Original paper

The first record of signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) and its projected expansion in Serbia under global climate change

Mladen HORVATOVIĆ*, Milica RANKOVIĆ, Dubravka MILIĆ, Branko MILJANOVIĆ, Aleksandar BAJIĆ

University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

Summary. The signal crayfish invasion through the Drava River towards the Danube and Serbia poses a significant threat to the indigenous astacofauna of the region. Our research aimed to determine whether it had already reached the Danube, and predict its future spread in Serbia. We recorded one specimen for the first time in the Serbian Danube at 1280 rkm. With the rate of 152 km in two years, the invasion was seemingly extremely fast. It seems likely that signal crayfish reached the Danube as early as 2014-2015 due to accelerated expansion through the Drava. Using Species Distribution Modeling, we predicted its potential distribution in Serbia until 2050 and 2070, based on climate change models. Scenarios show its expansion along the Danube and its tributaries, almost across Serbia, with the most substantial impact in the northern and central regions and slightly weaker in the south. In addition to already weakened indigenous *Pontastacus leptodactylus* and *Astacus astacus*, signal crayfish could reach habitats of so far isolated *Austropotamobius torrentium*. New waves of crayfish plague, combined with competition, could have a devastating impact on Serbian astacofauna and astacofauna of all Eastern Europe, the region with arguably the richest crayfish diversity in Europe.

Keywords: Danube, dispersal, native astacofauna, non-indigenous crayfish, Species Distribution Modeling.

INTRODUCTION

Indigenous crayfish species (ICS) in European inland waters are among the most endangered invertebrates, particularly threatened by the introduction and spread of non-indigenous crayfish species (NICS) (Holdich et al. 2009). Many European native crayfish populations entirely disappeared after the introduction of North American crayfish species in the XIX century (Lodge et al. 2000). Trends of population disappearance and decreasing ICS abundance still exists (Maguire et al. 2011). The leading cause is mostly not competition but the "crayfish plague", a disease transmitted by NICS native to North America (Alderman 1997). The causative agent is oomycete *Aphanomyces astaci* Schikora, 1906 to which American species are highly resistant, while for the European

ICS, it is deadly (Edgerton et al. 2004). Recent studies suggest that some ICS populations may develop a certain degree of resistance to crayfish plague (Kušar et al. 2013; Kokko et al. 2018). However, in the ecosystems from which ICS has disappeared or weakened, free ecological niches are soon occupied by NICS, making the return of ICS very difficult or impossible (Johnsen and Taugbol 2010).

After the first epidemic of the crayfish plague in Europe in 1860, the economic losses of the crayfish industry encouraged the introduction of alternative North American species resistant to the disease: spiny-cheek crayfish, *Faxonius limosus* (Rafinesque, 1817) in 1890; signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) in 1959; and red swamp crayfish, *Procambarus clarkii* (Girard, 1852) in 1973 (Weiperth et al. 2020). The practice continued until the 1980s, although they

E-mail: mladen.horvatovic@dbe.uns.ac.rs

have been shown to be permanent and latent carriers of the pathogen. Further introduction of NICS was banned from the late 1980s onwards (Edsman 2004) and the species mentioned above are classified as invasive (EU 2014, 2016). However, the spread of NICS in the Europe has continued, mostly by spontaneous dispersal in which watercourses played the most prominent role, especially the great European rivers (Johnsen and Taugbol 2010). Unfortunately, the human-mediated illegal introduction is still present, frequently due to releases of pet-traded individuals, kept for ornamental purposes (Weiperth et al. 2019). In 2009 ten NICS were recorded in Europe. At least one of them is present in most European countries (Holdich et al. 2009). There are only six described crayfish species native to Europe, all widely distributed (except endemic *Austropotamobius bihariensis* Pârvulescu, 2019 known to exist only in Romania). Much greater species diversity is expected in Eastern Europe, due to unresolved taxonomic problems of the genus *Pontastacus* Bott, 1950 (Pârvulescu 2019; Weiperth et al. 2020). That makes Eastern Europe particularly vulnerable to NICS expansion. Serbian freshwater ecosystems harbors three native crayfish species: narrow-clawed crayfish, *Pontastacus leptodactylus* (Eschscholtz, 1823) inhabits lowland rivers; noble crayfish, *Astacus astacus* (Linnaeus, 1758) only in unpolluted streams, rivers and lakes; and stone crayfish, *Austropotamobius torrentium* (Schrank, 1803) distributed in streams and small rivers at higher altitudes. Noble and stone crayfish are protected by national (SGRS 2016) and international legislation (Bern Convention Appendix III), while the spread of NICS highly endangers all three species.

Twentynine non-indigenous species of macroinvertebrates have been recorded in Serbian freshwater ecosystems so far. Most of them have been introduced by natural dispersal through the Danube River from neighbouring countries (Zorić et al. 2020b). So far, the presence of one NICS, *Faxonius limosus*, has been recorded in Serbian waters. The first record was in 2002 near the border with neighbouring Hungary (Karaman and Machino 2004). To date spinycheek crayfish has spread through the Serbian Danube and its tributaries (Zorić et al. 2020a) and continued expansion in Romania, towards the Danube delta (Pârvulescu et al. 2009, 2012; Todorov et al. 2020).

Pacifastacus leniusculus is considered the most successful crayfish invader and the greatest threat to the native astacofauna across Europe (Hudina et al. 2017; Svoboda et al. 2017; Dragičević et al. 2020). It has a substantial negative impact mainly due to the spread of the crayfish plague (Filipová et al. 2013). Considering its adaptability, fecundity, early maturation, considerable growth and aggressiveness, it also has a substantial advantage in direct competition with ICS (Westman et al. 2002; Hudina et al. 2013). The native range

of signal crayfish in the Northwestern USA and Southwestern Canada covers a broad range of habitats: small streams to large rivers and lakes, coastal brackish waters, lowland to sub-alpine regions (Holdich et al. 1997). Distribution in Europe comprises the same range of habitats (Souty-Grosset et al. 2006) which potentially allows it to come into contact with all European ICS. The signal crayfish is currently the most widespread NICS in Europe, registered in 30 European countries so far (Souty-Grosset et al. 2006; Kouba et al. 2014; Trožić-Borovac et al. 2019). Although this species has not yet been recorded in Serbia, its presence in neighbouring countries: Hungary (Illés 2001), Croatia (Maguire et al. 2008) and Bosnia and Herzegovina (Trožić-Borovac et al. 2019) indicates imminent emergence.

