

1 **Only one can remain? Environmental and spatial factors influencing habitat**
2 **partitioning among invasive and native crayfishes in the Pannonian Ecoregion**
3 **(Hungary).**

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25 **Abstract**

26 Biological invasions have increasingly threatened indigenous species, influence
27 metacommunity organization and consequently, global biodiversity. World-wide expansion of
28 non-indigenous crayfish (NICS) is associated with dramatic changes in species poor
29 indigenous crayfish (ICS) assemblages challenging conservation planning. We analysed long-
30 term changes of crayfish occurrences from the pre-invasion state, through the first appearance
31 of non-indigenous crayfish species (NICS), to their intensive spread in Hungarian waters.
32 Further, we analysed present-day crayfish metacommunity patterns for co-occurrences and
33 influence of spatial and environmental factors. Historic data revealed a marked pre-invasion
34 decline in indigenous noble crayfish *Astacus astacus* and stone crayfish *Austropotamobius*
35 *torrentium* populations, but not in the narrow-clawed crayfish *Pontastacus leptodactylus*.
36 Historic data provided no direct evidence for the impact of NICS on ICS, rather it supported
37 that NICS often entered areas where ICS had been extinct or were not present at all. Crayfish
38 species extremely rarely co-occurred which could indicate their strong competition and be
39 related to utilization of empty sites by NICS. Crayfish metacommunities were predominantly
40 spatially structured indicating the primary influence of ongoing invasion. Crayfish species
41 also exhibited different environmental preferences mainly along the altitude and temperature
42 gradients. We conclude that the invasion is still in the expanding phase and without an
43 effective conservational program the future of ICS is doubtful in Hungary. Conservation
44 policy should focus on the preservation and reintroduction of the stone and noble crayfishes in
45 highland refuges. Expansion of NICS should be prevented in refuge areas by utilizing
46 possibilities provided by natural and artificial barriers, and education and strict ban should be
47 simultaneously applied to prevent further illegal releases by aquarists.

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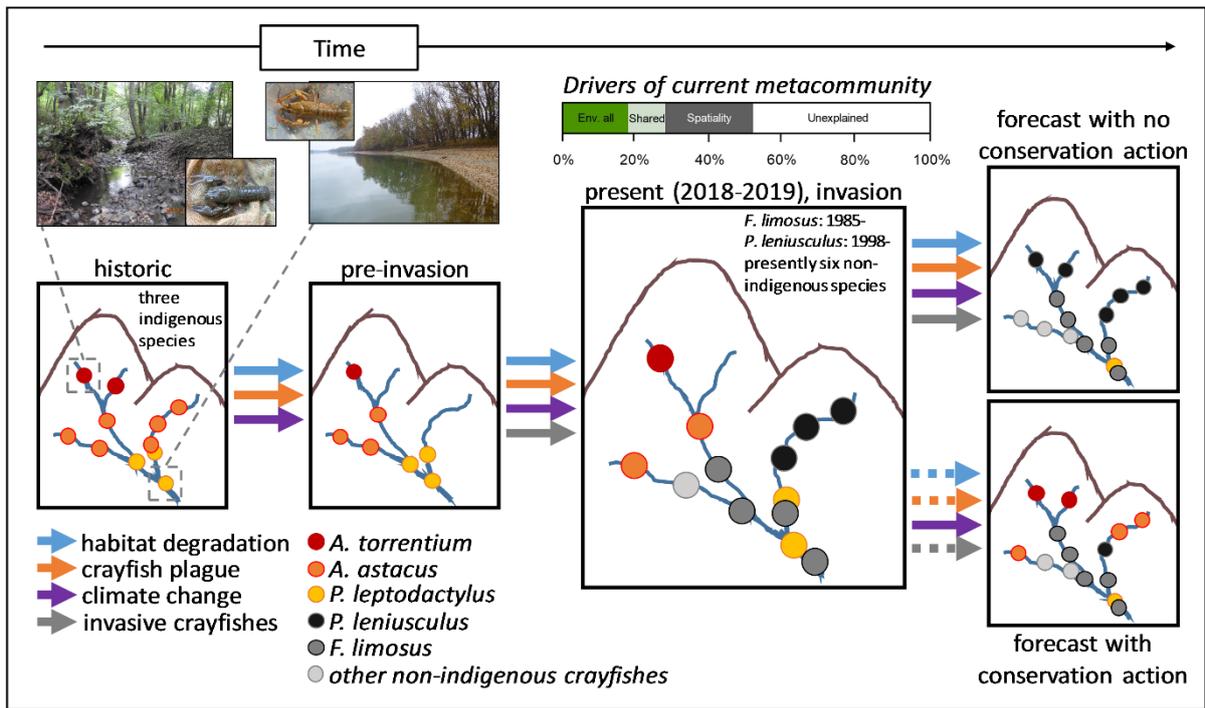
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50 **Keywords:**

51 Alien species, Biological invasion, Biotic interactions, Crayfish conservation, Environmental
52 drivers, Freshwater.

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57 **Highlights**

58 • Freshwater crayfishes decline parallel with spreading of invasive congeners globally

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60 • Indigenous crayfish populations started to deteriorate prior to invasion in Hungary

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62 • Crayfishes rarely co-occur indicating colonisation of empty sites and competition

63

64 • Spatiality predominate over environmental filtering in present crayfish distributions

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66 • Instant conservation actions are needed to prevent extinction of indigenous species

67

68 **1. Introduction**

69 Biological invasions and their impacts have been identified as one of the main drivers of
70 biodiversity loss globally (Mazor et al., 2018). Accordingly, a huge research effort is focused
71 on understanding fundamental mechanisms and consequences of invasions from population to
72 ecosystem scales (Chabrerie et al., 2019) and to develop effective conservation planning
73 frameworks which also consider potential effect of alien invasive species (Mačić et al., 2018).
74 Although plenty of sound concepts have been proposed to characterize and forecast the
75 outcome of invasion events, integrative studies have pointed out that settlement success,
76 expansion rate and impact of a potential invader is widely species-specific and depends on the
77 status of the recipient ecosystem (Gallien and Carboni, 2017; Chabrerie et al., 2019). The
78 purpose of our study is, therefore, to provide a detailed community ecological analysis and
79 conservational prospect on an ongoing invasion where a species poor indigenous crayfish
80 community is being increasingly threatened by multiple invasive species.

81 Freshwater crayfish (Decapoda: Astacidea; hereafter: crayfish) are distributed almost
82 world-wide, and they can be found practically in all types of permanent and periodic
83 freshwater habitats (Scholtz, 2002). Crayfish are keystone trophic regulators and ecological
84 engineers, as well as biodiversity indicators in many habitats where they present in high
85 densities (Reynolds et al., 2013). However, almost one-third of world's crayfish species are
86 threatened with extinction, including four of the five European Astacidae species as well
87 (Richman et al., 2015). Indigenous crayfish species (ICS) are exposed to several
88 anthropogenic stressors – e.g. habitat degradation, climate change, harvesting, introduced
89 alien predators, pollution –, among which probably one of the most global and severe threat is
90 the introduction and spread of non-indigenous, often invasive crayfish species (NICS) and
91 diseases they transmit (Capinha et al., 2013; Richman et al., 2015).

92 Invasion events in crayfish are often facilitated and their impacts are intensified by the
93 resilient status of ICS assemblages and the superior competitive properties of invaders. For
94 example, assemblages of European ICS comprise few, often just a sole tightly adapted species
95 (Holdich, 2002). Such assemblages are more sensitive to invasion due to their limited
96 functional diversity, the probable existence of weakly utilized resources and the lack of
97 redundant functional elements representing diversified environmental tolerance (Levine and
98 D'Antonio, 1999; Fargione and Tilman, 2005). Considerable proportion of habitats inhabited
99 by crayfish is exposed to anthropogenic degradation and climate change. Such areas often
100 become suboptimal or unsuitable for the resident community (Capinha et al., 2013, Římalová
101 et al., 2014; Chucholl and Schrimpf, 2016) which become therefore less resistant to invasions
102 as well. On the other hand, NICS often possess beneficial features assisting their invasion
103 success. Numerous NICS considered invasive are highly resistant to the crayfish plague
104 (*Aphanomyces astaci*), a parasite oomycete which they can carry and transmit to other, highly
105 sensitive crayfish species, amongst them to the European ICS (Kozubíková et al., 2010;
106 Filipová et al., 2013). They often show aggressive behaviour and can win one-against-one
107 fights with ICS (Söderbäck, 1995; Stucki and Romer, 2001; Hudina et al, 2016). Moreover,
108 several invasive NICS have higher temperature optima and tolerances as well as they are
109 more resistant to temporal droughts than many of their native congeners, properties which are
110 highly advantageous during the present climate change (Capinha et al., 2013; Kouba et al.,
111 2016). Correspondingly, invasions in crayfish relatively often accompanied with the
112 displacement of the resident species (Söderbäck, 1995; Westman et al., 2002; Holdich et al.,
113 2009; Chucholl and Schrimpf, 2016). Nevertheless, it is not always evident that the extinction
114 of the indigenous species relates directly to the invasive species (competitive displacement) or
115 it is due to other stressors (e.g. climate change, habitat degradation, disease) and the invasive
116 species has just benefited from the remaining vacant niche (Herbold and Moyle, 1986;

117 Chucholl, 2016). From the point of view of conservation planning, it is thus important to
118 understand the mechanisms that are responsible for the deterioration of ICS assemblages.

