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PRIMARY RESEARCH PAPER

Invasive dynamics of the signal crayfish *Pacifastacus leniusculus* in a protected area

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Abstract Invasive species have been drivers of biodiversity loss and functional changes in aquatic ecosystems, including in protected areas. Therefore, monitoring population invasion dynamics and biological traits is fundamental to better understand their ecological and economic impacts and for management actions development. We followed signal crayfish (*Pacifastacus leniusculus*) invasion in Rabaçal River upper reach at Montesinho Natural Park, Portugal. We collected information on the spread and biological traits (abundance, size, weight, physical condition, sex ratio, and aggressiveness) to assess differences between invasion core and front areas and among years. Signal crayfish population remained restricted since first reports in 2013 in the invasion core until 2017. After 2019, signal crayfish population has been spreading downstream, decreasing abundance at invasion core but increasing at invasion front. Significant higher number of crayfish with claw loss indicate potential higher signs of aggressiveness in the invasion front. Results also demonstrate a significant dominance of females although sex ratio is closer to 1:1 at the invasion front. Overall, results indicate signal crayfish is spreading and increasing their abundance at Rabaçal River highlighting the need for immediate management actions to hold dispersion and mitigate possible impacts.

Keywords Population dynamics · Invasive non-native species · Autecology · Establishment · Spread · Traits

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Introduction

Invasive non-native species are one of the major drivers of global change across ecosystems causing a multi-million dollar impact on the global economy (Epanchin-Niell, 2017; Diagne et al., 2021; Lovell et al., 2021). Although biological invasions appear

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to follow a similar pattern (e.g., related to the propagule pressure and environmental match; Lockwood et al., 2009), the invasion process has a complex dynamics where spatial heterogeneity, temporal variability, inter- and intraspecific interactions, or evolution might play an important role that may contribute to idiosyncrasy (Hastings et al., 2005; Haubrock et al., 2024; Sousa et al., 2024). In the last decades, the large majority of studies about invasive non-native species has focused on the causes of biological invasions or their impacts (Lowry et al., 2013), neglecting comprehensive long-term studies that focus on the invasion dynamics through time. The lack of generalizations associated with invasion patterns increases the difficulty in the development of management strategies and highlights the need for a deeper understanding of the autecology of the target invasive non-native species (Maguire, 2004; Arim et al., 2006; Haubrock et al., 2024).

The efficiency of the management of invasive non-native species is highly dependent on the understanding of the dynamics of the invasion process (Simberloff et al., 2013; Haubrock et al., 2024). In fact, many non-native species after establishment may remain in a lag phase before they become invasive and cause important impacts on ecosystems (Crooks, 2005; Strayer et al., 2017; Coutts et al., 2018). In this vein, the development of life history and demographic models has been pointed as crucial for the design of effective management strategies at different invasion stages (Sakai et al., 2001). However, these models need to be fueled and refined by in situ field data on species population dynamics and key biological traits, such as abundance, biomass, sex ratio, growth, mortality, and body condition, which may change along the invasion process (Sousa et al., 2024). This information will help to better predict the magnitude of the impacts and to develop targeted and effective control strategies and realistic estimation of management costs (Jardine & Sanchirico, 2018; Pergl et al., 2020). Because assessments need multiple years of sampling, which are constrained by the duration of funded research projects, they are difficult to implement and such studies are nowadays not considered cutting-edge science, although they are fundamental to understand and potentially decrease the impacts of the target invasive non-native species (see Reinke et al., 2019).

Freshwater non-indigenous crayfish species (NICS) are successful invaders responsible for negative impacts on biodiversity and ecosystem processes globally (Twardochleb et al., 2013). Due to their omnivory, these species are able to disrupt food webs through predation, competition (by outcompeting native species for basal resources), or by carrying novel diseases that affect native crayfish populations (Lodge et al., 2012). Several traditional or innovative methodologies have been used to detect and control NICS in aquatic ecosystems (for a review see Manfrin et al., 2019), and models have been developed to forecast their spread and control NICS populations (e.g., Filipe et al., 2017; Messager & Olden, 2018). However, efforts to control NICS have largely been unsuccessful, including the application of sterile male release technique SMRT in the field (Green et al., 2022). Consequently, the management of crayfish invasions continues to have a severe impact on the global economy, particularly in Europe where their management causes a significant cost of US$60 million per year (Kouba et al., 2022).

Recent advances have greatly improved our understanding of invasive crayfish dynamics. This research focused on crayfish movement behavior, the differences between front areas (recently invaded regions and invasion edges) and core areas (where the species was initially introduced and established a stable population), as well as their evolutionary patters (e.g., Johnson et al., 2006; Rubenson & Olden, 2017). Important biological traits might change the way that invasive non-native species disperse and affect ecosystems. For example, crayfish individuals in the invasion core and front might have different behavior (i.e., more aggressive at the core; Hudina et al., 2015). Biological traits, such as sex, body size, or physiological condition, may act as important drivers on the spread dynamics of invasive species (Phillips et al., 2006) and can alter the impacts of crayfish on key ecological processes along the invasion gradient (Carvalho et al., 2018; Alves et al., submitted). In the same vein, these impacts may vary seasonally, and this may differentially affect native communities through the years and even can have evolutionary consequences for native biodiversity (Mathers et al., 2016; Sousa et al., 2019a; Carvalho et al., 2022). Therefore, monitoring the patterns and dynamics of NICS populations through time can guide the implementation of specific management strategies at optimal time periods and decrease the magnitude of their impacts (Olden et al., 2006; Rogowski et al., 2013).

