Received: 13 November 2020 | Revised: 27 May 2021 | Accepted: 4 June 2021

DOI: 10.1111/fwb.13792

ORIGINAL ARTICLE

Freshwater Biology WILEY

Patterns and determinants of phenotypic variability within two invasive crayfish species

Iris Lang[1,2] | **Ivan Paz-Vinas**[1] | **Julien Cucherousset**[1] | **Géraldine Loot**[1]

[1]Laboratoire Évolution et Diversité Biologique (EDB UMR 5174), Université de Toulouse, CNRS, UPS, Toulouse, France

[2]Conservatoire d'Espaces Naturels d'Occitanie, Toulouse, France

Correspondence
Iris Lang. Conservatoire d'Espaces Naturels d'Occitanie, 75 voie du TOEC, BP 57611, F-31076 Toulouse Cedex 3, France.
Email: iris.lang@hotmail.fr

Funding information
Université Toulouse III - Paul Sabatier; Centre National de la Recherche Scientifique; Office français de la biodiversité

Abstract

1. Knowledge on the ecological determinants and evolutionary processes shaping intraspecific variability in the wild remains scarce. It is particularly needed in the context of biological invasions to fully understand the consequences of invasive species on the functioning of recipient ecosystems.
2. Using geometric morphometrics, stable isotopes, and elemental composition analyses, we quantified phenotypic variability (morphological, trophic, and stoichiometric traits) within and among invasive populations of two crayfish species with distinct invasion histories over 23 gravel pit lakes in southwest France. We sampled 12 populations of the red swamp crayfish (*Procambarus clarkii* Girard, 1852) and 11 populations of the spiny-cheek crayfish (*Faxonius limosus* Rafinesque, 1817). We aimed at unravelling the ecological determinants and the mechanisms (neutral or adaptive) underlying the phenotypic variability among invasive populations.
3. We demonstrate that, for each group of traits, *P. clarkii* and *F. limosus* display contrasting patterns of variance distribution across three ecological scales (population, sex, individual). Then, we demonstrate that *P. clarkii* trait variation in body morphology and stoichiometry is associated with both ecological and historical determinants (i.e. predation pressure, intraspecific invasion, and invasion age), and morphological traits in *F. limosus* vary with ecological factors only (i.e. predation pressure and abundance of *P. clarkii*). Finally, we highlight that different combinations of neutral and adaptive processes shaped the phenotypic variability in the two species, with a higher contribution of adaptive processes in *F. limosus*.
4. Overall, these results indicate that *F. limosus* has already gone through local adaptation in the meta-population while this has not yet occurred for *P. clarkii*, which was introduced later. This highlights that these two invasive species might have contrasting effects across ecological scales.
5. Our study emphasises that studying invasive species can provide great knowledge on intraspecific variability and its ecological determinants and evolutionary processes in the wild. Our results also stress the need to focus on intraspecific variability in the context of biological invasions as it can be substantial across wide geographic areas.

KEYWORDS
Faxonius limosus, geometric morphometrics, intraspecific variation, Procambarus clarkii, stable isotopes, stoichiometry

1 | INTRODUCTION

Intraspecific variability is increasingly studied within a multidisciplinary approach combining both population genetic and functional ecology, hence fostering our capacity to understand patterns of biodiversity (Mims et al., 2017; Vellend et al., 2014). Such multi-facetted integrative studies highlight the eco-evolutionary processes underlying intraspecific variability patterns at multiple organisational levels (e.g. population, community, or landscape levels; Hendry et al., 2011; Lowe et al., 2017), which can cause a heterogeneous distribution of intraspecific variation across ecological scales (Evangelista et al., 2019; McGill, 2008; Messier et al., 2010). These patterns generally result from: (1) adaptive processes (e.g. selection, plasticity) under environmental gradients (i.e. linked to spatio-temporal dynamics of organisms and ecosystems; Ackerly, 2003; Araújo et al., 2011; Holt & Gaines, 1992; Prunier et al., 2018); and (2) neutral processes (e.g. genetic drift) arising during range expansions (i.e. through surfing mutations and/or demographic processes like founder effects and population bottlenecks; Bélouard et al., 2019; Excoffier & Ray, 2008; Klopfstein et al., 2006). Because intraspecific variability can impact community structure and ecosystem functioning (Blanchet et al., 2020; Des Roches et al., 2018; Raffard et al., 2019, 2021) through different contributions of individuals to trophic interactions, ecosystem productivity, nutrient cycling, and/or through ecosystem engineering (Bassar et al., 2012; Harmon et al., 2009), there is a need to understand how it may vary across spatial and temporal scales.

In the context of biological invasions, intraspecific variability characterisation may help predict the ecosystem consequences of invasive individuals, which can intrinsically have important ecological effects (Simberloff et al., 2013). For example, ecological effects induced by sexual dimorphism can be modulated by sex-ratio variation (Fryxell et al., 2015). Biological invasions can be viewed as filtering processes during which invasive individuals pass through a succession of stages and environmental filters (Blackburn et al., 2011). The resulting founder effects and the adaptation of successful individuals to novel environments can lead to phenotypic differentiation between populations at the core of the invasion and those at the front, hence following the colonisation gradient (Huey, 2000; Juette et al., 2014; Strubbe et al., 2013). In recently established populations (i.e. toward the invasion front), individuals may thus exhibit a higher range of phenotypic characteristics (i.e. high intraspecific variability) compared to populations established earlier and that have already experienced changes in population size and adaptation to local environments (Aubret & Shine, 2009; Green, 2016; Gutowsky & Fox, 2012; Rey et al., 2016). The phenotypic variability may also be shaped locally by spatio-temporal variations of habitat characteristics (e.g. available trophic resources), abiotic environmental conditions, and the presence of intra- or interspecific competitors and/or natural enemies (Araújo et al., 2011; Kinnison et al., 2008; Mack et al., 2000; Shea & Chesson, 2002). Finally, the management of invaders might also lead to a change in their phenotypic traits (Závorka et al., 2020), by selecting avoidance or smaller body-size phenotypes (Côté et al., 2014; Evangelista et al., 2015). All these potential sources of intraspecific variation make invasive species perfect candidates to study the effect of ecological determinants and evolutionary processes at short time-scales (Hairston et al., 2005; Kinnison et al., 2008).

In this study, we investigated the patterns and the determinants of phenotypic variability of two invasive crayfish species: the spiny-cheek crayfish (*Faxonius limosus* Rafinesque, 1817) and the red swamp crayfish (*Procambarus clarkii* Girard, 1852). *Faxonius limosus* first occurred in the study area (i.e. the Garonne River basin area in south-western France) in 1988, while *P. clarkii* was first documented in 1995. Considering that they have distinct introduction histories and contrasting life histories, we expected to reveal contrasting patterns of phenotypic variability across the invaded landscape. Specifically, we first quantified—for each species—phenotypic variability (measured for morphological, trophic, and stoichiometric traits) among populations, along with the distribution of its variance across different levels of within-species organisation (i.e. individual, sex and population levels). Since *F. limosus* has been present in the study area for a longer period than *P. clarkii*, we expected that *F. limosus* will display lower morphological variability at the individual level (i.e. within populations) compared to the population level (i.e. among populations). We also expected a high proportion of variance at sex level for both species, since they display both sexual dimorphism (Chybowski, 2007; Malavé et al., 2018). For both species, we expected a higher variance of trophic traits at the population level, as they depend on local resources for generalist omnivorous species (Evangelista et al., 2019), and we expected a higher variance of stoichiometric traits at the individual level since they rely mainly on ontogeny (Bertram et al., 2008). Second, we investigated the environmental and historical determinants of each trait variability among populations for each species (Vellend & Geber, 2005). We predicted that phenotypic variability would be mostly explained by environmental characteristics (i.e. lake productivity, predation pressure, the coexistence of the two crayfish species, anthropogenic pressure) for *F. limosus*, and mostly explained by the colonisation history (invasion time span) for *P. clarkii*, which might not have already undergone local adaptation due to the recent establishment of populations (<20 years). Finally, we aimed to unravel the mechanisms (neutral or adaptive) underlying the phenotypic variability within and among populations (Leinonen et al., 2013). Considering the lag time between both species' introductions in the study area, we expected that phenotypic divergences would be mainly explained by adaptive processes in *F. limosus*, which might already have experienced adaptation to local resources, and by neutral processes (i.e. genetic drift) in *P. clarkii* because of its recent establishment.

2 | METHODS

2.1 | Study system and model species

The study was conducted in 23 gravel pit lakes ranging from 0.7 to 27 ha and located in a narrow geographical range: 12–55 km (Euclidean distance) away from Toulouse city along the Garonne River, in south-western France (Figure 1; see also Alp et al., 2016; Zhao et al., 2016). These lakes are disconnected from the hydrographic network and their characteristics differ according to environmental and human pressure gradients: recent lakes are globally further away from Toulouse than mature lakes (i.e. more productive lakes), and under lighter anthropogenic pressure (i.e. fishing management; Zhao et al., 2016). While trapping by the public is rare in the study area, some lakes are managed to control invasive populations of fish and crayfish (Závorka et al., 2020). The invasion process is relatively recent in the studied area, since the presence of *F. limosus* and *P. clarkii* in the Garonne floodplain were first documented in 1988 and 1995, respectively (Changeux, 2003; Magnier & Petit, 2016).

Our field observations revealed that colonisation of gravel pit lakes usually occurs rapidly, i.e. within a few months after gravel extraction has started. We henceforth computed a proxy of invasion time span using the following formula:

$$\text{Invasion time - span} = \text{sampling date} - \text{invasion date}$$

where the sampling date is 2016, 2017, or 2018 and the invasion date is 1988 or 1995 for *F. limosus* and *P. clarkii*, respectively, for the lakes formed before the first recorded occurrence of each species. For the lakes formed after 1988 and 1995 we used the following formula:

$$\text{Invasion time - span} = \text{sampling date} - \text{year of lake creation}$$

with the year of lake creation determined using aerial pictures (IGN 2019; see details in Table 1).

Native in North America, *P. clarkii* and *F. limosus* are among the most invasive crayfish species worldwide (Holdich et al., 2009; Lodge et al., 2012; Oficialdegui et al., 2019; Souty-Grosset

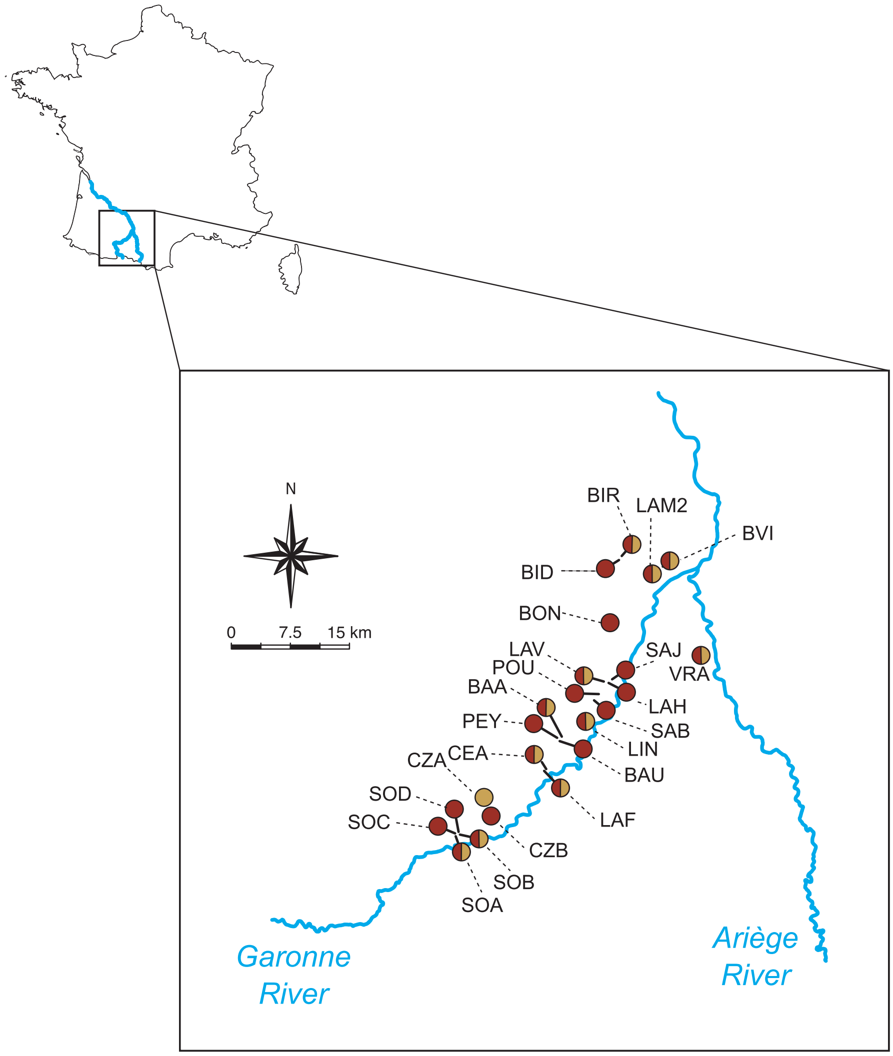

FIGURE 1 Map of the 23 studied gravel pit lakes, south-western Toulouse, France. Red circles represent *Procambarus clarkii* populations, dark orange circles represent *Faxonius limosus* populations, and coexisting populations are represented with red and dark orange circles

