Research paper

Spatial Expansion and Ecological Correlates of Invasive Crayfish *Procambarus clarkii* and *Pacifastacus leniusculus* in Mediterranean Rivers of Catalonia (NE Iberian Peninsula)

Rafel Rocaspana[1], Quim Pou-Rovira[2] and Enric Aparicio[3]

[1] Gesna Estudis Ambientals, S.L., Linyola, Catalonia, Spain; rafel@gesna.net
[2] Sorelló, estudis al medi aquàtic, Ed. Monturiol, Parc Científic i Tecnològic de la UdG, 17003 Girona, Catalonia, Spain
[3] GRECO, Institute of Aquatic Ecology, University of Girona, E-17071 Girona, Catalonia, Spain

Abstract: Invasive freshwater crayfish are among the most impactful non-native taxa in European river systems, yet quantitative assessments of their spatial expansion and environmental drivers remain scarce in Mediterranean regions. We investigated the expansion dynamics and environmental drivers of two invasive North American crayfish, *Procambarus clarkii* and *Pacifastacus leniusculus*, in rivers of Catalonia (NE Iberian Peninsula). Using occurrence data from official biological monitoring programs and global repositories aggregated into 10 x 10 km grids, we quantified expansion rates and modeled current distributions against geomorphological and anthropogenic factors, including a composite Environmental Disturbance Score. Results indicate significant range expansions for both species, with *P. clarkii* spreading more rapidly (slope = 5.8) and extensively (>100 grids) than *P. leniusculus* (slope = 1.4). Generalized Linear Models (GLMs) identified anthropogenic pressure and altitude as the primary predictors for *P. clarkii* occurrence, confirming its high occupancy in degraded, lowland reaches. Conversely, *P. leniusculus* trended toward cooler, higher-altitude reaches with lower disturbance, though associations were statistically less pronounced. This spatial segregation highlights how longitudinal gradients and human-induced disturbances jointly structure crayfish invasions in Mediterranean river systems. The advance of *P. leniusculus* into headwaters may pose a critical threat to the last refugia of the native white-clawed crayfish (*Austropotamobius pallipes*) through the transmission of crayfish plague. Accordingly, we advocate for differentiated management strategies: impact mitigation in degraded lowland reaches and proactive prevention in high-quality headwater systems.

Keywords: freshwater invertebrates; distribution modeling; habitat degradation; ecological gradients

1. Introduction

Biological invasions are a leading driver of biodiversity loss and ecosystem modification worldwide, with freshwater ecosystems among the most vulnerable due to their high levels of endemism and spatial isolation (Dudgeon *et al.*, 2006). Rivers and wetlands are particularly sensitive to biological invasions because they are subject to multiple, often interacting anthropogenic pressures, including hydrological alteration, pollution, habitat fragmentation and water abstraction, which reduce ecosystem resistance to non-native species establishment (Didham *et al.*, 2005, 2007).

The Mediterranean Basin, and the Iberian Peninsula in particular, constitutes a global hotspot of freshwater biodiversity and endemism (Hermoso *et al.*, 2015), but also one of the regions most affected by biological invasions (Muñoz-Mas & García-Berthou, 2020). The spread and impact of non-native freshwater species in this region are exacerbated by prolonged droughts, flow intermittency, intensive water abstraction, land-use change and widespread hydromorphological alteration (Sabater *et al.*, 2018). In addition, historical trade routes, aquaculture activities and climate change have facilitated the introduction and establishment of alien species in Mediterranean river systems (Gherardi *et al.*, 2009). Once established, non-native species often cause profound ecological changes through competition, predation, disease transmission and habitat alteration, frequently leading to population declines or local extinctions of native fauna (Vaeßen & Hollert, 2015; Morand, 2017; Haubrock *et al.*, 2025).

Among aquatic invaders, freshwater crayfish are widely recognized as particularly successful and damaging. Two North American species, the red swamp crayfish *Procambarus clarkii* (Girard, 1852) and the signal crayfish *Pacifastacus leniusculus* (Dana, 1852), are listed among the world's worst invasive species (Nentwig *et al.*, 2018). Their invasion success is linked to a combination of high fecundity, broad environmental tolerance and behavioural traits that allow them to exploit degraded and fluctuating habitats (Alcorlo *et al.*, 2008; Maceda-Veiga *et al.*, 2013).

Procambarus clarkii, introduced to the Iberian Peninsula in the 1970s for aquaculture and harvest, has since become widespread in Mediterranean wetlands and rivers (Cruz & Rebelo, 2007; Acevedo-Limón *et al.*, 2020). This species tolerates low oxygen concentrations, high temperatures, elevated salinity and severe organic pollution, conditions that often exclude native crayfish and other sensitive taxa (Gherardi, 2006; Maceda-Veiga *et al.*, 2013). Moreover, *P. clarkii* is an active and prolific burrower, excavating riverbanks and littoral sediments. Its burrowing and foraging activities increase bank erosion, resuspend fine sediments, reduce macrophyte cover and modify nutrient cycling, often promoting turbid, eutrophic conditions (Barbaresi *et al.*, 2004; Lodge *et al.*, 2012). These habitat alterations can negatively affect a wide range of aquatic organisms, including macroinvertebrates, amphibians and fish (Gherardi *et al.*, 2001; Geiger *et al.*, 2005; Lodge *et al.*, 2012; Martín-Torrijos *et al.*, 2019).

