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Trophic response of brown trout to signal crayfish invasion in a protected mountain river

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ABSTRACT

We investigated the dietary response of brown trout (*Salmo trutta*) to the invasion of the signal crayfish (*Pacifastacus leniusculus*) in the Baceiro River (Montesinho Natural Park, Portugal). We analyzed 1012 stomach contents across six sites (five invaded and one non-invaded) over the year of 2023. A total of 49 distinct dietary taxa were identified. Results reveal strong spatial and temporal variation in trout diets. While aquatic invertebrates (e.g., Baetidae, Heptageniidae, Simuliidae, oligochaetes, and signal crayfish) dominated in the spring and early summer, a shift toward terrestrial insects was observed during summer. Generalized Linear Models indicated that signal crayfish biomass significantly influenced prey richness, biomass, and diversity, while multivariate analyses confirmed distinct prey assemblages across space and time. Our study also showed that signal crayfish were consumed occasionally (2.09 % of all prey items) but accounted for 17.68 % of total prey biomass. This situation suggests limited, but non-negligible, trophic incorporation of the invasive crayfish on the brown trout diet, with possible implications for brown trout condition and food web structure. This study highlights the dynamic nature of trophic interactions following biological invasions and underscores the need for integrated management strategies to mitigate the impacts of *P. leniusculus* in protected freshwater ecosystems.

1. Introduction

Brown trout (*Salmo trutta*) is one of the most well-known and widely distributed freshwater fish species, playing a crucial role in both ecological and recreational contexts (Lobón-Cerviá and Sanz, 2018). Its diet is highly generalist, varying based on habitat, season, and prey availability, which allows it to thrive in a wide range of environments. Typically, brown trout feed on a variety of prey, including aquatic invertebrates, terrestrial insects, and small fish (Bridcut and Giller, 1995; Klemetsen et al., 2003). Their opportunistic feeding behavior helps brown trout adapt to fluctuating ecological conditions, including seasonal changes in prey abundance (Bridcut and Giller, 1995; Blanchfield et al., 2023). While many studies have explored the dietary habits of brown trout (e.g., Sánchez-Hernández et al., 2015; Cada et al., 1987; Orzavol et al., 2011; French et al., 2014), few have considered how these diets change over time (but see Sánchez-Hernández et al., 2022), particularly in response to the introduction of non-native species. The temporal variation in brown trout diets is an important aspect to consider in mountainous rivers, as it can provide insights into how a top predator fish adapts to shifts in the availability of different prey items over different seasons (Ringler, 1985; Johnson et al., 2007) .

Non-native species are one of the most pressing threats to freshwater biodiversity (Simberloff et al., 2013; Pyšek et al., 2020; Roy et al., 2024). Among the most impactful non-native species in European freshwater ecosystems, including in Portugal, is the signal crayfish (*Pacifastacus leniusculus*) (Anastácio et al., 2019; Krieg et al., 2020). Native to North America, signal crayfish was introduced into Europe in the second half of the 20th century for aquaculture and commercial purposes (Holdich et al., 2014). Since then, the species has rapidly spread across rivers and lakes, displacing native species, altering food webs, and modifying habitats. Their generalist diet, which includes both plants and animals, along with their aggressive behavior, makes signal crayfish particularly disruptive in introduced ecosystems (Alves et al., 2025; Carvalho et al., 2022; Galib et al., 2021; Sanders et al., 2022). Therefore, the signal crayfish may alter biotic interactions such as competition, predation, and parasitism, among others, with native species, including brown

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trout. These biotic changes may alter the structure and function of freshwater ecosystems (Alves et al., 2025; Galib et al., 2021).

The presence of non-native species can influence the diet of native species in various ways. On one hand, non-native species often disrupt food resources by outcompeting or preying on native organisms, thereby altering natural food webs (Rogers et al., 2017). Their introduction can reduce the availability of essential prey or plants for native predators, leading to population declines, ecosystem imbalances, and loss of biodiversity, ultimately threatening ecological stability and resilience (Ehrenfeld, 2010; Simberloff et al., 2013). On the other hand, non-native species can sometimes serve as alternative food resources for rare native species, supporting their survival when traditional prey or plants decline (Schlaepfer et al., 2011; Sax et al., 2022).

In the particular case of the signal crayfish, this species can directly compete with brown trout for food resources (e.g., macroinvertebrates), especially in environments where food availability is limited (Galib et al., 2021). On the other hand, and in the last years, several studies have highlight how non-native crayfish such as *Procambarus clarkii* and *Faxonius limosus* serve as an important prey for several species of vertebrates (Tablado et al., 2010; Giordano et al., 2025a, 2025b). Therefore, it is possible that the signal crayfish serve as a potential food source for brown trout, as trout are known to consume crayfish in certain conditions (Halkhoums, 2017; Matos et al., 2025; Momot, 1967). However, the incorporation of signal crayfish into the diet of brown trout may depend on several factors, including the size and behavior of both species, the abundance of crayfish, and environmental conditions such as water temperature (Carlsson et al., 2009; Matos et al., 2025). Given the significant impacts of signal crayfish on freshwater ecosystems and the potential role of brown trout as a predator, understanding how the presence of signal crayfish influences the dietary habits of brown trout is essential for effective ecosystem management and conservation.

A key question is whether the introduction of signal crayfish into freshwater ecosystems leads to measurable changes in the diet of brown trout. Specifically, it is important to determine if the presence of signal crayfish influences the availability of other prey species for brown trout, thereby affecting their overall diet. Furthermore, it is essential to investigate whether the brown trout in invaded areas exhibit different feeding behaviors compared to non-invaded areas, and whether the incorporation of signal crayfish into the trout diet occurs in a significant and consistent manner throughout the year.

Given this background, this study aims to investigate the dietary habits of brown trout in the Baceiro River, a protected river system in northeastern Portugal, in invaded and non-invaded sites along an entire year. The Baceiro River provides an ideal setting for this research, as it has experienced the invasion of signal crayfish in recent years (first record in 2013; Sousa et al., 2015). This study focused on comparing the diet of brown trout in areas with and without signal crayfish. The primary objectives of this study were: (i) to determine whether the presence of signal crayfish influences the diet of brown trout, (ii) to assess possible temporal shifts in the brown trout diet, and (iii) to assess the extent to which signal crayfish is incorporated into the diet of brown trout compared to other prey taxa. The findings of this study will contribute to the broader understanding of how non-native species, particularly signal crayfish, influence the feeding behavior of a native fish predator. Additionally, the results will have implications for the management of both brown trout and signal crayfish populations, helping to guide future efforts in ecosystem restoration and species conservation.

2. Material and methods

2.1. Study area

This study was conducted in the Baceiro River. This river originates in Spain, has a total length of about 60 km and is a tributary of the Tuela River (Douro basin) (Sousa et al., 2019). Almost all basin is located within the Montesinho Natural Park in northeastern Portugal. This protected area was established in 1979 and is well known in Portugal for its high conservation status and high biodiversity, hosting around 80 % of mammal species described for the country.

