

PREPRINT

Author-formatted, not peer-reviewed document posted on 21/05/2024

DOI: <https://doi.org/10.3897/arphapreprints.e127932>

The hidden side of the intrapopulation level in biological invasions

 **Ronaldo Sousa, Henrique Alves, Diana Gonçalves, Janeide Padilha, Amílcar Teixeira**

1 **The hidden side of the intrapopulation level in biological invasions**

2

3 Henrique Alves ^{1†}, Diana Gonçalves^{1†}, Amílcar Teixeira², Janeide Padilha¹ and Ronaldo

4 Sousa^{1*}

5

6 ¹ CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of
7 Minho, Campus Gualtar, 4710-057 Braga, Portugal.

8 ² Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa
9 Apolónia, 5300-253 Bragança, Portugal.

10 *Corresponding author: rg.eco.sousa@gmail.com

11 [†] Both authors contributed equally as the first author

12

13

14

15

16

17

18

19

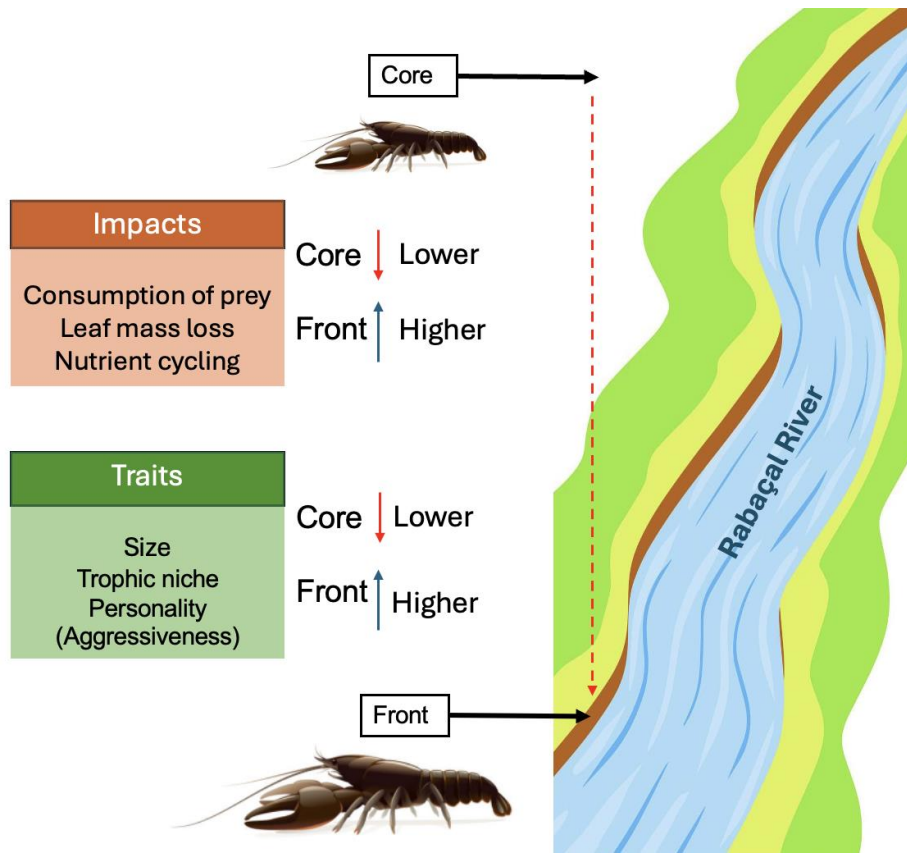
20

21

22

23

24 Graphical abstract



25

26

27

28

29

30

31

32

33

34

Abstract

35 Individual variation assessments are essential to better understand population and
36 community dynamics, and ecosystem functioning. Although researchers have long
37 recognized this aspect only recently has evidence accumulated about the ecological
38 importance of variation within species. The incorporation of individual variation provides
39 a more complete description of the effects a species may have on ecosystems and this
40 detailed ecological knowledge can be especially important in the context of biological
41 invasions. In this study, we used an invasion gradient of the signal crayfish *Pacifastacus*
42 *leniusculus* to assess possible intrapopulation differences in key biological traits and
43 evaluate possible changes in ecological impacts. For this, we collect individuals from the
44 core and front of an invasion gradient to characterize several biological traits such as
45 abundance, size, sex-ratio, physiological condition, personality, and trophic niche. In
46 addition, we perform two laboratory experiments to assess possible differences regarding
47 the consumption of prey, leaf mass loss, and nutrient cycling. Crayfish from the invasion
48 front are predominantly male and display lower abundance. However, they are larger,
49 exhibit superior physiological condition, demonstrate increased boldness and aggression,
50 and have elevated nitrogen values. In addition, crayfish from the front consume more prey
51 and more leafs and increase the concentration of nitrates and phosphates. Overall, the
52 signal crayfish has different biological traits and has distinct ecological impacts along the
53 invasion gradient. This study demonstrates the importance of assessing biological traits
54 and impacts of invasive species at the intrapopulation level. Such an approach could
55 improve and refine impact assessments with potential pay-offs in the development of
56 more effective conservation and management strategies.

57

58 Key words: biological traits; ecological impacts; invasive species; *Pacifastacus*
59 *leniusculus*; signal crayfish.

60

61

62

63 **Introduction**

64 Humans are key drivers of global environmental change (Dirzo et al. 2014) and
65 anthropogenic activities have redistributed the world's biota and mediated species
66 colonization of regions beyond their native range (Seebens et al. 2017; Pysek et al. 2020).
67 The consequences of these biological introductions are severe. Invasive species can
68 disturb ecological communities and alter ecosystem functions, drive population declines
69 and species extinctions, and continue to cost the global economy billions of euros every
70 year given their detrimental negative impacts on several key ecosystem services
71 (Ehrenfeld 2010; Strayer 2012; Simberloff et al. 2013; Diagne et al. 2021; Gallardo et al.
72 2024). Given the myriad of detrimental ecological and economic impacts attributed to
73 invasive species and limited possibilities for total eradication, it is not surprising that their
74 study is one of the most prominent areas of research in ecology (Lockwood et al. 2013).
75 Although all ecosystems are susceptible to introductions, freshwater ecosystems are
76 especially vulnerable (Strayer 2010). This situation presents a challenge for the
77 management and conservation of freshwater biodiversity because accelerated
78 introduction rates may have detrimental consequences, including the erosion of
79 biodiversity and as such, the disruption of key ecological processes and functions (Strayer
80 2010; Gallardo et al. 2016; Dudgeon, 2019).

81 Understanding the species-level traits associated with invasiveness has been a primary
82 focus of biological invasions, with attributes such as high dispersal rates, high fecundity,
83 and broad physiological tolerance among the key predictors of success (Pysek et al. 2020).
84 However, this may be an oversimplification (i.e. assessing the impacts, dispersal, or other
85 topics at the species level) because a species can be very invasive in one region while
86 simultaneously being almost innocuous a few kilometers away (Haubrock et al. 2024).
87 Moreover, a growing accumulation of evidence is showing that most of these impacts
88 happen at the population level, not only due to the environmental context or biotic
89 resistance of the invaded ecosystem (Blackburn et al. 2011), but also because intraspecific
90 variation is increasingly recognized as an important driver of invasion dynamics (Biro
91 and Stamps 2008; Haubrock et al. 2024). Individual variation in ecological assessments
92 is essential to understanding population and community dynamics, and ecosystem
93 functioning (Bolnick et al. 2011; Violle et al. 2012; Des Roches et al. 2018; Raffard et al.
94 2019). Although researchers have long recognized this aspect (e.g. Charles Darwin and
95 Russel Wallace use these intraspecific variations as the central foundation of the theory

96 of evolution) only recently has evidence accumulated about the ecological importance of
97 variation within species (Post et al. 2008; Des Roches et al. 2018; Raffard et al. 2019).
98 This last aspect may be highly related to variation in biological traits (e.g. size, sex,
99 boldness, sociability, activity, aggression) and associated behavioural syndromes, i.e.
100 correlations between these traits (Chapple et al. 2012). For example, personality, defined
101 as individual differences in behaviours that are stable over time and context (Sih et al.
102 2004), can affect any stage of the invasion process (Juette et al. 2014). This is because
103 traits that maximize the probability of being introduced outside the native range may also
104 promote successful establishment and spread within the recipient ecosystem (Myles-
105 Gonzalez et al. 2015; but see Chapple et al. 2012). These biological traits are also likely
106 to influence interactions with native species and ecosystems and thus may play an
107 important role in determining the intensity of ecological impacts (Juette et al. 2014).
108 Despite the theoretical relevance, the fact is that very few studies have demonstrated that
109 biological traits may vary along the invasion gradient with possible consequences for the
110 generated ecological impacts (Cote et al. 2010). However, biological traits, such as sex,
111 body size or physiological condition, may act as important drivers on the spread dynamics
112 of invasive species (Phillips et al. 2006) and may be responsible for distinct ecological
113 impacts as shown by Fryxell et al. (2015) where the effects of an introduced freshwater
114 fish on zooplankton and phytoplankton abundance, as well as productivity, were found to
115 be influenced by variations in sex ratio and sexual dimorphism, triggering pelagic trophic
116 cascades. Finally, these biological traits and impacts may vary not only spatially but also
117 through time and this may differentially affect native communities over the years and
118 may even have evolutionary consequences for native biodiversity (Mathers et al. 2016;
119 Carvalho et al. 2022; Haubrock et al. 2024).

120 Therefore, it seems reasonable to assume that the incorporation of individual variation
121 provides a more real and complete description of the invaded population, community, and
122 ecosystem under study. Their downplay in empirical studies is probably related to the
123 increased complexity of the analyses and due to additional costs in laboratory and/or field
124 sampling (Toscano et al. 2016). Even so, some studies already demonstrate the
125 importance of these intraspecific peculiarities in biological invasions, being the cane toad
126 *Rhinella marina* invasion in Australia the iconic textbook example (Shine, 2010). In this
127 study, we use the recent invasion of the signal crayfish *Pacifastacus leniusculus* (Dana,
128 1852) in a Portuguese river to assess possible differences in biological traits and
129 ecological impacts at the intrapopulation level. *Pacifastacus leniusculus* is native to North

130 America and was first introduced in Europe, primarily in Scandinavia, in the 1960s for
131 stocking purposes and replacement of the native crayfish species, which suffered great
132 declines due to the crayfish plague (Dunn 2012). *Pacifastacus leniusculus* is characterized
133 by high reproductive rates, fast-growing populations, wide environmental preferences,
134 and high dispersal abilities, making this species a very successful invader in Europe
135 (Dunn 2012). *Pacifastacus leniusculus* is an omnivorous species and displays generalist
136 and opportunistic feeding habits (Olsson et al. 2009). The feeding habits of *P. leniusculus*
137 may include plants and large quantities of detritus (e.g. leaf litter) but may also feed on
138 other animals such as benthic invertebrates, fishes, and even other crayfishes (Guan and
139 Wiles 1998; Henttonen and Huner 2017; Meira et al. 2019). The signal crayfish also
140 represent a food source for higher trophic levels such as birds, fishes, and mammals
141 (Britton et al. 2017). This species can also function as ecosystem engineers, altering
142 community structure, water quality, and nutrient dynamics (Carvalho et al. 2022).
143 Ultimately, the signal crayfish may play a crucial role in various ecosystems, holding a
144 central position in food webs, where it functions both as predator and prey, affecting
145 different trophic levels and being able to have top-down and bottom-up cascading effects
146 (Britton et al. 2017).
147 Given the high abundance and widespread distribution, the signal crayfish is responsible
148 for several ecological and economic impacts on invaded ecosystems, but these impacts
149 may be highly context-dependent even considering the same population. Therefore, the
150 main aim of this study was to assess possible intrapopulation differences in key biological
151 traits (abundance, size, sex-ratio, behavior, and trophic niche) and ecological impacts
152 (consumption of prey, leaf mass loss, and nutrient cycling) using the signal crayfish along
153 an invasion gradient.