Pacifastacus leniusculus was introduced in several European countries to compensate for the decline of native crayfish fisheries (Everard *et al.*, 2009). Although it occupies cooler and less eutrophic waters than *P. clarkii*, it shows strong competitive ability and high resistance to pathogens, which has facilitated its spread in many temperate river systems (Lodge *et al.*, 2012). Most importantly, both *P. clarkii* and *P. leniusculus* act as chronic carriers of the crayfish plague (*Aphanomyces astaci*), an oomycete pathogen that is largely asymptomatic in North American crayfish but highly lethal to native European species (Grandjean *et al.*, 2017; Svoboda *et al.*, 2017). The introduction and spread of this disease has been a major driver of the dramatic decline of native white-clawed crayfish (*Austropotamobius pallipes* species complex) throughout Europe, including the Iberian Peninsula (Martín-Torrijos *et al.*, 2019).

Catalonia (NE Spain) represents a particularly suitable region for studying the invasion dynamics of alien crayfish. Its river network spans a wide range of environmental and anthropogenic conditions, from highly modified coastal streams and lowland rivers affected by eutrophication and flow regulation to relatively well-preserved headwater systems in the Pyrenees. Freshwater ecosystems in Catalonia are among the most heavily invaded in the Iberian Peninsula, especially by non-native fish and crayfish species (Maceda-Veiga *et al.*, 2013; Muñoz-Mas & García-Berthou, 2020). Based on an extensive survey across Catalan river basins, Maceda-Veiga *et al.* (2013) demonstrated that the presence of *P. clarkii* is strongly associated with downstream reaches, high levels of eutrophication, increased water mineralization and altered fish assemblages dominated by tolerant and non-native species. Their results suggest that hydrological alteration and water quality degradation increase ecosystem vulnerability to invasion, whereas the preservation of headwater streams and the restoration of natural flow regimes may limit crayfish spread and provide refugia for native species. Despite the well-documented presence of invasive *P. clarkii* in Catalonia, quantitative assessments of temporal changes in their distribution, as well as in that of *P. leniusculus*, and of the relative contribution of environmental versus anthropogenic drivers remain scarce. Accurate knowledge of distributional trends and environmental constraints is essential to prioritize management actions, including early detection of new infestations, prevention of further spread and evaluation of potential ecological impacts.

This study aims to: (1) quantify recent changes in the distribution ranges of *P. clarkii* and *P. leniusculus* in selected Catalan river basins over a 15-year period; (2) identify and compare the environmental and anthropogenic factors associated with their current distribution; and (3) discuss the implications for management in a highly invaded Mediterranean region. We hypothesize that both species have significantly expanded their ranges, but that their distributions are governed by different environmental filters: *P. clarkii* primarily by warmer, more eutrophic and hydrologically altered conditions, and *P. leniusculus* by cooler waters and intentional stocking practices.

2. Materials and Methods

2.2. Study Area

Catalonia (NE Spain) features a Mediterranean climate characterized by hot, dry summers and mild, wet winters. Elevation ranges from sea level to over 3,000 m in the Pyrenees, producing diverse hydrological regimes. The study was conducted in the Catalan River Basin District (CRBD), which covers approximately 16,500 km² and comprises several small to medium-sized river basins managed by the Catalan Water Agency (Agència Catalana de l'Aigua, ACA) (Fig.1). The region contains more than 300 monitoring stations operated by the ACA, which provide long-term ecological and physicochemical data. Its rivers are characterized by flashy hydrology, pronounced summer droughts, and recurrent winter floods.

Catalonia's rivers are heavily modified: over 60% are classified as having moderate to poor ecological status under the EU Water Framework Directive (ACA, 2023). Human pressures include extensive irrigation, urban expansion, hydropower infrastructure, and recreational activities. The area has experienced a mean warming of +1.3 °C since 1980 (Lana *et al.*, 2022), increasing thermal suitability for warm-adapted exotic species.

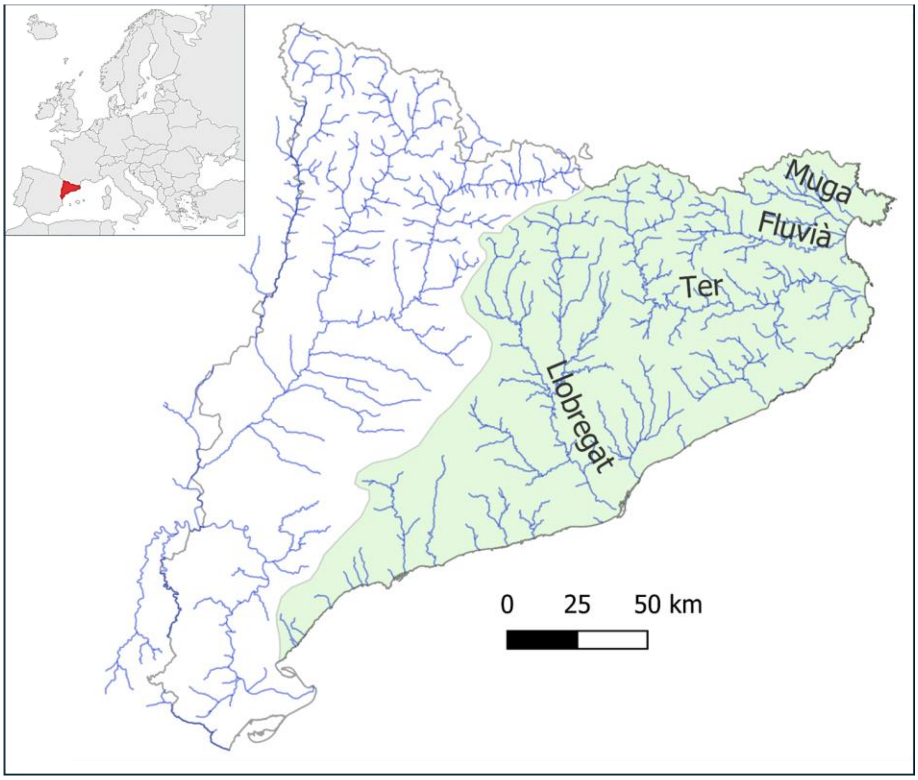

Figure 1. Geographic location of the study area within Europe. Catalonia is highlighted in red, and the main river basins within the Catalan River Basin District (background green layer) are indicated.

