0	˝	0
Gammarus roeselii	0	0	0	**3.6** (2.4-4.8)	**344.2** (0.4-1567.0)	**2.4**	**344.6** (0.4-1567.0)	0
(DR14)	0	0	0	**0.1** (0.04-0.1)	**5.4** (0.01-16.1)	**0.04**	**4.5** (0.01-16.1)	0
Niphargus sp.	0	**0.4**	˝	0	0	0	0	0
(SA16)	0	/	/	0	0	0	0	0
Synurella ambulans	**525.1** (157.3-1156.8)	0	˝	0	**25.8** (1.3-34.8)	**12.2** (7.5-16.8)	**22.4** (1.3-34.8)	0
(SA4, DR19)	**8.6** (3.3-17.1)		˝		**0.5** (0.04-1.8)	**0.3** (0.3-0.3)	**0.5** (0.04-1.8)	
Isopoda								
Jaera sarsi	0	**34.8** (1.5-199.0)	˝	0	**889.2** (669.6-1224.0)	**93.0** (14.0-184.8)	**331.9** (14.0-1224.0)	**75.7** (8.9-186.8)
(SA7, DR10)	0	**2.1** (0.04-11.4)	˝	0	**15.0** (12.0-16.9)	**1.3** (0.2-2.4)	**5.4** (0.2-16.9)	**4.3** (0.5-10.5)
Asellus aquaticus	0	**2.7** (2.0-3.4)	˝	0	**4.3** (1.2-9.6)	**7.5**	**4.7** (1.2-9.6)	0
(SA16, DR19)	0	**0.1** (0.03-0.2)	˝	0	**0.1**	**0.3** (0.03-0.3)	**0.1**	0
Mysida								
Limnomysis benedeni	0	0	0		0	**4.9** (0.4-9.5)	˝	**2.1** (0.4-4.4)
(DR16)	0	0	0	0	0	**0.1** (0.03.-0.4)	˝	**0.2** (0.1-0.3)
Katamysis warpachowskyi	0	0	0	0	0	0	0	**0.4**
	0	0	0	0	0	0	0	**0.02**
Total number of species (native/alien)	2/0	2/5	4/5	2/0	4/3	3/6	4/6	0/10

Data analyses

Densities (ind./m^2) and proportions in total macroinvertebrate densities were calculated for every recorded peracarid crustacean species by site, river segment (i.e. river type), and river. Average densities and proportions of peracarids in macroinvertebrate assemblages were calculated only for sites where a particular species or group of species (alien or native) were recorded. Biocontamination was assessed for all collected samples (N = 94) according to Arbačiauskas et al. (2008), where a site-specific biocontamination index (SBCI-Fam) was derived from two metrics: the abundance contamination index (ACI) and the richness contamination index (RCI-Fam) at family rank. The obtained SBCI-Fam classifies the sampling site into one of five classes ranging from 0 (no contamination) to 4 (severe contamination). Peracarida assemblages were analysed using non-parametric multidimensional scaling (NMDS) and PERMANOVA with the software packages PRIMER Version 6.1.13. and PERMANOVA Version 1.0.3 (PRIMER-E Ltd 2009). The average abundance of all Peracarida taxa for all 94 samples was square-root transformed to control the influence of dominant species, and the Bray-Curtis index of similarity was used to calculate the similarity matrix. Differences between site groups extracted from the NMDS plots were tested using PERMANOVA and SIMPER analysis. SIMPER analysis was used to identify taxa that contribute the most to the dissimilarity between tested groups.

Environmental variables were averaged for each site (N = 8 months, four in 2015 and four in 2016), log-transformed, and then analysed using different univariate and multivariate analyses. Pearson correlation coefficients and scatter plots between all pairs of environmental variables were examined to eliminate co-linear variables and reduce redundancy, resulting in 18 selected environmental variables (of the total 27) used for analyses. To examine spatial differences between sites, rivers, and river sections, Principal Component Analysis (PCA) was used. BIOENV analyses in PRIMER and Canonical Correspondence Analyses (CCA) using CANOCO 5 (Ter Braak & Šmilauer, 2012) were used to examine the relationship between peracarid crustacean assemblages and environmental factors. Preliminary Detrended Correspondence Analysis (DCA) in CANOCO 5 showed a unimodal rather than linear response of species data. Accordingly, CCA was used instead of constrained multivariate analyses like RDA or dbRDA that assume a linear response (Lepš & Šmilauer, 2003). Although 18 environmental variables and 16 species were initially used for CCA, a final subset of 12 environmental variables (with inflation > 5) and 14 species (10 alien and four native species, with two localized species excluded) was used for the samples+species+environmental factors triplot. The statistical significance of the first (CCA1) axis and all axes in CCA were tested using the Monte Carlo test (9999 permutations) to verify the significance of the models (Lepš & Šmilauer, 2003).

RESULTS

In total, 576 024 macroinvertebrate specimens were collected at the 48 sites of the four large rivers in Croatia, of which 90 395 (15.7 %) specimens were alien and 45 896 (8.0 %) native peracarid crustacean. In all, 16 peracarid species were found (Table 1): five native (Amphipoda-4, Isopoda-1) and 11 alien (Amphipoda-8, Isopoda-1, Mysida-2). Of the alien species, six (Amphipoda-5, Isopoda-1) were widespread along the Sava and/or Drava Rivers and considered invasive. The highest number of species (10), all alien, was found in the Danube River, while subsets of alien species were found in the Sava (5) and Drava Rivers (6). Only native peracarids were found in the most upstream reaches of the Sava River (first six sites, SA1-SA6), the first two sites of the Drava River (DR1-DR2) and at all three sites in the Mura River (Table 1, Fig. 1 and 2). At most sites in the Lower Sava, only alien species were found in both years, except the site SA6 where only the native species *G. fossarum* and *A. aquaticus* were found. At two sites in Lower Sava (SA9 and SA16), the native isopod *A. aquaticus* was found in low density with dominant alien peracarids. In the Middle Drava, native species (*G. fossarum* and *Synurella ambulans*) co-occurred with dominant aliens at sites DR10 and DR11 in 2015, while in 2017, native species (*G. fossarum*, *S. ambulans* and *A. aquaticus*) appeared in higher