154

155 **Material and Methods**

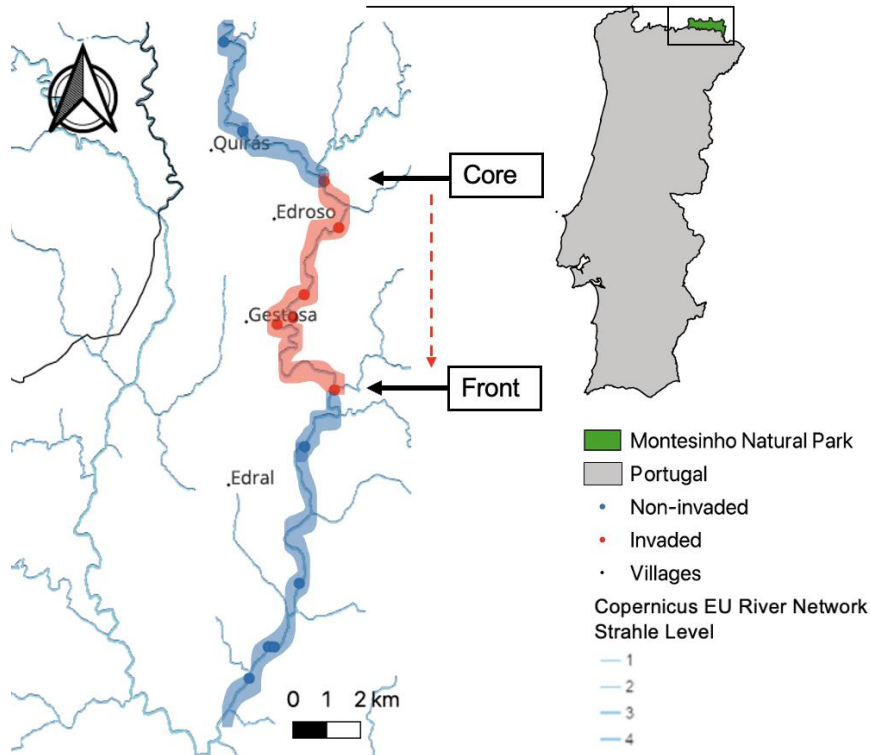
156 *Study Area*

157 This study was carried out in the Rabaçal River, at the Montesinho Natural Park and
158 adjacent downstream areas (Figure 1), located in NE Portugal. This river has a total length
159 of 88 km and its hydrological basin is subjected to a typically Mediterranean climate with
160 an Atlantic influence, characterized by high seasonal variability in terms of temperature
161 and precipitation (Oliveira et al. 2012). This variability in precipitation (between 1.000

162 and 1.600 mm) is responsible for abrupt alterations in river flow, with maximum values
163 being registered during winter/early spring and minimum values in late summer/early
164 autumn (Sousa et al. 2018).

165 The low human density in the Montesinho Natural Park, as well as the land use mainly
166 related to forest and subsistence agricultural activities (Nogueira et al. 2021a), makes this
167 hydrological basin one of the less disturbed in Portugal, containing important habitats for
168 many endangered freshwater species such as the pearl mussel, *Margaritifera*
169 *margaritifera*, the dragonfly *Macromia spendens*, the Northern Iberian spined loach
170 *Cobitis calderoni*, the Iberian desman (*Galemys pyrenaicus*), among many others (Sousa
171 et al. 2015 and 2019). However, in the last 15 years, the populations of these threatened
172 species suffered a reduction in abundance due to the impacts of droughts, habitat loss and
173 fragmentation, and the introduction of invasive species, such as the signal crayfish (Sousa
174 et al. 2019 and 2020; Nogueira et al. 2021b; Lopes-Lima et al. 2023). In Portugal, the
175 signal crayfish was first detected in 1997 in the Maçãs River (Bernardo et al. 2011;
176 Anastácio et al. 2019). In the next years, the species rapidly spread from the Maçãs River
177 to almost the entire Sabor basin (Meira et al. 2019). In 2013 was for the first time detected
178 in the Rabaçal and Tuela River basins (Sousa et al. 2015). In the Rabaçal River, the
179 species has been monitored annually since 2017 to assess their spread and some basic
180 autecological features (Carvalho et al. accepted). This monitoring program allows us to
181 confidently establish the core and front of the invasion gradient in the present study
182 (Figure 1). It should be also noted, that besides the recent introduction of the signal
183 crayfish, there are no records of any native or non-native crayfish species in the studied
184 area (Sousa et al. 2019).

185



186

187

188

189

190 **Figure 1** Map of the Rabaçal River with the location of both sites (core and front).

191

192 *Environmental characterization*

193 In both sites, temperature, conductivity, dissolved oxygen, and pH were measured using

194 an YSI EXO 2 multi-parameter probe in August 2023. Water samples were also collected

195 to determine total suspended solids (TSS) as well as total organic suspended solids (OSS).

196 For this, water volumes of 1 L were filtered using GFC filters, which were dried at 60 °C

197 for 48 h and then heated in a muffle at 550 °C for 8 h. TSS and OSS were determined by

198 weight difference following Zieritz et al. (2018). These measurements and water samples

199 were made/collected in the middle of the river near to the bottom at the end of the

200 morning.

201 Sediment samples were also collected in each site near (2m) the left bank using a cylinder

202 with 10 cm of diameter. Granulometry and organic matter in the sediment of each site

203 were determined following Sousa et al. (2020). Both sites were also subjected to an in

204 situ River Habitat Survey (RHS) to collect information related to the physical structure
205 of both sites, including the type of substrate in the channels, the complexity of the
206 structure of the vegetation on the banks, the type of aquatic vegetation, the characteristics
207 of the habitat and the type of artificial modification found in the channel and on the banks.
208 For this, the standard length of 500 metres of the river channel was used for data collection
209 at both sites following Raven et al. (1998). All this data was amassed in order to obtain
210 the Habitat Modification Score (HMS) and Habitat Quality Assessment (HQA) indices,
211 which are important for ascertaining the degree of disturbance in both sites

212

213 *Biological traits at the intrapopulation level*

214 To test possible intrapopulation differences in biological traits between individuals from
215 the core and the front of the invasion gradient in the Rabaçal River, abundance, sex-ratio,
216 size, physiological condition, behaviour, and trophic niche were evaluated. At each site
217 (core and front), in August 2023, we selected a river stretch of 100m that comprises
218 habitats including pools, runs, and riffles. Crayfish were captured by placing, at each site,
219 8 funnel traps, specifically used for decapod crustaceans, all cylindrical (43 cm d , 22 cm
220 h ; 1.5 cm mesh) and baited with fish remains, for 24 hours. The captured individuals were
221 counted, weighted, and measured from the rostrum tip to the telson rear edge (total
222 length), and sex was determined following Sousa et al. (2013). The abundance of the
223 signal crayfish per site was expressed as the total number of individuals per catch per unit
224 of effort (ind. CPUE/24 h). In addition, the physiological condition of organisms was
225 assessed using individuals randomly collected in the field with a total length between 8
226 and 11 cm in order to reduce possible bias. For this, Fulton's condition factor was used,
227 and it was calculated with the equation $K_c = 100 * W / L^3$, where W is the total weight of
228 signal crayfish and L is the total length.

229 Regarding behavior, four tests were conducted using unique sets of 16 randomly selected
230 individuals (8 females and 8 males) with a total length varying between 8 and 11cm from
231 both the core and the front of the invasion gradient. These individuals were acclimated in
232 tanks without food for three days at a water temperature similar to field conditions (18°C).
233 The experimental assessments were performed in an environmentally controlled room set
234 at 18°C in a tank with 46 x 63 x 32 cm (total volume of 60 L). The aims of the first and
235 second tests were to evaluate risk-taking behaviors associated with the presence of food

236 and exploratory behaviors in novel environments, respectively. Before testing, crayfish
237 were allowed a 5-minute acclimatization period within a shelter. After this period, the
238 shelter's lid was removed to initiate the test. Both tests measured the time taken to initiate
239 movement towards stimuli in seconds. In the first test, the time taken to exit the shelter
240 with a food source (cat food) positioned at the opposite end of the aquarium was recorded.
241 In the second test, a similarly arranged setup featured a container filled with stones and
242 shells to simulate a novel environment, and the time to exit the shelter was recorded in
243 seconds.

244 The third and fourth tests assessed aggression by comparing combat groups of individuals
245 from the core and the front of the invasion gradient: core female vs. front female, core
246 male vs. front male, core female vs. front male, and front female vs. core male. Individuals
247 of similar sizes were chosen for each combat. In the third test, crayfish were positioned
248 face to face for 15 minutes without any food present, while in the fourth test, food was
249 placed equidistantly. Each combat was conducted eight times with different individuals.
250 In the fourth test, the time each individual reached the food was recorded in seconds.
251 Subsequently, the winner of each encounter was determined based on dominance over
252 access to space and food, providing a measure of competitive aggression in scenarios with
253 and without immediate resource incentives.

254 To explore the trophic niche between the core and front of the invasion, a total of 15 males
255 and 15 females were randomly collected in both sites. The animals were measured (total
256 length varied between 6.5 and 10.5 cm in the core and between 7.0 and 11.8 cm in the
257 front) and immediately sacrificed by freezing and were kept at $-20\text{ }^{\circ}\text{C}$ until the day of
258 analysis. For stable isotope analysis, the animals were thawed; the abdominal muscle was
259 extracted, dried in an oven for 24 hours at 40°C , and then macerated using a ceramic
260 mortar. The elemental and isotopic composition analyses were conducted using a Thermo
261 Scientific Flash 2000 model Organic Elemental Analyzer (EA), which is linked to a Delta
262 V Advantage Isotope Ratio Mass Spectrometer (IRMS) via ConFlo IV. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
263 values of the samples and standards underwent normalization with certified reference
264 materials for each element—IAEA-N-1, IAEA-N-2, and IAEA-NO3 for nitrogen, and
265 USGS-24 and USGS-40 for carbon, achieving an analytical error margin of about 0.1%.
266 The isotopic composition of animal tissue samples was determined with precision, using
267 an internal standard of sea bass, which was read after every 12 analyses to ensure
268 analytical control. Corrections for the mass effect on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were made

269 according to a calibration curve derived from the delta values of N and C, which in turn
270 were based on the peak amplitudes of various masses within the caffeine standard.
271 Furthermore, the elemental composition was ascertained utilizing the K factor of
272 *Chlorella*, with samples being analyzed in duplicates to maintain a coefficient of variation
273 below 10%.