The signal crayfish was introduced into the Danube basin in 1970, when it was imported from California (USA) and released into several water bodies in Austria (Johnsen and Taugbol 2010). After reaching the Mura River, it easily spread through Austria and Slovenia due to its natural dispersal (Govedič 2006). In 2008 this species had reached Croatia through the Mura River (Maguire et al. 2008). Three years later, in 2011, it was recorded in the Drava River, 20 km downstream from the Mura River confluence with the Drava (Maguire et al. 2011). Its downstream dispersal rate in the Croatian part of the Mura River was 18-24.4 km/yr, among the highest in Europe (Hudina et al. 2009). In 2018 the signal crayfish were found in the Drava River only 50 km upstream from the confluence with the Danube (Dragičević et al. 2020) (Fig. 1). The downstream dispersal rate along 128 km of the Drava course was 21.3 km/yr. It was very high and in line with predictions of Hudina et al. from 2009. We can assume: if the dispersal rate remains at the same level, signal crayfish from the Drava will reach the Danube and Serbian waters probably in 2020-2021. The second direction of its invasion could be through the Sava River (Fig. 1), the largest right tributary of the Danube in Serbia. The species was illegally introduced into the Sava River system in 2012 in Korana River (Croatia) (Hudina et al. 2013) and in 2018 in Una River (Bosnia and Herzegovina) (Trožić-Borovac et al. 2019). So far, there is no information that signal crayfish reached the Sava River from mentioned tributaries. Although this will be a slower route, its downstream progression and consequently getting the Serbian waters can be expected soon.

Species Distribution Modeling (SDM) is one of the most commonly used techniques for modeling species' current and potential future distribution. It is widely used to determine the locations where invasive species are most likely to be found and dispersed (Peterson 2003; Andersen et al. 2004; Taucare-Ríos et al. 2016; etc.). Also, this technique has already been used to predict the potential global distribution for some invasive crayfish species, including *P. leniusculus*
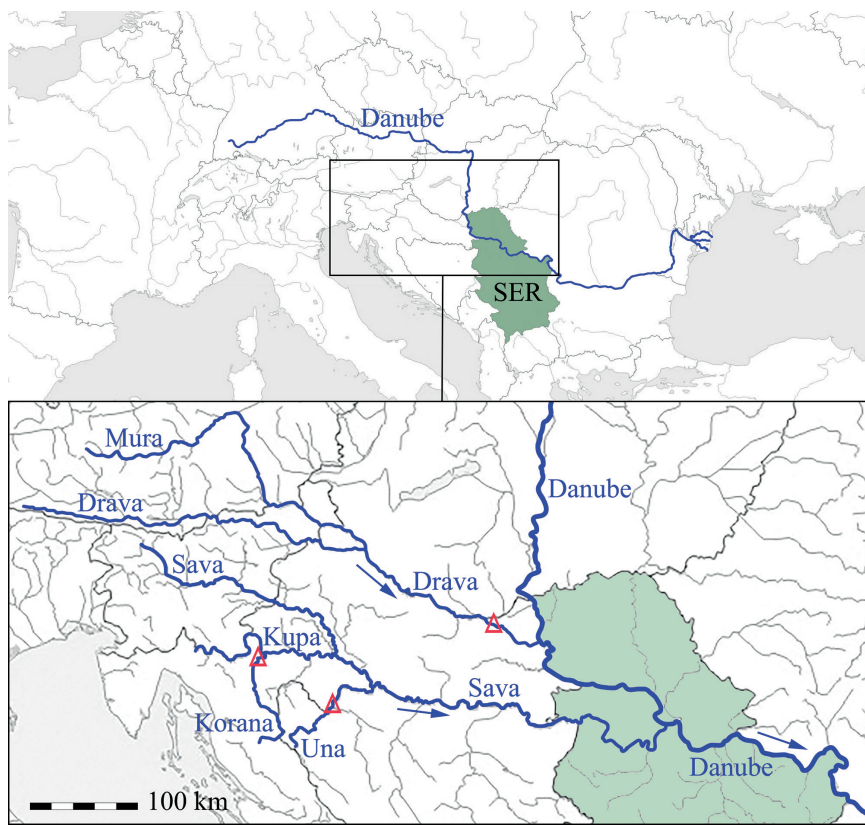
Fig. 1. Signal crayfish invasion routes towards the Danube and Serbia (SER). Red triangles indicate the sites of the last known records.

(Zhang et al. 2020). Given the significant threat that signal crayfish poses to native astacofauna throughout the region, information on possible dispersion routes and potential future distribution could be used to target invasion risk areas, help develop a prevention and management plan, monitor risk areas and eventually try to slow or stop further spread of signal crayfish in some watercourses.

Since *P. leniusculus* has not been recorded in Serbia, the first aim of our research was to determine whether it had already reached the Danube and consequently Serbian freshwater ecosystems. After finding we have given several assumptions to explain its swift progression through the Danube so far. Given the importance of our finding, the second aim of this paper was to provide the spread projection and future potential distribution under projected climate change of the *P. leniusculus* in Serbia. We also pointed out the significant threat that signal crayfish poses to the indigenous astacofauna in Serbia.

MATERIAL AND METHODS

Study area

The Danube is the second-longest river in Europe and the most important freshwater corridor connecting ten European countries, with its drainage extending into nine more.

It runs through much of Central and Southeastern Europe, and belongs to the Black Sea catchment area. The Danube enters Serbia from neighbouring Hungary at 1433 rkm. Its course through Serbia is 588 km long, of which the first 138 km makes the border between Croatia and Serbia. In that area, at 1382 rkm of the Danube course, the largest tributary from the territory of Croatia, the Drava River discharges into the Danube. In its course through Croatia, the Drava River has numerous floodgates for regulating occasional torrential waters, but it belongs to lowland rivers according to its hydromorfological characteristics. The Danube is a typical lowland river. A total of 92% of all watercourses in Serbia belong to the Danube basin.