Signal crayfish were first recorded in the Baceiro River in 2013 near to their confluence with the Tuela River (Sousa et al., 2015). After that, the species is spreading in the upstream direction (Sousa et al., 2019). This study focuses on six sampling sites along the river, including 5 invaded and 1 non-invaded site (Fig. 1). All sites have similar width (around 6 m), and the altitude varies between 594 m in the most downstream site (B1) and 835 m in the most upstream site (B6). The very low level of human disturbance makes this river an ideal area for studying predator-prey interactions, as there are no significant human impacts other than biological invasions (Sousa et al., 2019, 2020).

2.2. Environmental characterization

Environmental parameters, including pH, total dissolved solids (mg/L), conductivity (μS/cm), dissolved oxygen (mg/L), and temperature (°C), were measured at each sampling site using a HACH HQ2200 multiparameter probe. This environmental characterization was carried out consistently (i.e., near the bottom of the river and always at the middle of the channel) across all six sampling sites and over nine sampling periods between April and December of 2023.

2.3. Brown trout and signal crayfish data collection

Salmo trutta were captured using electrofishing (Hans Grassl™ ELT60II-GI; 300–600 V, DC, 2200W) for a period of 30-min at each sampling site, covering a standardized length of 150 m of the river in each site, comprising an area of about 1000 m^2. To minimize the risk of overlapping brown trout between locations, the sampling sites were spaced at least 1 km apart. This distance ensures site independence, as brown trout typically exhibit high site fidelity, generally remaining within a few hundred meters of their preferred habitats throughout much of the year (Höjesjö et al., 2015). Brown trout feed preferentially in the early hours of the day, so collection was always carried out in the morning to coincide with their natural feeding behavior (Sbragaglia and Breithaupt, 2022).

Brown trout were also measured and weighed to assess the ecophysiological condition index using Fulton's index ($Kf = \frac{W}{L^3}$), which relates the weight (W) to total length (L).

For signal crayfish, 8 to 10 traps per site were left underwater for 24 h, using as bait dead marine fish (*Trachurus trachurus*) and following the methodology described in Sousa et al. (2019). These cylindrical traps (43 cm diameter, 22 cm height, 1.5 cm mesh) were placed strategically in pools, riffles, near riverbanks, and across the central river channel.

The biotic characterization was carried out consistently across the six sampling sites from April to December of 2023. Due to heavy precipitation and high river flow between January and March, it was not possible to conduct electrofishing or set traps to monitor the populations of brown trout and signal crayfish, respectively.

The relative abundance of both species per site was expressed as the total number of individuals per catch per unit of effort (ind. CPUE).

2.4. Brown trout diet

To assess the brown trout diet, a total of 1012 stomach contents were analyzed. This was achieved using a non-lethal method, where water was injected into the trout's stomach to induce regurgitation, following the technique described by Sánchez-Hernández et al. (2010). Brown trouts were immediately returned to the river after sampling to reduce stress. Prey items found in the stomach were identified using Tachet et al. (2010), counted, and then dried at 60 °C for 48 h to determine their biomass (dry weight).

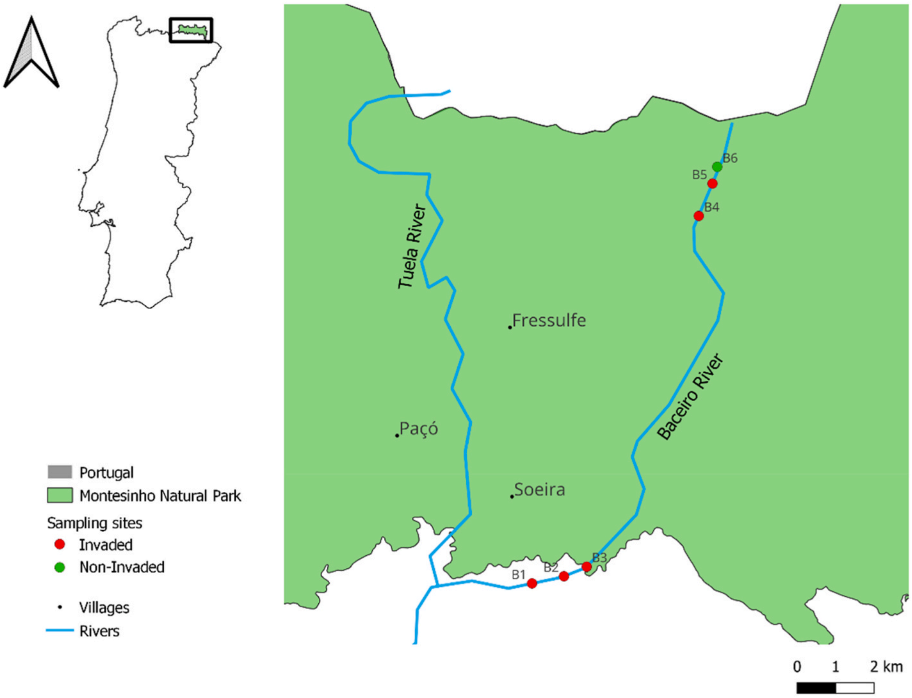

Fig. 1. Map of the surveyed area showing the location of the 6 sampling sites in Baceiro River. Map was produced using QGIS software (QGIS Development Team, 2022).

2.5. Data analysis

All statistical analyses were carried out using the R Studio software (R Core RStudio Team, 2022).

A Principal Component Analysis (PCA) was conducted to assess how the measured environmental factors vary across the six sampling sites and over time. The variables were normalized prior to analysis, enabling the classification of sampling sites based on their environmental characteristics.

To evaluate spatial and temporal variations in the relative abundances of brown trout and signal crayfish, two-way Analysis of Variance (ANOVA) was performed to test the effects of "Site" and "Julian Day" (JD) on abundance, as well as their interaction. Post-hoc pairwise comparisons were carried out using Tukey's Honest Significant Difference (HSD) test, based on estimated marginal means (EMMs), to identify significant differences between groups for both factors and their interaction.

Bar plots were used to analyze the composition and relative abundance of prey in the stomach contents of brown trout across the six sampling sites. These plots showed the proportional distribution of prey families over time, highlighting temporal and spatial variations in diet composition. The relative biomass index was also applied to assess the contribution of each prey family to the brown trout diet.

To assess whether abiotic and biotic factors influenced brown trout diet composition, three Generalized Linear Models (GLMs) were fitted with different response variables: prey richness, prey biomass, and Shannon diversity index. Prey richness was modeled with a Poisson distribution and log link function, while prey biomass and Shannon diversity index were modeled with Gaussian distributions and identity link functions. Predictor variables included trout length (cm), water temperature (°C), conductivity (μS/cm), pH, crayfish biomass (g), sampling site (categorical), and Julian day (categorical). Multicollinearity was assessed using the Variance Inflation Factor (VIF), with values below 7 considered acceptable. All continuous predictors were checked for redundancy; conductivity was retained over TDS due to a strong correlation between the two variables ($r > 0.95$). Model performance was evaluated using Akaike's Information Criterion (AIC), and residual diagnostics were visually inspected to confirm model adequacy.