274

275 *Ecological effects at the intrapopulation level*

276 Individuals of *Physella acuta* (Drapanaurd, 1805) were used to assess consumption rates
277 between crayfish individuals from the front and core of the invasion. The individuals of
278 *P. acuta* were captured using a hand net in the Fervença River. We use this species since
279 it is non-native in the study region (in this way we reduce the possible ethical problems
280 as a result of such high mortality, see results) and because gastropod species are usually
281 a preferred food item for signal crayfish (Rosewarne et al. 2016). The collected animals
282 were rapidly transported to the laboratory and individuals were measured based on the
283 shell length (from the apex to the tip of the last whorl), in millimeters using a caliper
284 (following Spyra et al., 2019). The shell length varied between 6mm and 10mm, with an
285 average (\pm SD) length of 6.97 (\pm 1.12) mm. A total of 70 male signal crayfish captured
286 in the Rabaçal River (core and front sites; Figure 1) were also used in this experiment.
287 The selected individuals had a size that varied between 7.5 cm and 9.5 cm, with an
288 average length (\pm SD) of 8.16 (\pm 0.50) cm and 8.35 (\pm 0.53) cm for signal crayfish from
289 the core and front of the invasion, respectively.

290 A total of 7 treatments with different abundances (2; 4; 8; 16; 32; 64; and 128 snails) of
291 *P. acuta* were prepared. For each treatment, 5 replicates were used. Then, individuals of
292 *P. acuta* were introduced into aquariums with no sediment, each one filled with 10 liters
293 of water and properly aerated. Subsequently, 1 signal crayfish individual was introduced
294 per aquarium. The signal crayfish individuals remained inside the aquariums for 24 hours
295 at a room temperature of 18°C, and at the end of this period, the number of *P. acuta*
296 individuals consumed were determined. The control treatment consisted of aquariums
297 with *P. acuta* using the same abundances as described above but without adding crayfish.

298 Another laboratory experiment was conducted to assess possible differences in leaf mass
299 loss. A total of 18 aquariums were prepared to replicate the most natural environment
300 possible for the crayfish, so that their behavior was changed as little as possible. To this

301 end, they were placed in a room with a temperature of 18°C with an aerification system
302 and, in each aquarium, stones were placed to provide a hiding refuge for the crayfish. A
303 total of 3 different treatments were considered: 6 aquariums act as a control, where no
304 crayfish was placed; 6 represent the core of the invasion; and the other 6 represent the
305 front of the invasion. A total of 20 liters of water were added to each aquarium. After 24h
306 of aerification of the aquariums, the crayfish were placed in the aquariums and left 24h
307 without food. We only used male crayfish of approximately the same size (size of 7.81
308 (± 0.52) cm and 8.05 (± 0.62) cm in the core and front treatments, respectively).
309 Simultaneously to this preparation of the aquariums, bags with 4g of dry alder leaves
310 *Alnus lusitanica* (Vít et al., 2017) were arranged. In addition, small bags were previously
311 placed in the Rabaçal River for one week for the leaves to be colonized by
312 microorganisms (fungi and bacteria). These small bags were then placed next to the 4g
313 food bags for 24 hours. After these 24 hours, the leaves were removed from the bags and
314 placed in the aquariums for a period of 17 days. At the end of this period, the remaining
315 leaves were collected with the aid of a 500 μm sieve and then placed in an oven at 60°C
316 for 48h. Finally, the leaves were weighed and the difference between the initial weight
317 (4g) and the final weight for each aquarium was calculated and then converted into a
318 percentage of leaf mass loss. At the end of the experiment, two water samples of 50ml
319 from each aquarium were collected to assess and compare the nutrient levels (ammonia,
320 nitrite, nitrate, and phosphate) between the control, front, and core treatments. For each
321 nutrient, specific protocols were followed, utilizing colorimetric methods (for details see
322 Grasshoff et al., 1999). The readings were taken on a spectrophotometer.

323

324 *Data analysis*

325

326 Abundance variation between the core and the front of the invasion was analyzed by non-
327 parametric Kruskal-Wallis's tests, since the statistical models used do not meet
328 homoscedasticity and/or normality assumptions, even after being transformed. Variations
329 in size and physiological condition between the core and the front of the invasion were
330 compared by parametric one-way ANOVAs. This type of analyses were also used to
331 compare leaf litter decomposition and nutrients between treatments in the laboratorial
332 experiment. ANOVAs were preceded by Shapiro-Wilk to check the normality of the
333 residuals and the Bartlett test to check for homoscedasticity, or normality was assumed if

334 the number of observations satisfied the assumptions of the central limit theorem (Zar
335 1999). Chi-squared analysis was used to calculate the significance level of sex-ratio for
336 core and front treatments. Two-way ANOVA was performed to evaluate gastropod
337 consumption between the core and the front of the invasion in the different abundance
338 levels. The investigation into the ecological niches of the front and core signal crayfish
339 individuals employed the SIBER method (Stable Isotope Bayesian Ellipses in R), as
340 introduced by Jackson (2011). This method entails the computation of ellipse areas,
341 refined by SEAC adjustments and extended through Bayesian modeling to generate the
342 SEAb (Bayesian Standard Ellipse Area). Such a model is crucial for intergroup
343 comparisons. The application of SEAb serves as a gauge for the relative scope of
344 ecological niches within groups, inferred from the dimensions of the modeled ellipses and
345 their predicted posterior distributions. Groups with corresponding SEAb figures indicate
346 a parallel in the width of their isotopic niches, implying a similar range in their dietary
347 choices.

348 All of these statistical analyses were carried out using the software Rstudio Team (2022),
349 using packages “SIBER” “ggplot2”, “cowplot”, “pgirmess”, “phia” and “multcomp”.

350

351 **Results**

352 *Environmental characterization*

353 The sampled sites are distanced by 15 km and have very similar climate and
354 environmental conditions (Table S1). Both sites are subjected to a very low human
355 disturbance and the RHS survey gives very similar results for both sites (Table S1).

356 Consequently, we assume that possible differences in biological traits and ecological
357 impacts (see below) are not related to possible distinct environmental conditions between
358 core and front sampling sites.

359

360

361

362 *Biological traits at the intrapopulation level*

363 Significant differences in abundance were found between the two sites (Kruskal-Wallis
364 test, $\chi^2 = 8.1483$, $p=0.00431$), with the core of the invasion showing higher abundance
365 (17.25 ± 13.38 CPUE) when compared to the front of the invasion (4.38 ± 4.47 CPUE)
366 (Figure 2A).

367 When comparing the size between the core and the front of the invasion, it was also noted
368 significant differences between the two sites (One-way ANOVA, $F=131.9$, $p<0.001$).
369 Signal crayfish individuals in the front of the invasion were larger than in the core (Figure
370 2B) with an average length (\pm SD) value of $9.63 (\pm 0.92)$ cm and $8.11 (\pm 0.63)$ cm,
371 respectively.

372 The analysis of sex-ratio revealed that there is a significant difference between the
373 percentage of females and males in the core of the invasion ($\chi^2=24.377$, $p<0.001$).
374 Females represent 71% of the signal crayfish individuals at the core while males represent
375 29% (Figure 2C). On the front of the invasion, there is no difference between the
376 percentage of females and males ($\chi^2=0.714$, $p=0.398$). Females represent 42.9% while
377 males represent 57.1% (Figure 2C).

378 When assessing the signal crayfish physiological condition using the Fulton's Condition
379 Index, it was possible to observe a significant difference between individuals from the
380 core and front of the invasion (One-way ANOVA, $p=0.00909$), with individuals from the
381 front presenting higher values (Figure 2D).

382

383

384

385

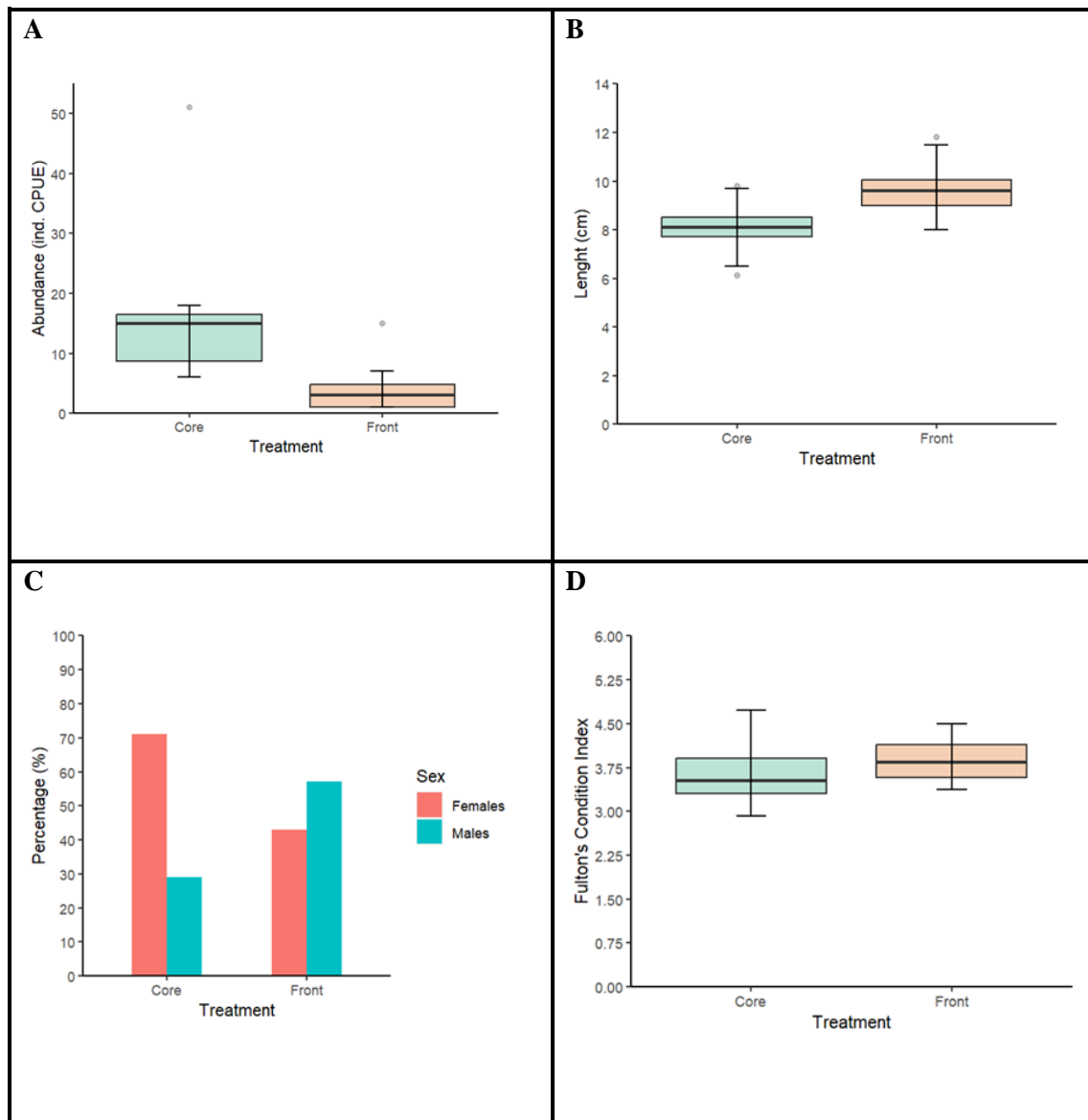
386

387

388

389

390



391

392 **Figure 2** Abundance (A), length (B), percentage of males and females (C), and Fulton's condition index
 393 (D) for signal crayfish (*Pacifastacus leniusculus*) in the core and front of the invasion gradient. Boxplots
 394 show median values (central line), the range from the 25th to 75th percentile (box), and the largest and
 395 lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers),
 396 and dots represent extreme values.