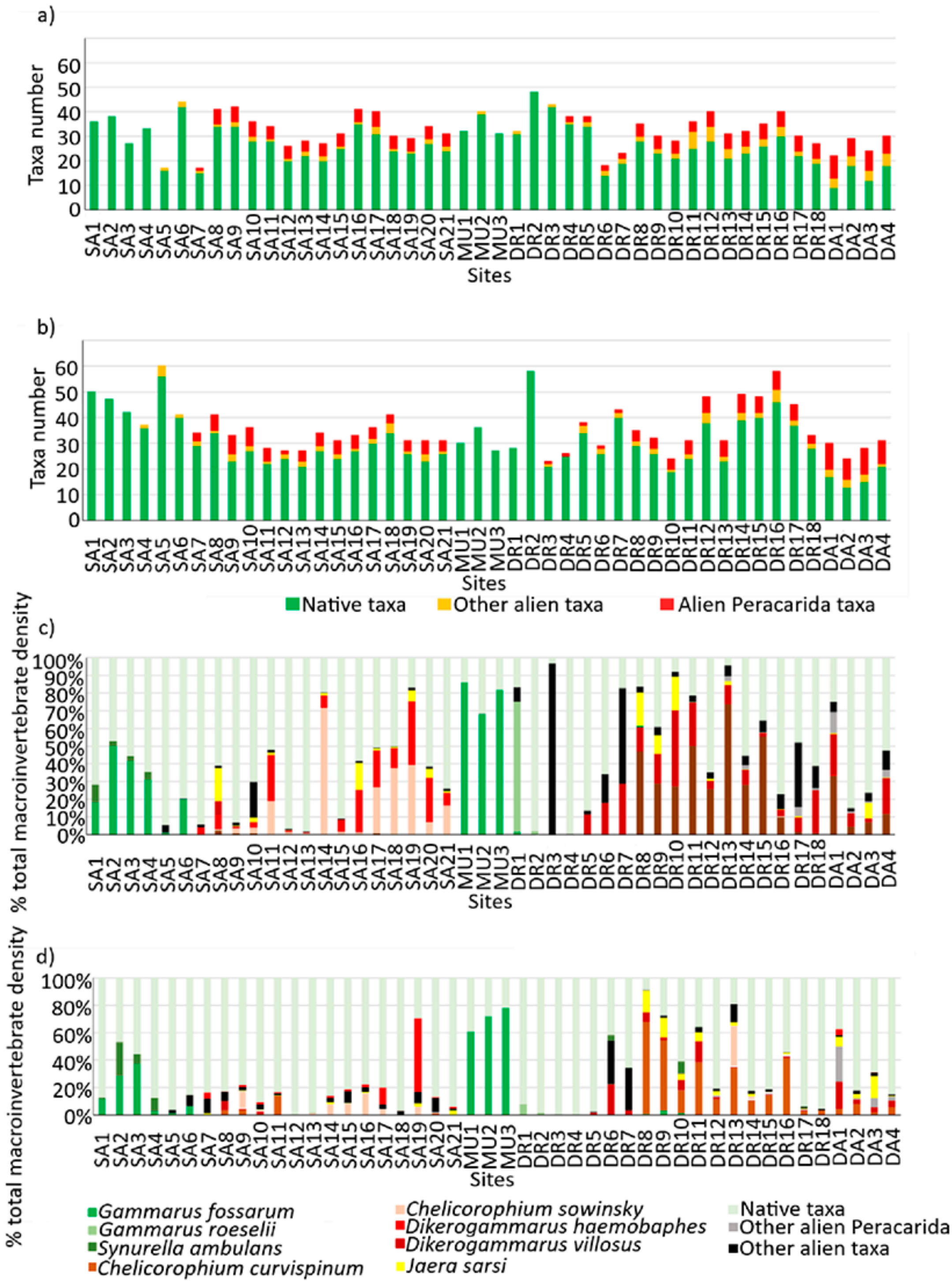

Figure 2. Taxa number in three groups (all native taxa, alien Peracarida and other alien taxa) for each site at all four rivers in 2015 (a) or 2016/2017 (b). The proportion of three native and six invasive peracarid species in the total abundance of macroinvertebrates at all sites in 2015 (c) and in 2016/2017 (d). *Número de taxones en tres grupos (todos los taxones nativos, Peracarida exótica y otros taxones exóticos) para cada punto en los cuatro ríos, en 2015 (a) o 2016/2017 (b). La proporción de tres especies peracáridas nativas y seis invasoras en la abundancia total de macroinvertebrados en todos los puntos en 2015 (c) y en 2016/2017 (d).*

density and proportion at four additional sites of Middle Drava and one site of Lower Drava (Fig. 2). At site DR5 in the littoral of the Dubrava Reservoir, *Dikerogammarus villosus* and *A. aquaticus* were present in both years sampled. On the other hand, in the littoral of the Čakovec Reservoir (DR3), *G. roeselii* and *A. aquaticus* were found in 2015, but were subsequently replaced by invasive *D. villosus* in 2017. At 15 sites of the Lower Sava, from one to five (average: 4) alien peracarid species per site were recorded, while at 18 sites on the Drava River, from one to six alien (avg: 3) species per site were recorded. At four Danube sites, from five to nine (avg: 8) alien species per site were recorded. The most widespread alien species, considered invasive, were the isopod *Jaera sarsi* (29 sites), and amphipods *D. villosus* (24 sites), *Chelicorophium curvispinum* (22 sites) and *C. sowinskyi* (19 sites). Two other invasive amphipods were found, *Dikerogammarus haemobaphes* in the Sava and the Danube Rivers and *Echinogammarus ischnus* in the Drava and the Danube Rivers. The most widespread native species was *G. fossarum* (14 sites). The furthest upstream distributed alien species were *D. haemobaphes* and *J. sarsi* in the Sava River at 596 rkm (site SA7) and *D. villosus* in the Drava River at 276 rkm (site DR3). The most upstream sites for all other alien peracarids in the Sava and Drava Rivers are shown in Table 1. In 2016, the invasive isopod *J. sarsi* was found at one site (SA7), which is 17 km upstream from the most upstream finding at site SA8 in 2015. In the Drava River, the invasive amphipod *D. villosus* was found at site DR3 in 2016, which is 6 km upstream from its most upstream finding at site DR4 in 2015.

Peracarid crustaceans were present on nearly all analysed sites, except DR7 in the deeper part of the Dubrava Reservoir. Their proportion in the total abundance of benthic macroinvertebrates ranged from 0.01 % (DR3) to 90.1 % (DR8), with an average proportion of 23.7 %. Native peracarids had the highest proportions in the macroinvertebrate assemblages in the Mura River, with an average proportion of 73.8 %, a relatively low average proportion of 16.7 % in the Sava River, and the lowest average proportion of 2.0 % in the Drava River. Alien peracarids had the highest average proportion in macroinvertebrate assemblages of 24.4 % in the Drava River, and lower in the Danube (20.0 %) and the Sava (17.7 %) Rivers (Fig. 2b, d). The contribution of alien Peracarida to the Abundance Contamination Index was (average; range): Sava (78.1 %; 36.2–96.0 %); Drava (74.5 %; 0.01–98.3 %); Danube (86.9 %; 83.0–95.0%); all three rivers (77.5%; 0.01–98.3%). The contribution of alien Peracarida to the richness contamination index (RCI-Fam) was high, on average 67.6 % and ranged from 25–100 % (average; range): Sava (72.3 %; 50–85.7 %); Drava (61.9 %; 25–100 %); Danube (71.8 %; 58.3–90 %). Among other alien macroinvertebrates, molluscs contributed the most to biocontamination (average values: Sava 33.7 %, Drava 28.9 %, Danube 11.9 %). (Fig. 2a, c).

The average total density of alien peracarids from 35 sites was 1291.4 ind./m^2 and ranged from 2.4 ind./m^2 (DR3) to 6379.2 ind./m^2 (DR15) (Fig. 2). Average densities of alien peracarids were highest in the Drava (2339.7 ind./m^2) and lower in the Sava (412.3 ind./m^2) and Danube Rivers (395.2 ind./m^2). The invasive amphipod *C. curvispinum* had similarly high densities in the Middle and Lower Drava, with the highest proportions in the macroinvertebrate assemblages in the Middle Drava, while its densities were lower in the Danube and Sava Rivers, though with much higher average proportions in the Danube (Table 1). *C. sowinskyi* was found in two river sections, Lower Sava at 15 sites and Lower Drava at five sites with similar average densities but much lower average proportions in the Drava (Table 1). The invasive amphipod *D. villosus* had the highest average densities and proportions in the Middle and Lower Drava, and medium densities in the Danube River, while at four sites in the Lower Sava it occurred in low densities (Table 1). Another invasive amphipod, *D. haemobaphes*, was found in the highest densities and proportions in the Lower Sava. It was not found in the Drava River, while in the Danube River, it was found at only two sites in very low densities (Table 1).

The total density of native peracarids for 11 sites, where they occurred without alien species, averaged 2059.6 ind./m^2 (range: 209.6–4339 ind./m^2). The average density of native peracarids at 12 sites where they co-occurred with alien species was 24.4 ind./m^2 (range: 2.0–

99.6 ind./m2). Among native species, the amphipod *G. fossarum* had the highest average densities and proportions in total benthic communities in the Mura River. It was abundant in the Middle Sava, but had low densities in the Middle Drava (Table 1). On the other hand, *G. roeselii* was not found in the Sava River and had the highest average densities in the Middle Drava, while it was found in low densities at two sites in the Mura River and one site in the Lower Drava. The native amphipod *S. ambulans* was abundant in the Middle Sava (4 sites), but also occurred in low densities in the Middle (6 sites) and Lower Drava (2 sites). In the Drava River, it was found at eight sites, of which it co-occurred with native peracarids at two sites, and was found with alien peracarids at six sites.