TABLE 1 Environmental, historical and management characteristics of the 23 sampled lakes

Lake	Latitude	Longitude	Year of lake creation	Productivity μg/L	CPUE *Procambarus clarkii* (ind. trap^{-1} hr^{-1})	CPUE *Faxonius limosus* (ind. trap^{-1} hr^{-1})	BPUE predation (g gillnet^{-1} hr^{-1})	Management type	Anthropogenic pressure
BAA	43.1920456	1.1207434	1990	21.78	0.44	0.61	184	Federal	3
BAU	43.1901757	1.1156223	2000	2.03	4.28	–	18,184	Private	1
BID	43.3132063	1.1707778	1992	10.90	0.67	–	12	Federal	3
BIR	43.3149184	1.1723150	1992	3.43	7.00	0.67	2,155	Federal	3
BON	43.2713507	1.1625212	1990	5.00	0.33	–	582	Communal	2
BVI	43.3109127	1.2116333	1963	73.92	2.94	3.72	5,259	Federal	3
CEA	43.1705748	1.1043968	1971	0.85	3.97	1.44	408	Private	1
CZA	43.1336084	1.0536924	1973	2.95	–	–[a]	64	Private	1
CZB	43.1347129	1.0540092	2008	2.80	0.11	–	–	Private	1
LAF	43.1657828	1.1032664	1982	1.63	1.69	2.00	2,107	Private	1
LAH	43.2305593	1.1624622	1972	1.32	14.72	0.06	596	Private	1
LAM2	43.3021334	1.2028925	1987	10.45	60.22	–	1,324	Federal	3
LAV	43.2311011	1.1557208	1992	1.78	8.56	0.11	190	Communal	2
LIN	43.2035470	1.1337380	1990	43.80	0.75	0.83	263	Federal	3
PEY	43.1915169	1.1141565	2007	4.20	5.67	–	1,377	Communal	2
POU	43.2218941	1.1530503	1996	2.68	3.33	–	361	Communal	2
SAB	43.2152621	1.1504806	2006	8.02	4.86	–	29	Communal	2
SAJ	43.2320905	1.1644074	1983	10.18	3.28	–	–	Private	1
SOA	43.1220218	1.0224061	1993	36.90	5.11	3.00	1,443	Private	1
SOB	43.1227472	1.0247659	1998	0.68	10.50	1.61	842	Private	1
SOC	43.1232123	1.0220173	2005	4.38	4.39	–	1,469	Private	1
SOD	43.1238749	1.0239614	2007	2.53	3.11	–	400	Private	1
VRA	43.2445421	1.2510805	1982	38.05	0.39	0.56	1591	Private	1

[a] *F. limosus* does occur in this lake. CPUE, catch per unit effort; BPUE, biomass per unit effort

et al., 2006). Both species strongly impact ecosystem functioning and native organisms through consumption (e.g. macroinvertebrates, fish, macrophytes; Correia & Anastácio, 2008; Vojkovská et al., 2014), disease transmission (Changeux, 2003), and ecological engineering, with *P. clarkii* displaying larger burrowing activity than *F. limosus* (Kouba et al., 2016). Veselý et al. (2021) recently demonstrated that these two species had distinctive trophic niches, with *P. clarkii* constraining the trophic niche of *F. limosus* when co-occurring. The high invasiveness potential of *P. clarkii* might be attributable to its ability to disperse overland (Cruz & Rebelo, 2007; Kerby et al., 2005; Thomas et al., 2019) and to its important agonistic behaviour (Gherardi & Cioni, 2004). *Faxonius limosus* tend to exhibit less aggressive interactions (Chucholl et al., 2008; Hudina & Hock, 2012) and individuals have been sighted dispersing overland on extremely rare occasions (Herrmann et al., 2018; Puky, 2014). As observed elsewhere, *P. clarkii* reproduces twice a year in autumn and spring (Alcorlo et al., 2008; Souty-Grosset et al., 2006). To maximise their reproductive success, both species are suspected to combine sexual and asexual modes of reproduction (i.e. parthenogenesis; Buřič et al., 2011, 2013; Yue et al., 2008). *Faxonius limosus* is also known to experience two peaks of mating activity throughout the year with long-term storage of sperm (Aklehnovich & Razlutskij, 2013; Buřič et al., 2013; Holdich & Black, 2007). In the studied system, previous works highlighted the existence of substantial phenotypic variability (morphology, trophic ecology) among (Evangelista, Lecerf, et al., 2019; Jackson et al., 2017) and within populations of *P. clarkii* (Lang et al., 2020; Raffard et al., 2017). This suggests contrasting impacts of invasive individuals on ecosystem functioning among populations of *P. clarkii* (Alp et al., 2016; Evangelista, Lecerf, et al., 2019). No study has yet focused on *F. limosus* intraspecific variability within our sampling area.

2.2 | Sampling and environmental characteristics

Faxonius limosus and *P. clarkii* were sampled from mid-September to mid-October 2016 in 15 lakes, from mid-September to mid-October 2017 in seven lakes and in September 2018 in two lakes using pairs of baited traps (one cylindrical trap: 62 cm × 34 cm × 34 cm, mesh size: 10 mm; one rectangular trap: 95 cm × 20 cm × 20 cm, mesh size: 4 mm) set overnight ($n = 12$ traps) and during the day ($n = 8$ traps) in the littoral habitat. To reduce the temporal bias, all sites were randomly distributed across sampling year. Only one lake was sampled on two following years to collect enough *F. limosus* individuals (lake CZA; $n = 2$ and $n = 10$ in 2017 and 2018, respectively). *Faxonius limosus* and *P. clarkii* coexisted in 11 lakes (i.e. sympatric populations) and were isolated in one lake for *F. limosus* and 11 lakes for *P. clarkii* (i.e. allopatric populations; Table 1). When needed, additional trapping, electrofishing (Deka 7000; Deka) and hand netting were performed along the shoreline to collect the desired number of adult crayfish, i.e. 24 individuals *per* species *per* lake, to robustly capture intraspecific variability in the studied phenotypic traits (Evangelista, Cucherousset, et al., 2019; Fourtune et al., 2018). Following capture, crayfish were sexed, measured for carapace length (±0.01 mm) and were euthanised on ice. A fresh muscle tissue sample from the abdomen was collected on each individual and stored in 70% ethanol at −20°C for subsequent genetic analyses, and each individual was placed in a labelled plastic bag and frozen in the laboratory. Stable isotope analyses were performed on an additional sample of abdominal muscle collected on each specimen after defrosting, rinsed with distilled water and oven-dried (60°C for 48 hr).

During our crayfish sampling campaign, we also collected—for each lake—putative trophic resources of *F. limosus* and *P. clarkii* at three different locations representative of the littoral habitat of the lake. Specifically, periphyton (i.e. resource from aquatic origin) and leaves of black poplar (*Populus nigra*; i.e. resource from terrestrial origin) were collected from the littoral since both species have a preference for vegetable diet at the adult stage (Jackson et al., 2017; Vojkovská et al., 2014). Periphyton and poplar leave samples were freeze-dried (−50°C for 5 days) and oven-dried (60°C for 48 hr), respectively (further details available in Jackson et al., 2017).

The same day, lake productivity was assessed by measuring chlorophyll-*a* concentration (μg/L; with an AlgaeTorch device, BBE moldaenke GmbH) at three locations within each lake. These three values were averaged to account for within-lake variability in environmental conditions (Table 1). For each species, crayfish abundance was estimated in each lake as the number of individuals trapped over 24 hr (catch per unit effort expressed in ind trap^{-1} hr^{-1}; Table 1). The predation pressure in each lake was assessed as the biomass of predator fish trapped in a set of gillnets randomly distributed, over 1 hr (biomass per unit effort expressed in g gillnet^{-1} hr^{-1}; Table 1; see further details in Lang et al., 2020 and Zhao et al., 2016).

For each lake, the level of anthropogenic pressure was assessed using management type as a proxy: private, communal and federal lakes were considered under low (coded 1), medium (2), and strong (3) anthropogenic pressure, respectively (Table 1).

2.3 | Morphological, stable isotope, and stoichiometric analyses

The morphological variation of crayfish bodies was analysed using a geometric morphometric technique (Zelditch et al., 2012) based on landmark analysis. Following Evangelista, Cucherousset, et al. (2019), 19 homologous landmarks were digitised on *F. limosus* and *P. clarkii* individuals (i.e. cephalothorax and abdomen) using TpsDig2 v.2.17 (Rohlf, 2015). To avoid any distortion bias, individuals were photographed dorsally directly after defrosting and before proceeding further. We used a full-Procrustes fit implemented in Morpho J v.1.06d to analyse the digitised coordinates by superimposing individual shapes (i.e. to remove bias due to different sizes, positions and orientations among individuals; Klingenberg, 2011). We used the products of the full-Procrustes fit (i.e. new Procrustes coordinates) and their centroid size (i.e. the square root of the summed squared distances of each landmark from their centroid) to characterise individuals.

Oven-dried muscle samples were ground to a fine powder and analysed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes at the Cornell Isotope Laboratory (COIL, New York). The terrestrial reliance (α) of crayfish was computed as:

$$\alpha = (\delta^{13}C_{\text{crayfish}} - \delta^{13}C_{\text{base1}})/(\delta^{13}C_{\text{base1}} - \delta^{13}C_{\text{base2}}).$$

and the trophic position (TP_{crayfish}) of each individual was computed following the two-sources model (i.e. aquatic vs. terrestrial source) of Post (2002):

$$TP_{\text{crayfish}} = TP_{\text{baseline}} + (\delta^{15}N_{\text{crayfish}} - [\delta^{15}N_{\text{base1}} \times \alpha + \delta^{15}N_{\text{base2}} \times (1 - \alpha)])/3.4$$

where baseline organisms common to all studied lakes are poplar leaves (base 1) and periphyton (base 2), $TP_{\text{baseline}} = 1$, and 3.4 is the fractionation coefficient between trophic levels (Post, 2002). These baselines were chosen to ensure robust comparisons between studied lakes.

Finally, for stoichiometric analyses, guts were removed for each crayfish prior to freeze-drying (Christ Martin™ Alpha 1-4 Ldplus Freeze Dryer), and individuals (whole-body) were finely grounded with two successive grindings using a grinder (Waring WSG30E) and an oscillating ball mill (Retsch MM200). A subsample of ground crayfish was analysed for carbon (C) and nitrogen (N) contents using an organic elemental analyser (Flash 2000 Thermofisher), and a mineralised replicate subsample (121℃ for 2 hr in sodium persulfate) was used to analyse dissolved phosphorus (P) contents using spectrophotometry (molybdate method; Parsons et al., 1984). All elemental ratios (C:N, C:P, N:P) are expressed as molar ratios.

2.4 | Genetic analyses

2.4.1 | DNA extraction, polymerase chain reaction protocol

Faxonius limosus neutral genetic variation was assessed using 9 microsatellites selected from Jiang et al. (2015) (loci PCSH0005, PCSH0006, PCSH0011, PCSH0038, PCSH0042, PCSH0054, PCSH0077, PCSH0089) and from Hulák et al. (2010) (locus 3.1). *Procambarus clarkii* neutral genetic variation was assessed using 14 microsatellites following Lang et al. (2020). We extracted DNA from the abdomen muscle of crayfish using a modified salt-extraction protocol (Aljanabi & Martinez, 1997). Three optimised multiplexed sets of loci for *P. clarkii*, and two for *F. limosus*, were co-amplified by polymerase chain reaction (PCR) in final volumes of 10 μl, containing 10–20 ng of genomic DNA, 5 μl of QIAGEN multiplex PCR master mix and locus-specific combination of primers (see Figure S1 for more details). PCRs were performed following the procedure described in Lang et al. (2020) (see Figure S1 for the description of the multiplex used in this study). Amplified fragments were analysed on an ABI PRISM 3730 capillary sequencer (Applied Biosystems) at the Génopole Toulouse Midi-Pyrénées.