397

398 Behavioral tests (Figure 3) reveal the dominance of the front group concerning
 399 exploratory behavior and aggressiveness. Regarding the food risk-taking test (Figure 3A),
 400 significant differences between groups were detected, with individuals from the front

401 exiting the shelter significantly faster ($t = 3.29$; $df = 25.64$; $p\text{-value} = 0.003$). Conversely,
402 no significant difference was observed between males and females ($t = -0.54$; $df = 26.75$;
403 $p\text{-value} = 0.59$). Concerning the exploration behavior (Figure 3B), no significant
404 differences between groups were detected for the time individuals take to exit the shelter
405 ($t = 1.58$; $df = 27.87$; $p\text{-value} = 0.13$). However, significant differences were observed
406 between sexes, with males exiting the shelter faster than females ($t = 3.19$; $df = 27.94$; $p\text{-}$
407 $\text{value} < 0.01$). A significant difference was noted between Front Female and Core Male
408 (chi-square test, $p\text{-value} = 0.01$), highlighting notable variance in competitive behavior
409 concerning resource acquisition (Figure 3C). Significant differences were also observed
410 in the outcomes of combats between Front Male and Core Male (chi-square test, $p\text{-value}$
411 < 0.01), as well as Front Female and Core Female (chi-square test, $p\text{-value} = 0.03$),
412 indicating spatial dominance by the individuals from the front of the invasion gradient
413 (Figure 3 D).

414

415

416

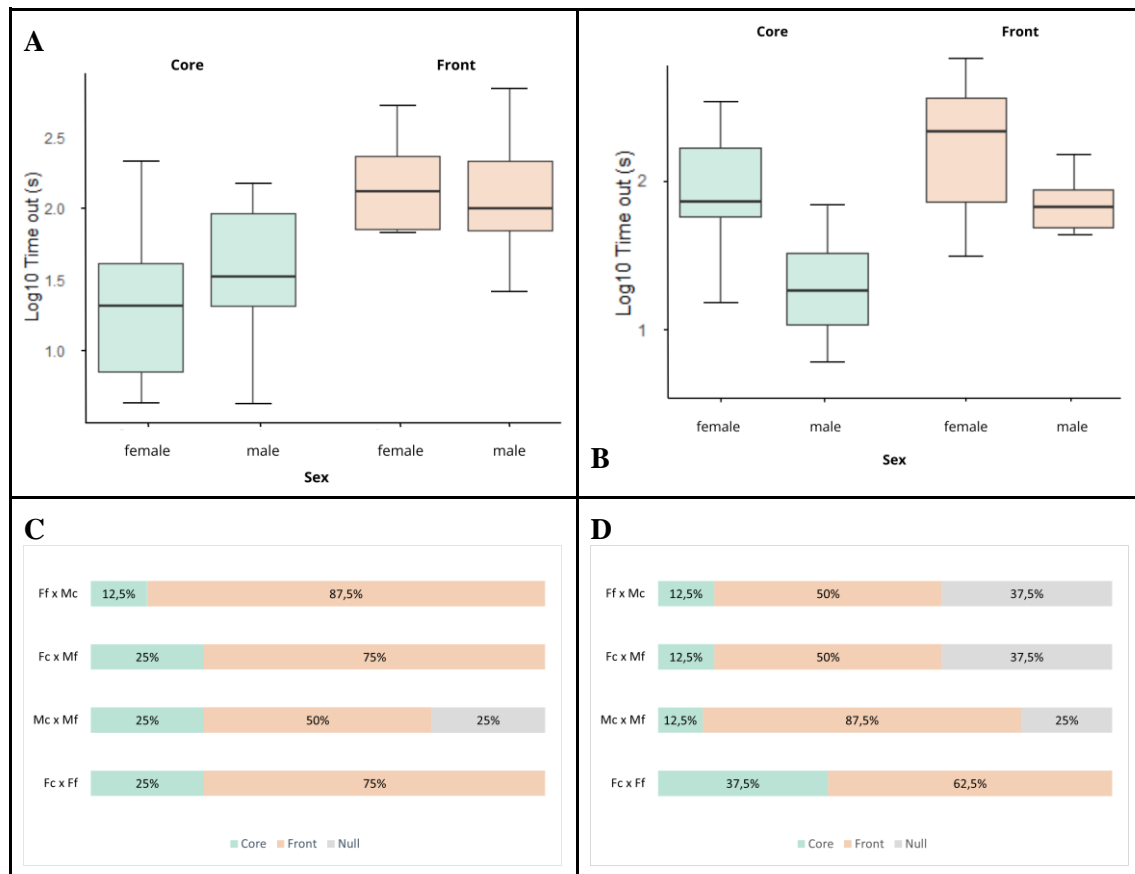
417

418

419

420

421



422

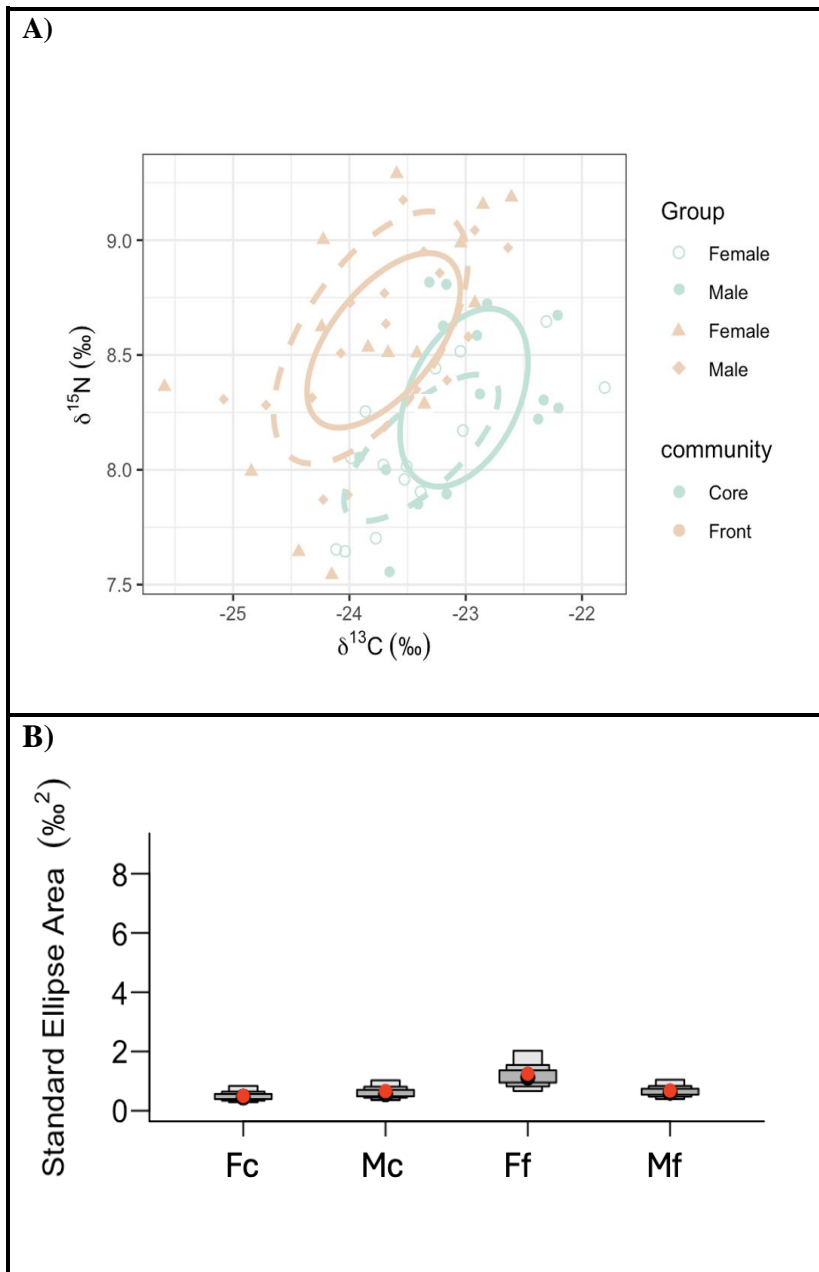
423 **Figure 3** Behavioral tests among groups (core and front) of signal crayfish (*Pacifastacus leniusculus*): A)
 424 Risk-taking with food; B) Exploration behavior; C) First to reach food; and D) Competition for space.
 425 Abbreviations: Ff (Female Front), Mf (Male Front), Fc (Female Core), Mc (Male Core).

426

427 For the stable isotope values, the ANOVA test results indicate a significant difference
 428 between the core and front groups ($F_{(1, 58)} = 11.27, p = 0.001$). The Tukey HSD posthoc
 429 test further confirms this, showing that the mean value for the core is significantly lower
 430 than that for the front group (95% CI: -0.58 to -0.15, $p = 0.001$). The SIBER results
 431 highlight a separation between the isotopic niches at the invasion core and front (Figure
 432 4A). The isotopic niche overlap between males from the core and the front was notably
 433 small at $0.03\%^{02}$, equating to 2% of the area. For females, the overlap was even less, at
 434 $0.005\%^{02}$ (0.3% of the area). When considering overlaps within the same group, at the
 435 front, the overlap was significant for both carbon and nitrogen isotopes, at $0.68\%^{02}$,
 436 making up 54% of the cumulative area, while at the core, a moderate male-female overlap
 437 was $0.30\%^{02}$, or 36% of the area. The areas of the standard ellipses, which correspond to

438 each group, varied, with ranges from 1.25‰² to 0.68‰² for females and males at the
 439 front, respectively, and from 0.50‰² to 0.66‰² for females and males at the core (Figure
 440 4B).

