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Research paper

The signal crayfish *Pacifastacus leniusculus* (Dana, 1852) (Crustacea, Decapoda) is threatening the near future of *Margaritifera margaritifera* Linnaeus, 1758 (Bivalvia, Unionoida) in the Negro River (NW Zamora, Spain)

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Abstract

The signal crayfish Pacifastacus leniusculus *(Dana, 1852) (Crustacea, Decapoda) is threatening the near future of* Margaritifera margaritifera *Linnaeus, 1758 (Bivalvia, Unionoida) in the Negro River (NW Zamora, Spain).* We studied the incidence of the signal crayfish on a population of *Margaritifera margaritifera* in the Negro River (Zamora, Spain) during the summers of 2019 to 2022. The incidence of predation was assessed as a mortality factor in relation to floods and the hydrology of the river. The crayfish were trapped in a small plot of high pearl mussels density monitored since 2002, and collected each summer since 2019, during which time their abundance increased (65 % in three years). Simultaneously, we counted the shells carried by the floods to the gravel riverbanks. The incidence of floods was stable between 2019 and 2022 (10.3 % to 19.4 % of mortality), while at the bottom of the river the mortality of pearl mussels increased due to predation from 2.7 % to 43.3 %. During the 2022 dry season, 29 pearl mussels that had recently died and whose shell edges were widely gnawed by crayfishes were collected from the plot. The shells appeared bitten only in the contour exposed above the gravel, ruling out the possibility that the marks could be the effect of the scavenging of dead specimens by the crayfish. Low intensity trapping barely affected the crayfish population, since the following year their abundance in the controlled section had recovered.

Key words: Predation, Invasive species, Crayfish trapping, Freshwater pearl mussels, Duero basin

Resumen

El cangrejo señal, Pacifastacus leniusculus *(Dana, 1852) (Crustacea, Decapoda), es una amenaza para el futuro próximo de* Margaritifera margaritifera *Linnaeus, 1758 (Bivalvia, Unionoida) en el río Negro (al noroeste de Zamora, España).* Se estudió la incidencia del cangrejo señal en una población de *Margaritifera margaritifera* en el río Negro (Zamora, España) durante los veranos de 2019 a 2022. Se evaluó la incidencia de la depredación como factor de mortalidad en relación con las riadas y la hidrología del río. Los cangrejos fueron capturados todos los veranos desde 2019 en una pequeña parcela con una alta densidad de náyades a la que se estaba dando seguimiento desde 2002, a pesar de lo cual su abundancia creció (un 65 % en tres años). Simultáneamente, se contaron las conchas arrastradas por las riadas hasta los depósitos de grava de las orillas. La incidencia de las riadas resultó estable entre 2019 y 2022 (del 10,3 % al 19,4 % de la mortalidad), mientras que en el fondo del río la mortalidad de náyades se incrementó por depredación del 2,7 % al 43,3 %. Durante el estiaje de 2022, se recogieron en la parcela 29 náyades recién muertas y cuyas conchas tenían los bordes notablemente roídos por los cangrejos. Las conchas aparecen mordidas únicamente en el contorno que asoma en la superficie de la grava, lo que descarta que las marcas puedan ser efecto del carroñeo de ejemplares muertos por los cangrejos. La captura de baja intensidad de cangrejos afectó poco a su población, dado que al año siguiente su abundancia en el tramo controlado se había recuperado.

Palabras clave: Depredación, Especies invasoras, Captura de cangrejos, Náyades, Cuenca del Duero

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Introduction

The signal crayfish *Pacifastacus leniusculus* (Dana, 1852) is a native species of North America introduced in Spain since 1974 (Diéguez-Uribeondo 2006, Martín-Torrijos et al 2021). In Europe, the species was first recorded in Sweden in 1959 (Dunn 2012) and thereafter in many other countries, and it is now widespread across the continent. *P. leniusculus* is an invasive alien species (IAS) with a high impact on the aquatic biota and the habitat where they live (Griffiths et al 2004, Souty-Grosset et al 2006, Galib et al 2019). In addition, it is a common vector of viruses, ectosymbiont branchiobdellidans and fungi, including the oomycete *Aphanomyces astaci* (Schikora, 1906) that causes the crayfish plague (Dunn 2012, Martín-Torrijos et al 2019).

