

# Temperature and Interspecific Competition Alter the Impacts of Two Invasive Species on a Key Ecosystem Process

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## Research Article

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# Abstract

Climate change is expected to alter impacts of invasive alien species (IAS). As omnivorous and ectotherms, invasive crayfish species (ICS) can be particularly influenced by altered thermal regimes with possible impacts on native species and key ecosystem processes, such as leaf-litter breakdown. We performed a controlled study using a multi-trophic approach to assess the individual and combined effects between two ICS (*Pacifastacus leniusculus* and *Procambarus clarkii*) on leaf-litter breakdown under temperature increase (15 and 18°C). We used one or two species combinations of ICS (2-individuals *per* aquarium) and 3-individuals of a native invertebrate detritivore (*Sericostoma* sp.) trapped inside a transparent cage and a fine-mesh bag containing microbially-colonized oak leaves. Oak leaves were added to assess direct and indirect impacts of crayfish on leaf-litter breakdown and fine particulate organic matter (FPOM) production. Leaf-litter breakdown by microbes and *Sericostoma* was affected by temperature but not by the influence of ICS. Increasing temperature enhanced leaf-litter breakdown and FPOM production by *P. clarkii*, but not by *P. leniusculus*. At 15°C, leaf breakdown was lower in treatments with both ICS than the expected from their individual performances, indicating competitive interactions between crayfish species. FPOM production was significantly correlated with leaf-litter breakdown but not in treatments with both ICS. Our results highlight that competitive interactions between ICS may occur and potentially attenuate their impacts on key ecological processes. However, predicted increases in temperature may change the performance of IAS and increase the magnitude of their combined effects.

## Introduction

Ecosystems have been facing tremendous changes in biodiversity and ecological functions due to global change, including climate change and introduction of invasive alien species (IAS) (Sala et al. 2000; Hellmann et al. 2008). Although impacts are expected to affect all types of ecosystems (Bowler et al. 2020), biodiversity in freshwaters have been particularly affected over the last decades (Woodward et al. 2010; He et al. 2019; Reid et al. 2019). Changes in water temperature may occur naturally or as a result from climate change (Caissie 2006). Because most freshwater species are ectothermic, thermal changes may easily affect their physiology, behaviour or biogeography (Rahel 2002). On one hand, changes in thermal regimes threaten native aquatic biodiversity by affecting, for example, their phenology, survival or functional performances (Horwitz and Richardson 1986; Elliott and Hurley 1997; Sousa et al. 2020) leading to changes in ecosystem functions and stability (Traill et al. 2010). On the other hand, altered thermal and streamflow regimes may also affect aquatic invasive species, by altering pathways of species introductions, influencing their establishment or mediating their impacts on native biodiversity and ecosystem functions (Hobbs and Huenneke 1992; Facon et al. 2006; Rahel and Olden 2008). Although the effects of climate change and IAS may occur simultaneously, their assessments have been mostly studied individually making it difficult to predict their combined effects.

Shifts in temperature may increase the success of species introductions, if climatic conditions on the invaded ecosystems move towards the habitat optimal conditions of the invasive species, while natives are moved away from their optimal conditions (Byers 2002). This may also affect introduced species that

remain in their lag phase, if climatic changes trigger their spread and activity, consequently amplifying their impacts (Hellmann et al. 2008). Beyond that, the complexity of the interaction of IAS with climate change may even increase when multiple invasive species co-occur. Individual performance or magnitude of effects of IAS might change due to antagonistic or synergistic effects among invaders (Jackson 2015). Interactions between multiple IAS may potentially increase their ecological impacts due to facilitative interactions among species triggering their establishment and spread (e.g., Simberloff and von Holle 1999; Simberloff 2006). However, IAS may interact through predation or competition, decreasing their impacts on native biodiversity (Ross et al. 2004). These potential interactions among multiple species and drivers have challenged scientists to develop more comprehensive approaches to detect effects that differ from species individual effects (Brook et al. 2008). Nevertheless, new insights on the interactions among invaders may unveil the net effect of IAS on native communities and ecosystem functions (Johnson et al. 2009), and climate scenarios should be considered (Rahel and Olden 2008).

Freshwater crayfish are successful invaders that affect native communities and ecosystem functions leading to important ecological and economic impacts (Lodge et al. 2012; Twardochleb et al. 2013; Kouba et al. 2021). As ectotherms, crayfish can be affected by altered thermal regimes (Hossain et al. 2018) outperforming native species (Madzivanzira et al. 2021). Increases in temperature can increase crayfish aggressiveness and metabolic rates (Diez et al. 2012), changing their feeding behaviour (Rodríguez Valido et al. 2021), altering their trophic role by shifting their diet (Carreira et al. 2017), with possible impacts on biotic interactions (predation, competition, diseases, Diez et al. 2012). The signal crayfish *Pacifastacus leniusculus* (Dana) and the red swamp crayfish *Procambarus clarkii* (Girard) represent two of the worst global invasive species (Anastácio et al. 2019) that co-occur in NE Portugal. The red swamp crayfish was first described in Portugal in 1979 in Caia River (Ramos and Pereira 1981) and is widely spread, while the signal crayfish was first described in 1997 in Maças River (Bernardo et al. 2011), it is restricted to the NE Portugal but is currently spreading (Sousa et al. 2019).

In streams, leaf-litter breakdown is a key ecosystem process where microbial and invertebrate (mainly detritivores) communities decompose organic matter from riparian zones and facilitate nutrient and carbon transfer to higher trophic levels (Graça 2001; Pascoal et al. 2021). In this key ecosystem process, some species, including omnivores such as ICS, may disrupt food web dynamics (Gessner et al. 2010). *P. clarkii* and *P. leniusculus* showed strong impacts on native invertebrate communities by reducing biodiversity through predation or competition for basal resources (leaf litter), and contributing to changes in the dynamics of leaf breakdown in freshwaters (Carvalho et al. 2016, 2018; Carvalho et al. *in press*). Because both species are recently co-occurring in Portugal (Filipe et al. 2017; Anastácio et al. 2019), it is urgent to assess how interactions among them may alter ecosystem functioning and how climate change may mediate their impacts.

In this study, we aimed to assess how increasing temperature and interactions between invasive crayfish species (ICS) impact leaf-litter breakdown directly, or indirectly by affecting the native detritivore invertebrates that feed on leaf litter. We hypothesised that: i) crayfish presence leads to a decrease in invertebrate leaf consumption due to the risk of predation and, consequently, may decrease leaf-litter

breakdown; ii) increasing temperature increases the impacts of ICS on leaf-litter breakdown and FPOM production but this might be species specific; and iii) the direct impacts of *P. clarkii* and *P. leniusculus* on leaf-litter breakdown decrease in sympatry if competitive relationships predominate. We expected that temperature increase may enhance the activity, foraging and feeding of ICS and consequently increase leaf-litter breakdown and FPOM production. We also expected that interspecific interactions between ICS may decrease their impacts on leaf-litter breakdown.