Our study focused on the invasion dynamics of the signal crayfish [*Pacifastacus leniusculus* (Dana, 1852)] in the free-flowing upper reach of Rabaçal River in the Montesinho Natural Park (NE Portugal). Crayfish presence in Portuguese freshwaters is limited to the red-swamp-crayfish [*Procambarus clarkii* (Girard, 1852)], which is widespread in most river basins across the country (but not in the study area) and the signal crayfish, which is restricted to the NE region of Portugal. Without native taxonomic relatives, NICS may have significant impacts on Portuguese freshwater ecosystems and species by having top-down and bottom-up effects on trophic webs through predation (also serving as prey for several fish, bird and mammal species), competition or by changing nutrient and energy dynamics acting as ecosystem engineers (e.g., Carvalho et al., 2022; Sousa et al., 2019a, b), particularly the signal crayfish, which is mainly found in the low-impacted rivers inside or close to the Montesinho Natural Park. Predation pressure in the area is mainly imposed by larger brown trout (*Salmo trutta* Linnaeus, 1758) individuals and Eurasian otter [*Lutra lutra* (Linnaeus, 1758)]. Nevertheless signal crayfish population is thriving and spreading in that area in the last decade. We aimed to assess population dynamics of this invasive non-native species by (i) examining changes in species range between 2017 and 2022 along 25 km in the upper reach of Rabaçal river and (ii) characterizing key biological traits, such as abundance, size structure, sex ratio, and physiological condition along the years and the invasion gradient. We predicted that (i) invasive signal crayfish population range is increasing over time; (ii) abundance is higher in the invasion core than in the front area, (iii) size and physiological condition of invasive crayfish is higher in the invasion front than in the core areas; and (iv) a sex ratio of 1:1 is maintained at invasion front and core areas.

Materials and methods

Study area

The Montesinho Natural Park located in NE Portugal is a 79,000 ha protected area created in 1979 (Nogueira et al., 2021). The primary area and the buffer region of Montesinho Natural Park are characterized by low anthropogenic disturbance, with land use in the area primarily related to forestry and agricultural activities (Nogueira et al., 2021). Montesinho Natural Park hosts an important biodiversity across different taxa and has particular focus on the conservation of plants, terrestrial vertebrates, and birds (Cabrita et al., 2000) although it contains important habitats for many aquatic species of conservation interest (see below). The climate and hydrological regime in the region is characterized by high seasonal variability in terms of temperature and precipitation (Oliveira et al., 2012; Sousa et al., 2019b). This variation in precipitation is responsible for pronounced changes in river flow that range from minimum values during late summer/early autumn and maximum values during autumn and winter and early spring (mean 27.07m^3/s in the Rabaçal River; Oliveira et al., 2012; Sousa et al., 2018).

The Rabaçal River is tributary of the Tua River (Douro basin) with a total extension of 88 km (Fig. 1). Although its spring is in Spain, the majority of the Rabaçal River basin area is located in NE Portugal. The study area focused on the upper section of the Rabaçal River, restricted downstream by the Rebordelo dam and within the protected area of the Montesinho Natural Park. Because the dam acts as a physical barrier to organism spread and upstream of the dam is the most undisturbed section of the river, we focused our sampling in the 25 km of the upper reach. The Rabaçal River is an important habitat for many species of conservation interest, which includes the dragonfly *Macromia splendens* (Pictet, 1843) (IUCN: Vulnerable; Annexes II and IV of Habitats directive) or the Iberian desman *Galemys pyrenaicus* (É. Geoffroy Saint-Hilaire, 1811) (IUCN: Endangered; Annexes II and IV of Habitats directive) and hosts the most well-preserved population of the freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus,1758) (IUCN: Endangered; Annexes II and V of Habitats directive) in Portugal (Sousa et al., 2015, 2020). Fish biodiversity is characterized by the Northern Iberian spined loach *Cobitis calderoni* Băcescu, 1962 (IUCN: Endangered; Annex II of Habitats directive) from the Cobitidae family, brown trout *Salmo trutta* from the Salmonidae family and few minnows such as Northern straight-mouth nase *Pseudochondrostoma duriense* (Coelho,

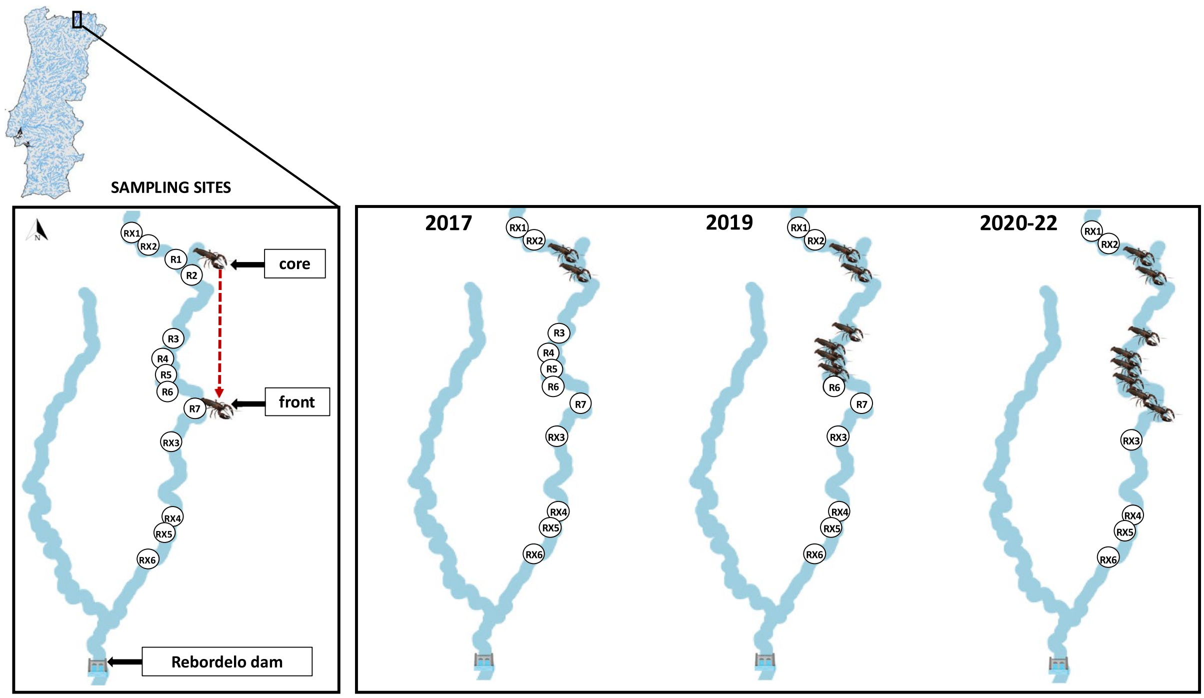

Fig. 1 Map of the sampling area and sampling sites In Rabaçal river upstream Rebordelo dam at Montesinho National Park. Invasion core corresponds to sites R1 and R2, while the invasion front to recently invaded sites from R3 to the invasion edge R7. Signal crayfish was present at 2 sites in 2017 (R1; R2), 5 sites in 2019 (R1–R5), and 7 sites in 2020–2022 (R1–R7)

1985) (IUCN: Vulnerable), calandino *Squalius alburnoides* (Steindachner, 1866) (IUCN: Vulnerable), Iberian barbel *Luciobarbus bocagei* (Steindachner, 1864), and northern Iberian chub *Squalius carolitertii* (Doadrio, 1988) (Sousa et al., 2020). No native crayfish is or has been present in the area. The presence of the invasive signal crayfish (*P. leniusculus*) was first detected in Portugal in 1997 in a tributary of the Sabor River in the Douro basin (Bernardo et al., 2011) and described in the study area in 2013 (Sousa et al., 2015). Since then, the species has been spreading and is considered one the major threats for local biodiversity and ecosystem functioning (Anastácio et al., 2019; Meira et al., 2019; Sousa et al., 2019a; Carvalho et al., 2022).