441



442

443 **Figure 4** Isotopic niche (A) widths for adult signal crayfish muscle tissue from the core (blue) and front
 444 (red) individuals, with their sample-size corrected standard ellipses (SEAc). Standard ellipse areas (SEAb)
 445 (B) for the core: female (Fc) and male (Mc); and front: female (Ff) and male (Mf). The boxes represent the
 446 95, 75, and 50% credible intervals, with the mode indicated by the black circles. The maximum likelihood
 447 estimate for the corresponding SEAc is indicated by the red circle.

448 *Ecological impacts at the intrapopulation level*

449 Any gastropod was found dead in the control treatment (data not shown). When analysing
450 *P. leniusculus* gastropod consumption, it was verified that there is a significant interaction
451 between abundance level and the type of treatment ($F=8.5143$, $p<0.001$), which means
452 that their effects are not independent. Because of this, the simple main effects of
453 treatments at each abundance level were scrutinized. In mesocosms with 2, 4, 8, 16, and
454 32 *P. acuta* individuals, practically all of them were consumed by the signal crayfish from
455 both the core and front of the invasion (Figure 5A). Statistical analysis shows that there
456 are no significant differences between core and front for these levels of abundance (Table
457 S2). However, for abundance levels of 64 and 128, there was a significant difference
458 between the core and the front of the invasion ($\chi^2=18.1458$, $p<0.001$; $\chi^2=52.7391$,
459 $p<0.001$, respectively) (Table S2). For these two levels, signal crayfish individuals from
460 the front consumed more gastropods than signal crayfish individuals from the core
461 (Figure 3A).

462 Significant differences in the percentage of leaf mass loss were also detected (One-way
463 ANOVA, $F=70.3$, $p<0.001$), presenting the front treatment with the highest values,
464 followed by the core treatment (Figure 5B). Both front and core were significantly
465 different from the control treatment (Tukey *post hoc* test, $p<0.001$ and $p=0.00157$,
466 respectively). Tukey *post hoc* test also showed that there is a significant difference in the
467 percentage of leaf mass loss between the front and the core treatments ($p<0.001$).

468

469

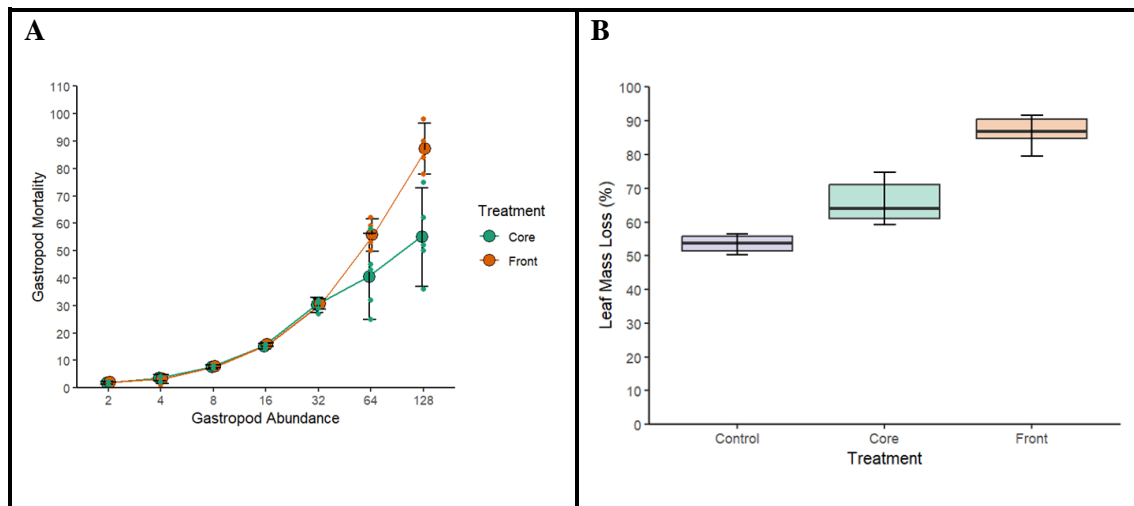
470

471

472

473

474



475

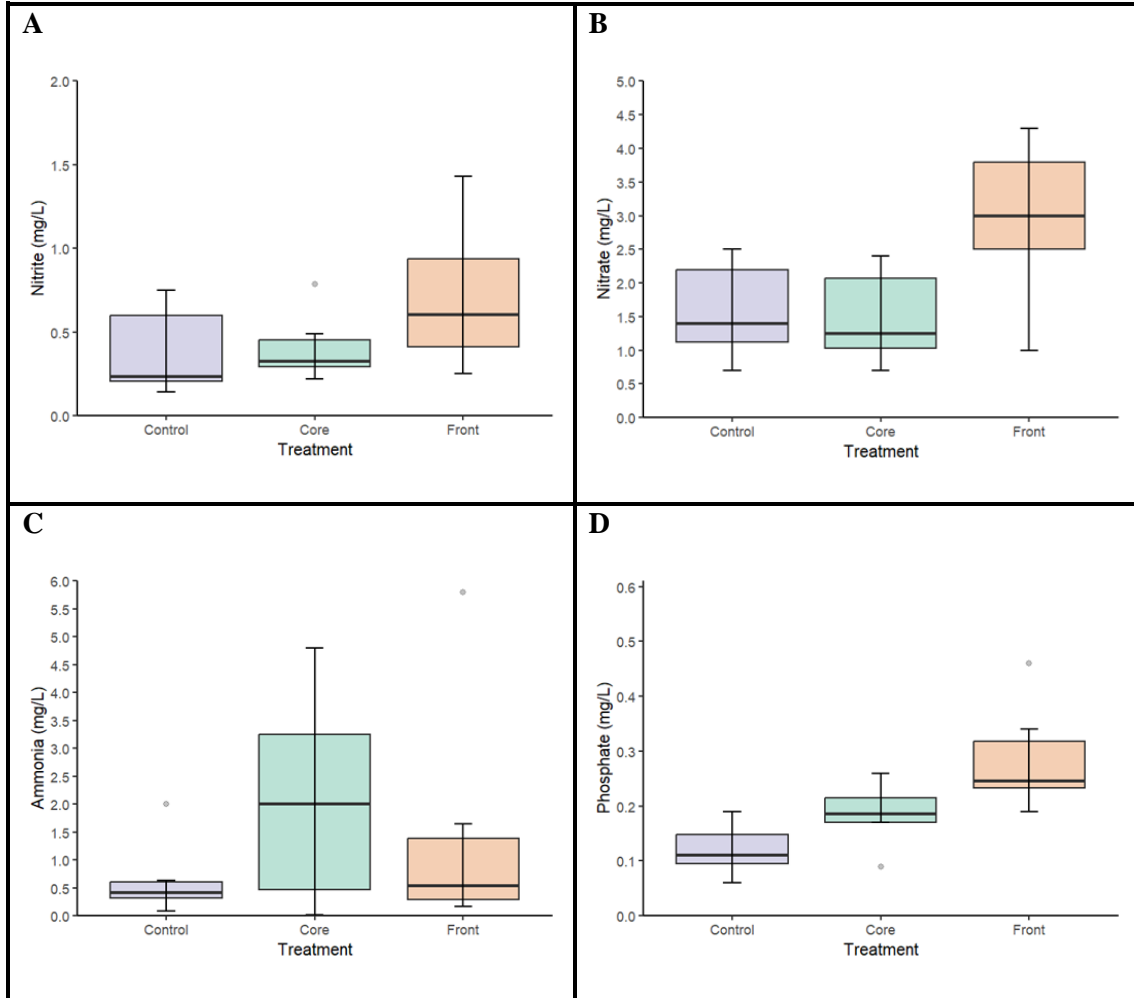
476 **Figure 5** Number of gastropods consumed by the signal crayfish (*Pacifastacus leniusculus*) in each
 477 abundance level for core and front treatments (A) and leaf mass loss (%) for control, core and front
 478 treatments (B). In A the largest dots represent the mean deaths in each abundance level for core (in red) and
 479 front (in blue) treatments. Smaller red dots represent the number of consumed gastropods at core treatment
 480 in each abundance level replicates and smaller blue dots represent the number of consumed gastropods at
 481 front treatment in each abundance level replicates. In B, boxplots show median values (central line), the
 482 range from the 25th to 75th percentile (box), and the largest and lowest value within 1.5 times interquartile
 483 range below and above the 25th and 75th percentile (whiskers).

484

485 No significant differences were found between the three treatments considering the nitrite
 486 concentrations (One-way ANOVA, $p > 0.05$) (Table S3), although the front treatment
 487 presented higher values (Figure 6A). Significant differences were detected for nitrate
 488 between the three treatments (One-way ANOVA, $p = 0.0228$) (Table S3), with the front
 489 presenting higher values (Figure 6B). These significant differences were detected
 490 between front and control treatments (Tukey *post hoc* test, $p = 0.0482$) (Table S4) and
 491 between core and front treatments (Tukey *post hoc* test, $p = 0.0336$) (Table S4). Ammonia
 492 concentration showed no significant differences between treatments (One-way ANOVA,
 493 $p > 0.05$) (Table S3; Figure 6C). Significant differences between treatments were detected
 494 for phosphate (One-way ANOVA, $p = 0.0041$) (Table S3), with higher values in the front
 495 treatment (Figure 6D). The comparison between front and control treatments revealed
 496 these differences (Tukey *post hoc* test, $p = 0.00222$) (Table S4), and an almost significant

497 difference was noted between the core and front treatments (Tukey *post hoc* test,
 498 $p=0.06938$) (Table S4).

499



500

501 **Figure 6** Concentration (mg/L) of nitrite (A), nitrate (B), ammonia (C) and phosphate (D) in control, core
 502 and front treatments. Boxplots show median values (central line), the range from the 25th to 75th percentile
 503 (box), and the largest and lowest value within 1.5 times interquartile range below and above the 25th and
 504 75th percentile (whiskers), and dots represent extreme values.

505

506 **Discussion**

507 Natural populations consist of phenotypically diverse individuals who exhibit variation
 508 in their biological traits (Bolnick et al. 2011). However, these differences are usually
 509 neglected in biological invasion studies and most work is devoted to assess ecological

510 impacts at the species level (Haubrock et al. 2024). In this study, we highlight that several
511 biological traits and ecological impacts may vary along an invasion gradient with clear
512 differences established at the intrapopulation level. In the particular case of the signal
513 crayfish in the Rabaçal River, we clearly establish that abundance was higher in the core;
514 larger individuals and a higher physiological condition were detected in the front of the
515 invasion and females were dominant in the core but males were dominant in the front.
516 Regarding behaviour, we demonstrated that the animals in the front are more aggressive,
517 bold, and active. In the same vein, individuals from the core and front present a clear
518 distinct trophic niche, with individuals from the front presenting higher nitrogen values.
519 These differences in biological traits are consistent with distinct ecological impacts, with
520 higher consumption of prey, leaf mass loss, and excretion of nutrients in individuals from
521 the front of the invasion gradient.

