

Research Article

Trait variability of the signal crayfish (*Pacifastacus leniusculus*) in a recently invaded region reflects potential benefits and trade-offs during dispersal

Fran Rebrina¹⁺, Josip Skejo¹, Andreja Lucić² and Sandra Hudina^{2*+}

¹Biology students association - BIUS, Rooseveltov trg 6., Croatia

²Faculty of Science, University of Zagreb, Zagreb, Croatia

E-mail: sandra.hudina@biol.pmf.hr (SH), rebrinafran@gmail.com (FR), skejo.josip@gmail.com (JS), andreja.lucic@biol.pmf.hr (AL)

*Corresponding author

+Equal contribution

Received: 5 May 2014 / Accepted: 19 September 2014 / Published online: 20 October 2014

Handling editor: Christoph Chucholl

Abstract

Rapid range expansion is one of the major attributes of invasion success. During range expansion, dispersing individuals face a novel environment where trade-offs between benefits of reduced conspecific density and pressure from niche competitors may differentially affect life-history traits essential for population growth. In this study, we examined differences in sex ratio, body condition, physiological condition and capacity for energetic investment into reproduction along an actively expanding range of one of the most successful crayfish invaders in Europe, the signal crayfish (*Pacifastacus leniusculus*). In the examined invasive range, signal crayfish faces competition by a native crayfish, *Astacus leptodactylus*. By comparing measured parameters between invasion fronts and invasion core sites, we analyzed whether the potential benefits of dispersal to areas with low conspecific density are outweighed by the competitive pressure from a native crayfish. Caught signal crayfish of both sexes from invasion fronts were in a better body condition compared to those from the invasion core, while females from invasion fronts also exhibited better energetic status of hepatopancreas and gonads. At the same time, *A. leptodactylus* from the contact zones at invasion fronts were in poorer body condition compared to those outside the invaded range. Thus, interspecific competition at invasion fronts affected the native *A. leptodactylus* more strongly than the signal crayfish, which still benefited from the reduced conspecific density in the presence of a competitor. This study highlights the importance of examining trait variability as invader advances its range, since a shift between the rate of population growth and counteracting pressure from other niche competitors could represent one of the crucial determinants of a lag between establishment and expansion of invasive species.

Key words: trait variation, range expansion, interspecific competition, density, freshwater invasion

Introduction

Invasive alien species are a major component of human-induced rapid environmental change (Sih et al. 2011; Simberloff et al. 2013) and one of the most important drivers of global biodiversity loss and ecosystem degradation (Sala et al. 2000; Pyšek and Richardson 2010). Such adverse impacts are created by a tiny fraction of all introduced species worldwide, which are able to make successful transitions through all stages of the invasion process to become successful invaders (cf. Williamson and Fitter 1996; Lockwood et al. 2007). Successful invaders are usually characterized with a suite of traits such as generalist feeding strategy, ability for rapid range expansion as well as traits that facilitate population establishment and growth following dispersal to novel

environments (Kolar and Lodge 2001; Marchetti et al. 2004; Olden et al. 2006). Recent studies suggest that the plasticity in behavioral, morphological and life history traits enhances invasion success as it allows invaders to easily adapt to different contexts encountered within the invasion process as well as within the recipient environment (Groen et al. 2012; Gutowsky and Fox 2012; Lopez et al. 2012). Moreover, traits which are beneficial for range expansion and population establishment could become increasingly prevalent at expanding range edges and consequently exhibit a spatial gradient along the invasion pathway (cf. Shine et al. 2011).

During range expansion, dispersing individuals are faced with a novel environment where trade-offs between benefits of reduced conspecific density and pressure from niche competitors may

differentially affect life-history traits essential for population growth (Burton et al. 2010). Dispersing individuals at an invasion front are expected to benefit from extremely reduced conspecific density, which, along with potentially more abundant resources at population edges could lead to fitness advantages (Burton et al. 2010). Such circumstances which improve the condition of individuals at the distribution edge may ultimately elevate the rates of population growth (Burton et al. 2010; Phillips 2009; Phillips et al. 2010) either by increasing individual growth rates (i.e. reducing the time needed to reach sexual maturity; Gutowsky and Fox 2012; Brandner et al. 2013) or by increasing reproductive fitness (i.e. raising the reproductive investment capacity; Bøhn et al. 2004; Lopez et al. 2012). On the other hand, theoretical studies suggest that a strong selection for reproduction and dispersal occurs at the expanding edges at the expense of competitive ability (Burton et al. 2010). Thus, the presence and pressure from a niche competitor may require selection for increased competitive ability at the expense of reproduction or dispersal.

In the present study, we look at changes in sex structure, body condition, physiological condition and potential for energetic investment into reproduction of the most widespread crayfish invader in Europe (Holdich et al. 2009), the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), along its actively expanding range in the Korana River, Croatia. Non-indigenous crayfish have been recognized as a major threat to freshwater ecosystems around the world (Twardochleb et al. 2013). Signal crayfish displaces the native crayfish through transmission of diseases such as crayfish plague (*Aphanomyces astaci*; e.g. Dieguez-Uribeondo 2006), as well as through competitive exclusion (e.g. Söderbäck 1995; Westman et al. 2002). Additionally, signal crayfish exhibits high dispersal rates (up to 18–24.4 km/yr; Hudina et al. 2009), which facilitated its widespread distribution in many parts of the world. The ongoing context of the range expansion in a recently invaded region of Croatia (Hudina et al. 2013) allowed us to sample and compare individuals that are at the very edge of the range expansion front where they face a native competitor (*Astacus leptodactylus* Eschscholtz, 1823), with those from established populations at invasion core where they face only intraspecific competition from conspecifics (Hudina et al. 2013). Signal crayfish from the invaded range in the Korana River are considered plague-free (A0 level of infection; Maguire et al. 2013), thus only interspecific competition is

expected to influence crayfish distribution/presence within the invaded range.

