

CORRECTED PROOF

Research Article

The current status of *Pacifastacus leniusculus* (Dana, 1852) and their effect on aquatic macroinvertebrate communities in Hungarian watercourses

Mercédesz Ludányi^{1,*}, Edwin T.H.M. Peeters², Béla Kiss¹, Ákos Gáspár¹, Ivo Roessink³, Tibor Magura^{4,5} and Zoltán Müller¹

¹BioAqua Pro Ltd. 4032 Debrecen, Soó Rezső utca 21, Hungary

²Wageningen University and Research, Aquatic Ecology and Water Quality Management Group, 6700AA Wageningen PO Box 47, The Netherlands

³Wageningen Environmental Research, Environmental Risk Assessment group, 6700AA Wageningen PO Box 47, The Netherlands

⁴University of Debrecen, Faculty of Science and Technology, Department of Ecology, 4032 Debrecen, Egyetem tér 1, Hungary

⁵ELKH-DE Anthropocene Ecology Research Group, 4032 Debrecen, Egyetem tér 1, Hungary

*Corresponding author

E-mail: ludanyimercedesz@gmail.com

Citation: Ludányi M, Peeters ETHM, Kiss B, Gáspár Á, Roessink I, Magura T, Müller Z (2022) The current status of *Pacifastacus leniusculus* (Dana, 1852) and their effect on aquatic macroinvertebrate communities in Hungarian watercourses. *Aquatic Invasions* 17 (in press)

Received: 12 April 2021

Accepted: 16 April 2022

Published: 22 August 2022

Handling editor: Kęstutis Arbačiauskas

Thematic editor: Elena Tricarico

Copyright: © Ludányi et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0).

OPEN ACCESS

Abstract

The freshwater crayfish *Pacifastacus leniusculus* is among the most widespread invasive crayfish species in Europe. *Pacifastacus leniusculus* invaded Hungary around 1998 and here we investigated the recent expansion of this species and its impact on other aquatic macroinvertebrates. The colonization of watercourses throughout Europe by the signal crayfish resulted in negative impacts on the present aquatic communities. Our investigation (i.e. in Rába, Pinka, Gyöngyös-stream, Répce, Arany-stream and Strém systems) revealed that the distribution range of signal crayfish is still in expansion in the western part of Hungary and in all likelihood impacting the aquatic communities in these watercourses. Our results obviously demonstrated that signal crayfish densities were highest in habitats with gravel or coarse particulate organic matter, which seems to reflect a species-specific habitat preference. Our investigation proved that the presence of *P. leniusculus* had negative effects on a number of protected species such as *Calopteryx virgo* and *Onychogomphus forcipatus* next to a significant negative effect on the Odonata and Trichoptera species richness as well as on the abundances of Ephemeroptera, Odonata and Trichoptera. Our study in Hungary supports the notion of the significant negative impact of signal crayfish on native freshwater invertebrate communities throughout Europe. In order to assess whether these impacts are restricted to fast flowing waters only, an adequate monitoring plan providing more knowledge on this species with respect to biotic and abiotic preferences and aquatic macroinvertebrate composition is required.

Key words: habitat preferences, signal crayfish, invasion, impact on aquatic invertebrates, protected macrofauna species

Introduction

Since 1959, *Pacifastacus leniusculus* has been introduced to over 20 European countries (Kouba et al. 2014) and its range expansion is the widest among invasive crayfish in Europe (Chucholl 2016; Ruokonen et al. 2018). The first observation of *P. leniusculus* in Hungary was made in 1998 in the western part of the country, e.g Gyöngyös-stream (Kovács et al. 2005, Puky and Schád 2006). The species arrived in Hungary most probably through

the Rába river from Austria (Bódis et al. 2012; Ludányi et al. 2016) where the species had a widespread abundance (Weinländer and Füreder 2009).

Crayfish species, like *P. leniusculus*, affect their physical and chemical environment (Reynolds and Souty-Grosset 2012; Freeland-Riggert et al. 2016). They modify abiotic conditions through either bioturbation (Albertson and Daniels 2016; Lodge et al. 2012; Early et al. 2016) and burrowing (Holdich et al. 2009; Pöckl 1999; Kholodkevich et al. 2005). As signal crayfish feed on plants, detritus, zoobenthos, fish, and other crayfish (Guan and Wiles 1998; Crawford et al. 2006; Twardochleb et al. 2013; James et al. 2015), their effects may occur at several levels of the aquatic ecosystem through predation and competition.

The most recent publication on the distribution of this species in Hungary contains data for the period up to 2015 (Ludányi et al. 2016) and according to this investigation the species mainly occurred in calcic, upland-hilly, medium and large sized watercourses in the western part of the country. That study also mentioned that signal crayfish prefer habitats where current velocity is between 0.3–0.4 ms⁻¹ (Ludányi et al. 2016). As *P. leniusculus* continues to colonize many watercourses, negative effects on aquatic macroinvertebrate communities are also expected as has been observed in the rivers Gwash, Chater and Clyde in the UK after colonization by this species (Mathers et al. 2018; Crawford et al. 2006). Turley et al. (2017) and Ruokonen et al. (2018) also revealed that *P. leniusculus* can negatively impact the species richness of other aquatic macroinvertebrates. Usually, invasive crayfish impact macroinvertebrate communities via direct predation (Nyström et al. 2001; Jackson et al. 2014; James et al. 2014) and indirectly through destruction of habitats (Griffiths et al. 2004). To what extent these effects also occur in Hungarian watercourses is still unknown, which is troublesome as observations by BioAqua Pro Ltd. show that the signal crayfish occurs in locations where many protected macroinvertebrate species used to occur (BioAqua Pro Ltd. database).

In order to investigate the potential impact of *P. leniusculus* on aquatic macroinvertebrates and protected species in particular we analysed both their occurrences in time and space using the database of BioAqua Pro Ltd. During different projects in Hungary in the last decades, macroinvertebrate data were collected and stored in the BioAqua Pro Ltd database and this information allowed us to: i) update the distribution area of the invasive freshwater crayfish *P. leniusculus* in Hungary, ii) to determine the relationship between crayfish abundance and abiotic and biotic conditions and iii) to evaluate possible threats of *P. leniusculus* to aquatic macroinvertebrate communities in general and to protected species in Hungary specifically.

Materials and methods

Data sets

The dataset of BioAqua Pro Ltd contained 378 sampling events at 137 locations throughout the western part of Hungary since 1993. We selected

macroinvertebrate samples from different water types ranging from calcic to siliceous bedrock, upland-hilly and lowland waters, and varying in size from very small to very large. These features have been derived from the Hungarian river basin management plan (<http://www2.vizeink.hu/>). From this database, we selected abiotic and biotic features of the sampling location, the presence or absence of *P. leniusculus* and macroinvertebrate abundance data. Out of the 137 locations, 81 comprised calcic, upland-hilly medium sized watercourses, 47 locations comprised calcic, upland-hilly large sized watercourses and 9 locations comprised upland-hilly siliceous small watercourses. We investigated 18 different abiotic and biotic habitat types based on the protocol of AQEM consortium (2002).