Field sampling

To assess the distribution and population dynamics of signal crayfish, we sampled a total of 13 sites between 2017 and 2022 (except for 2018) in late summer (end of August–beginning of September). Sampling sites were kept along the years, although some new sites were added each year to follow the downstream spread (Fig. 1). In 2017, 13 sampling sites were selected to assess the distribution of *P. leniusculus* in the Rabaçal River. In 2019–2022, we sampled a total of 10 sites: the 2 sites where signal crayfish was detected in 2017, 2 upstream and 6 downstream sites to follow their spread. At each site, we selected a river stretch 50–100 m and covered all the available habitats including pools, runs and riffles from banks to the center of the river channel. Crayfish were captured by placing 10—13 funnel traps at each site, two rectangular (50 × 30 × 20 cm; 0.5 cm mesh pore) and the others cylindrical (43 cm *d*, 22 cm h; 1.5 cm mesh) for 24 h. The abundance, size structure, and sex characterization were assessed at each trap per site every year. Relative abundance at each sampling site and year was calculated as CPUE (the number of individuals captured by number of traps by time; following Sousa et al., 2013, 2019a). For each sampling year, traps were set on a single occasion at each sampling site. Individuals were measured from the rostrum tip to telson rear edge (total length) and sex determined following Sousa et al. (2013; 2019a). Absence of claws in each individual was also registered as an indirect measure of aggressiveness. Claw injuries can be related with aggressive behavior between conspecifics (e.g., Kawai et al., 1994; Kouba et al., 2011) and also with population density (Savolainen et al., 2004; Ramalho et al., 2008). Weight was only collected in 2019, 2021, and 2022. Fulton's Condition Index was calculated for the years 2019, 2021, and 2022 using the formula:

$$\text{Fulton's Condition Index} = \frac{w}{TL^3} \times 100,$$

where w = weight of the individual and TL = total length of the individuals and following (Anastácio & Marques, 1998; Tarandek et al., 2023). None of the individuals captured was released back to water, following the Portuguese law (Decreto-lei No. 565/99 de 21 de Dezembro) and ICNF (Institute of Forests and Nature Conservation) dispositions.

Data analysis

To address differences in crayfish abundance, sex, size, and condition index between different invasion areas, we used "dispersion" as a variable. To that end, we split sampling sites into invasion zones "core" and "front." We considered as "core" areas the sampling sites where the signal crayfish was already present in 2017, which was restricted to a 500 m river stretch, and as "front" areas the sites invaded by the crayfish after 2017. In total, we considered 1139 crayfish in the invasion "core" and 1945 crayfish in the invasion "front." The year 2017 was not considered since crayfish presence was constrained to 2 sites considered as "core." Variations in relative abundance, biological (i.e., size), and physiological traits (i.e., condition index) among sampling sites or among years were compared by parametric one-way ANOVAs followed by Holm's post hoc tests. ANOVAs were preceded by Shapiro–Wilk to check the normality of the residuals and Bartlett test to check for homoscedasticity or normality was assumed if the number of observations satisfied the assumptions of the central limit theorem (Zar, 2013). Non-parametric Kruskal–Wallis tests were used when data failed to meet those assumptions. Trait variations (e.g., abundance, size, and condition index) between invasion zones were tested by Welch's *t* tests. Variations between crayfish sex and claw loss between invasion zones and sites were compared by Chi-square tests. All statistics and plots were performed in R software (Team, 2020; version 3.6.3) using the packages "car," "dplyr," "psych" for statistics and "ggplot2," 'ggstatsplot,' and "maptools" for visualization.

Results

The invasive range of signal crayfish at Rabaçal River increased from 0.5 km in 2017 to 10.7 km in 2020–2022 at a dispersion rate of ca. 2.04 km year^{-1} downstream (Fig. 1). In the first sampling year (2017), signal crayfish was only present at 2 upstream sites restricted in a 500 m reach (R1 and R2). In 2019, *P. leniusculus* was found at 5 sites (7.1 km), while in 2020–2022 at 7 sites (10.7 km) (Fig. 1). In 2022, signal crayfish invasion covered 42.9% of the total length of the sampling reach and was absent in 6 out of 13 sampled sites. Signal crayfish was absent upstream the invasion core at RX1 and RX2 sites and downstream the invasion front at RX3 to RX6 (Fig. 1).

During the study, a total of 3084 crayfish were captured (297 in 2017, 531 in 2019, 482 in 2020, 761 in 2021, and 1013 in 2022). Crayfish relative abundance was higher in 2017 (23.2 individuals CPUE) considering the 2 invaded sites. In 2019, mean crayfish relative abundance was 9.5 ind. CPUE at the 5 invaded sites. Since 2020, relative abundance increased from 6.8 ind. CPUE to 11.3 in 2021 and 19.1 in 2022 across the 7 invaded sites (Fig. 2A). In the invasion core, maximum relative abundance decreased from 26.3 ind. CPUE in 2017 to 13.5, while in the invasion front increased from 12.7 ind. CPUE in 2019 to 40.5 in 2022 (Fig. 2B). In the invasion core, mean relative abundance was 15.83 ind. CPUE, while in the invasion front was 11.63. Among invaded sites, crayfish mean relative abundance was highest at R2 (17.61 ind. CPUE) and R4 (15.27 ind. CPUE) (Fig. S.1). At R4 where crayfish were absent in 2017, relative abundance attained the highest value of 40.5 ind. CPUE in 2022.