Our surveys were conducted along the 37 km of the Danube course, upstream from the city of Novi Sad. Seven sites were investigated: (1) Novi Sad at 1262 rkm (45°13′17.20″N 19°47′33.90″E), (2) Beočinska ada at 1270 rkm (45°13′35.80″N 19°41′30.40″E), (3) Banoštor at 1275 rkm (45°13′06.80″N 19°38′09.30″E), (4) Koruška at 1280 rkm (45°12′43.40″N 19°34′16.60″E), (5) Susek at 1284 rkm (45°14′46.40″N 19°33′21.80″E), (6) Neštinska ada at 1289 rkm (45°13′49.20″N 19°30′27.80″E), and (7) Bačka Palanka at 1299 rkm (45°14′01.50″N 19°22′05.90″E) (Fig. 2).

Field sampling

Field surveys were conducted from April to December 2020, as part of regular hydrobiological monitoring of the Danube River in the south Bačka region, Vojvodina Province, Serbia. Investigated seven sites listed above have been examined more than once. Field research was of a general hydrobiological type, not exclusively dedicated to finding the crayfish. Therefore, no baited or shelter traps were used for sampling. Crayfish were caught by hands among the stones in shallow, by gillnets from the boat, and by electrofishing. Captured crayfish were determined to species and sexed. Indigenous crayfish were released at the capture point upon identification, while NICS specimens were removed from the water body and fixed in 70% ethanol. Some of the collected specimens were photographed. The material was deposited in the Zoological Collection of the Department of Biology and Ecology (ZZDBE), Faculty of Sciences, University of Novi Sad, Serbia. During field research the basic physical and chemical parameters of water were recorded: temperature, dissolved oxygen concentration, oxygen saturation, conductivity, pH value and water transparency.

Ministry of Environmental Protection, Mining and Spatial Planning, Republic of Serbia, approved the field research and issued the appropriate fishing permit for scientific research purposes.

Species distribution data used for SDM

Data on signal crayfish distribution in Croatia were taken from the literature sources (Hudina et al. 2009; Maguire et al. 2018; Dragičević et al. 2020) and data were provided by Ivana Maguire and Sandra Hudina (pers. comm. 2021). Data of the first record from Serbia can be found in the Results section. For the purpose of this study, all occurrence points were georeferenced.

Environmental variables (SDM)

Current bioclimatic data and future climate projections were obtained from the WorldClim database at 30 arc-seconds resolution (Hijmans et al. 2005). We derived the future projection of bioclimatic predictors from CNRM-CM5, IPSL-CM5A-LR and CCSM4 climate models. These models were three of the models that were used in the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (AR5) and the associated cycle of the fifth phase of the CMIP5 (IPCC 2014). For each future projection, we used two Representative Concentration Pathways (RCPs) - 2.6 and 8.5 for 2050 (averaged for 2041-2060) and 2070 (averaged
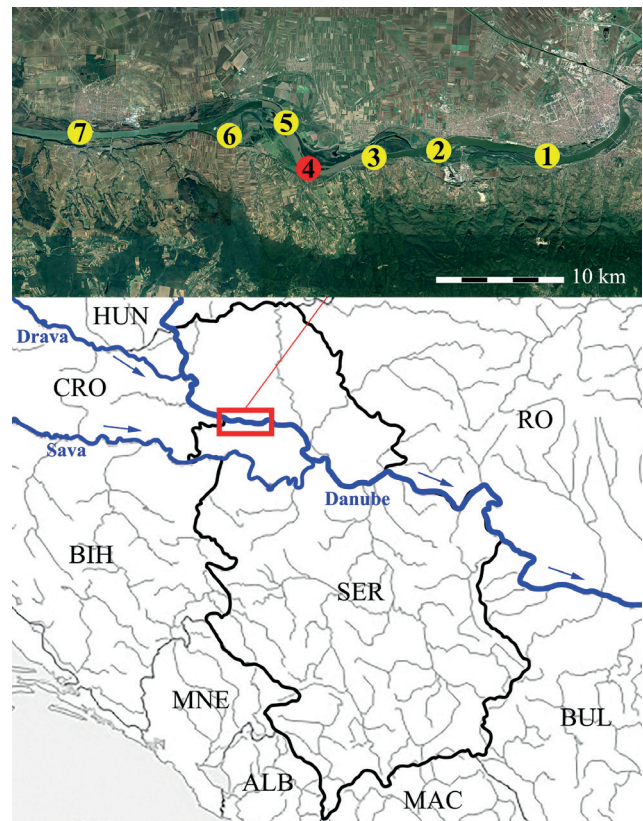
Fig. 2. Field research sites in 2020 on the Danube, upstream from the city of Novi Sad. 1 - Novi Sad, 2 - Beočinska ada, 3 - Banoštor, 4 - Koruška, 5 - Susek, 6 - Neštinska ada, 7 - Bačka Palanka. Red labeled number indicates the finding site of the first signal crayfish in Serbia (image source: https://earth.google.com [March 21, 2022]).

for 2061-2080) time frames (Van Vuuren et al. 2011). Each of these RCPs represents the possible trajectories for greenhouse gas emissions (Moss et al. 2008). For instance, RCP 2.6 presents the minimum greenhouse gas emission scenario; on the contrary, RCP 8.5 presents the maximum greenhouse gas emission scenario. Firstly, we perform a multicollinearity test on all 19 environmental variables using variance inflation factors (VIF) analysis in the R platform (R Core Team 2019). For this purpose, we used the USDM package (Naimi 2015). In the models were incorporated only remaining variables that had VIFs values under 5 (Mean Diurnal Range (BIO2), Max. Temperature of Warmest Month (BIO5), Mean Temperature of Driest Quarter (BIO9) and Precipitation of Warmest Quarter (BIO18)). All other environmental variables with high multicollinearity were removed.

Species Distribution Modeling procedure

To calculate the potential species distribution we used the Machine learning algorithm Maxent (Maximum entropy algorithm) using dismo R package (Hijmans et al.

2017). Maxent is one of the most popular and commonly used SDMs tools (Phillips and Dudik 2008; Warren and Seifert 2011), which uses only a presence data for modeling potential distribution. As a machine learning technique, it is based on the principle of maximum entropy. In order to approximate the potential geographic distribution, Maxent combines environmental variables and species occurrence (Phillips et al. 2006).

