

Biological Invasions

Multifaceted implications of the competition between native and invasive crayfish: a glimmer of hope for the native's long-term survival

--Manuscript Draft--

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Corresponding Author:	Parvulescu Lucian, Dr. West University of Timisoara Timisoara, Timis ROMANIA	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	West University of Timisoara	
Corresponding Author's Secondary Institution:		
First Author:	Octavian Pacioglu	
First Author Secondary Information:		
Order of Authors:	Octavian Pacioglu	
	Kathrin THEISSINGER	
	Andreea Alexa	
	Corina Samoilă	
	Ovidiu I Sîrbu	
	Anne Schrimpf	
	Jochen P Zubrod	
	Ralf Schulz	
	Mălina Pîrvu	
	Sandra F Lele	
	John I Jones	
	Parvulescu Lucian, Dr.	
Order of Authors Secondary Information:		
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Abstract:	<p>Biological invasions represent a complex phenomenon driven by multiple factors. In this study, a real-time invasion process between a native (<i>Pontastacus leptodactylus</i>) and an invasive (<i>Faxonius limosus</i>) crayfish species was investigated in the Lower Danube (South-East Europe) through an interdisciplinary approach, by measuring various ecological, genetic, physiological and biometric endpoints. The results revealed that the prolonged competition in old invaded sites of the river (at least a decade) either drove the native species to extinction, or, unexpectedly, allowed its survival as highly fragmented populations. However, for the latter situation, several biological and ecological traits differed in the remnant populations: increased trophic position and elemental imbalance for two major macronutrients (C: N molar ratio), low growth, as strongly contracted trophic niche widths and low overlap degree with the invasive</p>	

	<p>crayfish. The data suggest that the prolonged competition induced potential resource partitioning between species, potentially driving their coexistence, as the development of larger and heavier claws within the native males' population. On the contrary, in more recently invaded sectors of the Lower Danube (three years), the trophic niche of the native species was significantly larger compared to old invaded sites and characterised by high level of niche overlap, indicating almost identical diet with the invasive crayfish, but characterised by the lowest trophic position compared to other invasion sectors. The genetic diversity of the native crayfish populations was strongly reduced in the invaded sectors of the river, but without signs of genetic bottleneck, which may be explained by a drift-mutational equilibrium reached as a consequence of diminishing population size. Our findings suggest strong coexistence potential in the future for both species in the Lower Danube.</p>
<p>Response to Reviewers:</p>	<p>COMMENTS TO THE AUTHOR:</p> <p>This MS has attracted contrasting reviews, but the paper may be publishable after extensive revision, especially following carefully the comments from Reviewer 1. I thus invite the authors to provide a revision and detailed letter of responses before any final decision.</p> <p>Reviewer #1: This study aimed to document patterns of divergence in a native crayfish species following invasion by the invasive spiny cheeked crayfish. The authors took advantage of knowing the invasion history of four sites along the lower Danube to compare populations of both the native and invasive species pre and post-invasion (pre-invasion for NCC only). Authors use a combination of genetic data, stable isotope data to document diet, niche width, etc. and morphology data to identify how populations differ with invasion history. For full transparency, I do not have a genetics background and thus, cannot evaluate this part of the study. However, the rest of the data I feel comfortable reviewing. Strengths of this paper include the biogeographic/space for time substitution approach to identify patterns in how traits have changed in both the native and invasive species, along with the multiple traits assed in the study (e.g. genetic, diet, morphology). Trait divergence in response to anthropogenic stressors is of general interest and an important area of research. However, the paper could be greatly improved by more clearly and thoroughly stating the general research question at the start of the introduction and then more broadly introducing the study, stating objectives, hypotheses and predictions. There is a lot of theoretical and empirical work that could be drawn from (e.g. strength competition driving differences in niche width, invasion/genetic bottlenecks) to set the study up in a much stronger way.</p> <p>R: We would like to thank the reviewer for the thorough review that helped us to improve the manuscript. Please find below the answers to both your specific and general comments. All answers are written in red, to make the reading easier. We have undertaken all changes required in the text. We have stated more clearly the general research questions in the Introduction section (Lines: 58-78) and the hypotheses (Lines: 101-115). Moreover, the text was improved and rewritten as required by the reviewer (please see below).</p> <p>My other main suggestion is to consider being more reserved in the conclusions drawn. Yes, traits have shifted but given the study design (e.g. field study/correlational data/patterns) I'd suggest that the paper could be improved by discussing the data as such, rather than implying this is a direct consequence of invasion.</p> <p>R: In the revised version of the manuscript we have followed closely the reviewer suggestion. Therefore, we have discussed the trophic endpoints (i.e. trophic position, omnivory, elemental imbalance) and growth rate as rather correlational and not as a direct consequence of invasion (Lines: 389-392, 400-403). However, regarding the decrease in genetic diversity and the occurrence of bigger, heavier and wider claws and cephalothoraxes within male population from recovery zone, these are aspects that in our opinion could be directly related to invasion and interspecific interactions related changes. The reason for this is that the observed patterns for decreased genetic diversity as a result of habitat fragmentation (Watterson 1984; Canales-Delgadillo et al. 2012; Harrisson et al. 2014; Larsson et al. 2008) and different morphology (Bøhn et al. 2008; Cattau et al. 2018; Carroll et al. 2005; Huey et al. 2005; Strauss et al. 2006; Messenger and Olden 2019; Houdina et al. 2012; Ooue et al. 2019) were documented before in the literature for both crayfish and other taxa. Nevertheless, concerning the finding of this study with respect to morphology, we have added a cautionary statement in extrapolating the findings further, given the observational and not the causality nature of the results (Lines: 452-454).</p> <p>Specific comments:</p>

* The manuscript could be greatly improved by more clearly stating and setting up the general research question being addressed in the study. The paper could also be significantly improved by clearly stating objectives of the study, along with hypotheses and predictions of how the authors expect the two species and populations within each species to differ with respect to invasion history. For example, how do you expect trophic position to change with invasion history, how do you expect it to differ between the two species?

R: As stated above, the manuscript was improved by stating clearly the expected research questions, and updating the hypotheses (Lines: 101-115) as well as the way how different traits are related and influenced by invasion. Regarding the explanation for the change in trophic position, supplementary and better documented information was furthermore provided (Lines: 405-412), based on the findings of this study.

* I think it'd greatly help the reader if the author's referred to their sampling sites in a more descriptive manner rather than using the site abbreviations, e.g. "NCC", "NID". For example, consider referring to the sites in a way that describes the invasion status/history. Maybe instead of "AID" use the word "active invasion" or "active" site.

R: The four invasion sectors were renamed 'old invasion', 'recovery zone', 'active invasion' and 'non-invaded' throughout the manuscript.

* The authors might also consider being consistent with how the two crayfish species are referred to. For example, sometimes the abbreviations are used, other times "native species" and "invasive species" are used. I might also suggest using "native" and "invasive" instead of abbreviations. I think it might help the reader keep better track of the results.

R: Following the reviewer's recommendation, we have replaced the crayfish's abbreviations with 'native' and 'invasive' throughout the manuscript.

* Figure 4: I'd suggest denoting significant differences between groups on the figures if possible.

R: The significant differences were denoted with small (native) and capital (invasive) letters on all four panels.

* Figure 5: Indicate which pie charts belong to which species & what are the differences between the 6 pie charts?

R: Done as requested.

* Lines 326 - 340: The data collected and presented on the abundance of both species of crayfish is the "relative abundance" of the two species, e.g. proportion of the total catch that were SCC or NCC. In the discussion the authors discuss the data as "population abundance" and discuss how population abundance is, for example, low or high relative to invasion status. I don't believe this is the correct way to interpret and discuss the data because absolute abundance was not the data reported. I would suggest that the authors discuss the relative abundance of the two species to be consistent with the data presented.

R: We apologise for this inadvertence. We have replaced the incorrect wording 'population abundance' with 'relative abundance' (Line: 357) and discussed the results accordingly (Lines 354-360).

* Lines 363-366: I would suggest tempering this interpretation and conclusion of the lower growth rate and higher omnivore index & elemental imbalance for NCC where the two species appear to coexist. The data presented are all correlational and I'd suggest being careful to imply cause/effect. Additionally, lower growth rate doesn't necessarily mean that a population is in "distress" (I'd also caution the readers against anthropomorphic word choices). Sure it's likely a consequence of the shift in diet, but as long as the population can maintain positive per capita growth, survive and reproduce, it can persist and isn't necessarily "distressed".

R: The reviewer is correct. We have rewritten the text as such as to discuss the emphasised trophic endpoints as rather interrelated and not as a consequence of invasion (Lines: 382-384 and 389-393).

* Lines 374-382: The references and ideas brought up in this paragraph might be particularly good ones to draw on to set up the introduction and outline hypotheses & predications of the study. I think discussing how niche width may change as a consequence of increased competition with an invasive species would improve the introduction and nicely set some clear hypotheses and predictions.

R: We agree and have now added this information to the introduction. The second hypothesis of the revised manuscript version is related to potential opposite patterns in trophic niche widths and degree of overlap as a consequence of short versus long-term coexistence between the native and the invasive species (Lines 104-112).

* Line 399: Consider revising the topic sentences in the discussion to make a

stronger claim (almost like the heading of a newspaper article) and then have the rest of the paragraph follow with supporting information from this study & others to support or refute that claim. I think this would strength the points discussed rather than just stating "Another very interesting result...". Why is it interesting? Maybe revise to lead in with that.

R: As suggested by the reviewer, we have revised the topic sentences as headlines, suggesting the main findings of the study as statements or questions (Lines: 351, 379, 426, 456, 492).

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Reviewer #2: Revision BINV-D-18-00491

The paper is well written and discusses an interesting topic on competition between native and alien crayfish under field conditions. I have a few small remarks/ questions listed below.

R: We would like to thank the reviewer for the thorough review that helped us to improve the manuscript. Please find below the answers to your both specific and general comments. All answers are written in red, to make the reading easier.

In the result section it is somewhat difficult to follow at a certain point all the abbreviations. In the discussion this is much better to follow. I wonder if the authors can make this more clear in the result section as well.

