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Brown trout as a potential biological control of signal crayfish

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Abstract

This study evaluates the potential of brown trout (*Salmo trutta*) as a biological control of a recently established signal crayfish (*Pacifastacus leniusculus*) population in a protected area (Baceiro River, Montesinho Natural Park, Portugal). Five sampling sites were monitored over an entire year. Results indicated that brown trout was able to predate on signal crayfish but in an infrequent manner since only 12.24 % of the samples showed signs of signal crayfish in their stomachs. The number of signal crayfish in the stomach contents of brown trout was also low (only 2.13% of all prey items) but accounted for 17.70% of the total biomass of all retrieved prey items. Predation was higher in the warmer months and was size-dependent, with larger fish more able to predate on this non-native crayfish species. These findings highlight that, although brown trout can prey on signal crayfish, their effectiveness as a biological control agent is limited due to their lower abundance and predation rates. A multi-faceted management approach integrating other control strategies (mechanical removal) is recommended to decrease the abundance and biomass of the signal crayfish, and potentially their impacts, to maintain the ecosystem balance in this protected area.

Keywords: Biocontrol; freshwater ecosystems; non-native species; Montesinho Natural Park; *Pacifastacus leniusculus*, *Salmo trutta*

Introduction

Non-native species are a major threat to biodiversity, particularly in freshwater ecosystems (Strayer, 2010; Gallardo et al., 2016). Among these, the signal crayfish (*Pacifastacus leniusculus*) stands out as one of the most ecologically disruptive non-native species in Europe (Krieg et al., 2020). Native to North America, this species was introduced primarily for aquaculture and commercial reasons (Holdich et al., 2014) but has rapidly expanded its range, interacting with native species for resources and habitat use and altering key ecological processes (Galib et al., 2021; Alves et al., 2025). Its presence leads to cascading effects on freshwater ecosystems, including changes in trophic interactions, reductions in native biodiversity, and habitat degradation (Carvalho et al., 2022a, 2025). The first documented occurrence of signal crayfish in Portugal dates back to 1997 in the Maçãs River (Bernardo et al., 2011), and since then, this non-native species has been increasing in abundance and expanding its distribution in the northeast part of the country (Anastácio et al., 2019; Meira et al., 2019; Carvalho et al., 2025).

Traditional control measures applied to signal crayfish, such as mechanical removal and chemical treatments, often fail to provide long-term solutions and can have unintended ecological consequences (Peay et al., 2019; Moorhouse et al., 2014). A promising alternative is the use of native predators such as eels (*Anguilla anguilla*) to regulate non-native crayfish populations, leveraging natural ecological interactions to restore balance within affected ecosystems (Aquiloni et al., 2010; Musseau et al., 2015). Given the ecological impact of signal crayfish and the challenges of traditional control methods in mountainous oligotrophic rivers, investigating the role of potential native predators, such as brown trout (*Salmo trutta*), is crucial to understanding whether they can contribute as a biological control of this non-native species. The brown trout, a widely distributed species in European freshwater ecosystems, is known for its opportunistic feeding behavior, which may include crayfish species (Bridcut and Giller, 1995). As a visual predator, brown trout primarily rely on sight to capture prey, with feeding activity peaking at dawn and dusk (Klemetsen et al., 2003). This behavioral pattern aligns with the described nocturnal activity of signal crayfish (Sbragaglia and Breithaupt, 2022), making this non-native species potentially vulnerable to predation during crepuscular hours. Additionally, larger brown trout have been observed to incorporate progressively larger prey into their diet, suggesting that adult individuals could exert significant predation pressure on crayfish populations (Klemetsen et al., 2003).