A two-dimensional Non-metric Multidimensional Scaling (nMDS) was performed on square-root–transformed data using a Bray–Curtis similarity matrix to visualize spatial and temporal patterns in prey assemblages. Afterwards, a PERMANOVA was run with 9999 permutations under a reduced model on the same transformed data to test for differences among sampling sites and JDs. Post-hoc pairwise PERMANOVA comparisons were conducted to explore differences between sampling sites and JDs, and estimates of components of variation were calculated to quantify each factor's contribution to overall variance. Within- and between-group Bray–Curtis similarities were calculated to assess the cohesion and dissimilarity of prey assemblages. Finally, a similarity percentage (SIMPER) analysis with a cut-off of 90 % was also used to verify the species that had the most influence on the dissimilarity between sampling sites and JDs.

3. Results

3.1. Environmental characterization

The PCA based on the environmental characteristics divided the sampling sites into two major groups (Fig. 2). The first group, which includes sites B1, B2, and B3, was characterized by higher conductivity and TDS values, while the second group, with sites B4, B5, and B6, showed lower values for these variables. PC1 explained 55.6 % of the total variance, with the primary contributions coming from temperature, conductivity, and TDS. PC2 explained 22.9 % of the total variance and was strongly influenced by JDs. The detailed environmental characterization for each site and JDs is provided in Table S1.

3.2. Brown trout and signal crayfish population dynamics

The abundance of brown trout varied significantly across space and time (Fig. 3). Sites B4, B5, and B6 showed a sharp increase in abundance, peaking around JD 226 before declining, while sites B1, B2, and B3 exhibited more gradual increases with generally lower abundance. No significant differences in brown trout abundance were observed between sites ($F(5, 48) = 2.216$, $p = 0.070$). ANOVA confirmed a

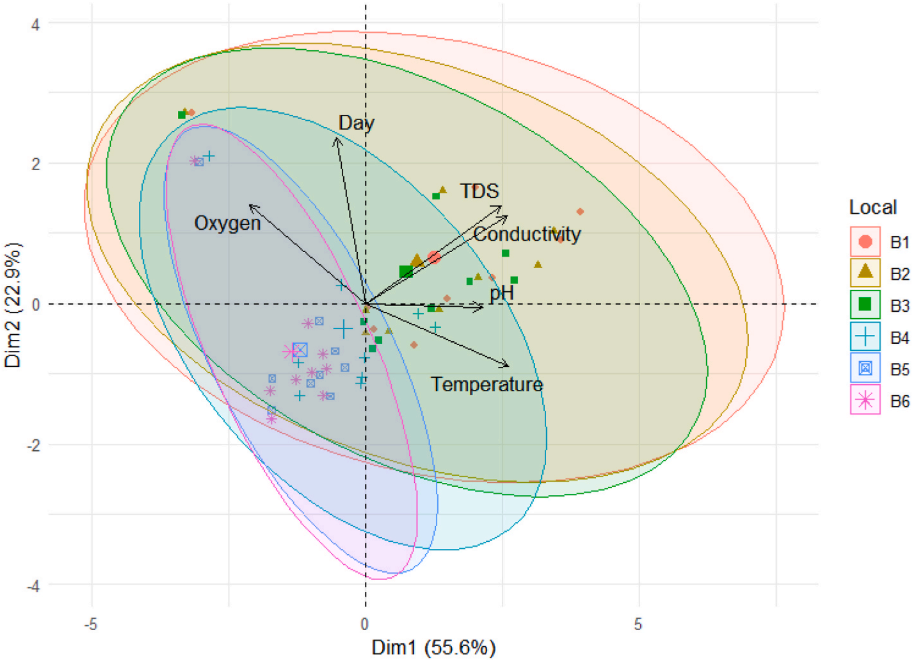

Fig. 2. Principal Components Analysis (PCA) showing the arrangement of the six sampling sites based on the abiotic factors measured throughout the year. PC1 explains 55.6 % of all variances and PC2 22.9 %.

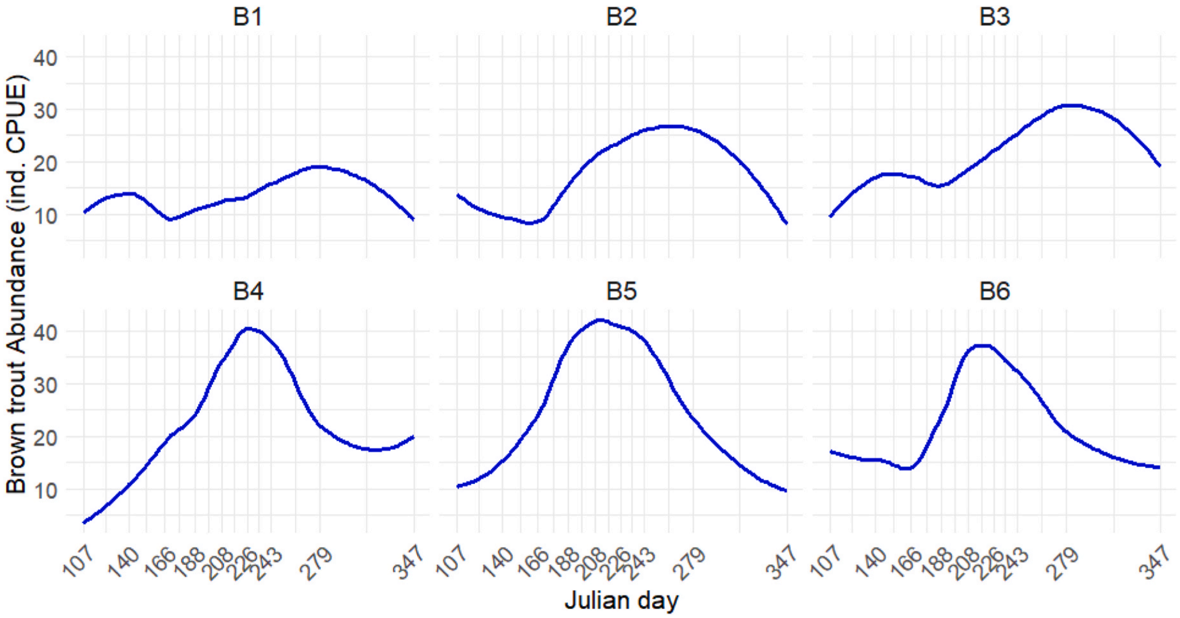

Fig. 3. Abundance of brown trout over time and sampling sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

significant effect of Julian Day (JD) on brown trout abundance (F(8, 45) = 4.86, $p < 0.001$). Tukey's test revealed higher abundance on JD 208 ($p = 0.037$), JD 226 ($p = 0.002$), and JD 243 ($p = 0.014$) compared to JD 107. Additionally, JD 226 had significantly higher abundance than JD 166 ($p = 0.014$) and JD 347 ($p = 0.012$). The lengths of the brown trout varied between 4.0 and 29.5 cm (data not shown).

The IC of brown trout revealed significant effects of site ($\chi^2 = 88.60$, df = 5, $p < 0.001$) and JD ($\chi^2 = 338.38$, df = 8, $p < 0.001$) (Fig. 4). Post-hoc pairwise Wilcoxon tests showed that IC was significantly higher at downstream sites (B1 and B2) compared to upstream sites (B4, B5, and B6) ($p < 0.001$ for all comparisons). Temporally, IC values declined progressively throughout the sampling period, with significantly higher values on early Julian days (e.g., JD 107) than on later dates such as JD 226 or JD 347 ($p < 0.001$). JD 347 exhibited significantly lower IC values than all other sampling dates (all $p < 0.001$, Wilcoxon post-hoc tests, Bonferroni-corrected).