R: Following the reviewer suggestion, both sampling regions (and sampling sites), as crayfish name abbreviations were replaced in the Results section as follows: the four invasion sectors were renamed 'old invasion', 'recovery zone', 'active invasion' and 'non-invaded' and the crayfish names were replaced with 'native' and 'invasive' throughout the manuscript.

In the discussion the authors mention the possible reasons for competition and also the impact of the alien species on the native, but I wonder what really drives the native to extinction at some sites and at others allows coexistence. Are it external

environmental factors others than the ones measured in this study? Do the authors have an idea on this? Are there other examples of this coexistence or examples of where only the alien crayfish wins the competition? Is it that if environmental conditions are good coexistence is possible irrespective of changes in the food and size/genetics of the native species?

R: There were two additional factors, besides the ones measured in this study, that are most likely equally involved in driving both species to either coexistence or the trigger replacement of the native by the invasive crayfish. These other two aspects are the crayfish plague, that can spread even in the absence of its carrier (i.e. the invasive species), and contrasting reproductive strategies (the native K type versus the invasive r type). We agree with the reviewer that the invasion success is a combination of potentially all these aspects, which are interlinked. Following the reviewer's suggestion, we have considered in the Discussion section a separate paragraph (Lines: 459-490), entitled "Supplementary mechanisms that make the invasive species successful in replacing the native crayfish" (Lines: 456-457). Therefore, we conclude that the invasion process is, indeed, as the reviewer suggested a multifaceted process. The authors conclude that this study might inform ecosystem management and protection programs for native crayfish, but I wonder how the authors actually see this? What could a water manager do to protect native crayfish or to actually restore native populations? This is not really clear.

R: Potential management measures to be implemented for this case-study were more thoroughly described in a special section (Lines: 494-510) entitled "Management implications" (Lines: 492).

See also pdf for some small remarks.

R: Former Lines 59: answer provided in Line 80, by stating the introduction of the invasive crayfish "on the continent".

Former Line 78: the sentence reworded, term 'capitalised' removed (Line: 96).

[Click here to view linked References](#)

1 revised manuscript: BINV-D-18-00491

2 **Multifaceted implications of the competition between native and invasive**
3 **crayfish: a glimmer of hope for the native's long-term survival**

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7 **Running title: Invasion dynamics**

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13 8 Octavian PACIOGLU¹, Kathrin THEISSINGER², Andreea ALEXA³, Corina SAMOILĂ³,
14 9 Ovidiu-Ioan SÎRBU³, Anne SCHRIMPF², Jochen P. ZUBROD², Ralf SCHULZ², Mălina
15 10 PÎRVU⁴, Sandra-Florina LELE⁴, John I. JONES⁵ and Lucian PÂRVULESCU^{4*}

16
17
18
19 12 ¹ National Institute of Research and Development for Biological Sciences, 060031 Bucharest,
20 13 Romania

21
22 14 ² Institute for Environmental Sciences, University of Koblenz-Landau, 76829 Landau,
23 15 Germany

24
25 16 ³ Department of Biochemistry and Pharmacology, Faculty of Medicine, “Victor Babeș”
26 17 University of Medicine and Pharmacy Timisoara, 300041 Timisoara, Romania

27
28 18 ⁴ Department of Biology-Chemistry, Faculty of Chemistry, Biology, Geography, West
29 19 University of Timisoara, 300115 Timisoara, Romania

30
31 20 ⁵ School of Biological and Chemical Sciences, Queen Mary University of London, E1 4NS
32 21 London, UK

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34
35 22
36
37 23 * Corresponding author: Lucian Pârvulescu, e-mail lucian.parvulescu@e-uvt.ro

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25 **Abstract**

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2 26 Biological invasions represent a complex phenomenon driven by multiple factors. In this
3 27 study, a real-time invasion process between a native (*Pontastacus leptodactylus*) and an
4 28 invasive (*Faxonius limosus*) crayfish species was investigated in the Lower Danube (South-
5 29 East Europe) through an interdisciplinary approach, by measuring various ecological,
6 30 genetic, physiological and biometric endpoints. The results revealed that the prolonged
7 31 competition in old invaded sites of the river (at least a decade) either drove the native
8 32 species to extinction, or, unexpectedly, allowed its survival as highly fragmented
9 33 populations. However, for the latter situation, several biological and ecological traits
10 34 differed in the remnant populations: increased trophic position and elemental imbalance for
11 35 two major macronutrients (C: N molar ratio), low growth, as strongly contracted trophic
12 36 niche widths and low overlap degree with the invasive crayfish. The data suggest that the
13 37 prolonged competition induced potential resource partitioning between species, potentially
14 38 driving their coexistence, as the development of larger and heavier claws within the native
15 39 males' population. On the contrary, in more recently invaded sectors of the Lower Danube
16 40 (three years), the trophic niche of the native species was significantly larger compared to old
17 41 invaded sites and characterised by high level of niche overlap, indicating almost identical
18 42 diet with the invasive crayfish, but characterised by the lowest trophic position compared to
19 43 other invasion sectors. The genetic diversity of the native crayfish populations was strongly
20 44 reduced in the invaded sectors of the river, but without signs of genetic bottleneck, which
21 45 may be explained by a drift-mutational equilibrium reached as a consequence of
22 46 diminishing population size. Our findings suggest strong coexistence potential in the future
23 47 for both species in the Lower Danube.
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32 49 **Key-words:** *Pontastacus leptodactylus*; *Faxonius limosus*; Biological invasions; Invasive
33 50 species; Populations genetics; Stable isotopes
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52 Introduction

53 The introduction of invasive species into new ecosystems and their population expansion
54 represent a growing threat to biodiversity, drastically altering ecosystems' structure and
55 functionality (Girdner et al. 2018). Invasive crayfish, for instance, are often associated with
56 negative effects on autochthonous fauna (Gherardi et al. 2011) and their expansion in new
57 territories frequently correlates with the decline of native crayfish species (Olden et al. 2006),
58 other macroinvertebrates (Lodge et al. 2012), macrophytes (van der Wal et al. 2013) and even
59 fish (Fitzsimons et al. 2007). However, the underlying mechanisms of native crayfish
60 recovery following biological invasions or their coexistence with invasive species are not
61 clearly determined (Nyström et al. 2001; Kats et al. 2003; Rodriguez 2006). It was suggested
62 that the success of invasive over native crayfish represents the synergic interaction of
63 multiple factors. One aspect is the superior competition for resources of the invasive *versus*
64 native crayfish (Olsson et al. 2009). The consequences of such interactions are trophic niche
65 shifts (Jackson and Britton 2014), decreases in trophic position of the native species (Vander-
66 Zanden et al. 1999) or changes in elemental imbalance (i.e. dissimilarity in nutrient content
67 between consumers and their food) for major macronutrients (González et al. 2010) and
68 growth rate (Olsson et al. 2008). Moreover, the invasion success is frequently associated with
69 geographic disjunct distribution of native populations, which usually leads to lower genetic
70 diversity and, in the extreme case of a population approaching elimination, to a genetic
71 bottleneck (Fitzpatrick et al. 2012). Another suspected consequence of biological invasions is
72 represented by the fast evolution of phenotypic morphological traits in the natives'
73 populations, in order to cope with the new competition exerted by the invasive taxa (Strauss
74 et al. 2006; Hudina et al. 2012). Changes in population structure, morphology and physiology
75 have already been reported between old and new invasion sectors for crayfish (Hudina et al.
76 2012; Pârvulescu et al. 2015; Rebrina et al. 2015). At large geographical scales, differences in
77 growth and feeding habits were registered between invasive and native crayfish, further
78 demonstrating the potential phenotypic changes brought by the invasion process (Pintor and
79 Sih 2009; Reisinger et al. 2017; Glon et al. 2018).

80 A very successful invader in Europe is the spiny-cheek crayfish *Faxonius limosus*
81 Rafinesque 1817, initially introduced for commercial purposes in 1890 to the continent
82 (Filipová et al. 2011). After reaching the basin of the Lower Danube, it started to expand
83 within the whole catchment and the lowland sections of its tributaries (Puky and Schád
84 2006). This crayfish colonises the Lower Danube at a speed of approximately 16 km a year
85 and competes with the indigenous narrow-clawed crayfish *Pontastacus leptodactylus*
86 Eschscholtz 1823 (Pârvulescu et al. 2012). As a consequence of this, the Lower Danube can
87 be divided into three different sectors, according to the invasion stage of the spiny-cheek
88 crayfish and the proportion of both species (*sensu* Pârvulescu et al. 2015). 1) An old-invaded
89 sector encompassed an area where the invasive crayfish is present for at least ten years and
90 has mostly displaced the native competitor, except for one native crayfish population
91 (Pârvulescu et al. 2015). 2) An active invasion sector comprises an area where both species
92 coexisted for maximum three years. 3) A third sector is the non-invaded Danube, only
93 inhabited by the native crayfish (Pârvulescu et al. 2012). Given the presence of a well-
94 established native crayfish population within the old-invaded sector (sector 1), coexistent
95 with the invasive crayfish, a supplementary fourth area (herein recovery zone) was defined in
96 this survey.