Sampling

For our analyses, we used both quantitative and qualitative macroinvertebrate samples that have been collected since 2005 by BioAqua Pro Ltd. The quantitative aquatic macroinvertebrate samples were taken by the company in line with the protocol of National Biodiversity Monitoring System, which is in fact a “multihabitat sampling” method (Juhász et al. 2009; Cheshmedijev et al. 2011; Nieuwenhuis 2005). In short, prior to the sampling we determined the relative abundance of the different microhabitats and sampled different microhabitats in proportion to their presence at the sampling location and determined prior to the sampling. We applied the “kick and sweep” technique (Juhász et al. 2009; Letovsky et al. 2012) with a standard dip net (with a 950 µm mesh fabric and a 25 × 25 cm metal frame) to collect the animals. Every location contained three independent samples (= 3 sections). Relative abundances of the collected species were expressed as the average of their abundance values in the three sections.

A standard hand net was used to collect qualitative samples, but not taking into account the size of the sampled area, thus only a species list was generated with presence and absence data.

In every case, when quantitative samples were taken, information was recorded on a field form about the abiotic and biotic habitat composition (Table 1), water depth, water velocity. The following taxonomic groups were collected from the sampled material for further identification up to the species level in the laboratory: Hirudinea, Ephemeroptera, Gastropoda, Bivalvia, Trichoptera, Coleoptera, Heteroptera, Plecoptera, Odonata, and Malacostraca. The bigger sized animals (native crayfishes, bivalves, snails) were identified in the field and returned to the water, while smaller animals were classified in the field into groups and bottles containing 70% ethanol. These invertebrates were identified in the laboratory using a Nikon SMZ 1000 microscope.

Data analysis

We used Quantum GIS 2.18 software to map the observations of *P. leniusculus* in the three different water types mentioned above. To visualize the expansion

Table 1. The investigated habitat types and their explanation.

| habitat type | habitat subtype | explanation | size fractions |
|------------------|--------------------------------------|--|------------------|
| abiotic habitats | hydropetric | bedrock outcrop | |
| | megalithal | large cobbles, boulders, blocks, bedrock | > 40 cm |
| | natural macrolithal | coarse blocks, head-sized cobbles | > 20–40 cm |
| | artificial macrolithic constructions | coarse blocks, head-sized cobbles | > 20–40 cm |
| | mezolithal | fist to hand-sized cobbles | > 6–20 cm |
| | microlithal | coarse gravel – size of a pigeon egg to child’s fist | > 2 cm–6 cm |
| | akal | fine to medium-sized gravel | > 2 mm–2 cm |
| | psammal / psammopelal | sand / sand with mud | > 6 μ m–2 mm |
| | argyllal | silt, loam, clay | < 6 μ m |
| biotic habitats | macro-algae | | |
| | micro-algae | | |
| | submerged macrophytes | | |
| | emergent macrophytes | | |
| | living parts of terrestrial plants | roots | |
| | xylal | deadwood, (roots, branches) | |
| | CPOM | coarse particles organic matter | |
| | FPOM | fine particles of organic matter | |
| | debris | mussel and snail shells | |

of the species, we compared the observations from 2015 onwards with those reported earlier (Ludányi et al. 2016).

We applied stepwise linear regression with stepwise selection (combination of forward and backward selections) using the *MASS* packages (Venables and Ripley 2002) to find the best model by AIC, predicting the relationship between the crayfish relative abundance and the abiotic and biotic habitat variables (cover of artificial macrolithic constructions, microlithal, akal, psammal, argyllal, xylal elements, CPOM and FPOM). The response variable (relative abundance of the *P. leniusculus*) was log transformed to achieve normality. In the generalized linear models (GLMs) sampling year was entered as a random factor. For this analysis we had the quantitative sampling data of *P. leniusculus* 29 sections of 15 locations from 8 years which means that we used only habitat information of those sections where *P. leniusculus* occurred to assess the habitat parameters affecting the abundance of the species. Visualizing significant relationships *jtools* package (Long 2020) was used.

We used Generalized Linear Models (GLMs) to analyse the impact of the presence of *P. leniusculus* on the relative abundance of the protected species, as well as on the richness and the relative abundance of the selected taxonomic groups (Bivalvia, Coleoptera, Ephemeroptera, Gastropoda, Heteroptera, Hirudinea, Malacostraca, Odonata, Plecoptera and Trichoptera) using the *lme4* package (Bates et al. 2015). Prior the analyses the distribution that best fits the data were identified using the *car* (Fox and Weisberg 2019) and the *MASS* packages (Venables and Ripley 2002). Relative abundance was followed a normal distribution, while richness was followed a Poisson distribution. We used a log link function and standard linear predictor in the case of Poisson distribution. Models on richness with overdispersed data, where greater variability (statistical dispersion) was

expected based on the model (models on overall richness, as well as on richness of the Ephemeroptera and the Trichoptera), were refitted using the sample location as a random factor, as proposed by Harrison (2014). Because of the multiple tests on the same set of data on the richness and the relative abundance of the selected taxonomic groups, we applied Bonferroni correction for the significance values (Dunn 1961). For these analyses data from 13 sampling locations of two upland-hilly, medium sized watercourses that differed in the presence of the crayfish were used (eight locations from the invaded Gyöngyös and five from the signal crayfish-free Kerca with a total of 104 sampling events). In all the models, sampling year was treated as a random factor. All analyses were completed in the R programming environment (R Core Team 2017).

Results

Distribution of P. leniusculus

Our data showed that *P. leniusculus* has moved further into Hungary since 2015 (Figure 1). *Pacifastacus leniusculus* occurred in 47 sampling locations corresponding to roughly 12.5% of all sampled locations and in at least 10 watercourses in western Hungary from July 2015 onwards. *P. leniusculus* had colonized a total of 20 sampling locations in 6 watercourses before 2015 (Ludányi et al. 2016) indicating that *P. leniusculus* has more than doubled its range in the last 5 years. The distribution pattern showed a clear spreading route from the river Rába towards the upper sections of the river Pinka. In addition, signal crayfish distribution in the Gyöngyös-stream, Répce, Arany-stream and Strém expanded from the upstream sections from Austria towards the downstream sections deeper into Hungary (Supplementary material Table S1).

Effect of habitat features

Our best model included four habitat variables (akal, psammal, CPOM and FPOM) and predicted a significant positive correlation between akal cover (%) and crayfish relative abundance ($t = 2.576$, $df = 13$, $p < 0.01$) and between CPOM cover (%) and crayfish relative abundance ($t = 3.614$, $df = 13$, $p < 0.001$) (Table 2, Figures 2, 3). The relationship between the crayfish relative abundance and psammal and FPOM cover was not significant.