Although predation by brown trout may contribute to the control of signal crayfish, its effectiveness depends on various ecological factors, including prey availability, environmental conditions, and the adaptability of both species (Carlsson et al., 2009). Moreover, there may be a lag period before native predators recognize non-native crayfish as a viable food source, potentially allowing their populations to expand before significant predation pressure is exerted (Carroll, 2007; Cox, 2013). Given this background, and because prevention (signal crayfish is already in the system) and eradication (signal crayfish is already widespread in the studied protected area) are no longer viable solutions, understanding the potential role of brown trout as a natural predator is essential for assessing the viability of biological control as a sustainable management strategy (e.g. better conserve or even re-stock brown trout populations in invaded areas). Therefore, this study aimed to: i) assess the degree of predation of brown trout on the signal crayfish; ii) assess possible spatial and temporal differences in predation rates; and iii) evaluate if this predation is size-dependent. We hypothesise that: i) brown trout will predate on signal crayfish, with predation rates being higher in sites where crayfish are more abundant; ii) predation rates will increase in warmer months due to greater activity of both species; and iii) larger trout will consume more crayfish.

Material and Methods

Study Area

Our study area comprised five sampling sites along the Baceiro River (Figure 1), within the Montesinho Natural Park (NE Portugal), a protected area created in 1979 (Castro et al., 2010). This protected area has a high biodiversity, hosting, for example, about 80% of Portugal's mammal species. The Baceiro River, with a total length of 60 km, originates in Spain and belongs to the Douro basin (Sousa et al., 2019). The area has very low human disturbance and harbours a rich aquatic biodiversity with high conservation status (e.g. Pyrenean desman *Galemys pyrenaicus*). Regarding the fish community, the brown trout (*Salmo trutta*), the Northern straight-mouth nase (*Pseudochondrostoma duriense*), the Iberian barbel (*Luciobarbus bocagei*), and the Northern Iberian chub (*Squalius carolitertii*) are present in the study area (Oliveira et al., in press). This river is an ideal area for studying predator-prey interactions given the lack of other (besides biological invasions) significant human disturbances, such as pollution and the presence of dams (Sousa et al., 2019, 2020). The signal crayfish was first detected in the Baceiro River in 2013 (Sousa et al., 2015).

Environmental characterization

The following environmental parameters were measured using a HACH HQ2200 multi-parameter probe at every sampling site and throughout the study period: pH, total dissolved solids, conductivity, dissolved oxygen, and temperature.

Brown trout and signal crayfish population dynamics

Brown trout feed, preferably, in the early hours of the day, so their collection was carried always in the morning to coincide with their natural feeding behavior. Fish were captured using electrofishing (Hans Grassl™ ELT60II-GI; 300-600 V, DC, 2200W) for a period of 30-minutes at each sampling site along a standard length of 150 meters of the river channel, comprising an area of about 1000 m2 in each site. Therefore, the abundance of brown trout per site was expressed as the total number of individuals per catch per unit of effort (CPUE/30 min).

A total of 8 to 10 traps were used per site to capture signal crayfish, baited with fish, and left underwater for 24 hours. Traps were strategically placed in areas such as pools, riffles, areas near the banks, and the central part of the river channel. The abundance of signal crayfish per site was expressed as the total number of individuals per catch per unit of effort (ind. CPUE/24 h).

Brown trout diet

A total of 825 stomach contents of brown trout were analyzed to determine whether the signal crayfish was present in the brown trout's diet. This was done through a simple, non-lethal technique, which involved squirting water into the stomach to induce regurgitation. Once the samples were collected, the sampled trout were carefully returned to the river to minimize stress and mortality. Individuals present in the stomach content were identified using Tachet et al. (2010) and were counted. Then, individuals identified for each taxon were dried at 60 ºC for 48h to record their biomass (dry weight). This analysis provided valuable insights into the dietary habits of brown trout and their role in controlling the signal crayfish population, contributing to a better understanding of trophic interactions within the ecosystem.

Data analysis

A Principal Component Analysis (PCA) was conducted to assess how abiotic factors, including temperature, oxygen, conductivity, total dissolved solids (TDS), and pH, vary across the five sampling sites over time. The variables were normalized and analyzed enabling the categorization of sites based on their environmental characteristics.

To assess the spatial and temporal changes in brown trout and signal crayfish abundances, a two-way ANOVA was performed to assess the effects of Site and Julian Day and their interaction. Post hoc pairwise comparisons were conducted using Tukey's Honest Significant Difference (HSD) test based on estimated marginal means (EMMs) for Site, Julian Day, and their interaction to explore differences between groups.