2.4.2 | Genotyping, quality control, and genetic variability assessment

We tested for the presence of null alleles and other potential genotyping errors with Microchecker v.2.2.3 (Van Oosterhout et al., 2004) at the sampling site level and for each locus. We then tested for the presence of significant deviations from Hardy–Weinberg equilibrium using Genepop v 4.0 (Rousset, 2008) and for the presence of significant linkage disequilibrium among loci within populations with Fstat v2.9.3.2 (Goudet, 2002). Levels of significance for Hardy–Weinberg equilibrium tests were corrected with Bonferroni corrections. Finally, we tested the neutrality of the microsatellite loci we used using BayeScan v.2.1 (Foll & Gaggiotti, 2008). We performed four independent Markov chain Monte Carlo (MCMC) analyses considering 20 pilot runs of 5,000 iterations per analysis, burning periods of 50,000 iterations and sample sizes of 10,000 (with thinning intervals of 50). We also considered prior odds for the neutral model equal to 10. The convergence of the four MCMC chains was verified visually and by conducting a Gelman and Rubin analysis (Gelman & Rubin, 1992), using the R v.3.6.0 statistical software (R Core Team, 2018) and a modified script from Paz-Vinas et al. (2013) based on the packages *boa* (Smith, 2007) and *coda* (Plummer et al., 2006). We considered that chains reached convergence when values less than 1.1 were obtained (Gelman & Hill, 2007). We quantified genetic diversity within lakes using observed (Hobs) and expected (Hexp) heterozygosity, computed with Genetix v4.05 (Belkhir et al., 1996), and allelic richness, mean number of alleles per locus, and Wright fixation indices, computed with Fstat v2.9.3.2 (Goudet, 2002; see details in Supporting Information and Table S1). The global genetic differentiation across lakes (F_{st}) and its 95% confidence interval were computed for each species, using Fstat v2.9.3.2 (Goudet, 2002).

2.5 | Statistical analyses

2.5.1 | Phenotypic variability

For each species, the allometry effect on body shape variation was removed using a regression of the Procrustes coordinates against log10-transformed centroid sizes, which are a proxy of individuals' body sizes (Klingenberg, 2016). The covariance matrix of the regression residuals was used to run two principal component (PC) analyses, to assess the intraspecific body shape variation. All these analyses were implemented in Morpho J. For each species, the first two PC axes were subsequently used as morphological scores (PC1 and PC2 scores) to characterise individuals' body shapes in further statistical analyses. PC1 and PC2 explained 33.1% and 21.6% of the

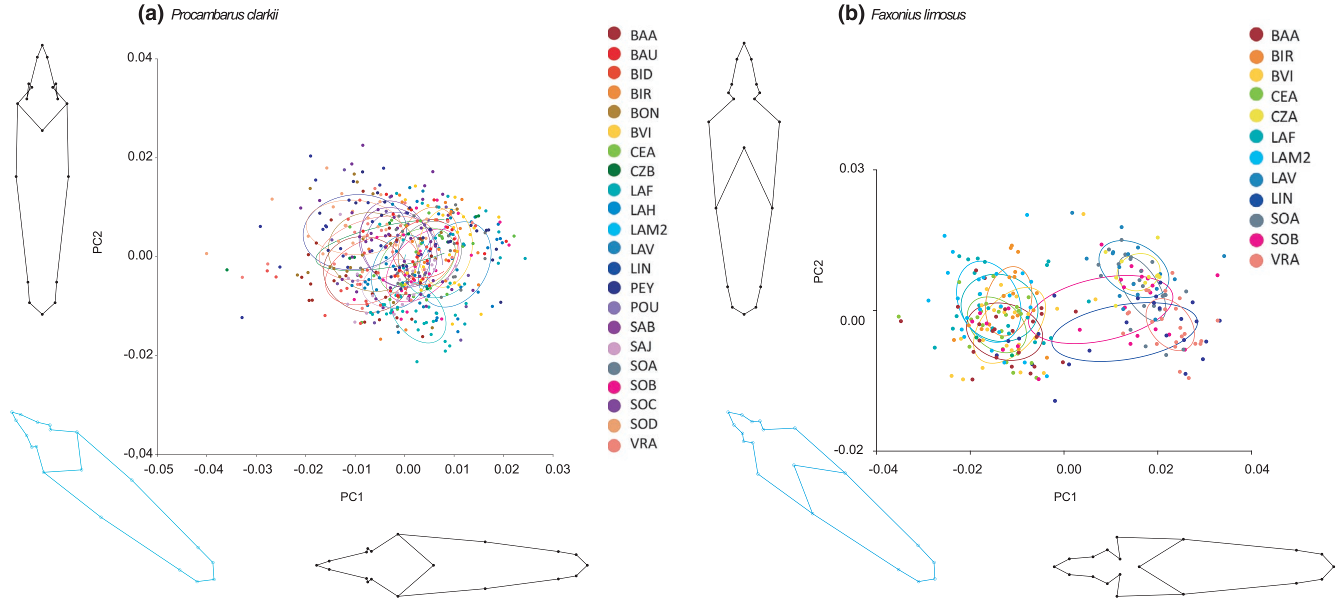

FIGURE 2 Principal component (PC) analysis based on the covariance matrix of allometry regression residuals for (a) *Procambarus clarkii* and (b) *Faxonius limosus* showing the 22 populations and 12 populations, respectively. Body shapes variations (scaling factor: 0.1) along PC1 and PC2 axes are displayed. Populations are coloured. Confidence ellipses represent 40% of the variance for each population

body morphological variations in *P. clarkii*, and 54.9% and 11.5% of the variations in *F. limosus*, respectively (Figure 2). Concerning *P. clarkii*, increasing PC1 scores were associated with stockier body, i.e. shortened abdomen and wider cephalothorax and rostrum, and increasing PC2 scores were associated with a streamlined body morphology, i.e. narrow cephalothorax and more elongated abdomen (Figure 2a). Concerning *F. limosus*, increasing PC1 scores were associated with stockier body, shortened cephalothorax, and more prominent rostrum, and increasing PC2 scores were associated with bigger rostrum, larger cephalothorax, and stockier abdomen (Figure 2b).

Linear models were run using PC1 and PC2 scores, carapace length, trophic position, terrestrial reliance, C:N, C:P, and N:P ratios as response variables and *sex* and *population* as explanatory variables, to assess the phenotypic variations among populations and sexes for each species.

2.5.2 | Variance partitioning

As phenotypic traits may vary according to individuals, their sex and their respective population (Evangelista, Cucherousset, et al., 2019; Malavé et al., 2018), analyses of variance component of PC1 and PC2 scores, carapace length, morphological scores, trophic position, terrestrial reliance, and C:N:P ratios were performed for each species using the *varcomp* function from the *ape* R package (v.5.3, Paradis et al., 2004) to quantify the distribution of variance for each trait across intraspecific ecological scales. *Individual* level was nested within *Sex* level, and nested within *Population* level, i.e. the studied lake. Variance partitioning was computed on linear mixed models (LMMs; *nlme* package v.3.1.142; Pinheiro et al., 2019). 95% confidence intervals of variance components were computed using a bootstrap procedure based on 200 iterations.

2.5.3 | Environmental determinants

To summarise lakes characteristics, a multiple factor analysis was performed on *productivity* (chlorophyll-a concentration in μg/L), *predation pressure* (biomass per unit effort in g gillnet^{-1} hr^{-1}), *anthropogenic pressure* (1, 2, 3 for low, medium and strong anthropogenic pressure, respectively), *coexistence* (Yes or No for allopatric and sympatric populations, respectively), *P. clarkii abundance*, and *F. limosus abundance* (catch per unit effort in ind. trap^{-1} hr^{-1}). The first and second multiple factor analysis axes explained 36.8% and 27.7% of the total variance, respectively. Positive values on the first axis were associated with the coexistence of the two crayfish species (and negative values with non-coexistence), and increasing values were associated with increasing productivity, increasing abundance of *F. limosus*, and increasing anthropogenic pressure. Increasing values on the second axis were associated with increasing predation pressure and decreasing *P. clarkii* abundance (Figure S2). These two axes were used as synthetising explanatory variables (environmental variables 1 and 2) in the subsequent analyses.

Linear mixed models were run to assess the effect of environmental characteristics (environmental variable 1, environmental variable 2) and the effect of the invasion time span, on PC1 and PC2 morphological scores, trophic position, terrestrial reliance, C:N:P ratios, using *population* as a random effect for each species (n = 501 and $n = 256$ for *P. clarkii* and *F. limosus*, respectively). For each full model, interactions were removed when non-significant using a backward procedure. Type II ANOVA implemented in the *car* R package (v.3.0.5; Fox & Weisberg, 2019) was used to test the significance of each factor.

2.5.4 | Neutral versus adaptive processes

To identify the neutral or adaptive character of the processes underlying the phenotypic variability among sympatric populations of *P. clarkii* and *F. limosus* ($n = 11$), we compared neutral genetic (F_{st}) and phenotypic (P_{st}) differentiation within each species (Leinonen et al., 2006). P_{st} was computed for morphology, diet and stoichiometry as:

$$P_{st_X} = \sigma^2_{\text{betweenpops}} / (\sigma^2_{\text{betweenpops}} + 2h^2\sigma^2_{\text{withinpop}})$$

where σ^2 is the variance of the phenotypic trait X (i.e. carapace length, morphological scores from PC1 and PC2 axis, trophic position, terrestrial reliance or C:N:P ratios) and h^2 is the heritability of X defined as the proportion of phenotypic variance with a genetic origin, set to 0.5 to avoid overestimating P_{st} (Leinonen et al., 2006; Lutz & Wolters, 1989). Traits evolve neutrally when P_{st} and F_{st} are equal, while different P_{st} and F_{st} imply adaptive processes (adaptive phenotype divergence for $P_{st} > F_{st}$, or a homogenising adaptation if $P_{st} < F_{st}$). For each lake, the global phenotypic uniqueness was computed as the mean population-specific P_{st} estimates of all traits (i.e. analogous to genetic uniqueness). All analyses were performed using the R v.3.6.0 statistical software (R Core Team, 2018).

3 | RESULTS

3.1 | Phenotypic variability

We found high population- and sex-dependent morphological variations for both species (Figure 2; Table S2). Concerning *P. clarkii*, morphological traits (PC1 and PC2 scores, carapace length) were significantly different among populations and between sexes (Table S2). More specifically, females had lower PC1 scores and higher PC2 scores than males, indicating that females had streamlined bodies compared to males, and they displayed higher carapace lengths than males (mean = 46.42 ± 0.30 *SE* and mean = 45.83 ± 0.38 *SE*, respectively; $F_{1,478} = 14.454$, $p < 0.001$). Concerning *F. limosus*, the interaction between sex and population effects on PC2 scores and carapace length was significant (interaction $\text{term}_{\text{PC2 score}}$: $F_{11,232} = 2.988$, $p < 0.001$; interaction $\text{term}_{\text{Carapace length}}$: $F_{11,232} = 2.359$, $p = 0.009$; Table S2). This indicated that the extent of sexual dimorphism in *F. limosus* varied between populations. Trophic traits (trophic position and terrestrial reliance) varied significantly between populations for both species (Table S2; Figure 3). Sex tended to have an effect on trophic position for *F. limosus* ($F_{1,243} = 3.861$, $p = 0.051$), with males displaying slightly higher trophic position than females (mean = 3.04 ± 0.05 *SE* and mean = 3.03 ± 0.03 *SE*, for males and females, respectively). Stoichiometric traits (C:N:P ratios) were highly context-dependent for *P. clarkii* and *F. limosus*: the interaction between sex and population was significant in all models except for N:P ratio in *P. clarkii*, which differed significantly among populations ($F_{21,478} = 13.162$, $p < 0.001$; Table S2, Figure 4) and between sexes ($F_{1,478} = 11.367$, $p < 0.001$; Table S2). Females had higher body N:P than males (mean = 12.87 ± 0.13 *SE* and mean = 12.66 ± 0.16 *SE*, for males and females, respectively).