Impact on protected species

In order to assess the potential impact of *P. leniusculus* on protected species, the occurrences of these species were compared between the invaded Gyöngyös-stream and the non-invaded Kerca stream. Despite the invasion of *P. leniusculus*, the Gyöngyös-stream still harboured many protected species comprising *Agneta elegantula*, *Aquarius najas*, *Calopteryx virgo*, *Cordulegaster bidentata*, *Cordulegaster heros*, *Gomphus vulgatissimus*,

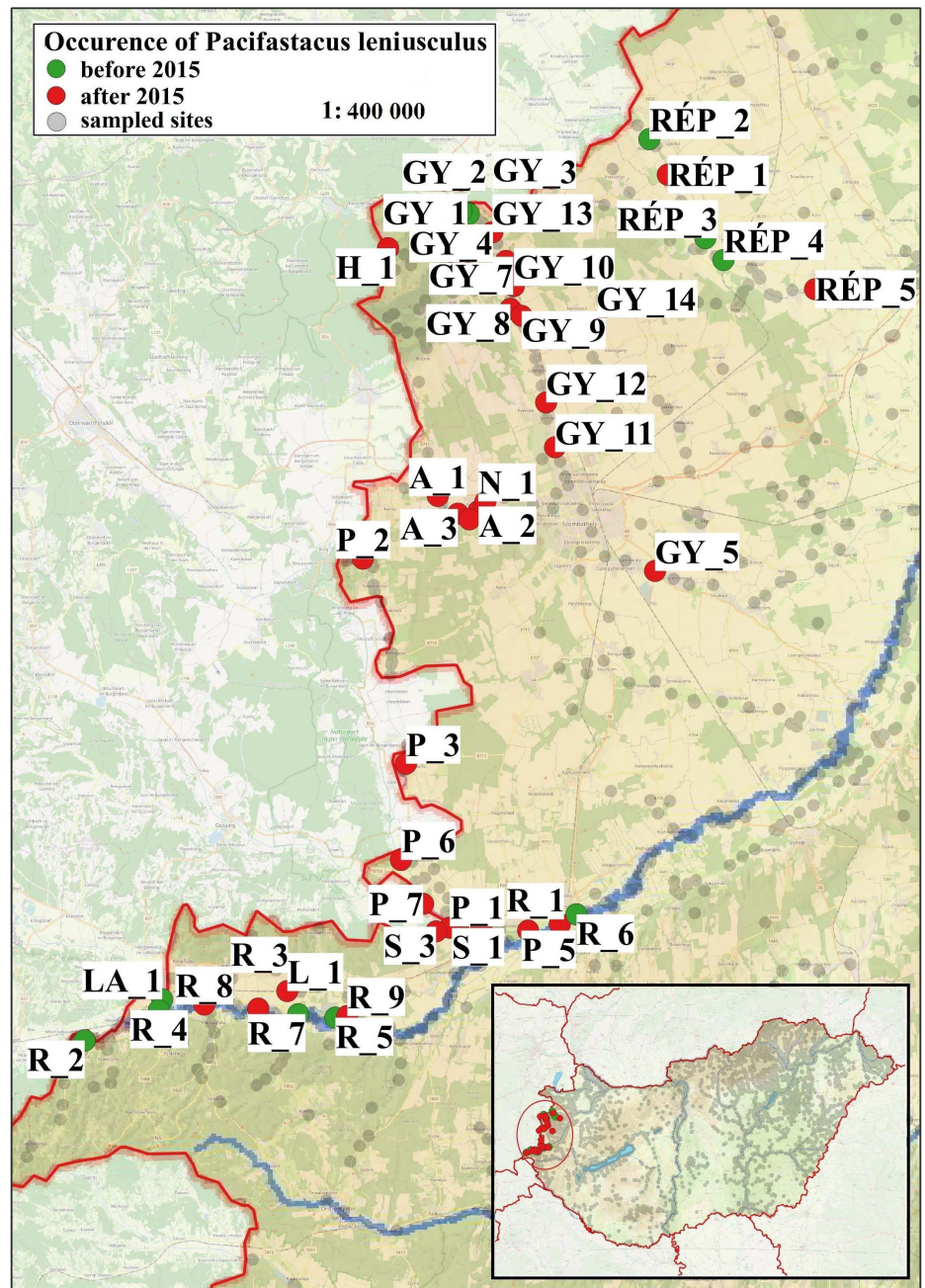


Figure 1. Distribution of *P. leniusculus* before and after 2015.

Table 2. The results of the best model by AIC predicting the relationship between the *P. leniusculus* relative abundance (individuals/m²) and habitat features based on 29 samples

| Habitat feature | Estimate | SE | df | t-value | p-value | |
|--------------------------|--|---------|--------|---------|---------|--------|
| (Intercept) | 1.1305 | 0.2990 | 25 | 3.781 | 0.0009 | |
| Abiotic habitat features | > 2 mm–2 cm gravel (akal) | 0.0143 | 0.0062 | 25 | 2.305 | 0.0297 |
| | > 6 µm–2 mm sand with mud (psammal) | –0.0105 | 0.0065 | 25 | –1.601 | 0.1218 |
| Biotic habitat feature | coarse particle of organic matter (CPOM) | 0.0241 | 0.0078 | 25 | 3.082 | 0.0050 |

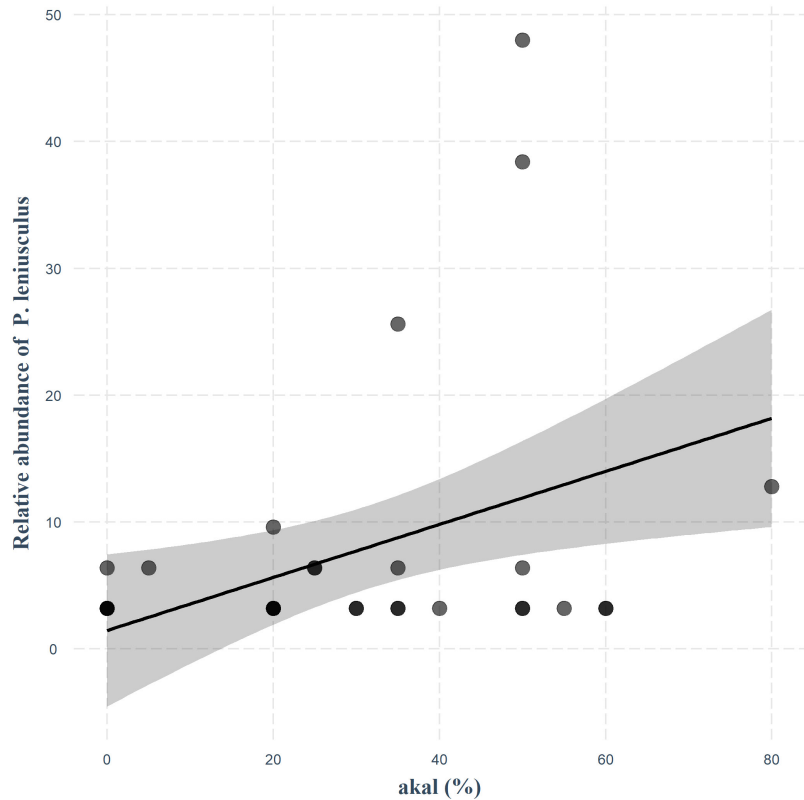


Figure 2. The relative abundance of *P. leniusculus* in proportion of akal cover (grey area: confidence interval; black line: direction of the relationship between the variables; y-axis scale is individuals/m²).