## Materials And Methods

### Animal collection and maintenance

We collected males of *P. clarkii* and *P. leniusculus* in the Minho River (41°57'N, 8°44'W) and Tuela River (41°51'N, 6°55'W), respectively with approximately the same size (*P. clarkii* 94 mm ± 6.55 SD; *P. leniusculus* 88 mm ± 7.33 SD total length) by using funnel traps (43 cm diameter; 22 cm height and 1.5 cm mesh). After being transported to the laboratory crayfishes were transferred to two tanks (1 x 0.6 x 0.65 m), one for each species. Native invertebrates from the genus *Sericostoma* sp. (a common native freshwater shredder in Northern Portugal) were also collected at Tuela River (41°51'N, 6°55'W), transferred to the laboratory and kept in aquariums. Both invasive crayfish individuals and native invertebrate shredders were collected from sites where species do not exist in sympatry to avoid biased response behaviour during the experiment. Animals were acclimated to laboratory conditions 15 days before the experiment (15°C water temperature, aeration and 12/12h photoperiod) and kept under starvation 24h before the experiment.

### Laboratorial setup

A laboratory experiment using a multi-trophic approach was performed to assess direct and indirect effects of two crayfish species (*P. leniusculus* and *P. clarkii*) alone and in combination, on leaf-litter breakdown and associated biota (invertebrate shredders and microorganisms), at two temperatures (15°C and 18°C). Aquariums (40 x 20 x 20 cm) containing two crayfish individuals per replicate (2 of each species in single species and 1 *per* species in two species treatments) were used. Indirect impacts of crayfish on native invertebrates (risk of predation) were assessed by trapping 3 *Sericostoma* sp. individuals inside a transparent cylindrical cage per replicate, where crayfish were not allowed to prey on invertebrates. Invertebrate cages were covered on the sides by fine mesh to allow water to circulate and potential detection of crayfish presence by native invertebrates. Each treatment (including the control – without crayfish) contained one fine-mesh bag with leaf discs previously colonized by microbes in a stream (10 days). In total, we had 4 treatments: Control – microbes and invertebrates; *P. clarkii* + microbes and invertebrates; *P. leniusculus* + microbes and invertebrates; and *P. clarkii* vs *P. leniusculus* + microbes and invertebrates (Fig. 1). Hereafter *P. clarkii* and *P. leniusculus* refer to intraspecific treatments while interspecific treatments refer to the ones where both crayfish species were interacting.

To measure crayfish direct impacts on leaf-litter breakdown and FPOM production, 5±0.03 g (dry weight) of oak leaves were introduced in the aquariums. To measure leaf breakdown by invertebrates and the

indirect effects of crayfish presence,  $1 \pm 0.02$  g (dry weight) of oak leaves was introduced inside invertebrate cages. To measure the contribution of microbes to leaf-litter breakdown 10 oak leaf discs (12 mm diameter,  $38 \pm 4.81$  mg dry weight) were introduced in fine-mesh bags. Leaf litter was prepared in separate bags and kept in a container to allow microbial colonization 7 days prior to the experiment. After that, leaf litter was introduced in each compartment (aquariums and cages) 24 hours before the experiment. Aquariums were filled with 5 L of water, gravel and pebbles (size  $850 \mu\text{m}$ – $60 \text{ mm}$ , layer of 2 cm) previously washed and autoclaved ( $120^\circ\text{C}$ , 20 min) and equipped with an aeration system. Half of the water volume of each aquarium was renewed on day 7. Temperature controlled rooms and temperature data loggers were used during the experiment. Each treatment had 4 replicates ( $N=32$ ) and the experiment ran for 15 days.

## Leaf-litter breakdown and FPOM production

At the end of the experiment, leaf litter remaining inside the aquariums (crayfish), cylindrical cages (invertebrates) and fine-mesh bags (microbes) was separated, washed and lyophilized for 48h, and weighted to the nearest 0.01 mg. Leaf-litter breakdown was calculated as leaf mass loss during the experiment. Percentage of leaf mass loss ( $L_c$ ) was obtained as  $L_c = (L_i - L_f) \times (100/L_i)$ , where  $L_i$  and  $L_f$  are the initial and final dry mass (DM, g) of leaves (following Carvalho et al. 2018). Leaf mass loss per animal body mass was calculated as  $g_L g_{AM}^{-1}$  where  $g_L$  is the mass of leaf mass loss and  $g_{AM}$  is the total animal body mass per replicate. The retrieved water (from the renewal and the end of the experiment) was collected and filtered using a combination of sieves (size range  $>0.45 \mu\text{m}$  to  $<1000 \mu\text{m}$ ) to collect FPOM (Hutchens Jr et al. 2017). The remaining FPOM of each replicate was then centrifuged (10 min, 14,000 rpm; Sigma 4 z16 K), and the pellet lyophilized (Biolblock Scientific-Christ Alpha 2-4 LD Plus) for 48 h, before weighed to the nearest 0.01 mg (following Carvalho et al. 2018). FPOM production by animal body mass was calculated by dividing grams of FPOM produced by animal body mass.

## Statistical analysis

Two-way ANOVAs were used to test the individual and combined effects of crayfish treatments (control, 1 and 2 species combination) and temperature (15 and  $18^\circ\text{C}$ ) on leaf-litter breakdown and FPOM production by microbes, native invertebrates and crayfish (Zar 2009; Supplementary material, Table S1). All ANOVAs were preceded by Shapiro-Wilk to check the normality of the residuals and Bartlett test to check for homoscedasticity (Zar 2009). To achieve a normal distribution and homoscedasticity, microbial and invertebrates leaf-litter breakdown by animal mass were log transformed. To test for putative interspecific interactions, we compared *observed* performances (leaf-litter breakdown and FPOM production) in two crayfish species treatments with the *expected* based on the sum of individual crayfish species treatments. The difference between *observed* and *expected* performances was tested against the null hypothesis that the average difference equaled 0 (t-test). Pearson's correlations between leaf-litter breakdown and FPOM production were calculated (Benesty et al. 2009). All the statistics were performed in R software (R Core Team 2021; version 3.6.3) using the “stats”, “multcomp” (Hothorn et al. 2016) and “psych” (Revelle 2021) packages and data visualization performed on “ggplot2” (Wickham 2016) and “ggpubr” (Kassambara and Kassambara 2020) packages.