97 In this study, a real-time invasion process was investigated through an
98 interdisciplinary approach, by measuring various ecologic, genetic, physiological and
99 morphological endpoints for both of the native and invasive crayfish in the Lower Danube
100 (South-Eastern Europe). To explore reasons for the native crayfish replacement, we examined

101 potential direct and indirect mechanisms that may have contributed to its decline. Therefore,
1 102 three hypotheses were tested. First, we hypothesized that the degree of genetic diversity of
2 103 the native crayfish populations decreased with the invasion age, with signs of a genetic
3 104 bottleneck in the sector where both species coexisted for at least ten years (i.e. the recovery
4 105 zone). Second, we predicted that the competition will induce diet shifts in both species, in
5 106 particular, a low degree of niche overlap (Jackson and Britton 2014) and low niche widths for
6 107 both species as a consequence of trophic segregation in the recovery zone (Sjödin et al.
7 108 2018). Moreover, these trophic endpoints will be accompanied by the native species'
8 109 decrease of trophic position, omnivorous feeding behaviour (Jackson et al., 2011) and growth
9 110 rate (Olsson et al. 2008), but with the increase of the elemental imbalance (González et al.,
10 111 2010) according to the invasion history. At the active invasion front, however, we expected a
11 112 higher degree of niche overlap between both species and greater niche width for the native
12 113 species due the short-term coexistence (Olsson et al. 2009). The third hypothesis predicted
13 114 that the native species will present adaptive phenotypic changes, such as increased
14 115 dimensions and mass of claws, as a consequence of prolonged competition with the invasive
15 116 crayfish in invaded sectors as opposed to non-invaded areas of the river (Hudina et al. 2012).
16 117

17 118 **Materials and Methods**

18 119 *Sampling design*

19 120 In each invasion sector, three sampling sites were selected following the design of Pârvulescu
20 121 et al. (2015), excepting the recovery zone, where only one sampling site was chosen (for site
21 122 abbreviations see Fig. 1 and Annex 1). In each sampling site 10 traps were used, with a
22 123 distance of 50 m in-between. The crayfish were caught in the littoral area from each site with
23 124 bait-traps (Pirate type, with double entrance), left over night. All crayfish were captured,
24 125 euthanized according to animal welfare regulations and their relative abundance in each
25 126 sampling site calculated.
26 127

27 128 *Population genetics of the native crayfish*

28 129 DNA was purified from muscle tissue of a leg using the 'High Salt DNA Extraction Protocol
29 130 for removable samples' (Aljanabi and Martinez 1997) from seven selected native populations
30 131 and three invasion sectors (see Fig. 1). To genotype the samples, 12 microsatellite loci were
31 132 used according to Gross et al. (2017). PCR was conducted in two multiplex batches (Batch A:
32 133 Aast4_5, Aast4_12, Aast4_24, Aast4_32, Aast4_40, Aast4_48; Batch B: Aast4_8, Aast4_16,
33 134 Aast4_26, Aast4_30, Aast4_34, Aast4_43) using the Type-it MicSat PCR Kit (Qiagen,
34 135 Hilden, Germany). PCR was carried out on a Primus 96 Cycler (Peqlab Biotechnologie
35 136 GmbH, Erlangen, Germany) under the following conditions: an initial denaturation at 95°C
36 137 for 5 min, followed by 30 cycles of 30 s at 94°C, 90 s at 57°C, 60 s at 72°C, and a final
37 138 extension of 30 min at 60°C. 2 µL PCR-product were added to 27.7 µL SLS-Buffer
38 139 (Beckman Coulter, Krefeld, Germany) and 0.3 µL 400 bp standard. The fragment analysis
39 140 was performed on a Beckman Coulter CEQ 8000 eight capillary sequencer. Loci were scored
40 141 using the software GeneMarker version 2.4.0 (State College, Pennsylvania, USA).
41 142

42 143 Micro-Checker version 2.2.3 was applied to test for scoring error due to stuttering,
43 144 large allele dropout and null alleles (Van Oosterhout 2004). All loci were tested for linkage
44 145 disequilibrium with ARLEQUIN version 3.5.1.3 (Excoffier and Lischer 2010). The average
45 146 and mean numbers of alleles per locus per population (A and A_m , respectively) as well as the
46 147 expected (H_E) and observed (H_O) heterozygosity were calculated as a measure of genetic
47 148 variability in ARLEQUIN version 3.5.1.3 (Excoffier and Lischer 2010). Each population was
48 149 tested for deviations of the Hardy-Weinberg equilibrium with Genepop on the web (Raymond
49 150

150 and Rousset 1995). The number of private alleles (A_P , i.e., allele endemic to populations or
1 151 sectors) per population was calculated with the GenAEx 6.5 software (Peakall and Smouse
2 152 2012). The genetic variation among all populations and invasion sectors was visualised by a
3 153 factorial correspondence analysis (FCA) conducted with the default settings in GENETIX
4 154 4.05 (Belkhir et al. 1996-2004).

6 155 All populations were tested for a recent reduction in the effective population size
7 156 (bottleneck event) using the coalescent-based software Bottleneck 1.2.02 (Cornuet and
8 157 Luikart 1997), which calculates the distribution of heterozygosity for each population and
9 158 locus under three microsatellite mutation models (infinite allele model, IAM; stepwise
10 159 mutation model, SMM, two phase model, TPM). To determine whether a population
11 160 exhibited a significant number of loci with heterozygosity excess, a "sign test", a
12 161 "standardized differences test", and a "Wilcoxon sign-rank test" were applied. The expected
13 162 heterozygosity (H_e) was compared to the observed heterozygosity (H_o) to establish whether
14 163 there was an excess or deficit of heterozygosity across all loci. The distribution obtained
15 164 through coalescent simulations enabled the computation of a P value for the observed
16 165 heterozygosity. P values < 0.05 indicated significant heterozygote excesses and thus signs for
17 166 a recent bottleneck under the respective mutation model. The allele frequency distribution
18 167 was applied to test whether it is approximately L-shaped (as expected under mutation-drift
19 168 equilibrium) or not (indicating a recent bottleneck). The software was used with 100.000
20 169 simulations and default settings.

25 171 *Trophic interactions, elemental imbalance and growth rate*

26 172 All sampling stations (Fig. 1) were initially screened for several criteria to assure the
27 173 selection of representative sites (one within each invasion sector) for the assessment of
28 174 interspecific relations between crayfish. The selection criteria were the following: similar
29 175 macroinvertebrate communities, at least 40% macrophyte coverage and the presence of
30 176 riparian trees on the shores, to assure comparable habitat conditions for the analysed crayfish.
31 177 Therefore, random 30-s kick samples for macroinvertebrates were collected using a 250- μ m
32 178 mesh pond net in each site ($n=5$). The invertebrates were identified in the laboratory to order
33 179 level or higher and community taxonomic composition was compared across sites with the
34 180 aid of one-way ANOSIM test (software PAST 2.01). The degree of coverage with
35 181 macrophytes and riparian trees was estimated visually in each sampling site.

36 182 Fresh subsamples of abdominal tissues from both species of crayfish ($n=15-20$
37 183 individuals/ species/site) and putative food resources ($n=3$ for each type) were collected in
38 184 the field, with a pond net or by hand and classified to taxonomic groups in place. In the
39 185 laboratory, the samples were oven dried (60°C for 48 h) before being homogenized and
40 186 wrapped into tin combustion capsules. The analysis for bulk stable carbon (^{12}C and ^{13}C) and
41 187 nitrogen (^{14}N and ^{15}N) isotopes in the samples and of their elemental composition was
42 188 performed using a Flash 2000 HT elemental analyser coupled via a ConFlo IV interface to a
43 189 Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen,
44 190 Germany). The reported stable carbon and nitrogen isotope values are expressed as δ (‰)
45 191 relative to the international reference standards Vienna PeeDee Belemnite and atmospheric
46 192 nitrogen, respectively.

47 193 To allow comparisons of diet variability among populations, stable isotope values
48 194 were corrected using resource baseline values (following Jackson and Britton, 2014). We
49 195 estimated the relative contribution (%) of each resource to the diet of both species of crayfish
50 196 using the Bayesian mixing model SIAR in R (Parnell et al. 2010; R Development Core Team,
51 197 2015). Fractionation factors between crayfish and resources were calculated according to
52 198 Jackson et al. (2017). The mean estimated proportional contribution of each resource to the

199 diet of each individual was then used to calculate an index of individual omnivory and
 200 trophic position, according to Jackson et al. (2017). The omnivory index represents an
 201 indicator of the diversity of trophic levels consumed by crayfish and, along with their trophic
 202 position, it gives a more comprehensive image of the role played by a major top-consumer in
 203 a local food-web (Christensen and Walters 2004). Moreover, isotope values were used to
 204 calculate the isotopic niche width of each population using SIBER in the SIAR package
 205 (trophic niche, *sensu* Jackson et al. 2012). Bayesian standard ellipse areas (SEA_b) were
 206 calculated as a measure of the isotopic niche width and to obtain confidence intervals for
 207 isotopic niche areas. These confidence intervals allow for statistical comparisons of the sizes
 208 of isotope niche widths among populations (Jackson et al. 2011). The SEA_b's were then used
 209 to calculate the degree of isotopic niche overlap, representing a quantitative measure of
 210 dietary similarity between populations (Guzzo et al. 2013).

211 The elemental imbalance (based on C: N ratio) between crayfish and their food
 212 sources was derived from the original formula of Cross et al. (2003). To comply with the
 213 omnivorous feeding habit of crayfish (Olsson et al. 2008) we proposed in this study a more
 214 equitable approach to calculate the elemental imbalance for C: N ratio, by employing the
 215 percentage of various food items assimilated in their biomass and the elemental molar ratio of
 216 targeted macronutrients (eq. 1). Therefore, the elemental imbalance (EI) for C: N ratio
 217 between both species of crayfish and their food sources was calculated as follows:

$$EI = \left| \ln \left(\frac{\sum_{i=1}^n \%resource_i \times C : X_i}{C : X_c} \right) \right| \quad (\text{eq. 1})$$

218
 219 where $\%resource_i$ represents the mean estimated proportional contribution of each resource
 220 to the diet according to Bayesian model output, $C : X_i$ represents the mean C: N molar ratio of
 221 that i food resource, $C : X_c$ represents the mean C: N ratio of crayfish and n the total amount
 222 of resources as indicated by the SIAR mixing model. All values are in module.

223
 224 The crayfish growth rate was estimated from the RNA/DNA ratio measurements
 225 (*sensu* Ollson et al. 2008). Aliquots of approximately 15 mg of abdominal tissue were
 226 extracted in the field and stored in RNALater (ThermoFisher, AM7020). Later, the samples
 227 were digested for 2 h in 75 mL proteinase K (10 mg/ml) digestion mixture at 55° C. The
 228 samples were added each 1 mL of Phe: CHCl₃ mixture (Ambion, 9721G), centrifuged for 10
 229 min at 13.000 rpm at 4°C and the aqueous layer further separated and transferred to a Spin III
 230 C column of the ZR-Duet DNA/RNA Miniprep kit (Zymo Research, ZRC 187609) for DNA
 231 and RNA purification, according to the kit protocol. All DNA and RNA samples were
 232 quantified and characterized (260/280 and 260/230 ratios) using a Nano-Drop ND-1000 UV-
 233 Vis Spectrophotometer.

