

Research Article

Dynamics of range expansion of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region in Croatia

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Abstract

The signal crayfish, *Pacifastacus leniusculus*, is the most widespread and one of the most successful crayfish invaders in Europe. In 2012 it was recorded in a karstic river in the continental part of Croatia, the Korana River, where it was illegally introduced. Here, it presents an alarming threat to native crayfish diversity, since three native crayfish species have viable populations in the Korana River and its tributaries. We explored the dynamics of signal crayfish range expansion in the Korana River, by i) examining the increase in its range, ii) analysing changes in its relative abundance, size and sex structure at former invasion fronts in 3 years (since the first study), and iii) examining the status of the native narrow-clawed crayfish (*Astacus leptodactylus*) in the area. Signal crayfish range increased 2.5 times since 2012, and currently covers 18.7% of the entire watercourse. Recorded dispersal rate (2.23 – 2.84 km/year) was similar in upstream and downstream direction. At both former invasion fronts, increase in the relative signal crayfish abundance by 4.9 – 6.1 times resulted in significant decrease in the size of signal crayfish individuals, demonstrating the prominent effect of density and competition intensity on the size structure its populations. The native *A. leptodactylus* was completely displaced from mixed populations at former invasion fronts, while at new invasion fronts no narrow-clawed crayfish were recorded. Abundant *A. leptodactylus* populations are still present in the Mrežnica and Kupa Rivers, and outside the invaded area in the Korana River. We discuss these findings in the context of potential management actions and legal requirements for such actions under EU Regulations.

Key words: range expansion, size structure, population abundance, invasive species, freshwater

Introduction

Non-indigenous crayfish species (NICS) represent one of the greatest threats to indigenous crayfish species (ICS). Their introduction, often followed by rapid range expansion, frequently results in population declines or extinction of ICS and negative impacts on freshwater biodiversity in general (Twardochleb et al. 2013). The signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), has invaded 29 European countries, and is the most widespread NICS in Europe (Kouba et al. 2014). Its success stems from its advantageous life-history traits, such as high fecundity and early maturation, high dispersal rates and high aggressiveness (Hudina et al. 2013). Signal

crayfish displaces European ICS primarily through disease transmission (transmission of the crayfish plague agent, *Aphanomyces astaci*; Filipová et al. 2013), but also through competitive exclusion (e.g., Söderbäck 1995; Westman et al. 2002). Also, it may exhibit adverse impacts on freshwater ecosystems through negative effects on macroinvertebrates, fish and other freshwater biota (Griffiths et al. 2004; Crawford et al. 2006; Twardochleb et al. 2013), and through direct effects on the physical environment of rivers by burrowing and bioturbation (Johnson et al. 2011; Harvey et al. 2013).

The signal crayfish has recently been illegally introduced to a karstic river in the continental part of Croatia, the Korana River (Hudina et al. 2013). Here,

it presents an alarming threat to native crayfish diversity, since three native crayfish species have viable populations in the Korana River and its tributaries (Maguire et al. 2011). In 2012, signal crayfish distribution in the Korana River was localized within 10 km of the river's lower reach, where it coexisted with the native *Astacus leptodactylus* (Eschscholtz, 1823 species-complex) at both upstream and downstream invasion fronts (Hudina et al. 2013). Recent research showed that *P. leniusculus* individuals at both invasion fronts were in a better body condition and had superior energetic status of organs (i.e. better physiological condition) compared to the individuals sampled from the invasion core, where signal crayfish density was much higher (Rebrina et al. 2015). Also, females from invasion fronts had a better energetic status of gonads, indicating higher potential for energetic investment into reproduction (Rebrina et al. 2015). This pinpoints the prominent role of intraspecific competition and density on population dynamics of the expanding signal crayfish population in the Korana River.

Range expansion of non-indigenous species represents a crucial element of their invasion success; however, identifying exact mechanisms of such process is a complex issue. This limits our ability to predict the dynamics of biological invasions and manage them successfully (Hudina et al. 2014). Thus, in this study, we explore range expansion dynamics of the signal crayfish in the Korana River, by i) examining the increase in its range and the downstream and upstream dispersal rate within the 3 years since the first study (Hudina et al. 2013), ii) analysing potential changes in the relative abundance, sex and size structure of signal crayfish populations at former invasion fronts within 3 years, and iii) by examining presence and relative abundance of the native narrow-clawed crayfish at former invasion fronts 3 years after the first study. All obtained results are discussed in the context of potential implications for signal crayfish management in the invaded karstic river.

Methods

Study area

The Korana River is a karstic river situated in central Croatia. Its springs form the famous Plitvice Lakes National Park. The Korana River (134 km river length, catchment area 2297 km², gradient of 2.7 meters per km) is the largest tributary of the Kupa River, and belongs to the Black Sea drainage system. The hydrological regime is characterized by discharge peaks during spring and autumn (Gajić-Čapka and Zaninović 2004), while the climate is

predominantly mild-continental (average annual temperature 11.1 °C, average rainfall 700–1100 mm per year). The Korana River has numerous natural and man-made cascades along the whole course of its length. Artificial cascades are less than one meter high and are a remnant of the many water-mills that existed on the river in the past. Due to its karstic character and the man-made cascades, the flow between cascades is slow and averages approximately 86 m³/s.