The freshwater pearl mussel (FPM) *Margaritifera margaritifera* Linnaeus, 1758 is a species catalogued as endangered 'EN' in Spain and occurs exclusively in a limited number of rivers of the northwest Iberian Peninsula. In the most recent FPM regional survey, carried out in 2018 (Morales 2022), the first data on the negative pressure exerted by the signal crayfish on the mussels –and on the Negro River ecology–, were assessed, one decade after the release of this invasive species. In this context, the predation exerted by the crayfish on the mussels in nearby basins of northern Portugal was assessed elsewhere (Meira et al 2019, Sousa et al 2019) based on direct observations of clamping on siphons, presence of cutting marks at the edge of the shells, and the bullying of mussels by crayfish.

The aim of this study is to report on the introduction of *P. leniusculus* in the Negro River catchment (NW Spain) and further investigate the potential effects of this invasive species (Morales 2023) on the most significant FPM population remaining in the Spanish Duero basin (Morales et al 2004). This is the first study of the lethal effect of this invasive crayfish species on an endangered freshwater mussel in a river of a mountainous area of Castilla and León.

Material and methods

Study area

The study was carried out in the main course of the Negro River, on the northern Iberian Plateau, which has a completely natural discharge regime. The total river length spans 52 km, draining an area of 376 km^2 with an altitudinal range spanning from 1,815 to 783 m above sea level, and an average gradient of 2.1 %, although in the sections studied the average gradient was only 0.4 %, with a predominance of coarse gravels. Sampling was carried out along a stretch of about 6.6 km (range: 794-892 m a.s.l). Waters were oligotrophic and lowly mineralized, with the FPM population distributed in low-density patches. Rainfall in the basin area varies between 800 and 1,000 mm/year, and in the mountain, ranges can frequently reach up to 1,500 mm/year (Morales and Lizana 2014, Morales 2022). The precise location of sampling station N2/7, located at 834 m a.s.l. is reserved to preserve the integrity of the mussel population.

The study area is nevertheless included in the Natura2000 network within the SAC 'Riverside Sub-basins of the Tera River' (ES4190067), and since 2015 it has been included in the 'Fluvial Nature Reserve of the Negro River and tributaries' (ES020RNF029). The riverbanks are covered with a dense mature riparian forest composed of alder *Alnus glutinosa* with ash trees: *Fraxinus excelsior* and *F. angustifolia*, and willows: *S. salviifolia*, *S. alba* and *S. atrocinerea*. This forest has a low lateral development but plays an important role in protecting against erosion of the fluvial banks during flooding (Morales et al 2004, Morales and Lizana 2014).

Methodology

The predation impact exerted by crayfish was assessed on plot N2/7, a stretch of gravel and riffles monitored closely since 2002 (Morales and Lizana 2014). The arrival of the signal crayfish to this exceptionally sensitive area was first detected in 2016 and from 2019 onwards they were trapped and removed during periods of maximum drought.

The FPM settlement was studied by means of visual counts of the adults –both dead and alive– present on the river bottom using glass-bottom bathyscopes in wadable stretches, and of shells left in the gravel deposits of the riverbanks after floods. From 2019 onwards, marks present on mussel shells were inspected in detail, as evidence of predation by crayfish.

The extent of the rear part of the shell left exposed above the substrate (where the siphons are located) was calculated using the angle dAv as in figure 1, where 'A' is the most anterior point of the shell´s longitudinal axis, 'd' is the dorsal edge where the anus protrudes into the mantle, and 'v' is the point of contact of the mantle with the foot at the shell ventral edge (v). The angle dAv estimated in 67 shells averaged 36° 5'. On each shell, the length and location of the margin chewed by crayfish was measured under 8x magnification. In order to calculate the extent of the chewed area, an angle was measured with vertex at point A and the furthest marks located at the dorsal edge (a) and ventral edge (b) of each valve of the predated animal, using the 29 specimens collected in 2022. The angular section dAv was estimated using AngleMeter version 1.9 (1) software. Total length (TL in fig. 1) and height (H) of FPM shells were measured in situ using calipers up to 0.1 mm precision, and shells were stored in the laboratory for eventual ulterior inspection.