234 The trophic positions, omnivory indices, elemental imbalance for C/N ratios and
 235 growth rates (DNA/RNA ratio) for each species were compared across sites with Kruskal-
 236 Wallis tests, followed by pairwise post-hoc Mann-Witney tests with Bonferroni corrections.
 237 Statistical tests were performed using SPSS version 17 (SPSS Inc., Chicago, USA) for
 238 Windows.

239 **Biometric measurements**

240
 241 The collected crayfish were preserved in 4% formaldehyde and later measured in the
 242 laboratory for several allometric parameters: total body length, cephalothorax length and
 243 width, abdomen width, left and right claws' length and width as well as dactylus length, using
 244 a digital calliper of 0.01 mm accuracy (Stanley Black and Decker, New Britain, Connecticut,
 245 USA). The body mass without claws and separately for each claw was measured individually

246 with a balance of 0.01 grams accuracy (Kern and Sohn GmbH, Balingen, Baden-
1 247 Württemberg, Germany). The Crayfish Condition Factor was determined for collected
2 248 individuals according to Adegboye (1981). We decided to weigh separately the crayfish body
3 249 and their claws to avoid biases caused by claws' size variability (Streissl and Hödl 2002).
4 250 Also, to avoid influences of physiological variances during pre- or post-reproductive stages
5 251 of females, we selected only males in this study. Injured individuals or those missing one or
6 252 both chelae were not considered.

8 253 Before statistically analysing the data, the total length was *a priori* compared across
9 254 sites to test for its variability, to assure the objectivity of employed sampling strategy for
10 255 selecting comparable data sets. Total length is considered a surrogate of crayfish age (Jackson
11 256 et al. 2017); therefore, it was used in this study as a reference variable to assure the
12 257 consistency of biometric representativeness across sampled sites. The allometric
13 258 measurements were compared across invasion sectors with one-way analysis of variance
14 259 (ANOVA), followed by post-hoc Tukey tests for each species of crayfish. Statistical tests
15 260 were performed using SPSS version 17 (SPSS Inc., Chicago, USA) for Windows.

18 261 **Results**

19 262 ***Invasion status***

20 263 The monitoring of crayfish caught in traps in the summer of 2016 revealed that the old-
21 264 invaded sector (comprising sampling sites BAV, BAZ and COR, for abbreviations see Fig. 1
22 265 and Annex 1) is populated solely by the invasive crayfish, excepting the sampling site STA
23 266 (i.e. recovery zone, Fig. 1), where the proportion of both species is approximately equal (Fig.
24 267 2). Also, in the active invasion sector (comprising sampling sites SVI, DUB and ORS, see
25 268 Fig. 1) the frequency of both species is approximately equal, however with a tendency for the
26 269 invasive domination in the first two sampling sites (Fig. 2). The non-invaded sector,
27 270 comprising the sampling sites BEC, GIU and DD, is populated solely by the native species
28 271 (Fig. 1 and 2).

29 272 ***Population genetics of the native crayfish***

30 273 In total, 252 native crayfish from seven populations and three invasion sectors were
31 274 successfully genotyped across 12 loci. The Micro-Checker analysis provided evidence for
32 275 putative null alleles for five loci in four populations (Table 1). No pair of loci showed
33 276 significant linkage disequilibrium. Six out of seven populations deviated significantly from
34 277 the Hardy-Weinberg equilibrium (Table 1). Among the sampled sectors, the number of alleles
35 278 and private alleles per population, as well as expected and observed heterozygosity are of the
36 279 same order of magnitude (Table 1). While the allele numbers and heterozygosity had a
37 280 similar range among all populations (A_m : 2.33 - 4.08; H_e : 0.324 - 0.428; Table 1), the number
38 281 of private alleles was much higher in the non-invaded sector (A_p : 3 - 10) than in the active
39 282 invasion and the recovery zone (A_p : 0 - 2). An FCA analysis showed that the native crayfish
40 283 populations from the non-invaded sector were genetically more diverse (wider spread scatter
41 284 plot) compared to the populations from the invaded sectors of the river (Fig. 3). Most
42 285 populations exhibited no signs of a recent bottleneck event (Table 3), except for population
43 286 SVI ("standardized difference test": $P_{IAM} = 0.016$; active invasion sector); GIU ("sign
44 287 test": $P_{TPM} = 0.047$, $P_{SMM} = 0.008$; "Wilcoxon sign-rank test": $P_{SMM} = 0.004$; non-
45 288 invaded sector) and DD ("Wilcoxon sign-rank test": $P_{SMM} = 0.013$; non-invaded sector).
46 289 However, for no population the allele frequency distribution differed significantly from an L-
47 290 shaped distribution, as expected under the mutation-drift equilibrium (Table 2).

48 291 ***Trophic interactions and growth rate***

295 The ANOSIM test indicated that overall, the macroinvertebrate communities were similar in
1 296 all ten sites ($P > 0.05$). Moreover, sampling sites STA, COR, ORS and GIU were
2 297 characterised as well by similar macrophyte coverage ($> 40\%$ in average) and had an
3 298 extensive undergrowth of riparian trees on the shores (Annex 1). Therefore, these sites were
4 299 considered representative for each invasion sector, with similar habitat conditions to allow
5 300 comparison of trophic interactions and growth rate assessment for both species of crayfish
6 301 (Fig. 1).

7 302 The trophic position of the native was highest in the non-invaded sector, intermediary
8 303 in the recovery zone and the lowest in the active invasion (Fig. 4A and Table 3, $P < 0.016$
9 304 pairwise Mann-Whitney tests), whereas the invasive crayfish featured similar trophic
10 305 positions in the old-invasion sectors, but significantly higher than in the active invasion (Fig.
11 306 4A and Table 3, $P < 0.016$ pairwise Mann-Whitney tests). The omnivory index of the native
12 307 species was the lowest in the active invasion, but similar in the non-invaded and the recovery
13 308 zone (Fig. 4B and Table 3, $P < 0.016$ pairwise Mann-Whitney). The invasive crayfish showed
14 309 a decrease of the omnivory index with the invasion history (Fig. 4B and Table 3, $P < 0.016$
15 310 pairwise Mann-Whitney tests). The elemental imbalance of C: N ratio for the native species
16 311 was significantly higher in the recovery zone compared to other invasion sectors, whereas for
17 312 the invasive crayfish it did not differ significantly across regions (Fig. 4C and Table 3, $P <$
18 313 0.016 pairwise Mann-Whitney tests for the native). The growth rate (RNA/DNA ratio) for the
19 314 native increased significantly with the invasion progress, whereas for the alien crayfish it was
20 315 significantly higher in the active invasion compared to other sectors (Fig. 4D and Table 3, P
21 316 < 0.016 pairwise Mann-Whitney tests).

22 317 The trophic niche width of the native in the recovery zone was significantly smaller
23 318 compared to the invasive species, which was twice as large; the niche width of the latter
24 319 species was similar in size to the one from the active invasion (Fig. 5 and Table 4). The
25 320 trophic niche width of the native in non-invaded and active invasion sectors was seven and
26 321 four times, respectively, larger compared to recovery zone (Table 4). The trophic niches of
27 322 both species overlapped when coexisting (Fig. 5), with the overlap being much smaller in the
28 323 recovery zone (16.2%) compared to active invasion (62%).

29 324 The mean percentage of food items assimilated by both species of crayfish varied
30 325 considerably among invasion sectors (Fig. 5). In the recovery zone approximately two-thirds
31 326 of both species' diets comprised detritus and primary producers (macrophytes and
32 327 filamentous algae). However, the last third was dominated by small fish (Fam. Gobiidae) in
33 328 invasive diet, whereas for the native it comprised macroinvertebrates (Fig. 5). In the old-
34 329 invaded sector, the invasive diet comprised a third detritus and a third macroinvertebrates,
35 330 being complemented by primary producers and small fish (Fig. 5). In the active invasion, the
36 331 diet of both species of crayfish was remarkably similar, comprising mainly primary producers
37 332 and macroinvertebrates, and only a small proportion (approx. 15%) represented by detritus
38 333 and small fish (Fig. 5). Most of the native's diet (90%) in the non-invaded comprised
39 334 secondary consumers (an equal proportion of small fish and macroinvertebrates) and only 10
40 335 % primary producers and detritus (Fig. 5).

51 337 ***Biometric measurements***

52 338 In total, 301 native and 318 invasive males were captured and measured in the laboratory.
53 339 Total body length of both species did not differ statistically significantly among sampling
54 340 sites (Table 3). However, the cephalothorax width, width and mass of both chelae and length
55 341 of both dactyli of the native species were significantly higher in the recovery zone compared
56 342 to both active invasion and non-invaded sectors (Table 3; one-way ANOVA, $P < 0.001$, *post*
57 343 *hoc* pairwise comparisons). The length of both chelae was significantly higher in the recovery

344 zone and active invasion compared to non-invaded sector (Table 3; one-way ANOVA, $P <$
1 345 0.05, *post hoc* pairwise comparisons). The mass without chelae, cephalothorax length,
2 346 abdomen width and the Crayfish Condition Factor of the native crayfish did not differ among
3 347 different invasion sectors (Table 3). The same allometric measurements and biomass of the
4 348 invasive species showed no significant differences among invasion sectors (Table 3).

7 350 Discussion

8 351
9 352 *Native crayfish populations registered a decreased genetic diversity corresponding to*
10 353 *invasion history*

12 354 Corroborating the results of this study with those of the survey between 2011 and 2014
13 355 (Pârvolescu et al. 2015), it was observed that the invasive species is gradually advancing in
14 356 the active invasion sector, slowly replacing the native crayfish (Fig. 2). Moreover, we found
15 357 that the reduction of the relative abundance of the native species in the active invasion sector
16 358 went along with diminished genetic diversity compared to individuals from the non-invaded
17 359 region (Fig. 3). This finding suggests that the pressure induced by the recent interaction was
18 360 sufficiently strong to affect the gene pool of the native species.