The Korana River has a rich crayfish fauna, with 3 out of 4 indigenous crayfish species for Croatia present in its drainage. The noble crayfish, *Astacus astacus* (Linnaeus, 1758), and the stone crayfish, *Austropotamobius torrentium* (Schrank, 1803), were recorded in the upper course and the tributaries, while the narrow clawed crayfish (*A. leptodactylus*) was recorded in recent decades in the lower course of the Korana River. Its presence in the Korana River is the result of its upstream dispersal from the Kupa and Mrežnica Rivers (Maguire et al. 2011).

Signal crayfish were recently deliberately introduced to the Korana River from an unknown source (Hudina et al. 2013). The presumed introduction site is located approximately 30 kilometers from the confluence of the Korana and Kupa rivers (Hudina et al. 2013). From there, it spread both upstream and downstream at a similar rate, successfully passing the cascades present in this section of the river. In 2012, the invasive range of the signal crayfish covered approximately 10 kilometers of the lower reach of the Korana River (Hudina et al. 2013). At both upstream and downstream invasion fronts, signal crayfish co-occurred with native *A. leptodactylus*, but seemed to be gradually displacing it from the invaded area (Hudina et al. 2013; Rebrina et al. 2015).

Distribution changes

Changes in signal crayfish distribution in the Korana River were analyzed in 2015 from July to November 2015 (4 months). We examined 23 sites during our study: 14 sites in the Korana River (2 former invasion fronts from 2012 and 6 sites upstream and 6 downstream from these former fronts – including the mouth of the Korana and Mrežnica rivers), 1 site in the Radonja River, 2 sites in the Mrežnica River and 6 sites in the Kupa River and its tributaries. Adjacent rivers (altogether 8 sites in 3 rivers and their tributaries; Figure 1) were also monitored to account for the eventual dispersal of the signal crayfish to these waterbodies from the Korana River. Some of the examined sites (7 out of 14) in the Korana River were identical to those investigated in 2012 (Figure 1, sites with overlapping symbols of the same shape)

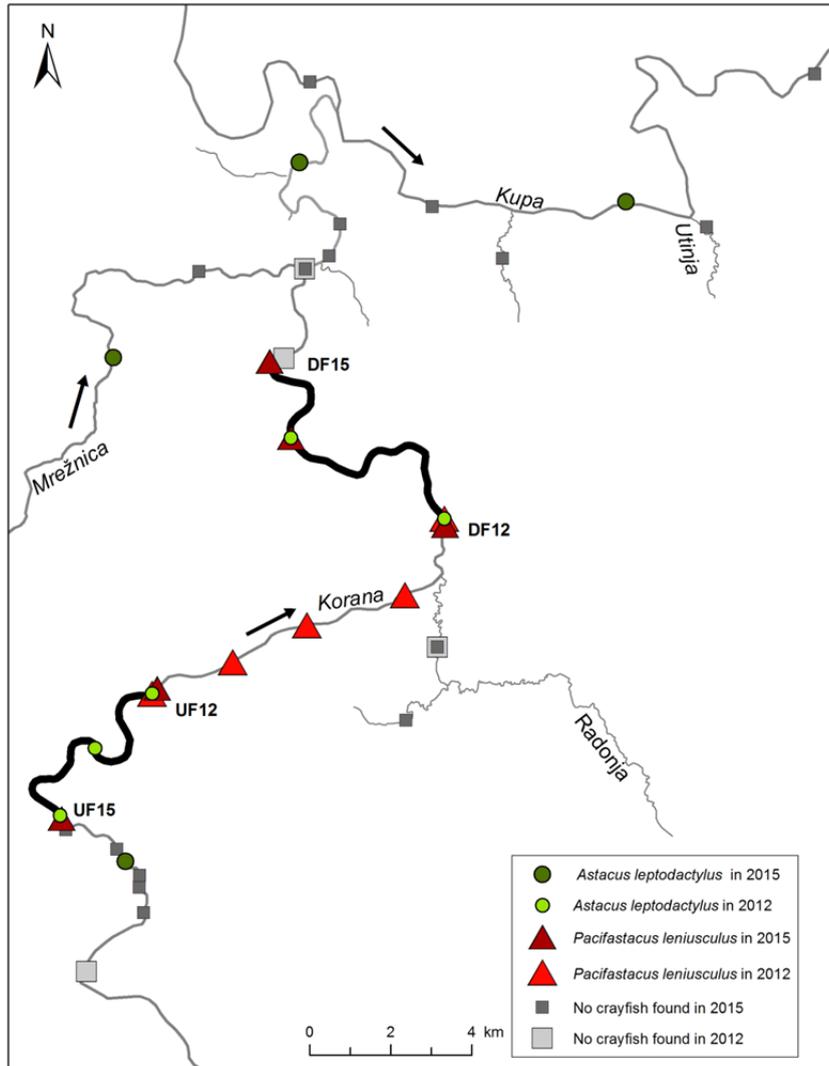


Figure 1. Changes in the distribution of invasive *P. leniusculus* and native *A. leptodactylus* in the Korana River and the status in adjacent rivers in 3 years (2012–2015). Arrows represent the direction of flow and bold parts of the watercourse represent the extent of range expansion in 3 years. (DF12 = former downstream invasion front in 2012, DF15 = downstream invasion front in 2015, UF12 = former upstream invasion front in 2012, UF15 = upstream invasion front in 2015).