119 In this study we focus on the crayfish fauna of the Hungarian part of the Danube
120 catchment (Carpathian basin: Pannonian Ecoregion), which comprises three ICS: noble
121 crayfish *Astacus astacus* (Linnaeus, 1758), narrow-clawed crayfish *Pontastacus leptodactylus*
122 (Eschscholz, 1823) and stone crayfish *Austropotamobius torrentium* (Schrank, 1803) (Entz,
123 1909; Puky et al., 2005). The first documented decrease in the crayfish populations and their
124 distributions was related to the appearance of the crayfish plague in the Carpathian Basin in
125 the late 19th century (Entz, 1909). Afterwards a significant effort was made to reintroduce the
126 most impacted stocks, primary that of the noble crayfish, at the end of the 19th and in the first
127 half of the 20th century (Thuránszky and Forró, 1987). However, in the 20th century,
128 populations of the ICS continued to deteriorate due to the dramatic environmental changes
129 caused by regulation of their natural habitats, pollution and other types of habitat degradation
130 (Thuránszky and Forró, 1987). Further, the Pannonian Ecoregion represents an appropriate
131 precedent of NICS invasion and simultaneous deterioration of ICS. The first NICS in natural
132 waters of Hungary was the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817),
133 appeared in the Danube near Budapest, in 1985 (Thuránszky and Forró, 1987). Thirteen years
134 later, in 1998 the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) was found in a stream
135 near the Austrian boundary (Kovács et al. 2005). Since that, several other crayfish species
136 have been introduced, mainly by illegal releases of pet-traded ornamental species (Weiperth et
137 al., 2019). Meanwhile, the first NICS, especially the spiny-cheek crayfish, have expanded
138 their ranges considerably (Ludányi et al., 2016; Weiperth et al., 2020). Parallel to the
139 expansion of NICS, a decrease in the area of the ICS was reported (Puky et al., 2005; Ludányi
140 et al., 2016).

141 The goal of our study is to highlight parallel changes in ICS and NICS distributions from
142 the onset of the invasion, and to quantify the influence of various factors on the present
143 distribution of crayfishes in the Hungarian part of the Danube catchment. We set a series of
144 specific aims and hypotheses to evaluate. First, based on historic data, we examined: (1)
145 whether there is an indication that ICS were displaced by the NICS in the invaded areas; and
146 (2) whether NICS have invaded areas where ICS were either not present at all or became
147 extinct prior to the invasion. Then, based on data of a recent country-wide crayfish survey, we
148 analysed: (3) whether present species co-occurrence patterns support the existence of a sharp
149 interspecific competition; (4) the relative influence of spatial, climatic, local environmental
150 and land cover properties on metacommunity assembly; and (5) which factors are the best
151 predictors of presence-absence of the predominant species. We believe that the identification
152 of drivers of crayfish distributions will support the assessment of vulnerability and potential
153 residuary area of ICS, the potential spread and impact of different NICS, and accordingly,
154 base conservation planning.

155

156

157 **2. Material and methods**

158 *2.1. Study area*

159 The survey covered the whole territory of Hungary (45° 48' - 48° 35' N, 16° 5' - 22° 58'
160 E), which belongs to the Pannonian Ecoregion in the Danube River catchment within the
161 Carpathian Basin (Fig. 1). Hungary lies in the temperate zone (mean annual air temperature:
162 10 – 11 °C; annual precipitation: 500-750 mm). It has a forested area of about 21.5% and
163 intensive agricultural area of ca. 49%. Most of streams and their riparian zone in the region
164 are regulated and exposed to human impacts to various extents. We selected sampling sites to
165 represent the whole range of stream habitats from first order streams to large river (Danube),

166 including some reservoirs. Specifically, investigated sites represented entire gradients in
167 stream size (range of channel width: 0.4 – 500 m) and altitude (75 – 491 m a.s.l.) with
168 permanent streams, and that of other influential climatic, environmental and land cover
169 properties characteristic in the area.

170

171 *2.2. Historical crayfish data and their mapping*

172 We searched scientific and grey literature (only those published by acknowledged
173 experts) for crayfish occurrence data in the study area. Then, we plotted 50 km × 50 km EOVS
174 (plane projection system used uniformly for the Hungarian maps) cell distribution of
175 crayfishes for three consecutive periods. The period from the late 1800s to 1990 was
176 considered to represent the pre-invasion distribution of ICS in the region, with only a single
177 report of NICS spiny-cheek crayfish occurrence at one specific location. While, the period
178 from 1991 to 2010 was considered to represent the early phase and the period from 2011 to
179 2019 was considered to represent the intensifying phase of the NICS invasion. Distribution
180 data obtained from our recent crayfish survey presented below were also included to this
181 long-term analysis.

182

183 *2.3. Crayfish survey*

184 Within the frame of the Country-wide Crayfish Survey project coordinated by the
185 Research Institute for Fisheries and Aquaculture, we examined altogether 949 sites for
186 occurrence of crayfish between October 2016 and December 2018 (Fig. 1b). We assessed
187 presence-absence of crayfish using various sampling methods adjusted to the characteristics
188 and size of different habitat types. In the smallest streams, we hand sampled potential crayfish
189 shelters during daylight and performed visual searches over the stream bed using headlights at
190 night along 100-200 m long stream sections. In wadeable streams with water depth \geq ~40 cm

191 or with limited water transparency we set non-baited crayfish traps (type LiNi, length 900
192 mm, diameter 450 mm, mesh size 5 mm) overnight and where the water transparency
193 allowed, we performed electric fishing (equipments: Samus 725 MP and Samus 1000) along
194 100-200 m long stream sections. While in non-wadeable streams and rivers, we used non-
195 baited crayfish traps, and where applicable (long sections with clear stream bottom), we
196 performed trawling with electrified bottom trawls (width 160-210 cm, length 400 cm, mesh
197 size 6 mm) along a 200-500 m long stream section. In regard the diversity of sampling
198 method, calculation of uniform catch-per-unit-effort data was not possible, and thus, we used
199 percentage relative abundance data for the analyses.

200

201 *2.4. Environmental and spatial data*

202 For the characterisation of the sampled habitats we assessed a series of climatic, local
203 environmental and land cover properties (see Appendix A in Electronic Supplementary
204 Material) that have been found to influence distribution and structure of European freshwater
205 crayfish assemblages (Pârvulescu et al, 2011; Pârvulescu and Zaharia, 2014; Římalová et al.,
206 2014; Chucholl, 2016; Chucholl and Schrimpf, 2016).

207 Climatic variables included altitude measured on site using GPS devices, and mean
208 annual air temperature and annual precipitation data provided by Hungarian Meteorological
209 Service and interpolated to a 1 km radius circle around each site using the Meteorological
210 Interpolation based on Surface Homogenized Data Basis (Szentimrey and Bihari, 2015).

211 Parallel to the crayfish sampling, we assessed a series of local environmental properties
212 related to morphology, bank structure, substratum composition and aquatic vegetation of the
213 sampled stream section. Wetted stream width, water depth and water current were measured
214 and averaged along 6-15 transects perpendicular to the channel. Bank structure was
215 characterised by the percentage coverage of trees, other vegetation and concrete along each

216 sampling section. Percentage composition of streambed substratum was visually assessed
217 based on fractions of silt (< 0.06 mm), sand (0.06-2 mm), gravel (2-60 mm), stone (60-400
218 mm), rock (> 400 mm) and concrete. Substratum composition was inspected directly in
219 transparent, wadeable streams and from dredged substratum samples in other habitats.
220 Percentage of macrophyte-free wetted area, and areas covered by emergent, submerged and
221 floating leaved macrophytes and filamentous algae were also assessed visually. Since
222 submerged macrophytes occurred only in highly transparent waters, therefore, their
223 occurrences could be assessed visually as well at all studied sites. Note that several sampling
224 teams contributed to this country-wide survey. However, since comparable assessment of
225 some environmental properties - e.g. bank structure, substratum compositions, macrophyte
226 coverage - requires specific experience, therefore, detailed local environmental data were
227 collected only for 628 sites visited by our most trained team members.

228 Information on land-use within a 1 km radius circle around each site was obtained from
229 the CORINE Land Cover 2018 database (European Environmental Agency, 2020) and
230 condensed into six comprehensive land cover variables – artificial surface (CORINE land
231 cover categories, CLC 1.1 – 1.4), agricultural area excluding pasture (CLC 2.1, 2.2, 2.4),
232 pasture (CLC 2.3), forest (CLC 3.1), other semi natural terrestrial area (CLC 3.2, 3.3) and
233 wetland and open water (CLC 4 – 5) (Appendix A in Electronic Supplementary Material).