Signal crayfish abundance showed significant spatial (F(5, 410) = 22.87, $p < 0.0001$) and temporal (F(8, 407) = 17.79, $p < 0.001$) variation (Fig. 5). Tukey's test revealed significantly higher signal crayfish abundance at sites B2 and B3 compared to site B5 ($p < 0.001$), while B1 and B4 displayed intermediate values. Tukey's post-hoc test indicated that abundance increased substantially from JD 140 onward, peaking on JD 226 before sharply declining. Crayfish abundance on JD 226 was significantly higher than on JD 107, JD 140, and JD 166 ($p < 0.0001$ for

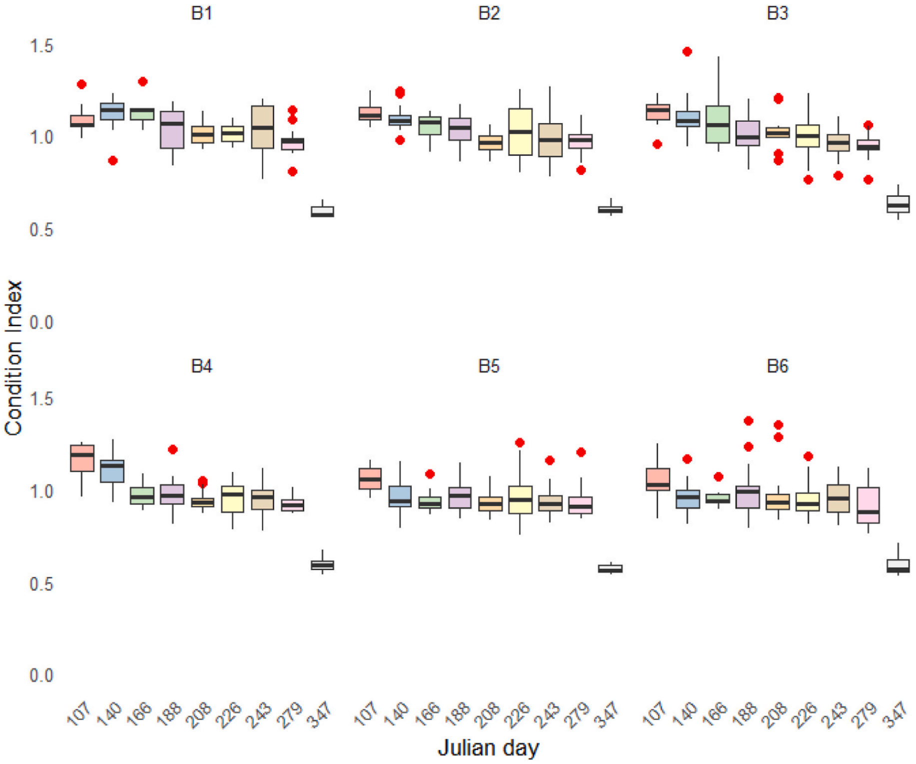

Fig. 4. Boxplot of ecophysiological condition index of brown trout over time and sampling sites. Boxplots show median values (central line), the range from the 25th to 75th percentile (box), and the largest and lowest value within 1.5 times the interquartile range below and above the 25th and 75th percentile (whiskers). Dots represent extreme values. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

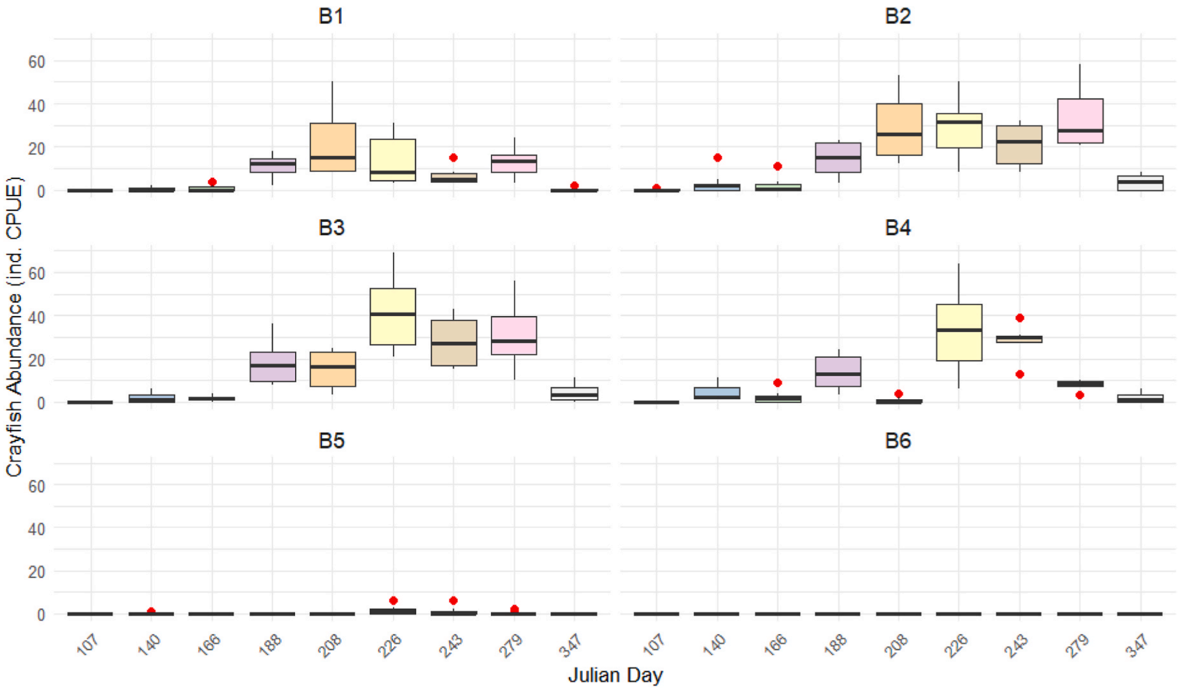

Fig. 5. Abundance of signal crayfish over time and sampling sites.

all comparisons). By JD 347, abundance decreased markedly relative to the summer peak (e.g., JD 347 vs. JD 226 and JD 279: $p < 0.0001$ for both), returning to levels comparable to those observed early in the season (JD 107–166) (see Fig. 5).