2.1. Data Collection

Occurrence data for invasive freshwater bivalves and gastropods were compiled from several open-access biodiversity and environmental repositories spanning both global and regional scales. Records were obtained from the Global Biodiversity Information Facility (GBIF; https://www.gbif.org/), the EXOCAT database of exotic species of Catalonia (https://exocat.creaf.cat/), the Biodiversity Data Bank of Catalonia (BDBC; http://biodiver.bio.ub.es/bdbc/), and the biological monitoring datasets of the ACA. Data extraction from GBIF involved querying the target species within the geographic extent of Catalonia and retrieving all available records with georeferenced information. Regional occurrence data from EXOCAT and the BDBC were incorporated to enhance coverage and to include records not indexed in global repositories. Additional occurrences and ecological information were sourced from the ACA bio-monitoring programme, which provides standardized biological and environmental data collected under official monitoring protocols.

All retrieved records were integrated into a unified database and harmonised using standard Darwin Core terms to ensure consistency across sources. Records lacking geographic coordinates or with clearly erroneous spatial information were excluded from further analyses. The final dataset therefore represents a harmonised compilation of *P. clarkii* and *P. leniusculus* occurrences in the CRBD, suitable for subsequent analyses of spatial distribution, temporal trends in range dynamics, and relationships with environmental variables and anthropogenic pressures.

2.2. Spatial Analysis

To perform a standardised spatial analysis, geographic coordinates were projected using QGIS (QGIS Development Team, 2025) into the Universal Transverse Mercator (UTM) coordinate system, specifically Zone 31N, which is the standard projection for the study area in Catalonia. A grid-based approach was adopted to mitigate sampling bias and account for spatial uncertainty. Occurrences were aggregated into a 10x10 km UTM grid. This resolution is consistent with international standards for calculating the Area of Occupancy (AOO) in biodiversity assessments.

To evaluate the expansion or contraction of the species' ranges, we calculated the cumulative occupancy. Unlike annual occupancy, which can be heavily influenced by fluctuations in sampling effort, cumulative occupancy represents the total number of unique 10x10 km grid cells where a species has been recorded up to a given year. This metric is particularly robust for monitoring the colonisation process of invasive or expanding species.

The rate of expansion was quantified using linear regression models, where the year was the independent variable and the cumulative number of occupied grid cells was the dependent variable. The slope of the regression line was used as a proxy for the expansion rate (new grid cells colonised per year). Statistical significance was determined using a *p*-value threshold of $\alpha = 0.05$. A significant positive slope indicated an increasing trend, a slope close to zero denoted stability.

2.3. Environmental and anthropogenic correlates

To investigate the association between environmental and anthropogenic factors and the occurrence of alien crayfish, we compiled several site-specific hydrological and climatic descriptors. Catchment attributes—including altitude (m a.s.l.), drainage area (km^2), and reach slope ($m\ km^{-1}$)—were extracted from publicly available geographic datasets, and mean air temperature was obtained from the BioClim database (Karger *et al.*, 2017). To assess environmental degradation at each site, we used an Environmental Disturbance Score (EDS) that integrates multiple categories of anthropogenic pressure: nutrient enrichment, non-natural land use, channel morphology alteration, connectivity disruption, and flow regulation. Disturbance levels for each category were assigned based on expert judgement and adapted from the classification framework proposed by Faro *et al.* (2024). Each variable was scored according to its deviation from minimally disturbed conditions, ranging from 1 (no deviation) to 4 (highly degraded). For each site, the five individual scores were summed to produce a composite EDS.

Differences in environmental and degradation variables between sites of presence and absence were initially assessed using the non-parametric Mann-Whitney *U* test, as most variables did not follow a normal distribution.

To identify the main drivers of species distribution, Generalized Linear Models (GLM) with a binomial error distribution and a logit link function were constructed. Prior to modelling, multicollinearity among predictors was evaluated using the Variance Inflation Factor (VIF). Variables exhibiting high redundancy were removed, retaining a final subset of predictors with VIF < 2 to ensure model stability. All predictor variables were standardized (mean = 0, SD = 1) before analysis to allow for a direct comparison of effect sizes through the estimated regression coefficients. The significance of predictors was assessed using the Wald test ($p < 0.05$), and the overall model fit was evaluated using McFadden's Pseudo-R^2. To visualize the independent effect of the most significant predictors, we calculated marginal response curves based on the fitted GLMs. Predicted probabilities were estimated across the range of the focal variable while holding all other predictors constant at their mean values. All statistical analyses were conducted in R version 4.4.0 (R Core Team, 2025).