522

523 *Biological traits at the intra-population level*

524 Biological traits were distinct along the invasion gradient. Abundance was lower in the
525 front of the invasion compared to the core and signal crayfish individuals were larger at
526 the front of the invasion. On a simplistic level, it is obvious that in the newly invaded
527 sites, there will be lower abundance, given that signal crayfish individuals are still arriving
528 at the front of the invasion. Similar differences in abundance were also reported in many
529 other animal invasions (see Wolf and Weissing 2012; Raffard et al. 2022). Differences in
530 size could be explained by the fact that larger individuals can move faster and thus, are
531 the ones who lead the invasion. Similar findings regarding the influence of size in
532 dispersal were recorded in a study with cane toads, *Bufo marinus*, in Australia, where
533 toads with longer legs were the faster ones and the first to reach new sites and so doing
534 lead the invasion front (Phillips et al. 2006). Another factor possibly contributing to the
535 differences in size between the front and the core is the possible low level of recruitment
536 in the front of the invasion given the low abundance of crayfish in this site. This may also
537 contribute to a higher average size in the front.

538 Differences in sex ratio were also detected between the core and front of the invasion. At
539 the core, the percentage of females was significantly higher than males but at the front,
540 the percentage of males was higher than females (although not significant). Higher male
541 percentage in the front of the invasion were also reported for round goby (*Neogobius*
542 *melanostomus*) in Canada (Gutowski and Fox 2011), or for other signal crayfish
543 populations in Europe (Capurro et al. 2007, Wutz and Geist 2013), and those results,
544 according to the authors, were related to recent, not fully established invasive populations.
545 The sex ratio is important since it may interfere with some ecological roles. For example,
546 female-biased populations of western mosquitofish *Gambusia affinis*, are able to induce
547 stronger pelagic trophic cascades compared with male-biased populations, causing larger
548 impacts on communities and ecosystems (Fryxell et al. 2015). Other studies with the
549 invasive freshwater crayfish *Procambarus clarkii* also showed that space is used
550 differently by both sexes being females more nomads (Barbaresi et al. 2004). The higher
551 percentage of females in the core of the invasion might also be related to a higher number
552 of encounters and fights among males as a result of higher crayfish abundance and a
553 consequent decrease in available space and resources (Sousa et al. 2013). This might
554 result in the death of some male individuals or the exclusion of the weakest in sites with
555 higher abundance. In alternative, it can be related to bolder male crayfish individuals that
556 will be more prone to explore downstream areas (Raffard et al. 2022).

557 It was also possible to observe that signal crayfish individuals in the front of the invasion
558 had a higher physiological condition when compared with the individuals in the core.
559 This may be related to the fact that there may be less competition for resources at the
560 front, which allows these individuals to have easier access to food (see below further
561 discussion on trophic niche) and refuges and in doing so they increase their fitness.
562 Although not studied, another explanation for these results may be related to a lower level
563 of parasitism in the front when compared to the core. Studies with cane toads in Australia
564 showed that pathogens and parasites lag about 2 years behind the front of toad invasion,
565 due to stochastic events that lead to local extinctions or transmission failure of the
566 pathogens/parasites in the front of the invasion (Phillips et al. 2010; Brown et al. 2013).
567 However, this last aspect was not assessed and future studies should explore this topic.

568 Several organisms rapidly change their behavioural traits to expand their distribution area
569 and be able to make decisions that involve risks (Yagound et al. 2022) due to the new
570 challenges imposed by the environment and the pressures arising from their expansion

571 (Biro and Stamps 2008). What we observe in this study is a behaviour change in
572 geographical terms, with the animals at the front being more exploratory and willing to
573 take risks than those at the core of the invasion (Gruber et al. 2018). The results of the
574 behavioural tests showed that the individuals at the front of the invasion are more
575 aggressive, dominate for space, and reach food more quickly. In earlier studies by Groen
576 et al. (2012) and Myles-Gonzalez et al. (2015), authors observed that the individuals at
577 the front took more risks and moved faster, as occurred in our trials of risk-taking for
578 food, with individuals from the front first reaching the food and winning for space.
579 Several studies have also concluded that individuals at the front of the invasion tend to
580 be more exploratory than those at the core (Liebl and Martin 2012 and 2014; Atwell et al.
581 2012), but our results were different. We observed that for *Pacifastacus leniusculus*,
582 females are more exploratory but there were no significant differences between
583 individuals at the front and core of the invasion. This divergence between studies may
584 have been due to the dominance of females over males, as has already been documented
585 for this species (Peeke et al. 1995). For example, in the study by Atkinson et al. (2023),
586 they concluded that sex has a strong effect on exploration behaviour.

587 In addition to differences in several biological traits as discussed above, we also observed
588 higher nitrogen values ($\delta^{15}\text{N}$) in signal crayfish individuals from the front compared to
589 individuals from the core. Earlier results from Olsson et al. (2009) reveal that signal
590 crayfish exhibit a significantly broader niche width compared to native noble crayfish
591 (*Astacus astacus*), demonstrating greater plasticity in habitat use and feeding strategies,
592 which could explain their successful invasion and the observed differences in trophic
593 levels and aggressive behavior. These findings contrast with other studies on invasive
594 crayfish species, such as the rusty crayfish (*Faxonius rusticus*), where individuals at the
595 invasion front displayed less competitive morphologies and occupied lower trophic levels
596 (Messenger and Olden 2019). The increased aggression and higher nitrogen levels
597 observed in signal crayfish at the front could suggest a different adaptive strategy
598 compared to rusty crayfish. In addition, the elevated nitrogen values observed in signal
599 crayfish at the invasion front suggest a dietary shift towards a higher reliance on higher
600 trophic-level prey. This shift in diet is likely an adaptive response to the specific
601 ecological conditions present at the invasion front, where competition for resources is
602 presumably less intense compared to the core areas, where crayfish abundance is
603 significantly higher (see also Hudina et al. 2017). Although direct sources of dietary

604 information were not collected in this study, it is plausible that crayfish in core areas
605 consume more submerged vegetation and detritus. Notably, female crayfish in the core
606 areas exhibited a smaller niche area, suggesting that the heightened competition for
607 limited food resources compels them to consume a lower range of dietary items at lower
608 trophic levels. In contrast, crayfish at the invasion front may have greater access to
609 invertebrates and other higher trophic-level prey, which are readily available in the initial
610 phase following their arrival. The relatively low abundance of crayfish at the invasion
611 front likely reduces competition, facilitating this opportunistic feeding behavior (Bubb et
612 al. 2004; Greenhalgh et al. 2022). Increased aggression among crayfish at the invasion
613 front could further enhance their ability to exploit these available resources, thereby
614 providing a competitive advantage in newly invaded territories.

615

616 *Ecological impacts at the intrapopulation level*

617 In addition to biological traits, this study highlighted that the ecological impacts may vary
618 along the invasion gradient with clear differences established at the intrapopulation level.
619 In the particular case of the signal crayfish in the Rabaçal River, the consumption of
620 gastropods, leaf mass loss, and the concentration of nitrates and phosphates were
621 significantly higher at the front of the invasion.

622 Dispersal is one fundamental ecological process where these intraspecific variations,
623 particularly the personality traits (e.g. activity, boldness, aggression, and exploration)
624 may play a crucial role (Daniels and Kemp 2022). Personality traits are often correlated
625 with dispersal and this correlation is also known as a dispersal syndrome (Biro and
626 Stamps 2008; Galib et al. 2022; Raffard et al. 2022). These dispersal syndromes have
627 been reported in a wide range of taxa such as great tits, *Parus major* (Dingemanse et al.
628 2003); common lizards, *Lacerta vivipara* (Cote and Clobert 2007); North American red
629 squirrels, *Tamiasciurus hudsonicus* (Cooper et al. 2017); mud crabs, *Panopeus herbstii*
630 (Belgrad and Griffen 2018), among others. Activity, boldness, and exploration traits are
631 often linked with better fitness, which makes that individuals who have these enhanced
632 traits are expected to disperse further (Juetter et al. 2014; Galib et al. 2022), and so doing
633 they lead the invasion front. Some of these personality traits have previously been
634 observed in the signal crayfish (Galib et al. 2022), and our results (see above) showed
635 that signal crayfish individuals in the front of the invasion were more active, aggressive
636 and bolder than individuals in the core.

637 Since individuals with this type of behaviour are more prone to disperse, this movement
638 can be associated with higher metabolic rates. To counterbalance energy expenditure,
639 metabolism can induce higher consumption rates (Raffard et al. 2022). This could be the
640 main reason explaining our results regarding gastropod consumption, where we see no
641 differences between core and front individuals in the low abundance treatments but in the
642 high abundance treatments, we observe a much higher consumption in the crayfish from
643 the front. This situation may demonstrate a higher voracity of crayfish from the front as
644 a result of their higher metabolism (Raffard et al. 2022).

645 This study also made it possible to verify that the signal crayfish interferes in key
646 ecosystem processes, as is the case of leaf litter processing and nutrient cycling. Here, it
647 was possible to observe that signal crayfish had an impressive effect in leaf litter
648 processing. Similar results have been reported for other invasive species, for example, the
649 red swamp crayfish, *Procambarus clarkii* (Carvalho et al. 2016 and 2018). Since leaf
650 litter processing is driven by a group of animals that control the flux of carbon and energy
651 in aquatic food webs (Carvalho et al. 2018), fundamental processes like nutrient cycling
652 and organic matter turnover (Jackson et al. 2014), could be affected by the invasion of
653 signal crayfish, even at the intrapopulation level. In addition, higher nutrient
654 concentrations were also found at the front corroborating an earlier study by Villéger et
655 al. (2012) where *Salmo trutta* individuals that were capable of dispersing further were the
656 ones that displayed a higher excretion rate of nitrogen and phosphorus.

657 Overall, we found a higher consumption of gastropods and leaf litter, and a higher
658 concentration of nutrients, namely nitrates and phosphates, in the front of the invasion,
659 where the bigger and most aggressive, active, and bolder individuals, supposedly with
660 higher metabolic rates, were present. Therefore, these intrapopulation interactions can
661 cascade to the population and community levels due to a different consumption of certain
662 preys or leaf litter and being also responsible for nutrient-mediated effects, modifying
663 nutrient influx at the ecosystem level. However, and as clearly demonstrated here,
664 although the per capita consumption of prey and excretion rates were significantly
665 different between the core and front of the invasion it should be noted that the abundance
666 in the two sites are quite distinct, being much higher in the core.