Since the signal crayfish is in close contact with the native crayfish at both upstream and downstream invasion fronts, we explore the potential effects of interspecific competition on spatial distribution of examined traits in signal crayfish individuals from core and front populations. If interspecific competition does not present a significant challenge to expanding signal crayfish (i.e. if the benefits of reduced conspecific density outweigh the potential costs of interspecific competition), we expected to observe a distinct spatial trait pattern in which individuals from invasion fronts would exhibit better condition and higher reproductive capacity compared to those from the invasion core (Gutowsky and Fox 2012; Lopez et al. 2012; Brandner et al. 2013). On the other hand, if interspecific competition poses a significant challenge to the expanding signal crayfish, we expected that such spatial pattern would be lacking due to trade-offs between competitive ability and population growth (cf. Burton et al. 2010). In such case, we expected no significant differences in condition and reproductive capacity between invasion front and core populations of the signal crayfish. Such shift in balance between the rate of population growth and counteracting pressure from other populations or niche competitors could represent one of the crucial determinants of a lag between the establishment and expansion of introduced species.

Materials and methods

Study area

The Korana River is a 134 km long karstic river in the central Croatia. With a catchment area of 2297 km² and an average discharge of 29 m³s⁻¹, it is the largest tributary of the Kupa River in the Black Sea drainage system. The hydrological regime is characterized by higher discharge during spring and autumn and lower discharge in the summer and winter period (cf. Hudina et al. 2013). The climate is predominantly mild-continental and partly humid, with an average annual temperature of 11.1°C and an average rainfall of 700–1100 mm per year. The karstic character of the Korana River makes it an important biodiversity hotspot with a relatively high number of endemic species (State Institute for Nature Protection 2009). Moreover, the Korana River has a rich native crayfish fauna, with the noble crayfish, *Astacus astacus* (Linnaeus, 1758) and the stone crayfish,

Austropotamobius torrentium (Schrank, 1803) present in the upper course and tributaries, and the narrow clawed crayfish (*A. leptodactylus*) present in the lower course.

Signal crayfish occurrence in the Korana River is the result of illegal introductions which occurred approximately 5 years ago from an unknown source (Hudina et al. 2013). The presumed introduction site is located approximately 30 kilometers from the confluence of the Korana with the Kupa River. Signal crayfish expanded both upstream and downstream from the presumed introduction site at a similar rate (Hudina et al. 2013). Today, the invasive range of the signal crayfish in the Korana covers approximately 10 kilometers of its lower reach. Native *A. leptodactylus* occurs at both invasion fronts as well as outside the invaded area (Hudina et al. 2013).

Field sampling

The study covered the entire invasive range of the signal crayfish in the Korana River. Sampling was performed in July 2012, in the period of increased signal crayfish activity (Souty-Grosset et al. 2006). Based on previous studies on signal crayfish distribution (Hudina et al. 2013), we identified two distributional points across the Korana River: 1) the invasion front at both upstream and downstream edge of an actively expanding range and 2) the invasion core established several generations ago (Hudina et al. 2013; Figure 1). Samples were taken from a total of 4 sites (approximate site length: 150 m) using baited crayfish traps. Two sites were located at the upstream and downstream invasion front, while two additional sites were located towards the center of the signal crayfish distribution, and were identified in a previous study (Hudina et al. 2013) as established populations, i.e. invasion core (Figure 1). Additionally, the area downstream of the invasive range was also examined within the research period (Outside IR; Figure 1). Since baited traps are size selective (Hogger 1988), all caught signal crayfish were above minimal size at maturity (> 6 cm total length; Souty-Grosset et al. 2006).

A similar number of traps (approximately 15) were simultaneously exposed at all localities for the same time period (5 days). At each site, traps were exposed in similar microhabitats and were checked daily. All caught crayfish were identified to the species level and their sex was determined, after which they were weighted (W) and 3 morphometric parameters were measured to the

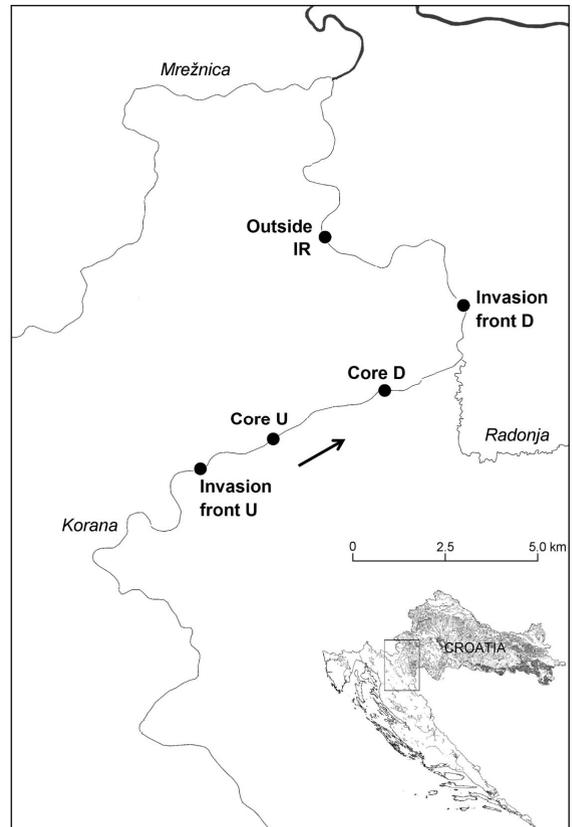


Figure 1. Invaded range of the signal crayfish in the Korana River. Letters U and D depict upstream (U) or downstream (D) site. 'Outside IR' marks the area outside invaded range, where only native crayfish were captured. Arrow depicts the direction of flow.

nearest mm using a digital caliper: total length (TL), carapace length (CL) and carapace width (CW). Upon measurements, native crayfish were released at the exact point of their capture, while signal crayfish were removed from the watercourse. A sample of signal crayfish specimens from each site was frozen at -20 °C immediately after sampling in the field and were transferred in electric car freezer to the Department of Zoology, Faculty of Science in Zagreb for the analyses of physiological condition and capacity for energetic investment into reproduction. Furthermore, for every trapping session, we recorded the catch per unit effort (CPUE; equal to the number of caught crayfish per trap per number of trapping nights) for each species at each site. As CPUE is a frequently used measure of relative crayfish abundance (Dana et al. 2010) and exhibits positive correlation with other measures of population density (Dorn et al. 2005), obtained CPUE was used to compare relative abundance/density of

sampled populations between the sites (invasion front and core).