3. Results

The analysis of cumulative distribution records indicate that the two invasive crayfish species exhibited significant range expansions over the study period (2007–2025), but with contrasting patterns (Fig.2). *Procambarus clarkii* showed a rapid and continuous expansion, as indicated by the steep slope of the fitted linear model (slope = 5.8). The number of occupied 10x10 km grids for this species increased exponentially, rising from fewer than 10 grids in 2007 to over 100 by 2025 (Fig.3). In contrast, *P. leniusculus* showed a more gradual increase in distribution (Slope: 1.4), reaching approximately 25 occupied grid cells by the end of the period (Fig.3). These results indicate that while both species are spreading, *P. clarkii* is colonizing new areas faster and more extensively than *P. leniusculus*.

Univariate analysis revealed clear differences in environmental conditions between presence and absence sites for *P. clarkii*, whereas patterns for *P. leniusculus* were less consistent (Table 1). Sites occupied by *P. clarkii* were associated with significantly larger catchment areas, lower altitudes and gentler slopes compared to absence sites (all $p < 0.001$). Presence sites also exhibited higher mean air temperatures and significantly higher values of anthropogenic pressure (EDS) than unoccupied sites ($p < 0.001$ in both cases). In contrast, *P. leniusculus* showed only modest univariate differences: presence sites were characterized by slightly smaller catchment areas and higher altitudes than absence sites, with these differences being statistically significant ($p = 0.013$ and $p = 0.049$, respectively), while slope, mean air temperature and anthropogenic pressure did not differ significantly between presence and absence sites ($p > 0.05$). Overall, these results suggest that *P. clarkii* is strongly associated with lowland, warmer and more anthropogenically impacted environments, whereas *P. leniusculus* shows a less clearly defined environmental profile.

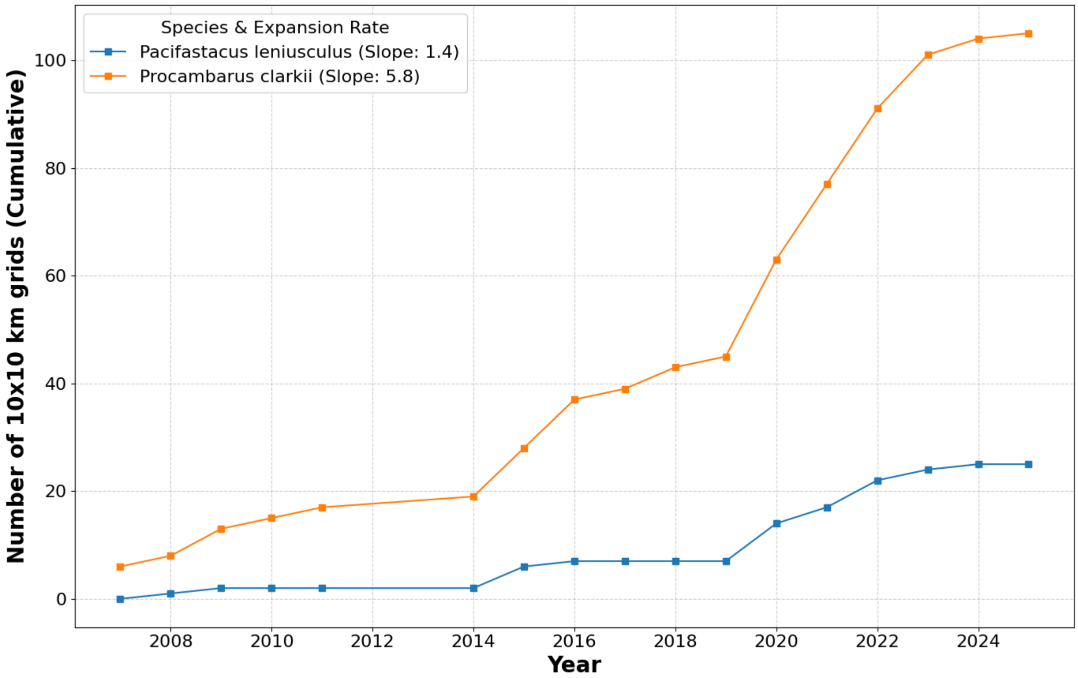

Figure 2. Temporal expansion trends of the invasive crayfish species *Procambarus clarkii* and *Pacifastacus leniusculus* in the Catalan River Basin District. The plot displays the cumulative increase in the Area of Occupancy (AOO), measured as the number of occupied 10×10 km UTM grid cells over time. The slope of the linear regression is provided in the legend.