For selecting the best potential distribution model, the ENMeval package (Muscarella et al. 2014) in R Studio, is used to define the best combination of Maxent's feature classes and regularization multipliers. As a result of using this package, we get 48 different models, with all possible combinations of feature types (L, LQ, LQH, LQHP, LQHPT) and multipliers (from 0.5 to 4), along with the lowest Akaike's information criteria (AICc) values (ΔAICc = 0), to select the best model candidate (Burnham et al. 2004; Muscarella et al. 2014). We also used Kappa statistics and delta AICc to evaluate the best model candidate prediction. Model performance was assessed through metrics the true skill statistics (TSS). The value of TSS ranges from -1 to +1, in accordance with which the higher values are indicators of better model performance (Allouche et al. 2006).

Potential distributions of the species under different climate change models and scenarios were predicted and projected in the territory of Serbia and future range size changes were calculated.

RESULTS

Field survey

During our research we recorded for the first time the presence of *Pacifastacus leniusculus,* the second NICS in Serbian waters. At the site named Koruška (45°12′43.40″N 19°34′16.60″E) (Fig. 2) at 1280 km of the Danube watercourse (102 km downstream of the Drava River confluence) on November 26, 2020 an adult male of the signal crayfish was caught (Fig. 3) by a net set on a clay bottom with riprap embankment stones, near the right bank, in a relatively strong current, at a depth of around 3 m. Two specimens of

Fig. 3. The first specimen of signal crayfish recorded in Serbia, Danube (left - dorsal, right - ventral view) (photo A. Bajić)

invasive *Faxonius limosus* were also caught at the same place.

Species Distribution Modeling

The results of the ENMeval package (TSS values of 0.6932) showed that models with LQHPT feature types, which use a regularization multiplier of 1.5 and 6 parameters, fit well. All three climate models and scenarios showed that signal crayfish could extend its range (by up to 67.71%) mainly to the north and central Serbia, for both 2050 and 2070 years (Fig. 4, Tab. 1). The CCSM4 model showed the lowest dispersion dependence on climate change. The CNRM-CM5 and IPSL-CM5A-LR models gave similar projections of the signal crayfish future dispersion, but showed a slightly higher dependence on climate change.

DISCUSSION

Our finding confirmed that *Pacifastacus leniusculus* has already reached the Danube River on Serbia's territory. Its appearance was expected, but expansion was incredibly fast - in just two years, since 2018 it was registered in the Drava River 50 km upstream from its confluence with the Danube, the signal crayfish has reached as much as 102 km downstream along the Danube, where we register it. Its invasive front has advanced 152 km downstream, with unbelievable a 76 km/yr dispersal rate in two years. This could lead to the conclusion that the dispersal rate in the lower section of the Drava and the Danube was actually 3-4 times higher than previously was determined (Hudina et al. 2009; Dragičević et al. 2020) for the Mura and Drava Rivers through entire Croatia. Trying to explain our findings we can only offer several assumptions.

The signal crayfish occurrence in the Danube in Serbia could be a result of illegal introductions. It seems unlikely, although such an example of illegal introduction is known from Croatia, Korana River in 2012 (Hudina et al. 2013). It has also been suggested that the previous expansion of signal crayfish along the Drava may have been aided by illegal translocations (Dragičević et al. 2020). But, the species has not been recorded in Serbia so far and there is no water body from which individuals could be transferred to the Danube. This species cannot be found in pet shops, or grown for ornamental purposes. However, illegal translocations in the Drava River may have been a significant factor in the downstream expansion and much earlier arrival to the Danube than we have recorded.

The Drava-Danube spread could have been accelerated or significantly aided by human intermediation, e.g. transport in ships' ballast water. This assumption should not be rejected entirely although it seems unlikely due to low frequency of freight river traffic (transport ships with ballast tanks) between Drava and Danube. A similar assumption

has been provided as one of the possible explanations for the rapid downstream expansion of spiny-cheek crayfish along the Danube in Serbia (Pavlović et al. 2006). However, the river traffic on the Danube is incomparably higher than mentioned on the Drava-Danube route. But, further expansion along the Danube could be accelerated by the ships' ballast water.

It seems the most plausible that the spread of signal crayfish through the Drava was somehow accelerated and occurred a few years earlier, but it was not registered. According to Dragičević et al. (2020) just three specimens were recorded while monitoring of the invasive front progress downstream along the Drava River in 2018. It should not be ruled out that some individuals passed earlier and unnoticed. High water levels in 2013 could have played a significant role in this. Increased water flow could have accelerated expansion, taking some small individuals far downstream along the Drava. Our assumption is consistent with the observation that the dispersal rate can be increased due to a change in river characteristics, e.g. higher water flow can lead to a higher dispersal rate (Hudina et al. 2017). Further, if the dispersal rate in the Danube so far has been similar to that mentioned earlier for the Drava River (21.3 km/yr spontaneous, without acceleration), then signal crayfish took about 5-6 years to expand 102 km downstream along the Danube. That would lead to the conclusion that signal crayfish have probably reached the Danube as early as 2014 or 2015. That could explain our finding and seemingly extraordinary rapid expansion. Also, we cannot completely rule out the possibility that the progression in the Danube so far has been accelerated somehow. Due to only one specimen we have registered, it is difficult to claim this is an actual invasive front of signal crayfish in the Danube. Urgent systematic and very careful field research is needed to be able to draw conclusions that go beyond assumptions.

Given the recent fast expansion of the invasive spiny-cheek crayfish through the Danube, it is reasonable to expect further signal crayfish expansion could be rapid. In addition to the already mentioned possible expansion acceleration mediated by ships' ballast water, another possibility of human-mediated expansion is emerging. In Serbia, commercial net fishing on lowland rivers is highly represented. Fishermen ("alasi" in Serbian) mostly catch fish on larger stretches of the river course. Crayfish are often entangled in submerged nets left in the river for several hours (author's observation). By moving the net to another position, the crayfish can be carried several kilometres from the original place in a short period.