A total of 1932 females and 1152 males were captured. The percentage of females was significantly higher than males both in the invasion core (65% and 35%, respectively) and in the invasion front (61–39%, females and males, respectively) ($\chi^2 = 5.16$, $P < 0.05$;

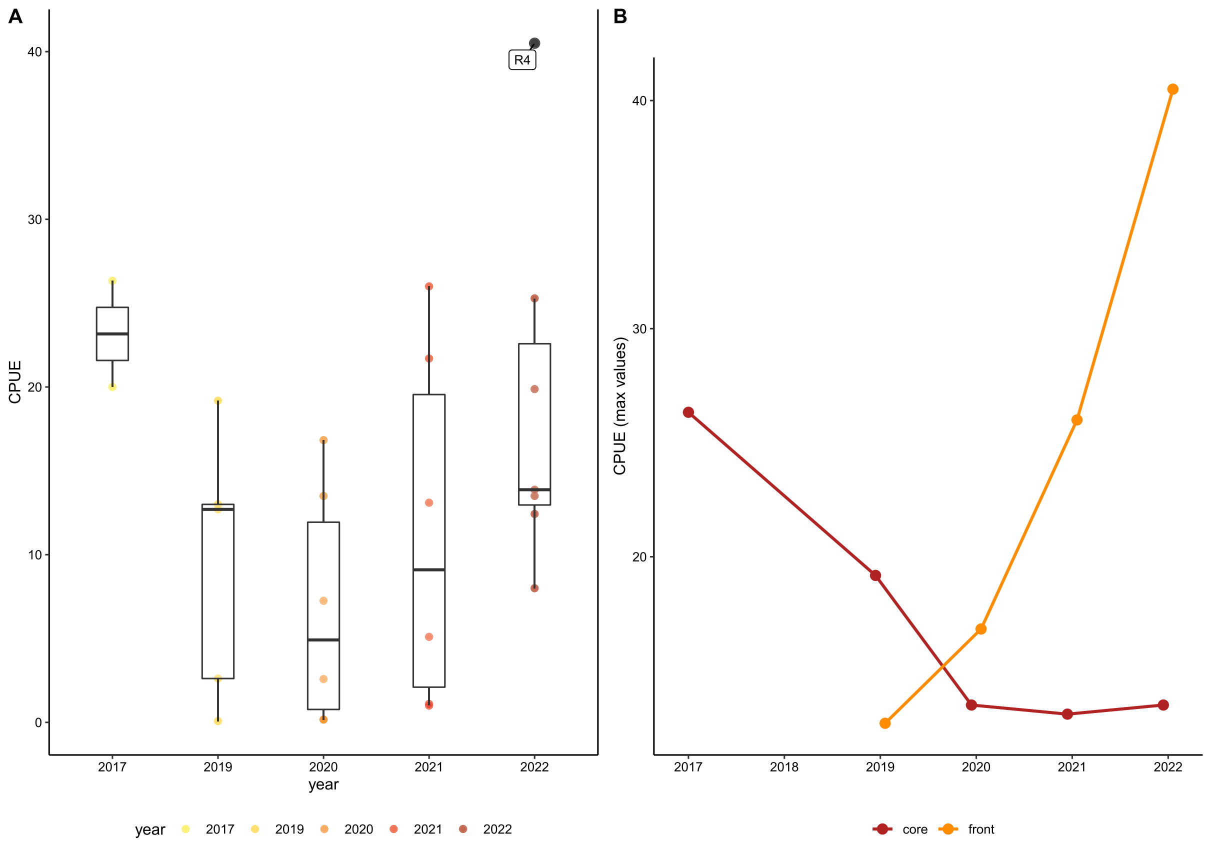

Fig. 2 Signal crayfish relative abundance (CPUE) through the years (**A**) and CPUE max values between invasion core and invasion front (**B**)

Fig. 3A). Proportion of females was significantly higher at upstream sites closer to the invasion core which were firstly invaded until 2019 (R1—1.6:1; R2—2.4:1; R3—2.1:1; R4—1.3:1; R5—1.8:1), but not at downstream sites which were only invaded after 2019 (R6—1.3:1; R7—1:1; $P > 0.05$; Fig. 3B).

Signal crayfish size ranged from 3.5 to 13.5 cm and mean values decreased significantly along the 5-year campaign ($F_{(4,\ 1134.46)} = 90.71$, $P < 0.001$; Supplementary Material, Fig. S.2). In 2017, mean crayfish size was significantly higher than in all the other years (Holm post hoc, $P < 0.001$; Supplementary Material Fig. S.2). Male sizes ranged between 4.8 and 13.5 cm, while female sizes ranged between 3.5 and 12.5 cm. Both male and female crayfish in the invasion core were significantly larger than in the invasion front ($t_{(2048.17)} = 5.39$, $P < 0.001$, Fig. 4A, C; Supplementary Material; Fig. S.3). Crayfish size was significantly lower in the R4 site (7.56 cm) than in all the other sites along the invasion gradient (Supplementary Material, Fig. S.4). Mean crayfish size was higher in site R2 (8.59 cm) at the invasion core, followed by R7 (8.56 cm). Crayfish size was higher in invasion front edge (R7) than in R4 (Holm post hoc, $P < 0.001$) and R5 (Holm post hoc, $P > 0.05$). At R2 (invasion core) mean crayfish size was higher than R1 (Holm post hoc, $P < 0.001$), R3 (Holm post hoc, $P < 0.001$), R4 (Holm post hoc, $P < 0.01$), and R5 (Holm post hoc, $P < 0.001$) (Supplementary Material, Fig. S.4). Male sizes ranged between 8.47 cm in site R7 at the invasion front edge and 7.33 cm in the site R4, a recently invaded site. Males and females size was significantly lower at site R4 than all the other sites (Holm post hoc, Supplementary Material, Fig. S.5). Males size was significantly higher in site R7 at the invasion front edge than site R1 at the invasion core (Holm post hoc, $P < 0.001$). Female sizes ranged