To assess whether abiotic and biotic factors influenced crayfish predation by brown trout, we used a Generalized Linear Model (GLM) with a binomial distribution and logit link function. The response variable was crayfish presence in brown trout stomach contents (0 = absent, 1 = present), and predictor variables included brown trout length (cm), water temperature (°C), total dissolved solids (TDS, mg/L), pH, crayfish abundance (ind. CPUE/24h), sampling site (categorical), and Julian day. We tested for multicollinearity among predictor variables using the Variance Inflation Factor (VIF), considering values >5 as indicative of multicollinearity issues. Due to a strong negative correlation between temperature and dissolved oxygen ($r = -0.89$), we excluded dissolved oxygen from the final model to avoid redundancy. Similarly, total dissolved solids (TDS) showed a very high correlation with conductivity ($r = 0.99$) and so conductivity was also removed to prevent collinearity issues. Model diagnostics were performed by examining residual plots to ensure the appropriateness of the GLM. All analyses were conducted in R using the glm() function from the base stats package.

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

Results

Environmental characterization

The results of the abiotic characterization per site and over time can be seen in Table S1. The PCA categorized the sampling sites into two major groups (Figure 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 and B5, showed lower values for these variables. PC1 explains 68.59% of the total variance, with the primary contributions coming from conductivity and TDS (negative side). PC2 accounted for 18.68% of the total variance and was strongly influenced by temperature. Dissolved oxygen and pH had minimal influence.

Brown trout and signal crayfish population dynamics

Brown trout abundance varied across space and time. Sites B4 and B5 showed a sharp increase, peaking around Julian day 226, followed by a decline (Figure 3). In contrast, sites B1, B2 and B3 exhibited more gradual increases with generally lower abundance (Figure 3). ANOVA indicated a marginal effect of sampling sites on trout abundance ($p = 0.0566$), with post-hoc comparisons revealing that site B5 had significantly higher abundance than site B1 ($p = 0.046$), while differences among other sites were not statistically significant. ANOVA confirmed a significant effect of sampling day on brown trout abundance ($F_{(8, 45)} = 4.86$, $p < 0.001$), with Tukey's test revealing higher abundance on Julian days 208 ($p = 0.037$), 226 ($p = 0.002$), and 243 ($p = 0.014$) compared to Julian day 107. Additionally, Julian day 226 had a significantly higher abundance than Julian day 166 ($p = 0.018$) but a lower abundance than Julian day 347 ($p = 0.012$), reinforcing the trend of declining brown trout abundance toward the later stages of the study period. Brown trout length varied between 4.0 and 28.7cm.

Signal crayfish abundance also showed significant spatial ($F_{(5, 362)} = 22.99$, $p < 0.001$) and temporal ($F_{(1, 366)} = 79.3$, $p < 0.001$) differences (Figure 4). Tukey's test indicated significantly higher crayfish abundances in sites B2 and B3 compared to site B5 ($p < 0.001$), site B1 compared to site B2 ($p = 0.0038$), B3 ($p = 0.0014$) and B5 ($p = 0.0052$). Additionally, B2 ($p = 0.0032$) and B3 ($p < 0.001$) had significantly higher crayfish abundance than B1. Temporal analysis revealed an increase in abundance after Julian day 140, peaking around Julian day 226, followed by a sharp decline (Figure 4), with a significantly lower crayfish abundance on Julian day 107 compared to Julian days 188, 208, 226, 243, and 279 (all $p < 0.05$).

Brown trout as a biological control of signal crayfish

The presence of signal crayfish in the stomach contents of brown trout was low. Among the sampled brown trouts, 12.24% showed evidence of signal crayfish in their stomachs, with crayfish representing only 2.13% of the total prey items sampled. However, signal crayfish accounted for 17.70% of the total biomass of the prey found in the stomach content.

The GLM results indicated that brown trout length ($p < 0.001$), water temperature ($p < 0.001$), and Julian day ($p = 0.002$) were significant predictors of signal crayfish predation. Larger trout had a higher probability of consuming crayfish (Table 1). Water temperature was positively associated with signal crayfish consumption. Conversely, Julian day showed a negative relationship with signal crayfish predation, indicating a decline in consumption over time. Spatial variation also played a role, as sites B4 ($p = 0.007$) and B5 ($p = 0.001$) showed significantly lower predation rates (Table 1). Signal crayfish abundance, however, was not a significant predictor of predation ($p = 0.394$) (Table 1). Model diagnostics indicated no severe multicollinearity (all VIF < 5), and residual analysis suggested an adequate model fit (Figure S1).