234 To enable the inclusion of possible effects of some important spatial constraints (i.e.
235 dispersal limitation and infection hotspots) in our analysis, we generated a set of theoretical
236 spatial variables modelling the relative position of each site within the study system. For this
237 purpose, we followed the modified approach of Borcard et al. (2004). Namely, geographical
238 distances were calculated from GPS coordinates for all possible pairwise site combinations,
239 distance data were $\log(x+1)$ transformed and then, the between sites distance matrix was
240 subjected to a principal coordinate analysis using Past 2.17 software (Hammer et al., 2001).

241 Because spatial variables with very low explanatory power presumably have little influence
242 on metacommunity processes, of the 948 obtained spatial variables we retained only the first
243 19 variables with $> 0.5\%$ eigenvalues for the further analyses.

244

245 *2.5. Statistical analysis*

246 Chi-square test of independence and long-term distribution data were used to evaluate
247 whether the probability of ICS extinction from $50 \text{ km} \times 50 \text{ km}$ EOV cells differ before and
248 after the appearance of NICS.

249 In order to assess whether there is an indication of non-random co-occurrence of crayfish
250 species, we calculated the four commonly used co-occurrence indices based on the presence-
251 absence species data of the country wide survey, and then, we tested them for significant
252 deviation from randomized assemblage patterns using the EcoSim 7.72 software (Gotelli and
253 Entsminger, 2011). The considered indices were (1) the checkerboard score (C-score), which
254 measures the association between species pairs based on the number of checkerboard units
255 (Stone and Roberts, 1990). C-score ranges from zero (species are maximally aggregated) to a
256 maximum of number of sites with species A multiplied by maximum number of sites with
257 species B (species are maximally segregated with no shared sites). (2) The variance-ratio (V-
258 ratio) measures the average covariance between all possible species pairs. This index indicates
259 species aggregation when its value is much larger than 1 and species segregation when its
260 value is much smaller than 1 (Schluter, 1984). (3) The number of species pairs forming
261 perfect checkerboards (N-checkerboard), and (4) the number of unique species combinations
262 (N-unique). Reference distributions of the four indices were generated by randomizing the
263 species presence-absence data matrix 5,000 times according to the sim2 algorithm of Gotelli
264 (2000). In this procedure, data units are reshuffled within each row (representing site data of

265 one species), which means that species occurrence frequencies are preserved, but all sites are
266 considered equiprobable.

267 To evaluate association between crayfish relative abundances and spatial and
268 environmental (climatic, local environmental and land cover) variables, we performed partial
269 direct gradient analysis and variance partitioning (Cushman and McGarigal, 2002). In order to
270 approximate normality and decrease load of extreme values, we $\arcsin\sqrt{x}$ transformed relative
271 abundance data and environmental variables scaled in percentages, and $\log(x+1)$ transformed
272 all other environmental variables. Spatial variables were left untreated. To avoid collinearity,
273 we excluded the less meaningful variable of each correlating (at $r \geq 0.7$) variable pairs from
274 the analysis (Appendix A in Electronic Supplementary Material). Because detrended
275 correspondence analysis (DCA) indicated a long gradient (12.2 in S.D. units) in crayfish data,
276 we chose canonical correspondence analysis (CCA) for the constrained ordination (Lepš and
277 Šmilauer, 2003). We performed a forward stepwise selection based on Monte Carlo
278 randomization test with 9,999 unrestricted permutations to reduce the number of explanatory
279 variables only to those with significant ($P < 0.05$) contribution to the final CCA model. For
280 quantification of unique and shared effects of spatial and environmental variable groups (i.e.
281 climate, local environment and land cover) on the relative abundance patterns of crayfish
282 metacommunities, we conducted a series of CCAs and partial CCAs based on the retained
283 explanatory variables of the final model (Cushman and McGarigal, 2002). DCA and CCA
284 were processed using CANOCO version 5 software (Šmilauer and Lepš, 2014).

285 We modelled presence-absence probabilities of the two most abundant ICS – noble
286 crayfish and narrow-clawed crayfish –, and the two most abundant NICS – spiny cheek
287 crayfish and signal crayfish – in relation to climatic, local environmental, land cover and
288 spatial variables by using logistic regression analysis (LRA) (Peng et al., 2002; Hosmer et al.,
289 2013). We treated potential explanatory variables similarly as in the CCA (Appendix A in

290 Electronic Supplementary Material). To find the most parsimonious LRA model that still
291 accurately predicts the response variable, first we filtered potential explanatory variables by
292 using a forward stepwise selection approach based on the score statistics and the likelihood
293 ratio test at $P < 0.05$. Then, for each preselected explanatory variable, we also checked
294 whether their removal from this preliminary set of variables could cause a significant drop in
295 model fit based on change in model likelihood at $P < 0.05$ as well. We performed these
296 procedures both with and without a constant term, and the inclusion of a constant to the final
297 model was decided based on the difference in likelihood between the best alternative models.
298 Finally, we checked whether the inclusion of any of the interactions among the variables in
299 the main effects model could improve the model fit. Evaluation of the final model was based
300 on the likelihood ratio test, the Pearson χ^2 goodness of fit statistics (Hosmer-Lemeshow test),
301 the Nagelkerke pseudo- R^2 and the classification success. The importance of each explanatory
302 variable as well as the constant term and interactions between main effects (if included) in the
303 final model was characterised by their individual regression coefficients β , the odds ratio (e^β)
304 and the Wald statistics. Positive and negative β values represent an increase and a decrease,
305 respectively, in the probability of the presence of the modelled crayfish species with the
306 increase of the value of the particular explanatory variable. Whereas, the odds ratio indicates
307 the rate of change of the probability of presence of the modelled crayfish along the “gradient”
308 of the particular explanatory variable. We performed LRA with SPSS version 27 software
309 (IBM Co.).

310

311 **3. Results**

312 *3.1. Non-indigenous crayfishes enter both ICS and ICS-free areas*

313 Long-term changes in the distribution of crayfishes in Hungary is presented in Fig. 2.

314 Historic data representative for the period from the late 1800s to 1990 show that of the ICS

315 the noble crayfish originally occurred in the whole territory of Hungary, the narrow-clawed
316 crayfish populated the whole plane area and the stone crayfish was present only in some
317 highland areas (Fig. 2a). Dramatic changes in the crayfish fauna has started in the late 1980s.
318 For example, the distribution area of the noble crayfish has decreased substantially, and the
319 beginning of these alterations roughly coincided with the appearance of the first NICS, the
320 spiny-cheek crayfish (Fig. 2b). From this time period several NICS has appeared and started
321 to spread. By now, area of the noble crayfish decreased by at least fifty percent and the stone
322 crayfish has lost a significant part of its original area, while no change in the distribution area
323 of the narrow-clawed crayfish could be evidenced (Fig. 2c). Meanwhile the spiny-cheek
324 crayfish has expanded to majority of lowland areas, signal crayfish colonized larger streams
325 in the western part of the country and few other NICS, namely the marbled crayfish
326 *Procambarus virginalis* Lyko, 2017, the red swamp crayfish *Procambarus clarkii* (Girard,
327 1852), the red claw crayfish *Cherax quadricarinatus* (Martens, 1868) and the Mexican dwarf
328 crayfish *Cambarellus patzcuarensis* Villalobos, 1943) have appeared at sporadic locations.

329 At least at rough historic scale, deterioration of the ICS fauna could not evidently be
330 related to the invasion of NICS. Statistical evaluation revealed that noble crayfish was likely
331 to become extinct before the arrival of NICS (chi-square test of independence, d.f. = 1, $N =$
332 46, $\chi^2 = 28.4$, $P < 0.001$). Namely, out of the 46 EOVS cells (50 km × 50 km) where the noble
333 crayfish was documented historically, this species likely became extinct in 24 EOVS cells
334 before, and only in one EOVS cell after the arrival of NICS. On the other hand, present
335 occurrences of NICS and ICS overlaps markedly. Out of the 39 EOVS cells where NICS are
336 present, there are ICS in 33 cells, as well (Fig. 2c).

337

338 *3.2. There are twice as many invasive than native species*

339 Altogether 3170 individuals and nine crayfish species were captured at 304 sites (32.0%),
340 while no crayfish was found at 645 sites (68.0%; Table 1). Beside the three ICS (noble
341 crayfish, narrow-clawed crayfish, stone crayfish), six NICS (spiny-cheek crayfish, signal
342 crayfish, marbled crayfish, red swamp crayfish, red claw crayfish and Mexican dwarf
343 crayfish) were detected, and NICS occurred at more sites (181) and at higher total number
344 (2078) than ICS (143 sites and 1092 individuals; sign test, $z = 2.25$, $P = 0.024$ and Mann-
345 Whitney U test, $z = -2.94$, $P = 0.003$, respectively).