Crayfish trapping was undertaken coinciding with the minimum discharge level of the river, between 6th August and 6th October 2019, 2021 and 2022. Passive trapping of adult crayfish was carried out using telescopic mesh pots (30 mm mesh size and 4 tunnel entries) and Finnish 'Pirat'™ rigid traps (35 x 10 mm light and 2 tunnel-type entries). Traps were set in groups of four and baited with fish remains, or alternatively with cat food pills. Trapping timing varied from a minimum of 18 to a maximum of 92 active hours. A separate trapping survey was used for the two different methods. Hourly water temperature data were gathered with an HOBBO Onset submersible

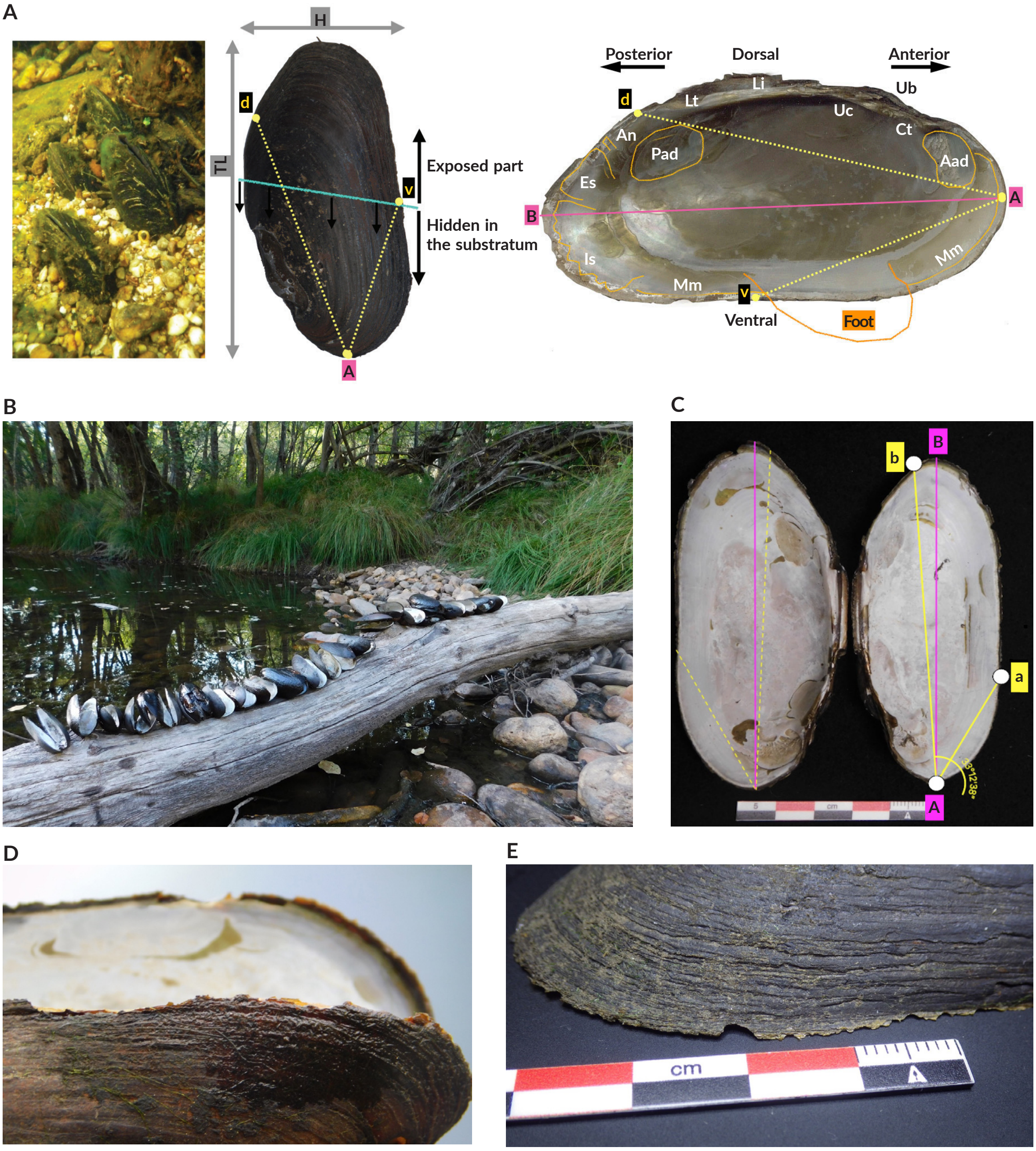

Fig. 1. Photo-gallery of gnawed shells of freshwater pearl mussels predated by signal crayfish, and main biometric shell's features: A, habitus of FPM adults placed on the river bottom and the main points of the shell; area of shell that remain hidden and within reach of signal crayfish are remarked; the contour v-d of the shell shows the mantle margin and the siphons with the orifices through which the animals exchange water with the river and excrete, which they must inevitably remain outside the substratum: Per, periostracum; Aad, anterior adductor muscle; Pad, posterior adductor muscle; Is, incurrent siphon; Es, excurrent siphon; An, anus; Mm, mantle margin; Li, ligament; Ub, umbo; Uc, umbo cavity; Lt, lateral teeth; Ct, cardinal teeth; B, mussels predated in Negro River by signal crayfish at sampling station N2/7 (04/09/2022) during extreme low water conditions; C, technique for measuring the gnawed contour of a shell from a specimen that has been recently killed; D, scraping caused by crayfish claws on the shell of an FPM recently dead; E, detail of the marks caused by the crayfish claws.