Discussion

Despite the presence of signal crayfish in brown trout stomach contents, their occurrence was infrequent, though they contributed significantly to total prey biomass consumed by brown trout. This situation, in addition to the low density of brown trout in comparison with other European rivers (Eklöv et al., 1999), even in the Iberian Peninsula (Nicola et al., 2008), suggests that this predator is unlikely to serve as an effective and sole biological control for signal crayfish in the Baceiro River.

Environmental characterization

The results of the environmental characterization showed clear spatial and temporal differences. Downstream sites (B1, B2, and B3) located at a lower altitude had higher conductivity and TDS compared to upstream sites (B4 and B5), with temperature playing a significant role in seasonal variation. Dissolved oxygen and pH remained relatively stable across sites and time of the year. These environmental patterns are particularly relevant because conductivity and TDS, which were higher in downstream sites, may influence habitat preferences of brown trout and signal crayfish. Likewise, temperature variation across sites aligns with observed seasonal trends in both brown trout and crayfish abundance. Anyway, it should be noted that all sites present very low human disturbance, being the Baceiro River an oligotrophic system characterized by clear waters and low nutrient concentrations.

Brown Trout and Signal Crayfish Population Dynamics

The peak in brown trout abundance was in the mid-to-late stages of the study period and aligns with seasonal patterns of salmonid populations that are influenced by environmental factors such as temperature and food availability (Lobón-Cerviá, 2009; Blanchfield et al., 2023). This observation is in line with the work of Elliott and Elliott (2010), who emphasized the importance of seasonal temperature changes in regulating brown trout population dynamics. However, it should be noted that in these mid-to-late stages of the study period, the river flow is also lower, which may increase the efficiency of electrofishing, contributing to higher abundance values. The spatial variation in brown trout abundance, particularly the lower overall abundance in sites B1, B2, and B3, could be attributed to differences in habitat quality, food availability, the presence of a higher abundance of the non-native signal crayfish, and the higher fishing pressure (Oliveira et al., in press).

The temporal variations in signal crayfish abundance suggest a strong temperature dependence, with crayfish captures increasing during warmer periods and declining during colder months due to reduced activity. As ectothermic organisms, their metabolism and behavior are temperature-dependent (Rodríguez Valido et al., 2021). Crayfish abundance increased after Julian day 140, peaking around day 226, before declining, reflecting typical seasonal activity patterns (Hudina et al., 2014). The observed increase in abundance during warmer periods (Julian days 166-226) likely corresponds to elevated metabolic rates and heightened activity of signal crayfish in response to higher temperatures and so increasing the chances of being captured (Rikardsen et al. 2006; Sousa et al., 2013). The subsequent decline in the autumn and early winter is likely due to decreased activity and burrowing behaviour in colder months, providing thermal stability (Hudina et al., 2014; Payette et al., 2003). Signal crayfish abundance also varied across sites, suggesting that local factors such as food availability, predation pressure, habitat conditions or interspecific interactions influence distribution (Yarra and Magoulick, 2018). Differences may also relate to invasion gradients, where invasion fronts exhibit lower abundances compared to core areas (Hudina et al., 2012; Alves et al., 2025; Carvalho et al., 2025). It should be noted that the Baceiro River site B1 was the first to be colonized by this non-native species and the spread was in the upstream direction, being the current invasion front located at site B5 (first records in this site at the end of the summer 2022; Ronaldo Sousa personal observation).