and the fieldwork covered 42 km of the lower course of the Korana River and 32 km of the Kupa and Mrežnica Rivers. At each examined site, crayfish were trapped using a similar number (6–8) of baited LiNi traps (Westman et al. 1978). Even though the width of the rivers differed (river width of the Kupa river approximately 95 m, river width of the Korana and Mrežnica rivers approximately 65 m), traps were always exposed on one river bank and in stretches of similar length (100 m). Furthermore, traps were set at comparable mesohabitats at each site (shaded areas, slow current, and presence of large stone blocks/travertine barriers or close to artificial

cascades). Captured crayfish were identified to species level, their sex was determined and total length (TL: from the tip of the rostrum to the end of the telson) was measured to the nearest 1 mm using a Vernier digital caliper. Native crayfish were released at the site of their capture, while signal crayfish individuals were removed from the river.

Temporal changes in crayfish abundance and population structure at former invasion fronts

For 3 years, either upstream (2013) or downstream (2014) or both (2015) former invasion fronts (sites

UF12 and DF12 in Figure 1) were monitored in order to gain insight into population dynamics/growth at the edges of the expanding range. Each year in August, a similar numbers of LiNi traps (8–10) were exposed at the same locations at downstream and upstream invasion fronts. Caught crayfish were identified to species level, sexed and measured (TL). August was chosen as it is the period of increased crayfish activity. Based on the number of caught crayfish we calculated catch per unit effort (CPUE; the number of crayfish captured per LiNi trap per trapping night) for each site. Since CPUE is a frequently used measure of relative crayfish abundance (Dana et al. 2010) and also shows positive correlation with other measures of population density (Dorn et al. 2005), we used CPUE to compare changes in the relative abundance crayfish at former invasion fronts (sites DF12 and UF12, Figure 1) throughout 3 years. We monitored changes in both native (*A. leptodactylus*) and invasive (*P. leniusculus*) relative abundances, which were comparable between years since trapping was performed in the same period (summer–autumn) and with a similar number of traps exposed at the same sites of the same stretch length (100 m).

Furthermore, we compared size structure (measured as total length) and sex ratio of signal crayfish at both upstream (UF12, Figure 1) and downstream former invasion fronts (DF12, Figure 1) throughout the sampling years (2012–2015). This enabled us to analyze potential changes in population structure in relation to changes in relative population abundance.

Data analyses

Crayfish distribution was recorded using a Garmin GPS map 60CSx (projection HTRS96) and the data were mapped using ArcMap in the ArcGIS 10.1 program package (ESRI Inc.). Upstream and downstream dispersal rates of signal crayfish were calculated by recording the distance of the downstream and upstream signal crayfish records in 2015 to the former invasion fronts (in 2012).

Statistical analyses were performed using R statistical software by R Core Team (2015). Changes in the sex ratio at former invasion fronts between years were analyzed using a chi-square test. Changes in size structure were analyzed using a generalized linear model (GLM) (with normal error), after ensuring that the assumptions of normality and homoscedasticity were met. Crayfish size (total length: TL) was the dependent variable, while year and relative crayfish abundance were used as predictors. Since no significant differences in TL were established between sexes at both sites, data for males and females at each site were pooled together.

Results

Distribution changes

In 3 years, the invasive range of the signal crayfish in the Korana River expanded 6.70 kilometres upstream (dispersal rate: 2.23 km/year) and 8.51 kilometres downstream (dispersal rate: 2.84 km/year) from the former invasion fronts (Figure 1). Thus, the invasive range has increased by 2.5 times since 2012, and currently covers approximately 25 km of the lower course of the Korana River (18.7% of the entire watercourse). The current downstream invasion front is only 3.07 kilometres upstream from the confluence with the Mrežnica River. No native *A. leptodactylus* individuals were recorded at the new invasion fronts (Figure 1). Additionally, at each new invasion front (upstream and downstream), only one male signal crayfish individual was recorded.

In the Mrežnica and Kupa Rivers (and Kupa's tributaries) no signal crayfish were recorded. At some of the examined sites (Figure 1; 1 out of 3 in the Mrežnica, and 2 out of 6 in the Kupa and its tributaries), a relatively high number of native *A. leptodactylus* were caught (*A. leptodactylus*: $CPUE_{Mreznica} = 4$; $CPUE_{Kupa} = 0.6$). Furthermore, *A. leptodactylus* was also recorded 4.67 kilometres downstream from the confluence of Mrežnica and Korana Rivers, in the town of Karlovac (Figure 1).