Data from other European countries show high dispersal rates during the spontaneous invasive progression of signal crayfish in the upstream direction, in addition to
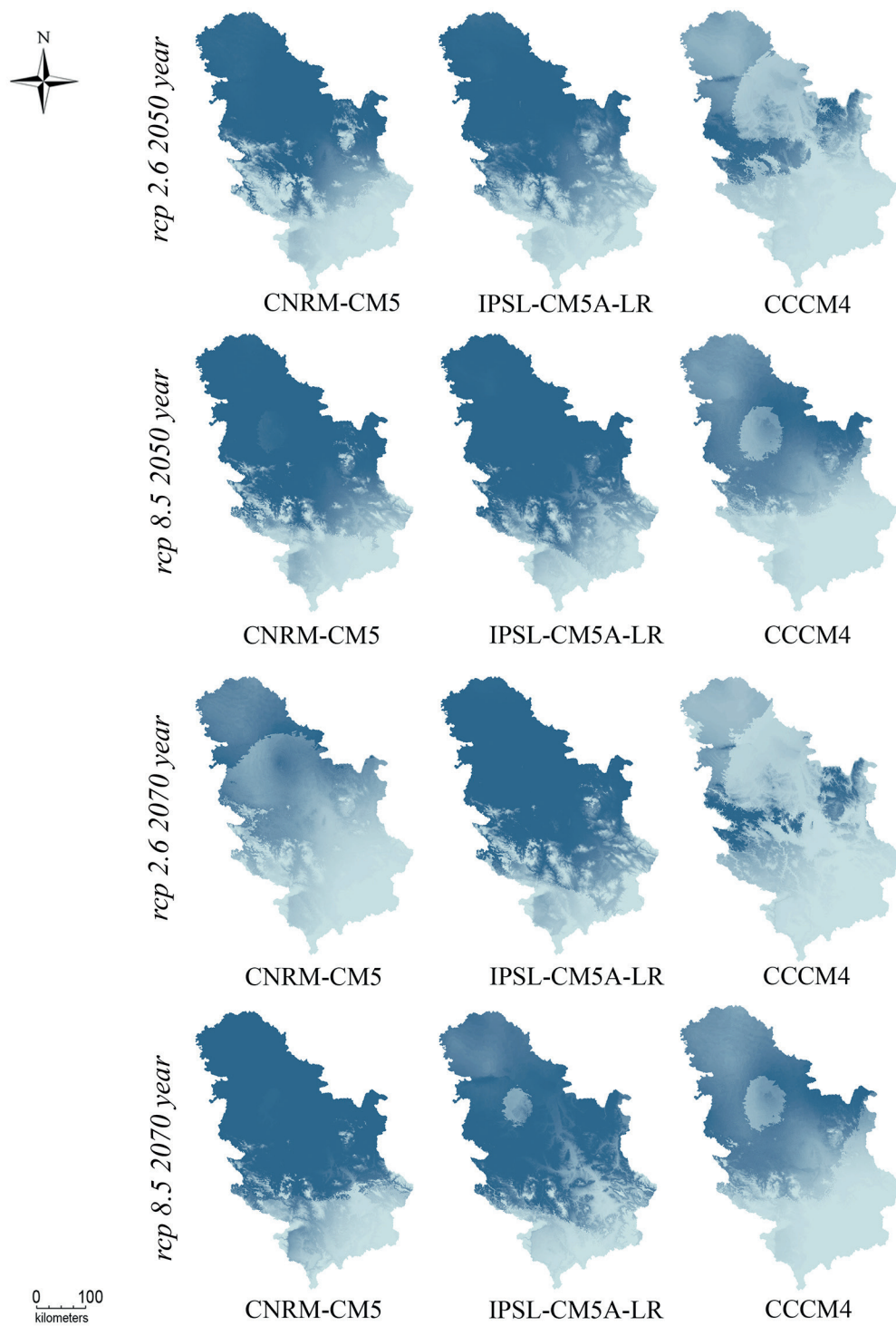
Fig. 4. Projected distribution under the future climatic models and all scenarios for *Pacifastacus leniusculus* in Serbia. The intensity of the color indicates predicted species distribution ranging between light blue - low, and dark blue - a high probability.

Table 1. The range reduction/expansion percentage for the investigated signal crayfish, based on predictions of the HadGEM2-ES and CCSM4 climate models and four rcp scenarios.

Climate model	rcp	2050	2070
CNRM-CM5	2.6	53.47	30.77
	8.5	61.42	67.71
IPSL-CM5A-LR	2.6	55.28	63.36
	8.5	64.82	54.81
CCSM4	2.6	8.48	6.44
	8.5	13.43	14.15

the expected high downstream dispersal rates (Hudina et al. 2009, 2017; Maguire et al. 2018). An alarming example is from the Korana River where even numerous limestone cascades did not slow down its upstream advance, which was at the same level as downstream (Hudina et al. 2017). Upstream expansion in the Danube tributaries on the territory of Serbia seems inevitable, as well as its further expansion into other river systems of the Danube basin. The spread of signal crayfish through the Danube and its tributaries on the territory of Serbia will potentially endanger 92% of Serbian watercourses, as much as belongs to the Black Sea catchment basin. Its further advance downstream towards the Danube delta will threaten the Eastern European freshwater habitats and arguably their endemic crayfish species (Pârvulescu 2019; Weiperth et al. 2020).

The second potential direction of signal crayfish invasion into Serbian waters, across the Sava River, should be mentioned. The signal crayfish were illegally introduced into the rivers Korana in 2012 and Una in 2018, both belonging to the Sava River system. According to available data, it has not yet reached the Sava River, but it is only a matter of time. We can assume that its downstream progression in the Sava will be at an approximate rate as in the Drava or Danube due to similar hydrological characteristics. The possibility of accelerated progression caused by human mediation should not be ruled out, due to developed freight river traffic and intensive commercial net fishing along the Sava River. A future invasion through the Sava River looks pretty plausible and could significantly accelerate the expansion of signal crayfish in Serbian waters.

So far, invasive *Faxonius limosus* in Serbia has come into contact with two of three indigenous crayfish species: *Pontastacus leptodactylus* and *Astacus astacus* (Zorić et al. 2020a, b). Although full impact study has not yet been done, significant increase in *F. limosus* abundance and decrease of *P. leptodactylus* in the Danube is noticeable (author's observation). In 2011, just three years after the first appearance of

F. limosus in the Romanian part of the Danube, a significant abundance decline of indigenous *P. leptodactylus* was observed (Pârvulescu et al. 2012). The most extreme example from the region is in eastern Croatia, where *F. limosus* has almost wholly displaced *P. leptodactylus* (Maguire et al. 2018).