3.3. *Brown trout diet*

The diet of brown trout varied significantly across the sampling sites and JD, reflecting both spatial and temporal variations in prey availability and consumption (Fig. 6), with certain orders like Ephemeroptera

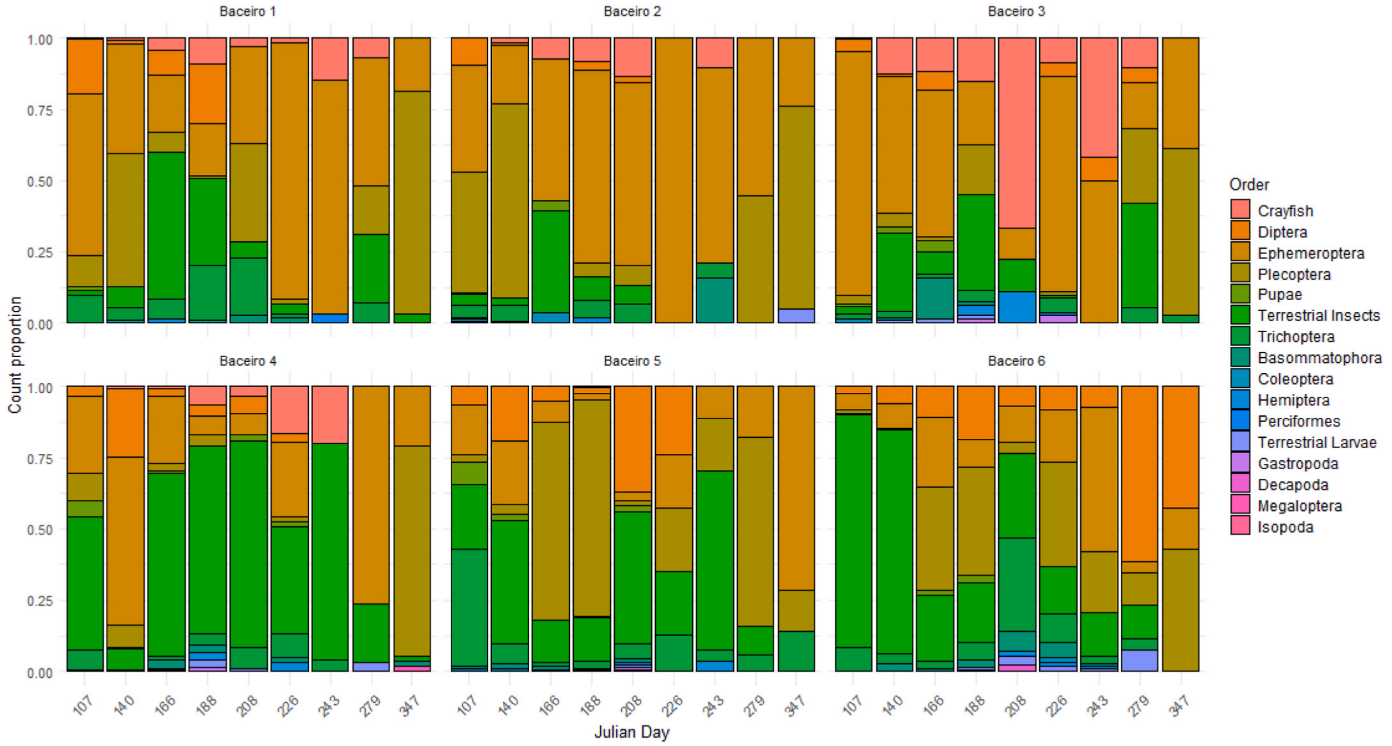

Fig. 6. Count proportion of taxonomic orders present on brown trout stomach contents over time and sampling sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(mainly Baetidae and Heptageniidae) consistently dominating the brown trout's diet in earlier JD (e.g., JD 107 and 140). In later JD there was a clear shift from aquatic-dominated prey compositions to more terrestrial insect dominance (JD 188 to 226). Signal crayfish appeared in the stomach contents of brown trout from sampling sites B1 to B4 during midsummer (JD 188–243), always in low percentages (<5 %). The detailed diet characterization for each site and JDs is provided in Table S2.

The Relative Biomass Index (Fig. 7) highlights the contribution of each prey type to the overall brown trout diet, confirming the dominance of certain prey groups when considering their contribution by dry weight. Terrestrial insects accounted for the highest percentage (22.5 %) of the total biomass consumed, followed by Baetidae (15.5 %), Simuliidae (11.4 %), and Heptageniidae (10.9 %). Despite their low occurrence, crayfish represented 5.5 % of the relative biomass index. However, when considering the total biomass summed across all samples, signal crayfish accounted for 17.7 % of the total prey biomass in invaded sites.

The GLM analysis revealed that multiple factors significantly influenced the structure of prey assemblages found in brown trout stomachs. Species richness increased with trout length ($p < 0.001$), while conductivity showed a marginal negative effect ($p < 0.052$). Sites B3 ($p = 0.002$), B4 ($p < 0.001$), B5 ($p = 0.033$), and B6 ($p = 0.002$) showed significantly lower richness compared to B1 (Table 1). Richness was also

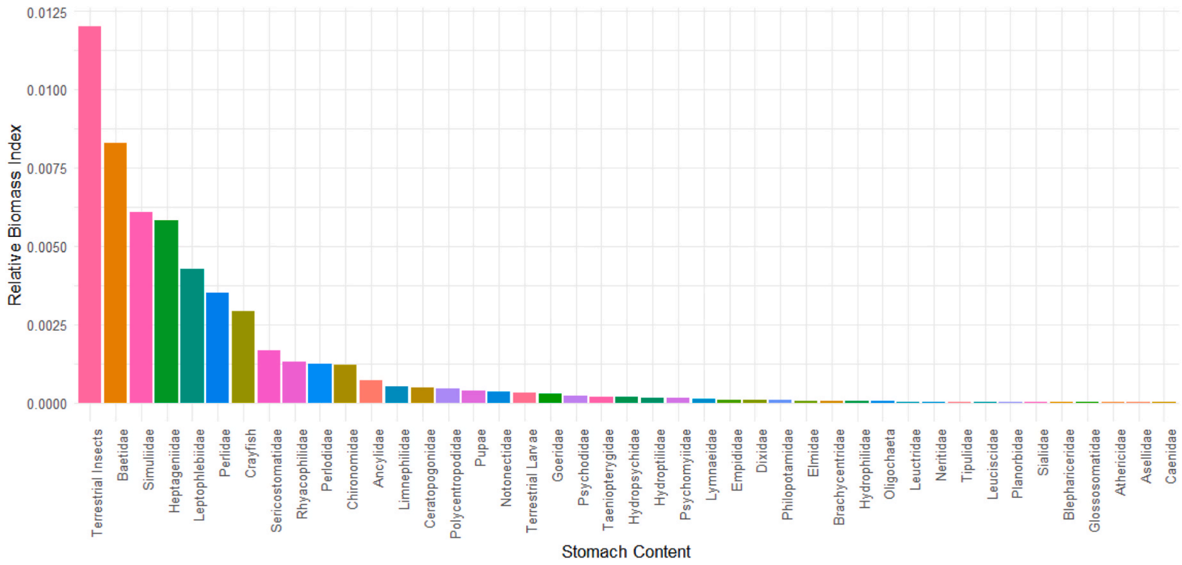

Fig. 7. Relative biomass index by brown trout stomach content.

Table 1

Summary of the generalized linear model (GLM) with a Poisson distribution and log link function. The response variable is prey richness (S) found in brown trout stomach contents. The predictor variables include trout length (cm), water temperature (°C), conductivity (μS/cm), pH, crayfish biomass (g), sampling site (factor with B1 as reference level), and Julian day. The table presents model estimates, standard errors, z-values, and p-values. Significant effects ($p < 0.05$) are marked with *, and highly significant effects ($p < 0.001$) with ***.