## Results

### Leaf-litter breakdown by microbes and invertebrates

Leaf-litter breakdown assessed as leaf mass loss was 2.05 g at 15°C, and 2.26 g at 18°C. Leaf-litter breakdown by microbes increased at the higher temperature (Two-way ANOVA,  $F_{1,3} = 6.403$ ,  $p < 0.05$ ; Fig. 2A) and was not affected by crayfish presence (Two-way ANOVA,  $p > 0.05$ ; Fig. 2A). Leaf-litter breakdown by native invertebrates (weighed by animal body mass) increased with temperature from a mean of 2.3 g to 2.8 g (Two-way ANOVA,  $F_{1,3} = 5.469$ ,  $p < 0.05$ ) but did not change significantly between crayfish treatments and the control with invertebrates only (Two-way ANOVA,  $p > 0.05$ ; Fig. 2B).

### Leaf-litter breakdown by crayfish

Leaf-litter breakdown by crayfish was significantly affected by temperature (Two-way ANOVA,  $F_{1,2} = 6.314$ ,  $p < 0.05$ ) and crayfish treatment (Two-way ANOVA,  $F_{1,2} = 3.784$ ,  $p < 0.05$ ). Leaf-litter breakdown assessed as leaf mass loss was lowest with two crayfish species at lower temperature (0.04 g) and higher with *P. clarkii* at higher temperature (0.07 g; Fig. 2A). Leaf-litter breakdown by *P. clarkii* at higher temperature was significantly higher than in two crayfish species treatments at lower temperature (Fig. 3A, Tukey post-hoc,  $p < 0.01$ ). Leaf-litter breakdown per crayfish body mass was higher in the presence than in the absence of crayfish (Kruskal-Wallis non-parametric test,  $p < 0.001$ ).

The difference between leaf-litter breakdown in interspecific treatments and the expected values based on individual performances of *P. clarkii* and *P. leniusculus* was significantly lower than zero ( $t(3) = -3.34$ ,  $p = 0.04$ ; Fig. 3B), but the effect did not differ from 0 at higher temperature ( $t(3) = -1.34$ ,  $p = 0.27$ ).

### FPOM production

FPOM production was higher in all crayfish treatments than in the control (Dunn's test  $p < 0.001$ ). FPOM production was significantly affected by temperature (Two-way ANOVA,  $F_{1,2} = 12.67$ ,  $p < 0.01$ ) and crayfish treatments (Two-way ANOVA,  $F_{1,2} = 7.42$ ,  $p < 0.01$ ). FPOM production was lower in all crayfish treatments at 15°C than in treatments with *P. clarkii* at higher temperature (Tukey post-hoc, *P. clarkii*  $p < 0.05$ ; *P. leniusculus*  $p < 0.01$ ; interspecific  $p < 0.001$ ). At higher temperature, FPOM by animal weight by *P. clarkii* was significantly higher than *P. leniusculus* (Tukey post-hoc,  $p < 0.05$ ; Fig. 4A). The difference between the observed FPOM production with two crayfish species treatments and the expected results based on the individual performance of *P. clarkii* and *P. leniusculus* did not differ significantly from 0 at 15°C ( $t(3) = -2.56$ ,  $p = 0.08$ ) neither at 18°C ( $t(3) = -1.07$ ,  $p = 0.36$ ) (not shown).

FPOM production was positively correlated with leaf breakdown on individual treatments by *P. clarkii* ( $r = 0.86$ ,  $p = 0.006$ ) and *P. leniusculus* ( $r = 0.90$ ,  $p = 0.003$ ) but not in interspecific treatments ( $r = 0.70$ ,  $p = 0.06$ ; Fig. 4B).

## Discussion

Our study highlights that altered thermal regimes change the interaction among ICS and their impacts on leaf-litter breakdown in streams. As hypothesized, temperature increase enhanced leaf-litter breakdown and FPOM production by ICS, but this effect was species specific because it was only observed for *P. clarkii*. Interactions among ICS were antagonistic, consequently decreasing the impacts on this important ecosystem process. However, in a temperature increase scenario, the effects of both ICS in interspecific treatments became additive.

Contrary to our hypothesis, the indirect presence of ICS did not alter the activity of native invertebrates and microbes on leaf-litter breakdown. Because leaf decomposition is an ecosystem process where many species across multiple trophic levels are involved, some species, including omnivores, may display strong influence in the process dynamics by affecting biodiversity (through predation) or by reducing basal resources (Gessner et al. 2010; Carvalho et al. 2016). Invasive crayfish can, for example, change nutrient dynamics through their activity including feeding or burrowing behaviour (Shin-ichiro et al. 2009; Harvey et al. 2014) and consequently change nutrient concentrations affecting leaf-litter breakdown rates by freshwater microbes, particularly at higher temperatures (Fernandes et al. 2014). However, in our study, we were not able to detect differences in microbial leaf-litter breakdown among crayfish treatments. We used a short-term experiment and included a water renewal in the middle of the experiment, which probably led to low nutrient concentrations associated with crayfish activity and low accumulation of excreted products, consequently masking a potential response of microbial communities to crayfish. Microbial leaf-litter breakdown was enhanced by increasing temperature in our study. Other studies demonstrated that warming temperature might intensify leaf-litter breakdown by microbial communities in streams (Fernandes et al. 2014). Impacts of climate change have been widely studied particularly at individual or species population levels but few have used multi-trophic approaches (but see Woodward et al. 2010).

Also, native invertebrates (*Sericostoma* sp.) were only affected by temperature but not by the indirect risk of predation imposed by the crayfish presence. Naive responses of native invertebrates to invasive crayfish were previously reported in other studies (e.g. Carvalho et al. 2016). Exposure to predation risk may change the behaviour of native species leading to a decrease in their foraging activity or changing their strategies with potential indirect consequences for ecosystem functions (Klose and Cooper 2012). In our experiment, invertebrates were indeed protected from direct predation by crayfish and oak leaves inside their cages were able to provide shelter and food resources that could possibly explain no change in their behaviour. Beyond that, *Sericostoma* sp. individuals were captured at a site where they have low contact with the invasive crayfish and there are no native crayfish in those rivers. This situation is consistent with their naive behaviour. We should note that this lack of response might change in the future due to evolutionary pressures imposed by crayfish dispersion and population growth and co-occurrence between ICS with native invertebrates (e.g. Klose and Copper 2012; Carvalho et al. *in press*). Moreover, climate change is expected to increase co-occurrence between native and invasive species increasing the probability of competitive interactions for resource availability (Zeng and Yeo 2018). Because native invertebrates might have to compete for resources and avoid predation by invasive crayfish, this will probably constitute a challenge in the future. In this sense, extreme events such as

droughts, which are predicted to increase in number and intensity in the studied region (Santos et al. 2015; Sousa et al. 2018; Nogueira et al. 2021), may indirectly favour crayfish impacts due to habitat dysconnectivity (intermittence) where in limited space and lower volume of water crayfish can easily affect direct resource consumption or predate on native species (Martinez 2012).

