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PRIMARY RESEARCH PAPER

Effects of intrapopulation phenotypic traits of invasive crayfish on leaf litter processing

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Abstract The impact of invasive alien species (IAS) on an ecosystem is primarily studied at the species level, whereas the functional impacts of their genetic and phenotypic traits are poorly investigated. We used two laboratory based experiments to assess how intrapopulation phenotypic traits (size, sex, and number of claws) of the invasive red swamp crayfish *Procambarus clarkii* affect invertebrate shredders and leaf processing. Leaf consumption was significantly affected by the size and number of claws but not by the sex of the crayfish. Bigger animals presented a higher overall consumption but, in contrast, the mass of leaves consumed per unit crayfish body mass decreased with size. Indeed, the production of particulate organic matter followed the same trend, suggesting that the higher metabolic needs of smaller animals are responsible for their higher feeding activity on leaf litter. Claw loss in *P. clarkii* also led to increased leaf consumption and may be related to increased energy requirements for claw regeneration. Our results highlight the importance of also considering the phenotypic traits within populations for better understanding the impacts of IAS on ecosystem processes.

Keywords Ecosystem processes · Intraspecific variation · Invasive alien species · *Procambarus clarkii* · Streams

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Introduction

Natural populations are usually composed of genetically and phenotypically distinct organisms that exhibit variations in morphological, behavioral and/or physiological traits (Bolnick et al., 2011; Sih et al., 2012). This intraspecific phenotypic variation is widely studied in evolution since it constitutes the foundation for natural selection (Bolnick et al., 2011). However, it is often assumed that conspecific individuals are phenotypically uniform, and so they would affect ecosystem functioning in a similar way (Hairston & Hairston, 1993). This assumption may be misleading because intraspecific variations can alter

biotic interactions (Bolnick et al., 2011), although few studies have addressed their effects on ecosystem functioning (but see e.g., Post et al., 2008; Harmon et al., 2009; Palkovacs & Post, 2009; Bassar et al., 2010; Palkovacs et al., 2015). For example, sex ratio variation and sexual dimorphism were shown to shape the effects of an introduced freshwater fish on zooplankton and phytoplankton abundance and productivity, inducing pelagic trophic cascades (Fryxell et al., 2015). Also, synergistic interactions between body size and species identity of a predator dragonfly modulated its functional effects on communities (Rudolf et al., 2014).

The introduction of invasive alien species (IAS) is a major threat to biodiversity (Simberloff et al., 2013). IAS may change biotic interactions, and thus affect several attributes of native communities, with impacts on relevant ecosystem processes and functions (Sousa et al., 2011; Gutiérrez et al., 2014). Invasion biology has focused on functional diversity at or above the species level to explain possible ecological effects in the invaded areas (Hooper et al., 2005; Palkovacs et al., 2009). However, recent studies have demonstrated that intraspecific trait variations in IAS should also deserve attention as they may influence invasion success (Chapple et al., 2012; Forsman, 2014; González-Suárez et al., 2015) and/or modulate ecological impacts (Evangelista et al., 2017).

The red swamp crayfish *Procambarus clarkii* (Girard, 1852) is a widely distributed IAS in freshwater ecosystems. Native to southern United States of America and north-eastern Mexico, *P. clarkii* was successfully introduced in Europe in the 1970s (Gherardi, 2006). Its engineering activities and ability to alter biotic interactions make this IAS a cause of great concern in freshwater ecosystems (Loureiro et al., 2015; Souty-Grosset et al., 2016). For example, leaf litter processing is a key ecosystem process driven by microbial decomposers and invertebrate detritivores that control the flux of energy and carbon in aquatic food-webs (Suberkropp, 1998). The omnivorous *P. clarkii* can have a pivotal effect on leaf litter processing through consumption of both leaf litter (i.e., direct effect) and native invertebrate detritivores (i.e., indirect effect) (Alp et al., 2016; Carvalho et al., 2016). This ultimately affects native invertebrate biodiversity (Correia & Anastácio, 2008) and ecosystem functioning (e.g., organic matter turnover and nutrient cycling; Bernardo & Ilhéu, 1994; Jackson

et al., 2014). Some studies explored the effects of *P. clarkii* sex and size on native invertebrate communities (Correia, 2002, 2003), but the effects of their intrapopulation phenotypic traits on ecosystem functioning, including leaf litter processing, remain poorly studied.