Temporal changes in crayfish abundance and population structure at former invasion fronts

During the study period, a substantial increase in relative signal crayfish abundance was observed at both former invasion fronts (Figure 2a, b; Table 1). At the former upstream invasion front (UF12) relative population abundance increased 4.9 times, while at the former downstream front (DF12) it increased 6.1 times (Figure 2). In 2012, the abundance of the native *A. leptodactylus* at former fronts was low compared to signal crayfish abundance; however, by 2015, *A. leptodactylus* was completely absent from the catch (Figure 2a, b). Sex ratio did not exhibit any significant changes throughout the study period at both former fronts (Chi-square test: $p > 0.05$; Table 1). However, a significant difference in size (TL) of the caught signal crayfish individuals was recorded between years at the former upstream invasion front ($F_{(2,217)} = 46.32$, $p < 0.001$; Figure 3a) while the former downstream front showed a clear and significant decrease in size ($F_{(2, 176)} = 71.17$, $p < 0.001$; Figure 3b). There was also a significant negative effect of increased relative crayfish abundance (CPUE) on crayfish size in the population ($p < 0.001$ for both former invasion fronts).

Figure 2. Changes in relative crayfish abundance (CPUE) of invasive *P. leniusculus* (CPUE PL) and native *A. leptodactylus* (CPUE AL) through the years at (a) former upstream and (b) former downstream invasion fronts.

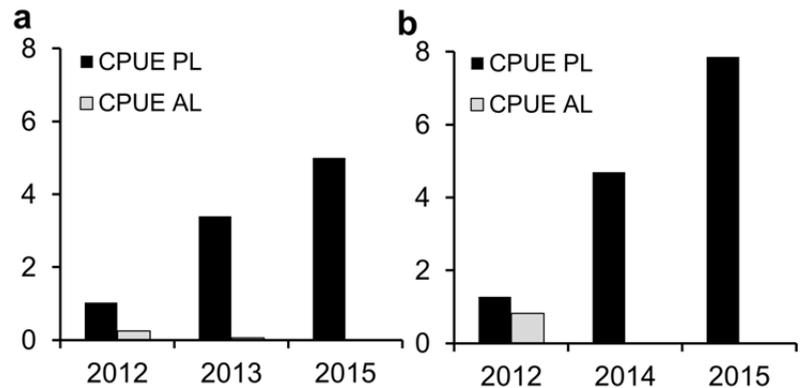


Figure 3. Changes in the size of individuals through years at (a) former upstream and (b) former downstream invasion fronts established using ANOVA of GLM coefficients. Significant differences between years are indicated by different letters on top of each box. Black lines represent medians, grey boxes interquartile ranges (IQR) and the whiskers 1.5xIQR.

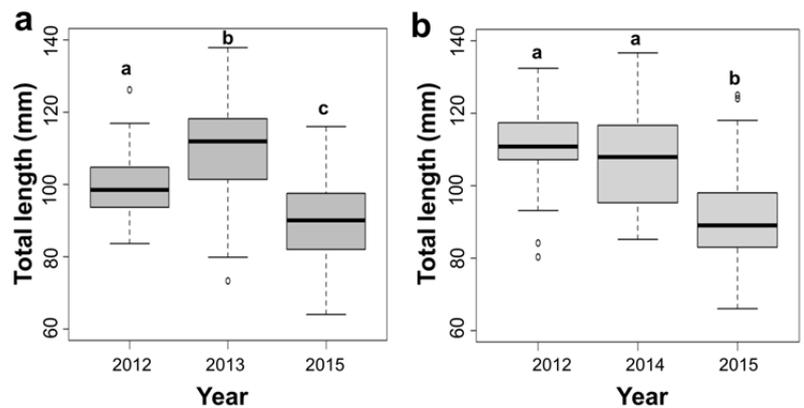


Table 1. Changes in crayfish abundance (CPUE), sex ratio (M : F ratio) and total length (TL) of caught signal crayfish in 3 years (2012–2015) at former invasion fronts.

Site	Year	N	CPUE	M:F ratio	TL (mm) \pm SD
Upstream front	2012	72	1.025	1 : 0.412	112.319 \pm 10.994
	2015	59	5	1 : 0.844	91.407 \pm 13.559
Downstream front	2012	38	1.28	1 : 0.583	98.515 \pm 9.977
	2015	40	7.857	1 : 0.667	89.400 \pm 11.562

Discussion

This study analysed the invasion dynamics of the most widespread crayfish invader in Europe, the signal crayfish, in a recently invaded area in Croatia (Korana River), where it is currently undergoing range expansion. In only 3 years the invasive range of the signal crayfish in the Korana River has increased 2.5 times and now covers 18.7% of the entire watercourse. The current dispersal rate is 2.23 km/year upstream and 2.84 km/year in the downstream

direction, which is in line with the dispersal rates recorded in other countries (0.4–7 km/year; reviewed in Hudina et al. 2009), except for the Croatian section of the Mura River, where recorded downstream dispersal rates were up to 8 times higher (Hudina et al. 2009). Such discrepancies in dispersal rates throughout the invasive range of the signal crayfish in Croatia are probably a result of differences in river characteristics (i.e. discharge is approximately 1.86 times higher in the 444 km long Mura River, compared to karstic and travertine dominated Korana River), but also potentially a result of an increase in

dispersal rates during continuous dispersal along the invasion pathway in the Mura River (Hudina et al. 2012). Several studies have suggested that during range expansions rapid increase in dispersal rates may occur due to plasticity (i.e. density-dependent dispersal) or evolutionary processes (rapid evolution of dispersive phenotypes at invasion fronts; Phillips et al. 2010; Lindstrom et al. 2013; Fronhofer and Altermatt 2015; Rollins et al. 2015). Thus, current dispersal rates in the Korana River could be expected to increase with time, and may represent underestimations of the speed of signal crayfish invasive range expansion in this waterbody in the future.