Temperature affected leaf-litter breakdown by crayfish, although those effects were species specific. Only *P. clarkii* increased leaf breakdown when compared with interspecific treatments at lower temperature. Because crayfish species are ectotherms, increasing temperatures are expected to enhance crayfish activity, including foraging and feeding behaviour (Rahel 2002). Invasive crayfish can tolerate temperatures around 30°C (Souty-Grosset et al. 2006) and may be highly tolerant to desiccation (Larson et al. 2009) but crayfish responses to temperature changes may be species specific. For example, annual mean temperature is the main driver in the projected distributions of *P. clarkii* and *P. leniusculus* for the future, but it will affect both species in different ways benefiting mostly *P. clarkii* in Europe (Zhang et al. 2020). Impacts of invasive crayfish on leaf-litter breakdown and the associated biota may also be species specific (Dunoyer et al. 2014). In our experiment, the effect of *P. leniusculus* on leaf-litter breakdown did not vary with increasing temperature. Invasive *P. leniusculus* captured in the UK showed increasing feeding rates at higher temperatures (Rodríguez Valido et al. 2021) but its maximum performance was reached at a higher temperature (24°C) than the maximum used in our experiment (18°C). Males of *P. leniusculus* showed its maximum feeding rate at 20°C (Simčič et al. 2014). No significant differences were found between the individual effects of both ICS, suggesting that both species have a strong impact on leaf-litter breakdown. Other studies suggested that ICS may functionally substitute vulnerable native invertebrate species (Stenroth and Nyström 2003). Our results show that *P. clarkii* significantly increased their leaf-litter breakdown at higher temperatures. Other studies demonstrated that heat waves may shift the diet of *P. clarkii* towards an increasing consumption of plant material (Carreira et al. 2017).

Interestingly, interactive effects between *P. leniusculus* and *P. clarkii* significantly decreased their expected individual impacts on leaf-litter consumption possible due to competition. *Procambarus clarkii* is expected to have a more aggressive and dominant behaviour when foraging for food sources when compared with *P. leniusculus* (Meira et al. 2019). Other studies showed that native *P. leniusculus* occupies a higher trophic position than *P. clarkii* in lake ecosystems (Larson et al. 2017). However, in our experiment, crayfish were only able to feed on leaf litter. Although the invasion meltdown hypothesis postulated that invasive species may benefit from other invasive by facilitation or mutualism leading to a synergistic or additive effect on ecosystems (Simberloff and von Holle 1999), interactions among multiple invaders might also be negative (antagonistic) particularly in freshwater ecosystems and are more severe in omnivore species (Jackson 2015). Our results show that these antagonistic interactions on leaf-litter breakdown change with increasing temperature where effects between both species become additive. Based on our data, we can hypothesize that this effect resulted from enhanced leaf-litter breakdown by *P. clarkii*. Nonetheless we should carefully interpret our results because in our experiment leaf litter was the only food source for ICS although food availability was not limited. However, in natural ecosystems niche partitioning between invasive species may also facilitate the spread of multiple

invasions and amplify their impacts on native biodiversity and ecosystems (Jackson et al. 2014). This indicates that although we used a simplified food web model, our results may help to clarify how these species interact and affect important ecosystem processes in freshwaters.

FPOM production by *P. clarkii* increased at higher temperature and was higher than that of *P. leniusculus* at higher temperature. ICS can functionally act as detritivores and play a significant role in detritus-based food webs contributing to produce FPOM, an important source of energy and carbon to stream biota (Carvalho et al. 2016). Again, we should carefully interpret our results because we limited food sources to leaf litter inside aquariums, oversimplifying natural conditions. For example, ICS may also have negative indirect impacts on detritus-based food webs through trophic cascades (Greig and McIntosh 2006). Our results showed expected positive and significant correlations between leaf-litter breakdown and FPOM production in intraspecific treatments, but interspecific crayfish interaction led to a non-significant correlation. Here competition may play an important role if crayfish increase the uptake of food resources to invest in body mass. Other studies reported increase in energy uptake and body mass in crustaceans when subjected to predation pressures (Glazier et al. 2020) and competition interaction may possibly lead to the same trends.

## Conclusion

Our results indicate that altered thermal regimes simulating climate change and interactions among ICS may have important ecological effects on detritus-based food webs. Although antagonistic effects on leaf-litter breakdown are expected at lower temperature, where both ICS co-exist, increasing temperature may change their impacts because their interaction might become additive. Future climate projections for Portugal predict air temperature increases that can reach in the worst case scenarios up to 8°C in summer and up to 2 and 4°C in winter and spring, respectively, which will consequently increase water temperature (Cardoso et al. 2019), and potentially affect the dynamic of ICS and their impacts. Some studies have highlighted that climate change will increase habitat suitability for ICS (e.g., Capinha et al. 2013). Further studies should address how interactions among ICS vary with population traits and increasing densities in natural conditions) where these omnivorous invaders may interfere directly and indirectly with food webs and affect important ecosystem processes. Realistic conservation management strategies should take into account how multiple invasive species interact, how they respond to climatic change and how both biotic and abiotic interactions mediate their impact on invaded ecosystems (Pyke et al. 2008; Rahel and Olden 2008). Even more urgent is to incorporate climate change scenarios (including the effects of extreme climatic events such as droughts and heatwaves) on invasive species management strategies to develop adequate regulations and policies and how this may interact with overall ecological effects.

## Declarations

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### **Data Availability Statement**

Data are available from the authors upon request.

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### **Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

### **Author Contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Francisco Carvalho. The first draft of the manuscript was written by Francisco Carvalho and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## **References**

1. Anastácio PM, Ribeiro F, Capinha C et al (2019) Non-native freshwater fauna in Portugal: A review. *Sci Total Environ* 650:1923–1934
2. Benesty J, Chen J, Huang Y, Cohen I (2009) Pearson correlation coefficient. *Noise reduction in speech processing*. Springer, pp 1–4
3. Bernardo JM, Costa AM, Bruxelas S, Teixeira A (2011) Dispersal and coexistence of two non-native crayfish species (*Pacifastacus leniusculus* and *Procambarus clarkii*) in NE Portugal over a 10-year period. *Knowledge and Management of Aquatic Ecosystems* 28
4. Bowler DE, Bjorkman AD, Dornelas M et al (2020) Mapping human pressures on biodiversity across the planet uncovers anthropogenic threat complexes. *People and Nature* 2:380–394
5. Brook BW, Sodhi NS, Bradshaw CJA (2008) Synergies among extinction drivers under global change. *Trends Ecol Evol* 23:453–460
6. Byers JE (2002) Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. *Oikos* 97:449–458
7. Caissie D (2006) The thermal regime of rivers: a review. *Freshw Biol* 51:1389–1406