Body condition

Following Streissl and Hödl (2002) and Maguire and Klobučar (2011), two body condition indices were calculated: Fulton's Condition Factor (FCF = W/TL^3); and the Crayfish Constant (CC = $W/(TL \times CL \times CW)$). Body condition is indicative of animal health and fitness (Peig and Green 2010) and is considered as a useful method for comparison of crayfish population status between sites/habitats (Maguire and Klobučar 2011). Since body condition indices are sex and size-dependent (Streissl and Hödl 2002), only similar sized individuals of the same species and same sex were compared within analyses. Additionally, only adult, intermoult and uninjured crayfish were used in body condition analyses. A total of 593 signal crayfish (275 males and 318 females) were used for body condition analyses.

Body condition indices FCF and CC were used to assess potential differences in condition of the signal crayfish between the invasion front and core sites. Additionally, we analysed potential differences in body condition of the native *A. leptodactylus* from the contact zone with the signal crayfish at invasion fronts and those outside the invaded area (downstream of the invasive range). Due to small sample size of *A. leptodactylus* males (3 males) at the upstream invasion front (Invasion front U; Figure 1), this site was excluded from body condition analyses. A total of 49 *A. leptodactylus* specimens (18 males and 31 females) were used in body condition analyses.

Physiological condition and capacity for energetic investment into reproduction

Comparison of physiological condition and capacity for energetic investment into reproduction between signal crayfish individuals from the invasion front and invasion core was performed by analyzing their organ moisture content. Organosomatic indices and organ moisture content are frequently used to evaluate crayfish condition, their energy reserves and potential effects of environmental stress (Huner et al. 1990; Jussila and Mannonen 1997). These indices depict key physiological processes such as energy storage (hepatosomatic and muscle moisture content) and reproduction capacity (gonad moisture content; Huner et al. 1988; Carmona-Osalde et al. 2004). Since these indices in decapods depend on seasonal dynamics and

year cycle (Yamaguchi 2001; Lucić and Erben 2005), sampling was performed within a short time period of 5 days in July 2012.

Upon arrival to the Department of Zoology in Zagreb, specimens frozen at $-20\text{ }^{\circ}\text{C}$ were thawed, weighted (total wet weight) and dissected. Digestive gland (hepatopancreas), gonads and abdominal muscle were carefully removed, placed in a previously prepared foil cup and immediately weighed to the nearest 0.001g using electronic balance, in order to obtain the weight of wet organ. Cups containing removed tissues were then dried at $80\text{ }^{\circ}\text{C}$ for 24 hours, after which they were weighed once more to obtain the weight of dry organ. Obtained measurements were then used to derive organ moisture content (OM):

$$\text{OM} = (W_{\text{wo}} - W_{\text{do}}) \times 100 / W_{\text{wo}};$$

where W_{wo} = weight of wet organ (g) and W_{do} = weight of dry organ (g). The obtained values are inversely proportional to the energetic status of those organs (Jussila 1997; Jussila and Mannonen 1997; Lucić et al. 2012). Since organ moisture is considered as the most reliable indicator of the energetic status of an animal (cf. Lucić et al. 2012), we used wet and dry weight of organs to derive organ moisture content of hepatopancreas (HM) and gonads (GM) and abdominal muscle (MM). These indices were compared between individuals from the invasion front and core. Following Lucić et al. (2012), and similar to studies on freshwater fish (e.g. Bøhn et al. 2004; Lopez et al. 2012), gonad moisture content was used as an indicator of potential for energetic investment into reproduction, and was measured for females only. Approximately 20 males and 20 females per site were used in all analyses, except for upstream invasion front. At this site only 14 animals (4 males and 10 females) were acquired due to low relative crayfish abundance. A total of 132 specimens ($TL_{\text{males}} = 102.8 \pm 12.1$; $TL_{\text{females}} = 107.5 \pm 10.5$) was used in analyses of organ moisture content.

Statistical analyses

Obtained data were tested to analyze whether they meet the assumptions for parametric tests (i.e. normality/homoscedasticity; Zar 1999). Since both raw and transformed data (logarithmic transformation of continuous data) violated these assumptions, their nonparametric analogues were used instead (Kruskal Wallis ANOVA with post-hoc multiple comparisons of the mean ranks, Mann-Whitney U test, Spearman Rank; Zar 1999).

Comparisons of sex structure between examined sites were performed using Yates' chi-square test (Zar 1999). Since all measured indices (except abdominal muscle moisture content) differed significantly between the sexes (Mann-Whitney U test; $p < 0.05$ for all parameters), males and females were analyzed separately. All analyses were performed in Statistica 12 Programme (Statsoft).

Results

Relative crayfish abundance and sex ratio

Within a 5 day sampling period, a total of 918 crayfish were caught, out of which 95.5% (862 individuals) were invasive signal crayfish. Native *A. leptodactylus* were absent from the two sampled core sites and were only captured at upstream and downstream invasion front (41 specimens in total; $CPUE_{\text{Invasion front U}} = 0.825$; $CPUE_{\text{Invasion front D}} = 0.025$), as well as outside the invasive range (15 individuals; $CPUE_{\text{Outside IR}} = 0.6$). All examined sites differed in the relative abundance of the signal crayfish, with core sites having higher catch per unit effort ($CPUE_{\text{Core U}} = 4.8$; $CPUE_{\text{Core D}} = 6.8$) than invasion fronts ($CPUE_{\text{Invasion front U}} = 0.3$; $CPUE_{\text{Invasion front D}} = 1.7$).