21 361 We would have expected to find even less alleles and lowered heterozygosity within
22 362 the recovery zone population compared to the active invasion and non-invaded sectors,
23 363 respectively, since the former area is comprised of individuals who potentially went through a
24 364 severe bottleneck event caused by long-term invasion and maintained by strong habitat
25 365 fragmentation and hindered gene flow. However, we could not detect statistically significant
26 366 evidence for a bottleneck event in this population. If a population gets abruptly contracted in
27 367 numbers, this usually induces a reduced genetic diversity. However, alleles are lost faster
28 368 than heterozygosity and theory predicts that a new mutation-drift equilibrium may be set
29 369 rapidly when effective population size becomes low (Watterson 1984), making the excess of
30 370 genetic diversity undetectable. This pattern was previously recorded for various animals
31 371 (Larsson et al. 2008; Canales-Delgado et al. 2012; Harrisson et al. 2014). Therefore, it
32 372 appears that for small population sizes new mutation-drift equilibrium is rapidly set, making
33 373 the detection of bottleneck events highly unlikely with this approach. Nevertheless, the
34 374 microsatellite analyses of the native crayfish showed that the genetic variability is reduced in
35 375 the invaded *versus* non-invaded sectors, which is reflected in the lower numbers of private
36 376 alleles (Table 1) and genetic diversity across sectors (Fig. 3).

42 379 *Trophic endpoints and growth rates of native crayfish species differ according to invasion*
43 380 *history*

45 381 The diversity of assimilated food items by the native species in the non-invaded sector
46 382 suggests a significantly more carnivorous feeding behaviour compared to the active invasion
47 383 sector, where almost half of its diet was based on macrophytes (Fig. 5). The almost identical
48 384 diet of native and the invasive species in the active invasion sector was reflected in a high
49 385 degree of trophic niche overlap (62%) and the widening of former's niche width compared to
50 386 other sites (Fig. 5). These findings suggest that the native crayfish was potentially forced by
51 387 the recently established competition to increase the percentage of assimilated primary
52 388 producers in this sector of the river. The increased fraction of primary producers in the
53 389 native's species diet, which are of lower quality compared to secondary producers (Cross et
54 390 al. 2003) comprising most of its trophic spectrum in the non-invaded sector can explain the
55 391 decrease of its trophic position, omnivory index and growth rate in the active invasion sector
56 392 (Fig. 3A, 3B and 3D). Previous studies found that following biological invasions, when top-

393 predators managed to coexist, significant changes in the trophic position, degree of omnivory
1 394 and values of trophic niche widths were detectable for the native species (Vander-Zanden et
2 395 al. 1999; Bøhn et al. 2008; Olsson et al. 2009; Jackson and Britton 2014). In crayfish, a
3 396 broader trophic niche that expanded towards lower trophic levels may have afforded
4 397 competitive advantages to the invasive *versus* native crayfish species in active invaded
5 398 regions (Olsson et al. 2009; Messenger and Olden 2019).

7 399 An exceptional situation was found in the recovery zone, where both species
8 400 coexisted for at least ten years in roughly equal proportions (STA site, Fig. 2). Whilst the
9 401 growth rate and trophic position was the lowest for the native species in this sector compared
10 402 to all others (Fig. 3A and 3D), the elemental imbalance for C: N ratio was the highest (Fig.
11 403 3C). The native crayfish appears to be feeding higher in the recovery zone' food web when
12 404 compared to conspecifics located in the active invasion sector. Recent findings related to
13 405 potential changes of trophic positions are, nevertheless, equivocal. Whilst the invasive
14 406 crayfish *Faxonius rusticus* in the USA showed similar patterns with both species in the
15 407 current case-study (i.e. lower trophic position in active *versus* old-invaded regions, Messenger
16 408 and Olden 2019), other studies found the opposite. Round gobies at the edge of their
17 409 expanding range had higher $\delta^{15}\text{N}$ signatures than the previous year front (Brandner et al.
18 410 2013) and invasion front bloody red mysid shrimp showed greater ability to locate and
19 411 capture zooplankton prey than those shrimp in core populations (Iacarella et al. 2015).

23 412 Moreover, the low degree of trophic niche overlap with the invasive species (16.2%)
24 413 and the smallest recorded size of the niche width in the recovery zone concur with the
25 414 different assimilated food items: whilst the fraction of animal tissue from the native's diet
26 415 was based on macroinvertebrates, the invasive species relied on small fish (Fig. 5). The idea
27 416 that a very important aspect in the success of the invasive species is a potentially broader diet
28 417 than the native species was emphasised before (Moyle and Light 1996; Olsson et al. 2009).
29 418 Thus, the invasive crayfish niche width being larger than their native counterparts is fitting
30 419 well with this theory. An increase of trophic niche width can be achieved either by shifting
31 420 the diet towards a wider array of food items or by resource partitioning (Bolnick et al. 2007;
32 421 Svanbäck and Bolnick 2007). The findings of this study suggest that the latter mechanism
33 422 may have occurred and likely represents one of the key-factors that allowed the coexistence
34 423 of both species of crayfish in this old invaded sector of the river.

39 425 *The native species developed phenotypic traits as a result of long-term competition*

40 426 The males of the native species showed significantly longer, wider and heavier claws as well
41 427 as wider cephalothoraxes in the recovery zone compared to other invasion sectors (Table 3).
42 428 Claws represent one of the major fitness determinants in adult male crayfish (Lele and
43 429 Pârvulescu 2019). Larger chelae are not only advantageous in foraging, interactions and
44 430 defence against predators, but also in competition for females and copulation, resulting in a
45 431 higher reproductive success (Houdina et al. 2012). This assumption is corroborated by
46 432 previous studies that showed changes in body morphology induced by competition or
47 433 changes of foraging habits (Carroll et al. 2005; Huey et al. 2005; Bøhn et al. 2008; Cattau et
48 434 al. 2018). The native crayfish' population size and structure have important consequences for
49 435 whether an evolutionary response can occur (Strauss et al. 2006). For example, if an invasive
50 436 species reduces the population size of the native through competition, then native populations
51 437 that have been invaded may be demographic sinks relative to those without invaders (Fox et
52 438 al. 1997; Woodworth et al. 2005). If there is optimal gene flow among invaded and non-
53 439 invaded populations, the selective pressures and evolutionary responses in the former regions
54 440 may be buffered by potential greater population growth rates of genotypes from non-invaded
55 441 populations (Strauss et al. 2006). As recently observed, the crayfish *Pacifastacus leniusculus*

442 presented bigger claws in rivers whilst coexisting with native species in Japan (Ooue et al.
1 443 2019) and Croatia (Houdina et al. 2012), and similar results were found for *F. rusticus* in the
2 444 USA (Messenger and Olden 2019). However, the interactions between native and invasive
3 445 species does not necessarily result in the development of phenotypic traits as response to
4 446 invasion, given short coexistence, because the autochthonous taxa may possess
5 447 pre- adaptations that allow species coexistence (Solarz and Newman 2001; Agrawal and
6 448 Kotanen 2003). This could explain the lack of any morphologic differences in native
7 449 populations between active invasion and non-invaded sectors of the Lower Danube (Table 3).
8 450 However, without knowledge of the heritability of the crayfish traits examined in this study,
9 451 inference regarding the specific mechanisms in operation is still limited, demanding caution
10 452 for further extrapolations (Kamran and Moore 2015).

13 453 14 454 *Supplementary mechanisms that make the invasive species successful in replacing the native* 15 455 *crayfish*

16 456 There might be additional factors, besides the ones measured by us in this study, that are
17 457 more than likely equally involved in driving both species to either coexistence or the
18 458 replacement of the native by the invasive crayfish. American crayfish invaders can
19 459 successfully exploit new ecosystems through disease-mediated invasions, where pathogens
20 460 are found by over-spill mechanisms in the absence of their carriers (Strauss et al. 2012).
21 461 Accordingly, *Aphanomyces astaci*, the oomycete pathogen responsible for the crayfish
22 462 plague, was discovered well ahead of the invasive species in several sites from the non-
23 463 invaded Danube (Pârvulescu et al. 2012; Schrimpf et al. 2012). Although European crayfish
24 464 species are susceptible to die after infection, resistant populations of the native species were
25 465 recorded before (Kokko et al. 2012; Schrimpf et al. 2012). The coexistence of *A. astaci* and
26 466 the native crayfish may be facilitated by adaptations of both pathogens and hosts, because the
27 467 oomycete haplotype groups identified in the non-invaded Danube are possibly relics from an
28 468 19th century outbreaks (Panteleit et al. 2018). Remnant populations surviving the infestation
29 469 with this pathogen were documented even for more sensitive European crayfish species such
30 470 as *Astacus astacus* LINNAEUS, 1758 (Makkonen et al. 2012), *Austropotamobius pallipes*
31 471 LEREBoullet, 1858 (Martín-Torrijos et al. 2017) and *A. torrentium* SCHRANK, 1803
32 472 (Kušar et al. 2013).

33 473 Another ecological mechanism possibly impacting on the invasion success is the
34 474 species reproduction strategy. The invasive crayfish is a typical r-strategist, whereas the
35 475 native is a typical K (Pârvulescu et al. 2015). The former species has a short life cycle of
36 476 around four years and reaches maturity in the second year (Henttonen and Huner 1999). It has
37 477 a relatively high fecundity, with an average number of eggs varying between 100 and 400
38 478 (Kozák et al. 2007) and is suspected of facultative parthenogenesis (Buřič et al. 2011).
39 479 Moreover, its reproductive strategy is very adaptable. It was discovered that following
40 480 invasion of the Lower Danube, a shift in sex ratio towards females emerged within the
41 481 populations from the active invasion sector, characterised by an increased number of eggs,
42 482 but with lower quantities of vitellus as compared to females from old-invasion sectors
43 483 (Pârvulescu et al. 2015).

44 484 Therefore, the output from this case-study and related studies (Pârvulescu et al. 2012,
45 485 2015; Panteleit et al. 2018) suggest that the replacement of the native crayfish from the
46 486 Lower Danube is caused by a multifaceted interaction between several factors, possible to
47 487 investigate thoroughly only through an interdisciplinary approach.