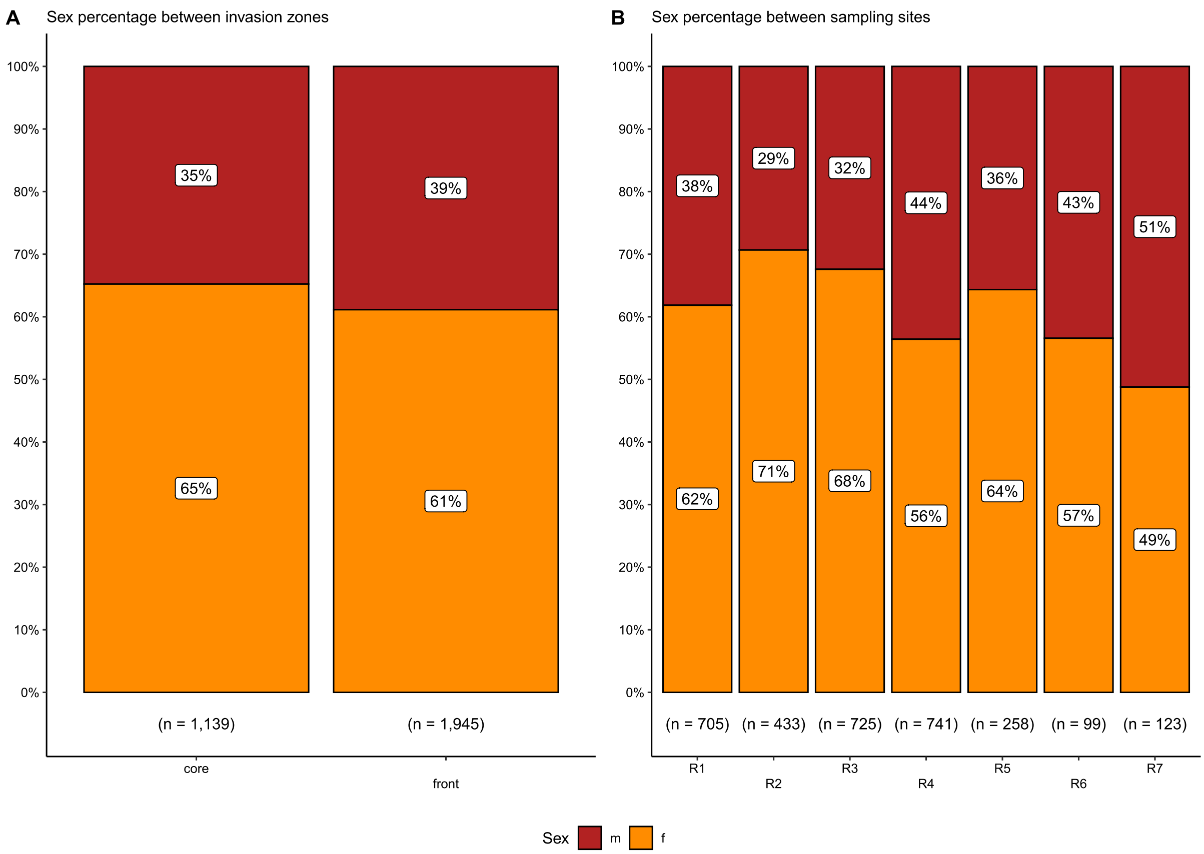

Fig. 3 Signal crayfish sex ratio (%) between invasion core and invasion front (**A**) and at different invaded sampling sites (**B**)

between 8.68 cm in site R2 at the invasion core and 7.74 cm in site R4.

Crayfish Fulton's body condition increased along the sampling years ($F_{(2,\ 622)} = 34.87$, $P < 0.001$; Supplementary Material, Fig. S.6) and significantly differed among sampling sites ($F_{(5,85.59} = 5.68$, $P < 0.001$). Crayfish Fulton's body condition was slightly significantly higher in the invasion core than in the invasion front ($t_{(521.32)} = 2.20$, $P < 0.05$; Fig. 4B, D). Both male and female crayfish had better body condition in the invasion core (Supplementary Material; Fig. S.7). Fulton's body condition was higher in R1 (3.73) at the invasion core, followed by site R7 (3.69), at the invasion front edge (Supplementary Material, Fig. S.8). Male Fulton's condition index ranged between 4.11 in site R1 at the invasion core and 3.73 at sites R4 and R6. Female Fulton's condition index ranged between 3.49 in site R1 at the invasion core and 3.18 in site R6. Males Fulton's body condition was significantly lower at site R4 than site R1 at the invasion core (Holm post hoc, $P < 0.05$; Supplementary Material, Fig. S.9). Crayfish average weight also increased significantly since the beginning of spread, from 17.9 g in 2019 to 20.0 in 2021 and 19.6 in 2022 ($F_{(2,\ 603)} = 5.81$, $P < 0.01$; Supplementary Material, Fig. S.10).

During the sampling campaign, we found 446 (14.5% of total) crayfish with claw injuries including broken claws ($N = 6$), missing claws (one, $N = 130$; both, $N = 12$), or regenerating claws (one, $N = 79$; both, $N = 3$). From those 58 crayfish with claw injuries were found in 2019, 94 in 2020, 79 in 2021, and 215 in 2022. Number of crayfish with claw injuries was significantly higher in the invasion front (67.5%) than the invasion core (32.5%) ($\chi^2 = 54.57$, $P < 0.001$). In 2020 ($\chi^2 = 5.15$, $P < 0.05$), 2021($\chi^2 = 10.65$, $P < 0.001$), and 2022 ($\chi^2 = 95.11$, $P < 0.001$), the number of crayfish with claw injuries was high in the invasion front than core, but in 2019 a higher number of crayfish with claw injuries was found in the