Brown trout as a biological control of signal crayfish

Only 12% of the brown trout analysed present signal crayfish in their stomach contents. The number of consumed crayfish in comparison to all items identified in the stomach contents was very low (2.13%), but their contribution to the biomass of consumed prey was more considerable (17.70%). The low prevalence and consumption rates of signal crayfish may be attributed to several ecological and behavioral factors. Crayfish possess hard exoskeletons and defensive behaviors, making them more difficult to capture and consume than softer-bodied prey (e.g., aquatic insects and small fish). Furthermore, previous studies have shown that fish predators often exhibit an initial lag in recognizing novel prey (Carroll, 2007; Cox, 2013), which may explain why brown trout do not yet effectively incorporate crayfish into their diet. Despite being an opportunistic feeder, whose hunting activity aligns with the crepuscular behavior of crayfish (Sbragaglia and Breithaupt, 2022), brown trout may still find it challenging to capture and handle this prey efficiently, resulting in its low occurrence in the diet.

Predation of signal crayfish was higher in warmer months and decreased over the year. Higher water temperatures are typically associated with increased metabolic rates and activity levels for both species (Rodríguez Valido et al., 2021; Rikardsen et al., 2006; Lobón-Cerviá, 2009; Carvalho et al., 2022b; Blanchfield et al., 2023). This likely leads to more frequent encounters between predators and preys, explaining the higher predation rates observed in summer.

Signal crayfish consumption was higher in large brown trouts, suggesting that only the largest individuals included crayfish in their diet. This pattern may be explained by morphological and behavioral factors, suggesting that morphological constraints, such as gape size and prey-handling ability, may limit predation in smaller trout, as shown in other fish species such as eels (Aquiloni et al., 2010) and pike *Esox lucius* (Elvira et al., 1996). Larger individuals are more capable of capturing and consuming signal crayfish, particularly juveniles, which are more vulnerable to predation (Momot, 1967; Faragher, 1983; Dorn et al., 1999). Additionally, a significant correlation was found between signal crayfish abundance and the percentage of empty trout stomachs (data not shown), implying an overall reduction in feeding efficiency or higher interspecific competition when crayfish population abundance was high, being this situation more effective in sites B1, B2 and B3 (Carvalho et al., 2022a).

We also found differences in predation rates along the sampling sites, but these differences mainly rely in a much lower predation in upstream sites B4 and B5. This situation is probably explained by the recent spread of the signal crayfish to these upstream sites, especially site B5 (first detection at the end of the summer of 2022) as could be seen by the very low abundance values presented by this non-native species.

Given the limited role of brown trout in controlling the signal crayfish population in the studied area, coupled with their low density, integrating their predation with that of other native predators may provide a more effective and robust approach to biological control. Among the native predators, European eels (*Anguilla anguilla*) and Eurasian otters (*Lutra lutra*) have been identified as key species that prey on invasive signal crayfish in European ecosystems. Studies have demonstrated that European eels actively prey on juvenile signal crayfish, attacking, killing, and consuming them, with their predation strategy relying more on stealth and ambush than active pursuit (Blake and Hart, 1995; Worley, 2022). However, eels are not present in our study area for decades, given the presence of downstream dams impeding their migration. Otters have been documented preying on signal crayfish, particularly during warmer months, and although they are opportunistic feeders, their seasonal predation could regulate signal crayfish populations over time (Britton et al., 2017). So, both brown trout and otters could play a role in controlling signal crayfish in the Baceiro River, though further research is needed to assess their long-term impact. Additionally, the recent introduction of the American mink (*Neogale vison*) in the Baceiro River could contribute to signal crayfish predation but may also alter overall biotic interactions, potentially causing cascading effects on overall biodiversity, including on brown trout and otters. However, further studies are needed to assess these possible interactions.

Conclusion

While brown trout, particularly larger individuals, can prey on signal crayfish, their overall impact on controlling crayfish abundance in the Baceiro River appears to be limited. This size-dependent predation may have management implications as currently, only trout over 20 cm can be harvested in the Baceiro River, and these larger fish are highly prized by anglers. Therefore, a potential conflict exists between anglers' interests and the role of brown trout as an effective biocontrol agent for signal crayfish. Other native predators, such as the Eurasian otter (*Lutra lutra*), also rely on signal crayfish as a primary food source. Therefore, integrating ecological knowledge with conservation measures (e.g., restocking brown trout, modifying fishing regulations) and leveraging native predators such as brown trout and otters could provide a more balanced and sustainable solution for mitigating the impact of signal crayfish in this protected area. Future management measures should also consider integrating multiple control methods (e.g., mechanical removal such as trapping) to mitigate the impact of signal crayfish while preserving the ecological balance of the Baceiro River ecosystem.