Variable	Estimate	Std. Error	z value	p-value
(Intercept)	6.0793	4.0213	1.512	0.130591
Trout Length (cm)	0.0323	0.0064	5.075	3.88e-07***
Water Temperature (°C)	0.0169	0.0129	1.313	0.189155
Conductivity	−0.0094	0.0024	−3.816	0.000135***
pH	−0.5742	0.5515	−1.041	0.297770
Crayfish biomass (g)	−0.6091	0.4196	−1.452	0.146594
Site B2	−0.0483	0.0974	−0.496	0.619653
Site B3	−0.3169	0.1027	−3.086	0.002026*
Site B4	−0.6864	0.1361	−5.045	4.53e-07***
Site B5	−0.5285	0.1728	−3.058	0.002229*
Site B6	−0.7396	0.1749	−4.229	2.35e-05***
Julian Day 140	−0.3877	0.0943	−4.108	3.99e-05 ***
Julian Day 166	−0.2346	0.1882	−1.247	0.21256
Julian Day 188	−0.1097	0.2882	−0.381	0.70356
Julian Day 208	−0.3259	0.2915	−1.118	0.26358
Julian Day 226	−0.4619	0.3205	−1.441	0.14949
Julian Day 243	−0.5910	0.3258	−1.814	0.06964
Julian Day 279	−0.8949	0.1947	−4.597	4.29e-06 ***
Julian Day 347	−1.3527	0.3433	−3.940	8.16e-05 ***

significantly lower on later sampling days, particularly JD 140 ($p < 0.001$), JD 279 ($p < 0.001$), and JD 347 ($p < 0.001$) (Table 1). Prey biomass was positively associated with crayfish biomass ($p < 0.001$), suggesting direct consumption of signal crayfish by trout in invaded sites (Table 2). Sampling day also played a role, with JD 140 ($p = 0.016$) and JD 279 ($p = 0.025$) exhibiting significantly lower biomass relative to JD 107 (Table 2). Shannon's diversity index was significantly influenced by trout length ($p < 0.001$) and was negatively related to crayfish biomass ($p = 0.006$) (Table 3). Diversity declined over time, with significantly lower values on JD 140 ($p = 0.009$), JD 279 ($p < 0.001$), and JD 347 ($p < 0.001$). Sites B3, B4, B5, and B6 also showed lower diversity compared to B1 ($p < 0.001$ for all) (Table 3).

Table 2

Summary of the generalized linear model (GLM) with a Gaussian distribution and identity link function. The response variable is total prey biomass (B) found in brown trout stomach contents. The predictor variables include trout length (cm), water temperature (°C), conductivity (μS/cm), pH, crayfish biomass (g), sampling site (factor with B1 as reference level), and Julian day. The table presents model estimates, standard errors, t-values, and p-values. Significant effects ($p < 0.05$) are marked with *, and highly significant effects ($p < 0.001$) with ***.

Variable	Estimate	Std. Error	t value	p-value
(Intercept)	2.9601	3.9021	0.759	0.448439
Trout Length (cm)	0.0002	0.0067	0.025	0.980247
Water Temperature (°C)	−0.0205	0.0130	−1.573	0.116404
Conductivity	0.0040	0.0026	1.544	0.123138
PH	−0.3648	0.5346	−0.682	0.495266
Crayfish biomass (g)	0.9987	0.2647	3.771	0.000181***
Site B2	−0.0192	0.1153	−0.167	0.867810
Site B3	−0.0185	0.1152	−0.161	0.872331
Site B4	0.1301	0.1464	0.889	0.374503
Site B5	0.1874	0.1882	0.996	0.319787
Site B6	0.3042	0.1878	1.619	0.105980
Julian Day 140	−0.2961	0.1221	−2.425	0.01564 *
Julian Day 166	−0.2894	0.2066	−1.401	0.16184
Julian Day 188	−0.4333	0.3056	−1.418	0.15690
Julian Day 208	−0.5111	0.3106	−1.645	0.10050
Julian Day 226	−0.6036	0.3387	−1.782	0.07532
Julian Day 243	−0.5966	0.3386	−1.762	0.07866
Julian Day 279	−0.4360	0.1935	−2.253	0.02465 *
Julian Day 347	−0.1530	0.3399	−0.450	0.65287

Table 3

Summary of the generalized linear model (GLM) with a Gaussian distribution and identity link function. The response variable is the Shannon diversity index (H) of prey items found in brown trout stomach contents. The predictor variables include trout length (cm), water temperature (°C), conductivity (μS/cm), pH, crayfish biomass (g), sampling site (factor with B1 as reference level), and Julian day. The table presents model estimates, standard errors, t-values, and p-values. Significant effects ($p < 0.05$) are marked with *, and highly significant effects ($p < 0.001$) with ***.

Variable	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	4.6940	2.6774	1.753	0.08014
Trout Length (cm)	0.0212	0.0046	4.584	5.68e-06 ***
Water Temperature (°C)	0.0199	0.0089	2.225	0.02648 *
Conductivity	−0.0078	0.0018	−4.435	1.12e-05 ***
PH	−0.4827	0.3668	−1.316	0.18876
Crayfish biomass (g)	−0.3456	0.1817	−1.918	0.05566
Site B2	−0.0635	0.0791	−0.803	0.42254
Site B3	−0.2274	0.0791	−2.877	0.00418*
Site B4	−0.5764	0.105	−5.738	1.60e-08 ***
Site B5	−0.4601	0.1291	−3.563	0.00040 ***
Site B6	−0.6003	0.1289	−4.657	4.05e-06 ***
Julian Day 140	−0.2146	0.0825	−2.601	0.009546 *
Julian Day 166	−0.0064	0.1396	−0.046	0.963520
Julian Day 188	0.2470	0.2065	1.196	0.232185
Julian Day 208	0.0434	0.2099	0.207	0.836468
Julian Day 226	−0.0581	0.2289	−0.254	0.799704
Julian Day 243	−0.1655	0.2288	−0.723	0.469695
Julian Day 279	−0.4748	0.1307	−3.632	0.000309 ***
Julian Day 347	−0.9893	0.2297	−4.306	1.98e-05 ***

The nMDS revealed distinct clustering of prey assemblages by sampling site and JD (Fig. 8). Early sampling days (e.g., JD 107, 140) grouped tightly and were characterized by aquatic prey taxa, while later days (e.g., JD 243, 279) showed a shift towards more terrestrial prey and occasional presence of crayfish. PERMANOVA results confirmed significant differences in prey assemblage composition among sampling sites ($F = 2.48$, $p = 0.005$) and JD ($F = 18.43$, $p < 0.001$). Interaction effects between sites and JD were also significant ($F = 1.64$, $p < 0.001$). Estimates of variation showed that JD explained the largest portion of dissimilarity (26.5 %), followed by the interaction term (11.5 %) and sampling site (2.2 %). Among the pairwise comparisons, B1 and B4 were significantly different in prey assemblages ($p = 0.022$). Significant differences were also found between B1 and B3 ($p = 0.030$), and between B3 and B4 for the evenness index ($p = 0.002$). Pairwise comparisons indicated significant dissimilarities between early and late JD as well as between downstream (e.g., B1) and upstream (e.g., B4–B6) sites (all $p < 0.05$).