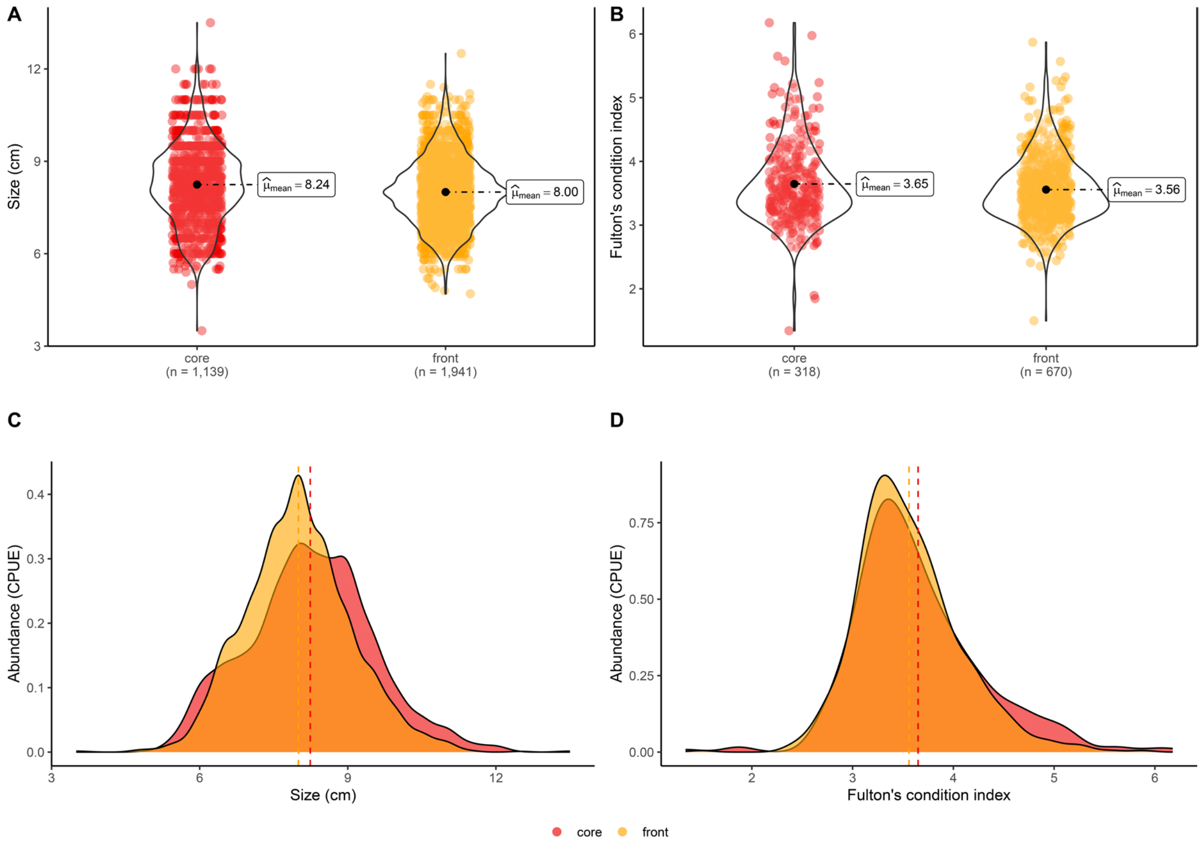

Fig. 4 Variation in signal crayfish body size (**A**, **C**) and Fulton's condition index (**B**, **D**) between the invasion core and invasion front

invasion core ($\chi^2 = 24.90$, $P < 0.001$). The number of crayfish individuals with claw injuries was higher in females than males ($\chi^2 = 67.88$, $P < 0.001$), 310 vs. 136, respectively. The number of crayfish individuals with claw injuries was significantly higher in females than males in 2020 ($\chi^2 = 15.36$, $P < 0.001$), 2021 ($\chi^2 = 4.57$, $P < 0.05$) and 2022 ($\chi^2 = 51.28$, $P < 0.001$), but not in 2019 ($\chi^2 = 2.48$, $P > 0.05$).

Discussion

Our study on population dynamics of the signal crayfish (*Pacifastacus leniusculus*) demonstrates that the species is well established at the Rabaçal River. As predicted, the crayfish population is spreading, but only in the downstream direction by increasing their abundance at new invaded sites (but decreasing at sites where the introduction was first recorded—invasion core). In addition, results demonstrate that biological traits varied between invasion zones. Crayfish size and body condition differed between the invasion core and front with slightly higher values recorded at the core; however, this situation needs to be interpreted with caution given that differences are not ecologically meaningful and probably result from the high number of individuals examined. Contrary to our hypothesis, sex ratio was different from that expected 1:1, being females clearly dominant.

Signal crayfish was first detected in Rabaçal River in late summer 2013 at sites R1 and R2 (Sousa et al., 2015). The most plausible explanation for this introduction was the illegal translocation of animals from the adjacent Sabor River basin, where the first record of this invasive non-native species in Portugal was in 1997 (Bernardo et al., 2011). Another possible explanation can be related to extreme high flows in 2010 (Quiraz 02O/01H, SNIRH, www.snirh.pt) that might have washed downstream some specimens from Spain before 2013 (Sousa et al., 2015). However, this last hypothesis seems implausible given the lack of crayfish in the upstream area (RX1 and RX2). These findings highlight that the legislative framework is not being effective in controlling the introduction of invasive species. Here, we suggest a clear investment in monitoring teams, particularly in areas of high conservation concern, such as the Montesinho Natural Park. A combination of surveillance and education actions with local communities would be recommendable to avoid future translocation events and new introductions. Anyway, results indicate that the species remained restricted to R1 and R2 between 2013 and 2017, which suggests that signal crayfish stayed in a lag phase before spreading in the following years, justifying the designation of these two sites as the invasion core. In our study, the crayfish population spread only toward the downstream direction, but other studies reported spreads toward the upstream direction or both directions (Anastácio et al., 2015; Hudina et al., 2017; Dragičević et al., 2021). The lack of upstream dispersal is not related with potential differences in microhabitat conditions as the river is still very pristine and diverse in terms of habitat quality. Only a few kilometers upstream large boulders could somehow impede crayfish movement. Additionally, fishing data for the area do not indicate notable differences in potential predation pressure upstream. However, it is important to note that dispersal events are relatively recent, and there remains a possibility that signal crayfish may attempt upstream movements in future. Similar results regarding a downstream spread were reported for other crayfish species, such as for the invasive rusty crayfish [*Faxonius rusticus* (Girard, 1852)] in NW USA (Messanger & Olden, 2019). The direction of dispersal might alter the velocity of spread since species moving downstream are expected to be facilitated by the river flow (e.g., Light, 2003; Bubb et al., 2005; Bernardo et al., 2011). However, some studies found no effect of high flows on downstream spread of crayfish (Bubb et al., 2002). Between 2017 and 2022, signal crayfish spread 10 km downstream. The velocity and direction of spread was similar to what was reported at Maças River in the adjacent Sabor River basin (Bernardo et al., 2011) and to other studies with the same species in Central Europe (e.g., Hudina et al., 2017). Many factors may explain the spread of NICS. The invasion process itself roughly follows a well-established sequence of stages, regardless the identity of the invasive non-native species, from an initial establishment phase, with low spread, followed by an expansion phase with increasing spread rates, and a final saturation phase when spread rates reach a threshold (Arim et al., 2006). Since 2020, signal crayfish is not spreading downstream but increasing their abundance at the new invaded areas. This population dynamics potentially indicates a dispersion cycle in our sampling area. According to our data, from 2013 to 2017, signal crayfish remained spatially restricted in the upper reach of the Rabaçal River. From 2017 to 2020, signal crayfish expanded downstream, and from 2020 to 2022, the population front became again spatially stationary while increasing their abundance. As reported for this species, population abundance, local habitat complexity, and resource availability are important determinants in promoting spread (Galib et al., 2022). In fact, some studies in the study area reported decreases in invertebrate abundance and diversity and other resources (e.g., leaf litter) with the increase of crayfish abundance (Sousa et al., 2019a; Carvalho et al., 2022). Limitation of habitat space and resources and the higher probability of encounters between individuals driven by population increase might force individuals to spread and colonize new downstream areas. Here, physical barriers could be an option to restrict both upstream and downstream dispersal. Other studies tested the effect of physical barriers to stop signal crayfish invasion in Germany with the results showing effectiveness for upstream dispersal (Chucholl et al., 2022). Because crayfish migration in lotic waters can be fast this requires immediate action and barriers should follow specific dimensions, promote water velocity of 0.65 m/s and be combined with removal of refuges that can be used by crayfish, together with periodical control measures (i.e., trapping) to reduce population density (Krieg & Zenker 2020).