In 2015, the invasive amphipod *D. villosus* was found in the Sava River (SA8, SA9, and SA15) in a low average density of 46.6 ind./m2 (range: 23.2–91.2 ind./m2) and proportion of 1.2 % (range: 1.1–7.8 %). In 2016, it was found at one additional site SA10, but was no longer found at SA15, and its densities and proportions in the total macroinvertebrate density at three sites had an average density of 50.5 ind./m2 (range: 48.0–55.4 ind./m2) with an average proportion of 2.0 % (range: 1.1–6.6 %).

The densities of all alien Peracarida were in positive and statistically significant correlations with the ACI (rs = 0.74, $p < 0.001$, n = 69) and SBCI-Fam (rs = 0.57, $p < 0.001$, n = 69) biocontamination indices. On the other hand, significant negative correlations were established between density of all native peracarids and all biocontamination indices: ACI (rs = -0.75, $p < 0.001$, n = 39), RCI-Fam (rs = -0.78, $p < 0.001$, n = 39) and SBCI-Fam (rs = -0.74, $p < 0.001$, n = 39) (Table 2). Since alien amphipods of the genera *Chelicorophium* and *Dikerogammarus* had the highest densities and proportions among alien taxa, they showed the highest positive correlations with biocontamination indices. The native amphipods *G. fossarum* and *S. ambulans* showed the highest negative correlations with biocontamination indices (Table 2). A significant negative correlation (rs = -0.43, $p < 0.005$) was established between alien peracarid species number and the number of native taxa for all sites (Fig. 3). Similarly, a negative correlation was established between the densities of total alien peracarids and all native taxa (rs = -0.32, $p < 0.005$). At sites without alien Peracarida species, the average number of native taxa (avg: 38, range: 16–58) was significantly higher (Mann-Whitney, U = 217, $p < 0.001$) than at sites with alien Peracarida (avg: 27, range: 9–56).

Table 2. Spearman correlations between peracarid densities and biocontamination indices (ACI – Abundance Contamination Index, RCI- Fam – Richness Contamination Index, SBCI-Fam – site-specific biocontamination index). Significant correlation *p* < 0.05 are shown in bold. All correlations were calculated only for sites where peracarids were present (sample size is shown in brackets). *Correlaciones de Spearman entre densidades de peracáridos e índices de biocontaminación (ACI - Índice de contaminación de abundancia, RCI - Fam - Índice de contaminación de riqueza, SBCI-Fam - índice de biocontaminación específico del punto). La correlación significativa* p *< 0.05 se muestra en negrita. Todas las correlaciones se calcularon solo para puntos donde estaban presentes los peracáridos (el tamaño de la muestra se muestra entre paréntesis).*

	Biocontamination indices		
	ACI	RCI-Fam	SBCI-Fam
Alien Peracarida (69)	**0.74**	0.22	**0.57**
Native Peracarida (39)	**-0.75**	**-0.78**	**-0.74**
Total Peracarida (92)	0.16	-0.13	0.03
C. curvispinum (40)	**0.52**	0.01	**0.49**
C. robustum (7)	**0.86**	0.39	0.43
C. sowinskyi (31)	**0.57**	-0.01	0.19
D. haemobaphes (30)	**0.47**	-0.29	0.20
D. villosus (45)	**0.62**	0.28	**0.57**
E. ischnus (19)	0.03	-0.41	-0.08
O. obesus (6)	-0.29	-0.64	-0.42
J. sarsi (49)	**0.45**	-0.13	**0.48**
G. fossarum (26)	**-0.60**	**-0.53**	**-0.51**
G. roeselii (9)	-0.44	-0.58	-0.65
S. ambulans (19)	**-0.66**	**-0.51**	**-0.58**
A. aquaticus (11)	-0.36	-0.04	-0.31
Peracarida taxa nu.	**0.59**	**0.74**	**0.59**
Alien Peracarida taxa nu.	**0.26**	**0.65**	0.20
Native Peracarida taxa nu.	**-0.51**	**-0.61**	**-0.50**
% Peracarida	**0.40**	0.04	**0.25**
% Alien Peracarida	**0.94**	**0.24**	**0.72**
% Native Peracarida	**-0.73**	**-0.73**	**-0.73**

In non-parametric multidimensional scaling (NMDS) analysis of peracarid assemblages at 47 sites (site DR7 excluded due to absence of peracarids) showed a clear separation of sites with only native species (Fig. 4), and this difference was significant (PERMANOVA, pseudo-F = 28.8 df = 1, $p = 0.001$). In the native species group, higher similarity and grouping of sites in the Sava and Mura Rivers was due to the dominance of *G. fossarum* at these sites, while sites in the up-

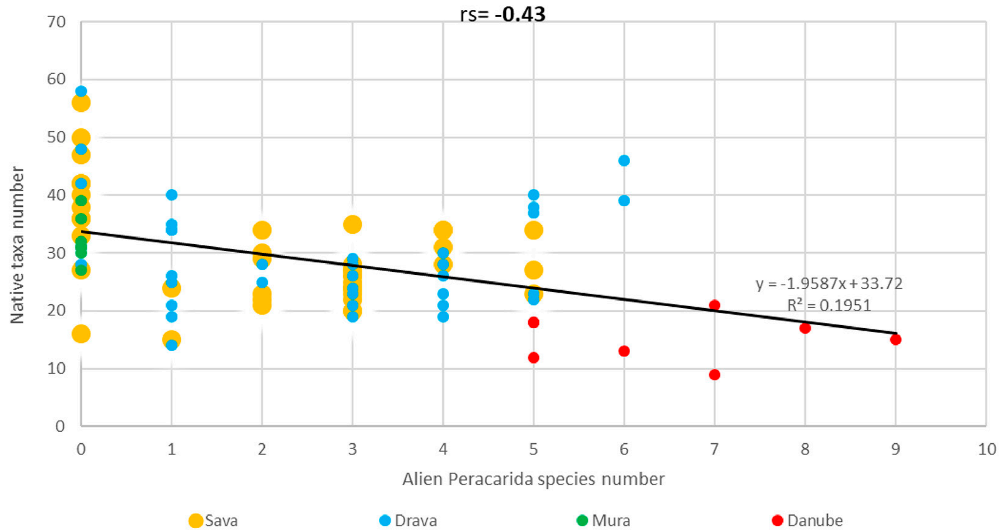

Figure 3. Relationships between the number of alien Peracarida species and the number of native taxa at 46 sites (excluding two sites, DR3 and DR5 in the deeper parts of the Drava reservoirs) in both years with linear regression for the four large rivers in Croatia (rs-Spearman correlation index with $p < 0.05$). *Relaciones entre el número de especies exóticas de peracáridos y el número de taxones nativos en 46 puntos (excluidos dos sitios, DR3 y DR5 en las partes más profundas de los embalses de Drava) en ambos años con regresión lineal para los cuatro grandes ríos de Croacia (índice de correlación de Spearman con* p < 0.05*).*