667 Results were clear demonstrating significant differences at the intrapopulation level but
668 some questions still remain unanswered and open the door for future studies. For example,
669 it would be interesting to assess the metabolic rates in individuals from the core and front

670 of the invasion as the possible key mechanism explaining the different consumption rates
671 and nutrient concentrations reported here. In the same vein, biological traits can also be
672 related to the expression of specific genes (Yagound et al. 2022) and future studies should
673 include transcriptomics in order to understand if genes related to phenotypic traits like
674 aggressiveness and boldness are being expressed in different ways in individuals in the
675 core and front of the invasion gradient. Finally, and because animals in the front of the
676 invasion may left behind their enemies, it would be interesting to assess the level of
677 parasitism in the core and front of the invasion gradient and their possible contribution to
678 the distinct ecological impacts (i.e., animals in the front consume more and excrete more
679 given their lower parasite load).

680

681 **Conclusion**

682 In this study, we clearly showed distinct biological traits and ecological impacts along an
683 invasion gradient of one of the most introduced freshwater organisms in Europe, the
684 signal crayfish, *Pacifastacus leniusculus*. These findings are in agreement with recent
685 studies showing substantial variability in dispersal and abundance trends in freshwater
686 invasive macroinvertebrates, indicating that levels of invasiveness and impacts may differ
687 markedly between populations (Haubrock et al. 2024). Our study, even introduced
688 another layer of variability, since we were able to demonstrate clear differences at the
689 intrapopulation level. Therefore, and recognizing that individual variation has important
690 ecological and evolutionary consequences, the assessment of biological traits at the
691 intrapopulation level may help better predict the success of dispersal and the ecological
692 impacts generated by invasive species, with eventual pay-offs in the implementation of
693 meaningful management strategies. Currently, theoretical and empirical studies in
694 biological invasions focus mainly on the species level (e.g. compilation of black lists),
695 especially those that have high ecological and economic impacts. However, as shown
696 here, biological traits and ecological impacts can be very different at the intrapopulation
697 level and for this reason, researchers and stakeholders should not perpetuate the
698 misconception that all individuals behave in a consistent and uniform manner along an
699 invasion gradient.

700

701 **Acknowledgments:** This study was supported by the Portuguese Foundation for Science and
702 Technology (FCT) through national funds under the project MULTI-CRASH: Multi-dimensional
703 ecological cascades triggered by an invasive species in pristine habitats (PTDC/CTA-
704 AMB/0510/2021) (<https://doi.org/10.54499/PTDC/CTA-AMB/0510/2021>) and the project
705 Scientific Employment Stimulus - 6th Edition (2023.07078.CEECIND).

706

707 **References**

708 Anastácio PM, Ribeiro F, Capinha C, Banha F, Gama M, Filipe AF, Rebelo R, Sousa R (2019) Non-native
709 freshwater fauna in Portugal: a review. *Science of the Total Environment*, 650: 1923 - 1934.

710

711 Atkinson S, Bryan E, Kruse J, Sarachick N, Mathews L (2023) Sex and reproductive status affect sheltering
712 and exploratory behaviours with high intra-individual variability in crayfish. *Behaviour* 160: 299-321.

713

714 Atwell JW, Cardoso GC, Whittaker DJ, Campbell-Nelson S, Robertson, KW, Ketterson ED (2012)
715 Boldness behavior and stress physiology in a novel urban environment suggest rapid correlated
716 evolutionary adaptation. *Behavioral Ecology* 23: 960-969.

717

718 Barbaresi S, Santini G, Tricarico E, Gherardi (2004) Ranging behaviour of the invasive crayfish,
719 *Procambarus clarkii* (Girard). *Journal of Natural History* 38: 2821-2832.

720

721 Belgrad BA, Griffen BD (2018) Personality interacts with habitat quality to govern individual mortality
722 and dispersal patterns. *Ecology and Evolution* 8: 7216-7227.

723

724 Bernardo JM, Costa AM, Bruxelles S, Teixeira A (2011) Dispersion et coexistence de deux écrevisses non-
725 natives (*Pacifastacus leniusculus* et *Procambarus clarkii*) au NE du Portugal sur une période de 10 ans.
726 *Knowledge and Management of Aquatic Ecosystems* 401: 1-13.

727

- 728 Biro PA, Stamps JA (2008) Are animal personality traits linked to life-history productivity?. Trends in
729 Ecology & Evolution 23: 361-368.
- 730
- 731 Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, ..., Richardson DM (2011) A
732 proposed unified framework for biological invasions. Trends in Ecology & Evolution 26: 333-339.
- 733
- 734 Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, ... Vasseur DA (2011) Why
735 intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution 26: 183-192.
- 736
- 737 Britton JR, Berry M, Sewell S, Lees C, Reading P (2017). Importance of small fishes and invasive crayfish
738 in otter *Lutra lutra* diet in an English chalk stream. Knowledge and Management of Aquatic Ecosystems
739 418: 13.
- 740
- 741 Brown GP, Kelehear C, Shine R (2013) The early toad gets the worm: cane toads at an invasion front
742 benefit from higher prey availability. Journal of Animal Ecology 82: 854-862.
- 743
- 744 Bubb DH, Thom TJ, Lucas MC (2004) Movement and dispersal of the invasive signal crayfish *Pacifastacus*
745 *leniusculus* in upland rivers. Freshwater Biology 49: 357-368.
- 746
- 747 Capurro M, Galli L, Mori M, Salvidio S, Arillo A (2007) The signal crayfish, *Pacifastacus leniusculus*
748 (Dana, 1852) [*Crustacea: Decapoda: Astacidae*], in the Brugnato Lake (Liguria, NW Italy). The beginning
749 of the invasion of the River Po watershed? Aquatic Invasions 2: 17-24.
- 750
- 751 Carvalho F, Pascoal C, Cássio F, Sousa R (2016) Direct and indirect effects of an invasive omnivore
752 crayfish on leaf litter decomposition. Science of the Total Environment 541: 714-720.
- 753
- 754 Carvalho F, Pascoal C, Cássio F, Sousa R (2018) Effects of intrapopulation phenotypic traits of invasive
755 crayfish on leaf litter processing. Hydrobiologia 819: 67-75.
- 756
- 757 Carvalho F, Sousa R, Cássio F, Pascoal C (2022) Temperature and interspecific competition alter the
758 impacts of two invasive crayfish species on a key ecosystem process. Biological Invasions 24: 3757-3768.
- 759

- 760 Carvalho F, Alves H, Pascoal C, Castro P, Miranda F, Teixeira A, Cássio F, Sousa R (accepted) Invasive
761 dynamics of the signal crayfish *Pacifastacus leniusculus* in a protected area. *Hydrobiologia*.
762
- 763 Chapple DG, Simmonds SM, Wong BB (2012) Can behavioral and personality traits influence the success
764 of unintentional species introductions?. *Trends in Ecology & Evolution* 27: 57-64.
765
- 766 Cooper EB, Taylor RW, Kelley AD, Martinig AR, Boutin S, Humphries MM, ... McAdam AG (2017)
767 Personality is correlated with natal dispersal in North American red squirrels (*Tamiasciurus hudsonicus*).
768 *Behaviour* 154: 939-961.
769
- 770 Cote J, Clobert J (2007) Social personalities influence natal dispersal in a lizard. *Proceedings of the Royal*
771 *Society of London B: Biological Sciences* 274: 383–390
772
- 773 Cote J, Clobert J, Brodin T, Fogarty S, Sih A (2010) Personality-dependent dispersal: characterization,
774 ontogeny and consequences for spatially structured populations. *Philosophical Transactions of the Royal*
775 *Society B: Biological Sciences* 365: 4065-4076.
776
- 777 Daniels JA, Kemp PS (2022) Personality-dependent passage behaviour of an aquatic invasive species at a
778 barrier to dispersal. *Animal Behaviour* 192: 63-94.
779
- 780 Des Roches S, Post DM, Turley NE, Bailey JK, Hendry AP, Kinnison MT, ... Palkovacs EP (2018) The
781 ecological importance of intraspecific variation. *Nature Ecology & Evolution* 2: 57-64.
782
- 783 Diagne C, Leroy B, Vaissière AC, Gozlan RE, Roiz D, Jarić I, ... Courchamp F (2021) High and rising
784 economic costs of biological invasions worldwide. *Nature* 592: 571-576.
785
- 786 Dingemanse NJ, Both , Van Noordwijk AJ, Rutten AL, Drent PJ (2003) Natal dispersal and personalities
787 in great tits (*Parus major*). *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:
788 741-747.
789
- 790 Dirzo R, Young HS, Galett M, Ceballos G, Isaac NJ, Collen B (2014) Defaunation in the
791 Anthropocene. *Science* 345: 401-406.

792

793 Dudgeon D (2019) Multiple threats imperil freshwater biodiversity in the Anthropocene. *Current*
794 *Biology* 29: R960-R967.

795

796 Dunn JC (2012) *Pacifastacus leniusculus* Dana (North American signal crayfish). In *A handbook of global*
797 *freshwater invasive species* (pp. 205-215). Routledge.

798

799 Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. *Annual Review of Ecology,*
800 *Evolution, and Systematics* 41: 59-80.

801

802 Fryxell DC, Arnett HA, Apgar T, Kinnison MT, Palkovacs EP (2015). Sex ratio variation shapes the
803 ecological effects of a globally introduced freshwater fish. *Proceedings of the Royal Society B: Biological*
804 *Sciences* 282: 20151970.

805

806 Galib SM, Sun J, Twiss SD, Lucas MC (2022) Personality, density and habitat drive the dispersal of
807 invasive crayfish. *Scientific Reports* 12 : 1114.

808

809 Gallardo B, Clavero M, Sánchez MI, Vilà M (2016) Global ecological impacts of invasive species in aquatic
810 ecosystems. *Global Change Biology* 22: 151-163.

811

812 Gallardo B, Bacher S, Barbosa AM, Gallien L, González-Moreno P, Martínez-Bolea V, ... Vilà M (2024)
813 Risks posed by invasive species to the provision of ecosystem services in Europe. *Nature*
814 *Communications* 15: 2631.

815

816 Grasshoff K, Kremling K, Erhardt M (1999) *Methods of Seawater Analysis* 3rd ed. Wiley-VCH.

817

818 Greenhalgh JA, Collins RA, Edgley DE, Genner MJ, Hindle J, Jones G, ... Battarbee RW (2022)
819 Environmental DNA-based methods detect the invasion front of an advancing signal crayfish population.
820 *Environmental DNA* 4: 596-607.

821

822 Groen M, Sopinka NM, Marentette JR, Reddon AR, Brownscombe JW, Fox MG, ... Balshine S (2012) Is
823 there a role for aggression in round goby invasion fronts?. Behaviour 149: 685-703.

824

825 Gruber J, Brown G, Whiting MJ, Shine R (2018) Behavioural divergence during biological invasions: a
826 study of cane toads (*Rhinella marina*) from contrasting environments in Hawai'i. Royal Society Open
827 Science 5: 180197.

828

829 Guan RZ, Wiles PR (1998) Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British
830 lowland river. Aquaculture 169: 177-193.