8. Capinha C, Larson ER, Tricarico E et al (2013) Effects of climate change, invasive species, and disease on the distribution of native European crayfishes. *Conserv Biol* 27:731–740
9. Cardoso RM, Soares PMM, Lima DCA, Miranda PMA (2019) Mean and extreme temperatures in a warming climate: EURO CORDEX and WRF regional climate high-resolution projections for Portugal. *Clim Dyn* 52:129–157
10. Carreira BM, Segurado P, Laurila A, Rebelo R (2017) Can heat waves change the trophic role of the world's most invasive crayfish? Diet shifts in *Procambarus clarkii*. *PLoS ONE* 12:e0183108
11. Carvalho F, Pascoal C, Cássio F, Sousa R (2018) Effects of intrapopulation phenotypic traits of invasive crayfish on leaf litter processing. *Hydrobiologia* 819:67–75
12. Carvalho F, Pascoal C, Cássio F, Sousa R (2016) Direct and indirect effects of an invasive omnivore crayfish on leaf litter decomposition. *Sci Total Environ* 541:714–720
13. Carvalho F, Pascoal C, Cássio F, Teixeira A, Sousa R (*in press*) Combined per-capita and abundance effects of an invasive species on native invertebrate diversity and a key ecosystem process. *Freshwater Biology*
14. Diez JM, D'Antonio CM, Dukes JS et al (2012) Will extreme climatic events facilitate biological invasions? *Front Ecol Environ* 10:249–257
15. Dunoyer L, Dijoux L, Bollache L, Lagrue C (2014) Effects of crayfish on leaf litter breakdown and shredder prey: are native and introduced species functionally redundant? *Biol Invasions* 16:1545–1555
16. Elliott JM, Hurley MA (1997) A functional model for maximum growth of Atlantic salmon parr, *Salmo salar*, from two populations in northwest England. *Funct Ecol* 11:592–603
17. Facon B, Genton BJ, Shykoff J et al (2006) A general eco-evolutionary framework for understanding bioinvasions. *Trends Ecol Evol* 21:130–135
18. Fernandes I, Seena S, Pascoal C, Cássio F (2014) Elevated temperature may intensify the positive effects of nutrients on microbial decomposition in streams. *Freshw Biol* 59:2390–2399
19. Filipe AF, Quaglietta L, Ferreira M et al (2017) Geostatistical distribution modelling of two invasive crayfish across dendritic stream networks. *Biol Invasions* 19:2899–2912
20. Gessner MO, Swan CM, Dang CK et al (2010) Diversity meets decomposition. *Trends Ecol Evol* 25:372–380
21. Glazier DS, Borrelli JJ, Hoffman CL (2020) Effects of fish predators on the mass-related energetics of a keystone freshwater crustacean. *Biology* 9:40
22. Graça MAS (2001) The role of invertebrates on leaf litter decomposition in streams—a review. *Int Rev Hydrobiol* 86:383–393
23. Harvey GL, Henshaw AJ, Moorhouse TP et al (2014) Invasive crayfish as drivers of fine sediment dynamics in rivers: field and laboratory evidence. *Earth Surf Proc Land* 39:259–271
24. He F, Zarfl C, Bremerich V et al (2019) The global decline of freshwater megafauna. *Glob Change Biol* 25:3883–3892

25. Hellmann JJ, Byers JE, Bierwagen BG, Dukes JS (2008) Five potential consequences of climate change for invasive species. *Conserv Biol* 22:534–543
26. Hobbs RJ, Huenneke LF (1992) Disturbance, diversity, and invasion: implications for conservation. *Conserv Biol* 6:324–337
27. Horwitz PHJ, Richardson AMM (1986) An ecological classification of the burrows of Australian freshwater crayfish. *Marine and Freshwater Research* 37:237–242
28. Hossain MA, Lahoz-Monfort JJ, Burgman MA et al (2018) Assessing the vulnerability of freshwater crayfish to climate change. *Divers Distrib* 24:1830–1843
29. Hothorn T, Bretz F, Westfall P et al (2016) Package ‘multcomp.’ Simultaneous inference in general parametric models Project for Statistical Computing, Vienna, Austria
30. Hutchens JJ Jr, Wallace JB, Grubaugh JW (2017) Transport and storage of fine particulate organic matter. *Methods in stream ecology*. Elsevier, pp 37–53
31. Jackson MC (2015) Interactions among multiple invasive animals. *Ecology* 96:2035–2041
32. Jackson MC, Jones T, Milligan M et al (2014) Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. *Freshw Biol* 59:1123–1135
33. Johnson PTJ, Olden JD, Solomon CT, vander Zanden MJ (2009) Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia* 159:161–170
34. Kassambara A, Kassambara MA (2020) Package ‘ggpubr’
35. Klose K, Cooper SD (2012) Contrasting effects of an invasive crayfish (*Procambarus clarkii*) on two temperate stream communities. *Freshw Biol* 57:526–540
36. Kouba A, Oficialdegui FJ, Cuthbert RN et al (2021) Identifying economic costs and knowledge gaps of invasive aquatic crustaceans. *Science of the Total Environment*
37. Larson ER, Magoulick DD, Turner C, Laycock KH (2009) Disturbance and species displacement: different tolerances to stream drying and desiccation in a native and an invasive crayfish. *Freshw Biol* 54:1899–1908
38. Larson ER, Twardochleb LA, Olden JD (2017) Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*. *Limnology* 18:275–286
39. Lodge DM, Deines A, Gherardi F et al (2012) Global introductions of crayfishes: evaluating the impact of species invasions on ecosystem services. *Annu Rev Ecol Evol Syst* 43:449–472
40. Madzivanzira TC, South J, Weyl OLF (2021) Invasive crayfish outperform Potamonautid crabs at higher temperatures. *Freshw Biol* 66:978–991
41. Martinez PJ (2012) Invasive crayfish in a high desert river: implications of concurrent invaders and climate change. *Aquat Invasions* 7:219–234
42. Meira A, Lopes-Lima M, Varandas S et al (2019) Invasive crayfishes as a threat to freshwater bivalves: Interspecific differences and conservation implications. *Sci Total Environ* 649:938–948