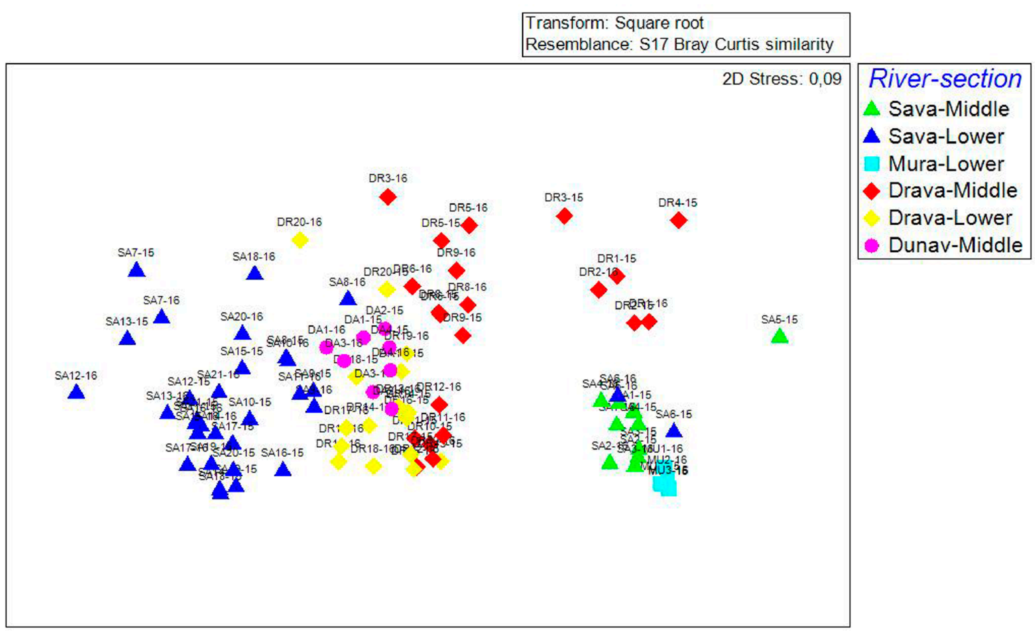

Figure 4. Non-parametric multidimensional scaling (NMDS) of all 48 sites in both sampling years based on the peracarid crustacean density matrix (Bray-Curtis similarity, fourth root transformation, river section as factors, site abbreviations: DA-Danube, DR-Drava, MU-Mura, SA-Sava). *Escalado multidimensional no paramétrico (NMDS) de los 48 puntos en ambos años de muestreo basado en la matriz de densidad de crustáceos peracáridos (similitud de Bray-Curtis, cuarta transformación de raíz, sección de río como factores. DA-Danubio, DR-Drava, MU-Mura, SA-Sava).*

per part of the Middle Drava were dominated by *G. roeseli*. In the alien species group, there was a clear separation of the Middle Sava and two Drava segments, with the Danube sites being in between. When the alien group of sites was analysed separately, a significant difference between these river reaches was established (PERMANOVA, pseudo-F = 13.5 df = 3, $p = 0.001$). Pair-wise tests revealed a significant difference between these three river sections (pseudo-F: 2.18-4.44, $p < 0.002$). SIMPER analysis showed that *D. haemobaphes* and *C. sowinskyi* were characteristic species for the Lower Sava, while *D. villosus* and *C. curvispinum* were characteristic species for the Middle and Lower Drava and the Middle Danube.

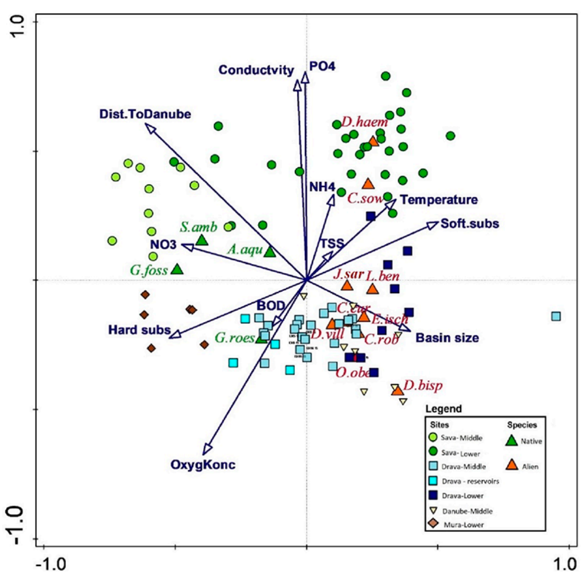

Figure 5. Canonical correspondence analysis (CCA) triplot of 92 samples (46 sites in two years), 12 explanatory environmental variables (PO4-Orthophosphates, NO3-nitrates, NH4-Ammonia, TSS-Total suspended solids, BOD-Biological oxygen demand, OxygKonc-Dissolved oxygen, Soft subs-Soft substrate, Hard subs-Hard substrate, Dist. to Danube-Distance to Danube) and 14 Peracarida species (*G. foss-G. fossarum*, *G. roes-G. roeseli*, *S. amb-S. ambulans*, *D. vill-D. villosus*, *D. haem-D. haemobaphes*, *D. bisp-D. bispinosus*, *C. sow-C. sowinskyi*, *C. cur-C. curvispinum*, *C. rob-C. robustum*, *E. isch-E. ischnus*, *O. obe-O. obesus*, *L. ben-L. benedeni*, *A. aqu-A. aquaticus*, *J. sar-J. sarsi*), the amount of peracarid assemblage variation explained by the explanatory variables was 52.5 %; Monte Carlo test: CCA1 $p = 0.0001$, all axes $p = 0.0001$. *Análisis de correspondencia canónica (CCA) de 92 muestras (46 puntos en dos años), 12 variables ambientales explicativas (PO4-Ortofosfatos, NO3-nitratos, NH4-Amoníaco, TSS-Sólidos suspendidos totales, DBO-Demanda biológica de oxígeno OxygKonc-Oxígeno disuelto, Substrato blando-Substrato blando, Substrato duro subs-Duro, Dist. Al Danubio-Distancia al Danubio) y 14 especies de Peracarida. La varianza explicada fue 52.5 %; Prueba de Monte Carlo: CCA1* p *= 0.0001, todos los ejes* p *= 0.0001.*

Using canonical correspondence analysis (CCA), we tested the connections between physicochemical parameters and peracarid crustaceans. Native species are likely to be found at a greater distance from the Danube River and with a higher concentration of nitrates. This is mostly due to the high densities of *G. fossarum* in the Mura River and Middle Sava, which had the highest nitrate concentrations. The amphipods *D. haemobaphes* and *C. sowinskyi* were present at sites in the Sava River with higher concentrations of phosphorus, ammonia and with higher water temperatures and soft substrates. Most other alien peracarids found in higher densities in the Drava and/or Danube Rivers were associated with higher oxygen concentrations and coarser substrate, and to basin size (as a proxy measure of discharge at the studied sites) (Fig. 5).

DISCUSSION

The Danube River is one of the main routes of spread for invasive species from the Ponto-Caspian region into Central Europe. It is part of the Southern Corridor (Bij de Vaate et al., 2002) and part of the European Invasive Network (Panov et al., 2009). The whole course of the Danube River is divided into three parts: Upper, Middle, and Lower Danube (Literáthy et al., 2002), where the section passing through Croatia belongs to the Middle Danube. In 2013, benthic macroinvertebrate sampling during the Joint Danube Survey (JDS 3) recorded a total of 28 peracarid species in this stretch of the river (Borza et al., 2015; Liška et al., 2015). In this study, a total of 16 species of peracarid crustaceans were found, of which 11 were alien species found in three of four of the Croatian large rivers. In the JDS 3 survey, all recorded alien species were found in the Middle Danube and all recorded alien Peracarida species in Croatian large rivers originated from Ponto-Caspian region. Six recorded species can be considered invasive due to their presence in high densities and their widespread distribution along the Sava and Drava Rivers. At most sites with invasive species, there were no native species or they were present only in low densities, as observed in studies of large rivers in France and Poland (Bollache et al., 2004; Grabowski et al., 2007a). In the Sava and Drava Rivers, invasive peracarids have been spreading upstream. In a previous study by Žganec et al. (2009), the most upstream sites of alien Peracarida species (*D. haemobaphes* and *C. curvispinum*) in the Sava River was at 474 rkm from the mouth, while in the present study, the most upstream sites with alien peracarids (*D. haemobaphes* and *J. sarsi*) was at rkm 594, indicating an upstream spread of 120 rkm in just 11 years (2005 to 2016). In the Drava River, the most upstream sites of *D. villosus* in the previous study were 239 rkm from the mouth in 2008, while in this study it was found 37 km upstream (rkm 276) in 2017. In the future, further upstream movement of invasive species can be expected in the Sava (*D. haemobaphes*, *C. sowinskyi*) and Drava Rivers (*D. villosus*, *C. curvispinum*). This could potentially lead to the disappearance of native amphipods (*G. fossarum*, *G. roeselii* and *S. ambulans*) from the most upstream reaches of these two rivers. The Danube is a source of invasive species that colonized the Sava and Drava Rivers. Passive dispersal by attachment on the surface of ships and small fishing boats (stowaway pathway, see Žganec et al., 2020) has likely been the main mechanism of upstream spread from the Danube River. The alien peracarid species *Echinogammarus* (*Trichogammarus*) *trichiatus* (present in the Upper and Middle Danube, but not yet in the Croatian section of the Danube; Borza et al., 2015) is a potential new invader of Croatian large rivers.