The aim of our study was to assess the effects of the intrapopulation traits of *P. clarkii* on leaf litter processing. We expected that differences in the size and sex of *P. clarkii* would lead to differences in its feeding behavior and leaf litter processing. We also expected that larger animals would consume more leaf litter (Gutiérrez-Yurrita et al., 1998) and consequently produce more particulate organic matter (POM). During the reproductive period, *P. clarkii* individuals are susceptible to lose claws due to increased aggressiveness (Bender, 1971; Juanes & Smith, 1995) and we hypothesized that this loss of claws would decrease their feeding activity, with consequences on leaf litter processing.

Materials and methods

Specimens collection

We collected males and females of *P. clarkii* with different total lengths (small < 69 mm ± 10 mm SE and big > 108 mm ± 4 mm SE) in the Minho River (41°57′N, 8°44′W), Portugal. Specimens were collected in a semi-enclosed area (depth variation between 1.5 and 3.0 m and 14 km upstream of the river mouth). A full description of the abiotic and biotic conditions of the collection site can be found in Sousa et al. (2013) and Mota et al. (2014). Crayfish were captured with fyke-nets (10-mm mesh, 0.7-m-diameter mouth, 7-m-long, 3.5-m central wing) during their active period (May and June 2013) as described in Sousa et al. (2013).

We collected *Sericostoma* sp. larvae (Trichoptera, Sericostomatidae), a common invertebrate shredder in northern Portugal, at the upper reach of the Cávado River in May and June 2013. This site is located 10 km downstream from the town of Montalegre (41°48′N, 7°51′W), Portugal, where the presence of crayfish (including *P. clarkii*) is not reported so far. *Sericostoma* sp. individuals had a total length of approximately 10 mm (case length).

Crayfish and *Sericostoma* sp. individuals were acclimated to laboratory conditions for 7 days before the experiment (15°C room temperature, 12/12-h photoperiod). During this period, individuals were kept in aquariums (crayfish were kept individually) with 3 l of aerated mineral water, and allowed to feed on alder *Alnus glutinosa* (L.) Gaertn leaves. Individuals of both species were put under starvation for 24 h before the beginning of the experiments.

Mesocosm experiments

For both laboratory mesocosm experiments, we used aquariums (40 × 23 × 25 cm) equipped with an aeration system. The mesocosms were filled with 3 l of mineral water and the bottoms were covered with sand, gravel, and pebbles (size 850 μm–60 mm grain size; layer of 2 cm), previously washed and autoclaved (120°C, 20 min). Sets of alder leaves, collected in autumn 2012 immediately after abscission, were weighed (mean = 4.01 g dry weight ± 0.01 SE), placed in mesh bags (green plastic 23 × 15 cm; 5 × 5 mm pore size) and submerged in deionized water for 36 h to promote the leaching of soluble compounds. The content of each litter bag was then transferred to each mesocosm.

Mesocosms also received leaf discs previously immersed in a stream to ensure the colonization of alder leaves by a natural microbial community. Sets of 10 alder discs (12 mm diameter) were immersed for 1 week in a low-order stream (Oliveira, 41°35′N, 8°13′W) and then placed in the mesocosms (one set/mesocosm) at the beginning of the experiment following Fernandes et al. (2015). Data from leaf discs were not used to calculate leaf litter processing since they were protected from *Sericostoma* sp and crayfish disturbance and consumption.