43. Nogueira JG, Teixeira A, Varandas S et al (2021) Assessment of a terrestrial protected area for the conservation of freshwater biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems* 31:520–530
44. Pascoal C, Fernandes IR, Mundiath SS et al (2021) Linking microbial decomposer diversity to plant litter decomposition and associated processes in streams
45. Pyke CR, Thomas R, Porter RD et al (2008) Current practices and future opportunities for policy on climate change and invasive species. *Conserv Biol* 22:585–592
46. R Core Team (2021) R: A language and environment for statistical computing. R Foundation
47. Rahel FJ (2002) Using current biogeographic limits to predict fish distributions following climate change. In: *American Fisheries Society Symposium*. American Fisheries Society, pp 99–112
48. Rahel FJ, Olden JD (2008) Assessing the effects of climate change on aquatic invasive species. *Conserv Biol* 22:521–533
49. Ramos MA, Pereira TG (1981) Um novo Astacidae para a fauna portuguesa: *Procambarus clarkii* (Girard, 1852). *Boletim do Instituto Nacional de Investigação das Pescas*, pp 37–47
50. Reid AJ, Carlson AK, Creed IF et al (2019) Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol Rev* 94:849–873
51. Revelle W (2021) How to use the psych package for mediation/moderation/regression analysis
52. Rodríguez Valido CA, Johnson MF, Dugdale SJ et al (2021) Thermal sensitivity of feeding and burrowing activity of an invasive crayfish in UK waters. *Ecohydrology* 14:e2258
53. Ross DJ, Johnson CR, Hewitt CL, Ruiz GM (2004) Interaction and impacts of two introduced species on a soft-sediment marine assemblage in SE Tasmania. *Mar Biol* 144:747–756
54. Greig HS, McIntosh AR (2006) Indirect effects of predatory trout on organic matter processing in detritus-based stream food webs. *Oikos* 112:31–40
55. Sala OE, Chapin FS, Armesto JJ et al (2000) Global biodiversity scenarios for the year 2100. *Science* 287
56. Santos RMB, Fernandes LFS, Varandas SGP et al (2015) Impacts of climate change and land-use scenarios on *Margaritifera margaritifera*, an environmental indicator and endangered species. *Sci Total Environ* 511:477–488
57. Shin-ichiro SM, Usio N, Takamura N, Washitani I (2009) Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. *Oecologia* 158:673–686
58. Simberloff D (2006) Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecol Lett* 9:912–919
59. Simberloff D, von Holle B (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biol Invasions* 1:21–32
60. Simčič T, Pajk F, Jaklič M et al (2014) The thermal tolerance of crayfish could be estimated from respiratory electron transport system activity. *J Therm Biol* 41:21–30

61. Sousa R, Ferreira A, Carvalho F et al (2020) Small hydropower plants as a threat to the endangered pearl mussel *Margaritifera margaritifera*. *Sci Total Environ* 719:137361
62. Sousa R, Ferreira A, Carvalho F et al (2018) Die-offs of the endangered pearl mussel *Margaritifera margaritifera* during an extreme drought. *Aquatic Conservation: Marine and Freshwater Ecosystems* 28:1244–1248
63. Sousa R, Nogueira JG, Ferreira A et al (2019) A tale of shells and claws: The signal crayfish as a threat to the pearl mussel *Margaritifera margaritifera* in Europe. *Sci Total Environ* 665:329–337
64. Souty-Grosset C, Holdich D, Noel P et al (2006) Atlas of crayfish in Europe. Muséum national d'Histoire naturelle
65. Stenroth P, Nyström P (2003) Exotic crayfish in a brown water stream: effects on juvenile trout, invertebrates and algae. *Freshw Biol* 48:466–475
66. Traill LW, Lim MLM, Sodhi NS, Bradshaw CJA (2010) Mechanisms driving change: altered species interactions and ecosystem function through global warming. *J Anim Ecol* 79:937–947
67. Twardochleb LA, Olden JD, Larson ER (2013) A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science* 32:1367–1382
68. Wickham H (2016) Programming with ggplot2. In: *ggplot2*. Springer, pp 241–253
69. Woodward G, Perkins DM, Brown LE (2010) Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365:2093–2106
70. Zar JH (2009) *Biostatistical analysis*. ed. 5ta. Ed, New Jersey, USA
71. Zeng Y, Yeo DCJ (2018) Assessing the aggregated risk of invasive crayfish and climate change to freshwater crabs: A Southeast Asian case study. *Biol Conserv* 223:58–67
72. Zhang Z, Capinha C, Usio N et al (2020) Impacts of climate change on the global potential distribution of two notorious invasive crayfishes. *Freshw Biol* 65:353–365