Within the invaded range, sex ratio of the signal crayfish differed significantly between examined sites ($\chi^2 = 27.6$, $p < 0.001$), with significantly higher proportion of males recorded at invasion fronts compared to the invasion core sites (Figure 2).

Body condition

Body condition analyses revealed significant statistical differences between examined sites in crayfish condition for both males (Kruskal Wallis ANOVA; FCF: $H_{(3,275)} = 93.2$, $p \ll 0.001$, CC: $H_{(3,275)} = 45.2$, $p \ll 0.001$; Figure 3a, b) and females (FCF: $H_{(3,318)} = 50.1$, $p \ll 0.001$, CC: $H_{(3,318)} = 13.8$, $p = 0.003$; Figure 3c, d). While post-hoc non-parametric tests showed that the upstream front and core did not always differ significantly in both body condition indices (Figure 3a - d), we recorded a significant negative correlation between relative crayfish abundance (CPUE) and measured body condition indices in both males (Spearman Rank: $R_{\text{FCF}} = -0.536$, $R_{\text{CC}} = -0.381$; $N = 275$; $p \ll 0.001$) and females ($R_{\text{FCF}} = -0.378$, $R_{\text{CC}} = -0.188$; $N = 318$; $p < 0.001$). This shows that animals at sites with lower crayfish abundance (invasion fronts) had a better body condition compared to animals at sites with higher crayfish abundance (invasion core).

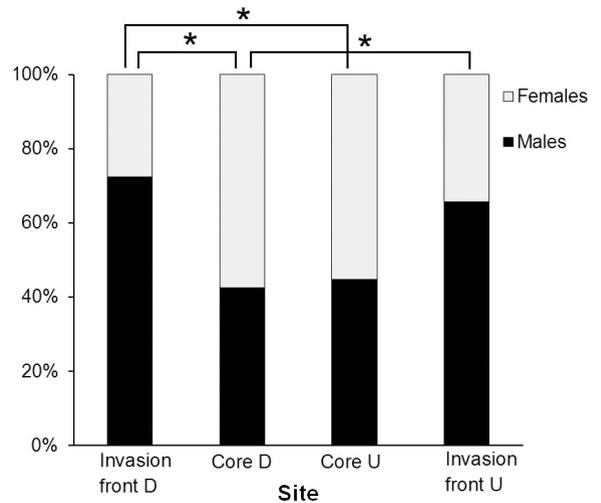


Figure 2. Sex ratio of the signal crayfish along the invaded range. Upstream (Invasion front U) and downstream (Invasion front D) invasion fronts had a significantly higher proportion of males compared to invasion core sites. Statistically significant differences in sex ratio between the sites are marked with *.

No significant differences in body condition of the native *A. leptodactylus* were recorded between examined invasion fronts and *A. letodactylus* populations outside the invasive range, both for males (Mann-Whitney U test, $p > 0.05$ for both parameters; Figure 4a, b) and females (Kruskal Wallis ANOVA, $p > 0.05$ for both indices; Figure 4c, d). However, in both sexes, examined indices exhibited a similar trend, with the highest median values for both indices recorded outside the invasive range (Figure 4a - d). As mentioned earlier, the upstream invasion front was excluded from the analyses of body condition of males due to small sample size.

Physiological condition and capacity for energetic investment into reproduction

Analyses of organ moisture content (HM and MM) in signal crayfish males showed no statistically significant differences between the sites (Kruskal Wallis ANOVA, $p > 0.05$), and no significant correlation between CPUE and examined indices (Spearman rank, $p > 0.05$). Females differed significantly between the sites in moisture content of hepatopancreas (HM: $H_{(3,65)} = 14.7$, $p = 0.002$; Figure 5a) and gonads (GM: $H_{(3,65)} = 12.4$, $p = 0.006$; Figure 5b), but, similar to males, exhibited no significant difference in moisture content of abdominal muscle (MM).

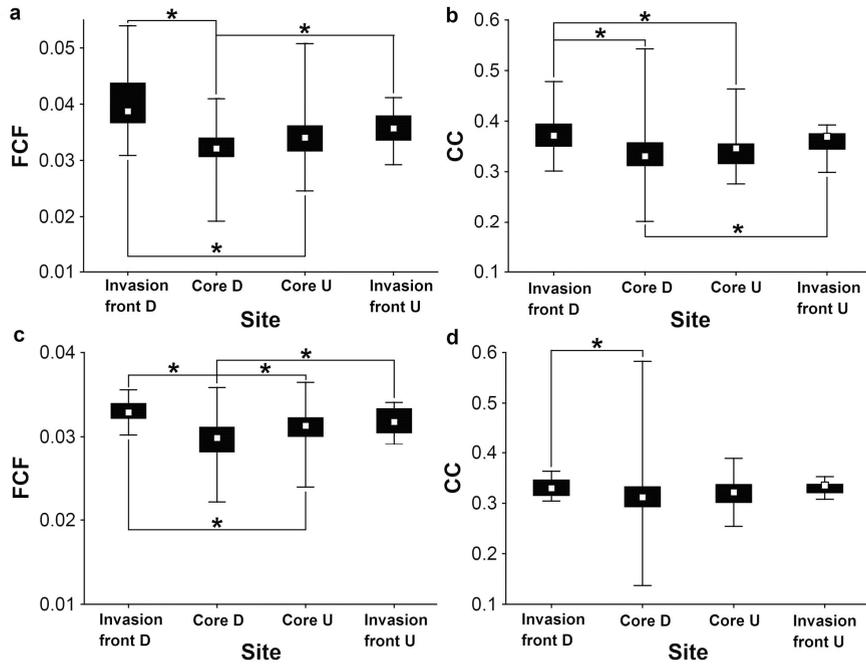


Figure 3. Comparison of body condition indices, Fulton’s condition factor (FCF) and crayfish constant (CC) of signal crayfish males (a, b) and females (c, d) at examined sites. The graphs show medians, quartiles, and non-outlier minima and maxima. Statistically significant differences established using nonparametric post hoc multiple comparisons of mean ranks are marked with *.