Characteristic alien species for the Sava River were the invasive amphipods *C. sowinskyi* and *D. haemobaphes*, while *D. villosus* and *C. curvispinum* were characteristic species in the Drava and Danube Rivers. Sites in the Lower Sava had softer substrates, with higher temperatures, conductivity and ammonia concentrations, and lower dissolved oxygen and orthophosphate concentrations versus sites on the Drava River. There are many possible reasons why *D. haemobaphes* and *C. sowinskyi* had higher densities in the Lower Sava than in the Lower and Middle Drava, where *D. villosus* and *C. curvispinum* dominate. The first two species may be better adapted to the conditions in the Lower Sava, while the stronger competitors *C. curvispinum* and *D. villosus* outcompeted them in the Lower and Middle Drava, which has abiotic conditions similar to the Danube. This is supported by the fact that *D. haemobaphes* is better adapted to a wider range of environmental conditions than *D. villosus* (Muskó, 1994), showing very high tolerance to higher temperatures (6–30°C) (Bij de Vaate et al., 2002) and low oxygen concentrations in water (Bacela et al., 2009), while *C. sowinskyi* prefers softer substrates with detritus (Jazdzewski & Konopacka, 1993), conditions found in the Lower Sava. Furthermore, conditions in the Middle and Lower Drava are more suitable for *D. villosus*, which prefers sites with a higher oxygen concentration in the water, lower conductivity (Boets et al., 2010), and an optimal temperature range of 20–23°C (Bruijs et al., 2001), and although it inhabits various substrates, it prefers hard substrates and embankments (Boets et al., 2010). *C. curvispinum* is also adapted to a wide range of ecological conditions. It can be found in different river reaches (Borza, 2011), but prefers sites with a higher oxygen concentration in the water (Van der Velde et al., 1998) and can be present on hard and soft substrates (Van den Brink et al., 1993). These traits, along with physicochemical parameters, could explain the differences in densities of the four invasive amphipods in the Sava and Drava Rivers. Further, the closer proximity of the Lower Drava to the Danube River, in combination with the competitive superiority of *D. villosus* and *C. curvispinum*, could also explain the domination of the later invaders in the Drava River. To date, they have not yet succeeded in outcompeting the first invaders *D. haemobaphes* and *C. sowinskyi* from the Lower Sava in Croatia, which is much more distant to the Danube. The co-occurrence of *D. villosus* and *D. haemobaphes* was found in the Drava River in previous research (Horvai et al., 2012; Žganec et al., 2009). However, in the present study, *D. haemobaphes* was not found at sites in the Drava River, suggesting that *D. villosus* has outcompeted *D. haemobaphes* from the Drava River. On the other hand, *C. sowinskyi* was likely the first invader, with the first record in Hungary in 1917, expanding its range from the Ponto-Caspian region in the Danube River (Borza, 2011; Borza et al., 2017). Therefore, *C. sowinskyi* was likely the first invader of the Sava and Drava, while *C. curvispinum* arrived second and colonized the Drava more successfully than the Lower Sava. It can be assumed that *C. curvispinum* did not succeed in outcompeting *C. sowinskyi* in the Lower Sava as in the Drava due to less suitable physico-chemical conditions for its survival in the Sava River.

The appearance of *D. villosus* in the Croatian part of the Sava River at three sites (SA8, SA9, SA15) in 2015 was reported and discussed in Žganec et al. (2018), while here we add a single finding of this species at site SA10 in 2016. Since it was also recorded in the Serbian part of the Sava River in 2011 and 2012, its dispersal from the Danube or Lower Sava may have been enabled by ships navigating the Sava River and transporting crude oil to the refinery at the Sisak port (at 594 rkm) (Komatina & Grošelj, 2015). In the present study, *D. villosus* and *D. haemobaphes* were found to co-occur at four sites in the Sava River, where *D. villosus* was more abundant at two sites (SA8 and SA9), and *D. haemobaphes* dominated at two sites (SA10 and SA15).

Interestingly, in 2015, alien and native peracarids in the Drava River co-occurred at only five sites, with a low density of native peracarids. However, in 2017, native species (*G. fossarum*, *G. roeselii*, *S. ambulans* and *A. aquaticus*) appeared at three more sites in the Drava with densities (8.8–199.2 ind./m^2) similar to or even higher than the co-occurring *D. villosus* at three sites. Densities of *D. villosus* decreased drastically at these three and other sites in the Drava, probably due to the extremely cold temperatures in January 2017, when parts of the Lower Drava were frozen (Meteorološki & Hidrološki bilten, 2017). These results suggest that native species co-occurred with alien species in the Drava River, but were present in low abundance at specific microhabitats or in deeper parts of the river and were not detected during sampling along the river bank in 2015. Low winter temperatures caused increased mortality and population decline of invasive *D. villosus*, while native species survived and immediately colonized the littoral parts of the Drava River, usually dominated by *D. villosus*. It is also possible that the reappearance of native species was caused by drift from small tributaries, and in situations with low densities of *D. villosu*s, they could better survive and thus appeared in higher densities at three sites in the Drava River.

The number of alien species and their density increased in the downstream direction along the Sava and Drava Rivers, while the number of native species and their density decreased in the same direction. Furthermore, a significant negative correlation was established between densities and taxa number of alien Peracarida versus all native taxa, suggesting that alien Peracarida had a potentially negative impact on the native taxa. Alien species, particularly *D. villosus*, due to their predatory impact or habitat overlap with native species, often can impact native species by reducing their densities or can cause the complete disappearance of native species (Dick & Platvoet, 2000; Krisp & Maier, 2005; Noordhuis et al., 2009). On the other hand, downstream sections of the Sava and Drava Rivers had higher organic and chemical pollution than upstream sections (Matoničkin et al., 1975; Meštrov et al., 1978, 1989), which could be responsible for the reduced densities and disappearance of native species, creating empty niches for the colonization of alien species that are generally better adapted to pollution (Gurevitch & Padilla, 2004; Riley et al., 2005). It is difficult to determine precisely whether alien peracarids were the drivers of changes in macroinvertebrate assemblages, or whether their success was facilitated more by the absence of native species (Didham et al., 2005; MacDougall & Turkington, 2005). The highly biocontaminated macroinvertebrate assemblages in the Croatian large rivers (Ćuk et al., 2019), with invasive Peracarida as the most abundant aliens, likely resulted from the synergistic effect of pollution that decreased densities of native species, and the negative impact of invasive peracarids on native macroinvertebrates.

Biocontamination level and the number of alien macroinvertebrate species in the rivers examined here were similar to parts of the Middle Danube (Paunović et al., 2007; Liška et al., 2015) and the French part of the Moselle River (Devin et al., 2005). However, higher biocontamination levels were recorded in Croatian large rivers (Ćuk et al., 2019; this study), than in other large rivers along three main corridors used for the spread of Ponto-Caspian macroinvertebrates in Europe (Arbačiauskas et al., 2008). In our case, alien Peracarida dominated in abundance compared to other alien taxa, and biocontamination indices showed high correlations with densities of invasive Peracarida. The same results were recorded in many other European rivers where peracarids from the Ponto-Caspian region also dominated the macroinvertebrate assemblages (Dnieper River -Mastitsky & Makarevich, 2007; Meuse River - Josens et al., 2005; Rhine River - Bernauer & Jansen, 2006; Bij de Vaate et al., 2002; Leuven et al., 2009; Van Riel et al., 2006; Elbe River - Hellmann et al., 2015; Vistula and Oder Rivers - Grabowski et al., 2007b; Wall River - Van Riel et al., 2006b). The highest density of alien Peracarida in our samples was seen in two genera, *Dikerogammarus* and *Chelicorophium*, which also contributed most to the biocontamination indices. Similar results were recorded in other European lotic systems, where peracarids were also the main contributor to biocontamination (Arbačiauskas et al., 2011; Jabłońska-Barna & Koszałka, 2020).