48 488 49 489 *Management implications*

490 In the recovery zone, the proportion of the native crayfish is roughly the same as for the
1 491 invasive species (Fig. 2). It is, however, difficult to deduce if the native crayfish population is
2 492 actually recovering, steady or just declining at a slower pace compared to other invaded areas
3 493 of the Lower Danube, as no historic data are available for this sector. Practical measures to
4 494 eradicate or stop the downstream invasion are, in our opinion, very difficult to implement.
5 495 Most cases of biological invasions with crayfish species were considered as disastrous for the
6 496 local habitats and biota and the implementation of management solutions for alien species
7 497 eradication prone to failure (Gherardi et al. 2011). In fact, the eradication methods are
8 498 applicable only for isolated habitats that can be controlled. Only in such cases eradication is
9 499 feasible and economically profitable, when compared to the enormous costs that the spiny-
10 500 cheek crayfish may request to be removed from the Danube, being the second largest
11 501 European river. However, previous studies on taxonomically related invasive and native
12 502 species within the same habitats indicate the potential for their long-term coexistence (Byers
13 503 2000; Wauters et al. 2002; Carroll et al. 2005; Huey et al. 2005; Bøhn et al. 2008; Cattau et
14 504 al. 2018). Therefore, we conclude that there is a fair chance for the native population to find
15 505 equilibrium with the invasive crayfish in the Lower Danube, albeit as strongly fragmented
16 506 populations.
17 507

508 **Conclusions**

509 In the present study, we assessed consequences of the interactions between a native and an
510 510 invasive crayfish species. Our findings suggest that one of the major causes for the native
511 511 crayfish replacement is that the invasive species is a superior competitor for food. Moreover,
512 512 we also showed that long-term coexistence of both species is possible, but with secondary
513 513 effects involved for the native crayfish, such as diminished genetic diversity, several trophic
514 514 endpoints and growth rate as a direct or indirect result of interspecific competition and
515 515 resource partitioning. With this knowledge in mind, larger assessment sectors should be more
516 516 thoroughly studied in the future, in order to implement efficient measures for invasive species
517 517 eradication in the Lower Danube, the second largest European river.
518 518

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525 525

526 **Author's contribution**

527 LP and OP conceived the study. OP, MP, LP and SFL provided field samples collection. KT
528 528 and AS provided genetic data and population genetic analyses. OP provided samples
529 529 preparation for stable isotope, nutrient mass, JPZ performed stable isotope and elemental
530 530 analyses, OP, RS and JIJ performed statistical analyses. AA, CS and OIS measured the
531 531 RNA/DNA ratio (growth rate). MP and SFL measured biometric parameters. OP, KT and LP
532 532 led the writing of the manuscript. All authors contributed and approved publication.
533 533
534 534

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741 **Figure captions**

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2 743 **Figure 1.** Map depicting the invasion sectors in the Lower Danube and sampling sites
3 744 selected for this study. The sampling sites were selected according to the invasion dynamics:
4 745 old invasion (OI), recovery zone (R), active invasion (AI) and non-invaded (NI) sectors.
5 746 Various sites are indicated using different geometric shapes. The sites sampled for stable
6 747 isotope analyses and growth rate assessments are indicated in bold. For sites' name,
7 748 abbreviation and geographic location see Annex 1.

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10 750 **Figure 2.** The relative abundances of native and invasive crayfish in various sampling sites,
11 751 caught with bait-traps. Sites abbreviation as in Figure 1 and Annex 1.

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14 753 **Figure 3.** Factorial Correspondence Analysis (FCA) showing the degree of genetic similarity
15 754 of 252 narrow-clawed crayfish individuals across 12 microsatellite loci based on the first two
16 755 dimensions (factors). Each point represents one individual. The distance between points
17 756 reflects the degree of genetic differentiation among individuals. The individuals are grouped
18 757 by population (N = 7; colours) and by invasion sectors (N = 3; symbols: Recovery zone =
19 758 diamond, Active Invasion = square, Non-invaded = circle).

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23 760 **Figure 4.** Trophic position (A), Omnivory index (B), Elemental imbalance for C/N ratio (C)
24 761 and growth rate (RNA/DNA) (D) for both narrow-clawed crayfish and spiny-cheek crayfish
25 762 competitors in the sampled invasion sectors. Error bars indicate $\pm 1SE$. Abbreviations: OI -
26 763 old invaded, R – Recovery zone, AI – Active Invasion, NI - Non-invaded sectors. Significant
27 764 differences ($P < 0.05$) symbolised by different letters between taxa (normal letters- the native
28 765 and capital letters-the invasive species).

30 766

31 767 **Figure 5.** Trophic niches of both native and invasive crayfish in the sampled invasion sectors
32 768 (expressed as SEA's) and mean frequencies of assimilated food types (expressed as pie-
33 769 charts). Abbreviations: OI - old invaded, R – Recovery zone, AI – Active Invasion, NI - Non-
34 770 invaded sectors.

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774 **Supplementary files**

1 775 **Annex 1:** Sampling sites abbreviation, geographic location (latitude and longitude),
2 776 taxonomic composition of macroinvertebrates within each sampling site, cover degree by
3 777 macrophytes and presence/absence of riparian trees on the river shores.
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779 **Tables**

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2 781 **Table 1.** Summarized microsatellite results across 12 loci. The table comprise the invasion
 3 782 sectors, population code (Pop), number of samples (n), alleles across all loci (A), mean
 4 783 number of alleles (A_m) and number of private alleles (A_p), expected (H_e) and observed (H_o)
 5 784 heterozygosity, as indication if the populations are in Hardy-Weinberg equilibrium (HWE).

Sector	Pop	n	A	A_m	A_p	H_e	H_o	HWE
Active	DUB	37	45	3.75	2	0.428	0.394	NO
Active	ORS	39	43	3.58	1	0.416	0.409	NO
Active	SVI	33	33	2.75	0	0.358	0.385	YES
Non-invaded	BEC	20	28	2.33	3	0.324	0.275	NO
Non-invaded	GIU	41	51	4.25	10	0.399	0.324	NO
Non-invaded	DD	37	49	4.08	6	0.395	0.371	NO
Recovery	STA	44	34	2.83	1	0.328	0.357	NO

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Table 2. Summarized Bottleneck results, with mutation model for each population (infinite allele model, IAM, stepwise mutation model, SMM and two phase model, TPM). Significant P values (< 0.05) are indicated in bold. For site abbreviations see Fig. 1 and Annex 1.

Sector	Site	Sign test P			Standardized differences test			Wilcoxon sign-rank test		
		IAM	TPM	SMM	IAM	TPM	SMM	IAM	TPM	SMM
Active	DUB	0.45	0.44	0.08	0.31	0.68	0.91	0.62	0.67	0.2
Active	ORS	0.25	0.24	0.07	0.28	0.76	0.97	0.56	0.51	0.05
Active	SVI	0.14	0.19	0.46	0.01	0.11	0.27	0.03	0.23	0.55
Non-invaded	BEC	0.43	0.52	0.44	0.28	0.61	0.78	0.55	0.84	0.49
Non-invaded	GIU	0.40	0.047	0.01	0.45	0.96	0.99	0.89	0.08	0.004
Non-invaded	DD	0.28	0.09	0.08	0.74	0.94	0.99	0.56	0.12	0.01
Recovery	STA	0.4	0.47	0.06	0.21	0.61	0.93	0.43	0.84	0.16

Table 3. F-values of one way-ANOVA for biometric data (in bold) and of χ^2 -values of Kruskal-Wallis tests for RNA/DNA ratio, trophic position, omnivory index and elemental imbalance (C: N ratio) for both crayfish species in different invasion sectors. Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.