## Figures

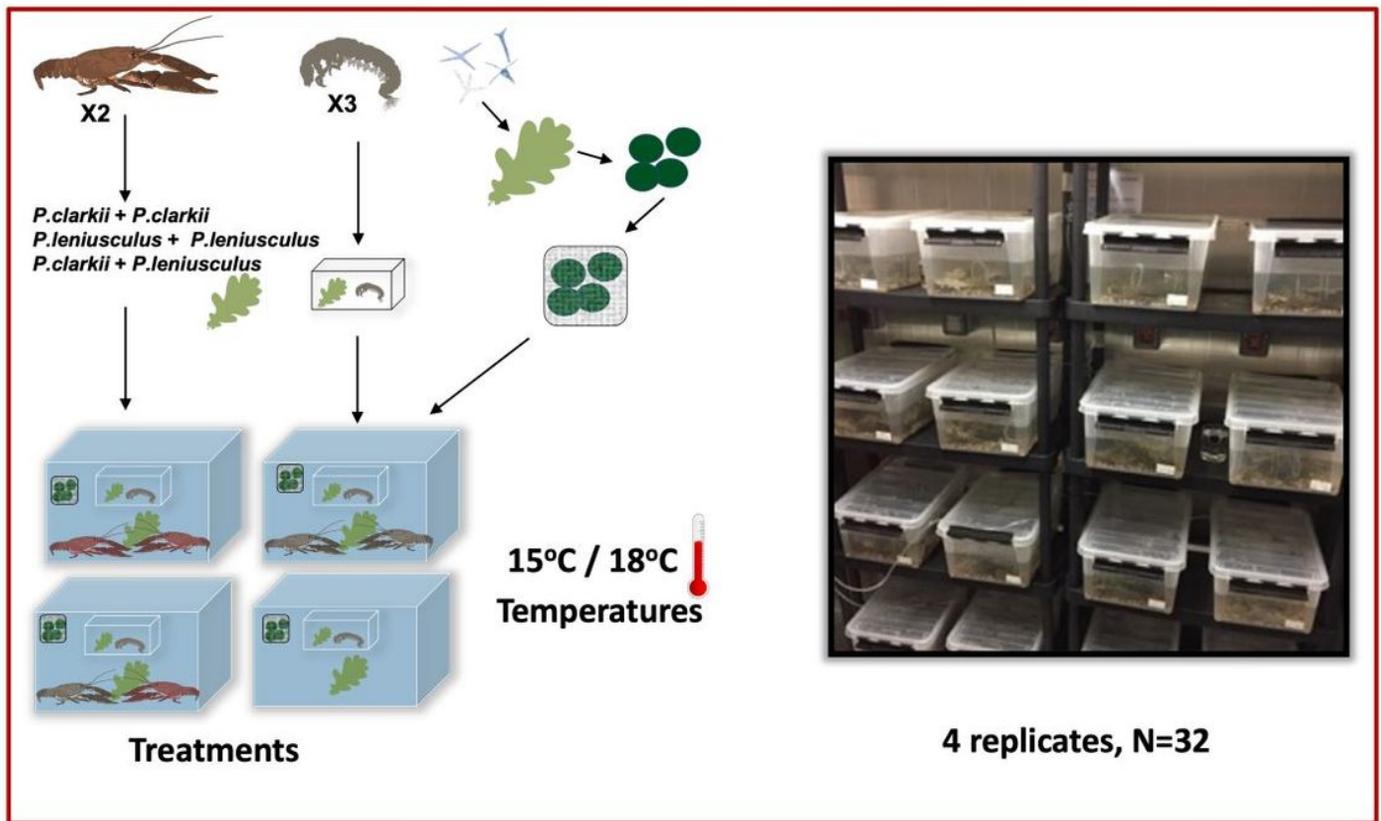
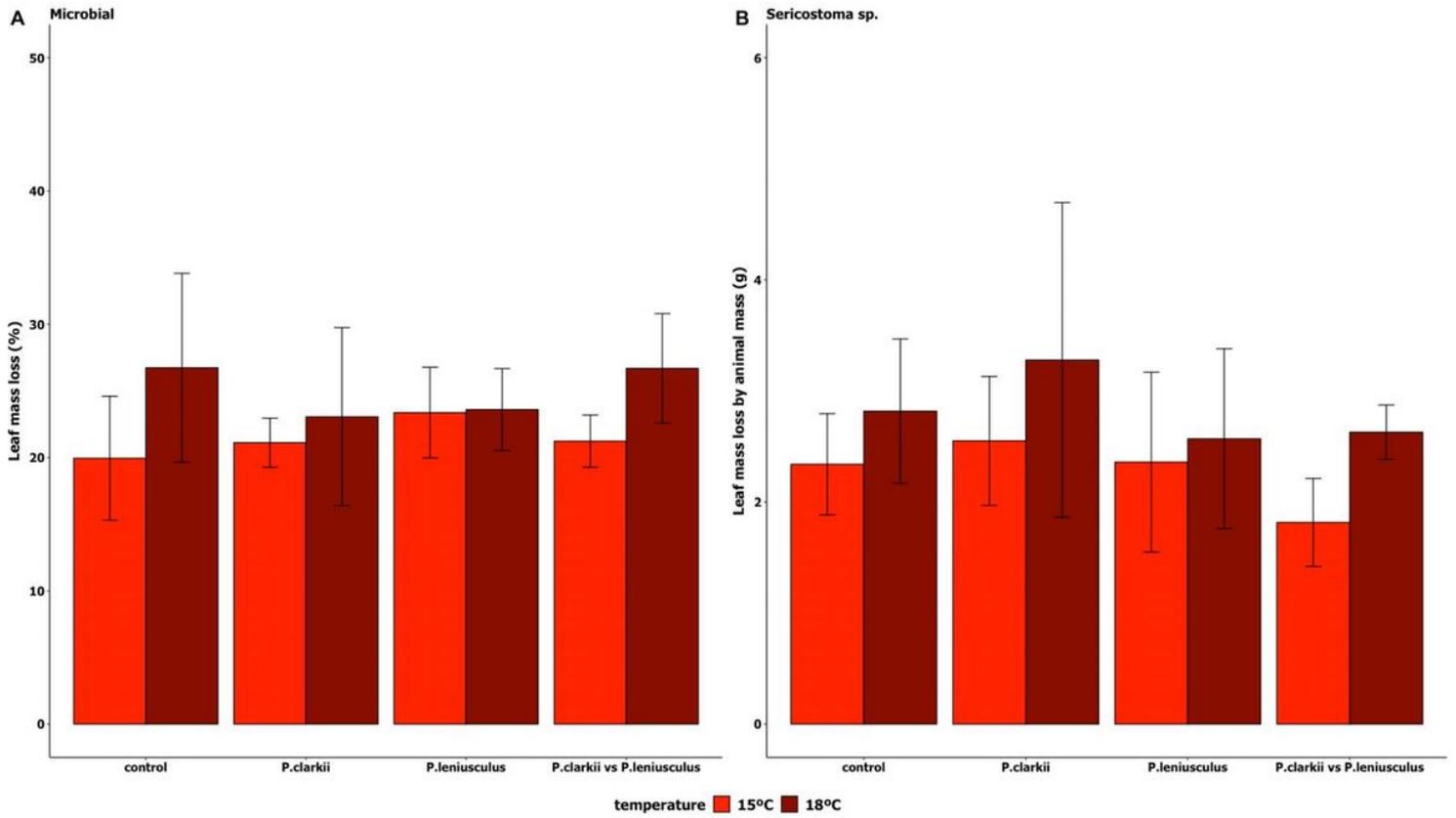