3.2 | Variance partitioning

For *P. clarkii*, morphological trait variation was higher at the individual level (67%, 62%, and 75% of the variance for carapace length, PC1, and PC2 scores, respectively; Figure 5a). For *F. limosus*, variation of two morphological traits was higher at the population level (47% and 72% for carapace length and PC1 scores, respectively), while PC2 scores variation was higher at sex and individual levels (48% and 47%, respectively; Figure 5b). For both species, the trophic trait variation was mainly explained at the population level and was almost non-existent at the sex scale (Figure 5a). For *F. limosus*, the distribution of trophic position variation was more balanced across populations and individual scales (44% and 53%, respectively; Figure 5b). For *P. clarkii*, stoichiometric variation was mainly explained at the individual level (70%, 57%, and 62% of the variance for C:N, C:P, and N:P ratios, respectively; Figure 5a). For *F. limosus*, C:P and N:P ratio variation was mainly explained at the individual level (52% and 58% of the variance). However, C:N ratio variation was equivalently explained at both individual and population levels (42% and 44%, respectively; Figure 5).

3.3 | Environmental determinants

For morphological traits in *P. clarkii*, we only found predictors for PC2 scores. PC2 scores divergence was significantly explained by the invasion time span (LMM, $F_{1,18.8} = 10.440$, $p = 0.005$) and the environmental variable 2 (LMM, $F_{1,17.4} = 19.633$, $p < 0.001$; Table S3). Specifically, PC2 scores increased with decreasing invasion time span, i.e. individuals had more elongated body morphology in recently established populations, and PC2 scores increased with increasing environmental variable 2, i.e. with increasing predation pressure and decreasing abundance of *P. clarkii*. For *F. limosus*, there was a significant effect of environmental variable 2 on carapace length (LMM, $F_{1,7.9} = 8.704$, $p = 0.019$; Table S3). Specifically, the carapace length of individuals increased with increasing predation pressure and decreasing abundance of *P. clarkii*. For the two species, there was no evidence for an association between trophic traits of individuals and historical or environmental determinants. For *P. clarkii*, there was a significant

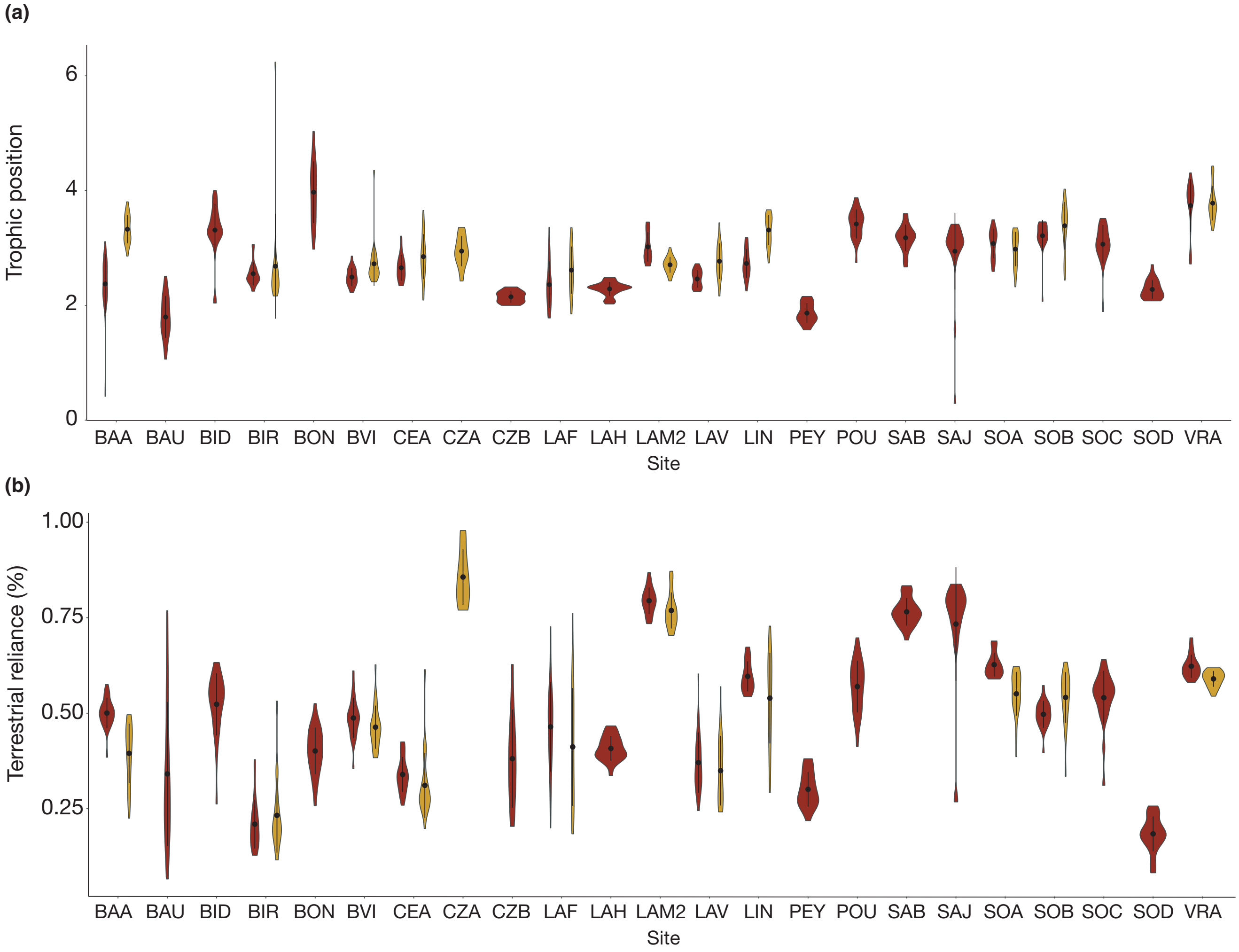

FIGURE 3 Violin plots representing (a) trophic position and (b) terrestrial reliance variations for *Procambarus clarkii* (red; $n = 501$) and *Faxonius limosus* (dark orange; $n = 256$). Mean trophic position = 2.77 ± 0.64 *SE* and mean = 3.03 ± 0.53 *SE* for *P. clarkii* and *F. limosus*, respectively. Mean terrestrial reliance = 0.48 ± 0.18 and mean = 0.49 ± 0.18 for *P. clarkii* and *F. limosus*, respectively

effect of invasion time span on C:P and N:P ratios of individuals (LMM, $F_{1,18.6} = 10.433$, $p = 0.005$ and LMM, $F_{1,18.6} = 8.695$, $p = 0.008$, respectively; Table S3). Specifically, C:P and N:P ratios of crayfish bodies decreased with increasing invasion time span. There was also a significant effect of environmental variable 2 on N:P ratio (LMM, $F_{1,17.6} = 4.640$, $p = 0.045$; Table S3). N:P ratio increased in *P. clarkii* individuals with increasing predation pressure and decreasing abundance of conspecifics. We detected no environmental or historical determinants neither for C:N ratio for *P. clarkii*, nor for all stoichiometric traits for *F. limosus*.

3.4 | Neutral versus adaptive processes

There was a global genetic differentiation among the studied lakes for *P. clarkii* ($F_{st} = 0.213$, $CI_{95\%}$: 0.191–0.237) and for *F. limosus* ($F_{st} = 0.209$, $CI_{95\%}$: 0.077–0.221). For *P. clarkii*, P_{st} for morphological traits and stoichiometric traits did not differ from F_{st}. This indicated that morphological and stoichiometric variations were due to neutral processes. Trophic position and terrestrial reliance variations were shaped by adaptive processes in *P. clarkii*, since their P_{st} were significantly higher than F_{st} ($P_{st_{\text{trophic position}}} = 0.652$ ($CI_{95\%}$: 0.386–0.771) and $P_{st_{\text{terrestrial reliance}}} = 0.882$ ($CI_{95\%}$: 0.701–0.952), respectively; Figure 6a). For *F. limosus*, all groups of traits (i.e. morphological, stoichiometric, and trophic traits) were shaped by a combination of adaptive and neutral processes (Figure 6b). Phenotypic differentiation in morphological and stoichiometric traits (measured by P_{st}) was higher for *F. limosus* than for *P. clarkii*, highlighting that the relative part of adaptive processes shaping the phenotypic variability was more important in *F. limosus* (Figure 7). However, the phenotypic differentiation (P_{st}) in *P. clarkii* was higher for the trophic position and terrestrial reliance, indicating that the relative importance of adaptive processes shaping this trait variation in *P. clarkii* was greater compared to *F. limosus* (Figure 7).

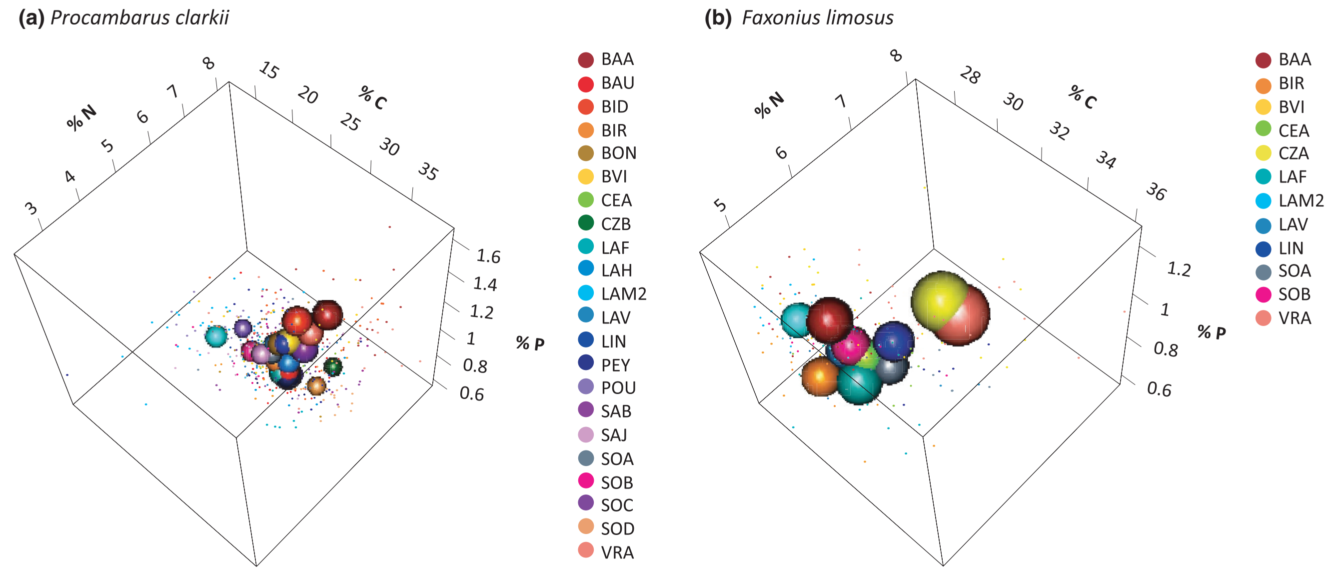

FIGURE 4 Stoichiometric niches for (a) *Procambarus clarkii* and (b) *Faxonius limosus*. Each sphere represents a population of crayfish, its position represents the mean elemental composition of the population and its volume corresponds to 40% of the variance