831

832 Gutowsky LFG, Fox MG (2011) Occupation, body size and sex ratio of round goby (*Neogobius*
833 *melanostomus*) in established and newly invaded areas of an Ontario river. Hydrobiologia 671: 27-37.

834

835 Haubrock PJ, Soto I, Ahmed DA,... Cuthbert (2024) Biological invasions are a population-level rather
836 than a species-level phenomenon. Global Change Biology 30: e17312.

837

838 Henttonen P, Huner JV (2017) The introduction of alien species of crayfish in Europe: A historical
839 introduction. In *Crayfish in Europe as Alien Species: How to make the best of a bad situation?*, 13-22.

840

841 Hudina S, Kutleša P, Trgovčić K, Duplić A (2017). Dynamics of range expansion of the signal crayfish
842 (*Pacifastacus leniusculus*) in a recently invaded region in Croatia. Aquatic Invasions 12: 67-75.

843

844 Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within
845 communities: SIBER—Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80: 595-602.

846

847 Jackson MC, Jones T, Milligan M, Sheath D, Taylor J, Ellis A, England J, Grey J (2014) Niche
848 differentiation among invasive crayfish and their impacts on ecosystem structure and functioning.
849 Freshwater Biology 59: 1123-1135.

850

851 Juette T, Cucherousset J, Cote J (2014) Animal personality and the ecological impacts of freshwater non-
852 native species. Current Zoology 60: 417-427.

853

854 Liebl AL, Martin LB (2012) Exploratory behaviour and stressor hyper-responsiveness facilitate range
855 expansion of an introduced songbird. *Proceedings of the Royal Society B: Biological Sciences* 279: 4375-
856 4381.

857

858 Liebl AL, Martin LB (2014) Living on the edge: range edge birds consume novel foods sooner than
859 established ones. *Behavioral Ecology* 25: 1089-1096.

860

861 Lockwood JL, Hoopes MF, Marchetti MP (2013) *Invasion ecology*. John Wiley & Sons.

862

863 Lopes-Lima M, Reis J, Alvarez MG, Anastácio PM, Banha F, Beja P, Castro P, Gama M, Gil MG, Gomes-
864 dos-Santos A, Miranda F, Nogueira JG, Sousa R, Teixeira A, Varandas S, Froufe E (2023) The silent
865 extinction of freshwater mussels in Portugal. *Biological Conservation* 285: 110244.

866

867 Mathers KL, Chadd RP, Dunbar MJ, Extence CA, Reeds J, Rice SP, Wood PJ (2016) The long-term effects
868 of invasive signal crayfish (*Pacifastacus leniusculus*) on instream macroinvertebrate communities. *Science*
869 *of the Total Environment* 556: 207-218.

870

871 Meira A, Lopes-Lima M, Varandas S, Teixeira A, Arenas F, Sousa R (2019) Invasive crayfishes as a threat
872 to freshwater bivalves: interspecific differences and conservation implications. *Science of the Total*
873 *Environment* 649: 938 - 948.

874

875 Messenger ML, Olden JD (2019) Phenotypic variability of rusty crayfish (*Faxonius rusticus*) at the leading
876 edge of its riverine invasion. *Freshwater Biology* 64: 1196-1209.

877

878 Myles-Gonzalez E, Burness G, Yavno S, Rooke A, Fox MG (2015) To boldly go where no goby has gone
879 before: boldness, dispersal tendency, and metabolism at the invasion front. *Behavioral Ecology* 26: 1083-
880 1090.

881

882 Nogueira JG, Teixeira A, Lopes-Lima M, Varandas S, Sousa R (2021a) Assessment of terrestrial protected
883 areas for the conservation of freshwater biodiversity. *Aquatic Conservation: Marine and Freshwater*
884 *Ecosystems* 31: 520-530.

885

886 Nogueira JG, Sousa R, Benaissa H, De Knijf G, Ferreira S, Ghamizi M, Gonçalves D, Lansdown R, Prié
887 V, Riccardi N, Seddon M, Urbańska M, Vikhrev I, Varandas S, Teixeira A, Lopes-Lima M (2021b)
888 Alarming decline of freshwater trigger species in western Mediterranean Key Biodiversity Areas.
889 Conservation Biology 35: 1367-1379.

890

891 Oliveira JM, Segurado P, Santos JM, Teixeira A, Ferreira MT, Cortes RV (2012) Modelling Stream-Fish
892 Functional Traits in Reference Conditions: Regional and Local Environmental Correlates. PLoS ONE 7:
893 e45787.

894

895 Olsson K, Stenroth P, Nyström PER, Granéli W (2009) Invasions and niche width: does niche width of an
896 introduced crayfish differ from a native crayfish?. Freshwater Biology 54: 1731-1740.

897

898 Peeke HV, Sippel J, Figler MH (1995) Prior residence effects in shelter defense in adult signal crayfish
899 (*Pacifastacus leniusculus* (Dana)): results in same-and mixed-sex dyads. Crustaceana 68: 873-881.

900

901 Phillips BL, Brown GP, Webb JK, Shin R (2006) Invasion and the evolution of speed in toads. Nature 439:
902 803-803.

903

904 Phillips BL, Kelehear C, Pizzatto L, Brown GP, Barton D, Shine R (2010) Parasites and pathogens lag
905 behind their host during periods of host range advance. Ecology 91: 872-881.

906

907 Post DM, Palkovacs EP, Schielke EG, Dodson SI (2008) Intraspecific variation in a predator affects
908 community structure and cascading trophic interactions. Ecology 89: 2019-2032.

909

910 Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, ... Richardson DM (2020)
911 Scientists' warning on invasive alien species. Biological Reviews 95: 1511-1534.

912

913 R Studio Team (2022) R: A Language and Environment for Statistical Computing R Foundation for
914 Statistical Computing, Vienna, Austria; URL: <https://www.R-project.org/>

915

- 916 Raffard A, Santoul F, Cucherousset J, Blanchet S (2019) The community and ecosystem consequences of
917 intraspecific diversity: A meta-analysis. *Biological Reviews* 94: 648–661.
- 918
- 919 Raven PJ, Holmes NTH, Dawson FH, Everard M (1998) Quality assessment using River Habitat Survey
920 data. *Aquatic Conservation: Marine and Freshwater Ecosystems* 8: 477–499.
- 921
- 922 Rosewarne PJ, Mortimer RJ, Newton RJ, Grocock C, Wing CD, Dunn AM (2016) Feeding behaviour,
923 predatory functional responses and trophic interactions of the invasive Chinese mitten crab (*Eriocheir*
924 *sinensis*) and signal crayfish (*Pacifastacus leniusculus*). *Freshwater Biology* 61: 426–443.
- 925
- 926 Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulm PE, Jeschke JM, ... Essl F (2017) No saturation in
927 the accumulation of alien species worldwide. *Nature Communications* 8: 14435.
- 928
- 929 Shine R (2010) The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly*
930 *Review of Biology* 85: 253–291.
- 931
- 932 Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: an ecological and evolutionary overview. *Trends*
933 *in Ecology & Evolution* 19: 372–378.
- 934
- 935 Simberloff D, Martin J-L, Genovesi P, Maris V, Wardle D, Aronson J, Courchamp F, Galil B, Garcia-
936 Berthou E, Pascal M, Pyšek P, Sousa R, Tabacchi E, Vilà M (2013) Impacts of biological invasions: what's
937 what and the way forward. *Trends in Ecology and Evolution* 28: 58–66.
- 938
- 939 Sousa R, Freitas F, Nogueira AJA, Mota M, Antunes C (2013). Invasive dynamics of the crayfish
940 *Procambarus clarkii* (Girard, 1852) at the international section of the River Minho (NW of the Iberian
941 Peninsula). *Aquatic Conservation: Marine and Freshwater Ecosystems* 23: 656–666.
- 942
- 943 Sousa R, Amorim A, Froufe E, Varandas S, Teixeira A, Lopes-Lima M (2015) Conservation status of the
944 freshwater pearl mussel *Margaritifera margaritifera* in Portugal. *Limnologica* 50: 4–10.
- 945
- 946 Sousa R, Ferreira A, Carvalho F, Lopes-Lima M, Varandas S, Teixeira A (2018) Die-offs of the endangered
947 pearl mussel *Margaritifera margaritifera* during an extreme drought. *Aquatic Conservation: Marine and*
948 *Freshwater Ecosystems* 28: 1244–1248.

949 Sousa R, Nogueira J, Ferreira A, Carvalho F, Lopes-Lima M, Varandas S, Teixeira A (2019) A tale of shells
950 and claws: the signal crayfish as a threat to the pearl mussel *Margaritifera margaritifera* in Europe. Science
951 of the Total Environment 665: 329 - 337.

952

953 Sousa R, Ferreira A, Carvalho F, Lopes-Lima M, Varandas S, Teixeira A, Gallardo B (2020) Small
954 hydropower plants as a threat to the endangered pearl mussel *Margaritifera margaritifera*. Science of the
955 Total Environment 719: 137361.

956

957 Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and
958 prospects for the future. Freshwater Biology 55: 152-174.

959

960 Strayer DL (2012) Eight questions about invasions and ecosystem functioning. Ecology Letters 15: 1199-
961 1210.

962

963 Toscano BJ, Gownaris NJ, Heerhartz SM, Monaco CJ (2016) Personality, foraging behavior and
964 specialization: integrating behavioral and food web ecology at the individual level. Oecologia 182: 55-69.

965

966 Villéger S, Grenouillet G, Suc V, Brosse S (2012) Intra- and interspecific differences in nutrient recycling
967 by European freshwater fish. Freshwater Biology 57: 2330-2341.

968

969 Violle C, Enquist BJ, McGill BJ, Jiang LIN, Albert CH, Hulshof C, ... Messier J (2012) The return of the
970 variance: intraspecific variability in community ecology. Trends in Ecology & Evolution 27: 244-252.

971

972 Wolf M, Weissing FJ (2012) Animal personalities: Consequences for ecology and evolution. Trends in
973 Ecology and Evolution 27: 452-461.

974

975 Wutz S, Geist J (2013) Sex- and size-specific migration patterns and habitat preferences of invasive signal
976 crayfish (*Pacifastacus leniusculus* Dana). Limnologia 43: 59-66.

977

978 Yagound B, West AJ, Richardson MF, Selechnik D, Shine R, Rollins LA (2022) Brain transcriptome
979 analysis reveals gene expression differences associated with dispersal behaviour between range-front and
980 range-core populations of invasive cane toads in Australia. *Molecular Ecology* 31: 1700-1715.

981

982 Zar JH (1999) *Biostatistical analysis*. Pearson Education India.

983

984 Zieritz A, Bogan AE, Rahim KA, Sousa R, Jainih L, Harun S, Razak NFA, Gallardo B, McGowan S,
985 Hassan R, Lopes-Lima M (2018) Changes and drivers of freshwater mussel diversity and distribution in
986 northern Borneo. *Biological Conservation* 219: 126–137.

987

988

989

990

991