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Table 1. Summary of the generalized linear model (GLM) with a binomial distribution and logit link function. The response variable is the presence/absence of signal crayfish in brown trout stomach contents (0 = absent, 1 = present). The predictor variables include trout length (cm), water temperature (°C), total dissolved solids (TDS, mg/L), pH, crayfish abundance (ind. CPUE/24h), 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)	27.547567	24.844294	1.109	0.26751
Trout Length (cm)	0.308655	0.032958	9.365	< 2e-16 ***
Water Temperature (°C)	0.318505	0.075412	4.224	2.4e-05 ***
TDS	-0.024971	0.018133	-1.377	0.16848
pH	-4.78158	3.434486	-1.392	0.16385
Crayfish Abundance	0.01555	0.018281	0.851	0.39499
Site B2	-0.777836	0.461591	-1.685	0.09197
Site B3	0.075695	0.472005	0.16	0.87259
Site B4	-1.935907	0.724084	-2.674	0.0075 *
Site B5	-4.323871	1.351895	-3.198	0.00138 *
Julian Day	-0.013684	0.004513	-3.032	0.00243 *

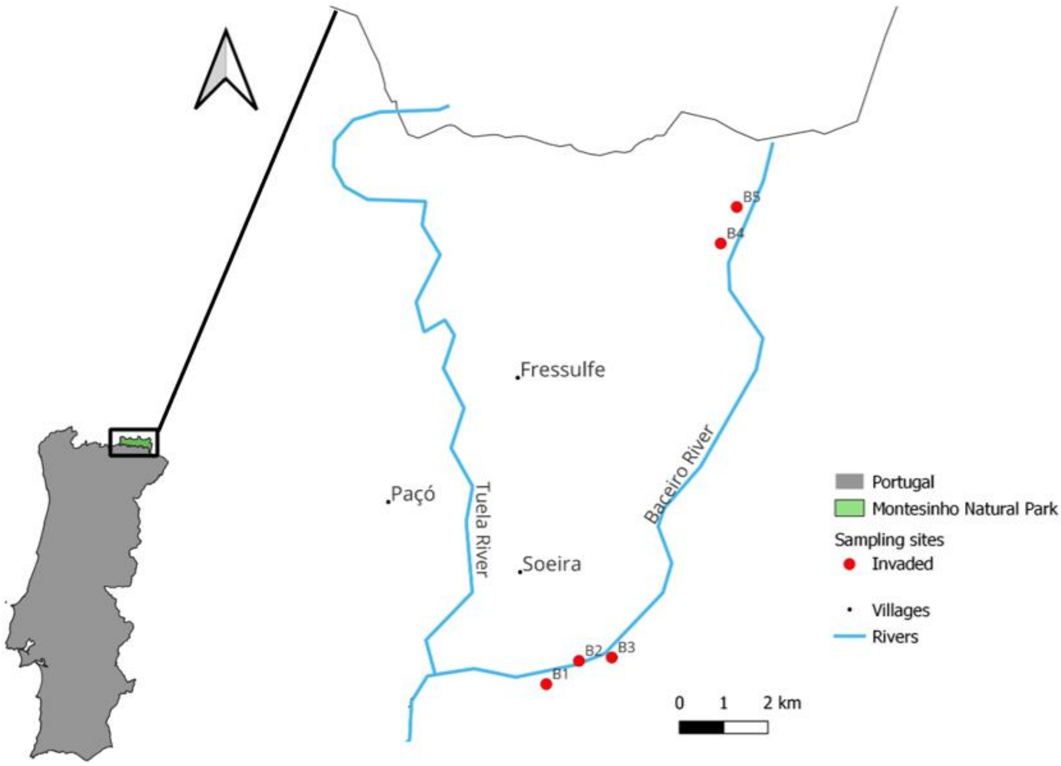

Figure 1 Map of the surveyed area showing the location of the 5 sampling sites in Baceiro River. Map was produced using QGIS software (QGIS Development Team, 2022).