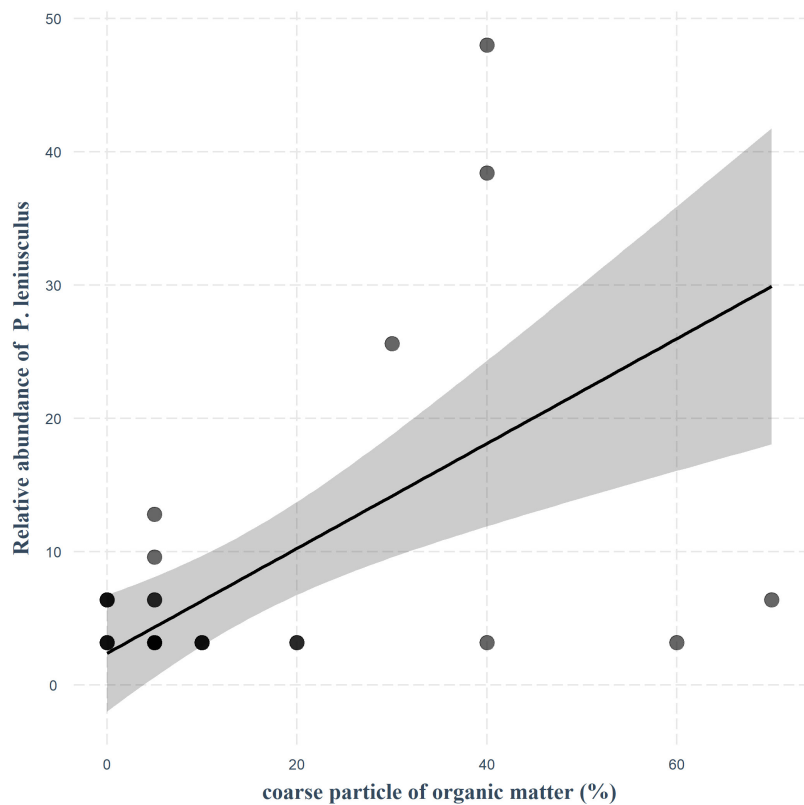


Figure 3. The relative abundance of *P. leniusculus* in proportion of CPOM cover (grey area: confidence interval; black line: direction of the relationship between the variables; y-axis scale is individuals/m²).

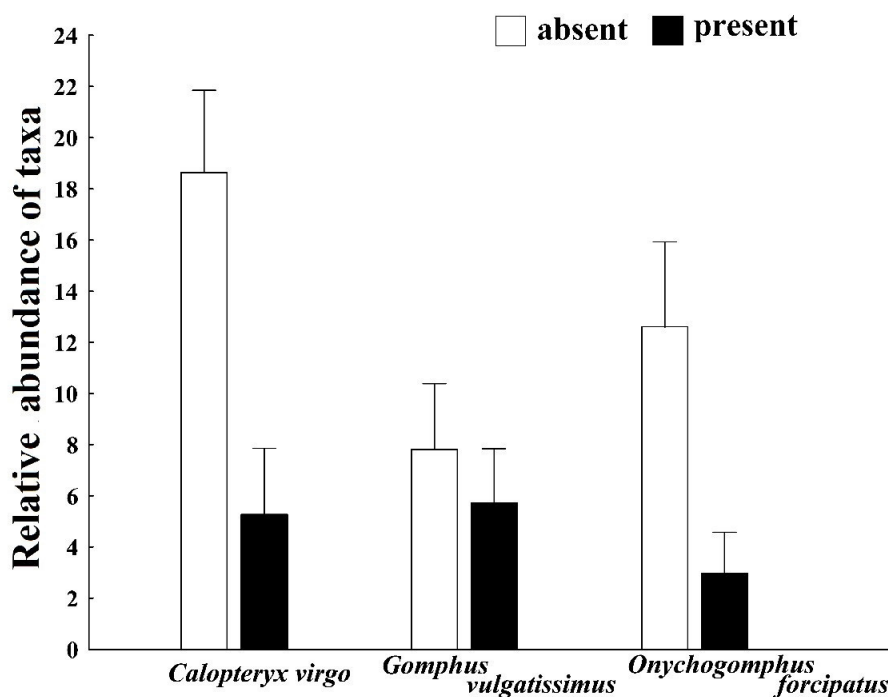


Figure 4. Relative abundance of *C. virgo*, *G. vulgatissimus* and *O. forcipatus* in the presence of *P. leniusculus* (\pm SE)).

Table 3. The results of generalized linear models regarding abundance of protected species (bold: significant differences $p < 0.05$, $n = 104$).

| Species | Estimate | SE | χ^2 | p |
|---------------------------------|----------------|--------------|---------------|-------------------|
| <i>Calopteryx virgo</i> | -14.470 | 3.866 | 14.007 | < 0.001 |
| <i>Gomphus vulgatissimus</i> | -0.395 | 2.129 | 0.0344 | 0.8528 |
| <i>Onychogomphus forcipatus</i> | -6.910 | 2.163 | 10.206 | 0.0014 |

Macronychus quadrituberculatus, *Oligoneuriella rhenana*, *Onychogomphus forcipatus*, *Ophiogomphus cecilia* and *Unio crassus*. In the Gyöngyös-stream concerned, the relative abundance of *Calopteryx virgo* ($2,94 \pm 1,08$ ind./m² \pm S.E.), *Gomphus vulgatissimus* ($4,32 \pm 1,66$ ind./m² \pm S.E.) and *Ophiogomphus cecilia* ($5,58 \pm 1,57$ ind./m² \pm S.E.) was the highest.

Calopteryx virgo, *C. heros*, *G. vulgatissimus*, *O. forcipatus*, *O. cecilia* and *U. crassus* occurred in the crayfish affected Gyöngyös and crayfish free Kerca as well. However, due to the low abundances of most of these protected species, a proper comparison was only possible for *C. virgo*, *G. vulgatissimus* and *O. forcipatus*. According to the GLM analysis, the relative abundance of *C. virgo* and *O. forcipatus* was significantly lower in the presence of *P. leniusculus*, while the relative abundance of *G. vulgatissimus* did not show significant relationship with the crayfish occurrence (Figure 4, Table 3).

Effect on other macroinvertebrates

The presence of crayfish had a significant, negative effect on the number of species of Odonata ($z = -4.669$, $n = 104$, $p < 0.0001$) and Trichoptera ($z = -5.368$, $n = 104$, $p < 0.0001$) and the total number of species ($z = -2.989$,

Table 4. The results of generalized linear models regarding number of taxa of main macroinvertebrate groups in the presence of the *P. leniusculus* (*: significant differences by Bonferroni correction, $n = 104$, the significance level is $0.05/11 = 0.0045$).

| Main taxonomic group | Estimate | SE | χ^2 | p |
|----------------------|----------|-------|----------|----------|
| Bivalvia | -0.781 | 0.615 | 1.6145 | 0.204 |
| Coleoptera | -0.779 | 0.429 | 3.2971 | 0.069 |
| Ephemeroptera | -1.392 | 0.589 | 5.5708 | 0.018 |
| Gastropoda | -1.139 | 0.633 | 3.2458 | 0.072 |
| Heteroptera | -0.636 | 0.474 | 1.7976 | 0.180 |
| Hirudinea | -0.802 | 0.485 | 2.738 | 0.098 |
| Malacostraca | -0.043 | 0.192 | 0.05 | 0.823 |
| Odonata | -0.883 | 0.189 | 21.796 | < 0.001* |
| Plecoptera | -0.146 | 0.315 | 0.2147 | 0.643 |
| Trichoptera | -2.292 | 0.427 | 28.817 | < 0.001* |
| Total | -1.569 | 0.525 | 8.9353 | 0.003* |