Fig. 1. Fotografías de conchas de náyades mordisqueadas por cangrejos señal y principales características biométricas de la concha: A, hábitus de ejemplares adultos de náyade fijados en el fondo del río y puntos principales de la concha; se observa la superficie de la concha que queda escondida y la que queda al alcance del cangrejo señal; en el contorno v-d de la concha se muestran el margen del manto y los sifones con los orificios a través de los cuales el animal intercambia agua con el río y ejerce la función excretora y que deben quedar inevitablemente fuera del sustrato: Per, periostraco; Aad, músculo aductor anterior; Pad, músculo aductor posterior; Is, sifón incurrente; Es, sifón excurrente; An, ano; Mm, margen del manto; Li, ligamento; Ub, umbo; Uc, cavidad umbo; Lt, dientes laterales; Ct, dientes cardinales; B, mejillones depredados en el río Negro por el cangrejo señal en la estación de muestreo N2/7 (04/09/2022) durante un episodio extremo de aguas bajas; C, técnica para medir el contorno mordisqueado en la concha de un ejemplar recientemente muerto; D, marcas en la concha de una náyade recientemente muerta provocadas por las pinzas del cangrejo; E, vista detallada de las marcas provocadas por las pinzas del cangrejo.

Table 1. Impact of the presence of signal crayfish and of major floods in consecutive FPM surveys at N2/7 plot. Data refer to individuals predated in the riverbed and shells deposited on the riverbanks.

Tabla 1. *Impacto de la presencia del cangrejo señal y de las grandes crecidas en muestreos consecutivos de FPM en la parcela N2/7. Datos de individuos depredados en el cauce y de conchas depositadas en las orillas.*

	Year					
	2002	2006	2016	2019	2021	2022
Count of live mussels	185	165	151	125	107	67
Count of shells found underwater	5	3	7	10	19	29
Mussels found dead in the river/live FPM (%)	2.7	1.8	4.6	9.6	17.8	43.3
Shells found in the river showing predation marks (%)	0,0	0,0	0,0	30.0	73.7	100.0
Crayfish (catches per unit effort, CPUE)	0	0	0	0.049	0.053	0.18
Count of shells found on the banks	43	17	21	16	11	13
Shells found on the banks after floods/live FPM (%)	23.2	10.3	13.9	12.8	10.3	19.4

datalogger set between shaded alder roots. Flow rates were derived from the gauging station CHD2113 of the Duero River Authority (https://www.saihduero.es/risr/EA113).