Interestingly, recorded dispersal rates were similar in both downstream and upstream directions. Thus, since 2012, upstream and downstream dispersal distances have remained similar despite severe floods that were recorded in the Korana and Kupa Rivers in last 3 years (Croatian Waters 2015). While in 2013 the floods occurred at the period of decreased crayfish activity (January), in other years they occurred at the period of high activity and mating (September and October respectively). It might be that the river geomorphology (numerous travertine barriers that provide an abundance of shelters to crayfish, artificial cascades that have increased the depth and reduced the current velocity in the majority of the river reaches between cascades) prevents significant downstream drift of invasive crayfish in such extreme events, and individuals that are transported during such floods probably die during transport.

Density-dependent growth in crayfish has been extensively studied in aquaculture (e.g. Ahvenharju et al. 2005), but also frequently observed in natural populations (cf. Guan and Wiles 1999; Moorhouse and Macdonald 2011b). Crayfish growth is affected by environmental conditions, among which temperature, resource availability and density are considered the most prominent (Guan and Wiles 1999; Westman and Savolainen 2002). Increased density raises competition intensity, which may in turn reduce foraging time, lower consumption rates and affect energy allocation (Guan and Wiles 1999; Corkum and Cronin 2004). Such processes may in turn reduce the per-moult increment (and growth rates), consequently resulting in lower abundance of large crayfish in a population (Olsson 2008). While such effect is probably more evident in closed ecosystems such as lakes, it also significantly affects populations in open water systems, where crayfish can also attain high densities due to their burrowing and homing behavior (Guan and Wiles 1999). In our case, the initial benefits of dispersal to areas with low conspecific density, which have resulted in better body condition

and higher energetic status of organs in individuals at the 2012 invasion fronts (Rebrina et al. 2015), have led to a relatively fast increase in signal crayfish abundance (an increase of 5–6 times in 3 years). Increased crayfish abundance in turn exhibited negative effects on signal crayfish growth, as a significant decrease in body size was observed at both examined sites within the three year period. Interestingly, at the former upstream invasion front in 2013 we recorded a significant increase in crayfish size, which was then followed by a significant decrease in 2015. In 2013, relative crayfish abundance increased 3 times compared to the abundance recorded in 2012. It seems that despite the threefold increase in abundance, individuals still benefited from lower resource competition compared to individuals at the invasion core, which in turn affected their growth rates. By 2015 relative crayfish abundance had increased almost 5 times compared to crayfish abundance in 2012 at this site, and such increase was translated to a significant reduction in crayfish size. Similarly, at the downstream invasion front, the increase in crayfish abundance of 3.5 times between 2012 and 2014 did not significantly influence population size as did the increase of population abundance in 2015 (6 fold increase since 2012). Thus for the Korana River population, the increase in crayfish abundance at invasion fronts of over 3.5 times exhibits a negative effect on crayfish growth and represents probably the tipping point at which resources become scarce and competition intensity increases. We also assume that the observed reduction in crayfish size is preceded by range expansion events (peristaltic spread of signal crayfish, Holdich et al. 2014).

Distribution of the native narrow-clawed crayfish at the edges of the invaded area has also changed within 3 years. At both former invasion fronts, *A. leptodactylus* was completely absent in 2015, while in 2012 it was present in low abundance (Hudina et al. 2013). In 2015 *A. leptodactylus* were not recorded at any examined site within the invaded area, but were found 7.74 km downstream and 2.39 km upstream from the new invasion fronts. The absence of *A. leptodactylus* from both current and former invasion fronts indicates that it is being displaced by a competitively superior signal crayfish (Hudina et al. 2016). Furthermore, while no infection by crayfish plague was recorded on signal crayfish individuals, one *A. leptodactylus* individual infected with the Ps genotype of crayfish plague pathogen (to which the signal crayfish is the natural host) was recorded in the Korana River (Maguire et al. 2016). This indicates that disease transmission (specifically of crayfish plague pathogen) is potentially a strong

contributing factor to the observed displacement of native narrow-clawed crayfish by invasive signal crayfish. While currently in extremely low abundance, the presence of *A. astaci* in the Korana River could negatively impact native crayfish populations downstream from the invaded area in a relatively short time period, since crayfish plague spores may spread downstream before their carrier as evident from the case study on *A. leptodactylus* and *O. limosus* in the Romanian part of the Danube (Pârvolescu et al. 2012). Since the invaded area is very close to the confluence of two rivers (the Mrežnica and Kupa Rivers), which host abundant narrow-clawed populations, there is a substantial threat of crayfish plague occurrence in these watercourses in the future, by either unintentional transport by fishing gear (Jussila et al. 2014) or by its downstream spread ahead of its host.