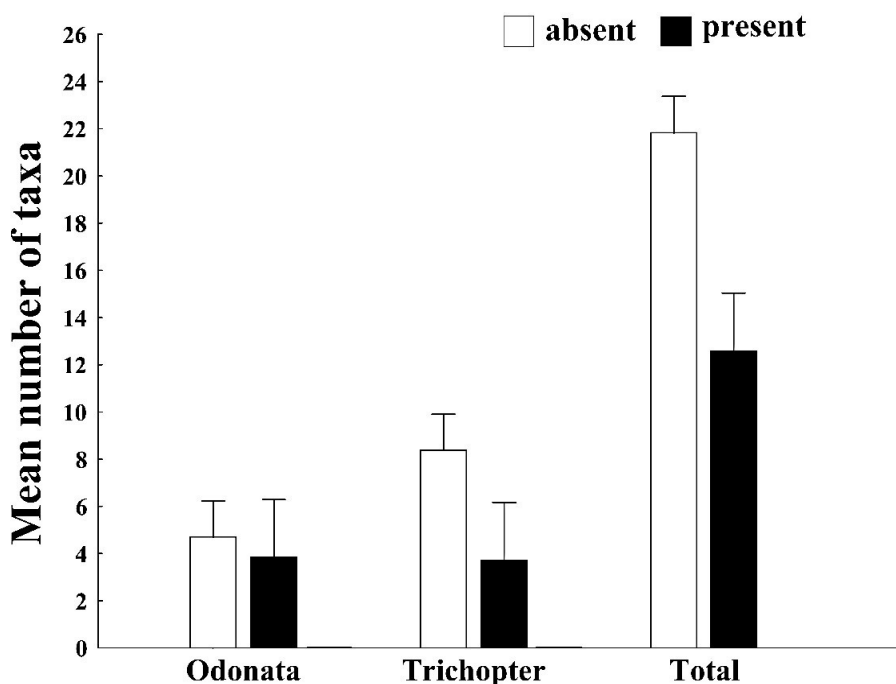


Figure 5. Mean number of taxa within Odonata, Trichoptera and total number of taxa in the absence/presence of *P. leniusculus* (\pm SE).

$n = 104$, $p < 0.001$) (Table 4, Figure 5). The relationship between crayfish presents and number of species of Bivalvia, Coleoptera, Gastropoda, Heteroptera, Hirudinea, Malacostraca and Plecoptera was not significant.

The presence of *P. leniusculus* resulted statistically significant declines in relative abundance of Ephemeroptera, Odonata, Trichoptera and total macroinvertebrate relative abundance. We did not find significant relationship between the crayfish present and the relative abundance of Bivalvia, Coleoptera, Gastropoda, Heteroptera, Malacostraca and Plecoptera (Table 5, Figure 6).

Discussion

Our analysis showed that the distribution area of *P. leniusculus* has expanded in Hungary since 2015 and this pattern is in line with observations in other

Table 5. The results of linear models regarding abundance of taxa of main macroinvertebrate groups (*: significant differences by Bonferroni correction, $n = 104$, the significance level is $0.05/11 = 0.0045$).

| Taxa | Estimate | SE | χ^2 | p |
|---------------|----------|--------|----------|----------|
| Bivalvia | -12.798 | 9.066 | 1.993 | 0.158 |
| Coleoptera | -5.727 | 2.318 | 6.104 | 0.014 |
| Ephemeroptera | -145.1 | 50.2 | 8.355 | 0.004* |
| Gastropoda | -1.01 | 0.542 | 3.473 | 0.063 |
| Heteroptera | 0.126 | 2.09 | 0.004 | 0.952 |
| Hirudinea | -3.683 | 1.629 | 5.112 | 0.024 |
| Malacostraca | -167 | 164.9 | 1.026 | 0.311 |
| Odonata | -38.01 | 9.19 | 17.068 | < 0.001* |
| Plecoptera | -2.915 | 14.773 | 0.039 | 0.844 |
| Trichoptera | -268.94 | 64.09 | 17.608 | < 0.001* |
| Total | -639.2 | 206.3+ | 9.604 | 0.002* |

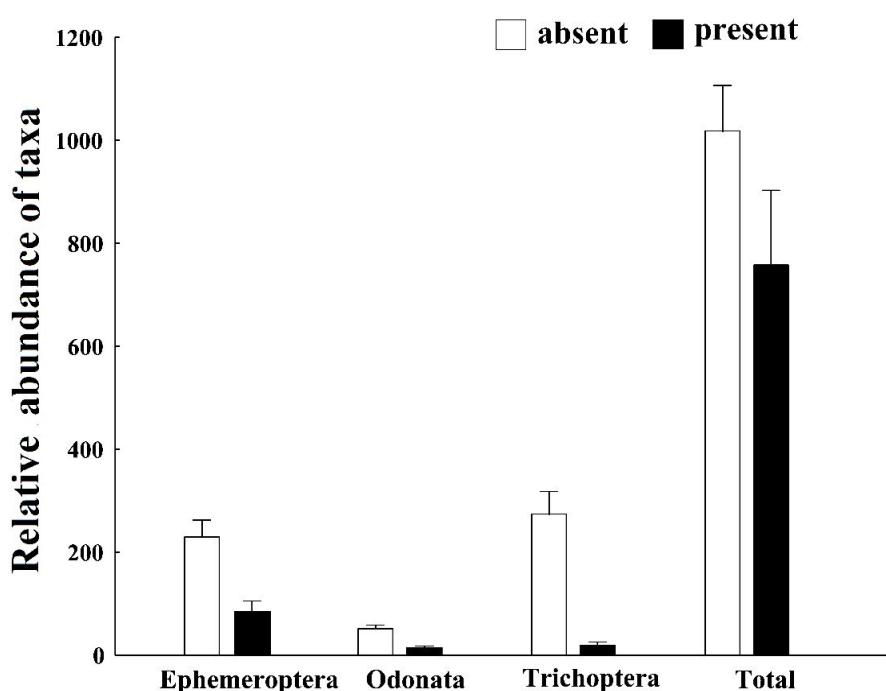


Figure 6. Relative abundance of Ephemeroptera, Odonata, Trichoptera and abundance of all taxa in the presence of *P. leniusculus* (\pm SE).

European countries like the Czech Republic, Austria and Latvia (Kouba et al. 2014; Hudina et al. 2017). Although the distribution area is still confined to the western part of the country, it is constantly expanding towards the Rába river and its tributaries (e.g. Pinka, Gyöngyös-stream). In addition, *P. leniusculus* was also found in the upper section of Danube and in its tributary, called Lajta (Weiperth et al. 2020). There are numerous reasons for the fast spreading of *P. leniusculus*, but one of the main reasons for the fast expansion is probably the human-mediated stocking (Weinländer and Füreder 2009). If we take into account this fact, detailed action plans and cross-border management strategies will be needed which should reduce the impact of this species on the native aquatic community (Jussila and Edsman 2020).



Figure 7. The typical habitats of *P. leniusculus* in Gyöngyös-stream (A) and Strém (B).

