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Genetic and phenotypic variability within two invasive species: patterns, determinants and consequences on freshwater ecosystem functioning

Iris Lang

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THÈSE

**En vue de l'obtention du
DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE**

Délivré par l'Université Toulouse 3 - Paul Sabatier

Présentée et soutenue par

Iris LANG

Le 4 décembre 2020

**Variabilité génétique et phénotypique de deux espèces
invasives : patrons, déterminants et implications pour le
fonctionnement des écosystèmes dulçaquicoles.**

Ecole doctorale : **SEVAB - Sciences Ecologiques, Vétérinaires, Agronomiques et
Bioingenieries**

Spécialité : **Ecologie, biodiversité et évolution**

Unité de recherche :

EDB - Evolution et Diversité Biologique

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« Elles se touchent toutes »
Petit Lac Lamartine, 19 juillet 2018

À grand-mémé.

Avant-propos

Cette thèse de l'école doctorale SEVAB a été réalisée au Laboratoire Évolution & Diversité Biologique au sein de l'équipe AQUAECO. Elle a été financée par une bourse doctorale ATP de l'Université Paul Sabatier – Toulouse III et s'est appuyée sur le projet ERADINVA (Office Français pour la Biodiversité - OFB), le projet DISPERSINVA (Agence de l'Eau Adour-Garonne et UNPG) et le projet EC2CO STOECHIOINVA (CNRS INEE).

Cette thèse est composée d'une Introduction générale (Chapitre I), d'une présentation des modèles et des sites d'étude (Chapitre II), de quatre chapitres sous la forme d'articles scientifiques (Chapitres III, IV, V et VI) et d'une Discussion générale (Chapitre VII).

Une partie de l'Introduction générale renvoie à un article publié dans *Frontiers in Ecology and the Environment* (Annexe I). Le Chapitre III est en cours de soumission dans la revue *Journal of Applied Ecology*. Le Chapitre IV est en cours de soumission dans la revue *Freshwater Biology*. Le Chapitre V a été publié dans la revue *Ecology and Evolution*. Enfin, le Chapitre VI est un manuscrit en cours de préparation.

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Chapitre I

Introduction générale

Depuis les années 90 l'intérêt scientifique pour les relations entre biodiversité et fonctionnement des écosystèmes est grandissant (Loreau et al., 2001; Reiss et al., 2009). Dans le contexte actuel de crise de la biodiversité, il est d'autant plus important de comprendre ces relations. Les études à la fois théoriques et expérimentales ont démontré l'importance de la biodiversité sur la dynamique des écosystèmes (Naeem et al., 1994; Tilman & Downing, 1994; Loreau, 2000; Griffin et al., 2009). De manière générale, il est reconnu qu'une biodiversité importante augmente la productivité des écosystèmes, ce qui peut favoriser la stabilité des processus écologiques, et offre une meilleure résistance et résilience des écosystèmes aux perturbations d'origine naturelle ou anthropique (Hooper et al., 2005). Un des mécanismes sous-jacents à cette capacité de résilience est la redondance fonctionnelle, c'est-à-dire la multiplicité d'espèces ayant des fonctions équivalentes dans l'écosystème (Chapin et al., 1992; Peterson et al., 1998). La redondance fonctionnelle permet donc de compenser la perte de certaines espèces par un effet tampon qui assure le maintien des processus écologiques au sein de l'écosystème perturbé (Hooper et al., 2005). Ceci illustre la complexité de la notion de biodiversité et l'importance de ne pas considérer uniquement la biodiversité taxonomique ou phylogénique pour comprendre les relations entre biodiversité et fonctionnement des écosystèmes, mais de considérer également la biodiversité fonctionnelle (Díaz et al., 2007).