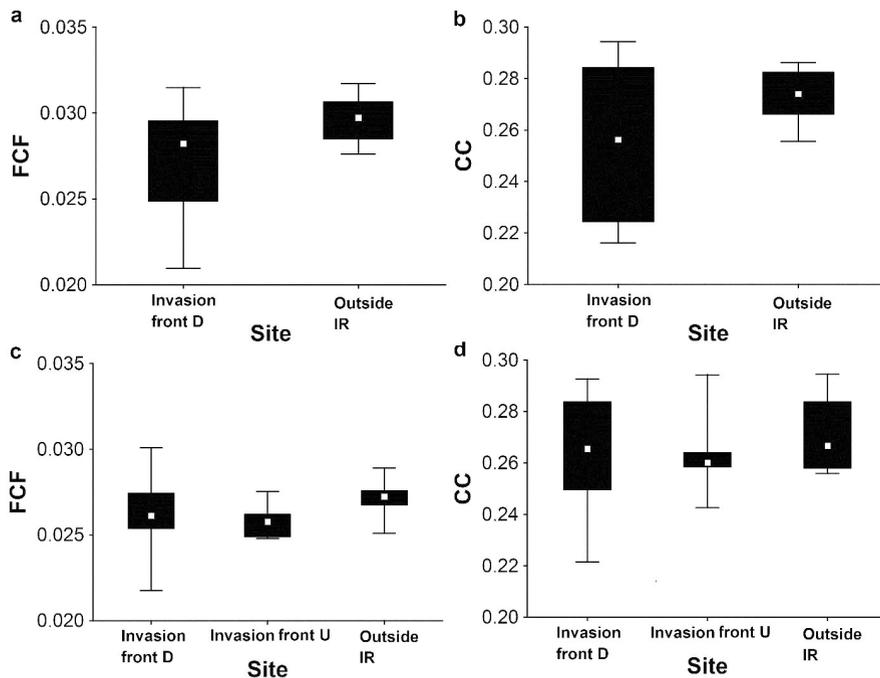
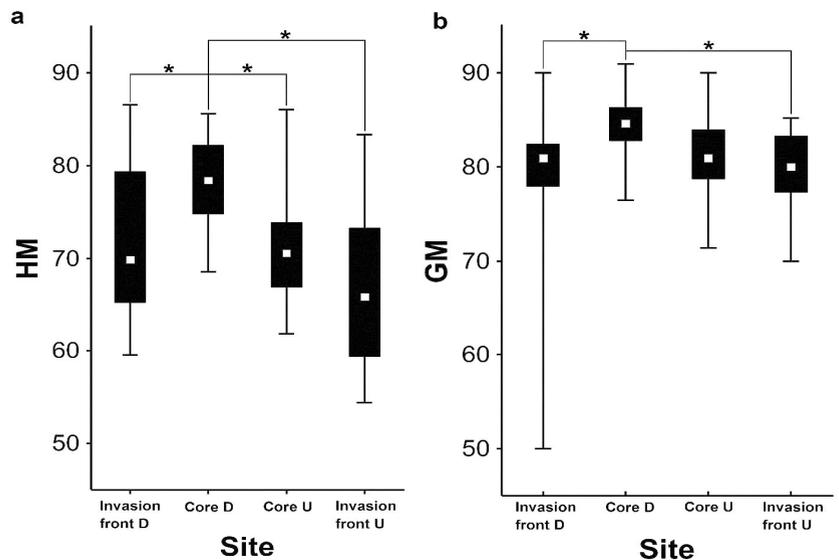


Figure 4. Comparison of body condition indices, Fulton’s condition factor (FCF) and crayfish constant (CC) of males (a, b) and females (c, d) of the native *A. leptodactylus* at invasion fronts where it occurs with the signal crayfish and outside invaded range of the signal crayfish. The graphs show medians, quartiles, and non-outlier minima, and maxima.

Figure 5. Comparison of moisture content of hepatopancreas, HM (a) and gonads, GM (b) in signal crayfish females at examined sites. The graphs show medians, quartiles, and non-outlier minima, and maxima. Statistically established significant differences established using nonparametric post hoc multiple comparisons of mean ranks are marked with *.



Furthermore, significant positive correlations between CPUE and HM and GM were recorded ($R_{HM}=0.378$, $R_{GM}=0.418$; $N=65$; $p < 0.001$) for females. Since organ moisture content indices are inversely proportional to the energetic status of those organs, obtained results show that females from sites with a lower crayfish abundance (invasion fronts) had lower moisture content, i.e. better energetic status of hepatopancreas and gonads than females from sites with a higher crayfish abundance (invasion core).

Discussion

The signal crayfish in a recently invaded region of Croatia exhibited differences between the invasion front and core sites in relative crayfish abundance, sex ratio, body condition and energetic status of hepatopancreas and gonads in females. Such results are in line with the suggested plasticity of freshwater invaders, established by analyses of trait variability across the invasive range of different species (i.e. round goby, African jewelfish; Gutowsky and Fox 2011; 2012; Lopez et al. 2012; Brandner et al. 2013), including signal crayfish (Hudina et al. 2012). As crayfish were sampled with size-biased baited traps (cf. Dorn et al. 2005), performed inter-population comparisons in this study were limited to adult samples of the signal crayfish populations. Even though the use of traps may lead to sampling biases (Price and Welch 2009), the standardization of the trapping period, number of traps, bait type and microhabitats where traps were set, ensured that performed

comparisons reflect potential differences between the signal crayfish population samples at examined sites.