The particularly negative effect on ICS populations has crayfish plague retention in the ecosystem. Even after the initial epidemic wave, the disease persists and occurs in periodic outbreaks wherever NICS populations are established. After each outbreak, ICS populations disappear or become significantly weakened (Holdich et al. 2009). We can assume that expansion of *P. leniusculus* will start new waves of the disease in Serbian waters, due to recorded presence of crayfish plague pathogen in the invaded range of signal crayfish in the Drava (Maguire et al. 2016). Combined with competitive superiority of *P. leniusculus* compared to indigenous *P. leptodactylus* and *A. astacus*, drastic decline in abundance of already weakened populations of native crayfish can be expected in Serbia. Recent examples from Croatia support this prediction. In the Korana River the native *P. leptodactilus* has completely disappeared within the affected areas in just three years since the introduction of *P. leniusculus* (Hudina et al. 2017; Maguire et al. 2018). The lack of native *P. leptodactylus* in the Drava River is significant and indicates its displacement (Dragičević et al. 2020).

The expansion of *P. leniusculus* poses a significant threat to the third indigenous crayfish species in Serbia, *Austropotamobius torrentium*. Since *A. torrentium* inhabits mainly mountain streams, it has not yet come into contact with invasive *F. limosus* (Zorić et al. 2020b) which does not prefer such habitats. However, *P. leniusculus* inhabits mountain streams as well. Its upstream expansion into the upper reaches (small rivers and streams) of the Danube tributaries seems inevitable. Except for the crayfish plague, it is to be expected that *A. torrentium* will be especially endangered by direct competition and predation, due to its much smaller growth than *P. leniusculus*. Another adverse effect, which *P. leniusculus* exhibits on the entire ecosystem, is habitat disturbance caused by burrowing and uplifting of the substrate, which leads to turbidity. That is particularly significant in smaller, clear mountain watercourses, where this negative effect can affect not only native crayfish, but also other aquatic invertebrates and even fishes (Crawford et al. 2006; Harvey et al. 2013). Due to its fecundity, large growth, opportunistic omnivorous feeding, aggressiveness and habitat effects, signal crayfish can significantly negatively impact the functioning of the entire aquatic ecosystems. Such impacts can be expected in Serbia, particularly in the fragile upper reaches of smaller watercourses.

Successful prediction of the progression and directions of the signal crayfish invasion can be of great importance

for Serbian ecosystems, especially those already weakened by other negative impacts, or harboring some endangered species (e.g. stone crayfish). Species Distribution Modeling (SDM) has proven to be a helpful tool widely used today. Using SDM we presented a projection of the future spread and distribution of *P. leniusculus* in Serbia until 2050 and 2070. In addition to undoubted benefits, this method still has certain weaknesses because it uses a limited number of factors in the assessment. Some significant variables, such as interactions with other crayfish species or other organisms, accelerating or slowing down expansion by human activity, changes in dispersal rate due to plasticity (density-dependent dispersal) or selection (favoring of dispersive phenotypes at invasion front) (Lindstrom et al. 2013; Rollins et al. 2015) are too complex and unpredictable to be involved in the assessment. Despite these modeling shortcomings, the obtained results largely coincide and support our predictions based on the literature data, documented examples, observations, and findings.

According to all three climate models and scenarios results, signal crayfish will extend their range through northern and central Serbia for both 2050 and 2070 years. In those regions its population settlement is almost inevitable. The invasion will also reach the other areas of Serbia, with a slightly weaker impact in the south. The signal crayfish further downstream and upstream expansion along the Danube as the main route will continue, and upstream into Danube tributaries and their river systems. According to SDM models, the degree of progression represents the projected spontaneous expansion, which in reality can be modified by various factors.

The current conditions of the native crayfish populations in Serbia and the impact of invasive *F. limosus* have had on them so far are poorly known. The emergence of another, even more aggressive invasive crayfish, *P. leniusculus* will lead to a huge additional negative pressure on the indigenous crayfish. Therefore, urgent scientific surveys of all crayfish (ICS and NICS) in Serbia are needed to gain actual data on their populations. It is crucial to obtain data on their current distribution and conduct constant monitoring of NICS invasion progression as soon as possible, also including citizen-science data. The mentioned second path of invasion across the Sava River system should also be observed. The abundance, population settlement, and impact on native astacofauna and freshwater ecosystems in Serbia must be scientifically monitored. At the same time, it is necessary to start with management actions aimed at its control. Although attempts to stop its spread seem futile, especially in the Danube and its larger tributaries, it is essential to implement measures that can at least slow down the invasion (e.g. setting traps and removing non-indigenous crayfish from

the water body). Or simply not speeding up the invasion (e.g. educate commercial fishermen to clean nets and remove non-indigenous crayfish; or even economically and ecologically better, use them as human or animal food). In smaller watercourses, an attempt could be made to completely stop the invasion front (e.g. setting up crayfish barriers). It is important to educate the local citizens and thus prevent the illegal introduction of NICS into small and isolated water bodies. With constant monitoring and timely response, such activities could yield results relatively fast.

ACKNOWLEDGEMENTS

REFERENCES

Alderman DJ. 1997. History of the spread of crayfish plague in Europe, in Crustaceans: Bacterial and fungal diseasaes. Scientific and Technical Review. 15:15–23.

Allouche O, Tsoar A, Kadmon R. 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). Journal of Applied Ecology. 43(6):1223–1232. https://doi.org/10.1111/j.1365-2664.2006.01214x.

Andersen MC, Adams H, Hope B, Powell M. 2004. Risk analysis for invasive species: general framework and research needs. Risk Analysis. 24:893–900.

Bern Convention Appendix III. European Treaty Series No. 104 of the Council of Europe, Convention on the Conservation of European Wildlife and Natural Habitats, Appendix III – Protected fauna species, 19 September 1979, Bern. https://www.coe.int/en/web/bern-convention/appendices.

Burnham KP, Anderson DR. 2004. Multimodel Inference: Understanding AIC and BIC in Model Selection. Sociological Methods and Research. 33:261–304. https://doi.org/10.1177/0049124104268644.

Crawford L, Yeomans WE, Adams CE. 2006. The impact of introduced signal crayfish *Pacifastacus leniusculus* on stream invertebrate communities. Aquatic Conservation: Marine and Freshwater Ecosystems. 16:611–621.