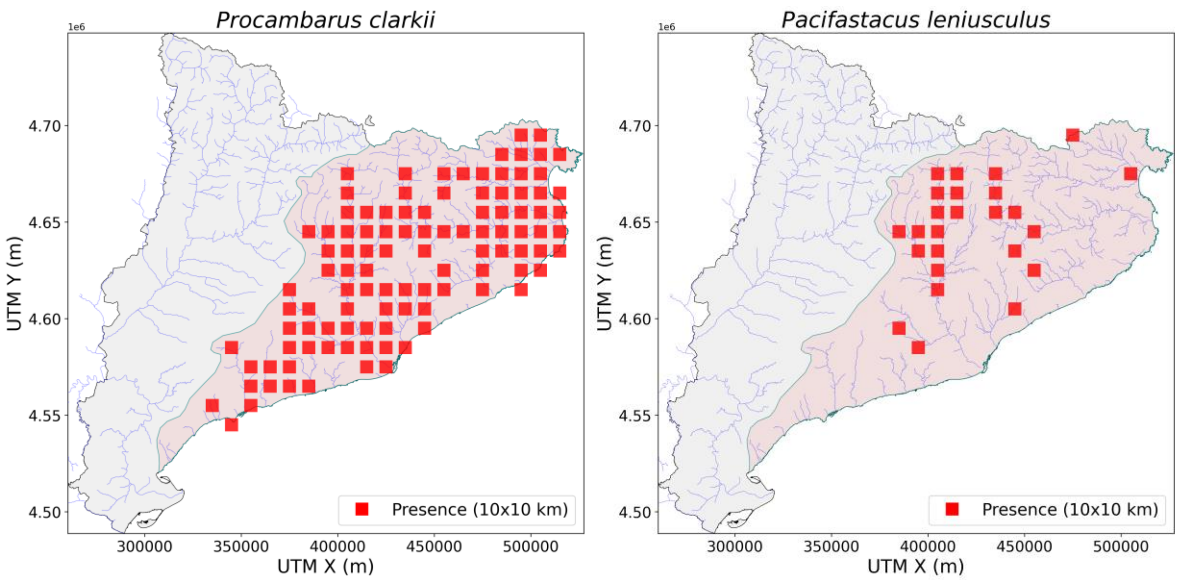

Figure 3. Distribution maps of the invasive species *Procambarus clarkii* and *Pacifastacus leniusculus* in the Catalan River Basin District Internal (background red layer). Presence is recorded in 10×10 km UTM grid cells (Zone 31N), integrating all available historical and current records.

Table 1. Summary statistics (mean ± SD) of environmental variables for presence and absence sites of *Procambarus clarkii* and *Pacifastacus leniusculus*, including *Mann-Whitney U* test results.

	Procambarus clarkii			*Pacifastacus leniusculus*		
Variable	**Presence (n=93)**	**Absence (n=76)**	***p***	**Presence (n=19)**	**Absence (n=143)**	***p***
Catchment Area (km^2)	528.8 ± 995.8	98.7 ± 354.1	**< 0.001**	317.1 ± 356.4	346.4 ± 855.5	**0.013**
Altitude (m a.s.l.)	247.7 ± 188.9	482.7 ± 350.0	**< 0.001**	428.7 ± 162.9	338.6 ± 305.8	**0.049**
Slope (m/km)	10.5 ± 10.4	23.3 ± 19.9	**< 0.001**	13.8 ± 10.5	16.3 ± 17.1	0.835
Mean Air Temperature (°C)	13.3 ±2.3	11.8 ± 2.9	**< 0.001**	12.6 ± 1.1	12.6 ± 2.8	0.194
EDS (Anthropogenic pressure)	13.8 ± 3.8	10.5 ± 3.1	**< 0.001**	11.7 ± 3.11	12.4 ± 4.0	0.538

The generalized linear model (GLM) identified anthropogenic pressure and altitude as the main predictors of *P. clarkii* occurrence (Table 2). Occurrence probability increased significantly with higher levels of anthropogenic pressure (EDS; $\beta = 0.652$, $p = 0.008$), with the odds of presence being nearly doubled for each standard deviation increase in EDS (OR = 1.92). In contrast, altitude showed a significant negative effect ($\beta = -0.534$, $p = 0.030$), indicating a lower probability of occurrence at higher elevations (OR = 0.59). Slope also exhibited a negative effect, although this relationship was only marginally non-significant ($\beta = -0.470$, $p = 0.064$). Catchment area and mean air temperature did not have a significant influence on occurrence probability ($p > 0.05$). Overall, these results suggest that *P. clarkii* occurrence is primarily associated with low-altitude, human-impacted environments, whereas broader-scale climatic and geomorphological variables play a secondary role once anthropogenic pressure is accounted for.

For *P. leniusculus*, the GLM model explained a limited proportion of the variance in occurrence and none of the evaluated predictors showed a statistically significant effect at $\alpha = 0.05$ (Table 3). However, several variables exhibited ecologically meaningful trends. Altitude showed a positive effect on occurrence probability ($\beta = 0.864 \pm 0.617$ SE), suggesting a tendency for higher presence at mid- to upper-catchment elevations. In contrast, slope displayed a negative effect ($\beta = -0.622 \pm 0.386$ SE), indicating reduced occurrence in steeper river sections. Anthropogenic pressure (EDS) showed a weak negative effect ($\beta = -0.336 \pm 0.322$ SE), while catchment area ($\beta = 0.110 \pm 0.317$ SE) and mean air temperature ($\beta = 0.613 \pm 0.871$ SE) had only minor influences on presence probability.

Response curves fitted on a common scale revealed strong ecological segregation between *P. clarkii* and *P. leniusculus* (Fig. 4). Occurrence probability of *P. clarkii* increased markedly with anthropogenic pressure (EDS) and declined with altitude, indicating a preference for lowland, human-altered river reaches. In contrast, *P. leniusculus* showed consistently low probabilities at high EDS values and a positive response to altitude, with occurrence concentrated in upper catchments. These contrasting responses highlight divergent ecological niches and reinforce the role of longitudinal gradients and anthropogenic pressure in structuring crayfish distributions.

Table 2. Results of the Generalized Linear Model (GLM, binomial distribution) explaining the occurrence probability of *Procambarus clarkii*. Predictor variables were standardized (z-score) prior to analysis to allow direct comparison of effect sizes.

Predictor	Estimate (β)	SE	*Z*-value	*p*-value	Odds Ratio (OR)
EDS (Anthropogenic pressure)	0.652	0.247	2.637	**0.008**	1.919
Altitude (m a.s.l.)	-0.534	0.246	-2.170	**0.030**	0.586
Slope (m/km)	-0.470	0.254	-1.849	0.064	0.625
Catchment Area (km^2)	0.362	0.509	0.711	0.477	1.436
Mean Air Temperature (°C)	0.258	0.195	1.326	0.185	1.294

Table 3. Results of the Generalized Linear Model (GLM, binomial distribution) explaining the occurrence probability of *Pacifastacus leniusculus*. Predictor variables were standardized (z-score) prior to analysis to allow direct comparison of effect sizes.