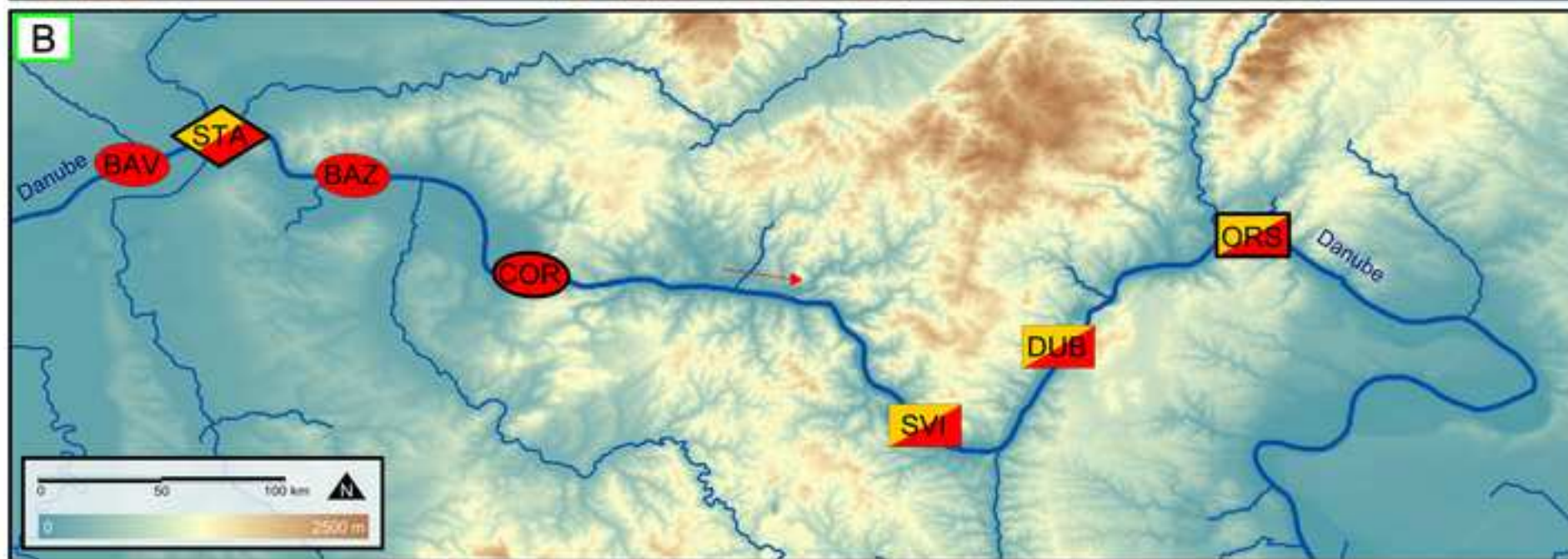
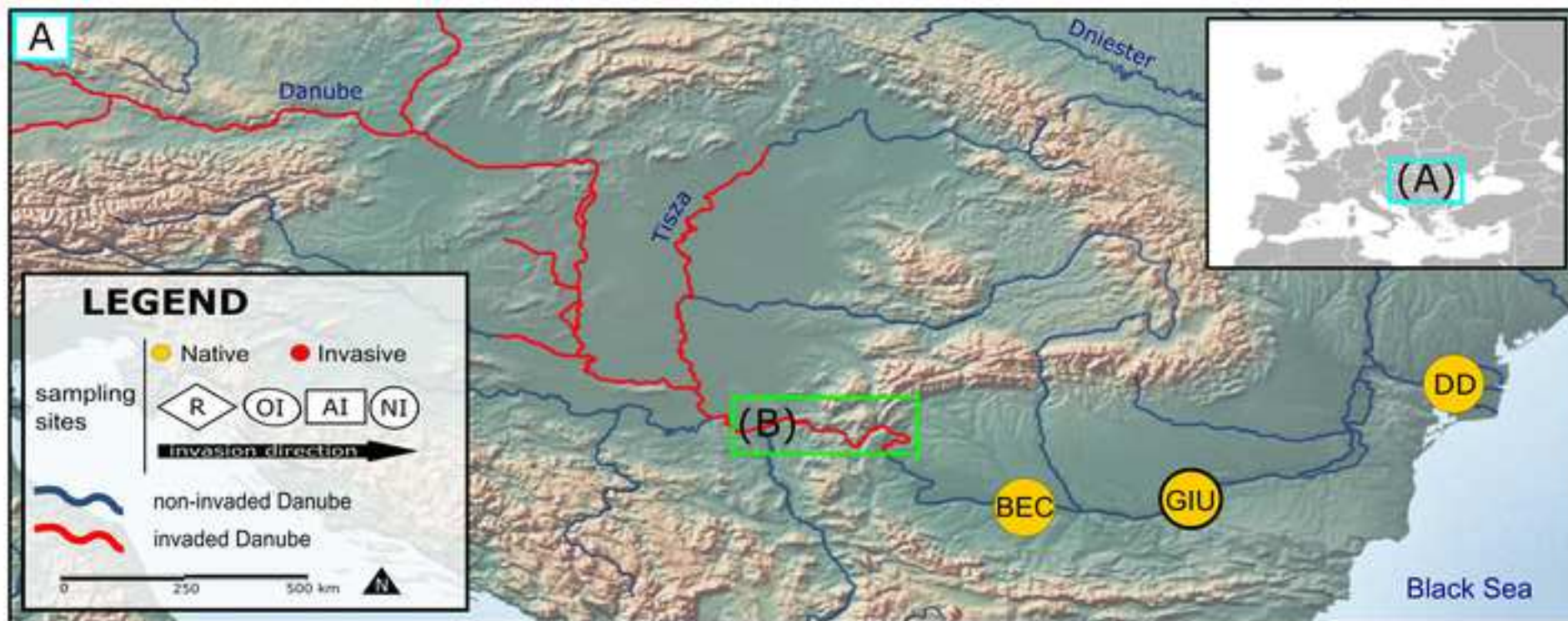
Variable	Native	Invasive
Total length	0.01	0.68
Cephalothorax length	0.05	0.56
Cephalothorax width	7.17***	1.19
Abdomen width	0.71	0.2
Left claw length	3.4*	0.01
Left dactylus length	9.63***	0.21
Left claw width	13.43***	0.46
Right claw length	3.66*	0.16
Right dactylus length	8.15***	0.29
Right claw width	25.28***	0.94
Mass without claws	0.55	0.003
Left chelae mass	18.62**	0.07
Right chelae mass	52.1***	0.07
Crayfish Condition Factor	2.8	7.09
Growth rate (RNA/DNA)	17.33***	0.29
Trophic position	34.8***	34.7***
Omnivory index	25.6**	41.9***
Elemental imbalance (C: N ratio)	33.6***	5.99

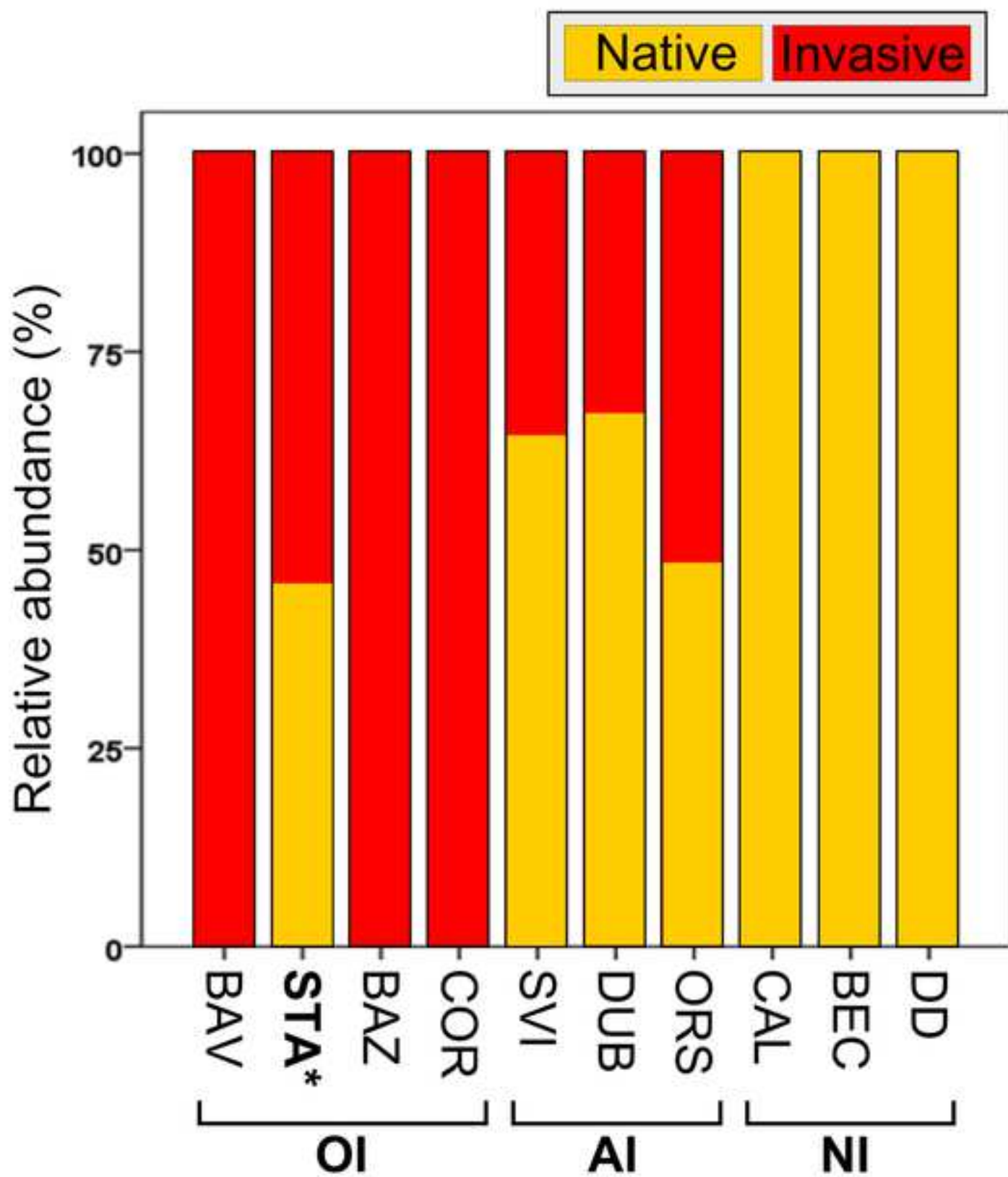
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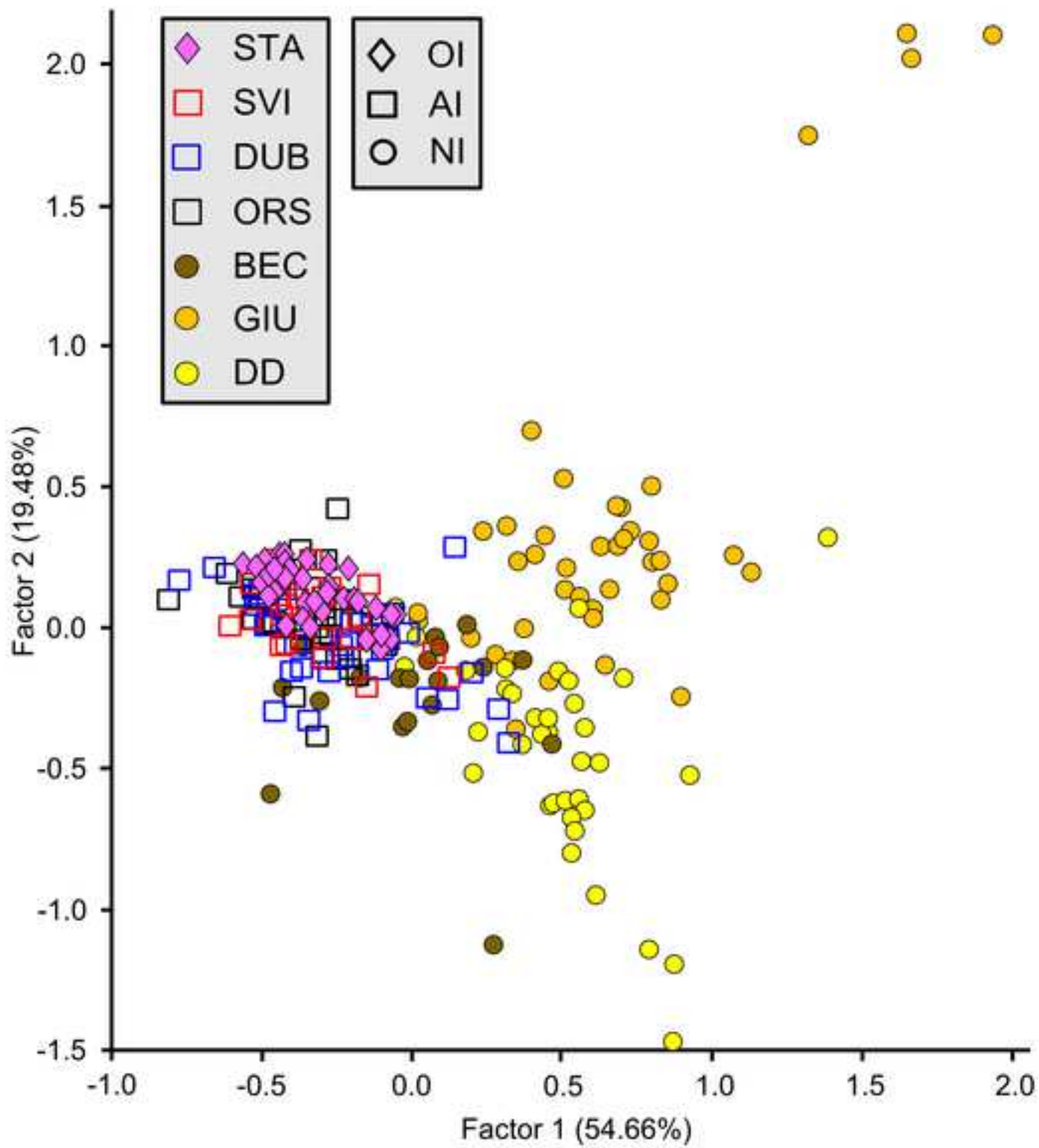
855 **Table 4.** Contingency table of posterior probability of Bayesian distribution of SEAb's
 856 (between brackets) for both species of crayfish and invasion sectors (expressed as area in
 857 trophic space, %²). The SEAb's were compared two by two for each possible combination of
 858 species x invasion sector and the significance P values stated.