The *P. leniusculus* populations originate from large, low-altitude rivers and show intolerance to streams with steeper slopes and higher water velocity. These kind of water types contains fine particulate fragments, like sand or mud and assume a higher amount of organic matter accumulation (Larson et al. 2012), like in Gyöngyös-stream and Strém (Figure 7). In parallel with this result, our analysis showed that the typical abiotic habitat consisted of akal (gravel of size 2–20 mm) and coarse particulate organic matter (from leaves and branches). Vedia et al. (2013) found that the abundance of *P. leniusculus* had a positive relationship with vegetation cover and with boulders. Remarkably, literature on habitat requirements for *P. leniusculus* is not unambiguous and this may be due to regional differences in their distribution area, like differences in topography, slope

features, bedrock properties and habitat composition. Our analyses also showed a relationship between the coarse particulate organic matter cover and *P. leniusculus* relative abundance. Accumulation of CPOM in watercourses automatically leads to higher food availability for aquatic macroinvertebrates feeding on this organic matter. Since macroinvertebrates are prey items for crayfish and crayfish may feed on organic matter (Doherty-Bone et al. 2018), this may be the reason for the positive correlation of crayfish densities with particulate organic matter.

Our study showed that although the signal crayfish still co-occurred with several protected species in the invaded Gyöngyös-stream, larvae of *Calopteryx virgo* and *O. forcipatus* had higher densities in stream without signal crayfish present. In consequences, some of these protected species are threatened by the presence of this crayfish. As far as we know, there are no specific studies available on the effects of *P. leniusculus* on protected species including aquatic macroinvertebrates, except for other crayfish species, like *Austropotamobius pallipes* (Ibbotson and Furse 1995; Martín-Torrijos et al. 2019). However, in the case of other Decapoda species, the biggest threat is formed by the crayfish plague (Diéguez-Uribeondo et al. 1997), which is caused by the oomycete *Aphanomyces astaci* (Schikora, 1903) and invaders like *P. leniusculus* are resistant, but it is extremely lethal for the native crayfish species (Vaefßen and Hollert 2015; Ludányi et al. 2016). As we realized, the effects of *P. leniusculus* on other protected species lacks extensive investigation, but admittedly they can yield new, interesting results. The conservation of protected species and their habitats is a current issue and information about the risk factors may help to develop protection plans for national parks or environmental protection agencies.

In their study, Ludányi et al. (2016) found that *P. leniusculus* mainly occurred in fast flowing watercourses in the western part of the country and therefore the signal crayfish mostly affected macroinvertebrates associated with this type of water. This means that 12.5% of the investigated locations are suitable for *P. leniusculus* in Hungary. Our study clearly showed that the presence of *P. leniusculus* has impact on different macroinvertebrate groups which is consistent with other investigations (Moorhouse et al. 2014; Ruokonen et al. 2014; Ercoli et al. 2015). However, the identity of the most affected groups and species depends on the water type under study. In our study, Coleoptera, Gastropoda, Bivalvia, Heteroptera, Hirudinea, Plecoptera and Malacostraca (without Decapoda) were not significantly affected by the presence of crayfish while the *P. leniusculus* had effect on groups of Ephemeroptera, Odonata and Trichoptera. No effects on molluscs were found in our study and it seems to be in line with the laboratory findings of Rosewarne et al. (2016) who found that gastropods were the least preferred prey by the signal crayfish. Our study investigated the impact of crayfish in their outdoor environment where they could actively select their food. The observed absence of any effect on

snails may indeed indicate that other organisms are preferred prey instead. Interestingly, there are studies with pronounced effects of signal crayfish on snails (Olden et al. 2009; Twardochleb et al. 2013; Rosewarne et al 2016) and possibly the amount of available, alternative food items play a role here with hardly any effect on snails when other food items are abundant but with strong snail effect when other food items are scarce.

The number of species of the Odonata, Trichoptera and the total aquatic macroinvertebrate community were significantly lower in watercourse with crayfish, than in locations which have not been invaded yet. In our study, populations of Ephemeroptera and Trichoptera had statistically significant lower densities in the invaded watercourse. This result is completely in line with the study of Guan and Wiles (1998). The observed effect on the Ephemeroptera and Plecoptera taxa may be caused by the fact that these taxa also prefer habitats characterized by fine sediment accumulation and coarse organic matter. As a result, they strongly co-occur with the signal crayfish in these locations and thereby become a preferred food item. Nyström and Strand (1996) found that *P. leniusculus* selectively predate on sedentary taxa such as leeches (Hirudinea) and snails (Gastropoda).

Our study showed that signal crayfish *P. leniusculus* had a significantly negative impact on the native freshwater invertebrate community in western Hungary. Hence, it can be stated that signal crayfish form a real threat to Hungarian aquatic macroinvertebrate communities and threaten most of the aquatic macroinvertebrate groups which is manifested in a lower number of species and a lower species relative abundance. *P. leniusculus* is on the European list of Invasive Alien Species of Union concern (<https://eunis.eea.europa.eu/species/258987>) indicating the severeness of invasions. Yet, it seems that there is no control over the expansion of signal crayfish in Hungary. In order to assess whether these impacts are restricted to faster flowing waters, which means that surface water velocity is between 0.3 and 0.4 ms⁻¹ (Ludányi et al. 2016), only an adequate monitoring plan is required, providing more knowledge on this species with respect to biotic and abiotic preferences and aquatic macroinvertebrate composition is required. This way the most valuable aquatic habitats and communities can be protected with cross-border measures, by investigating the most affected watercourses, the occurrence of protected and non-protected aquatic species, with the main focus on the spread of the *P. leniusculus*.

Cooperation between nations could be the most effective way to address the issue of invasive species. An excellent practical example for this kind of protection can be found in Fennoscandia, where it came to fruition on different levels. The so-called controlled spreading of alien species, like *P. leniusculus*, would assure that the spreading of the species is restricted and controlled and thereby protect the native and protected aquatic invertebrate stocks through blocking the direct interactions between native species and alien crayfish species (Jussila and Edsman 2020). According to

the Swedish legislation, a possible way to attenuate the negative effect of *P. leniusculus* is the preservation of the native *Astacus astacus* populations (Jussila and Edsman 2020). As Jussila and Edsman (2020) also mentioned, Hungary has to count with the fact that alien species once established, will be nearly impossible to eradicate. In the Hungarian context, the monitoring of the affected watercourses and its tributaries and the installation of ecological barriers (Cowart et al. 2018; Krieg et al. 2021) and application of physical removal (Moorhouse et al. 2014) would be the best onset to impede the spreading of *P. leniusculus*.

In conclusion, further studies on how to stop the spreading of *P. leniusculus* and more knowledge on habitat usage and the effects of this species, especially on protected species may give clue information for management in the future.

Acknowledgements

We are grateful to all members of BioAqua Pro Ltd, who made samplings in the survey area. Our grateful thanks are also extended to Rita Odler who has provided linguistic reviews. We deeply acknowledge the comments of reviewers, which greatly improved the manuscript.

Funding declaration

The survey activity in the investigated region is supported by the projects in National Biodiversity Monitoring System since 2005, the monitoring of Natura2000 species since 2011 and the INTERREG V-A Austria-Hungary Programme (reg. number: ATHU077). Tibor Magura was supported by the OTKA K-131459 grant.

Authors' contribution

ML: research conceptualization, investigation and data collection, data analysis and interpretation, writing, review and editing; ETHMP and IR: data analysis and interpretation, review and editing; BK and ZM: research conceptualization, sample design and methodology, investigation and data collection, funding provision; ÁG: systematization of data, database organization; TM: data analysis and interpretation, writing – review and editing.