In Experiment one, we assessed if size (small < 70 mm and big > 100 mm total length) and sex of *P. clarkii* affected leaf consumption and the production of POM. Experimental design consisted of (i) microbial control, without animals; (ii) invertebrate control, 6 *Sericostoma* sp.; (iii) 1 small crayfish male + 6 *Sericostoma* sp.; (iv) 1 small crayfish female + 6 *Sericostoma* sp.; (v) 1 big crayfish male + 6 *Sericostoma* sp.; and (vi) 1 big crayfish female + 6 *Sericostoma* sp. Crayfish were weighed at the beginning and the end of the experiment.

In Experiment two, we assessed if the number of claws of *P. clarkii* affected leaf consumption and POM production. We selected males of similar size and with two (mean = 98 mm ± 3 SE), one (mean = 96 mm ± 7 SE), or no claws (mean = 94 mm ± 4 SE). The crayfish size did not differ between treatments (one-way ANOVA; $P = 0.566$; $F = 1.12$;). Experimental design consisted of (i) microbial control; (ii) invertebrate control, 6 *Sericostoma* sp.; (iii) 1 crayfish without claws + 6 *Sericostoma* sp.; (iv) 1 crayfish with 1 claw + 6 *Sericostoma* sp.; (v) 1 crayfish with 2 claws + 6 *Sericostoma* sp.

Both experiments ran for 21 days and each treatment was replicated 4 times. One-third of the water volume of each mesocosm was renewed every 7 days. The retrieved water (from each renewal and final remaining) was filtered through a 53-μm sieve to collect small particles from water suspensions, mainly POM. Then, POM was centrifuged (10 min, 14,000 rpm; Sigma 4–16 K), and the pellet lyophilized (Biolblock Scientific-Christ Alpha 2–4 LD Plus) for 48 h, before being weighed to the nearest 0.01 mg. At the end of the experiments, the remaining leaf material was cleaned, dried at 60°C for 48 h, and weighed to the nearest 0.01 g. Percentage of leaf consumption (L_c) was quantified 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. Leaf consumed per gram of crayfish body mass was calculated as g L g C^{-1} where g L is the mass of leaf and g C is the biomass of crayfish.

Statistical analyses

Two-way analysis of variance (ANOVA) was used to test if crayfish size and sex affected leaf consumption and POM production (Zar, 2009). One-way ANOVA was used to test if the number of crayfish claws affected leaf consumption and POM production used (Zar, 2009). Moreover, leaf consumption and POM production by the crayfish were compared with microbial control and invertebrate control by one-way ANOVAs. ANOVAs were followed by Tukey´s post-tests to determine where significant differences occurred (Zar, 2009).

All ANOVAs were preceded by the Shapiro–Wilk test to check if the residuals of the models had a Gaussian distribution, and the Bartlett test to check for homoscedasticity (Zar, 2009). Residuals were

normally distributed and variances were homogenous. All statistical analyses were performed using STA-TISTICA 8 (StarSoft, USA).

Results

In experiment one, leaf consumption was significantly higher in the presence of big crayfish compared to microbial and invertebrate controls. ($P < 0.001$; $F = 13.63$). Leaf consumption by microbes (microbial control) was 41.4% ± 1.27 and by the *Sericostoma* sp. (invertebrate control) was 42.6% ± 1.24. Leaf consumption was affected by the crayfish size ($P = 0.004$; $F = 12.41$; Fig. 1A, Table 1) but not by their sex ($P = 0.779$; $F = 0.08$; Fig. 1A, Table 1). Big crayfish had higher leaf consumption (83.5% ± 7.58 in males and 83.4% ± 3.86 in females) than small crayfish (60.2% ± 5.75 in males; 63.8% ± 6.61 in females) (Fig. 1A). However, an opposite trend was found when leaf consumption was expressed as grams of leaf consumed per gram of crayfish body mass (Fig. 1B).