Based on the current extent of invasive range expansion, and the fact that signal crayfish is on a list of invasive alien species of Union concern for which effective management measures are required (Article 19, Regulation (EU) 1143/2014 of the European Parliament and of the Council and Commission implementing Regulation (EU) 2016/1141 adopting a list of invasive species of Union concern), we advocate urgent management actions aimed at control of signal crayfish further dispersal. Most studies report that eradication in a well-established signal crayfish population is almost impossible (Holdich et al. 2014), although there are several examples of relatively successful eradication efforts (cf. Peay et al. 2006; Freeman et al. 2009; Sandodden and Johnsen 2010) in small and isolated water bodies. Moreover, control of dispersal and abundance of signal crayfish populations is also considered a daunting task. Several studies suggest that either natural (e.g. Kerby et al. 2005) or specially designed artificial barriers (Frings et al. 2013) and electric fish barriers (Benejam et al. 2015) could be effective in controlling invasive crayfish dispersal. Artificial crayfish barriers require water flow above critical levels against which crayfish can swim, (Frings et al. 2013), while electric barriers require energy source and are more suitable to smaller waterbodies. In the Korana River, existing barriers are presumably too low to stop the invasion of the signal crayfish in any direction. Furthermore, in summer when water levels are low, the flow over these barriers is very limited, and further studies are required in order to establish whether crayfish barriers (Frings et al. 2013) could be installed on the river, i.e. whether flow requirements for these barriers are met. Finally, several studies have demonstrated that crayfish, including signal crayfish, can move overland and thus avoid all types of water barriers (Marques et al.

2015). Manual removal of crayfish by intensive trapping is often suggested as a control measure, however it is considered to be very time-consuming and should be maintained continuously for very long time periods (or indefinitely) in order to be effective (Dana et al. 2011; Holdich et al. 2014). Recent research does demonstrate that intensive trapping of signal crayfish at the margins of population may have localized effects in terms abundance and dispersal reductions (Moorhouse and Macdonald 2011a), as well as exert significant positive effects on abundance and diversity of sympatric aquatic macroinvertebrates (Moorhouse et al. 2014). A combination of intensive trapping with biological control by predatory fish (fish stocking) has been shown to be an effective method for substantial population reduction of the rusty crayfish (*Orconectes rusticus*) in a Wisconsin lake (Hein et al. 2007). However, such a management approach is applicable in enclosed water bodies such as lakes, while in continuous/connected habitats (rivers and lakes) its efficiency would be reduced due to the possibility of crayfish and fish migration.

Thus, we advocate a long term management based upon manual removal of crayfish through intensive trapping and also a further study of the effectiveness of the sterile-male release technique (Aquiloni et al. 2009) as a control measure, as well as its further testing in the field (Green et al. 2016). In the Korana River, such actions should be concentrated at population margins, especially at the upstream invasion front, since Plitvice Lakes National Park (Natura 2000 site HR5000020; SCI) with its unique geomorphological forms which also harbor populations of native (and protected) crayfish species (*A. astacus* and *A. torrentium*) is located approximately 98 km upstream at the springs of the Korana River. Also, *A. torrentium* is a target feature of the Natura 2000 sites in the Radonja River and its tributaries (HR2001391 and HR2001339; SCI), which are indirectly connected to the Korana River and located approximately 10 kilometers from the invaded area. Since removal efforts may be partially impeded by density-dependent elevation in body condition of remaining individuals (Moorhouse and Macdonald 2011b), a targeted approach and assessment of the most effective and cost-beneficial management option using spatial and population models is required (Taylor and Hastings 2004; Hock et al. 2016; Sieracki et al. 2014). Finally, due to the high potential of spread of crayfish plague spores by fishing gear (Jussila et al. 2014), and potential for its downstream dispersal through the water column to adjacent rivers before its host (Pârvolescu et al. 2012), actions preventing its unintentional transmission as well as

regular monitoring for crayfish plague agent presence are required at Plitvice Lakes National Park, Korana's tributaries and Kupa and Mrežnica Rivers. Due to foreseen management interactions for signal crayfish under Regulation (EU) No 1143/2014 on the prevention and management of the introduction and spread of invasive alien species, such a targeted approach to its management is vital for the success of these actions and their economic efficiency.