Results

Since 2019, underwater shells found in the riverbed started to appear gnawed by crayfish, showing a large part of their perimeter was chewed off (table 1). The maximum affectation was detected in 2022, when 27 out of 29 shells showed evidence of crayfish attacks (fig. 1). The incidence of predation on FPM was estimated from the fraction of shells that appeared gnawed relative to the angle dAv. On average (N = 58 valves) the value of the angle dAv (table 2) was 30° 8' (range 19° 1'-36° 4'), which represents between 83 % and 99 % of the shell perimeter left exposed to attacks (table 1). The shells examined had an average total length (LT) of 90.1 mm (range: 81.1–98.8 mm), and a height (H) of 42.3 mm (range: 37.7-48.1 mm).

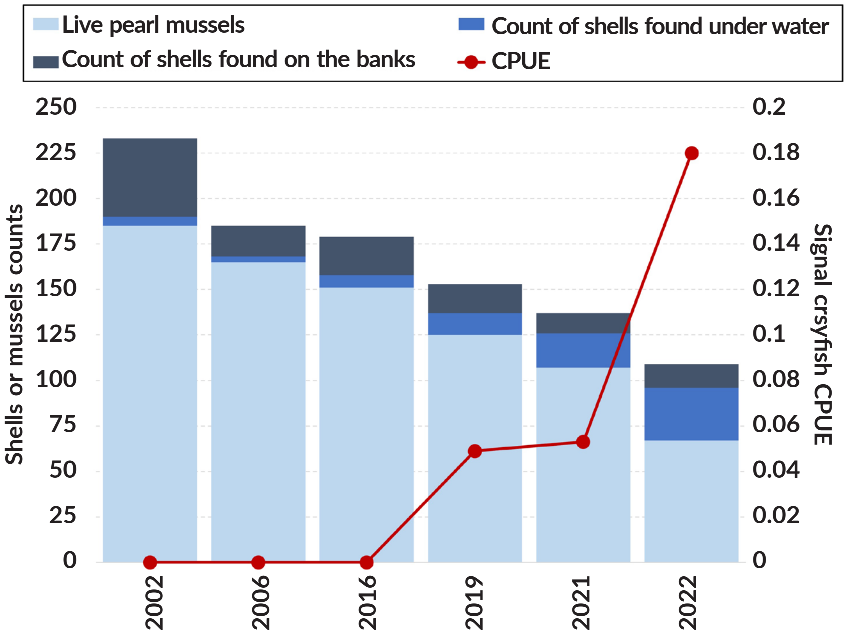

Fig. 2. Reduction in mussel population size over time in N2/7 plot due to hydrological causes and in relation to the intensification of signal crayfish catches per unit effort (CPUE).

Fig. 2. *Reducción del tamaño de la población de mejillones a lo largo del tiempo en la parcela N2/7 por causas hidrológicas y en relación con la intensificación de las capturas de cangrejo señal por unidad de esfuerzo (CPUE).*