Again, the crayfish size ($P = 0.002$; $F = 14.92$), but not the sex ($P = 0.673$; $F = 0.19$), had a significant effect on leaf consumption (Fig. 1B; Table 1). Leaf consumption per unit body mass decreased with size. Big crayfish processed 0.08 g/g ± 0.01 in males, 0.08 g/g ± 0.01 leaves in females, while small crayfish processed 0.28 g/g ± 0.05 in males and 0.33 g/g ± 0.10 in females.

POM production by big crayfish was significantly higher than that by microbes and *Sericostoma* sp. ($P < 0.001$; $F = 9.79$). The microbial control produced 0.15 g ± 0.02 of POM, and invertebrate control produced 0.11 g ± 0.02. POM production was affected by the crayfish size ($P = 0.003$; $F = 14.11$; Fig. 1C; Table 1) but not by the sex ($P = 0.486$; $F = 0.52$; Fig. 1C; Table 1), and it was significantly higher in the presence of big crayfish. POM production was 0.52 g ± 0.11 in big male crayfish, 0.62 g ± 0.10 in big female crayfish, 0.27 g ± 0.02 in small male crayfish, and 0.28 g ± 0.04 in small female crayfish (Fig. 1C). An opposite trend (i.e., POM production decreased with crayfish size) was
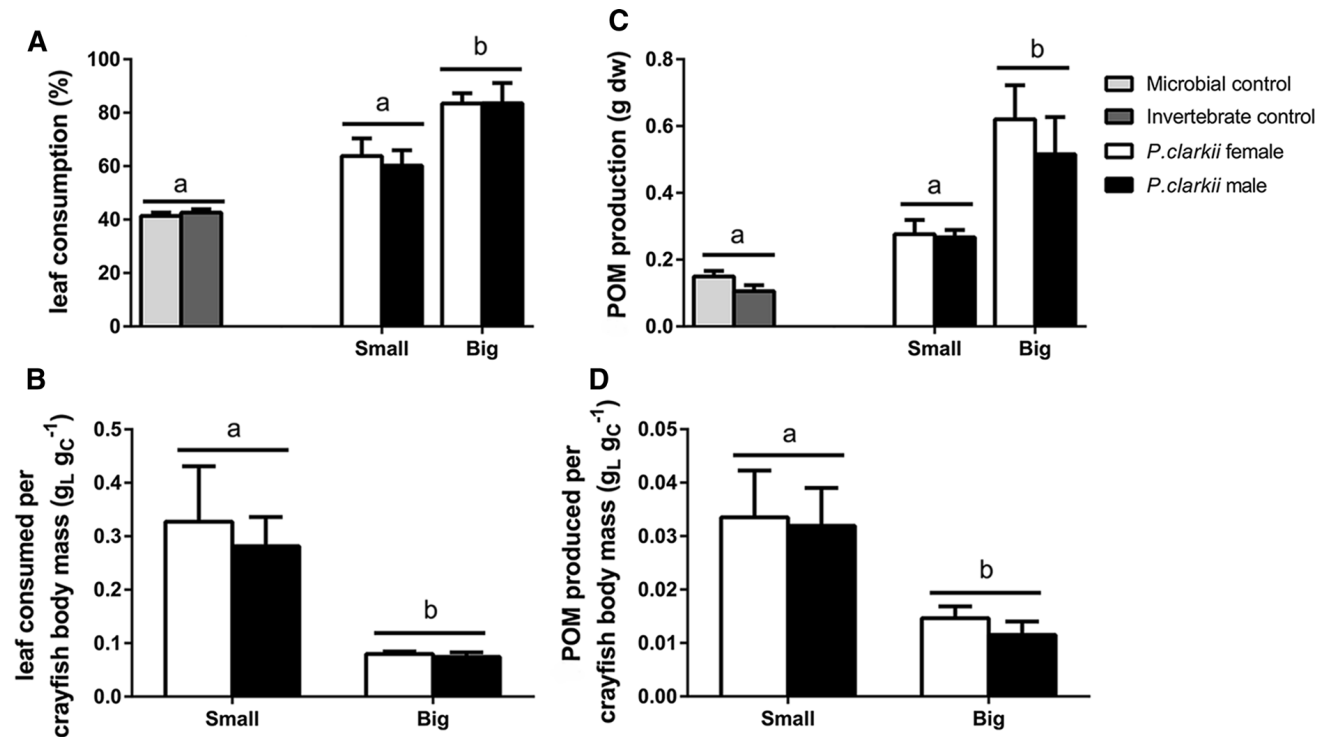