Variabilité intraspécifique

La complexité de la biodiversité réside également dans son organisation, puisqu'elle peut être mesurée à différents niveaux d'organisation biologique, allant du gène à l'écosystème. Pendant longtemps la variabilité intraspécifique a été négligée au détriment de la variabilité interspécifique : autrement dit, tous les individus d'une même espèce étaient considérés comme écologiquement équivalents (Díaz & Cabido, 2001; Lavorel & Garnier, 2002). Il est maintenant admis qu'au sein d'une même espèce les individus peuvent être spécialisés, c'est-à-dire occuper des niches écologiques différentes (Bolnick et al., 2003). En conséquence, les individus d'une même espèce peuvent avoir des effets différents sur le fonctionnement et les dynamiques écologiques et évolutives parce qu'ils présentent des génotypes et/ou des phénotypes distincts (Bolnick et al., 2003, 2011).

Variabilité phénotypique : variations et covariations de traits

Darwin considérait que les variations intraspécifiques étaient d'origine aléatoire, et qu'elles pouvaient faire l'objet d'une sélection naturelle favorisant les individus avec les caractères les plus avantageux pour la survie, c'est-à-dire les individus avec la plus forte valeur sélective (*fitness*¹ en anglais ; Arnold, 1983). La valeur sélective dans un environnement donné mesure donc la capacité d'un génotype à contribuer à la génération suivante et dépend de la survie, de la croissance et de la fécondité des individus. Ces propriétés, ainsi que toutes les caractéristiques morphologiques, physiologiques, phénologiques et comportementales des individus sont appelées traits, et forment le phénotype des individus (Bolnick et al., 2003; Violle et al., 2007). La variabilité phénotypique correspond donc aux variations de traits entre individus d'une même espèce. Quand les traits traduisent les performances écologiques des individus et ainsi leur valeur sélective, ils sont qualifiés de traits fonctionnels (McGill et al., 2006; Violle et al., 2007). Les traits de réponse traduisent la réponse des organismes à des variations environnementales et sont à distinguer des traits d'effet qui décrivent l'effet des individus sur le fonctionnement de l'écosystème dont ils font partie (Violle et al., 2012; Díaz et al., 2013). Certains traits sont à la fois considérés comme des traits de réponse et d'effet au regard des questions de recherche soulevées (Díaz et al., 2013). C'est par exemple le cas des traits dits stœchiométriques², qui caractérisent la composition élémentaire corporelle des organismes (e.g. ratios Carbone:Azote:Phosphore, noté C:N:P; Leal et al., 2017). Les traits stœchiométriques peuvent par exemple refléter le régime alimentaire et l'allocation des nutriments selon le stade de développement des organismes (trait de réponse; Vrede et al., 2011), ou encore les taux d'excrétion des individus qui affectent la disponibilité des nutriments dans le milieu pour les autres organismes (trait d'effet; Vanni & McIntyre, 2016).

Il existe des intercorrélations entre les traits fonctionnels au sein des individus (autrement appelées covariation, intégration ou syndrome phénotypiques), c'est-à-dire que les valeurs de traits dépendent des valeurs d'autres traits au sein d'un individu. Le polymorphisme de ressource, à savoir l'existence de variations d'utilisation des ressources spatiales (i.e. l'habitat) ou trophiques associées à des morphologies différentes des individus, constitue un exemple très simple de covariations de traits (Smith & Skúlason, 1996). Les covariations phénotypiques ont principalement fait l'objet d'études cherchant à expliquer l'interdépendance du comportement et des traits physiologiques des organismes (Careau et

¹ Dans le manuscrit sont précisés les termes anglais communément utilisés par les écologues francophones.

² La stœchiométrie correspond à la composition élémentaire corporelle des organismes (e.g. proportions de carbone, azote, phosphore et ratios entre ces éléments).

al., 2008; Biro & Stamps, 2010; Reale et al., 2010; Dingemanse et al., 2012). Ces covariations phénotypiques sont autant amenées à changer dans les populations naturelles sous l'effet de la sélection et de la plasticité, que les moyennes de traits et leurs variances (Pigliucci, 2003), et elles traduisent à la fois les contraintes environnementales et les contraintes ontogénétiques propre aux organismes (Murren, 2012; Armbruster et al., 2014; Laughlin & Messier, 2015). Tout comme les valeurs moyennes et les variances de traits, les covariations de traits varient donc entre espèces (variation interspécifique) mais également au sein d'une même espèce (variation intraspécifique ; Blanck & Lamouroux, 2007; Peiman & Robinson, 2017; Raffard et al., 2019a).