Predictor	Estimate (β)	SE	*Z*-value	*p*-value	Odds Ratio (OR)
EDS (Anthropogenic pressure)	-0.336	0.322	-1.04	0.297	0.71
Altitude (m a.s.l.)	0.864	0.617	1.40	0.162	2.37
Slope (m/km)	-0.622	0.386	-1.61	0.108	0.54
Catchment Area (km^2)	0.110	0.317	0.35	0.728	1.12
Mean Air Temperature (°C)	0.613	0.871	0.70	0.481	1.85

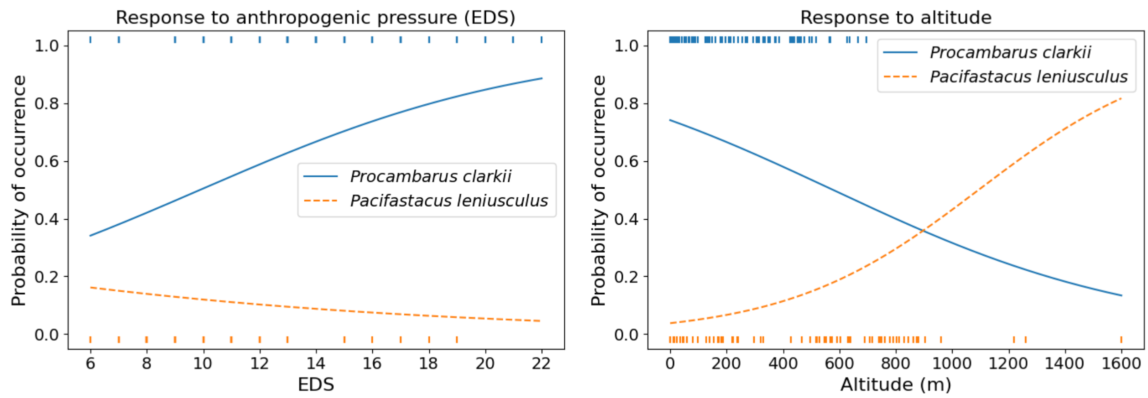

Figure 4. Predicted probability of occurrence along gradients of anthropogenic pressure (EDS) and altitude for *Procambarus clarkii* and *Pacifastacus leniusculus*. Response curves were derived from binomial GLMs, holding other predictors at their mean values. Rug plots indicate observed presences (upper ticks) and absences (lower ticks). Both species were plotted using the same scale to allow direct comparison.

4. Discussion

This study documents the continued expansion of two invasive North American crayfish species in the Mediterranean river systems of Catalonia, while also revealing clear differences in their invasion dynamics and environmental associations. The rapid increase in occupancy of *P. clarkii* contrasts sharply with the slower and more spatially restricted expansion of *P. leniusculus*, highlighting species-specific invasion pathways within the same regional context. Interpreting these expansion curves, however, requires careful consideration of sampling effort. The marked rise in detections from 2015 and again from

2020 coincides with the systematic implementation of river monitoring programmes in Catalonia required by the EU Water Framework Directive (Munné *et al.*, 2016). This temporal overlap suggests that a substantial part of the apparent spread may reflect increased sampling intensity rather than biological expansion alone. Prior to WFD implementation, aquatic macroinvertebrates received comparatively less monitoring attention than other groups (e.g. fish) across the Iberian Peninsula, likely leading to an underestimation of their historical distribution. Despite this sampling bias, the underlying biological trend remains clear. The expansion alien crayfish in Catalonia aligns with broader patterns reported across the Iberian Peninsula and Central Europe, where invasive crayfish continue to expand without clear signs of saturation (Lodge *et al.*, 2012; Haubrock *et al.*, 2023b).

The pronounced expansion of *P. clarkii* observed in this study is consistent with its well-documented ecological plasticity and capacity to exploit disturbed environments. This species has been shown to tolerate a wide range of physicochemical conditions, including high temperatures, low oxygen concentrations, elevated salinity and organic pollution, which are increasingly common in Mediterranean rivers subject to intense human pressure (Gherardi, 2006; Maceda-Veiga *et al.*, 2013). The strong positive association between *P. clarkii* occurrence and anthropogenic pressure detected by the GLM supports the view that habitat degradation and hydromorphological alteration facilitate its establishment and spread. Comparable relationships between *P. clarkii* presence, eutrophication, and altered fish assemblages have been documented across Catalonia and other regions of the Iberian Peninsula, indicating that human disturbance reduces biotic resistance and creates ecological opportunities for this opportunistic invader (Maceda-Veiga *et al.*, 2013).

The GLM also identified altitude as a key limiting factor for *P. clarkii*, with occurrence probability declining markedly at higher elevations. This pattern is consistent with previous studies indicating that the species is largely confined to lowland and mid-altitude reaches, where thermal regimes and hydrological conditions are more favourable (Maceda-Veiga *et al.*, 2013). Nevertheless, ongoing climate warming in the region may progressively reduce this limitation, potentially allowing *P. clarkii* to colonise higher elevations in the future, as has been suggested for other warm-adapted invasive taxa (Haubrock *et al.*, 2023a).