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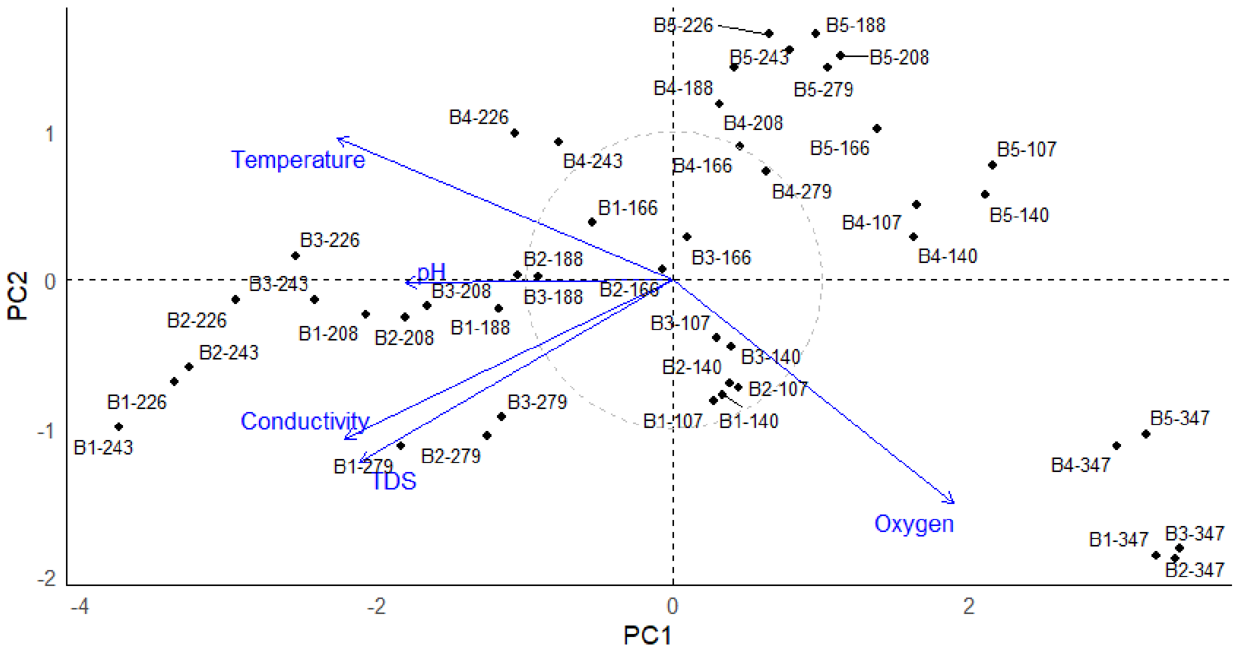

Figure 2 Principal Components Analysis (PCA) showing the arrangement of the five sampling sites based on the abiotic factors measured throughout the year. P (Julian Days). PC1 explains 68.59% of all variance and PC2 18.68%.