Our results also showed a higher number of crayfish with claw injuries in the invasion core than the front in 2019, which can be an indicator of potential competitive interactions among individuals. However, claw injuries may also occur during molting, severe weather conditions or predation (Kouba et al., 2011; Soto et al., 2024). This observation emphasizes the need for assessing invasive non-native species population dynamics and potential native predators at relevant temporal scales and their importance to support decision-making in the development of management measures. Future studies should be conducted to confirm this pattern over time.

Biological and physiological traits might play an important role in the spread dynamic of NICS. Our study demonstrated differences in sex ratio in invasion core and front. At the invasion core and front, the percentage of females was higher than males, but statistical differences were only found at sites closer to the invasion core. As the signal crayfish invasion moved downstream, the percentage of female and male crayfish was closer to 1:1. Higher male percentage at the invasion front areas were also reported for round goby [*Neogobius melanostomus* (Pallas, 1814)] in Canada (Gutowsky & Fox, 2011) or for signal crayfish in Europe (Capurro et al., 2007; Wutz and Geist, 2013; Rebrina et al., 2015) and those events were related to recent, not fully established invasive populations. Sex ratio is important for mediating the ecological roles of aquatic species. For example, female-biased populations of western mosquitofish *Gambusia affinis* (Baird & Girard, 1853) are able to induce stronger pelagic trophic cascades compared with male-biased populations, causing larger impacts on communities and ecosystems (Fryxell et al., 2015). Other studies on the invasive freshwater crayfish *Procambarus clarkii* have also shown that space is used differently by both sexes, with females being more nomadic that males potentially due to the reproduction phase (Barbaresi et al., 2004). Since our sampling was consistently conducted in late summer, it is possible that this timing does not capture the full annual cycle of the species and so observed sex ratio may be affected by seasonal variations in sex ratios and reproductive cycles. However, this is unlikely as the mating season occurs later in the year, where reproduction can play an important role in the behavior of males and females. The higher percentage of females in the invasive core area might also be related to a higher number of encounters and fights among males as a result of higher crayfish abundance and consequent decrease in available space and resources. This might result in death of some individuals or exclusion of the weakest. In alternative, it can be related with bolder crayfish individuals that will be more prone to explore downstream areas. In fact, in 2020–2022 among all captured crayfish specimens, those with higher total length were caught at the most downstream sites (R6 and R7—invasion front) although those specimens had no higher body weight or condition. Smaller crayfish can also be easily predated by brown trout *Salmo trutta* individuals and so we cannot exclude size-specific predation. Manual and mechanical sterile SMRT (male gonopods removal) has been tested to limit juvenile recruitment as and increase effectiveness in management actions. Although manual sterilization led to a decrease in copulation in laboratorial conditions (Johovic et al., 2020), mechanical sterilization demonstrated low efficiency in stopping reproduction both in laboratorial and natural systems (Green et al., 2022). Nevertheless, SMRT application has been proven its effectiveness in Italy with 87% reduction in population size (Aquiloni & Zanetti, 2014). Future research is recommended not only to enhance the efficiency of the method in reducing reproduction success but also to increase cost-effectiveness.

In our study, physiological conditions of signal crayfish differed between core and invasion front. However, these results should be interpreted with caution in terms of ecological relevance. Statistical differences are related to the high number of individuals and these differences among crayfish individuals between invasion core and front were low (mean difference of 0.2 cm in size) and Fulton's condition index was almost equal. In our study, body size was higher in 2017 than in the following years indicating that increased abundance leads to a decrease in size structure. This may further indicate that the crayfish population in the upper reach of the Rabaçal River inside Montesinho Natural Park has healthy juvenile recruitment that increases the probability of continuous population growth and spread in future. With population establishment and recruitment, it is also expected that population average size will decrease (firstly in core areas) based on the number of young individuals. Some studies indicate that biological traits, such as body size and fitness, can be drivers of invasive non-native species spread, suggesting that strong fitness provides higher competitive advantage (e.g., Shine et al., 2011; Lopez et al., 2012; Perkins & Nowak, 2013) that favors new habitat exploration. Other authors hypothesize that smaller animals in the invasion front may be a result of competitive disadvantages of niche space that force smaller individuals to disperse to forage for food (e.g., Hudina et al., 2014; Messager & Olden, 2019; Wood et al., 2021). In our study, crayfish body size and body condition were slightly lower in the invasion front than in the core areas. Smaller crayfish with lower body condition crayfish were found at a recently invaded site, where the first individuals detected in 2019. This site also had the highest relative abundance among all sampling locations. In contrast, larger individuals, especially males, were primarily found were at the invasion front edge.