References

- Albertson LK, Daniels MD (2016) Effects of invasive crayfish on fine sediment accumulation, gravel movement, and macroinvertebrate communities. *Freshwater Science* 35: 644–653, <https://doi.org/10.1086/685860>
- AQEM consortium (2002) Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 1.0, February 2002. <http://www.aqem.de>
- Bates D, Mächler M, Bolker B, Walker S (2015) “Fitting Linear Mixed-Effects Models Using lme4”. *Journal of Statistical Software* 67: 1–48, <https://doi.org/10.18637/jss.v067.i01>
- Bódis E, Borza P, Potyó I, Puky M, Weiperth A, Guti G (2012) Invasive mollusc, crustacean, fish, and reptile species along the Hungarian section of the River Danube and some connected waters. *Acta zoologica Academiae Scientiarum Hungaricae* 58 (Suppl.): 29–45
- Cheshmedijev S, Soufi R, Vidinova Y, Tyufekchieva V, Ya-neva I, Uzunov Y, Varadinova E (2011) Multi-habitat sampling method for benthic macroinvertebrate communities in different river types in Bulgaria. *Water Research and Management* 1(3): 55–58
- Chucholl C (2016) The bad and the super-bad: prioritising the threat of six invasive alien to three imperilled native crayfishes. *Biological Invasions* 7: 1967–1988, <https://doi.org/10.1007/s10530-016-1141-2>
- Cowart DA, Breedveld KG, Ellis MJ, Hull JM, Larson ER (2018) Environmental DNA (eDNA) applications for the conservation of imperiled crayfish (Decapoda: Astacidea) through monitoring of invasive species barriers and relocated populations. *Journal of Crustacean Biology* 38: 257–266, <https://doi.org/10.1093/jcabi/ruy007>

- Crawford L, Yeomans W, Adams C (2006) The impact of introduced signal crayfish *Pacifastacus leniusculus* on stream invertebrate communities. *Aquatic Conservation Marine and Freshwater Ecosystems* 16: 611–621, <https://doi.org/10.1002/aqc.761>
- Diéguez-Urbeondo J, Temino C, Muzquiz JL (1997) The crayfish plague fungus (*Aphanomyces astaci*) in Spain. *Bulletin Français de Pisciculture* 347: 753–763, <https://doi.org/10.1051/kmae/1997051>
- Doherty-Bone TM, Dunn AM, Liddell C, Brown LE (2018) Transformation of detritus by a European native and two invasive alien freshwater decapods. *Biological Invasions* 20: 1799–1808, <https://doi.org/10.1007/s10530-018-1661-z>
- Dunn, Olive Jean (1961) “Multiple Comparisons Among Means”. *Journal of the American Statistical Association* 56: 52–64, <https://doi.org/10.1080/01621459.1961.10482090>
- Early R, Bradley B, Dukes J, Lawler JJ, Olden J, Blumenthal D, Gonzales P, Grosholz E, Ibañez I, Miller L, Sorte C, Tatem A (2016) Global threats from invasive alien species in the twenty-first century and national response capacities. *Nature Communications* 7: 12485, <https://doi.org/10.1038/ncomms12485>
- Ercoli F, Ruokonen T, Erkamo E, Jones R, Hämäläinen H (2015) Comparing the effects of introduced signal crayfish and native noble crayfish on the littoral invertebrate assemblages of boreal lakes. *Freshwater Science* 34: 555–563, <https://doi.org/10.1086/680517>
- Fox J, Weisberg S (2019) An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA, <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- Freeland-Riggert BT, Cairns SH, Poulton BC, Riggert CM (2016) Differences Found in the Macroinvertebrate Community Composition in the Presence or Absence of the Invasive Alien Crayfish, *Orconectes hylas*. *PLoS ONE* 11: e0150199, <https://doi.org/10.1371/journal.pone.0150199>
- Griffiths SW, Collen P, Armstrong JD (2004) Competition for shelter among over-wintering signal crayfish and juvenile Atlantic salmon. *Journal of Fish Biology* 65: 436–447, <https://doi.org/10.1111/j.0022-1112.2004.00460.x>
- Guan RZ, Wiles PR (1998) Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. *Aquaculture* 169: 177–193, [https://doi.org/10.1016/S0044-8486\(98\)00377-9](https://doi.org/10.1016/S0044-8486(98)00377-9)
- Harrison XA (2014) Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* 2: e616, <https://doi.org/10.7717/peerj.616>
- Holdich DMM, Reynolds JDD, Souty-Grosset C, Sibley PJJ (2009) A review of the ever-increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* 394–395: 11, <https://doi.org/10.1051/kmae/2009025>
- Hudina S, Kutleša P, Trgovčić S., Duplić A (2017) Dynamics of range expansion of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region in Croatia. *Aquatic Invasions* 12: 67–75, <https://doi.org/10.3391/ai.2017.12.1.07>
- Ibbotson AT, Furse MT (1995) Literature review of the ecology of the signal crayfish *Pacifastacus leniusculus* and its impacts upon the white clawed crayfish *Austropotamobius pallipes*. Institute of Freshwater Ecology, 31 pp
- Jackson MC, Jones TA, Milligan M, Sheath D, Taylor J, Ellis A, England J, Grey J (2014) Niche differentiation among invasive crayfish and their impact on ecosystem structure and functioning. *Freshwater Biology* 59: 1123–1135, <https://doi.org/10.1111/fwb.12333>
- James J., Slater F, Cable J (2014). A.L.I.E.N. databases: addressing the lack in establishment of non-natives databases. *Crustaceana* 87: 1192–1199, <https://doi.org/10.1163/15685403-00003329>
- James J, Slater FM, Vaughan IP, Young KA, Cable J (2015) Comparing the ecological impacts of native and invasive crayfish: could native species’ translocation do more harm than good? *Oecologia* 178: 309–316, <https://doi.org/10.1007/s00442-014-3195-0>
- Juhász P, Kiss B, Müller Z (2009) Protocol for sampling and assessment of aquatic macroinvertebrates within the framework of National Biodiversity Monitoring System. In: Nature Protection Information System, Central Protocol, Debrecen, Hungary, pp 17–21
- Jussila J, Edsman L (2020) Relaxed attitude towards spreading of alien crayfish species affects protection of native crayfish species: case studies and lessons learnt from a Fennoscandian viewpoint. *Freshwater Crayfish* 25: 39–46, <https://doi.org/10.5869/fc.2020.v25-1.039>
- Kholodkevich SV, Shumilova TE, Fedotov VP, Zhuravlev DA (2005) Effects of the *Astacus astacus* L. Population on Biomass of Macrophytes in a Fresh Water Body. *Russian Journal of Ecology* 36: 271–276, <https://doi.org/10.1007/s11184-005-0072-4>
- Kouba A, Petrusek A, Kozák P (2014) Continental-wide distribution of crayfish species in Europe: update and maps. *Knowledge and Management of Aquatic Ecosystems* 413: 05, <https://doi.org/10.1051/kmae/2014007>
- Kovács T, Juhász P, Ambrus A (2005) Data to the distribution of crayfish in Hungary (Decapoda: Astacidae, Cambaridae). *Folio Historico-Naturalia Musei Matraensis* 29: 85–89
- Krieg R, King A, Zenker A (2021) Barriers against invasive crayfish species in natural waters and fish passes - Practical experience. *Global Ecology and Conservation* 25: e01421, <https://doi.org/10.1016/j.gecco.2020.e01421>
- Larson ER, Abbott CL, Usio N, Azuma N, Wood KA, Herborg LM, Olden JD (2012) The signal crayfish is not a single species: cryptic diversity and invasions in the Pacific