Fig. 1 Effects of sex and size of *Procambarus clarkii* on percentage of leaf consumption per mesocosm (**A**), leaf consumed per crayfish wet biomass (**B**), POM production per mesocosm (**C**), and POM production per crayfish wet biomass (**D**) from Experiment 1. Results were also compared to microbial control (mesocosms without *Sericostoma* sp. or *P.* *clarkii*) and invertebrate control (with *Sericostoma* sp. only, six individuals). Other mesocosms had 6 larvae of the invertebrate shredder *Sericostoma* sp. and 1 *P. clarkii* with different sex and size. Mean ± SEM, $n = 4$. Different letters indicate significant differences between treatments ($P < 0.05$)

Table 1 Summary statistics of two-way ANOVAs that tested for the effects of *Procambarus clarkii* sex and size on percentage of leaf consumption, leaf consumed per crayfish biomass and POM production in Experiment 1

Parameter	Effect	df	F	P
Percentage of leaf consumption	Crayfish sex	1	0.08	0.779
	Crayfish size	1	12.41	0.004
	Crayfish sex × Crayfish size	1	0.09	0.764
Leaf consumed per crayfish biomass	Crayfish sex	1	0.19	0.673
	Crayfish size	1	14.92	0.002
	Crayfish sex × Crayfish size	1	0.12	0.735
POM production	Crayfish sex	1	0.52	0.486
	Crayfish size	1	14.11	0.003
	Crayfish sex × Crayfish size	1	0.37	0.556
POM production per crayfish biomass	Crayfish sex	1	0.16	0.692
	Crayfish size	1	11.25	0.006
	Crayfish sex × Crayfish size	1	0.02	0.897

observed when POM production was expressed as POM produced per gram of crayfish body mass (Fig. 1D). Again, the crayfish size ($P = 0.006$; $F = 11.25$; Fig. 1D; Table 1), but not the sex $P = 0.692$; $F = 0.17$; Fig. 1D; Table 1), had a significant effect on POM. POM production was higher in small crayfish (0.03 g/g ± 0.01 in males and 0.03 g/g ± 0.01 in females) than in big crayfish (0.01 g/g ± 0.00 in males and 0.02 g/g ± 0.00 in females).

In experiment two, leaf consumption was higher in the presence of crayfish (with none, one or two claws) than in microbial and invertebrate controls ($P < 0.001$; $F = 44.6$; Fig. 2A). Leaf consumption was lower in the microbial control (without invertebrates or crayfish) (41.4% ± 1.27) and in invertebrate control (without crayfish) (46.0% ± 1.05), intermediate in mesocosms with crayfish with both claws (60.7% ± 1.22) and higher in mesocosms where crayfish had one (76.3% ± 4.48) or no claws (80.6% ± 3.19) (Tukey tests, $P < 0.05$). Claw loss significantly affected leaf consumption ($P = 0.005$; $F = 10.36$; Table 2). Crayfish without claws ($P = 0.005$, Tukey test, Fig. 2A) and crayfish with one claw ($P = 0.020$, Tukey test, Fig. 2A; Table 2) had higher leaf consumption than crayfish with both claws.

POM production was higher in the presence of crayfish (with none or one claw) than in microbial or invertebrate controls ($P = 0.002$; $F = 7.32$ Fig. 2B). POM production was 0.15 g ± 0.02 in the microbial control, 0.28 g ± 0.04 in invertebrate controls, 0.49 g ± 0.05 in crayfish with both claws, 0.59 g ± 0.12 in crayfish with one claw, and

0.60 g ± 0.10 in mesocosms with crayfish without claws. Crayfish claw loss had no effect on the amount of POM produced ($P = 0.646$; $F = 0.46$; Table 2).