SIMPER analysis revealed that only a few of the prey taxa were responsible for most of the spatial and temporal variation detected in the brown trout diet. Regarding the sampling sites, terrestrial insects contributed 27–40 % of the between-site variation, Baetidae 10–20 %, Heptageniidae 5–15 % and Simuliidae 6–14 %, with signal crayfish providing smaller but recurrent contributions (≤8 %) at invaded downstream sites. Regarding the temporal analysis, early JD (107–166) were contrasted with late ones (JD 188–347), and terrestrial insects, Baetidae, and Simuliidae, accounted for more than 70 % of that turnover.

4. Discussion

Brown trout exhibited marked spatial and temporal variation in diet composition in the Baceiro River, reflecting their opportunistic feeding behavior and the influence of both environmental gradients and seasonal dynamics. As a generalist predator, brown trout adjust their foraging strategies in response to local prey availability and environmental conditions, which in this study were shaped by variation in temperature, conductivity, and the presence of the invasive signal crayfish.

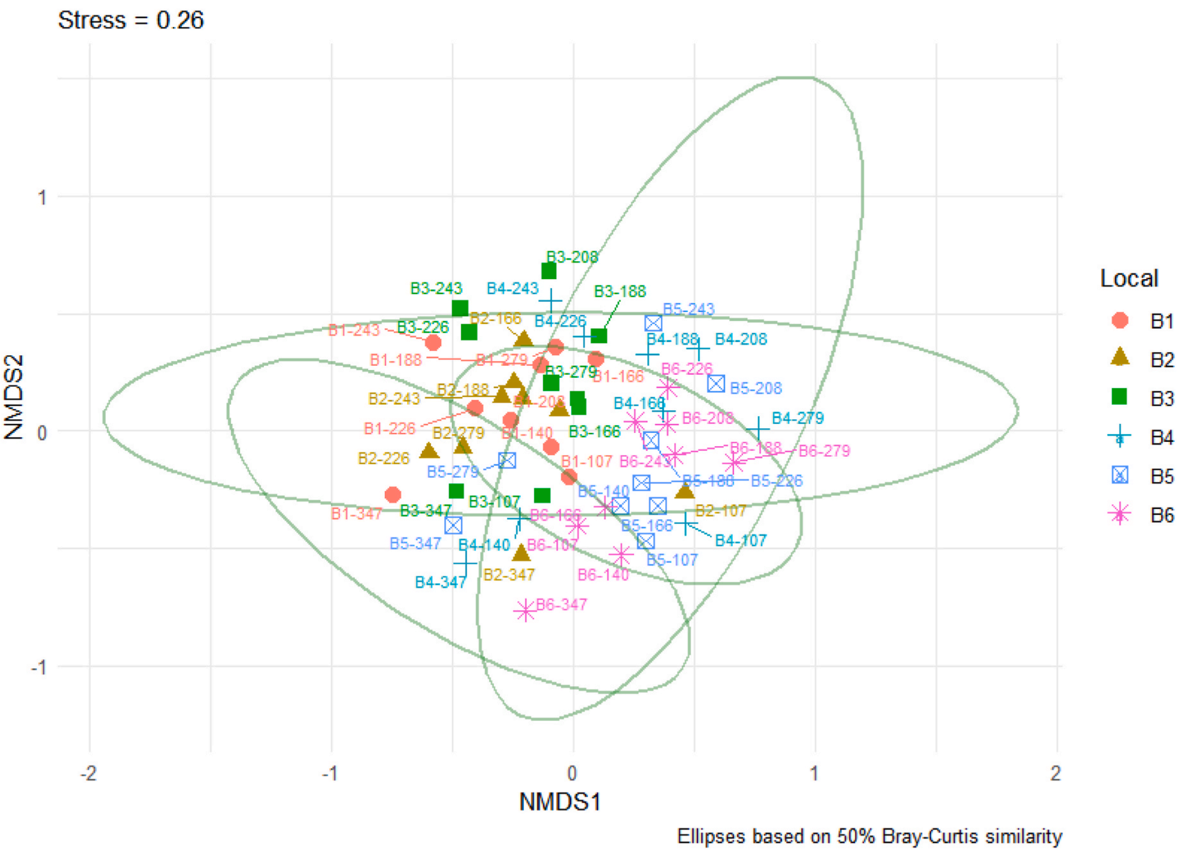

Fig. 8. Non-metric Multi-Dimensional Scaling (nMDS) of brown trout stomach content similarity over time and sampling sites. Sampled sites grouped by 50 % similarity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.1. *Environmental characterization*

The results of the environmental characterization showed clear spatial and temporal differences. Sampling sites B1, B2, and B3, located downstream at lower altitudes, exhibited consistently higher conductivity and TDS compared to upstream sites B4, B5, and B6, which may influence habitat preferences of brown trout and signal crayfish. The spatial variation suggests a natural longitudinal gradient, likely influenced by increasing mineral accumulation downstream (Vannote et al., 1980). Seasonal variation was also evident, with temperature contributing most strongly to temporal differences. These seasonal variations are typical of temperate freshwater ecosystems (Rikardsen et al., 2006; Sousa et al., 2007). In contrast, pH and dissolved oxygen showed relatively minor fluctuations across space and time. The observed environmental gradient aligns with patterns of variation in biological communities, supporting the river continuum concept for these latitudes, which posits that physical and chemical changes along a river's course drive predictable ecological shifts in structure and function (Vannote et al., 1980).

4.2. *Brown trout and signal crayfish population dynamics*

The observed temporal pattern of brown trout abundance, peaking around mid-to-late summer is consistent with seasonal fluctuations typically observed in salmonid populations, driven by temperature, prey availability, and reproductive cycles (Lobón-Cerviá, 2009; Elliott and Elliott, 2010; Blanchfield et al., 2023). The increase in brown trout abundance during warmer periods may also reflect increased detectability due to lower river flow, which enhances electrofishing efficiency, potentially contributing to elevated capture rates. Lower brown trout abundance in downstream sites (B1–B3) could be associated with local factors such as reduced habitat quality, higher fishing pressure, and higher signal crayfish abundance in these sections (Oliveira et al., 2025). The ecophysiological condition index (IC) of brown trout exhibited significant spatial and temporal variation, suggesting that both abiotic and biotic factors influence their physiological condition. Lower IC values observed in upstream sites B4, B5, and B6, particularly when compared to B1, may reflect increased intraspecific competition in these areas, where brown trout abundance was higher. In contrast, higher IC values in downstream sites (B1–B3) may be related to reduced intra-specific competition or could indicate that brown trout are benefiting from the presence of signal crayfish, because in these sites this non-native species has been present for a longer time. This interpretation is supported by the significant differences in the number of signal crayfish found in trout stomachs across sites, with higher counts recorded in B3 and B1 compared to B5, where crayfish were recently introduced and remain scarce. These findings suggest that brown trout in formerly invaded areas may be partially incorporating signal crayfish into their diet, which could positively influence their condition. The interaction between site and JD further emphasizes the complex interplay of local environmental conditions and seasonal dynamics affecting brown trout fitness. Such variation is consistent with previous studies highlighting the sensitivity of salmonid condition to spatial and temporal changes in food availability and habitat characteristics (Klemetsen et al., 2003; Milner et al., 2003; Blanchfield et al., 2023). This seasonal pattern may reflect changes in food availability, metabolic stress, or reproductive investment across the year. The drop in ecophysiological condition index observed around JD 347 aligns with the spawning period of *S. trutta,* which typically occurs between November and February in Iberian populations. Other studies showed similar reductions in condition following spawning, highlighting the substantial energetic costs associated with reproduction (Birnie-Gauvin et al., 2019). For instance, first-time spawning females were found to lose approximately 20 % of their total energy reserves during the reproductive period (Jonsson and Jonsson, 2006).