The future spread of invasive amphipods in the Sava and Drava Rivers will likely cause an increase in biocontamination and decrease native taxa richness and diversity in the most upstream reaches of the Sava, Drava, and Mura Rivers. To slow and prevent further upstream spreading of alien Peracarida in the future, it is essential to inform different groups of people of this issue, most importantly those who spend the most time on rivers, such as anglers and boat drivers. Further, "check, clean and dry" protocols (GB Non-native species secretariat, 2020) should urgently be included in the national regulations. In addition, future regular monitoring of the spread of invasive species in Croatian large rivers is necessary to ensure that the applied measures are working.

CONCLUSIONS

This study showed that alien peracarids were present in the Sava, Drava and Danube, while only native peracarids were present in the Mura and the most upstream sites in the Drava and Sava Rivers. The highest number of alien species were found in the Danube River (10 species), while the Sava had five alien species and Drava had six alien species. Distance to the Danube River, as the source of all alien peracarids, and physicochemical parameters explained the variation in peracarids assemblages between the studied rivers, along with the different colonization history of each river section. The most widespread alien species was *J. sarsi*, while the most abundant species was *C. curvispinum*. The most widespread and the most abundant native species was *G. fossarum*. Alien Peracarida contributed most to the biocontamination indices, i.e. to the abundance and richness contamination of macroinvertebrate assemblages. Measures that would slow down the upstream spread of invasive peracarids, such as education of the anglers and boat drives along with "check, clean and dry" protocols are urgently needed to protect the remaining native macroinvertebrate biodiversity in the most upstream segments of Croatian large rivers.

REFERENCES

AQEM CONSORTIUM (2002). Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive . Version 1.0, February 2002.

Arbačiauskas, K., Rakauskas, V., & Virbickas, T. (2010). Initial and long-term consequences of attempts to improve fish-food resources in Lithuanian waters by introducing alien peracaridan species: a retrospective overview. *Journal of Applied Ichthyology*, 26 (SUPPL. 2), 28–37. DOI: 10.1111/j.1439-0426.2010.01492.x

Arbačiauskas, K., Semenchenko, V., Grabowski, M., Leuven, R. S. E. W., Paunović, M., Son, M. O., Csányi, B., Gumuliauskaite, S., Konopacka, A., Nehring, S., Van der Velde, G., Vezhnovetz, V., & Panov, V. E. (2008). Assessment of biocontamination of benthic macroinvertebrate communities in European inland waterways. *Aquatic Invasions*, 3(2), 211–230. DOI: 10.3391/ai.2008.3.2.12

Arbačiauskas, K., Višinskiene, G., & Smilgevičiene, S. (2011). Non-indigenous macroinvertebrate species in Lithuanian fresh waters, Part 2: Macroinvertebrate assemblage deviation from naturalness in lotic systems and the consequent potential impacts on ecological quality assessment. *Knowledge and Management of Aquatic Ecosystems*, 402, 1–18. DOI: 10.1051/kmae/2011076

Argano, R. (1979). *Guide per il riconoscimento delle specie animali delle acque interne Italiane. 5. Isopodi (Crustacea, Isopoda)*. Consiglio nazionale delle ricerche. Impresso della stamperia Veldonega, Verona.

Bacela, K., Konopacka, A., & Grabowski, M. (2009). Reproductive biology of *Dikerogammarus haemobaphes*: An invasive gammarid (Crustacea: Amphipoda) colonizing running waters in Central Europe. *Biological Invasions*, 11(9), 2055–2066. DOI: 10.1007/s10530-009-9496-2

Bernauer, D., & Jansen, W. (2006). Recent invasions of alien macroinvertebrates and loss of native species in the upper Rhine River, Germany. *Aquatic Invasions*, 1(2), 55–71. DOI: 10.3391/ai.2006.1.2.2

Bij de Vaate, A., Jazdzewski, K., Ketelaars, H. A. ., Gollasch, S., & Van der Velde, G. (2002). Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, 59(7), 1159–1174. DOI: 10.1139/f02-098

Boets, P., Lock, K., Messiaen, M., & Goethals, P. L. M. (2010). Combining data-driven methods and lab studies to analyse the ecology of *Dikerogammarus villosus*. *Ecological Informatics*, 5(2), 133–139. DOI: 10.1016/j.ecoinf.2009.12.005

Bollache, L., Devin, S., Wattier, R., Chovet, M., Beisel, J.-N., Moreteau, J.-C., & Rigaud, T. (2004). Rapid range extension of the Ponto-Caspian amphipod *Dikerogammarus villosus* in France: potential consequences. *Archiv Für Hydrobiologie*, 160(1), 57–66. DOI: 10.1127/0003-9136/2004/0160-0057

Borza, P. (2011). Revision of invasion history, distributional patterns, and new records of Corophiidae (Crustacea: Amphipoda) in Hungary. *Acta Zoologica Academiae Scientiarum Hungaricae*, 57(1), 75–52.

Borza, P., Csányi, B., Huber, T., Leitner, P., Paunović, M., Remund, N., Szekeres, J., & Graf, W. (2015). Longitudinal distributional patterns of Peracarida (Crustacea, Malacostraca) in the River Danube. *Fundamental and Applied Limnology/Archiv Für Hydrobiologie*, 187(2), 113–126. DOI: 10.1127/fal/2015/0769

Borza, P., Huber, T., Leitner, P., Remund, N., & Graf, W. (2017). Current velocity shapes co-existence patterns among invasive *Dikerogammarus* species. *Freshwater Biology*, 62(2), 317–328. DOI: 10.1111/fwb.12869

Bruijs, M. C. M., Kelleher, B., Van der Velde, G., & Bij de Vaate, A. (2001). Oxygen consumption, temperature and salinity tolerance of the invasive amphipod *Dikerogammarus villosus*: indicators of further dispersal via ballast water transport. *Fundamental and Applied Limnology*, 152(4), 633–646. DOI: 10.1127/archiv-hydrobiol/152/2001/633

Cărăusu, S., Dobreanu, E., Manolache, C. (1955). *Fauna Republicii Populare Romîne, Crustacea, Amphipoda*. Volume IV. Academia Republicii Populare Romîne.

Ćuk, R., Miliša, M., Atanacković, A., Dekić, S., Blažeković, L., & Žganec, K. (2019). Biocontamination of benthic macroinvertebrate assemblages in Croatian major rivers and effects on ecological quality assessment. *Knowledge and Management of Aquatic Ecosystems*, 2019-Janua(420). DOI: 10.1051/kmae/2019003

Devin, S., Beisel, J. N., Usseglio-Polatera, P., & Moreteau, J. C. (2005). Changes in functional biodiversity in an invaded freshwater ecosystem: The Moselle River. *Hydrobiologia*, 542(1), 113–120. DOI: 10.1007/s10750-004-8771-6

Dick, J. T. A., & Platvoet, D. (2000). Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proceedings of the Royal Society B: Biological Sciences*, 267(1447), 977–983. DOI: 10.1098/rspb.2000.1099

Didham, R. K., Tylianakis, J. M., Hutchison, M. A., Ewers, R. M., & Gemmell, N. J. (2005). Are invasive species the drivers of ecological change? *Trends in Ecology and Evolution*, 20(9), 470–

474. DOI: 10.1016/j.tree.2005.07.006

Dobson, M. (2012). *Identifying Invasive Freshwater Shrimps and Isopods*, Freshwater Biological Association. Cumbria. England