Discussion

Our results clearly demonstrate that both *P. clarkii* size and number of claws, but not sex, affect leaf litter consumption and POM production. This highlights the importance of intrapopulation phenotypic trait variations of IAS as a driver of ecosystem processes and functions.

Our results suggest that crayfish size was an important trait for leaf processing. Although bigger animals consumed higher amounts of leaf litter, smaller animals showed higher levels of leaf consumption per unit mass. This can be explained by the higher metabolic demands of smaller animals that consume higher amounts of leaf litter per animal biomass as compared to bigger animals (Singer, 2004). Furthermore, since the small crayfish used in this study were not sexually mature, their energetic demands were probably mainly devoted to rapid growth. In addition, and consistent with the results for leaf consumption, bigger animals were found to also produce more POM in our study. Smaller crayfish produced higher POM per body mass, giving further support to the suggestion of higher metabolic and energetic demands for smaller animals. POM plays a key role in freshwater ecosystems as invertebrate collectors depend on this resource (Graça, 2001; Reiss et al., 2010) and our results suggest that alterations in
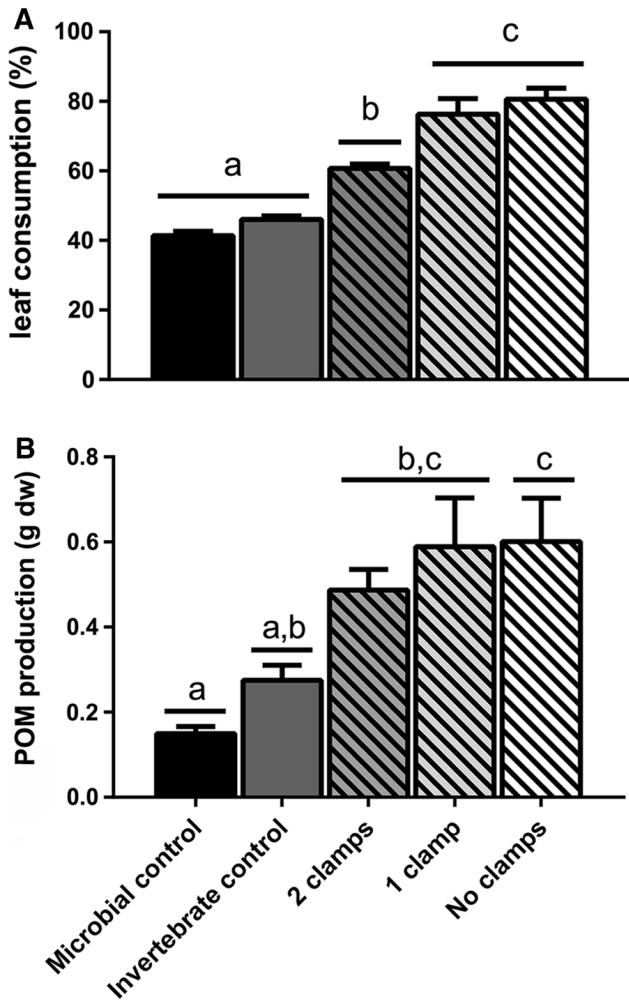
Fig. 2 Effects of claw loss of. *Procambarus clarkii* on percentage of leaf consumption (**A**) and POM production (**B**) per mesocosm from Experiment 2. Results were also compared to microbial control (mesocosms without *Sericostoma* sp. or *P. clarkii*) and invertebrate control (with *Sericostoma* sp. only, 6 individuals). Other mesocosms had *P. clarkii* with different numbers of claws (none, one, two) in the presence of 6 larvae of the invertebrate shredder *Sericostoma* sp. Mean ± SEM, $n = 4$. Bars with patterns indicate treatments with the presence of *P. clarkii*. Different letters indicate significant differences between treatments ($P < 0.05$)

Table 2 Summary statistics of one-way ANOVAs that tested for the effects of *Procambarus clarkii* claw loss on leaf consumption and FPOM in Experiment 2

Parameter	df	F	P
Leaf processing	2	10.36	0.005
POM production	2	0.46	0.646

POM production by the crayfish may affect aquatic ecosystems due to changes in quantity and/or quality of food supplies (Dietrich, 1997; Wipfli et al., 2007).