346

347 *3.3. Crayfish species rarely co-occur*

348 Occurrences of the nine crayfish species were highly separated. At vast majority of
349 crayfish sites only one species was present (283 sites, 93.1% of sites with crayfish). Two
350 species co-existed at 14 sites (4.6%), three species at six sites (2.0%) and six species at one
351 site (0.3%). Bootstrap-based analyses proved that species occurrences were much more
352 segregated (based on C-score and V-ratio, $P < 0.001$ for both) than expected by chance only
353 (Table 2). In addition, checkerboard species pairs were more numerous ($P = 0.029$), whereas
354 the number of unique species combinations was much fewer than expected by chance only (P
355 < 0.001).

356

357 *3.4. Spatial processes predominate over environmental filtering*

358 Based on the data of 201 sites with crayfish and detailed environmental data, variable
359 selection for the CCA multivariate analysis yielded 20 significant explanatory variables
360 representing each of the four variable groups (i.e. climate, local environmental, land cover and
361 spatial variables; see Appendix B in Electronic Supplementary Material). These variables
362 explained altogether 51.4% of the total variance in crayfish relative abundance patterns
363 (pseudo- $F = 9.5$, $P < 0.001$) (Fig. 3). Variance partitioning identified spatiality as the

364 predominant pattern (33.7% of the total variance) in crayfish metacommunities, followed by
365 the influence of local environment (16.7%), climate (13.2%) and land cover (7.5%). Spatial
366 variable group accounted for the highest pure effect (24.1%) as well, whereas a large part of
367 variance explained by climatic, local environmental and land cover variable groups proved to
368 be shared effect (i.e. patterns that are simultaneously explained by more variable groups).
369 Cumulated influence of all environmental properties, the climate, local environment and land
370 cover (17.7% of variance in crayfish relative abundance as pure effect), was still less than the
371 pure influence of spatiality. Of explanatory variables, altitude accounted for the highest
372 amount of variance (11.8% as total effect) in crayfish relative abundance data, while the
373 individual predictive power of other non-spatial variables was low (see Appendix B in
374 Electronic Supplementary Material).

375 The three ICS aligned far from each other in the CCA ordination space, which indicates
376 marked differences in their spatio-environmental preferences (Fig. 4). Along the first
377 ordination axis, which correlated most with altitude, noble crayfish and stone crayfish scored
378 positive (i.e. their relative abundance increased with altitude) and narrow-clawed crayfish
379 negative values (i.e. its relative abundance decreased with altitude). Separation of stone
380 crayfish was also clear from all NICS, which indicates the unique niche occupancy of this
381 species. NICS signal crayfish was positioned close to the noble crayfish along the first and
382 second ordination axes suggesting some overlap in environmental preferences and spatial
383 occurrence between the two species. Of ICS, occurrence constraints of narrow-clawed
384 crayfish proved to be most similar to some of the NICS, namely the spiny-cheek crayfish, the
385 red swamp crayfish and the red claw crayfish. Finally, the two thermophilous NICS, the
386 marbled crayfish and the Mexican dwarf crayfish received similar scores and separated from
387 all the other species.

388 Final logistic regression models assessing the occurrence of noble crayfish, narrow-clawed
389 crayfish, spiny cheek crayfish and signal crayfish were statistically significant ($\chi^2 = 646.4 -$
390 810.7 , d.f. = 7 – 11, $P < 0.001$) and explained 77.5 – 96.7% (Nagelkerke pseudo- R^2) of the
391 variance in presence-absence data of these species based on 628 sites with detailed
392 environmental information (Table 3). Models correctly classified between 86.6% (spiny-
393 cheek crayfish) and 98.1% (signal crayfish) of sites for presence or absence of the four
394 species. Neither the inclusion of a constant nor that of any of the pairwise interactions
395 between the main effects proved to significantly improve the models. Insignificant
396 interactions indicate that main effects were consistent and independent. Logistic regression
397 analysis ascertained that occurrence probabilities of these species were primarily spatially
398 arranged, but were influenced also by some climatic, local environmental and land cover
399 properties (Table 4). Probability of presence of the noble crayfish increased towards higher
400 altitudes (mean \pm 95% CI: 165.0 ± 15.4 m a.s.l. in sites with and 113.2 ± 3.4 m a.s.l. without
401 noble crayfish; t-test, $t = 6.4$, d.f. = 36, $P < 0.001$) and cooler annual mean air temperatures
402 (mean \pm 95% CI: 10.5 ± 0.3 °C in sites with and 11.0 ± 0.1 °C without noble crayfish; t-test, t
403 $= -3.7$, d.f. = 35, $P < 0.001$). On the contrary, probability of presence of narrow-clawed and
404 spiny-cheek crayfishes increased towards lower altitudes (mean \pm 95% CI: 97.0 ± 2.5 m a.s.l.
405 in sites with and 117.6 ± 3.6 m a.s.l. without narrow-clawed crayfish, t-test, $t = 8.0$, d.f. = 138,
406 $P < 0.001$; and 85.5 ± 1.7 m a.s.l. in sites with and 121.3 ± 3.9 m a.s.l. without spiny-cheek
407 crayfish; t-test, $t = -14.6$, d.f. = 626, $P < 0.001$), while signal crayfish was substantially more
408 likely to occur in areas with cooler annual mean temperatures (mean \pm 95% CI: 10.7 ± 0.1 °C
409 in sites with and 11.0 ± 0.1 °C without signal crayfish; t-test, $t = -4.3$, d.f. = 31, $P < 0.001$).

410

411

412 **4. Discussion**

413 Since the appearance of the first NICS, the spiny-cheek crayfish in the 1980s,
414 intensifying invasion resulted that NICS are now dominate over ICS both in species richness
415 and abundance in the Hungarian waters. Our results demonstrate that invasion of NICS is
416 likely also facilitated by the pre-invasion deterioration of ICS populations. Moreover, we
417 elucidated the ecological aspects of the restructuring process of crayfish metacommunities in
418 the Pannonian Ecoregion, such as strong spatial arrangement and the importance of upland
419 refugee sites in two of the three ICS.

420

421 *4.1. Historic data indicate pre-invasion deterioration of the ICS fauna*

422 Analysis of long-term distribution patterns supports findings of earlier studies that noble
423 crayfish and stone crayfish had already disappeared from large areas before the arrival of
424 NICS. Major identified causes of this decline of ICS populations are the crayfish plaque and
425 habitat degradation and loss (Thuránszky and Forró, 1987; Puky and Schád, 2006). Although
426 our analyses did not reveal a decrease in the distribution area of the narrow-clawed crayfish,
427 at least at the spatial resolution of historic data, other studies reported a pre-invasion decline
428 of local populations that was mainly related to the crayfish plaque and the intensive stocking
429 of European eel *Anguilla anguilla* (Linnaeus, 1758) from the 1960s to 1991 (Bíró, 1976;
430 Pintér and Thuránszky, 1983). We consider that this discrepancy between the distribution and
431 abundance patterns of narrow-clawed crayfish at least partly be related to differences in their
432 habitat use and population characteristics compared to the former two ICS. Namely, noble
433 crayfish and stone crayfish inhabit small to medium sized streams. Their populations are often
434 isolated from each other and their dispersal and recolonization is highly constrained by a
435 variety of natural and artificial barriers in this region (Erós et al., 2018; this study). Whereas,
436 narrow-clawed crayfish live in larger waterbodies that are unlikely become entirely degraded

437 or isolated, and form larger metapopulations that can more likely survive even in case of a
438 massive decline of local populations.

439 Deteriorated populations of ICS and the high proportion of potentially suitable sites with
440 no crayfish could assist and still promote further expansion of NICS in Hungary. However,
441 abundant crayfish free sites also represent a conservation possibility. Conservational
442 management actions aiming to block the dispersal of NICS through natural and man-made
443 barriers into uninfected and refugee areas of ICS could be an operative choice. Many of the
444 sites with no detected crayfish in our survey represent stream sections that are hardly
445 (re)colonisable due to man-made barriers, and thus, may be utilized for species conservation
446 attempts as potential reserve areas for recolonized or translocated ICS populations. The
447 efficiency of these reintroductions could be increased by discovering and breeding crayfish
448 plaque resistant stocks of noble crayfish and stone crayfish (c.f. Kokko et al., 2012;
449 Makkonen et al., 2012).

450

451 *4.2. Present-day crayfish metacommunity composed mainly of single species assemblages*

452 Co-occurrence analysis of local assemblages reveal that crayfish species rarely co-occur
453 in Hungarian waters. Interestingly, some ICS as well as ICS and NICS are not rarely reported
454 to co-occur (e.g. Stucki and Romer, 2001; Westman et al., 2002; Kadlecová et al., 2012;
455 Schrimpf et al., 2013; Pacioglu et al., 2020) and historic reports also mentioned several co-
456 occurring populations of ICS in the Pannonian Ecoregion (Entz, 1909, and references therein).
457 Therefore, the present distribution of the nine crayfish species represents an extreme situation,
458 which requires further investigation.