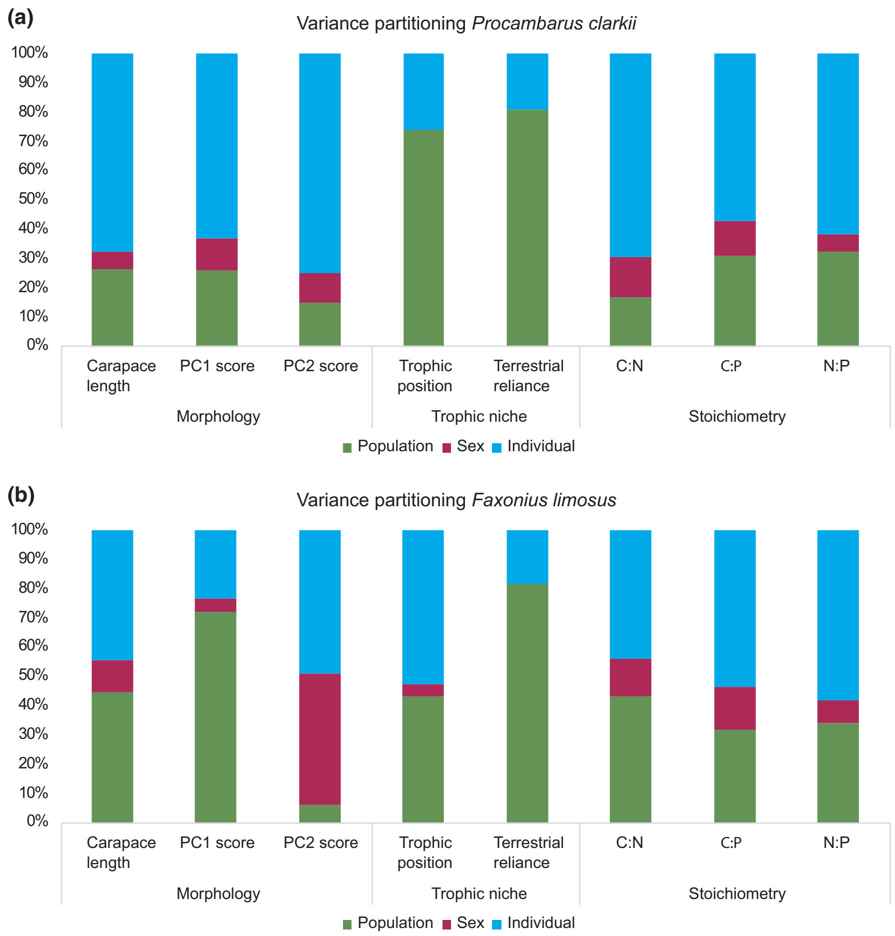

FIGURE 5 Partition of variance of the studied traits (PC1 and PC2 scores, carapace length, trophic position, terrestrial reliance, C:N:P ratios) for (a) *Procambarus clarkii* ($n = 501$) and (b) *Faxonius limosus* ($n = 256$)

4 | DISCUSSION

This study stresses the importance of investigating intraspecific trait variation across different ecological scales in invasive species to fully understand their ecological effects. Our results demonstrate that both *F. limosus* and *P. clarkii* display high intraspecific phenotypic variability (i.e morphological, trophic, and stoichiometric traits) within and among populations, and this was despite a moderate and low

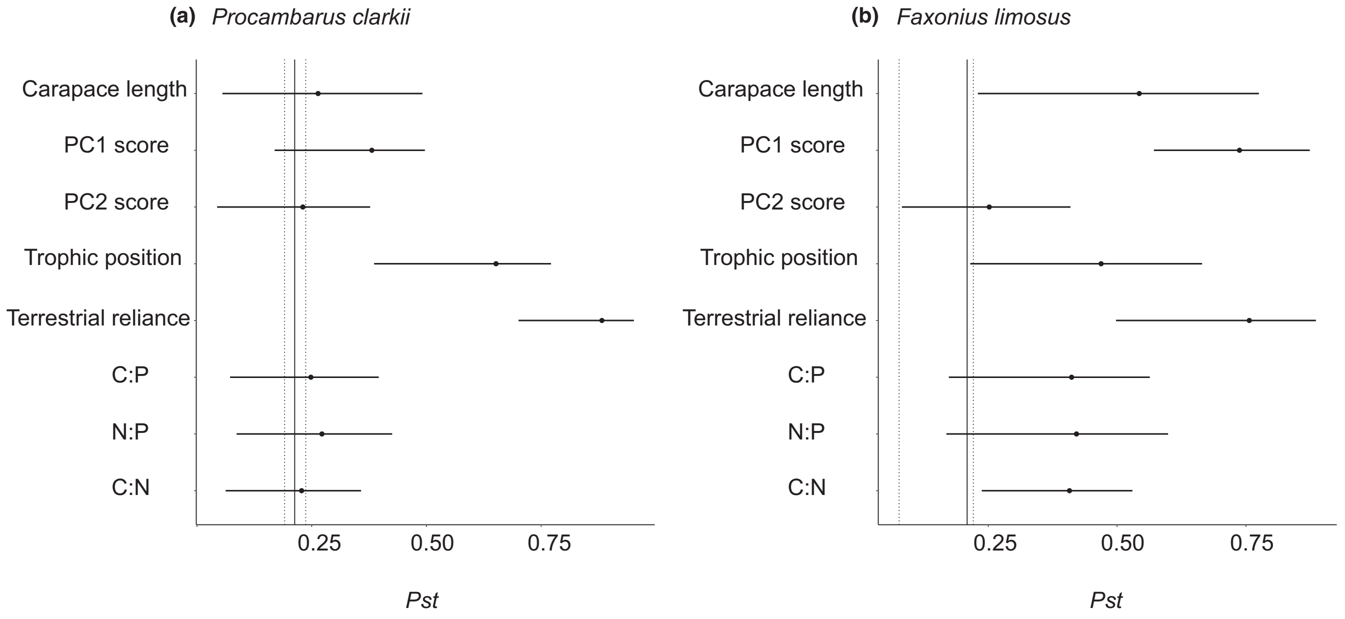

FIGURE 6 P_{st} estimates for each trait (carapace length, PC1 and PC2 scores, trophic position, terrestrial reliance, C:P, N:P, C:N ratios), and F_{st} (vertical straight line) on neutral microsatellite markers for (a) *Procambarus clarkii* ($n = 257$) and (b) *Faxonius limosus* ($n = 246$) from 11 sympatric populations. Horizontal bars represent 95% confident interval of P_{st}, and the vertical dotted line represents 95% confident interval of F_{st} estimated using a bootstrap procedure

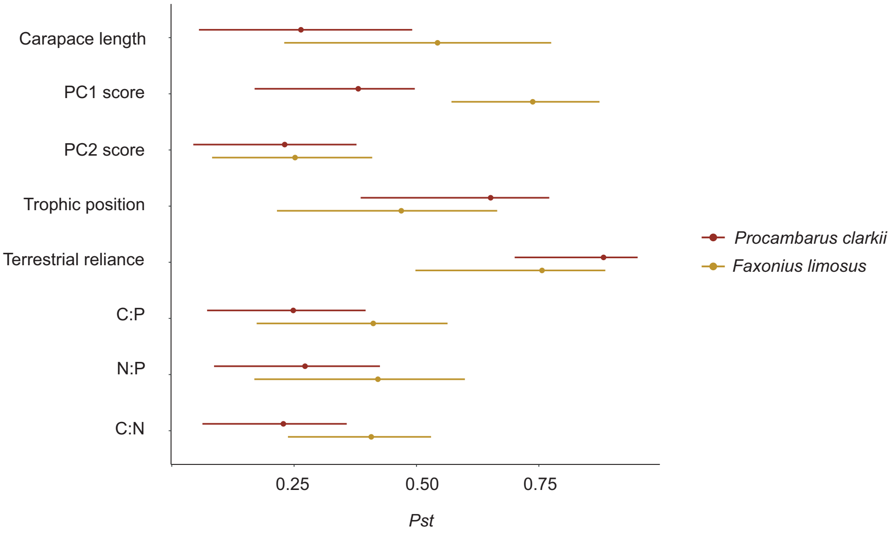

FIGURE 7 Comparison of P_{st} estimates for each trait (carapace length, PC1 and PC2 scores, trophic position, terrestrial reliance, C:P, N:P, C:N ratios), between *Procambarus clarkii* (red; $n = 257$) and *Faxonius limosus* (dark orange; $n = 246$) from 11 sympatric populations. Horizontal bars represent 95% confident interval of P_{st}, and the vertical dotted line represents 95% confident interval of F_{st} estimated using a bootstrap procedure

genetic variability for *P. clarkii* and *F. limosus*, respectively. We also highlighted sexual dimorphism in both species, which was more pronounced in *F. limosus*. As expected, variance in morphological traits was mainly explained at the individual level in *P. clarkii* and at the population level in *F. limosus*. Trophic traits were mainly explained at the population level and stoichiometric traits were mainly explained at the individual level for both species. Different factors explained the intraspecific variability of each species. For *P. clarkii*, morphological and stoichiometric traits were determined by both historical and environmental biotic determinants. For *F. limosus*, the morphology of individuals varied with environmental biotic conditions. Finally, we highlighted that different combinations of neutral and adaptive processes shaped intraspecific variability in *F. limosus* and *P. clarkii*. Globally, as expected, the relative importance of adaptive processes underlying the intraspecific variability in *F. limosus* was stronger compared to *P. clarkii*.

4.1 | Phenotypic variability

As expected, morphological trait variation was mainly explained at the individual level (within populations) for *P. clarkii* and at the population level (among populations) for *F. limosus*. This suggested that the fittest phenotypes might have been selected over time for *F. limosus* within populations, contrary to *P. clarkii*, which has been more recently established. Our results also revealed the existence of sexual dimorphism in morphological and stoichiometric traits for both crayfish species (Chybowski, 2007; Loureiro et al., 2015; Malavé et al., 2018), a dimorphism that seems to be associated with a trophic differentiation in *F. limosus*. Morphological differentiation between sexes supported the results from other studies that have explored morphological dimorphism on these two species: females displayed an elongated abdomen at equal size compared to males, as a result of carrying eggs under the abdomen (Chybowski, 2007; Wang et al., 2011). C:N:P composition of crayfish body is due to the balance between assimilation of elements from their environment, and excretion and egestion processes. The sexual dimorphism in stoichiometric traits might be due to different body proportions and different molting cycles between male and female individuals, and to differentiated elemental requirements in females for egg production (Buřič, Kouba, & Kozák, 2010a, 2010b; Færøvig & Hessen, 2003; Hamasaki et al., 2020).

For both species, stoichiometric traits and trophic traits seem highly context-dependent, suggesting that variability in the elemental composition of crayfish resulted from a strong environmental pressure due to local bioavailability of nutrients or contrasted trophic resources among populations. The greater trophic trait variance at the population level highlighted the opportunistic omnivorous diet of *P. clarkii* and *F. limosus*, which rely on available trophic resources (Correia, 2003; Larson et al., 2017). The sex effect on the trophic position was different than observed in previous studies, highlighting that sexual dimorphism varies among geographical areas (Larson et al., 2017). However, although the variation of the stoichiometric traits at the population level was notable (i.e. due to differences in available nutrients; El-Sabaawi, Kohler, et al., 2012; El-Sabaawi, Zandonà, et al., 2012), the major part of the variance was observed at the individual level for both species, which might be partly due to maturity and ontogenetic differences between individuals (González et al., 2011). Variations in the elemental composition can be considered as physiological plasticity, which may be reversible at relatively short time scales compared to morphological plasticity, which is fundamentally driven by physiological processes, but is more likely to be permanent (Bradshaw, 1965). The capacity of heterotrophic organisms to maintain their elemental composition in various environments when feeding on resources of different elemental composition is supposed to be high (Sterner & Elser, 2002). Our results highlight the non-strict homeostasis of *P. clarkii* and *F. limosus*. The high differences at the individual level suggest that individuals may have strongly different effects on the ecosystem functioning (i.e. through different excretion and egestion rates; Vanni & McIntyre, 2016).

4.2 | Environmental determinants

As expected, we found that morphological variation (carapace length) was explained by environmental characteristics for *F. limosus*, which might have undergone local adaptation. Body size adaptations generally maximise foraging efficiency and constrain the body-size distribution of organisms in the food web in return (Lundberg & Persson, 1993; Woodward & Warren, 2009). Here, carapace length of *F. limosus* individuals increased with increasing predation pressure and decreasing *P. clarkii* abundance (i.e. through density-dependent processes; Atkinson & Hirst, 2009). Larger individuals might have been selected over time since they might be less vulnerable to predation pressure through gape limitation (Garvey et al., 2003). Concerning *P. clarkii*, our results highlighted that both colonisation history (invasion time span) and environmental biotic determinants (predation pressure and abundance of *P. clarkii*) explained morphological variation, indicating that strong environmental pressure can rapidly lead to phenotypic variation in *P. clarkii*, following its establishment (<20 years; Evangelista, Lecerf, et al., 2019; Lang et al., 2020). Specifically, our results suggest that an elongated rostrum, a shortened cephalothorax, and a longer abdomen (i.e. deeper muscle involved in walking, Takahata et al., 1984) could facilitate overland dispersion and the colonisation of new environments. This hypothesis remains to be tested using quantitative genetics, by conducting a common garden study on genetically based phenotypic traits associated with fitness during range expansion (Keller & Taylor, 2008).