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References

- Ahvenharju T, Savolainen R, Tulonen J, Ruohonen K (2005) Effects of size grading on growth, survival and cheliped injuries of signal crayfish (*Pacifastacus leniusculus* Dana) summerlings (age 0+). *Aquaculture Research* 36: 857–867, <https://doi.org/10.1111/j.1365-2109.2005.01294.x>
- Aquiloni L, Becciolini A, Berti R, Porciani S, Trunfio C, Gherardi F (2009) Managing invasive crayfish: use of X-ray sterilisation of males. *Freshwater Biology* 54: 1510–1519, <https://doi.org/10.1111/j.1365-2427.2009.02169.x>
- Benejam L, Saura-Mas S, Montserrat J, Torres F, Macies M (2015) Could electric fish barriers help to manage native populations of European crayfish threatened by crayfish plague (*Aphanomyces astaci*)? *Management of Biological Invasions* 6: 307–310, <https://doi.org/10.3391/mbi.2015.6.3.10>
- Corkum LD, Cronin DJ (2004) Habitat complexity reduces aggression and enhances consumption in crayfish. *Journal of Ethology* 22: 23–27
- 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, <https://doi.org/10.1002/aqc.761>
- Croatian Waters (2015) Water levels of Sava, Kupa and Korana River Report: <http://www.voda.hr/hr/stanje-vodostaja-rijeke-save-kupe-korane> (accessed 7 July 2016).
- Dana ED, López-Santiago J, García-De-Lomas J, García-Ocaña DM, Gámez V, Ortega F (2010) Long-term management of the invasive *Pacifastacus leniusculus* (Dana, 1852) in a small mountain stream. *Aquatic Invasions* 5: 317–322, <https://doi.org/10.3391/ai.2010.5.3.10>
- Dom NJ, Urgelles R, Trexler JC (2005) Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland. *Journal of North American Benthological Society* 24: 346–356, <https://doi.org/10.1899/04-037.1>
- Filipová L, Petrušek 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, <https://doi.org/10.1371/journal.pone.0070157>
- Freeman M, Turnbull J, Yeomans W, Bean CW (2009) Prospects for management strategies of invasive crayfish populations with an emphasis on biological control. *Aquatic Conservation – Marine and Freshwater Ecosystems* 20: 211–223
- Frings RM, Vaeßen SCK, Groß H, Roger S, Schüttrumpf H, Hollert H (2013) A fish-passable barrier to stop the invasion of non-indigenous crayfish. *Biological Conservation* 159: 521–529, <https://doi.org/10.1016/j.biocon.2012.12.014>
- Fronhofer EA, Altermatt F (2015) Eco-evolutionary feedbacks during experimental range expansions. *Nature Communications* 6: 6844, <https://doi.org/10.1038/ncomms7844>
- Gajić-Čapka M, Zaninović K (2004) Climate conditions in the Sava, Drava and the Danube River basins. *Croatian Waters* 12: 297–312
- Green N, Stebbing P, Bentley M, Andreou D, Lane MR (2016) The River Barle Signal Crayfish Project: assessing the potential of male sterilisation as a signal crayfish control technique. 21st Symposium of the International Association of Asctacology – Book of Abstracts, September 5–8, 2016, Madrid, Spain. Real Jardín Botánico, Madrid, Spain, p 53
- 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 (1999) Growth and reproduction of the introduced crayfish *Pacifastacus leniusculus* in a British lowland river. *Fisheries Research* 42: 245–259, [https://doi.org/10.1016/S0165-7836\(99\)00044-2](https://doi.org/10.1016/S0165-7836(99)00044-2)
- 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, <https://doi.org/10.1002/esp.3486>
- Hein CL, Vander Zanden MJ, Manguson JJ (2007) Intensive trapping and increased fish predation cause massive population decline of an invasive crayfish. *Freshwater Biology* 52: 1134–1146, <https://doi.org/10.1111/j.1365-2427.2007.01741.x>
- Hock K, Wolff NH, Beeden R, Hoey J, Condie SA, Anthony KRN, Possingham HP, Mumby PJ (2016) Controlling range expansion in habitat networks by adaptively targeting source populations. *Conservation Biology* 30: 856–866, <https://doi.org/10.1111/cobi.12665>
- Holdich DM, James J, Jackson C, Peay S (2014) The North American signal crayfish, with particular reference to its success as an invasive species in Great Britain. *Ethology Ecology and Evolution* 26: 232–262, <https://doi.org/10.1080/03949370.2014.903380>
- Hudina S, Faller M, Lucić A, Klobučar G, Maguire I (2009) Distribution and dispersal of two invasive crayfish species in the Drava river basin, Croatia. *Knowledge and Management of Aquatic Ecosystems* 394–395: 1–11, <https://doi.org/10.1051/kmae/2009023>
- Hudina S, Hock K, Žganec K, Lucić A (2012) Changes in population characteristics and structure of the signal crayfish at the edge of its invasive range in a European river. *Annales de Limnologie - International Journal of Limnology* 48: 3–11, <https://doi.org/10.1051/limn/2011051>
- 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, <https://doi.org/10.5869/ffc.2013.v19.021>
- Hudina S, Hock K, Žganec K (2014) The role of aggression in range expansion and biological invasions. *Current Zoology* 60: 401–409, <https://doi.org/10.1093/czoolo/60.3.401>
- Hudina S, Hock K, Radović A, Klobučar G, Petković J, Jelić M, Maguire I (2016) Species-specific differences in dynamics of agonistic interactions may contribute to the competitive advantage of the invasive signal crayfish (*Pacifastacus leniusculus*) over the native narrow-clawed crayfish (*Astacus leptodactylus*). *Marine and Freshwater Behaviour and Physiology* 49: 147–157, <https://doi.org/10.1080/10236244.2016.1146448>