Although we found some trends in biological traits between invasion core and front areas, some studies with the invasive signal crayfish found no effect of size, sex, and claw loss during the spread process (e.g., Galib et al., 2022). This highlights the importance of context dependency and potential interactions with other environmental or biological factors that might influence the dynamic of biological invasions, favoring some intrapopulation biological traits depending on the ecosystem characteristics. Nevertheless, other traits might also be important for invasive crayfish spread. Personality traits in *P. leniusculus* are reported to play a role in promoting spread driven by individuals with higher boldness (Galib et al., 2022). Future studies on signal crayfish should address the importance of personality traits, variations among age classes and sex and their ability to trigger spread or mediate species impacts on ecosystems.

Studying population dynamics and biological traits of NICS might help us to predict and integrate the process of biological invasions and develop efficient management actions (Galib et al., 2022). Several factors may contribute to declines in invasive crayfish populations, which are important to consider for effective management. Habitat changes, predation, diseases, or even intensive trapping can be important drivers of crayfish number reductions (Hein et al., 2007; Larson et al., 2019). Also, management strategies must account for the impact of crayfish abundance and individual size contribute on cannibalism rates within populations, as higher densities and larger sizes can exacerbate cannibalistic behavior (Houghton et al., 2017). Understanding these dynamics is crucial for developing targeted management approaches that address both environmental conditions and population characteristics as the efficiency of control actions depends on the characteristics of the invaded water bodies. For example, trapping requires continuous efforts and combination between trapping and biological control by predators has only successfully eradicated invasive crayfish in closed systems (small lakes and pounds) but not in rivers (e.g., Hansen et al., 2017). Other studies in Europe demonstrated the potential effective use of native fish predators to control invasive crayfish species tested both in control environments and in situ (e.g., Aquiloni et al., 2010). Therefore, controlling invasive crayfish populations should consider context dependency and a wide ecological, social, and cultural approach. Continuous study on the population dynamics of signal crayfish at our study site, combined with a trophic approach will be fundamental to understand potential intrapopulation differences on their impacts and predict future patterns on spread at newly invaded areas. For example, in the last few years, we found signal crayfish in larger brown trout (*Salmo trutta*) stomach contents and Eurasian otter (*Lutra lutra*) feces in the Rabaçal River. This can be an indicator of natural population control; however, larger individuals of brown trout are also highly prized by sports fishermen, which may pose some conflicts between different stakeholders (i.e., in one hand bigger trouts have higher crayfish biocontrol potential, and on the other are the main target of sport fisherman). However, some studies also demonstrated that signal crayfish can have negative impacts on trout (e.g., Peay et al., 2009). We suggest some management measures focused on this native predator that can contribute to control signal crayfish invasion in that area. One possible action is to regulate fishing particularly where crayfish abundance is high. Implementing fishing regulations can promote growth on native brown trout individuals and populations and help to reduce crayfish abundance. Another management suggestion is to promote catch and release fishing, which does not compromise brown trout individuals' survival. We further suggest increasing restocking efforts in areas where crayfish are in high density. In this case, we suggest a social environmental engagement that includes multiple entities to maximize the potential use of brown trout as a native biocontrol of the signal crayfish. It is highly important to involve all stakeholders to minimize conflicts and maximize efficiency. Upstream the invasion core, continuous surveys and management actions should be implemented in the next few years to prevent upstream colonization by the invasive crayfish. Based on our results and according to signal crayfish spread velocity downstream, we suggest that a population control should be done in the next years to prevent or at least delay species colonization upstream the Rebordelo dam. In fact, crayfish intensive trapping can have positive consequences for native invertebrate communities and increased taxon richness, as reported for signal crayfish in the UK (Moorhouse et al., 2014). In this case, we suggest targeting crayfish female individuals with eggs before juvenile release, particularly in the autumn when the water is still at temperatures that promote crayfish higher activity (and therefore increase trapping efficiency). Integrated control management should consider mechanical, biological, physical, and chemical approaches (Guerardi et al., 2011) and assess their pros and cons. Because trapping and predation together have shown effectiveness in controlling but not eradicating species (Guerardi et al., 2011), combining the installation of artificial or natural barriers can also be effective but should be implemented with caution, as it might lead to hydrological changes and impact native species (Krieg et al., 2020). Nevertheless, the combination of these actions must be taken immediately to primarily focus on containing crayfish dispersal and reduce crayfish relative abundance. Emerging control methods should also be considered. For example, the use of sterile male release can be expensive but causes no harm to the environment neither to other non-target species and has demonstrated positive effects (e.g., Piazza et al., 2015). Other biotechnological techniques such as biocides or pheromones can also be efficient tools to control and eradicate NICS and should be considered in this area (Manfrin et al., 2021). However, controlling NICS populations might lead to unexpected indirect effects by opening space for other native (or non-native) species at the same trophic level (Hansen et al., 2013). The continuous monitoring of this area is also of pivotal importance and can help to develop emergent mathematical models that can help to identify cost-effective prevention, control, and eradication actions (Thompson et al., 2021). Consequently, management approaches require caution and long-term studies on the dynamic of biological invasions.

Conclusion

The spread of NICS impacts native biodiversity and ecosystem functioning with implications for ecosystem conservation, including in protected areas. Since its first report in the study area in 2013 (Sousa et al., 2015), invasive signal crayfish led to significant impacts on native invertebrate populations (Sousa et al., 2019a; Carvalho et al., 2022). High habitat quality, availability of refugee areas, and abundance and diversity of food provide favorable conditions for the continuous spread of the signal crayfish population in the Rabaçal River and therefore increase their impact. The Montesinho Natural Park and surrounding area harbor important freshwater ecosystems colonized by many species with high conservation value. Based on our findings, we recommend the urgent implementation of integrated management actions combining control and containment techniques to stop downstream dispersal, potential upstream dispersal, and population relative abundance reduction. Therefore, we advocate the continuation of the long-term study of the population dynamics and the assessment of multi-trophic interactions and ecosystem impacts of the signal crayfish in this protected area. This will be crucial to develop management actions aiming to stop their spread and reduce their abundance that may include intensive trapping or the protection of brown trout and Eurasian otter populations given their potential to naturally biocontrol the signal crayfish and to reduce their abundance at highly invaded areas.

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Data availability The data that support the findings of this study are available from the corresponding author, FCarvalho, upon reasonable request.

Declarations

Competing interests The authors have not disclosed any competing interests.

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