459 Assemblages with substantially less co-occurrences compared to random patterns are
460 generally considered to indicate interspecific competition or dispersal limitation (Diamond et
461 al., 2015; Dallas et al., 2018). By the end of the 20th century, general deterioration of ICS

462 populations caused their separation, with no co-occurrences known at the present in
463 Hungarian waters (Puky et al., 2005; this study). Whereas, regarding the limited co-
464 occurrence of ICS and NICS, two alternative mechanisms could be posed. First, single species
465 assemblages could be the consequence of a sharp interspecific competition and competitive
466 extinction, which is a common phenomenon in crayfishes (Söderbäck, 1995; Maguire et al.,
467 2018; Pacioglu et al., 2020). Second, the high abundance of crayfish free sites and the
468 evidence on pre-invasion deterioration of ICS validate that single species assemblages could
469 be due to the invasion of empty sites by NICS along different, still largely non-overlapping
470 invasions routes. Specifically, the spiny-cheek crayfish spreads along and from River Danube
471 and River Tisza, while the signal crayfish from the western boarder of the country along the
472 rivers Rába, Mura and Dráva (Ludányi et al., 2016; Lipták and Vitázková, 2014; this study).
473 While, occurrences of other NICS is spatially still quite limited and sporadic. However, if the
474 invasion processes, which is the most likely scenario, interactions between NICS will also
475 increasingly influence the spatial restructuring of both ICS and NICS populations. It is not yet
476 predictable that which NICS will be able to coexist on the long run or become the most
477 dominant species, but on the other hand, there are evidences that NICS may displace each
478 other as well (Hudina et al., 2011; James et al., 2016).

479 The few crayfish co-occurrences we observed were restricted to main invasion corridors
480 (i.e. River Danube and River Tisza) and to infection hotspots with repeated illegal
481 introductions (i.e. vicinity of thermal springs and large cities; Weiperth et al., 2019). Co-
482 occurrence of narrow-clawed crayfish and spiny-cheek crayfish populations was also
483 observed mainly in larger lowland streams and rivers. Compared to small streams, large
484 habitats provide more possibility for resource partitioning and physical separation of species
485 and therefore, even strong competitors like the crayfishes may coexist for a longer period (e.g.
486 Stucki and Romer, 2001; Pacioglu et al., 2020).

487 Dominance of single species assemblages has some important conservational aspects. On
488 one hand, loss or exterminative alteration of a given crayfish site could not result in the
489 extinction of local population of more than one ICS. On the other hand, due to the lack of
490 diversity hotspots (i.e. sites with multiple ICS), multi-species conservation efforts should be
491 dispersed across multiple sites representing a diverse set of crayfish habitats.

492

493 *4.3. Site position seems to be more important than habitat characteristics during the invasion*

494 Organisation of metacommunities is determined by environmental filtering (species
495 sorting) and dispersal mechanisms (Heino et al., 2015). Here we found that presently spatial
496 processes dominate over environmental filtering in crayfish metacommunity structuring in the
497 Hungarian stream network system. Since crayfish species, especially the European ICS, have
498 strictly defined environmental tolerances (Pârvolescu et al., 2011; Chucholl and Schrimpf,
499 2016; this study), a marked spatial arrangement in their metacommunity structure may
500 indicate the determinative influence of ongoing NICS invasion. Dispersal of NICS from
501 infection centres is connected to stream networks and thus, it is spatially arranged. Therefore,
502 distribution of NICS and their effect on ICS are spatially arranged as well. Man-made barriers
503 characterising most streams in this region are also likely to constrain (at least slow down) the
504 dispersal of crayfishes along the stream network and enhance spatiality in metacommunity
505 structure. We should note, however, that since more recently introduced NICS are pet-traded
506 (marbled crayfish, red swamp crayfish, red claw crayfish and Mexican dwarf crayfish), they
507 are introduced at multiple sites (Löökkös et al., 2016; Weiperth et al., 2019), and therefore,
508 their succeeding dispersal, if happens at all, is supposed to be spatially more balanced.
509 Further, we suppose that when crayfish metacommunities reach a new equilibrium,
510 mechanisms forcing their spatial arrangement will weaken whereas environmental
511 filtering/species sorting will be more pronounced than presently, in the midst of the invasion.

512 Since there is still a considerable gap in our knowledge about how the relative weight of main
513 drivers of metacommunity organisation could change during a forced community
514 restructuring, for example during a multispecies invasion, a long-term monitoring of this
515 crayfish invasion provides a favourable possibility to learn much about the rules of
516 metacommunity dynamics as well.

517

518 *4.4. The invasion of NICS is likely to continue*

519 As we argued above, the expansion of NICS is very likely to progress in the region and
520 both the infected area and the number of invasive NICS are supposed to increase further in the
521 future. A crucial point of conservation planning is therefore, assessing which NICS and to
522 what extent may invade ICS areas.

523 There are some areas in Hungary that have not yet reached by the spiny-cheek crayfish,
524 such as the western Pannonia and the upland headwaters. Considering the tendency of
525 expansion (Lipták and Vitázková, 2014; this study), this species will very likely populate the
526 whole area of Hungary. The only question is whether it will enter headwaters as well or not.
527 Based on the present and others results (Capinha et al., 2013), if the climate does not change
528 much, there may remain some upland habitats that may remain uninfected by the spiny-cheek
529 crayfish. However, if global warming continues as forecasted, then these climatic
530 impediments will diminish and spiny-cheek crayfish may enter the last refuges of noble
531 crayfish and stone crayfish as well (Capinha et al., 2013).

532 Signal crayfish is reported to have the widest environmental range and the highest
533 conservational concern present in European freshwaters (Chucholl, 2016). Signal crayfish has
534 occupied only a moderate part of potentially suitable habitats in the Pannonian Ecoregion yet.
535 However, a further significant expansion of signal crayfish in Hungary could represent a fatal
536 threat to native stone crayfish and noble crayfish. This is because signal crayfish has highly

537 overlapping environmental preferences with these ICS (Chucholl, 2016; partly this study), and
538 of NICS, signal crayfish has the highest habitude entering the headwater refuge habitats of
539 stone crayfish and noble crayfish (Hubert and Schubart, 2005; Chucholl, 2016). Therefore,
540 one of the most important conservation challenges is to prevent the entry of signal crayfish
541 into uninfected Pannonian highland areas.

542 Considering its massive invasion in lentic and slow flowing lotic habitats in some
543 European areas (Gherardi, 2006), the red swamp crayfish could be the next NICS to distribute
544 extensively in Hungarian waters. Considering its habitat preference and the relatively high
545 temperature optima (Maceda-Veiga et al., 2013), the red swamp crayfish would most likely
546 enter habitats of the narrow-clawed crayfish and unlikely the upland refugees of the noble
547 crayfish and the stone crayfish.

548 Perhaps, not all NICS are supposed to become invasive or even survive in the long run in
549 Hungarian natural waters. Thermophilous pet-traded species (e.g. red claw crayfish, Mexican
550 dwarf crayfish) introduced to some thermal springs are not likely to increase their areas in
551 Hungary. Canonical correspondence analysis also proved the distinct habitat preference of
552 thermophilous Mexican dwarf crayfish and marbled crayfish. However, the invasion potential
553 of the marbled crayfish in European temperate waters is still subject of debate (Chucholl,
554 2014). Marbled crayfish was originally found in thermal waters in Hungary (Lókkös et al.,
555 2016), but some of its populations were reported to show signs of cold acclimatization
556 (Vesely et al., 2015) and it has appeared in the Danube (Weiperth et al., 2015).

557 It is evident now that thermal habitats that are connected to the natural stream network
558 system (e.g. Lake Hévíz, several thermal springs in cities Budapest, Egerszalók and
559 Miskolctapolca; see also Weiperth et al., 2020) operate as the most dangerous infection sites
560 by attracting illegal pet releases. Therefore, it is of outmost importance to protect these sites
561 from illegal releases by using caution advertisement and strict sanctions.

562

563 *4.5. The long-term survival of ICS seems to be doubtful without an effective conservation*
564 *action*

565 Multiple concurring stressors threaten the long-term survival of ICS in the Pannonian
566 Ecoregion. The most endangered species is definitely the stone crayfish which has special
567 environmental tolerances (Pârvulescu and Zaharia, 2013; Chucholl and Schrimpf, 2016, this
568 study). This species has very limited remnant ranges restricted to uppermost sections of a few
569 small streams in only three highland areas. These very last refuge habitats have special
570 microclimate that is likely to alter or even drain under the forecasted climate change scenarios
571 (c.f. Capinha et al., 2013). Moreover, all these habitats are connected to NICS invaded
572 watersheds (i.e. to Danube and River Rába).

573 As our results demonstrate, the range of noble crayfish has already declined by at least
574 50% in the Pannonian Ecoregion. The rate of decline in distribution is similar or even higher
575 across the whole range of this species (Edsman et al., 2010; Richman et al., 2015). Further
576 decrease in distribution area and abundance of noble crayfish is forecasted because this
577 species is not likely to survive in coexistence with invasive NICS that potentially can carry
578 the crayfish plaque, and because noble crayfish is strongly affected by habitat alterations
579 related to global climate change and anthropogenic impacts (Capinha et al., 2013; Chucholl,
580 2016).