Interestingly, no differences in leaf consumption or POM production were detected according to animal sex under laboratory conditions. Typically, crayfish males are much more aggressive and active than females (Abrahamssom, 1966; Stein & Magnuson, 1976; Berril & Arsenault, 1984; Usio & Townsend, 2002), and this behavior could be expected to translate into higher feeding rates. Moreover, their higher activity and movements could be expected to cause abrasion and contribute to increased leaf processing. Nevertheless, in our case, and as reported by others (Gutiérrez et al., 1998; Correia, 2002, 2003), no differences were observed in diet preferences between *P. clarkii* males and females.

Interestingly, but contrary to that expected, our results showed that crayfish with one or no claws were able to consume more leaves than those with both claws. In addition, while not being significantly different, our results did also indicate that crayfish without both claws tended to produce more POM than crayfish with both claws. Crayfish use claws to grab and collect food resources (Stein, 1976), and thus those without claws would be expected to have a lower capacity for capturing food. Indeed, a previous study demonstrated that the blue crab *Callinectes sapidus* Rathbun, 1896, without both chelipeds, had lower feeding rates than those with both or just one cheliped (Smith & Hines, 1991) and this reduction in the feeding capacity continued during the regenerative process (Juanes & Smith, 1995). In fact, claws have already been shown to make a major contribution to the physiological fitness of adult crayfish (Hudina et al., 2012) and animals with larger claws have advantages in foraging, defense against predators, intraspecific competition, sexual competition, and reproductive success (Garvey & Stein, 1993; Lee, 1995; Streissl & Hödl, 2002). Other studies also showed that claw loss in decapod crustaceans affects diet by reducing resource consumption (e.g., Matheson & Gagnon, 2012), with consequences for growth and regeneration, and leading to changes in population dynamics and community processes (Juanes & Smith, 1995). Our results contradict these studies but it is possible that this discrepancy is related to the crayfish's capacity for regeneration of its body parts (Cooper, 1998). Indeed, the regeneration of claws,

which comprise a substantial portion of the total body weight of crayfish, is an energy intensive process (Powell et al., 1998). In addition, crayfish with one or no claws may compensate for this deficiency by using their walking legs to help in feeding (e.g., Smith & Hines, 1991) since major chelae are used mainly for agonistic encounters whereas minor chelae are used for prey capture and handling (Mariappan et al., 2000). Finally, in the collection site for the crayfish used in this study (River Minho, Iberian Peninsula), up to 40% of *P. clarkii* males have been reported to have lost one or two claws as a result of increased aggressiveness (Sousa et al., 2013). Therefore, it is conceivable that the crayfish increased feeding on leaf litter, with impacts on leaf processing. Nevertheless, these results should be interpreted with caution because the study was conducted under laboratory conditions, where crayfish were not subjected to competition or predation and were confined to aquarium mesocosms. Also, the crayfish had limited food resources (leaf litter and/ or invertebrate shredders), which is a simplified representation of food resources for an omnivore. Therefore, to better understand the impacts of intrapopulation traits on key ecosystem processes, further studies with *P. clarkii* should be conducted in field mesocosms under different environmental conditions.

In conclusion, our results demonstrate that in freshwater ecosystems, not only the identity of the species, but also their intrapopulation traits, are important predictors of key ecosystem processes including leaf litter consumption and POM production. Therefore, we advocate that intrapopulation trait variation should be considered when assessing the impacts of IAS on native species and ecosystem processes.

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