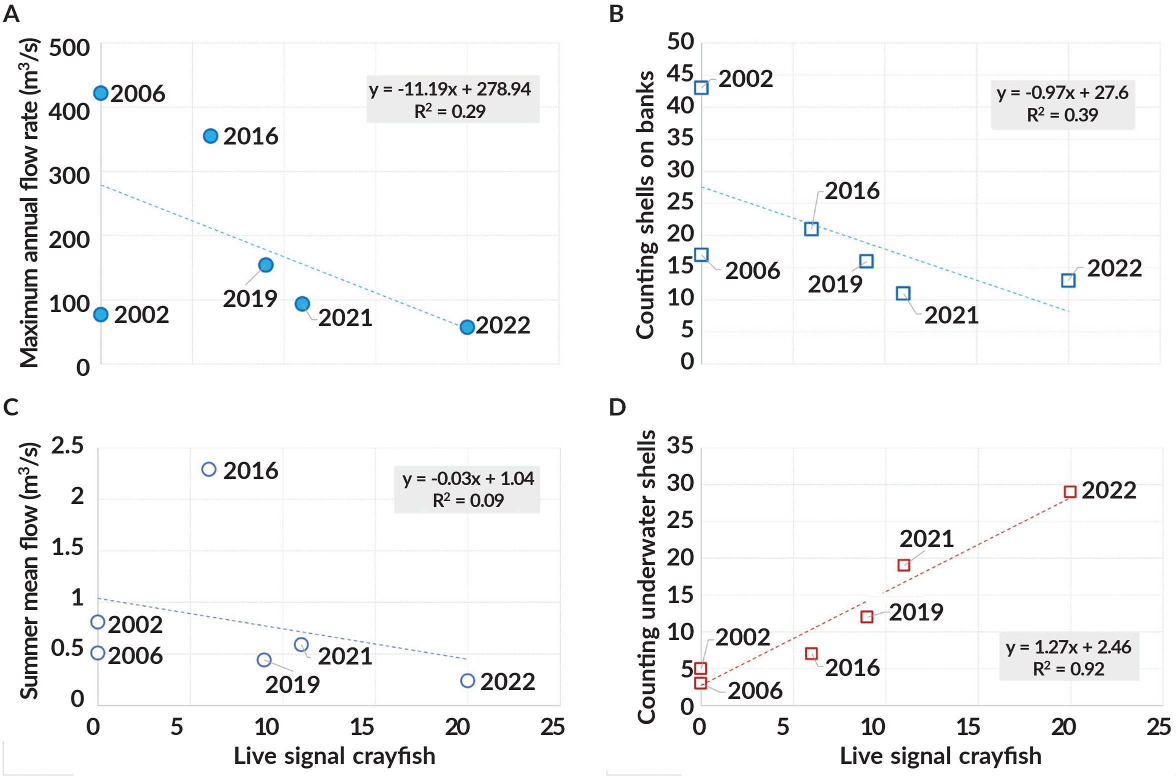

Fig. 3. Linear relationship between maximum and minimum water flow during the dry period (A, C) and the abundance of shells deposited on the gravel banks (B) and river bottom during the invasion of the stretch by signal crayfish (D). Flow rate data from the CHD2113 gauging station at Sta. Eulalia de Rionegro (https://www.saihduero.es/risr/EA113).

Fig. 3. *Relación lineal entre los caudales máximo y mínimo durante el período de estiaje (A, C) y la abundancia de conchas depositadas en los bancos de grava (B) y en el fondo del río durante la invasión del tramo por el cangrejo señal (D). Datos de caudal de la estación de aforo CHD2113 en Sta. Eulalia de Rionegro (https://www.saihduero.es/risr/EA113).*

From 2002 to 2022, the number of live FPM counted in plot N2/7 varied between 185 and 67 (fig. 2), representing a -65 % decline during that period, although the annual rates of decline were much higher in the most recent surveys. Thus, the reduction between 2016 and 2019 was estimated at -2 %, coinciding with the introduction of signal crayfish in the area. Between 2019 and 2021, and between 2021 and 2022 it remained constant at -20 %. Conversely, 7, 6 and 20 signal crayfish specimens were captured during the 2019, 2021 and 2022 trapping seasons, respectively (fig. 3), representing an increase in crayfish abundance of 65 % during this period.

Shells found on the riverbed and those found deposited on the riverbanks were counted separately (table 1). During the two-decade study, the number of submerged shells increased from 5 to 29, while on the banks they decreased from 43 to 13. While the mortality effect of floods remained almost constant at between 23.2 % and 19.4 % of the local FPM population during that period, the increase in underwater mortality rate increased from 2.7 % in 2002 to 43.3 % in 2022, coinciding with a significant reduction in the flow regime (fig. 3D). No shells on the gravel banks showed marks of crayfish attack or were found broken or showed a degraded margin.

During the dry season (considered to extend between 6 August and 6 October) the waterflow was very low in 2019 and 2021 (between 0.20 and 0.33 m^3/s, on a daily average), while the average daily water temperature at the N2/7 plot was 18.90 °C (2019) and 19.28 °C (2021).

Table 2. Descriptive statistics of measurements (N = 29) of shell's contour of freshwater pearl mussels affected by predation by signal crayfish on N2/7 plot: AV, angle [aAb] value; SC, percentage of cell contour exposed on the substrate gnawed by crayfish.