Dragičević P, Faller M, Kutleša P, Hudina S. 2020. Update on the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) range expansion in Croatia: a 10-year report. BioInvasions Records. 9(4):793–807. https://doi.org/10.3391/bir.2020.9.4.13.

Edgerton BF, Henttonen P, Jussila J, Mannonen A, Paasonen P, Taugbol T, Edsman L, Souty-Grosset C. 2004. Understanding

the causes of disease in European freshwater crayfish. Conservation Biology. 18:1466–1474.

Edsman L. 2004. The Swedish story about import of live crayfish. Bulletin Français de la Pêche et de la Pisciculture. 372-373:281–288.

EU. 2014. Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. Official Journal of the European Union. 57:35.

EU. 2016. Commission Implementing Regulation (EU) 2016/1141 of 13 July 2016 adopting a list of invasive alien species of Union concern pursuant to Regulation (EU) No 1143/2014 of the European Parliament and of the Council. Official Journal of the European Union. 189:4–8.

Filipová L, Petrusek A, Matasová K, Delaunay C, Grandjean F. 2013. Prevalence of the crayfish plague pathogen *Aphanomyces astaci* in populations of the signal crayfish *Pacifastacus leniusculus* in France: Evaluating the threat to native crayfish. PLoS One. 8:e70157.

Govedič M. 2006. Potočni raki Slovenije: razširjenost, ekologija, varstvo. Miklavž na Dravskem polju, Slovenia: Center za kartografijo favne in flore. 15 p.

Harvey GL, Henshaw AJ, Moorhouse TP, Clifford NJ, Holah H, Grey J, Macdonald DW. 2013. Invasive crayfish as drivers of fine sediment dynamics in rivers: field and laboratory evidence. Earth Surface Processes and Landforms. 39:259–271.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology. 25(15):1965–1978. https://doi.org/10.1002/joc.1276.

Hijmans RJ, Phillips S, Leathwick J. Elith J. 2017. Dismo: Species Distribution Modeling. R package version 1. 1–4.

Holdich DM, Harlioglu MM, Firkins I. 1997. Salinity adaptations of crayfish in Brittish Waters with particular reference to *Austropotamobius pallipes, Astacus leptodactylus* and *Pasifastacus leniusculus*. Estuarine, Coastal and Shelf Science. 44:147–154.

Holdich DM, Reynolds J, Souty-Grosset C, Sibley P. 2009. A review of the ever increasing threat to European crayfish from nonindigenous crayfish species. Knowledge and Management of Aquatic Ecosystems. 2009(394-395):11.

Hudina S, Faller M, Lucić A, Klobucar G, Maguire I. 2009. Distribution and dispersal of two invasive crayfish species in the Drava river basin, Croatia. Knowledge and Management of Aquatic Ecosystems. 2009(394-395):09. https://doi.org/10.1051/kmae/2009023.

Hudina S, Kutleša P, Trgovčić K, 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.

Hudina S, Žganec K, Lucić A, Trgovčić K, Maguire I. 2013. Recent invasion of the karstic river systems in Croatia through illegal introductions of the signal crayfish. Freshwater Crayfish. 19:21–27.

Illés P. 2002. A jelzőrák (*Pacifastacus leniusculus*) előfordulása Magyarországon. Cinege. 7:39–41.

IPCC. 2014. Fifth assessment report (AR5). Cambridge: Cambridge University Press.

Johnsen SI, Taugbol T. 2010. NOBANIS – Invasive Alien Species Fact Sheet – *Pacifastacus leniusculus*. Database of the European Network on Invasive Alien Species – NOBANIS [internet]. Acessed: 25/09/2021. www.nobanis.org.

Karaman I, Machino Y. 2004. Occurrence of the spiny-cheek crayfish (*Orconectes limosus*) and the Chinese mitten crab (*Eriocheir sinensis*) in Serbia. Crayfish News. 26:19–20.

Kokko H, Harlioglu MM, Aydin H, Makkonen J, Gökmen G, Aksu Ö, Jussila J. 2018. Observations of crayfish plague infections in commercially important narrow-clawed crayfish populations in Turkey. Knowledge and Management of Aquatic Ecosystems. 419:10. https://doi.org/10.1051/kmae/2018001.

Kouba A, Petrusek A, Kozák P. 2014. Continental-wide distribution of crayfish species in Europe: update and maps. Knowledge and Management of Aquatic Ecosystems. 2014(413):05. https://doi.org/10.1051/kmae/2014007.

Kušar D, Vrezec A, Ocepek M, Jenčič V. 2013. *Aphanomyces astaci* in wild crayfish populations in Slovenia: first report of persistent infection in a stone crayfish *Austropotamobius torrentium* population. Diseases of Aquatic Organisms. 103:157–169.

Lindstrom T, Brown GP, Sisson SA, Phillips BL, Shine R. 2013. Rapid shifts in dispersal behavior on an expanding range edge. Proceedings of the National Academy of Sciences of the United States of America. 110:13452–13456.

Lodge DM, Taylor CA, Holdich DM, Skurdal J. 2000. Nonindigenous crayfishes threaten North American freshwater biodiversity: Lessons from Europe. Fisheries. 25:7–20.

Maguire I, Jelić M, Klobučar G. 2011. Update on the distribution of freshwater crayfish in Croatia. Knowledge and Management of Aquatic Ecosystems. 2011(401):31. https://doi.org/10.1051/kmae/2011051.

Maguire I, Jelić M, Klobučar G, Delpy M, Delaunay C, Grandjean F. 2016. Prevalence of the pathogen *Aphanomyces astaci* in freshwater crayfish populations in Croatia. Diseases of Aquatic Organisms. 118:45–53.

Maguire I, Klobučar G, Marčić Z, Zanella D. 2008. The first record of *Pacifastacus leniusculus* in Croatia. Crayfish News: IAA Newsletter. 30(4):1–4.

Maguire I, Klobučar G, Žganec K, Jelić M, Lucić A, Hudina S. 2018. Recent changes in distribution pattern of freshwater crayfish in Croatia - threats and perspectives. Knowledge and Management of Aquatic Ecosystems. 2018(419):2. https://doi.org/10.1051/kmae/2017053.