In *P. clarkii*, stoichiometric trait variation was also explained by colonisation history (invasion time span) due to its recent establishment, and environmental biotic determinants (predation pressure and abundance of *P. clarkii*). C:P and N:P variation are likely to be due to P variation since C and N contents are relatively uniform in the different classes of molecules and cellular structures in organisms (Sterner & Elser, 2002). Elemental composition variation in crayfish might be due to ontogeny differences, or differences of resource quality within the ecosystem. For instance, variation in C:P and N:P ratios in food resources between more recent and older populations might be caused by the trophic status of the lake, which increases with lake age (Colas et al., 2021). However, we failed to detect the environmental and historical determinants of stoichiometric variability in *F. limosus*.

Surprisingly, we failed to detect the determinants driving trophic trait variation for both crayfish species, and this is certainly because local resource availability in each lake was not considered in our models. Invasion time span might not have been identified as a determinant because trophic traits of invasive crayfish are suspected to be consistent over time (Larson et al., 2017). It is noteworthy that the trophic traits computation we used is based on the assumption that crayfish mainly feed on primary producers which were the only sampled trophic resources consistent among all studied lakes. However, the trophic position of approximately 3 suggested that they feed on more than one trophic level (i.e. primary producers, fish larvae/eggs, invertebrates including crayfish; Alcorlo et al., 2004; Gutiérrez-Yurrita et al., 1998; Vojkovská et al., 2014). Our results contrast with recent studies which demonstrated that *P. clarkii* had a more carnivorous diet than *F. limosus*, and that the latter relied more on plant-based food (Veselý et al., 2021). By contrast, we found that *F. limosus* had a slightly higher trophic position than *P. clarkii*. However, our results showed that *F. limosus* terrestrial reliance tended to decrease in the presence of *P. clarkii*, suggesting that *P. clarkii* might have outcompeted *F. limosus* in the littoral habitat, a pattern that has already been observed in sympatric invasive populations (Kreps et al., 2016; Veselý et al., 2021).

4.3 | Neutral versus adaptive processes

A combination of adaptive and neutral processes shaped the phenotypic variability within *F. limosus* and *P. clarkii*. As expected, neutral processes mostly shaped morphological and stoichiometric trait variation for *P. clarkii*, which has been established more recently in our study area. Trophic trait variation was due to adaptive processes, supporting our previous interpretation: this opportunistic generalist species relies on available trophic resources, constrained by the environment (Alcorlo et al., 2004; Correia, 2003; Linzmaier et al., 2020). Surprisingly, for *F. limosus*, adaptive and neutral processes contributed equally to the variation in each group of traits (i.e. morphological, trophic, and stoichiometric traits). Results of F_{st}/P_{st} comparisons might be biased for *F. limosus*, since F_{st} values were relatively high between populations in the study area even though genetic diversity for this species was very low, probably due to a combination of historical (all European populations descend from a very low number of individuals introduced in Poland in 1890; Filipová et al., 2011) and biological factors. Indeed, the species was reported to perform facultative parthenogenesis in captivity, although this remains to be confirmed in the wild (Buřič et al., 2011). This differentiation might be due to the differential effects of genetic drift (stochastic fixation of different alleles in different lakes) among lakes following the founder effects produced when lakes are invaded.

If there are some limitations to F_{st}/P_{st} comparisons within each species (Edelaar et al., 2011; Hendry, 2002), P_{st} for a given trait are comparable between both species in order to compare the relative importance of adaptive processes shaping their intraspecific variability. As expected, we found that the relative importance of adaptive processes shaping the phenotypic variability was greater in *F. limosus*, which has been established in our study area before *P. clarkii* and might have already experienced local adaptation. This difference of adaptation capacity between both species might be even greater considering that *P. clarkii* seemed to reproduce twice a year in our study system (Souty-Grosset et al., 2006). Multiple reproductions per year would result in greater population turn-over, which should definitely provide a great adaptation of the species in new environments (MacArthur & Wilson, 1967). However, the mating of *F. limosus* and *P. clarkii* has not been studied in our studied area and this could differ among the studied lakes since breeding is generally synchronised within populations, but not necessarily among populations (*personal observations*). It is worth noting that newly created lakes are rapidly colonised by *P. clarkii* in our study area, a pattern that has not been observed for *F. limosus* (Julien Cucherousset, personal observations). Hence, invasion time span might have been overestimated for *F. limosus* and adaptive processes might have occurred even more rapidly following the invasion for this species compared to *P. clarkii*. Monitoring F_{st} and P_{st} at the population level over time would be insightful for describing the succession of processes involved in phenotypic differentiation within and between species. Combined with the study of their population dynamics, such an approach would be relevant to develop efficient local management strategies aimed at limiting their ecological and economic impacts in the studied region.

5 | CONCLUSION

We highlighted strong morphological, trophic, and stoichiometric variation among and within populations from two co-occurring invasive species, and more importantly, we found a contrasting distribution of variance for each trait across three ecological scales (sex, individual, population), depending on the species. This suggests that invasive individuals have highly diverse impacts on ecosystem functioning even at small geographical scales, supporting previous findings (Evangelista, Lecerf, et al., 2019; Juette et al., 2014; Phillips & Shine, 2006), and that these impacts may differ depending on the considered ecological scale and the considered species. It is of high importance to improve our understanding of the ecological and evolutionary mechanisms that shape genetic and phenotypic variation of invasive species because it can inform us on many relevant features such as their resistance to disturbance (i.e. global changes, removal attempt), their ability to expand their range and their potential impacts on ecosystem functioning.

DATA AVAILABILITY STATEMENT

All data used in this study are available on Figshare. https://doi.org/10.6084/m9.figshare.14562150.

ACKNOWLEDGMENTS

We are grateful to two anonymous reviewers for all the comments and suggestions that have helped to improve the manuscript. We are thankful to the gravière team, particularly to Rémy Lassus, Paul Millet, Julien Salanon, Camille Mulatero, Gary Williams, Jérémy Fraysse, Bastien Jorigné, and Thibault Pivetta, for their help during the fieldwork. We also thank Charlotte Veyssière and Héloïse Bossuat for their precious help in the laboratory. This work was supported by the OFB (Office français de la biodiversité) as part of the ERADINVA project and by CNRS as part of the EC2CO project STOECHIOINVA. I.L. was supported by a PhD grant ATP from the University Toulouse III—Paul Sabatier.

AUTHOR CONTRIBUTION

I.L., I.P.V., G.L., and J.C. designed the study; I.L. and J.C. performed the fieldwork and I.L. performed trait measurement; I.L. and G.L. processed the genetic samples; I.L. and I.P.V. analysed the genetic data and performed statistical analyses; I.L. drafted the first version of the manuscript; and I.P.V., J.C., and G.L. contributed to revisions. All authors approved the final version of the manuscript.

ORCID

Iris Lang https://orcid.org/0000-0001-5329-5023
Ivan Paz-Vinas https://orcid.org/0000-0002-0043-9289
Julien Cucherousset https://orcid.org/0000-0003-0533-9479

REFERENCES

Ackerly, D. D. (2003). Community assembly, niche conservatism, and adaptive evolution in changing environments. *International Journal of Plant Sciences*, *164*(S3), S165–S184. https://doi.org/10.1086/368401

Aklehnovich, A., & Razlutskij, V. (2013). Distribution and spread of spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817) in Belarus. *BioInvasions Records*, *2*(3), 221–225. https://doi.org/10.3391/bir.2013.2.3.08

Alcorlo, P., Geiger, W., & Otero, M. (2004). Feeding preferences and food selection of the red swamp crayfish, *Procambarus clarkii*, in habitats differing in food item diversity. *Crustaceana*, *77*(4), 435–453. https://doi.org/10.1163/1568540041643283

Alcorlo, P., Geiger, W., & Otero, M. (2008). Reproductive biology and life cycle of the invasive crayfish *Procambarus clarkii* (Crustacea: Decapoda) in diverse aquatic habitats of South-Western Spain: Implications for population control. *Fundamental and Applied Limnology*, *173*(3), 197–212. https://doi.org/10.1127/1863-9135/2008/0173-0197

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, *25*(22), 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Alp, M., Cucherousset, J., Buoro, M., & Lecerf, A. (2016). Phenological response of a key ecosystem function to biological invasion. *Ecology Letters*, *19*(5), 519–527. https://doi.org/10.1111/ele.12585

Araújo, M. S., Daniel Bolnick, I., & Layman, C. A. (2011). The ecological causes of individual specialisation. *Ecology Letters*, *14*(9), 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Atkinson, D., & Hirst, A. G. (2009). Life histories and body size. In A. G. Hildrew, D. G. Raffaelli, & R. Edmonds-Brown (Eds.), *Body size: The structure and function of aquatic ecosystems* (Pp. 33–54). Cambridge University Press.

Aubret, F., & Shine, R. (2009). Genetic assimilation and the postcolonization erosion of phenotypic plasticity in Island Tiger snakes. *Current Biology*, *19*(22), 1932–1936. https://doi.org/10.1016/j.cub.2009.09.061

Bassar, R. D., Ferriere, R., López-Sepulcre, A., Marshall, M. C., Travis, J., Pringle, C. M., & Reznick, D. N. (2012). Direct and indirect ecosystem effects of evolutionary adaptation in the Trinidadian Guppy (*Poecilia Reticulata*). *The American Naturalist*, *180*(2), 167–185. https://doi.org/10.1086/666611

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., & Bonhomme, F. (1996). *GENETIX 4.05, Logiciel Sous Windows TM Pour La Génétique Des Populations*. Montpellier: Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II.

Bélouard, N., Paillisson, J. M., Oger, A., Besnard, A. L., & Petit, E. J. (2019). Genetic drift during the spread phase of a biological invasion. *Molecular Ecology*, *28*(19), 4375–4387. https://doi.org/10.1111/mec.15238

Bertram, S. M., Bowen, M., Kyle, M., & Schade, J. D. (2008). Extensive natural intraspecific variation in stoichiometric (C:N:P) composition in two terrestrial insect species. *Journal of Insect Science*, *8*(26), 1–7. https://doi.org/10.1673/031.008.2601

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., … Richardson, D. M. (2011). A proposed unified framework for biological invasions. *Trends in Ecology and Evolution*, *26*(7), 333–339. https://doi.org/10.1016/j.tree.2011.03.023

Blanchet, S., Prunier, J. G., Paz-Vinas, I., Saint-Pé, K., Rey, O., Raffard, A., … Dubut, V. (2020). A river runs through it: The causes, consequences, and management of intraspecific diversity in river networks. *Evolutionary Applications*, *13*(6), 1195–1213. https://doi.org/10.1111/eva.12941

Bradshaw, A. D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, *13*, 115–155. https://doi.org/10.1016/S0065-2660(08)60048-6

Buřič, M., Hulák, M., Kouba, A., Petrusek, A., & Kozák, P. (2011). A successful crayfish invader is capable of facultative parthenogenesis: A novel reproductive mode in decapod crustaceans. *PLoS One*, *6*(5), e20281. https://doi.org/10.1371/journal.pone.0020281

Buřič, M., Kouba, A., & Kozák, P. (2010a). Intra-sex dimorphism in crayfish females. *Zoology*, *113*(5), 301–307. https://doi.org/10.1016/j.zool.2010.06.001

Buřič, M., Kouba, A., & Kozák, P. (2010b). Molting and growth in relation to form alternations in the male spiny-cheek crayfish *Orconectes limosus*. *Zoological Studies*, *49*(1), 28–38.

Buřič, M., Kouba, A., & Kozák, P. (2013). Reproductive plasticity in freshwater invader: From long-term sperm storage to parthenogenesis. *PLoS One*, *8*(10), e77597. https://doi.org/10.1371/journal.pone.0077597

Changeux, T. (2003). Évolution de la répartition des écrevisses en France métropolitaine selon les enquêtes nationales mensées par le conseil supérieur de la pêche de 1977 à 2001. *Bulletin Français De La Pêche Et De La Pisciculture*, *370–371*, 17–41.

Chucholl, C., Stich, H. B., & Maier, G. (2008). Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*. *Fundamental and Applied Limnology*, *172*(1), 27–36. https://doi.org/10.1127/1863-9135/2008/0172-0027

Chybowski, L. (2007). Morphometrics, fecundity, density, and feeding intensity of the spinycheek crayfish, *Orconectes limosus* (Raf.) in natural conditions. *Archives of Polish Fisheries*, *15*(3), 175–241.

Colas, F., Baudoin, J.-M., Bonin, P., Cabrol, L., Daufresne, M., Lassus, R., & Cucherousset, J. (2021). Ecosystem maturity modulates greenhouse gases fluxes from artificial lakes. *Science of the Total Environment*, *760*, 144046. https://doi.org/10.1016/j.scitotenv.2020.144046

Correia, A. M. (2003). Food choice by the introduced crayfish *Procambarus clarkii*. *Annales Zoologici Fennici*, *40*(6), 517–528.