Tabla 2. *Estadística descriptiva de las medidas (N = 29) del contorno de las conchas de náyades afectadas por la depredación del cangrejo señal en la parcela N2/7: AV, valor del ángulo [aAb]; SC, porcentaje de contorno celular expuesto sobre el sustrato roído por cangrejos de río.*

	AV	SC (%)
Average	30° 8’	94.4
Median	33° 4’	96.5
Maximum	36° 4’	99.9
Minimum	19° 1’	82.6

These values were much higher in 2022, with 19.98 °C average daily temperature and 0.15 m^3/s average flow; furthermore, the river remained almost completely dry (0.03 m^3/s) at the flow measurement station for 36 days. Historical flow data are shown in figure 3A, C. In 2002 the average daily flow was 0.73 m^3/s in the dry season, representing change of -79.5 % in two decades. The most important peak floods occurred in 2002 (378 m^3/s) and 2006 (422 m^3/s), while in the crayfish trapping years the maximum peak flood occurred in 2021 (57.34 m^3/s), representing a change of -84.8 %.

Discussion and conclusions

The signal crayfish is the most common invasive aquatic species in Spain in the rivers of Castilla and León. Its negative effect on freshwater pearl mussels through predation has been observed in several rivers of the Duero River basin (Meira et al 2019, Sousa et al 2019) and elsewhere in Europe (Machida and Akiyama 2013, Sanders and Mills 2022). Predation on FPM induces changes in their siphon-opening behavior, thus affecting their respiration, feeding and larval spawning, and eventually involves extraction of the FPM specimens from the substrate and lethal chewing. Elevated water temperatures cause low oxygen dissolution at times when the FPM gills are carrying the embryos, forcing them to expose their siphons to the outside, and making them more vulnerable to the attack of crayfish. The shells are only chewed on the contour emerging from the surface of the gravel, which excludes displayed marks as a by-product of scavenging. Still, the hypothesis that crayfish may chew on shells as a supply of carbonate and calcium is currently being investigated (Morales, unpublished data).

In addition, selective predation on glochidia (larvae) conglutinates has been observed to occur in the Negro River, thus directly affecting FPM reproduction. This is a particularly critical condition in this mussel population, which is known to have not recruited for decades (Morales et al 2004, Morales 2022), and could impede the population's recovery by the eventual release of individuals derived from captive breeding.

Preventing introduction of invasive species should be a priority for environmental administrations given that once an alien species is established in the wild it is very complex and economically expensive to apply eradication methods (Beisel and Lévêque 2010). Eradication methods must be specific so as not to adversely affect the native communities, so that actions to communicate the problems caused by these species and surveillance should be a priority and continued over time. This also requires periods of sustained effort to reduce the abundance of the invader, and as the species becomes rarer in the river, very intensive trapping campaigns may be required to catch the remaining very few specimens. If these practices are abandoned, there could be a return to the original conditions within a relatively short period of time (Dana et al 2010, 2011). This reaction of the invasive population is partly due, in addition to possible downstream migration, to the possibility that the traps selectively capture the adults, leaving the juvenile population intact. Galib et al (2019) remarked that signal crayfish, even at low densities, can seriously disturb several components of a river ecosystem. It is therefore necessary to minimize the spreading of the invasive crayfish within and between rivers.

During the research period, dry seasons and periods of flood discharge were much lower than those recorded in historical data; in addition, the water temperature has increased by 1 °C in only three years. These multi-annual cycles without occurrence of major floods and with long droughts, and in combination with warm water, make it difficult for FPM to survive alongside the increasing presence of invasive crayfish. Such new conservation pressure, long extreme drought, and crayfish predation currently represent the most important cause of mortality, exceeding the natural mortality expected to occur after major floods (Morales and Lizana 2014), and could accelerate the collapse of this population in the medium long-term.

At present, with, the effects of climate change, the dry season flow is minimal and the water is comparatively warm for many months each year, facilitating the establishment of the invasive species (Horvatović et al 2022) and adversely affecting survival of FPM, which is very sensitive to reduced levels of dissolved oxygen. Management, monitoring, and control measures are urgently needed to control the dispersal and prevent the permanent establishment of this plague (Stancliffe-Vaughan 2015), as this is incompatible with the long-term conservation of populations of native low-mobility species of benthic fauna, as is the case of bivalves or dragonflies naiads (Moorhouse et al 2014, Ludányi et al 2022).

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Conflicts of interest

No conflicts of interest declared

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