581 The most tolerant to the above mentioned stressors is narrow-clawed crayfish. This
582 species tolerates global climate change (Capinha et al., 2013), it is less sensitive to
583 anthropogenic habitat degradation (Maguire et al. 2018) and may become partially resistant to
584 crayfish plaque (Kokko et al., 2012). Further, although there are some indications that
585 populations of narrow-clawed crayfish could also be under pressure in areas invaded by the
586 spiny-cheek crayfish and signal crayfish (Maguire et al., 2018; Pacioglu et al., 2020), this

587 species seems to be able to coexist with NICS, especially with the spiny-cheek crayfish
588 (Pacioglu et al., 2020; this study).

589 We conclude that without an effective conservational program the future of ICS is
590 doubtful in Hungary. Present population and invasion trends designate the extinction of noble
591 crayfish and stone crayfish and make the long-term survival of narrow-clawed crayfish
592 ambiguous. As we outlined above, conservation policy should focus on the preservation of
593 highland refugees of the stone and noble crayfishes. Expansion of NICS to these areas should
594 be prevented by utilizing possibilities provided by natural and artificial barriers. Crayfish-free
595 areas in the upland region should be screened for potential sites of reintroduction
596 programmes. Since ICS are much more likely to survive the invasion of NICS when the effect
597 of crayfish plaque is excluded (Schrimpf et al., 2013), we should concentrate a considerable
598 effort on finding more resistance ICS stocks for breeding and reintroduction. Last but not
599 least, education and strict ban should be simultaneously applied for pet-trading and in the
600 vicinity of the identified infection hotspots to prevent further NICS introductions.

601

602 **CRedit authorship contribution statement**

603 **Attila Mozsár:** Conceptualization, Methodology, Investigation, Data curation, Visualization,
604 Writing - review & editing. **Diána Árva:** Methodology, Investigation, Writing - review &
605 editing. **Vilmos Józsa:** Funding acquisition, Methodology, Investigation, Writing - review &
606 editing. **Károly Györe:** Funding acquisition, Methodology, Investigation, Writing - review &
607 editing. **Balázs Kajári:** Methodology, Data curation, Investigation, Writing - review &
608 editing. **István Czeglédi:** Methodology, Investigation, Data curation, Writing - review &
609 editing. **Tibor Erős:** Methodology, Writing - review & editing. **András Weiperth:**
610 Methodology, Investigation, Writing - review & editing. **András Specziár:**

611 Conceptualization, Formal analysis, Visualization, Writing - original draft, Writing - review
612 & editing.

613

614 **Declaration of competing interest**

615 The authors declare that they have no known competing financial interests or personal
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- 857

858

859 Table 1. Observed number of individuals (N), number of sampling sites with detection and

860 frequency of occurrence (FO) of indigenous (ICS) and non-indigenous (NICS) crayfish

861 species.

862

	N	No. of sites	FO%
ICS			
Noble crayfish, <i>Astacus astacus</i> (Linnaeus, 1758)	628	76	8.0
Narrow-clawed crayfish, <i>Pontastacus leptodactylus</i> (Eschscholz, 1823)	341	50	5.3
Stone crayfish, <i>Austropotamobius torrentium</i> (Schrank, 1803)	123	17	1.8
NICS			
Spiny-cheek crayfish, <i>Faxonius limosus</i> (Rafinesque, 1817)	1272	147	15.5
Signal crayfish, <i>Pacifastacus leniusculus</i> (Dana, 1852)	136	25	2.6
Marbled crayfish, <i>Procambarus virginalis</i> Lyko, 2017	181	9	0.9
Red swamp crayfish, <i>Procambarus clarkii</i> (Girard, 1852)	444	3	0.3
Red claw crayfish <i>Cherax quadricarinatus</i> (Martens, 1868)	4	3	0.3
Mexican dwarf crayfish, <i>Cambarellus patzcuarensis</i> Villalobos, 1943	41	5	0.5
Sites with crayfish		304	32.0
Sites without crayfish		645	68.0

863

864

865 Table 2. Co-occurrence metrics indicate high segregation in crayfish species occurrences compared to simulated random distribution.

866

Metric	Observed value	Expected value				Standardized effect size	Conclusion relative to the expected pattern
		Mean	Variance	P (obs. ≤ exp.)	P (obs. ≥ exp.)		
C-score	968	596	818	1.000	<0.001	13.0	Less species co-occurrences More segregated species
V-ratio	0.256	1.001	0.005	<0.001	1.000	-10.3	occurrences
Number of checkerboard species pairs	19.0	14.7	4.1	0.994	0.029	2.12	More checkerboard species pairs
Number of unique species combinations	13.0	34.5	4.3	<0.001	1.000	-10.4	Less unique species combinations

867

868

869 Table 3. Logistic regression analysis final models' tests reveal good fitting between presence-absence data of four abundant crayfish species and
870 forward selected climatic, local environmental, land use and spatial explanatory variables (model variables are shown in Table 4).

871

	Likelihood ratio test			Pearson goodness-of-fit			Nagelkerke	Classification success
	χ^2	df	P	χ^2	df	P	pseudo-R ²	
Noble crayfish, <i>Astacus astacus</i>	715.5	9	<0.001	187.7	619	1.000	0.907	93.9%
Narrow-clawed crayfish, <i>Pontastacus leptodactylus</i>	662.3	9	<0.001	512.2	619	0.999	0.869	94.6%
Spiny-cheek crayfish, <i>Faxonius limosus</i>	546.4	11	<0.001	334.0	617	1.000	0.775	86.6%
Signal crayfish, <i>Pacifastacus leniusculus</i>	810.7	7	<0.001	84.4	621	1.000	0.967	98.1%

872

873

874 Table 4. Explanatory variables of final logistic regression models analysing the presence-
 875 absence patterns of four predominant crayfish species, their parameters and statistics.
 876

Explanatory variable	Likelihood ratio test			Parameter			Wald's χ^2			e^B (odds ratio)
	χ^2	df	P	B	SE	χ^2	df	P		
<i>Noble crayfish, Astacus astacus</i>										
Altitude	16.0	1	<0.001	6.3	1.6	15.0	1	<0.001	561.5	
Mean annual air temperature	21.3	1	<0.001	-16.0	3.7	18.8	1	<0.001	<0.001	
Wetted width	24.2	1	<0.001	-3.0	0.7	16.3	1	<0.001	0.052	
Substratum, gravel	10.1	1	0.001	1.8	0.6	9.3	1	0.002	6.2	
Spatial-1	16.5	1	<0.001	30.6	8.8	12.2	1	<0.001	>1000	
Spatial-8	4.0	1	0.047	35.4	17.4	4.1	1	0.042	>1000	
Spatial-10	15.1	1	<0.001	-27.0	7.6	12.8	1	<0.001	<0.001	
Spatial-12	7.9	1	0.005	20.6	7.7	7.2	1	0.007	>1000	
Spatial-13	4.8	1	0.029	14.3	6.6	4.8	1	0.029	>1000	
<i>Narrow-clawed crayfish, Pontastacus leptodactylus</i>										
Altitude	217.3	1	<0.001	-3.9	0.5	63.2	1	<0.001	0.020	
Wetted width	65.5	1	<0.001	2.8	0.5	32.2	1	<0.001	16.9	
Substratum, sand	7.7	1	0.006	-1.9	0.7	6.6	1	0.010	0.148	
Substratum, gravel	27.9	1	<0.001	2.9	0.6	22.4	1	<0.001	18.4	
other semi natural terrestrial	7.5	1	0.006	1.5	0.5	7.8	1	0.005	4.6	
Spatial-7	6.8	1	0.009	-17.3	6.8	6.4	1	0.012	<0.001	
Spatial-10	5.3	1	0.021	18.4	8.2	5.0	1	0.025	>1000	
Spatial-11	21.5	1	<0.001	32.3	7.4	19.0	1	<0.001	>1000	
Spatial-18	13.9	1	<0.001	-23.4	6.6	12.4	1	<0.001	<0.001	
<i>Spiny-cheek crayfish, Faxonius limosus</i>										
Altitude	174.8	1	<0.001	-2.4	0.3	64.7	1	<0.001	0.093	
Wetted width	18.6	1	<0.001	1.1	0.3	16.3	1	<0.001	2.9	