In contrast, *P. leniusculus* exhibited a much weaker and less clear association with environmental gradients. Neither anthropogenic pressure nor environmental variables were significant predictors of occurrence in the multivariate analysis, and univariate differences between presence and absence sites were modest. This lack of strong statistical relationships likely reflects several interacting factors. First, the relatively small number of occupied sites reduces statistical power and may mask subtle environmental effects. Second, the distribution of *P. leniusculus* in Catalonia may still reflect introduction history to a greater extent more than would be expected from present environmental conditions, a pattern widely reported across Europe (Everard *et al.*, 2009; Lodge *et al.*, 2012). Despite this, the tendency of *P. leniusculus* to occur at higher altitudes and under lower levels of anthropogenic pressure than *P. clarkii* is ecologically meaningful. This species generally requires better water quality and higher oxygen availability than *P. clarkii*, enabling it to dominate cooler and less degraded river reaches (Peay *et al.*, 2009). This pattern suggests a form of longitudinal segregation between the two invaders, with *P. clarkii* occupying degraded downstream reaches and *P. leniusculus* extending into mid- and upper-catchment sections.

This segregation has important conservation implications, as mid-altitude streams often constitute the last refugia for the native white-clawed crayfish (*Austropotamobius pallipes* species complex). Both invasive species act as chronic carriers of the crayfish plague (*Aphanomyces astaci*), and their spread has been identified as a major driver of native crayfish declines throughout the Iberian Peninsula (Martín-Torrijos *et al.*, 2019). In this context, the expansion of *P. leniusculus* into cooler headwaters may pose a particularly severe threat, even if its overall rate of spread is slower than that of *P. clarkii*.

Overall, the results indicate that anthropogenic pressure and longitudinal gradients jointly structure invasive crayfish distributions in Mediterranean rivers, but with contrasting responses between species. These findings underscore the need for differentiated management strategies: containment and impact mitigation in already degraded lowland systems dominated by *P. clarkii*, and proactive prevention, early detection and potential eradication efforts for *P. leniusculus* in higher-quality river reaches that still retain significant conservation value.

Funding: This research was funded by Gesna Estudis Ambientals, as part of the company's internal research and development activities.

Acknowledgments: The authors thank the Agència Catalana de l'Aigua for providing access to the biological and environmental monitoring data that partially supported this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

ACA (2023). 3r cicle de planificació (2022-2027). *Agència Catalana de l'Aigua*

Acevedo-Limón L., Oficialdegui F.J., Sánchez M.I. & Clavero M. (2020). Historical, human, and environmental drivers of genetic diversity in the red swamp crayfish (*Procambarus clarkii*) invading the Iberian Peninsula. *Freshwater Biology* **65**, 1460–1474. https://doi.org/10.1111/fwb.13513

Alcorlo P., Geiger W. & Otero M. (2008). Reproductive biology and life cycle of the invasive crayfish *Procambarus clarkii* (Crustacea: Decapoda) in diverse aquatic habitats of South-Western Spain: Implications for population control. *Fundamental and Applied Limnology / Archiv für Hydrobiologie* **173**, 197–212. https://doi.org/10.1127/1863-9135/2008/0173-0197

Barbaresi S., Tricarico E. & Gherardi F. (2004). Factors inducing the intense burrowing activity of the red-swamp crayfish, *Procambarus clarkii*, an invasive species. *Naturwissenschaften* **91**, 342–345. https://doi.org/10.1007/s00114-004-0533-9

Cruz M.J. & Rebelo R. (2007). Colonization of freshwater habitats by an introduced crayfish, *Procambarus clarkii*, in Southwest Iberian Peninsula. *Hydrobiologia* **575**, 191–201. https://doi.org/10.1007/s10750-006-0376-9

Didham R.K., Tylianakis J.M., Gemmell N.J., Rand T.A. & Ewers R.M. (2007). Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology & Evolution* **22**, 489–496. https://doi.org/10.1016/j.tree.2007.07.001

Didham R.K., Tylianakis J.M., Hutchison M.A., Ewers R.M. & Gemmell N.J. (2005). Are invasive species the drivers of ecological change? *Trends in Ecology & Evolution* **20**, 470–474. https://doi.org/10.1016/j.tree.2005.07.006

Dudgeon D., Arthington A.H., Gessner M.O., Kawabata Z.-I., Knowler D.J., Lévêque C., *et al.* (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**, 163–182. https://doi.org/10.1017/S1464793105006950

Everard M., Gray, Kindemba V. & Cowx I. (2009). Impacts of invasive species on ecosystem services: the case of the signal crayfish (*Pacifastacus leniusculus*). *Environmental Law & Management* **21**, 250

Faro A.T., Ferreira M.T., Oliveira J.M., Faro A.T., Ferreira M.T. & Oliveira J.M. (2024). A Fish-Based Tool for the Quality Assessment of Portuguese Large Rivers. *Fishes* **9**. https://doi.org/10.3390/fishes9050149

Geiger W., Alcorlo P., Baltanás A. & Montes C. (2005). Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions* **7**, 49–73. https://doi.org/10.1007/s10530-004-9635-8

Gherardi F. (2006). Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology* **39**, 175–191. https://doi.org/10.1080/10236240600869702

Gherardi F., Gollasch S., Minchin D., Olenin S. & Panov V.E. (2009). Alien Invertebrates and Fish in European Inland Waters. In: *Handbook of Alien Species in Europe*. pp. 81–92. Springer Netherlands, Dordrecht.