In the present study, upstream and downstream invasion front were male dominated and exhibited 4 to 16 times lower relative crayfish abundance compared to respective population samples at the invasion core. Reduction in conspecific density at expanding edges of population is an expected consequence of dispersal (Phillips et al. 2010). The prevalence of males in population samples at invasion fronts indicated that the initial dispersal of the signal crayfish may be biased towards males. Male biased sex ratios at the invasion front were already recorded for the signal crayfish (Hudina et al. 2012; but see Almeida et al. 2013 for analysis by population size-classes), while studies on freshwater invasive fish reported both male-biased (Gutowsky and Fox 2011) and female-biased sex ratios (cf. Brandner et al. 2013). As male-biased dispersal may emerge if the variance in reproductive success between patches is larger for males than females (Gros et al. 2009), males may be the first to disperse from the invasion core (i.e. area of high conspecific density) due to their high aggression levels. Previous studies have shown that the spatial distribution of crayfish is affected by their social status (Fero and Moore 2008). As males are more aggressive and compete more intensely than females over resources such as food, shelter and mates (cf. Fero et al. 2007; Berry and Breithaupt 2010), they may benefit more from dispersal to areas with reduced conspecific density.

Previous theoretical and field research highlighted the potential benefits of dispersal to areas with low conspecific density, which may result in exponential population growth and evolution of traits determining such growth (i.e. increase in individual growth rates and reproductive investment/fecundity; Phillips et al. 2010; Burton et al. 2010). Theoretical studies also highlighted potential trade-offs between traits determining population growth/dispersal and competitive ability in the presence of niche competitor (Burton et al. 2010). We hypothesized that competitive pressure posed by the niche competitor should create a distinct spatial pattern of variation in examined traits. If a pressure posed by the presence of interspecific competitor is weak, benefits of reduced conspecific density in the novel environment should outweigh the potential costs of interspecific competition. Such conditions should therefore lead to increase in condition and reproduction capacity of individuals at expanding edges. On the other hand, if interspecific competition poses a significant challenge to expanding signal crayfish, we expected that such spatial pattern would be lacking (cf. Burton et al. 2010).

Since no infection by crayfish plague was recorded in the invaded range in the Korana River at the time of sampling (Maguire et al. 2013), and since *A. leptodactylus* has demonstrated the ability to outcompete other crayfish invaders (e.g. *Orconectes limosus* in France; Laurent 2003), we consider it as a viable competitor to the signal crayfish. In this study, the signal crayfish from the invasion front were in a significantly better body condition compared to those from the invasion core. Both body condition indices revealed significant differences between examined sites in both males and females. Additionally, invasion front females also exhibited significantly better energetic status of both hepatopancreas, a major energy storage organ in crustaceans (Johnston et al. 1998), and gonads. Gonad moisture content (GM) indicated a significantly higher potential for energetic investment into reproduction in females from invasion front. Moreover, both body condition and organ moisture indices exhibited a significant correlation with relative crayfish abundance. Individuals from sites with lower relative crayfish abundance (upstream and downstream invasion front) had better body condition and higher energetic status of organs compared to those from sites with higher crayfish abundance (invasion core sites). Such results are in line with the findings of several

studies on invasive freshwater fish and amphibians, which demonstrated that individuals at invasion fronts benefit from reduced intraspecific density/competition intensity and elevated resource availability by exhibiting increase in individual growth rates (Phillips et al. 2010; Brandner et al. 2013), body condition (Bøhn et al. 2004; Gutowsky and Fox 2012; Lopez et al. 2012; Brandner et al. 2013) and potential for reproductive investment (Gutowsky and Fox 2012; Lopez et al. 2012; Brandner et al. 2013). Furthermore, since significant trait variation was recorded between the invasion front and core populations of the signal crayfish, and since populations of native *A. leptodactylus* at the edges of invasive range (upstream and downstream invasion front) were in a poorer (albeit not significantly) body condition than those outside the invasive range, we conclude that the competitive challenge posed by the native niche competitor was not strong. Moreover, interspecific competition at invasion fronts affected the native *A. leptodactylus* more strongly than the signal crayfish. As relative crayfish abundance of *A. leptodactylus* outside the invasive range was well below the CPUE of the signal crayfish from the invasion core, signal crayfish still benefited from the reduced conspecific density in the presence of an inferior competitor.

During range expansion, the benefits of reduced conspecific density in the novel environment, as well as external factors such as the presence and pressure from niche competitors, can contribute to trait variability along the invasion pathway. Identification of these internal and external drivers of trait variability during the invasion is essential for improving our understanding of the exceptional speed at which invasive species such as signal crayfish are able to expand their range. As such opposing drivers could create distinct spatial and temporal patterns in trait variation, our research highlights the importance of examining the changes in population characteristics as an invader advances its range. Such studies could contribute to the understanding of processes that occur between establishment and dispersal phase, since a shift between the rate of population growth and counteracting pressure from other niche competitors could represent one of the crucial determinants of a lag between the establishment and expansion of introduced species. These insights could have important implications for risk assessments and management of established populations of different invasive species, especially in vulnerable ecosystems (Dudgeon et al. 2006; Strayer 2010) such as freshwaters.

Acknowledgements

We thank Nina Jeran and Krešimir Trgovčić for their help during fieldwork, and Krešimir Žganec for fieldwork assistance as well as for helpful discussions of this manuscript. We are very grateful to NGO Sedra for technical assistance during sampling. Furthermore, we thank Ivana and Adam Maguire for proofreading the manuscript, as well as the editor and three anonymous reviewers for their helpful comments.