Substratum, stone	5.5	1	0.019	-1.5	0.7	4.9	1	0.028	0.234
Spatial-1	8.5	1	0.004	21.4	7.8	7.5	1	0.006	>1000
Spatial-2	58.0	1	<0.001	82.7	17.3	22.9	1	<0.001	>1000
Spatial-3	7.0	1	0.008	-23.4	9.0	6.8	1	0.009	<0.001
Spatial-7	44.2	1	<0.001	-44.6	8.8	25.9	1	<0.001	<0.001
Spatial-8	22.7	1	<0.001	-27.7	6.4	18.8	1	<0.001	<0.001
Spatial-10	4.9	1	0.027	-17.4	7.8	4.9	1	0.026	<0.001
Spatial-14	6.1	1	0.014	10.9	4.5	6.0	1	0.014	>1000
Spatial-16	10.9	1	0.001	-17.5	5.6	9.8	1	0.002	<0.001
<i>Signal crayfish, Pacifastacus leniusculus</i>									
Mean annual air temperature	168.7	1	<0.001	-42.5	12.8	11.0	1	0.001	<0.001
Wetted width	17.5	1	<0.001	3.3	1.0	10.2	1	0.001	27.5
Spatial-1	34.6	1	<0.001	755.3	252.0	9.0	1	0.003	>1000
Spatial-3	19.0	1	<0.001	-213.4	73.0	8.5	1	0.003	<0.001
Spatial-7	4.3	1	0.038	-151.2	77.2	3.8	1	0.050	<0.001
Spatial-12	28.9	1	<0.001	-140.2	44.2	10.1	1	0.001	<0.001
Spatial-14	8.4	1	0.004	101.0	40.0	6.4	1	0.012	>1000

878 **Legends to figures**

879

880 Fig. 1. Location of sampling sites in Hungary, with the indication of crayfish occurrences in
881 2018-2019.

882

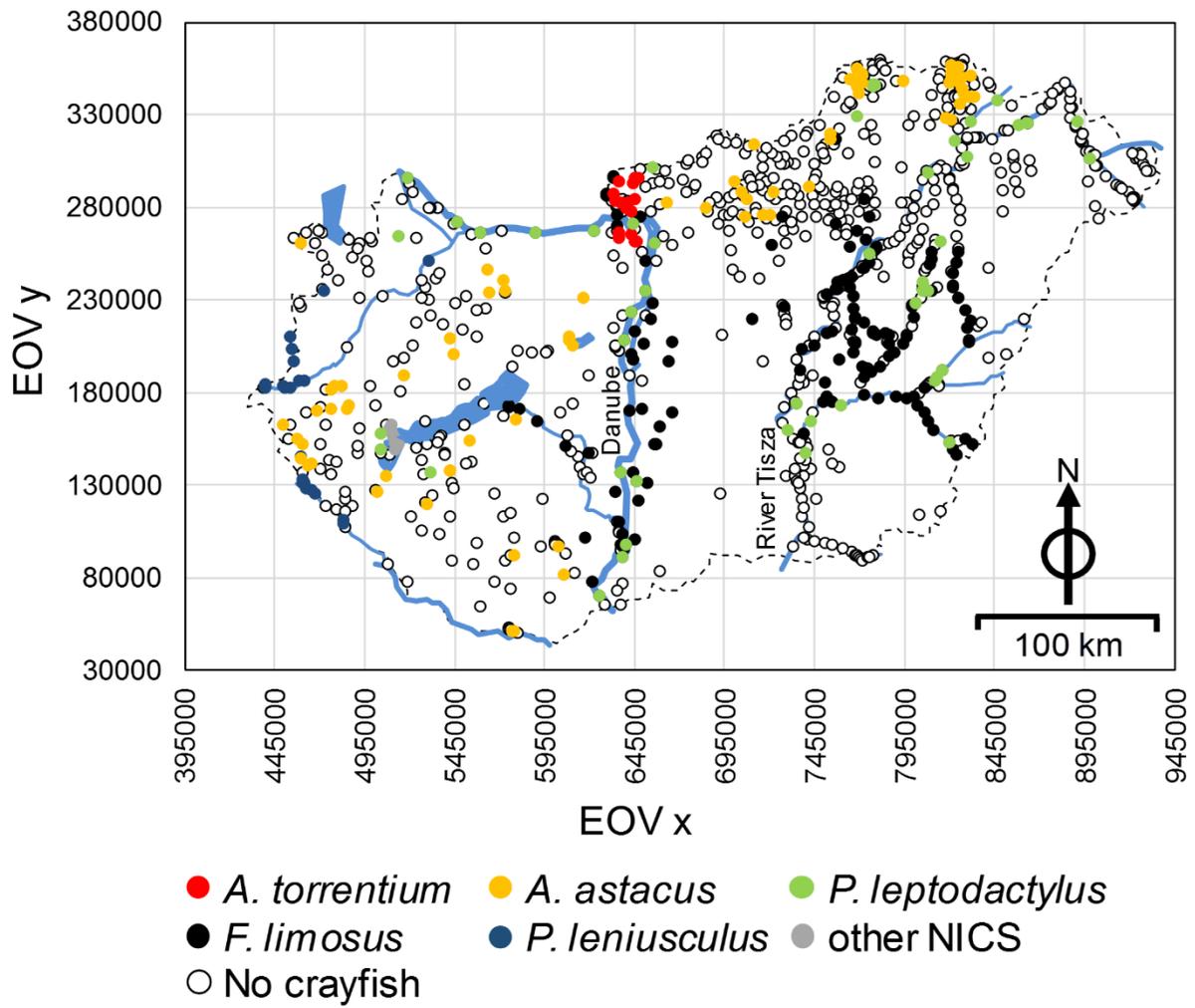
883 Fig. 2. Distribution of crayfish before and after the introduction of non-indigenous species
884 presented on 50 km × 50 km EOV (plane projection system used uniformly for the Hungarian
885 maps) grid. (a) From late 1800s to 1990; (b) between 1991 and 2010; (c) between 2011 and
886 2019.

887

888 Fig 3. Variance partitioning revealed the predominant influence of spatiality on
889 metacommunity organisation in crayfishes. Non-significant and negative partitions are not
890 presented. In the panel below is shown how cumulative influence of all environmental
891 properties (climate, local environment and land cover) relates to spatial effect.

892

893 Fig 4. Canonical correspondence analysis (CCA) biplots along the first and second (a) and
894 first and third axes (b) showing the relationship between crayfish assemblage composition and
895 climatic, local environmental, land use and spatial predictor variables in Hungary, during
896 2018-2019. Percentage variances represented by axes are indicated in brackets (of taxa data;
897 of taxa-explanatory variables relation) after the axis name. Red diamonds represent
898 indigenous and black diamonds represent non-indigenous crayfish species; green arrows
899 represent climatic, local environmental and land use explanatory variables (abbreviations are
900 explained in Appendix A in the Electronic Supplementary Material); while grey arrows
901 represent spatial variables. Note that for clarity we show only the most influential explanatory
902 variables.



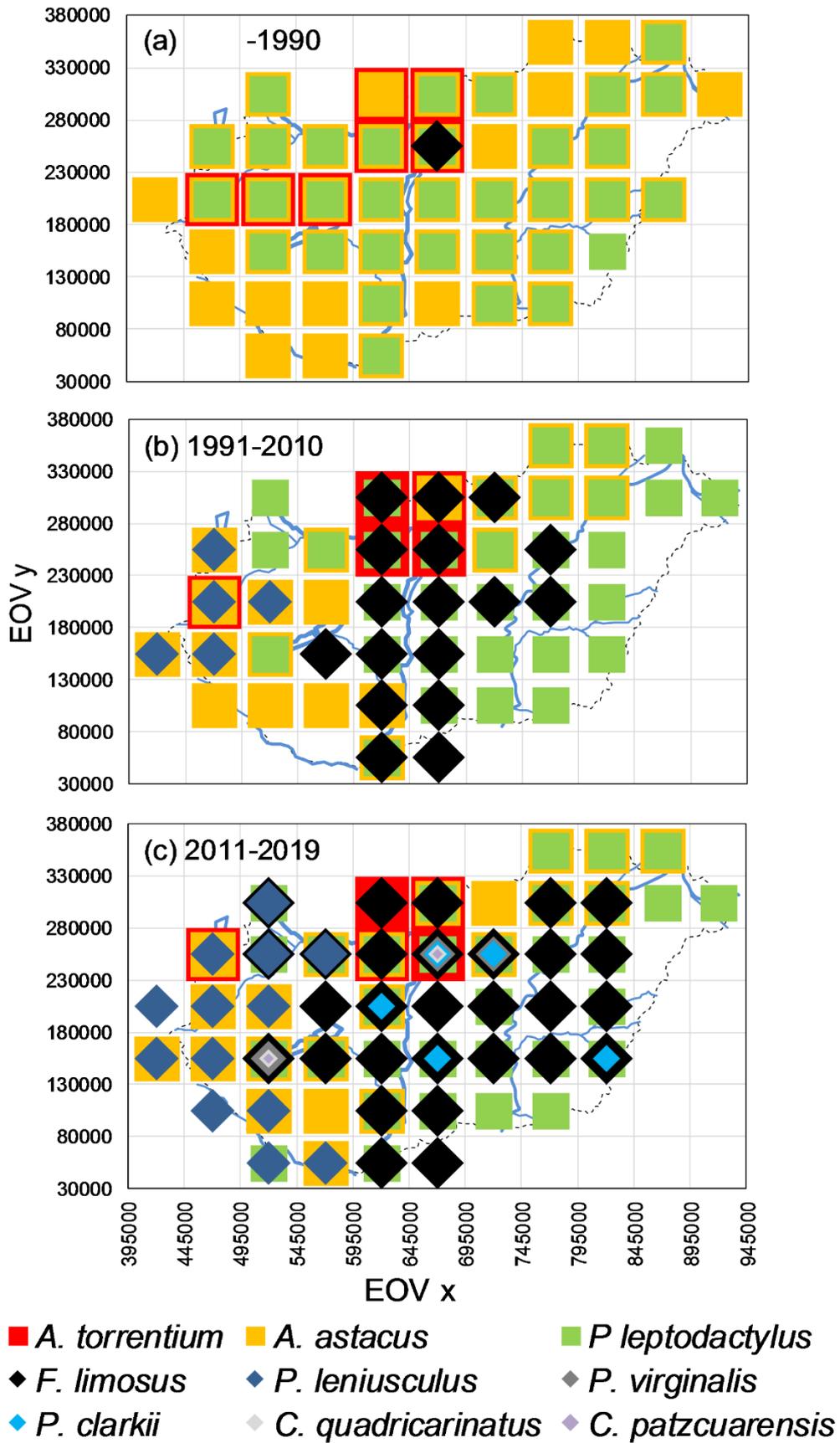
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Figure 1