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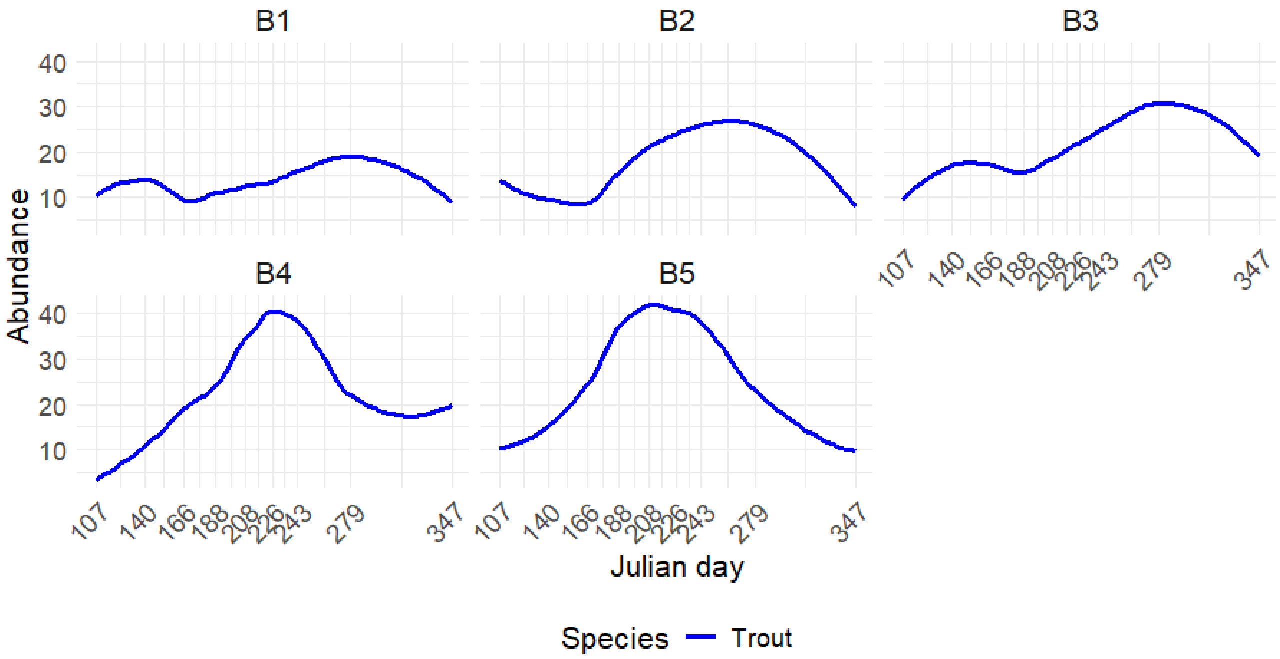

Figure 3 Abundance of brown trout over time and sampling sites.

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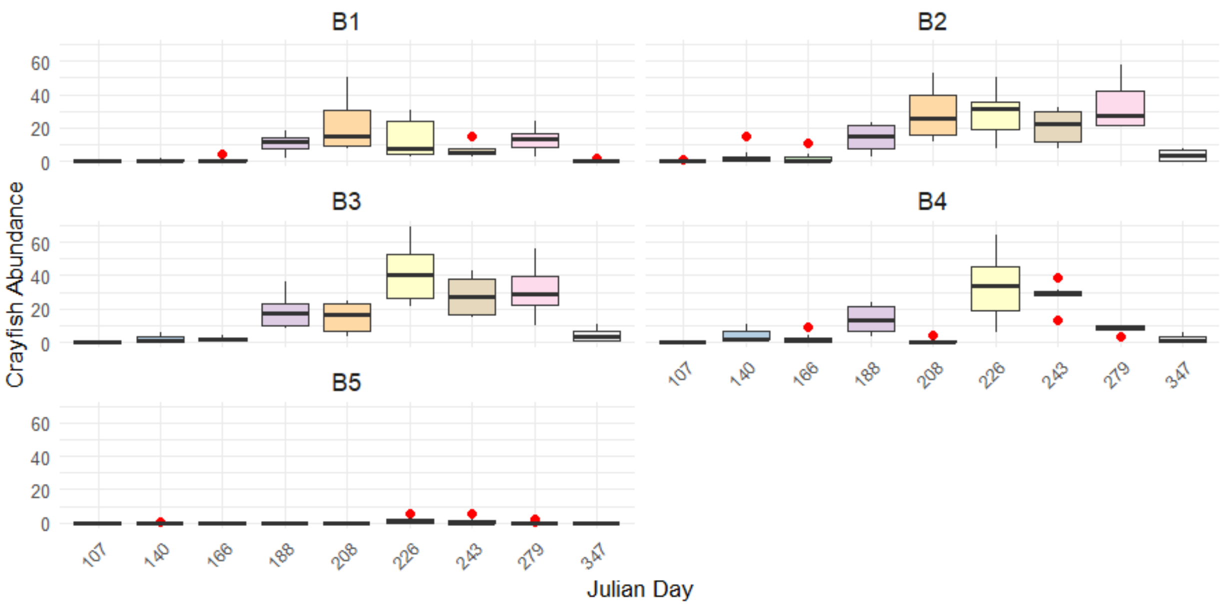

Figure 4 Abundance of signal crayfish over time and sampling sites.