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, 81(2), 163–182. DOI: 10.1017/S1464793105006950

Eggers, T. O., Martens, A. (2001). Bestimmungsschlüssel der Süßwasser-Amphipoda (Crustacea) Deutschlands, Lauterbornia, 42, 1-68

Elliott, M. (2003). Biological pollutants and biological pollution - An increasing cause for concern. *Marine Pollution Bulletin*, 46(3), 275–280. DOI: 10.1016/S0025-326X(02)00423-X

GB Non-native species secretariat (2020): Non Native Species Secretariat (NNSS) of Great Britain, Animal and Plant Health Agency, Sand Hutton, York, YO41 1LZ, http://www.nonnativespecies.org/checkcleandry/ (accessed 21 January 2020)

Gergs, R., & Rothhaupt, K. O. (2014). Invasive species as driving factors for the structure of benthic communities in Lake Constance, Germany. *Hydrobiologia*, 746(1), 245–254. DOI: 10.1007/s10750-014-1931-4

Ghetti, P. F. (1997). *I macroinvertebrati nel controllo della qualità di ambienti di acque correnti. Indice Biotico Esteso (I.B.E.), Manuale di applicazione, Provincia Autonoma di Trento.* Trento, Italy

Gollasch, S. (2007). Marine vs. freshwater invaders: is shipping the key vector for species introductions to Europe? In: F. Gherardi (ed.) *Biological Invaders in Inland Waters: Profiles, distribution, and threats*. Invading Nature - Springer Series In Invasion Ecology, vol 2. (p.p. 339-345). Springer, Dordrecht, Netherland

Grabowski, M., Bacela, K., & Konopacka, A. (2007a). How to be an invasive gammarid (Amphipoda: Gammaroidea) - Comparison of life history traits. *Hydrobiologia*, 590(1), 75–84. DOI: 10.1007/s10750-007-0759-6

Grabowski, M., Jazdzewski, K., & Konopacka, A. (2007b). Alien crustacea in polish waters - Amphipoda. *Aquatic Invasions*, 2(1), 25–38. DOI: 10.3391/ai.2007.2.1.3

Gurevitch, J., & Padilla, D. K. (2004). Are invasive species a major cause of extinctions? *Trends in Ecology and Evolution*, 19(9), 470–474. DOI: 10.1016/j.tree.2004.07.005

Haas, G., Brunke, M., Streit, B. (2002). Fast turnover in dominance of exotic species in the Rhine River determines biodiversity and ecosystem function: an affair between amphipods and mussels. In: E. Leppäkoski, S. Gollasch, S. Olenin (eds.) *Invasive Aquatic Species of Europe. Distribution, Impacts and Management* (p. p. 426-432). Kluwer Academic Publishers, Dordrecht, Netherland

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(4), 385–397. DOI: 10.3391/ai.2015.10.4.03

Holdich, D. M., & Pöckl, M. (2007). Invasive crustaceans in European inland waters. In: F. Gherardi (ed.) *Biological Invaders in Inland Waters: Profiles, Distribution, and Threats*, (p. p.29–75). Springer. DOI: 10.1007/978-1-4020-6029-8_2

Horvai, V., Czirok, A., Lőkkös, A., Borza, P., Bódis, E., & Deák, C. (2012). New faunistic data from the riparian zone of the Hungarian-Croatian reach of the Drava river. *Acta Biologica Debrecina. Supplementum Oecologica Hungarica,* 28(September 2015), 109–120.

Illies, J. (1978). *Limnofauna Europaea*. Gustav Fischer Verlag, Stuttgart, Germany

Jabłońska-Barna, I., & Koszałka, J. (2020). Biocontamination of the Aquatic Ecosystems of Northeastern Poland. *Handbook of Environmental Chemistry*, 87, 127–139. DOI: 10.1007/978-3-030-12139-6_6

Jazdzewski, K., Konopacka, A. (1993). Remarks on the morphology, taxonomy and distribution of *Corophium curvispinum* G.O. Sars, 1895 and *Corophium sowinsky* Martinov, 1924 (Crustacea, Amphipoda, *Corophiidae*). Boll., *Mus. civ. St. nat. Verona*, 20, 487-501

Jazdzewski, K., Konopacka, A., & Grabowski, M. (2004). Recent drastic changes in the gammarid fauna (Crustacea, Amphipoda) of the Vistula River deltaic system in Poland caused by alien invaders. *Diversity and Distributions*, 10(2), 81–87. DOI: 10.1111/j.1366-9516.2004.00062.x

Josens, G., Bij de Vaate, A., Usseglio-Polatera, P., Cammaerts, R., Chérot, F., Grisez, F., Verboonen, P., & Vanden Bossche, J. P. (2005). Native and exotic Amphipoda and other Peracarida in the River Meuse: New assemblages emerge from a fast changing fauna. *Hydrobiologia*, 542(1), 203–220. DOI: 10.1007/s10750-004-8930-9

Karaman, G. S., Pinkster, S. (1977a). Freshwater *Gammarus* species from Europe, North Africa, and adjacent regions of Asia (Crustacea – Amphipoda), *Gammarus pulex* group and related species, *Bijdr. Dierk.*, 47, 1–97

Karaman, G. S., Pinkster, S. (1977b). Freshwater *Gammarus* species from Europe, North Africa, and adjacent regions of Asia (Crustacea – Amphipoda), *Gammarus roeselii* group and related species, *Bijdr. Dierk.*, 47, 165-196

Komatina D., Grošelj, S. (2015). Transboundary water cooperation for sustainable development of the Sava River Basin, In: R. Milačić, J. Ščančar, M. Paunović (eds.) *The Sava River* (p. p. 1-28). Springer, Berlin/Heidelberg

Krisp, H., & Maier, G. (2005). Consumption of macroinvertebrates by invasive and native gammarids: A comparison. *Journal of Limnology*, 64(1), 55–59. DOI: 10.4081/jlimnol.2005.55

Lepš, J., Šmilauer, P. (2003). *Multivariate analysis of ecological data using CANOCO*. Cambridge University Press, Cambridge

Leuven, R. S. E. W., Van der Velde, G., Baijens, I., Snijders, J., Van der Zwart, C., Lenders, H. J. R., & Bij de Vaate, A. (2009). The river Rhine: A global highway for dispersal of aquatic invasive species. *Biological Invasions*, 11(9), 1989–2008. DOI: 10.1007/s10530-009-9491-7

Literáthy, P., Koller – Kreimel, V., Liška, I. (2002). *Joint Danube Survey, Final Report*. ICPDR – International Commission for the Protection of the Danube River. Vienna

Liška, I., Wagner, F., Sengl, M., Deutsch, K., Slobodnik, J. (2015). *Joint Danube Survey 3: A Comprehensive Analysis of Danube Water Quality, Final Report*. International Commission for the Protection of the Danube River (ICPDR). Vienna

MacDougall, A. S., & Turkington, R. (2005). Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, 86(1), 42–55. DOI: 10.1890/04-0669

MacNeil, C., Briffa, M., Leuven, R. S. E. W., Gell, F. R., & Selman, R. (2010). An appraisal of a biocontamination assessment method for freshwater macroinvertebrate assemblages; a practical way to measure a significant biological pressure? *Hydrobiologia*, 638(1), 151–159. DOI: 10.1007/s10750-009-0037-x

Mastitsky, S. E., & Makarevich, O. A. (2007). Distribution and abundance of Ponto-Caspian amphipods in the Belarusian section of the Dnieper River. *Aquatic Invasions*, 2(1), 39–44. DOI: 10.3391/ai.2007.2.1.4

Matoničkin, I., Pavletić, Z., Habdija, I., Stilonović, B. (1975). *A contribution to the valorisation of waters of the ecosystem of river Sava*. Sveučilišna naklada Liber. Zagreb, Croatia

Meteorološki i Hidrološki bilten 01/2017, Editors: Strelec Mahović, N., Dragojlović, D., Državni hidrometeorološki zavod, Croatia,