Moss R, Babiker W, Brinkman S, Calvo E, Carter T, Edmonds J, Elgizouli I, Emori S, Erda L, Hibbard K, et al. 2008. Towards new scenarios for analysis of emissions: climate change, impacts, and response strategies. IPCC Expert Meeting Report, IPCC, Geneva, Switzerland.

Muscarella R, Galante PJ, Soley-Guardia M. Boria RA, Kass JM, Uriarte M, Anderson RP. 2014. ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche models. Methods in Ecology and Evolution. 5:1198–1205. https://doi.org/10.1111/2041-210X.12261.

Naimi B. 2015. Usdm: Uncertainty Analysis for Species. Distribution models. R package version 1. 1–15.

Pârvulescu L. 2019. Introducing a new *Austropotamobius* crayfish species (Crustacea, Decapoda, Astacidae): A Miocene end-

emism of the Apuseni Mountains, Romania. Zoologischer Anzeiger. 279:94–102.

Pârvulescu L, Palos C, Molnar P. 2009. First record of the spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) (Crustacea: Decapoda: Cambaridae) in Romania. North-Western Journal of Zoology. 5:424–428.

Pârvulescu L, Schrimpf A, Kozubíková E, Resino S C, Vrålstad T, Petrusek A, Schulz R. 2012. Invasive crayfish and crayfish plague on the move: first detection of the plague agent *Aphanomyces astaci* in the Romanian Danube. Diseases of Aquatic Organisms. 98(1):85–94.

Pavlović SZ, Milošević SM, Borković SB, Simić VM, Paunović MM, Žikić RV, Saičić ZS. 2006. A report of *Orconectes* (*Faxonius*) *limosus* (Rafinesque, 1817) Crustacea: Decapoda: Astacidea: Cambaridae: *Orconectes*: Subgenus *Faxonius* in the Serbian part of the River Danube. Biotechnology and Biotechnological Equipment. 1:53–56.

Peterson AT. 2003. Predicting the geography of species invasions via ecological niche modeling. The Quarterly Review of Biology. 78:419–433.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling. 190:231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026.

Phillips SJ, Dudik M. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography. 31(2):161–175. https://doi.org/10.1111/j.0906-7590.2008.5203.x.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rollins LA, Richardson MF, Shine R. 2015. A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). Molecular Ecology. 24:2264–2276.

SGRS. 2016. Službeni glasnik RS 5/2010, 47/2011, 32/2016, 98/2016. Pravilnik o proglašenju i zaštiti strogo zaštićenih i zaštićenih divljih vrsta biljaka, životinja i gljiva (Prilog I, Strogo zaštićene divlje vrste biljaka, životinja i gljiva). Ministarstvo životne sredine i prostornog planiranja i Ministarstvo poljoprivrede, šumarstva i vodoprivrede, Beograd; 5.2.2010, 29.6.2011, 30.3.2016, 8.12.2016.

Souty-Grosset C, Holdich DM, Noël PY, Reynolds JD, Haffner P. 2006. Atlas of Crayfish in Europe, Muséum national d'Histoire naturelle, Paris, 187 p.

Svoboda J, Mrugała A, Kozubíková-Balcarová E, Petrusek A. 2017. Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. Journal of Fish Diseases. 40:127–140.

Taucare-Ríos A, Bizama G, Bustamante RO. 2016. Using global and regional species distribution models (SDM) to in-fer the invasive stage of *Latrodectus geometricus* (Araneae: Theridiidae) in the Americas. Environmental Entomology. 45(6):1379–1385.

Todorov M, Trichkova T, Hubenov Z, Jurajda P. 2020. *Faxonius limosus* (Rafinesque, 1817) (Decapoda: Cambaridae), a new invasive alien species of European Union concern in Bulgaria. Acta Zoologica Bulgarica. 72(1):113–121.

Trožić-Borovac S, Škrijelj R, Vesnić A, Đug S, Mušović A, Šljuka S, Borovac B, Gajević M. 2019. Negative effects of introducing allochthonous species *Pacifastacus leniusculus* (Dana, 1852) into aquatic ecosystems of Bosnia and Herzegovina. In: Ivković M, Stanković I, Matoničkin Kepčija R, Gračan R, editors. Book of Abstracts - 3rd Symposium of Freshwater Biology. Zagreb, Croatia: Croatian Association of Freshwater Ecologists. p. 33.

Van Vuuren DP, Edmonds J, Kainuma M, Riahi K, Thomson A, Hibbard K. Hurtt GC, Kram T, Krey V, Lamarque JF, et al. 2011. The representative concentration pathways: an overview. Climatic Change. 109(1):5–31. https://doi.org/10.1007/s10584-011-0148-z.

Warren DL, Seifert SN. 2011. Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. Ecological Applications. 21(2):335–342. https://doi.org/10.1890/10-1171.1.

Weiperth A, Bláha M, Szajbert B, Seprős R, Bányai Z, Patoka J, Kouba A. 2020. Hungary: a European hotspot of nonnative crayfish biodiversity. Knowledge and Management of Aquatic Ecosystems. 2020(421):43. https://doi.org/10.1051/kmae/2020035.

Weiperth A, Gál B, Kuříková P, Langrová I, Kouba A, Patoka J. 2019. Risk assessment of pet-traded decapod crustaceans in Hungary with evidences of *Cherax quadricarinatus* (von Martens) in the wild. North-Western Journal of Zoology. 14:42–47.

Westman K, Savolainen R, Julkunen M. 2002. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a small enclosed Finnish lake: a 30 year study. Ecography. 25:53–73.

Zhang Z, Capinha C, Usio N, Wetering R, Liu X, Li Y, Landeria J M, Zhou Yokota M. 2020. Impacts of climate change on the global potential distribution of two notorious invasive crayfishes. Freshwater Biology. 65(3):353–365.

Zorić K, Atanacković A, Ilić M, Csányi B, Paunović M. 2020a. The spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) (Decapoda: Cambaridae) invades new areas in Serbian inland waters. Acta Zoologica Bulgarica. 72(4):623–627.

Zorić K, Atanacković A, Tomović J, Vasiljević B, Tubić B, Paunović M. 2020b. Diversity of alien macroinvertebrate species in Serbian waters. Water. 12(12):3521.