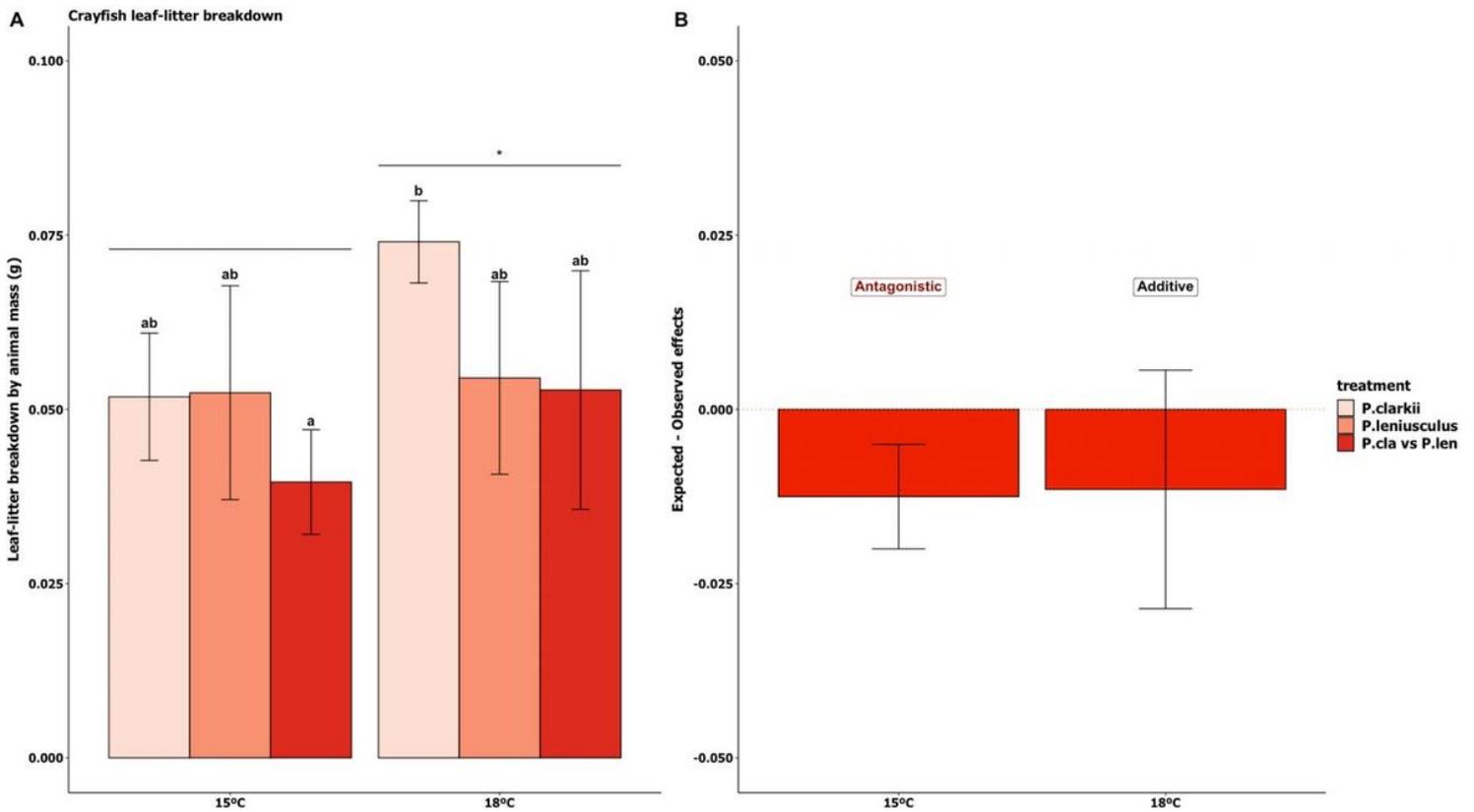
Figure 1

Experimental laboratory design where we assess the individual and combined effects between two invasive crayfish species (*Pacifastacus leniusculus* and *Procambarus clarkii*) on leaf-litter breakdown and associated biota under temperature increase (15 and 18°C).



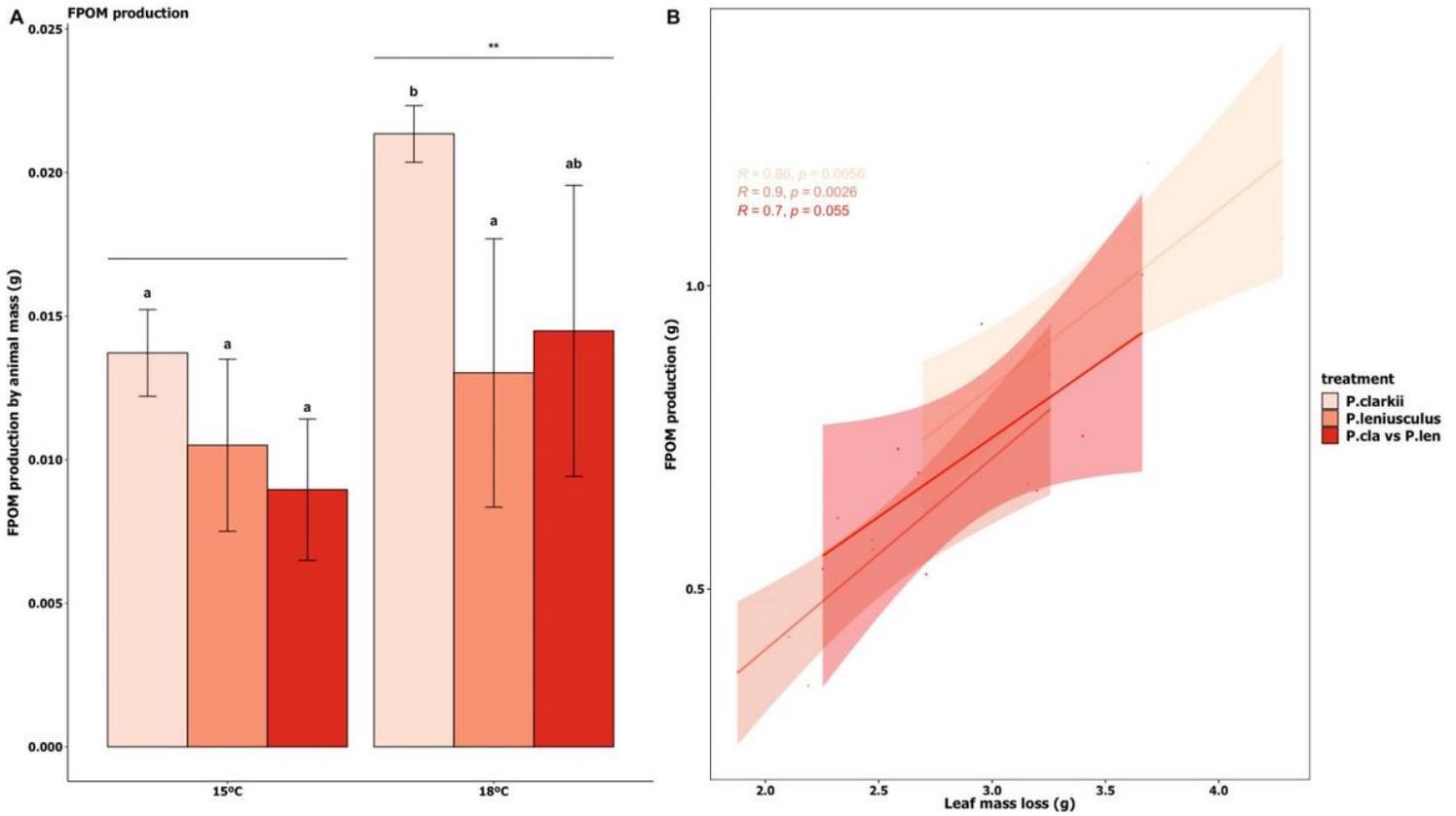
**Figure 2**

Leaf-litter breakdown by microbes (A) and *Sericostoma* sp. (B) in the absence (control) and presence of individual and combined effects of invasive crayfish species at different temperatures (15° and 18°C). Mean  $\pm$  SD, n=4.



**Figure 3**

Leaf-litter breakdown by crayfish weighed by invasive crayfish body mass (A) across individual and combined species treatments and different temperatures (15° and 18°C). Mean  $\pm$  SD, n=4. Expected-observed performance in interspecific treatments (B) calculated based on the difference between observed leaf breakdown in interspecific treatments and the expected value based on the weighed sum of individual mean performance of each species. Net effect was tested against 0. Asterisk corresponds to significant differences (t-test). Effects are additive if = 0, antagonistic if <0 and synergistic if > 0.



**Figure 4**

FPOM production by invasive crayfish body mass (A) across individual and combined species treatments and different temperatures (15° and 18°C). Mean  $\pm$  SD, n=4. Correlation between grams of leaf-breakdown and grams of FPOM production (B) across different individual and combined crayfish species treatments. Significant results were obtained by Pearson Correlation Coefficient, n=8.

## Supplementary Files

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