- Johnson MF, Rice S, Reid I (2011) Increase in coarse sediment transport associated with disturbance of gravel river beds by signal crayfish (*Pacifastacus leniusculus*). *Earth Surface Processes and Landforms* 36: 1680–1692, <https://doi.org/10.1002/esp.2192>
- Jussila J, Toljamo A, Makkonen J, Kukkonen H, Kokko H (2014) Practical disinfection chemicals for fishing and crayfishing gear against crayfish plague transfer. *Knowledge and Management of Aquatic Ecosystems* 413: 02, <https://doi.org/10.1051/kmae/2014002>
- Kerby JL, Riley SPD, Kats LB, Wilson P (2005) Barriers and flow as limiting factors in the spread of an invasive crayfish (*Procambarus clarkii*) in southern California streams. *Biological Conservation* 126: 402–409, <https://doi.org/10.1016/j.biocon.2005.06.020>
- Kouba A, Petrušek 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>
- 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 USA* 110: 13452–13456, <https://doi.org/10.1073/pnas.1303157110>
- Maguire I, Jelić M, Klobučar G (2011) Update on the distribution of freshwater crayfish in Croatia. *Knowledge and Management of Aquatic Ecosystems* 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, <https://doi.org/10.3354/dao02955>
- Marques M, Banha F, Águas M, Anastácio P (2015) Environmental cues during overland dispersal by three freshwater invaders: *Eriocheir sinensis*, *Pacifastacus leniusculus*, and *Procambarus clarkii* (Crustacea, Decapoda). *Hydrobiologia* 742: 81, <https://doi.org/10.1007/s10750-014-1968-4>
- Moorhouse TP, Macdonald DW (2011a) The effect of manual removal on movement distances in populations of signal crayfish (*Pacifastacus leniusculus*). *Freshwater Biology* 56: 2370–2377, <https://doi.org/10.1111/j.1365-2427.2011.02659.x>
- Moorhouse TP, Macdonald DW (2011b) The effect of removal by trapping on body condition in populations of signal crayfish. *Biological Conservation* 144: 1826–1831, <https://doi.org/10.1016/j.biocon.2011.03.017>
- 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>
- Olsson K (2008) Dynamics of omnivorous crayfish in freshwater ecosystems, PhD thesis, Lund University, Lund, Sweden, 35 pp
- Pârvulescu L, Schrimpf A, Kozubíková E, Cabanillas Resino S, Vrálstad T, Petrušek 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: 85–94, <https://doi.org/10.3354/dao02432>
- Peay S, Hiley PD, Collen P, Martin I (2006) Biocide treatment of ponds in Scotland to eradicate signal crayfish. *Bulletin Français de la Pêche et de la Pisciculture* 380–381: 1363–1379, <https://doi.org/10.1051/kmae/2006041>
- Phillips BL, Brown GP, Shine R (2010) Evolutionarily accelerated invasions: the rate of dispersal evolves upwards during the range advance of cane toads. *Journal of Evolutionary Biology* 23: 2595–2561, <https://doi.org/10.1111/j.1420-9101.2010.02118.x>
- Rebrina F, Skejo J, Lucić A, Hudina S (2015) Trait variability of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region reflects potential benefits and trade-offs during dispersal. *Aquatic Invasions* 10: 41–50, <https://doi.org/10.3391/ai.2015.10.1.04>
- Rollins LA, Richardson MF, Shine R (2015) A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Molecular Ecology* 24: 2264–2276, <https://doi.org/10.1111/mec.13184>
- Sandodden R, Johnsen SI (2010) Eradication of introduced signal crayfish *Pacifastacus leniusculus* using the pharmaceutical BETAMAX VET. *Aquatic Invasions* 5: 75–81, <https://doi.org/10.3391/ai.2010.5.1.9>
- Sieracki JL, Bossenbroek JM, Chadderton WL (2014) A Spatial Modeling Approach to Predicting the Secondary Spread of Invasive Species Due to Ballast Water Discharge. *PLoS ONE* 9: e114217, <https://doi.org/10.1371/journal.pone.0114217>
- Söderbäck B (1995) Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanisms. *Freshwater Biology* 33: 291–304, <https://doi.org/10.1111/j.1365-2427.1995.tb01168.x>
- Taylor CM, Hastings A (2004) Finding optimal control strategies for invasive species: a density-structured model for *Spartina alterniflora*. *Journal of Applied Ecology* 41: 1049–1057, <https://doi.org/10.1111/j.0021-8901.2004.00979.x>
- 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>
- Westman K, Pursiainen M, Vilkinen R (1978) A new folding trap model which prevents crayfish from escaping. *Freshwater Crayfish* 4: 235–242
- 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, <https://doi.org/10.1034/j.1600-0587.2002.250107.x>
- Westman K, Savolainen R (2002) Growth of the signal crayfish, *Pacifastacus leniusculus*, in a small forest lake in Finland. *Boreal Environment Research* 7: 53–61