Meštrov, M., Dešković, I., Tavčar, V. (1978). Pollution of the river Sava – according to several years of ecological research, *Ekologija*, 13, 61-79

Meštrov, M., Habdija, I., Futač, N. (1989). Biološko-ekološka valorizacija kvalitete vode rijeke Save- In: M. Meštrov (ed.) *Rijeka Sava-zaštita i korištenje voda* (p. p. 290-308 Zagreb, JAZU

Muskó, I. B. (1994). Occurrence of Amphipoda in Hungary since 1853. *Crustaceana*, 66, 144-152

Noordhuis, R., Van Schie, J., & Jaarsma, N. (2009). Colonization patterns and impacts of the invasive amphipods *Chelicorophium curvispinum* and *Dikerogammarus villosus* in the IJsselmeer area, the Netherlands. *Biological Invasions*, 11(9), 2067–2084. DOI: 10.1007/s10530-009-9487-3

Nunes, A. L., Tricarico, E., Panov, V. E., Cardoso, A. C., & Katsanevakis, S. (2015). Pathways and gateways of freshwater invasions in

Europe. *Aquatic Invasions*, 10(4), 359–370. DOI: 10.3391/ai.2015.10.4.01

Official Gazette (2013): No. 73/13, 151/14, 78/15, 61/16, 80/18, Regulation on Water Quality Standard

Panov, V. E., Alexandrov, B., Arbačiauskas, K., Binimelis, R., Copp, G. H., Grabowski, M., Lucy, F., Leuven, R. S., Nehring, S., Paunović, M., Semenchenko, V., & Son, M. O. (2009). Assessing the risks of aquatic species invasions via european inland waterways: From concepts to environmental indicators. *Integrated Environmental Assessment and Management*, 5(1), 110–126. DOI: 10.1897/IEAM_2008-034.1

Paunovic, M., Jakovcev-Todorovic, D. G., Simic, V. M., Stojanovic, B. D., & Cakic, P. D. (2007). Macroinvertebrates along the Serbian section of the Danube River (stream km 1429-925). *Biologia*, 62(2), 214–221. DOI: 10.2478/s11756-007-0032-5

Pinkster, S. (1993). *A revision of the genus Echinogammarus, Stebbing, 1899, with some notes on related genera (Crustacea, Amphipoda)*. Museo civico di storia naturale di Verona

Piscart, C., Kefford, B. J., & Beisel, J. N. (2011). Are salinity tolerances of non-native macroinvertebrates in France an indicator of potential for their translocation in a new area? *Limnologica*, 41(2), 107–112. DOI: 10.1016/j.limno.2010.09.002

Rewicz, T., Grabowski, M., Macneil, C., & Karolina, B. (2014). The profile of a ' perfect ' invader – the case of killer shrimp, *Dikerogammarus villosus*. *Aquatic Invasions*, 9(3), 267–288.

Ricciardi, A., & MacIsaac, H. J. (2000). Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. *Trends in Ecology and Evolution*, 15(2), 62–65. DOI: 10.1016/S0169-5347(99)01745-0

Ricciardi, A., Whoriskey, F. G., & Rasmussen, J. B. (1997). The role of the zebra mussel (*Dreissena polymorpha*) in structuring macroinvertebrate communities on hard substrata. *Canadian Journal of Fisheries and Aquatic Sciences*, 54(11), 2596–2608. DOI: 10.1139/f97-174

Riley, S. P. D., Busteed, G. T., Kats, L. B., Vandergon, T. L., Lee, L. F. S., Dagit, R. G., Kerby, J. L., Fisher, R. N., & Sauvajot, R. M. (2005). Effects of urbanization on the distribution and abundance of amphibians and invasive species in southern California streams. *Conservation Biology*, 19(6), 1894–1907. DOI: 10.1111/j.1523-1739.2005.00295.x

Santagata, S., Bacela, K., Reid, D. F., Mclean, K. A., Cohen, J. S., Cordell, J. R., Brown, C. W., Johengen, T. H., & Ruiz, G. M. (2009). Concentrated sodium chloride brine solutions as an additional treatment for preventing the introduction of nonindigenous species in the ballast tanks of ships declaring no ballast on board. *Environmental Toxicology and Chemistry*, 28(2), 346–353. DOI: 10.1897/08-140.1

Strayer, D. L. (2010). Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, 55(SUPPL. 1), 152–174. DOI: 10.1111/j.1365-2427.2009.02380.x

Ter Braak, C. J. F., Šmilauer, P. (2012). *Canoco reference manual and user's guide: software for ordination, version 5.0*. Microcomputer Power, Ithaca

Van den Brink, F. W. B., Van der Velde, G., & Bij de Vaate, A. (1993). Ecological aspects, explosive range extension and impact of a mass invader, *Corophium curvispinum* Sars, 1895 (Crustacea: Amphipoda), in the Lower Rhine (The Netherlands). *Oecologia*, 93(2), 224–232. DOI: 10.1007/BF00317675

Van den Brink, F. W. B., Van der Velde, G., Cazemier, W. G. (1990). The faunistic composition of the freshwater section of the River Rhine in The Netherlands: present state and changes since 1900. In: R. Kinzelbach, G. Friedrich (eds.) *Biologie des Rheins*. (p. p. 191-216). Limnol aktuell

Van der Velde, G, Rajagopal, S., Kelleher, B., Musko, I. B., & Bij de Vaate, A. (1998). Ecological impact of crustacean invaders: General considerations and examples from the Rhine River. *Biodiversity Crisis and Crustacea*, 12, 3–33.

Van Riel, M. C., Van der Velde, G., & Bij de Vaate, A. (2006a). To conquer and persist: Colonization and population development of the Ponto-Caspian amphipods *Dikerogammarus villosus* and *Chelicorophium curvispinum* on bare stone substrate in the main channel of the River Rhine. *Archiv Für Hydrobiologie*, 166(1), 23–39. DOI: 10.1127/0003-9136/2006/0166-0023

Van Riel, M. C., Van der Velde, G., Rajagopal, S., Marguillier, S., Dehairs, F., & Bij de Vaate, A. (2006b). Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. *Hydrobiologia*, 565(1 SPEC. ISS.), 39–58. DOI: 10.1007/s10750-005-1904-8

Veuille, M. (1979). L'évolution du Genre Jaera Leach (Isopodes; Asellotes) et ses Rapports avec L'histoire de la Méditerranée. *Bijdr. Dierk.*, 49, 195-217

Wawrzyniak-Wydrowska, B., & Gruszka, P. (2005). Population dynamics of alien gammarid species in the River Odra estuary. *Hydrobiologia*, 539(1), 13–25. DOI: 10.1007/s10750-004-3081-6

Wittmann, K. J., Ariani, A. P., Daneliya, M. (2016). The Mysidae (Crustacea: Peracarida: Mysida) in fresh and oligohaline waters of the Mediterranean. Taxonomy, biogeography, and bioinvasion, *Zootaxa*, 4142(1), 1–70. DOI: 10.11646/zootaxa.4142.1.1

Žganec, K., ćuk, R., Tomović, J., Lajtner, J., Gottstein, S., Kovačević, S., Hudina, S., Lucić, A., Mirt, M., Simić, V., Simčić, T., & Paunović, M. (2018). The longitudinal pattern of crustacean (Peracarida, Malacostraca) assemblages in a large south European river: Bank reinforcement structures as stepping stones of invasion. *Annales de Limnologie*, 54 (January). DOI: 10.1051/limn/2018008

Žganec, K., Gottstein, S., & Hudina, S. (2009). Ponto-Caspian amphipods in Croatian large rivers. *Aquatic Invasions*, 4(2), 327–335. DOI: 10.3391/ai.2009.4.2.4

Žganec, K., Lajtner, J., Ćuk, R., Crnčan, P., Pušić, I., Atanacković, A., Kralj, T., Valić, D., Jelić, M., & Maguire, I. (2020). Alien macroinvertebrates in Croatian freshwaters. *Aquatic Invasions*, 15(4), 593–615. DOI: 10.3391/ai.2020.15.4.04.