Gherardi F., Renai B. & Corti C. (2001). Crayfish predation on tadpoles: a comparison between a native (Austropotamobius pallipes) and an alien species (*Procambarus clarkii*). *Bulletin Français de la Pêche et de la Pisciculture*, 659–668. https://doi.org/10.1051/kmae:2001011

Grandjean F., Roques J., Delaunay C., Petrusek A., Becking T. & Collas M. (2017). Status of *Pacifastacus leniusculus* and its role in recent crayfish plague outbreaks in France: improving distribution and crayfish plague infection patterns. *Aquatic Invasions* **12**, 541–549. https://doi.org/10.3391/ai.2017.12.4.10

Haubrock P.J., Cuthbert R.N. & Haase P. (2023a). Long-term trends and drivers of biological invasion in Central European streams. *Science of The Total Environment* **876**, 162817. https://doi.org/10.1016/j.scitotenv.2023.162817

Haubrock P.J., Pilotto F., Soto I., Kühn I., Verreycken H., Seebens H., *et al.* (2023b). Long-term trends in abundances of non-native species across biomes, realms, and taxonomic groups in Europe. *Science of The Total Environment* **884**, 163808. https://doi.org/10.1016/j.scitotenv.2023.163808

Haubrock P.J., Soto I., Cuthbert R.N., Kurtul I. & Briski E. (2025). Analysing factors underlying the reporting of established non-native species. *Scientific Reports* **15**, 12337. https://doi.org/10.1038/s41598-025-96133-0

Hermoso V., Filipe A.F., Segurado P. & Beja P. (2015). Effectiveness of a large reserve network in protecting freshwater biodiversity: a test for the Iberian Peninsula. *Freshwater Biology* **60**, 698–710. https://doi.org/10.1111/fwb.12519

Karger D.N., Conrad O., Böhner J., Kawohl T., Kreft H., Soria-Auza R.W., *et al.* (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific Data* **4**, 170122. https://doi.org/10.1038/sdata.2017.122

Lana X., Serra C. & Martínez M.D. (2022). The effects of the climatic change on daily maximum and minimum temperatures along 102 years (1917–2018) recorded at the Fabra Observatory, Barcelona. *Theoretical and Applied Climatology* **149**, 1373–1390. https://doi.org/10.1007/s00704-022-04096-3

Lodge D.M., Deines A., Gherardi F., Yeo D.C.J., Arcella T., Baldridge A.K., *et al.* (2012). Global Introductions of Crayfishes: Evaluating the Impact of Species Invasions on Ecosystem Services. *Annual Review of Ecology, Evolution, and Systematics* **43**, 449–472. https://doi.org/10.1146/annurev-ecolsys-111511-103919

Maceda-Veiga A., De Sostoa A. & Sánchez-Espada S. (2013). Factors affecting the establishment of the invasive crayfish *Procambarus clarkii* (Crustacea, Decapoda) in the Mediterranean rivers of the northeastern Iberian Peninsula. *Hydrobiologia* **703**, 33–45. https://doi.org/10.1007/s10750-012-1335-2

Martín-Torrijos L., Kokko H., Makkonen J., Jussila J. & Diéguez-Uribeondo J. (2019). Mapping 15 years of crayfish plague in the Iberian Peninsula: The impact of two invasive species on the endangered native crayfish. *PLOS ONE* **14**, e0219223. https://doi.org/10.1371/journal.pone.0219223

Morand S. (2017). Infections and Diseases in Wildlife by Non-native Organisms. In: *Impact of Biological Invasions on Ecosystem Services*. (Eds M. Vilà & P.E. Hulme), pp. 177–190. Springer International Publishing, Cham.

Munné A., Ginebreda A. & Prat N. (2016). Water Status Assessment in the Catalan River Basin District: Experience Gathered After 15 Years with the Water Framework Directive (WFD). In: *Experiences from Surface Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I)*. (Eds A. Munné, A. Ginebreda & N. Prat), pp. 1–35. Springer International Publishing, Cham.

Muñoz-Mas R. & García-Berthou E. (2020). Alien animal introductions in Iberian inland waters: An update and analysis. *Science of The Total Environment* **703**, 134505. https://doi.org/10.1016/j.scitotenv.2019.134505

Nentwig W., Bacher S., Kumschick S., Pyšek P. & Vilà M. (2018). More than "100 worst" alien species in Europe. *Biological Invasions* **20**, 1611–1621. https://doi.org/10.1007/s10530-017-1651-6

Peay S., Guthrie N., Spees J., Nilsson E. & Bradley P. (2009). The impact of signal crayfish (*Pacifastacus leniusculus*) on the recruitment of salmonid fish in a headwater stream in Yorkshire, England. *Knowledge and Management of Aquatic Ecosystems*, 12. https://doi.org/10.1051/kmae/2010003

QGIS Development Team (2025). QGIS Geographic Information System (v. 3.40). Open Source Geospatial Foundation

R Core Team (2025). R: A Language and Environment for Statistical Computing

Sabater S., Elosegi A. & Ludwig R. (2018). *Multiple Stressors in River Ecosystems: Status, Impacts and Prospects for the Future*. Elsevier.

Svoboda J., Mrugała A., Kozubíková-Balcarová E. & Petrusek A. (2017). Hosts and transmission of the crayfish plague pathogen Aphanomyces astaci: a review. *Journal of Fish Diseases* **40**, 127–140. https://doi.org/10.1111/jfd.12472

Vaeßen S. & Hollert H. (2015). Impacts of the North American signal crayfish (*Pacifastacus leniusculus*) on European ecosystems. *Environmental Sciences Europe* **27**, 33. https://doi.org/10.1186/s12302-015-0065-2