Correia, A. M., & Anastácio, P. M. (2008). Shifts in aquatic macroinvertebrate biodiversity associated with the presence and size of an alien crayfish. *Ecological Research*, *23*(4), 729–734. https://doi.org/10.1007/s11284-007-0433-5

Côté, I. M., Darling, E. S., Malpica-Cruz, L., Smith, N. S., Green, S. J., Curtis-Quick, J., & Layman, C. (2014). What doesn't kill you makes you wary? Effect of repeated culling on the behaviour of an invasive predator. *PLoS One*, *9*(4), e94248. https://doi.org/10.1371/journal.pone.0094248

Cruz, M. J., & Rebelo, R. (2007). Colonization of freshwater habitats by an introduced crayfish, *Procambarus clarkii*, in Southwest Iberian Peninsula. *Hydrobiologia*, *575*(1), 191–201. https://doi.org/10.1007/s10750-006-0376-9

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., … Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology & Evolution*, *2*(1), 57–64. https://doi.org/10.1038/s41559-017-0402-5

Edelaar, P., Burraco, P., & Gomez-Mestre, I. (2011). Comparisons between QST and FST – How wrong have we been? *Molecular Ecology*, *20*(23), 4830–4839. https://doi.org/10.1111/j.1365-294X.2011.05333.x

El-Sabaawi, R. W., Kohler, T. J., Zandoná, E., Travis, J., Marshall, M. C., Thomas, S. A., … Flecker, A. S. (2012). Environmental and organismal predictors of intraspecific variation in the stoichiometry of a neotropical freshwater fish. *PLoS One*, *7*(3), e32713. https://doi.org/10.1371/journal.pone.0032713

El-Sabaawi, R. W., Zandonà, E., Kohler, T. J., Marshall, M. C., Moslemi, J. M., Travis, J., … Flecker, A. S. (2012). Widespread intraspecific organismal stoichiometry among populations of the Trinidadian guppy. *Functional Ecology*, *26*(3), 666–676. https://doi.org/10.1111/j.1365-2435.2012.01974.x

Evangelista, C., Britton, R. J., & Cucherousset, J. (2015). Impacts of invasive fish removal through angling on population characteristics and juvenile growth rate. *Ecology and Evolution*, *5*(11), 2193–2202. https://doi.org/10.1002/ece3.1471

Evangelista, C., Cucherousset, J., Olden, J. D., & Lecerf, A. (2019). Scale-dependent patterns of intraspecific trait variations in two globally invasive species. *Oecologia*, *189*(4), 1083–1094. https://doi.org/10.1007/s00442-019-04374-4

Evangelista, C., Lecerf, A., & Cucherousset, J. (2019). Contrasting ecological impacts of geographically close invasive populations. *Oecologia*, *189*(2), 529–536. https://doi.org/10.1007/s00442-018-04333-5

Excoffier, L., & Ray, N. (2008). Surfing during population expansions promotes genetic revolutions and structuration. *Trends in Ecology and Evolution*, *23*(7), 347–351. https://doi.org/10.1016/j.tree.2008.04.004

Færøvig, P. J., & Hessen, D. O. (2003). Allocation strategies in crustacean stoichiometry: The potential role of phosphorus in the limitation of reproduction. *Freshwater Biology*, *48*(10), 1782–1792. https://doi.org/10.1046/j.1365-2427.2003.01128.x

Filipová, L., Lieb, D. A., Grandjean, F., & Petrusek, A. (2011). Haplotype variation in the spiny-cheek crayfish *Orconectes limosus*: Colonization of Europe and genetic diversity of native stocks. *Journal of the North American Benthological Society*, *30*(4), 871–881. https://doi.org/10.1899/10-130.1

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, *180*(2), 977–993. https://doi.org/10.1534/genetics.108.092221

Fourtune, L., Prunier, J. G., Mathieu-Bégné, E., Canto, N., Veyssière, C., Loot, G., & Blanchet, S. (2018). Intraspecific genetic and phenotypic diversity: Parallel processes and correlated patterns? *BioRxiv* 288357. https://doi.org/10.1101/288357

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression*, 3rd edn. Thousand Oaks, CA: Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Fryxell, D. C., Arnett, H. A., Apgar, T. M., Kinnison, M. T., & Palkovacs, E. P. (2015). Sex ratio variation shapes the ecological effects of a globally introduced freshwater fish. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1817), 20151970. https://doi.org/10.1098/rspb.2015.1970

Garvey, J. E., Rettig, J. E., Stein, R. A., Lodge, D. M., & Klosiewski, S. P. (2003). Scale-dependent associations among fish predation, littoral habitat, and distributions of crayfish species. *Ecology*, *84*(12), 3339–3348. https://doi.org/10.1890/02-0444

Gelman, A., & Hill, J. (2007). *Data analysis using regression and multilevel/hierarchical models*. Cambridge University Press.

Gelman, A., & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statistical Science*, *7*(4), 457–472. https://doi.org/10.2307/2246134

Gherardi, F., & Cioni, A. (2004). Agonism and interference competition in freshwater decapods. *Behaviour*, *141*(10), 1297–1324. https://doi.org/10.1163/1568539042729702

González, A. L., Fariña, J. M., Kay, A. D., Pinto, R., & Marquet, P. A. (2011). Exploring patterns and mechanisms of interspecific and intraspecific variation in body elemental composition of desert consumers. *Oikos*, *120*(8), 1247–1255. https://doi.org/10.1111/j.1600-0706.2010.19151.x

Goudet, J. (2002). Fstat Vision (1.2): A computer program to calculate F-statistics. *Journal of Heredity*, *86*, 485–486.

Green, A. J. (2016). The importance of waterbirds as an overlooked pathway of invasion for alien species. *Diversity and Distributions*, *22*(2), 239–247. https://doi.org/10.1111/ddi.12392

Gutiérrez-Yurrita, P. J., Sancho, G., Bravo, M. Á., Baltanás, Á., & Montes, C. (1998). Diet of the red swamp crayfish *Procambarus Clarkii* in natural ecosystems of the Doñana National Park temporary fresh-water marsh (Spain). *Journal of Crustacean Biology*, *8*(1), 120–127. https://doi.org/10.2307/1549526

Gutowsky, L. F. G., & Fox, M. G. (2012). Intra-population variability of life-history traits and growth during range expansion of the invasive round goby, *Neogobius melanostomus*. *Fisheries Management and Ecology*, *19*(1), 78–88. https://doi.org/10.1111/j.1365-2400.2011.00831.x

Hairston, N. G., Ellner, S. P., Geber, M. A., Yoshida, T., & Fox, J. A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters*, *8*(10), 1114–1127. https://doi.org/10.1111/j.1461-0248.2005.00812.x

Hamasaki, K., Osabe, N., Nishimoto, S., Dan, S., & Kitada, S. (2020). Sexual dimorphism and reproductive status of the red swamp crayfish *Procambarus clarkii*. *Zoological Studies*, *59*(7), 1–14. https://doi.org/10.6620/ZS.2020.59-07

Harmon, L. J., Matthews, B., Roches, S. D., Chase, J. M., Shurin, J. B., & Schluter, D. (2009). Evolutionary diversification in stickleback affects ecosystem functioning. *Nature*, *458*(7242), 1167–1170. https://doi.org/10.1038/nature07974

Hendry, A. P. (2002). QST > = ≠ < FST? *Trends in Ecology & Evolution*, *17*(11), P502. https://doi.org/10.1016/S0169-5347(02)02603-4

Hendry, A. P., Kinnison, M. T., Heino, M., Day, T., Smith, T. B., Fitt, G., … Carroll, S. P. (2011). Evolutionary principles and their practical application. *Evolutionary Applications*, *4*(2), 159–183. https://doi.org/10.1111/j.1752-4571.2010.00165.x

Herrmann, A., Schnabler, A., & Martens, A. (2018). Phenology of overland dispersal in the invasive crayfish *Faxonius immunis* (Hagen) at the Upper Rhine River area. *Knowledge & Management of Aquatic Ecosystems*, *419*(30), https://doi.org/10.1051/kmae/2018018

Holdich, D. M., & Black, J. (2007). The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817) [Crustacea: Decapoda: Cambaridae], digs into the UK. *Aquatic Invasions*, *2*(1), 1–15. https://doi.org/10.3391/ai.2007.2.1.1

Holdich, D. M., Reynolds, J. D., Souty-Grosset, C., & Sibley, P. J. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems*, *394–395*, 11. https://doi.org/10.1051/kmae/2009025

Holt, R. D., & Gaines, M. S. (1992). Analysis of adaptation in heterogeneous landscapes: Implications for the evolution of fundamental niches. *Evolutionary Ecology*, *6*(5), 433–447. https://doi.org/10.1007/BF02270702

Hudina, S., & Hock, K. (2012). Behavioural determinants of agonistic success in invasive crayfish. *Behavioural Processes*, *91*(1), 77–81. https://doi.org/10.1016/j.beproc.2012.05.011

Huey, R. B. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science*, *287*(5451), 308–309. https://doi.org/10.1126/science.287.5451.308

Hulák, M., Kaspar, V., Kozák, P., Buric, M., Filipová, L., & Petrusek, A. (2010). Cross-species amplification of microsatellite markers in the invasive spiny-cheek crayfish (*Orconectes limosus*): Assessment and application. *Journal of Applied Genetics*, *51*(1), 73–78. https://doi.org/10.1007/BF03195713

Jackson, M. C., Evangelista, C., Zhao, T., Antoine Lecerf, J., Britton, R., & Cucherousset, J. (2017). Between-lake variation in the trophic ecology of an invasive crayfish. *Freshwater Biology*, *62*(9), 1501–1510. https://doi.org/10.1111/fwb.12957

Jiang, H., Qian, Z., Wei, L. U., Xing, Z., Hongwei, Y. U., & Li, J. (2015). Microsatellite marker identification from transcriptome derived sequences of the red swamp crawfish, *Procambarus clarkii*. *Conservation Genetics Resources*, *7*(3), 729–731. https://doi.org/10.1007/s12686-015-0447-1

Juette, T., Cucherousset, J., & Cote, J. (2014). Animal personality and the ecological impacts of freshwater non-native species. *Current Zoology*, *60*(3), 417–427. https://doi.org/10.1093/czoolo/60.3.417

Keller, S. R., & Taylor, D. R. (2008). History, chance and adaptation during biological invasion: Separating stochastic phenotypic evolution from response to selection. *Ecology Letters*, *11*(8), 852–866. https://doi.org/10.1111/j.1461-0248.2008.01188.x

Kerby, J. L., Riley, S. P. D., Kats, L. B., & Wilson, P. (2005). Barriers and flow as limiting factors in the spread of an invasive crayfish (*Procambarus clarkii*) in Southern California streams. *Biological Conservation*, *126*(3), 402–409. https://doi.org/10.1016/j.biocon.2005.06.020

Kinnison, M. T., Unwin, M. J., & Quinn, T. P. (2008). Eco-evolutionary vs. habitat contributions to invasion in salmon: Experimental evaluation in the wild. *Molecular Ecology*, *17*(1), 405–414. https://doi.org/10.1111/j.1365-294X.2007.03495.x

Klingenberg, C. P. (2011). MORPHO J: An integrated software package for geometric morphometrics. *Molecular Ecology Resources*, *11*, 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Klingenberg, C. P. (2016). Size, shape, and form: Concepts of allometry in geometric morphometrics. *Development Genes and Evolution*, *226*(3), 113–137. https://doi.org/10.1007/s00427-016-0539-2

Klopfstein, S., Currat, M., & Excoffier, L. (2006). The fate of mutations surfing on the wave of a range expansion. *Molecular Biology and Evolution*, *23*(3), 482–490. https://doi.org/10.1093/molbev/msj057

Kouba, A., Tíkal, J., Cisař, P., Veselý, L., Fořt, M., Příborský, J., ... Buřič, M. (2016). The significance of droughts for hyporheic dwellers: Evidence from freshwater crayfish. *Scientific Reports*, *6*, 26569. https://doi.org/10.1038/srep26569

Kreps, T. A., Larson, E. R., & Lodge, D. M. (2016). Do invasive rusty crayfish (*Orconectes rusticus*) decouple littoral and pelagic energy flows in lake food webs? *Freshwater Science*, *35*(1), 103–113. https://doi.org/10.1086/683358