905

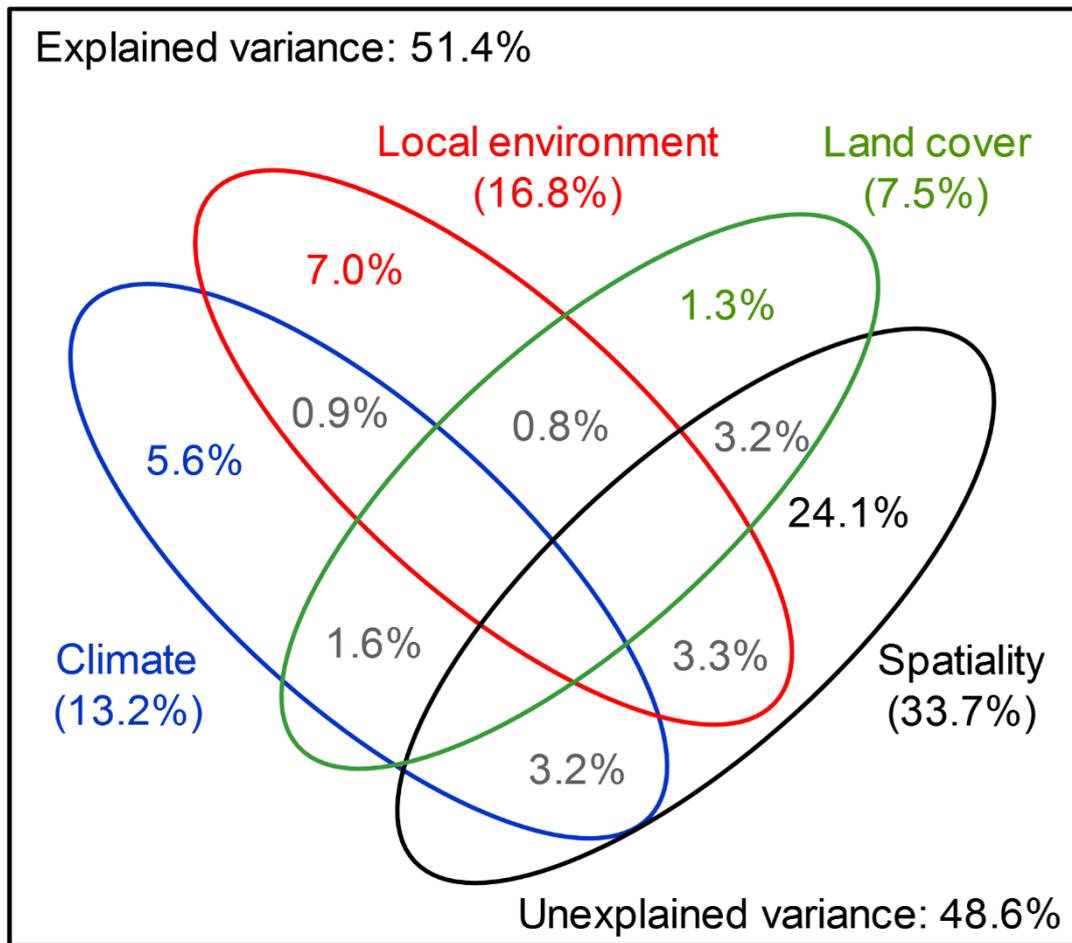
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Figure 2

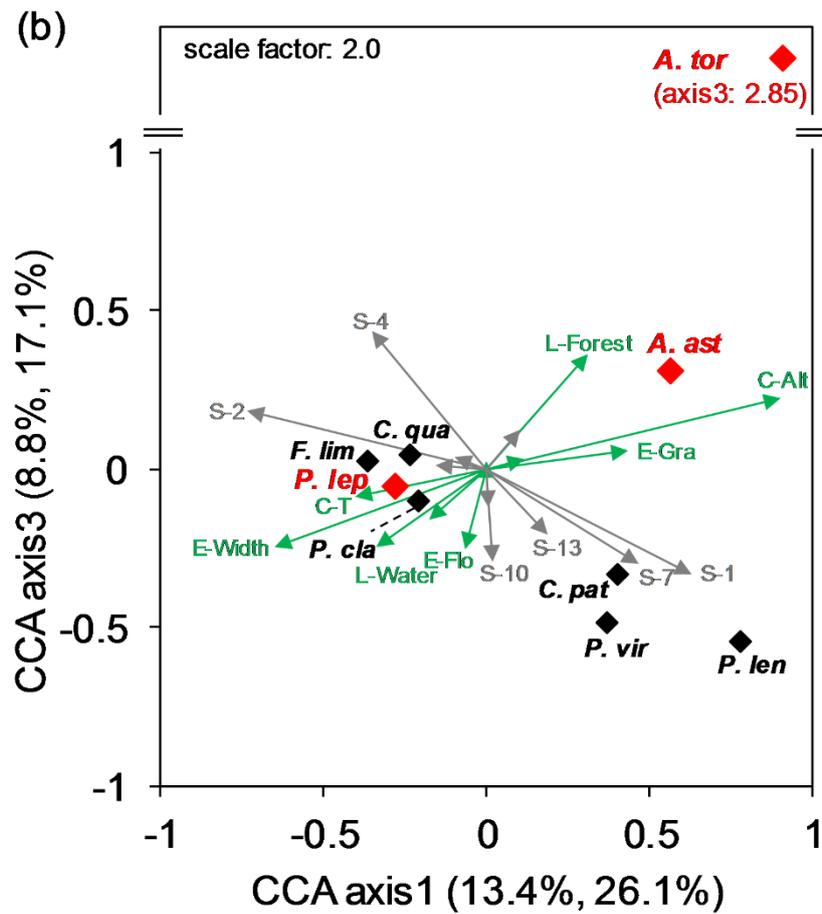
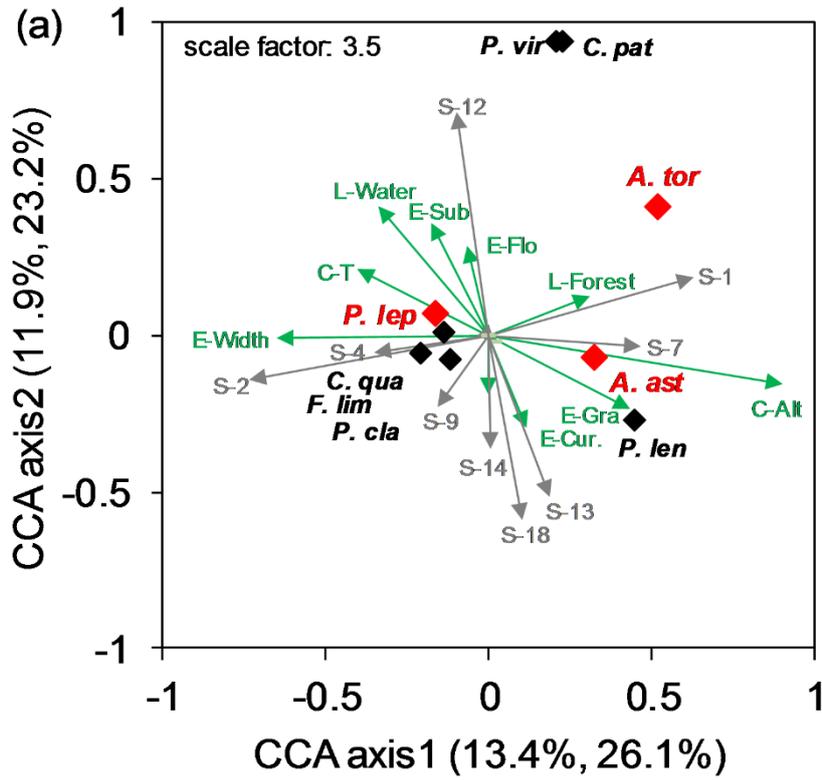


0% 20% 40% 60% 80% 100%

909

910

Figure 3



911

912

Figure 4