References

- Almeida D, Argent R, Ellis A, England J, Copp GH (2013) Environmental biology of an invasive population of signal crayfish in the River Stort catchment (southeastern England). *Limnologia - Ecology and Management of Inland Waters* 43: 177–184, <http://dx.doi.org/10.1016/j.limmo.2012.09.002>
- Berry FC, Breithaupt T (2010) To signal or not to signal? Chemical communication by urine-borne signals mirrors sexual conflict in crayfish. *BMC Biology* 8: 25, <http://dx.doi.org/10.1186/1741-7007-8-25>
- Bøhn T, Sandlund OT, Amundsen P, Primicerio R (2004) Rapidly changing life history during invasion. *Oikos* 106: 138–150, <http://dx.doi.org/10.1111/j.0030-1299.2004.13022.x>
- Brandner J, Cerwenka AF, Schliewen UK, Geist J (2013) Bigger is better: characteristics of round gobies forming an invasion front in the Danube river. *PLoS ONE* 8(9): e73036, <http://dx.doi.org/10.1371/journal.pone.0073036>
- Burton OJ, Phillips BL, Travis JMJ (2010) Trade-offs and the evolution of life-histories during range expansion. *Ecology Letters* 13: 1210–1220, <http://dx.doi.org/10.1111/j.1461-0248.2010.01505.x>
- Carmona-Osalde C, Rodriguez-Serna M, Olvera-Novoa M a, Gutierrez-Yurrita PJ (2004) Gonadal development, spawning, growth and survival of the crayfish *Procambarus llamasii* at three different water temperatures. *Aquaculture* 232: 305–316, [http://dx.doi.org/10.1016/S0044-8486\(03\)00527-1](http://dx.doi.org/10.1016/S0044-8486(03)00527-1)
- Dana E, López-Santiago J, García-de-Lomas J, García-Ocaña D, 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, <http://dx.doi.org/10.3391/ai.2010.5.3.10>
- Dieguez-Urbeondo J (2006) The dispersion of the *Aphanomyces astaci*-carrier *Pacifastacus leniusculus* by humans represents the main cause of disappearance of the indigenous crayfish *Austropotamobius pallipes* in Navarra. *Bulletin Français de la Pêche et de la Pisciculture* 380–381: 1303–1312, <http://dx.doi.org/10.1051/kmae:2006036>
- Dorn NJ, Urgelles R, Trexler JC (2005) Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland. *Journal of the North American Benthological Society* 24: 346–356, <http://dx.doi.org/10.1899/04-037.1>
- Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard A-H, Soto D, Stiassny MLJ, Sullivan CA (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological reviews of the Cambridge Philosophical Society* 81: 163–182, <http://dx.doi.org/10.1017/S1464793105006950>
- Fero K, Moore P (2008) Social spacing of crayfish in natural habitats: what role does dominance play? *Behavioral Ecology and Sociobiology* 62: 1119–1125, <http://dx.doi.org/10.1007/s00265-007-0540-x>
- Fero K, Simon JL, Jourdie V, Moore PA (2007) Consequences of social dominance on crayfish resource use. *Behaviour* 144: 61–82, <http://dx.doi.org/10.1163/15685390779947418>
- Groen M, Sopinka NM, Marentette JR, Reddon AR, Brownscombe JW, Fox MG, Marsh-Rollo SE, Balshine S (2012) Is there a role for aggression in round goby invasion fronts? *Behaviour* 149: 685–703, <http://dx.doi.org/10.1163/1568539X-00002998>
- Gros A, Poethke HJ, Hovestadt T (2009) Sex-specific spatio-temporal variability in reproductive success promotes the evolution of sex-biased dispersal. *Theoretical Population Biology* 76: 13–18, <http://dx.doi.org/10.1016/j.tpb.2009.03.002>
- Gutowsky LFG, Fox MG (2012) Intra-population variability of life-history traits and growth during range expansion of the invasive round goby, *Neogobius melanostomus*. *Fisheries Management and Ecology* 19: 78–88, <http://dx.doi.org/10.1111/j.1365-2400.2011.00831.x>
- Gutowsky LFG, Fox MG (2011) Occupation, body size and sex ratio of round goby (*Neogobius melanostomus*) in established and newly invaded areas of an Ontario river. *Hydrobiologia* 671: 27–37, <http://dx.doi.org/10.1007/s10750-011-0701-9>
- Hogger J (1988) Ecology, population biology and behaviour. In: Holdich DM, Lowery R (eds), *Freshwater crayfish: Biology, Management and Exploitation*. The University Press, Cambridge, UK, 498 pp
- Holdich DM, Reynolds JD, Souty-Grosset C, Sibley PJ (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, <http://dx.doi.org/10.1051/kmae/2009025>
- 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: 09, <http://dx.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, <http://dx.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, <http://dx.doi.org/10.5869/fc.2013.v19.021>
- Huner JV, Kõnönen H, Lindqvist OV (1990) Variation in body composition and exoskeleton mineralization as functions of the molt and reproductive cycles of the noble crayfish, *Astacus astacus* L. (Decapoda, Astacidae), from a pond in central Finland. *Comparative Biochemistry and Physiology Part A: Physiology* 96: 235–240, [http://dx.doi.org/10.1016/0300-9629\(90\)90071-Y](http://dx.doi.org/10.1016/0300-9629(90)90071-Y)
- Huner JV, Lindqvist OV, Kõnönen H (1988) Comparison of morphology and edible tissues of two important commercial crayfishes, the noble crayfish, *Astacus astacus* Linné, and the red swamp crayfish, *Procambarus clarkii* (Girard) (Decapoda, Astacidae and Cambaridae). *Aquaculture* 68: 45–57, [http://dx.doi.org/10.1016/0044-8486\(88\)90290-6](http://dx.doi.org/10.1016/0044-8486(88)90290-6)
- Johnston DJ, Alexander CG, Yellowhees D (1998) Epithelial cytology and function in the digestive gland of *Themus orientalis* Decapoda: Scyllaridae. *Journal of Crustacean Biology* 18: 271–278, <http://dx.doi.org/10.2307/1549320>
- Jussila J (1997) Carapace mineralization and hepatopancreatic indices in natural and cultured populations of marron (*Cherax tenuimanus*) in Western Australia. *Marine and Freshwater Research* 48: 67, <http://dx.doi.org/10.1071/MF96057>
- Jussila J, Mannonen A (1997) Marron (*Cherax tenuimanus*) and noble crayfish (*Astacus astacus*) hepatopancreas energy and its relationship to moisture content. *Aquaculture* 149: 157–161, [http://dx.doi.org/10.1016/S0044-8486\(96\)01425-1](http://dx.doi.org/10.1016/S0044-8486(96)01425-1)

- Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16: 199–204, [http://dx.doi.org/10.1016/S0169-5347\(01\)02101-2](http://dx.doi.org/10.1016/S0169-5347(01)02101-2)
- Laurent PJ (2003) Can *Astacus leptodactylus* Eschscholtz, 1823 stop the progress of *Orconectes limosus* Rafinesque, 1871? *Association des Astaciculteurs de France* 74: 8–16
- Lockwood JL, Hoopes MF, Marchetti MP (2007) *Invasion Ecology*, 1st edn. Blackwell Publishing, Oxford, 299 pp
- Lopez DP, Jungman AA, Rehage JS (2012) Nonnative African jewelfish are more fit but not bolder at the invasion front: a trait comparison across an Everglades range expansion. *Biological Invasions* 14: 2159–2174, <http://dx.doi.org/10.1007/s10530-012-0221-1>
- Lucić A, Erben R (2005) Seasonal changes of the condition indices and haemocyte counts of the *Austropotamobius torrentium* population in Kraljevec stream, Croatia. *Bulletin Français de la Pêche et de la Pisciculture* 367–377: 687–697
- Lucić A, Hudina S, Faller M, Cerjanec D (2012) A comparative study of the physiological condition of native and invasive crayfish in Croatian rivers. *Biologia* 67: 172–179, <http://dx.doi.org/10.2478/s11756-011-0151-x>
- Maguire I, Klobučar G (2011) Size structure, maturity size, growth and condition index of stone crayfish (*Austropotamobius torrentium*) in North-West Croatia. *Knowledge and Management of Aquatic Ecosystems* 401: 12, <http://dx.doi.org/10.1051/kmae/2011026>
- Maguire I, Jelić M, Klobučar G, Delaunay C, Grandjean F (2013) Prevalence of pathogen *Aphanomyces astaci* in freshwater crayfish populations in Croatia. Proceedings of the Regional European Crayfish meeting, Rovinj, Croatia, p 48
- Marchetti MP, Moyle PB, Levine R (2004) Alien fishes in California watersheds: characteristics of successful and failed invaders. *Ecological Applications* 14: 587–596, <http://dx.doi.org/10.1890/02-5301>
- Olden JD, Poff NL, Bestgen KR (2006) Life-history strategies predict fish invasions and extirpations in the Colorado River Basin. *Ecological Monographs* 76: 25–40, <http://dx.doi.org/10.1890/05-0330>
- Peig J, Green AJ (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Functional Ecology* 24: 1323–1332, <http://dx.doi.org/10.1111/j.1365-2435.2010.01751.x>
- Phillips B (2009) The evolution of growth rates on an expanding range edge. *Biology Letters* 5: 802–804, <http://dx.doi.org/10.1098/rsbl.2009.0367>
- Phillips B, Brown G, Shine R (2010) Life-history evolution in range-shifting populations. *Ecology* 91: 1617–1627, <http://dx.doi.org/10.1890/09-0910.1>
- Price JE, Welch SM (2009) Semi-quantitative methods for crayfish sampling: sex, size, and habitat bias. *Journal of Crustacean Biology* 29: 208–216, <http://dx.doi.org/10.1651/08-3018R.1>
- Pyšek P, Richardson DM (2010) Invasive Species, Environmental Change and Management, and Health. *Annual Review of Environment and Resources* 35: 25–55, <http://dx.doi.org/10.1146/annurev-environ-033009-095548>
- Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* 287: 1770–1774, <http://dx.doi.org/10.1126/science.287.5459.1770>
- Shine R, Brown GP, Phillips BL (2011) An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences of the United States of America* 108: 5708–5711, <http://dx.doi.org/10.1073/pnas.1018989108>
- Sih A, Ferrari MCO, Harris DJ (2011) Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications* 4: 367–387, <http://dx.doi.org/10.1111/j.1752-4571.2010.00166.x>
- Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle DA, Aronson J, Courchamp F, Galil B, Garcia-Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* 28: 58–66, <http://dx.doi.org/10.1016/j.tree.2012.07.013>
- 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, <http://dx.doi.org/10.1111/j.1365-2427.1995.tb01168.x>
- Souty-Grosset C, Holdich DM, Noel P, Reynolds JD, Haffner P (ed) (2006) Atlas of Crayfish in Europe. Museum National d'Histoire Naturelle, Paris, France, 188 pp
- State Institute for Nature Protection (2009) Biodiversity of Croatia. State Institute for Nature Protection, Zagreb, Croatia, 45 pp
- Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55: 152–174, <http://dx.doi.org/10.1111/j.1365-2427.2009.02380.x>
- Streissl F, Hödl W (2002) Growth, morphometrics, size at maturity, sexual dimorphism and condition index of *Austropotamobius torrentium* Schrank. *Hydrobiologia* 477: 201–208, <http://dx.doi.org/10.1023/A:1021046426577>
- Twardochleb L, Olden JD, Larson ER (2013) A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science* 32: 1367–1382, <http://dx.doi.org/10.1899/12-203.1>
- 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, <http://dx.doi.org/10.1034/j.1600-0587.2002.250107.x>
- Williamson MH, Fitter A (1996) The characters of successful invaders. *Biological Conservation* 78: 163–170, [http://dx.doi.org/10.1016/0006-3207\(96\)00025-0](http://dx.doi.org/10.1016/0006-3207(96)00025-0)
- Yamaguchi T (2001) Seasonal changes of the hepatopancreas index in females of the fiddler crab, *Uca lactea*. *Crustaceana* 74: 627–634, <http://dx.doi.org/10.1163/156854001750377902>
- Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, London, UK, 663 pp