- Northwest range of *Pacifastacus leniusculus*. *Freshwater Biology* 57: 1823–1838, <https://doi.org/10.1111/j.1365-2427.2012.02841.x>
- Letovsky E, Myers IE, Canepa A, McCabe DJ (2012). Differences between kick sampling techniques and short-term Hester-Dendy sampling for stream macroinvertebrates. *BioOne* 83: 47–55, <https://doi.org/10.1893/0005-3155-83.2.47>
- Lodge DM, Deines A, Gherardi F, Yeo DCJ, Arcella T, Baldrige AT, Barnes MA, Chadderton WL, Feder JL, Gantz CA, Howard GW, Jerde CL, Peters WB, Peters JA, Sargent WL, Turner CR, Wittmann ME, Zeng Y (2012) Global Introductions of Crayfishes: Evaluating the Impact of Species Invasions on Ecosystem Services. *Annual Review of Ecology, Evolution, and Systematics* 43: 449–472, <https://doi.org/10.1146/annurev-ecolsys-111511-103919>
- Long JA (2020) jtools: Analysis and Presentation of Social Scientific Data. <https://cran.r-project.org/package=jtools>
- Ludányi M, Peeters ETHM, Kiss B, Roessink I (2016) Distribution of crayfish species in Hungarian waters. *Global Ecology and Conservation* 8: 254–262, <https://doi.org/10.1016/j.gecco.2016.09.009>
- Martín-Torrijos L, Kokko H, Makkonen J, Jussila J, Diéguez-Urbeondo J (2019) Mapping 15 years of crayfish plague in the Iberian Peninsula: The impact of two invasive species on the endangered native crayfish. *PLoS ONE* 14: e0219223, <https://doi.org/10.1371/journal.pone.0219223>
- Mathers KL, Rice SP, Wood PJ (2018) Temporal variability in lotic macroinvertebrate communities associated with invasive signal crayfish (*Pacifastacus leniusculus*) activity levels and substrate character. *Biological Invasions* 20: 567–582, <https://doi.org/10.1007/s10530-017-1557-3>
- Moorhouse TP, Poole AE, Evans LC, Bradley DC, Macdonald DW (2014) Intensive removal of signal crayfish (*Pacifastacus leniusculus*) from rivers increases numbers and taxon richness of macroinvertebrate species. *Ecology and Evolution* 4: 494–504, <https://doi.org/10.1002/ece3.903>
- Nieuwenhuis R (2005) ECOSURV Manual for Sampling and Determination, Hungarian Ministry for Environment and Water, Budapest, pp 11–12
- Nyström P, Strand J (1996) Grazing by a native and an exotic crayfish on aquatic macrophytes. *Freshwater Biology* 36: 673–682, <https://doi.org/10.1046/j.1365-2427.1996.d01-508.x>
- Nyström P, Svensson O, Lardner B, Brönmark C, Granéli W (2001) The influence of multiple introduced predators on a littoral pond community. *Ecology* 82: 1023–1039, [https://doi.org/10.1890/0012-9658\(2001\)082\[1023:TIOMIP\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[1023:TIOMIP]2.0.CO;2)
- Olden JD, Larson ER, Mims MC (2009) Home-field advantage: native signal crayfish (*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese mystery snail (*Bellamya chinensis*). *Aquatic Ecology* 43: 1073, <https://doi.org/10.1007/s10452-009-9244-9>
- Pöckl M (1999) Distribution of crayfish species in Austria with special reference to introduced species. *Freshwater Crayfish* 12: 733–750
- Puky M, Schád P (2006) Distribution and conservation status of Decapod (Decapoda) species in Hungary. *Acta Biologica Debrecina-Supplementum Oecologica Hungarica* 14: 195–204
- Reynolds JD, Souty-Grosset C (2012) Management of fresh-water biodiversity: crayfish as bioindicators. Cambridge University Press, Cambridge, 384 pp, <https://doi.org/10.1017/CBO9781139031790>
- R Core Team (2017) R: A language and environment for statistical computing. - R Foundation for Statistical Computing: Vienna, Austria. <https://doi.org/http://www.R-project.org>
- Rosewarne PJ, Robert JG, Mortimer RJG, Newton RJ, Grocock C, Wing CD, Dunn AM (2016) Feeding behaviour, predatory functional responses and trophic interactions of the invasive Chinese mitten crab (*Eriocheir sinensis*) and signal crayfish (*Pacifastacus leniusculus*). *Freshwater Biology* 61: 426–443, <https://doi.org/10.1111/fwb.12717>
- Ruokonen TJ, Karjalainen J, Hämäläinen H (2014) Effects of an invasive crayfish on the littoral macroinvertebrates of large boreal lakes are habitat specific. *Freshwater Biology* 59: 12–25, <https://doi.org/10.1111/fwb.12242>
- Ruokonen JT, Sjövik R, Erkamo E, Tulonen J, Ercoli F, Kokko H, Jussila J (2018) Introduced alien signal crayfish (*Pacifastacus leniusculus*) in Finland – uncontrollable expansion despite numerous crayfisheries strategies. *Knowledge and Management of Aquatic Ecosystems* 419: 27, <https://doi.org/10.1051/kmae/2018016>
- Turley MD, Bilotta GS, Gasparrini A, Sera F, Mathers K, Humpheryes I, England J (2017) The effects of non-native signal crayfish (*Pacifastacus leniusculus*) on fine sediment and sediment-biomonitoring. *Science of the Total Environment* 601–602: 186–193, <https://doi.org/10.1016/j.scitotenv.2017.05.106>
- Twardochleb LA, Olden JD, Larson ER (2013) A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science* 32: 1367–1382, <https://doi.org/10.1899/12-203.1>
- Vaeßen S, Hollert H (2015) Impacts of the North American signal crayfish (*Pacifastacus leniusculus*) on European ecosystems. *Environmental Science Europe* 27: 33, <https://doi.org/10.1186/s12302-015-0065-2>

- Vedia I, Galicia D, Baquero E, Oscoz J, Miranda R (2013) Environmental factors influencing the distribution and abundance of the introduced signal crayfish in the north of Iberian Peninsula. *Marine and Freshwater Research* 68: 900–908, <https://doi.org/10.1071/MF16020>
- Venables WN, Ripley BD (2002) *Modern Applied Statistics with S*, Fourth edition. Springer, New York, USA, 498 pp, <https://doi.org/10.1007/978-0-387-21706-2>
- Weinländer M, Füreder L (2009) The continuing spread of *Pacifastacus leniusculus* in Carinthia (Austria). *Knowledge and Management of Aquatic Ecosystems* 394–395: 17, <https://doi.org/10.1051/kmae/20010011>
- Weiperth A, Kouba A, Csányi B, Danyik T, Farkas A, Gál B, Józsa V, Patoka J, Juhász V, Parvulescu L, Mozsár A, Seprós R, Staszny Á, Szajbert B, Ferincz Á (2020) Az idegenhonos tízlábú rákok (Crustacea: Decapoda) helyzete Magyarországon [The present status of non-native Decapod (Crustacea: Decapoda) species in Hungary]. *Tudomány-Science* 113(2): 61–69

Supplementary material

The following supplementary material is available for this article:

Table S1. Details of the distribution of *Pacifastacus leniusculus* in Hungarian watercourses.