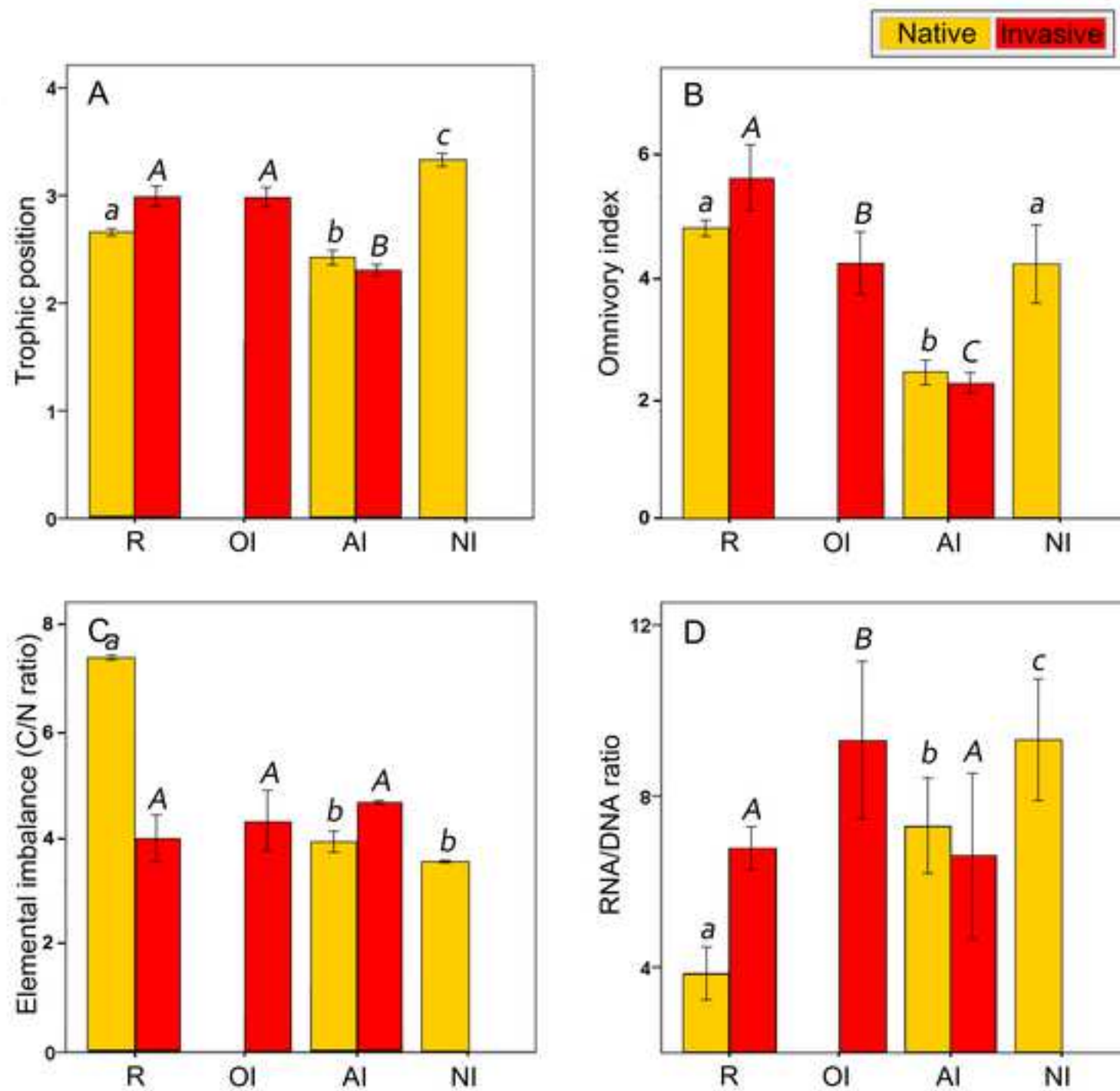
Taxa	Native Old- invaded	Native Active	Native Non- invaded	Invasive Old- invaded	Invasive Old- invaded	Invasive Active
Native Recovery (0.45)	-	<0.001	<0.001	<0.001	<0.001	<0.001
Native Active (3.39)	<0.001	-	<0.001	<0.001	0.13	<0.001
Native Non- invaded (1.68)	<0.001	<0.001	-	<0.001	<0.001	0.15
Invasive Recovery (0.96)	<0.001	<0.001	<0.001	-	0.85	<0.001
Invasive Old- invaded (4.12)	<0.001	0.13	<0.001	0.85	-	<0.001
Invasive Active (1.22)	<0.001	<0.001	0.15	<0.001	<0.001	-

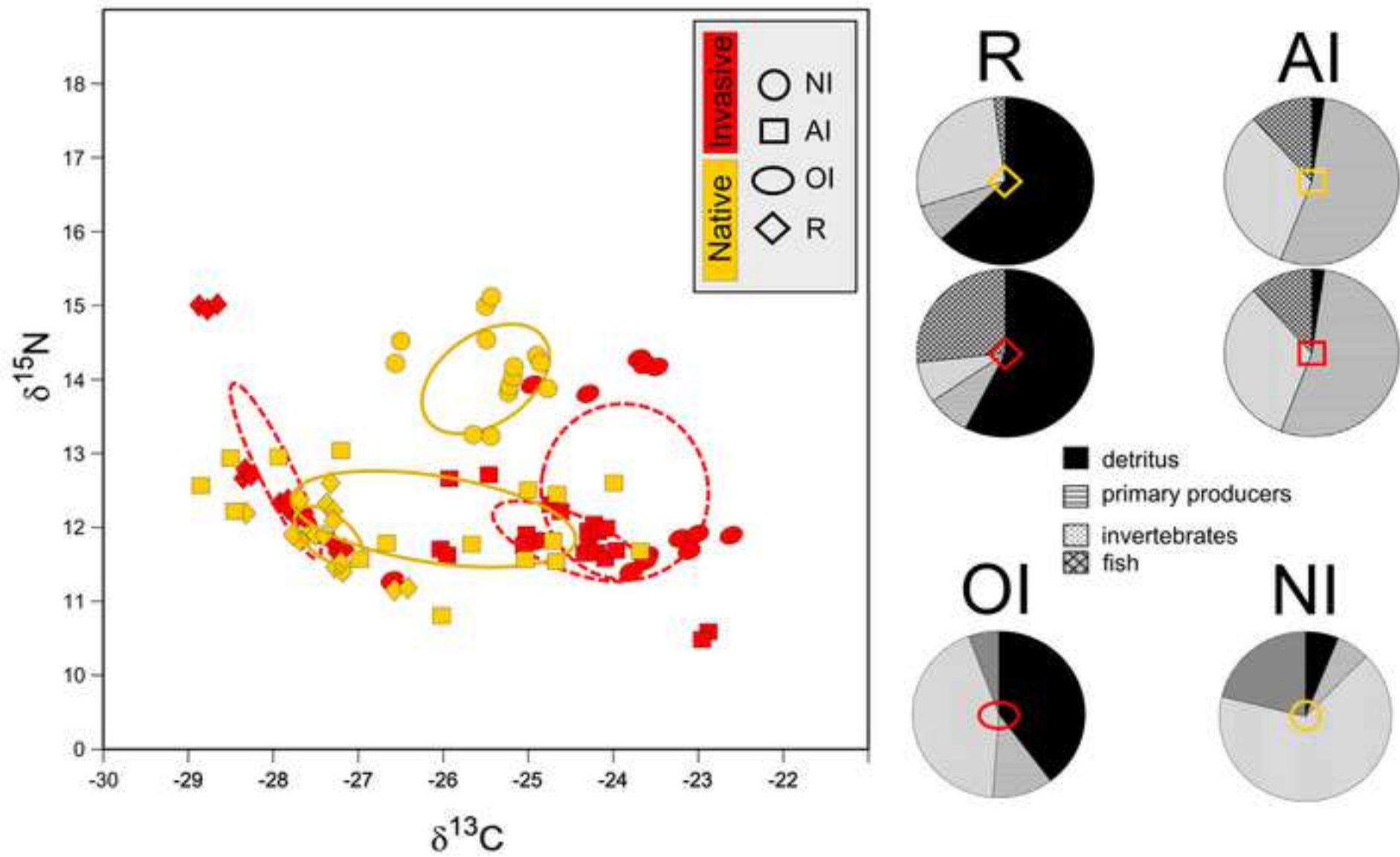
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