Lang, I., Evangelista, C., Everts, R. M., Loot, G., & Cucherousset, J. (2020). Stable resource polymorphism along the benthic littoral-pelagic axis in an invasive crayfish. *Ecology and Evolution*, *10*(5), 2650–2660. https://doi.org/10.1002/ece3.6095

Larson, E. R., Twardochleb, L. A., & Olden, J. D. (2017). Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*. *Limnology*, *18*(3), 275–286. https://doi.org/10.1007/s10201-016-0505-8

Leinonen, T., Cano, J. M., Mäkinen, H., & Merilä, J. (2006). Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology*, *19*(6), 1803–1812. https://doi.org/10.1111/j.1420-9101.2006.01182.x

Leinonen, T., McCairns, R. J. S., O'Hara, R. B., & Merilä, J. (2013). QST-FST comparisons: Evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews Genetics*, *14*(3), 179–190. https://doi.org/10.1038/nrg3395

Linzmaier, S. M., Musseau, C., Matern, S., & Jeschke, J. M. (2020). Trophic ecology of invasive marbled and spiny-cheek crayfish populations. *Biological Invasions*, *22*(11), 3339–3356. https://doi.org/10.1007/s10530-020-02328-z

Lodge, D. M., Deines, A., Gherardi, F., Yeo, D. C. J., Arcella, T., Baldridge, A. K., ... Zeng, Y. (2012). Global introductions of crayfishes: Evaluating the impact of species invasions on ecosystem services. *Annual Review of Ecology, Evolution, and Systematics*, *43*(1), 449–472. https://doi.org/10.1146/annurev-ecolsys-111511-103919

Loureiro, T. G., Manuel, P., Gentil, S., Araujo, P. B., & Almerão, M. P. (2015). Red swamp crayfish: Biology, ecology and invasion – An overview. *Nauplius*, *23*(1), 1–19. https://doi.org/10.1590/S0104-6497201400 2214

Lowe, W. H., Kovach, R. P., & Allendorf, F. W. (2017). Population genetics and demography unite ecology and evolution. *Trends in Ecology and Evolution*, *32*(2), 141–152. https://doi.org/10.1016/j.tree.2016.12.002

Lundberg, S., & Persson, L. (1993). Optimal body size and resource density. *Journal of Theoretical Biology*, *164*, 163–180. https://doi.org/10.1006/jtbi.1993.1146

Lutz, C. G., & Wolters, W. R. (1989). Estimation of heritabilities for growth, body size, and processing traits in red swamp crawfish, *Procambarus clarkii* (Girard). *Aquaculture*, *78*(1), 21–33. https://doi.org/10.1016/0044-8486(89)90003-3

MacArthur, R. H., & Wilson, E. O. (1967). *The theory of island biogeography*. Princeton University Press.

Mack, R. N., Daniel Simberloff, W., Lonsdale, M., Evans, H., Clout, M., & Bazzaz Fakhri, A. (2000). Biotic invasions, causes, epidemiology, global consequences and control. *Ecological Applications*, *10*(3), 689–710.

Magnier, J., & Petit, K. (2016). "L'enquête Nationale Sur Les Écrevisses." *Office International de l'eau*, *Note Technique*, 1–21.

Malavé, B. M., Styga, J. M., & Clotfelter, E. D. (2018). Size, shape, and sex-dependent variation in force production by crayfish chelae. *Journal of Morphology*, *279*, 312–318. https://doi.org/10.1002/jmor.20773

McGill, B. J. (2008). Exploring predictions of abundance from body mass using hierarchical comparative approaches. *American Naturalist*, *172*(1), 88–101. https://doi.org/10.1086/588044

Messier, J., McGill, B. J., & Lechowicz, M. J. (2010). How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters*, *13*(7), 838–848. https://doi.org/10.1111/j.1461-0248.2010.01476.x

Mims, M. C., Hartfield, E. E., Kirk, D. A., Lytle, D. A., & Olden, J. D. (2017). Traits-based approaches support the conservation relevance of landscape genetics. *Conservation Genetics*, *19*(1), 17–26. https://doi.org/10.1007/s10592-017-1028-5

Oficialdegui, F. J., Green, A. J., Clavero, M., Sánchez, M. I., Boyero, L., Michot, T. C., ... Lejeusne, C. (2019). Unravelling the global invasion routes of a worldwide invader, the red swamp crayfish (*Procambarus clarkii*). *Freshwater Biology*, *64*(2), 1–19. https://doi.org/10.1111/fwb.13312

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Parsons, T. R., Maita, Y., & Lalli, C. M. (1984). *A manual of chemical and biological methods for seawater analysis* (1st ed.). Pergamon Press.

Paz-Vinas, I., Quéméré, E., Chikhi, L., Loot, G., & Blanchet, S. (2013). The demographic history of populations experiencing asymmetric gene flow: Combining simulated and empirical data. *Molecular Ecology*, *22*(12), 3279–3291. https://doi.org/10.1111/mec.12321

Phillips, B. L., & Shine, R. (2006). An invasive species induces rapid adaptive change in a native predator: Cane toads and black snakes in Australia. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1593), 1545–1550. https://doi.org/10.1098/rspb.2006.3479

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2019). *Linear and Nonlinear Mixed Effects Models*. R package version 2.1-142.

Plummer, M., Best, N., Cowles, K., & Vines, K. (2006). CODA: Convergence diagnosis and output analysis for MCMC. *R News*, *6*(1), 7–11.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.2307/3071875

Prunier, J. G., Dubut, V., Loot, G., Tudesque, L., & Blanchet, S. (2018). The relative contribution of river network structure and anthropogenic stressors to spatial patterns of genetic diversity in two freshwater fishes: A multiple-stressors approach. *Freshwater Biology*, *63*(1), 6–21. https://doi.org/10.1111/fwb.13034

Puky, M. (2014). Invasive crayfish on land: *Orconectes limosus* (Rafinesque, 1817) (Decapoda: Cambaridae) crossed a terrestrial barrier to move from a side arm into the Danube River at Szeremle, Hungary. *Acta Zoologica Bulgarica*, *66*(S7), 143–146.

R Core Team. (2018). *R: A Language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Raffard, A., Cucherousset, J., Montoya, J. M., Richard, M., Acoca-Pidolle, S., Poésy, C., … Blanchet, S. (2021). Intraspecific diversity loss in a predator species alters prey community structure and ecosystem functions. *PLoS Biology*, *19*(3), e3001145. https://doi.org/10.1371/journal.pbio.3001145

Raffard, A., Lecerf, A., Cote, J., Buoro, M., Lassus, R., & Cucherousset, J. (2017). The functional syndrome: Linking individual trait variability to ecosystem functioning. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1868), 20171893. https://doi.org/10.1098/rspb.2017.1893

Raffard, A., Santoul, F., Cucherousset, J., & Blanchet, S. (2019). The community and ecosystem consequences of intraspecific diversity: A meta-analysis. *Biological Reviews*, *94*(2), 648–661. https://doi.org/10.1101/328112

Rey, O., Danchin, E., Mirouze, M., Loot, C., & Blanchet, S. (2016). Adaptation to global change: A transposable element-epigenetics perspective. *Trends in Ecology and Evolution*, *31*(7), 514–526. https://doi.org/10.1016/j.tree.2016.03.013

Rohlf, F. J. (2015). The Tps series of software. *Hystrix*, *the Italian Journal of Mammalogy*, *26*(1), 9–12. https://doi.org/10.4404/hystrix-26.1-11264

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Shea, K., & Chesson, P. (2002). Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution*, *17*(4), 170–176. https://doi.org/10.1016/S0169-5347(02)02495-3

Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., … Vilà, M. (2013). Impacts of biological invasions: What's what and the way forward. *Trends in Ecology and Evolution*, *28*(1), 58–66. https://doi.org/10.1016/j.tree.2012.07.013

Smith, B. J. (2007). Boa: An R PACKAGE for MCMC output convergence assessment and posterior inference. *Journal of Statistical Software*, *21*(11), 1–37. https://doi.org/10.18637/jss.v021.i11

Souty-Grosset, C., Holdich, D. M., Noël, P. Y., Reynolds, J. D., & Haffner, P. (2006). *Atlas of Crayfish in Europe*. Paris, France: Patrimoines naturels; 64. Muséum national d'Histoire naturelle.

Sterner, R. W., & Elser, J. J. (2002). *Ecological stoichiometry: The biology of elements from molecules to the biosphere*. Princeton University Press.

Strubbe, D., Broennimann, O., Chiron, F., & Matthysen, E. (2013). Niche conservatism in non-native birds in Europe: Niche unfilling rather than niche expansion. *Global Ecology and Biogeography*, *22*(8), 962–970. https://doi.org/10.1111/geb.12050

Takahata, M., Komatsu, H., & Hisada, M. (1984). Positional orientation determined by the behavioural context in *Procambarus clarkii* Girard (Decapoda: Macrura). *Behaviour*, *88*(3–4), 240–264. https://doi.org/10.1163/156853984X00344

Thomas, J. R., Masefield, S., Hunt, R., Wood, M. J., Hart, A. G., Hallam, J., … Cable, J. O. (2019). Terrestrial emigration behaviour of two invasive crayfish species. *Behavioural Processes*, *167*, 103917. https://doi.org/10.1016/j.beproc.2019.103917

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Vanni, M. J., & McIntyre, P. B. (2016). Predicting nutrient excretion of aquatic animals with metabolic ecology and ecological stoichiometry: A global synthesis. *Ecology*, *97*(12), 3460–3471. https://doi.org/10.1002/ecy.1582

Vellend, M., & Geber, M. A. (2005). Connections between species diversity and genetic diversity. *Ecology Letters*, *8*(7), 767–781. https://doi.org/10.1111/j.1461-0248.2005.00775.x

Vellend, M., Lajoie, G., Bourret, A., Múrria, C., Kembel, S. W., & Garant, D. (2014). Drawing ecological inferences from coincident patterns of population- and community-level biodiversity. *Molecular Ecology*, *23*(12), 2890–2901. https://doi.org/10.1111/mec.12756

Veselý, L., Ruokonen, T. J., Weiperth, A., Kubec, J., Szajbert, B., Guo, W., … Kouba, A. (2021). Trophic niches of three sympatric invasive crayfish of EU concern. *Hydrobiologia*, *848*(3), 727–737. https://doi.org/10.1007/s10750-020-04479-5

Vojkovská, R., Horká, I., & Ďuriš, Z. (2014). The diet of the spiny-cheek crayfish *Orconectes limosus* in the Czech Republic. *Central European Journal of Biology*, *9*(1), 58–69. https://doi.org/10.2478/s11535-013-0189-y

Wang, Q., Yang, J. X., Zhou, G. Q., Zhu, Y. A., & Shan, H. (2011). Length-weight and chelae length-width relationships of the crayfish *Procambarus clarkii* under culture conditions. *Journal of Freshwater Ecology*, *26*(2), 287–294. https://doi.org/10.1080/02705060.2011.564380

Woodward, G., & Warren, P. (2009). Body size and predatory interactions in freshwaters: Scaling from individuals to communities. In A. G. Hildrew, D. G. Raffaelli, & R. Edmonds-Brown (Eds.), *Body size: The structure and function of aquatic ecosystems* (Pp. 98–117). Cambridge University Press.

Yue, G. H., Wang, G. L., Zhu, B. Q., Wang, C. M., Zhu, Z. Y., & Lo, L. C. (2008). Discovery of four natural clones in a crayfish species *Procambarus clarkii*. *International Journal of Biological Sciences*, *4*(5), 279–282. https://doi.org/10.7150/ijbs.4.279

Závorka, L., Lassus, R., Britton, R., & Cucherousset, J. (2020). Phenotypic responses of invasive species to removals affect ecosystem functioning. *Global Change Biology*, *26*(10), 5693–5704. https://doi.org/10.1111/gcb.15271

Zelditch, M. L., Swiderski, D. L., & Sheets, H. D. (2012). *Geometric morphometrics for biologists: A primer* (2nd ed.). Academic Press.

Zhao, T., Grenouillet, G., Pool, T., Tudesque, L., & Cucherousset, J. (2016). Environmental determinants of fish community structure in gravel pit lakes. *Ecology of Freshwater Fish*, *25*, 412–421. https://doi.org/10.1111/eff.12222

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Lang, I., Paz-Vinas, I., Cucherousset, J., & Loot, G. (2021). Patterns and determinants of phenotypic variability within two invasive crayfish species. *Freshwater Biology*. 66, 1782–1798. https://doi.org/10.1111/fwb.13792