Signal crayfish abundance also showed pronounced spatial and temporal variations, with increased activity and capture rates during warmer months, particularly after JD 140, peaking around JD 226, before declining. This pattern is characteristic of the species' ectothermic physiology, with elevated activity during warmer months, corresponding to known periods of activity and foraging (Rodríguez Valido et al., 2021; Hudina et al., 2017), likely exacerbated by the elevated metabolic demands typical of summer conditions (Rikardsen et al., 2006; Sousa et al., 2013). The subsequent decline in abundance in colder periods is likely due to reduced activity and burrowing behavior, which provide thermal refuge (Hudina et al., 2017; Payette and McGaw, 2003). Spatial variation in crayfish abundance was also notable, with significantly higher abundance in downstream invaded sites (B1–B3) decreasing towards the upstream areas, consistent with an ongoing upstream invasion front. The Baceiro River provides a clear case of active spread, with B1 as the initial colonization point and site B5 currently representing the invasion front. This upstream gradient in crayfish abundance is consistent with known patterns of invasion fronts exhibiting lower densities compared to established core areas (Hudina et al., 2012; Alves et al., 2025; Carvalho et al., 2025).

4.3. Brown trout diet

The diet of *S. trutta* showed pronounced spatial and temporal variation, with clear separation between early (aquatic-dominated) and late (terrestrial-dominated) sampled periods, with JD explaining the largest proportion of variation. These results also reflect the species' opportunistic feeding behavior and seasonal changes in prey availability (Bridcut and Giller, 1995; Sánchez-Hernández et al., 2010). In early sampling periods (e.g., JD 107 and 140), the diet was dominated by aquatic invertebrates, particularly Ephemeroptera (Baetidae, Heptageniidae) and Diptera (Simuliidae), consistent with patterns reported for salmonids in similar mountain rivers (Bridcut and Giller, 1995; Sánchez-Hernández et al., 2010). A marked shift toward terrestrial insects was observed between JD 188 and JD 226, a transition commonly associated with increased input of allochthonous material during summer (Nakano and Murakami, 2001; Polis et al., 1997; Kawaguchi and Nakano, 2001).

Despite its low frequency in the diet (9.98 % of individuals; 2.09 % of all prey items), signal crayfish accounted for 5.5 % of the Relative Biomass Index and 17.68 % of the total summed prey biomass. This indicates that, although signal crayfish may not be a primary food source for brown trout, they can represent a substantial dietary contribution when present, likely due to their large individual mass. Larger brown trout were more likely to prey upon crayfish, suggesting a size-dependent feeding strategy, likely constrained by morphological limitations such as gape size and prey handling capacity (Aquiloni et al., 2010; Nyström et al., 2006). Predation occurred mostly during midsummer (JD 188–243), coinciding with peak crayfish activity, and was primarily detected at sites B1 to B4. The low occurrence of crayfish in brown trout diet may reflect behavioral or ecological factors, including handling difficulty due to the crayfish's hard exoskeleton and defensive behavior, or lag in prey recognition in recently invaded areas (Carroll, 2007; Cox, 2013; Sbragaglia and Breithaupt, 2022). Spatially, signal crayfish predation was higher in downstream sites (B1–B3), where this species is well established, and lower in B4–B5, likely due to the recent upstream colonization. This pattern suggests that time since invasion may influence prey-predator dynamics, supporting the notion that native predators gradually incorporate novel prey over time (Santamaría et al., 2022; Carlsson et al., 2009).

GLMs revealed that species richness, prey biomass, and Shannon diversity were significantly influenced by multiple predictors, including crayfish biomass, trout length, sampling site, and JD. Signal crayfish biomass was positively associated with total prey biomass but negatively associated with diversity indices, suggesting potential impacts of signal crayfish on the structure of prey assemblages (Carvalho et al., 2022). Richness and diversity declined significantly over time, particularly on later sampling days (JD 243, 279, and 347), a pattern consistent with seasonal reductions in aquatic invertebrate availability (Power, 1992; Teixeira and Cortes, 2006).

Lastly, environmental conditions, particularly temperature and water chemistry, may further modulate the interaction between brown trout and signal crayfish. Temperature is known to influence digestion rates in brown trout, potentially increasing their metabolic demand and feeding frequency during warmer periods (Elliot, 1972; Ovidio et al., 2002), which could in turn affect their capacity to consume signal crayfish. Simultaneously, elevated temperatures also stimulate the activity and foraging behavior of signal crayfish (Crawford et al., 2006; Nyström et al., 2001), potentially intensifying their ecological impact. These ecological impacts have been well documented in freshwater ecosystems, including reductions in benthic invertebrate diversity and macrophyte cover (Jackson et al., 2014; Mathers et al., 2016; Carvalho et al., 2018; Galib et al., 2021; Carvalho et al., 2022). In this study, downstream sites (e.g., B1–B3) with higher conductivity and temperature may therefore experience greater ecological pressure from signal crayfish, both directly and indirectly through altered food web dynamics.

5. Conclusion

This study underscores the spatial and temporal variability in the diet of *S. trutta* in a river system recently invaded by *P. leniusculus*. Brown trout displayed notable dietary plasticity, shifting from aquatic to terrestrial prey from spring to summer, consistent with their generalist feeding behavior. Although signal crayfish were consumed occasionally, particularly by larger trout and during warmer periods, their contribution to the diet was low. These results suggest that brown trout are unlikely to exert significant biotic resistance to signal crayfish under current conditions.

Long-term monitoring and further studies using complementary techniques such as stable isotope analysis or DNA metabarcoding are recommended to fully assess trophic interactions and track potential shifts in the diet of brown trout. This study could be used as a reference to compare future changes in brown trout diet in response to the invasion dynamics of the signal crayfish in an important protected area. Understanding how brown trout and other native predators, such as the Eurasian otter (*Lutra lutra*), interact with signal crayfish over time will be essential for guiding future conservation and management efforts in the Baceiro River and other invaded freshwater ecosystems. Effective protection of these native predators may help reduce signal crayfish abundance and biomass. Therefore, management actions that promote sustainable fishing, enhance in-stream structure, improve connectivity, and encourage local community involvement in conservation programs should be prioritized.

CRediT authorship contribution statement

Matilde Matos: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Amílcar Teixeira:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **António B. Nogueira:** Writing – review & editing, Investigation. **Janeide Padilha:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Ronaldo Sousa:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actao.2025.104135.

Data availability

Data will be made available on request.

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