Le cadre conceptuel le plus utilisé au cours des dernières années pour caractériser les covariations phénotypiques est sûrement celui de l'hypothèse du syndrome du rythme de vie (*Pace-of-life syndrome* ou *POLS* en anglais; Reale et al., 2010; Dammhahn et al., 2018). Cette hypothèse est fondée sur l'idée que les traits d'histoire de vie des individus (i.e. croissance, survie, reproduction) ainsi que leurs traits physiologiques, comportementaux et morphologiques ont co-évolué sous l'effet des contraintes environnementales. La connaissance des relations entre traits, et donc des compromis entre les différentes fonctions des organismes et de leurs contraintes physiologiques rend possible (i) la prédiction de valeurs de traits basée sur un nombre limité de traits mesurés et (ii) l'inférence des effets des individus sur le fonctionnement de l'écosystème, autrement dit, l'intégration fonctionnelle (Armbruster et al., 2014). C'est dans cette deuxième optique que Raffard et al. (2017) ont développé le concept du *syndrome fonctionnel*, pour une compréhension plus intégrative des effets des individus sur le fonctionnement des écosystèmes. Le *syndrome fonctionnel* se définit comme l'association d'une suite de traits de réponse (*syndrome de réponse*) et de traits d'effet (*syndrome d'effet*) corrélés entre eux, respectivement (Raffard et al., 2017). Dans cette étude, des traits physiologiques comme le métabolisme (e.g. consommation d'oxygène) et la stœchiométrie des individus semblent être les principaux facteurs expliquant les covariations entre traits et entre syndromes de réponse et d'effet.

Processus évolutifs et déterminants écologiques de la variabilité intraspécifique

La variabilité phénotypique présentée par les individus d'une même espèce peut souligner des différences génétiques entre ces individus. La variabilité génétique d'une population est façonnée par des flux de gènes et se traduit en général par sa richesse et sa fréquence allélique³. Actuellement il est accepté que la variabilité génétique d'une population résulte de

³ Allèles : différentes versions d'un même gène.

l'histoire démographique des individus considérés, ainsi que de processus évolutifs tels que (i) les mutations qui permettent la formation de nouveaux allèles, (ii) la migration qui entraîne des flux de gènes, (iii) la dérive génétique et (iv) la sélection qui conduisent à la perte ou à la fixation d'allèles, de manière aléatoire ou non, respectivement (Freeland et al., 2012; Box 1).

Box 1

Forces évolutives

Les mutations sont des événements ponctuels augmentant la variabilité génétique qui sont reconnus pour être majoritairement neutres vis-à-vis de la valeur sélective des individus. Leur maintien dans les populations sera déterminé par une combinaison entre hasard et sélection (Freeland et al., 2012).

La migration est souvent liée à la dispersion d'individus entre populations qui peut entraîner des flux de gènes si les individus produisent une descendance viable. Les flux de gènes augmentent la diversité génétique d'une population mais tendent à homogénéiser les populations entre elles.

La dérive génétique correspond à l'évolution aléatoire des fréquences alléliques dans une population, indépendamment des mutations et des flux de gènes. C'est une force évolutive très marquée dans les populations isolées et de petit effectif, pouvant conduire à une perte de diversité génétique de ces populations par la fixation et/ou la perte d'allèles (Hartl & Clark, 1997). À long terme, la dérive génétique peut entraîner des différenciations génétiques et phénotypiques entre populations (Rogell et al., 2010).

La sélection constitue un autre processus adaptatif clef des dynamiques éco-évolutives⁴ qui affecte directement les phénotypes indépendamment des génotypes associés. La variabilité génétique y est indirectement soumise, par sa relation aux traits phénotypiques affectant la valeur sélective des individus (Hendry, 2016). La sélection permet une adaptation locale des meilleurs phénotypes par une maximisation de la valeur sélective des individus dans un environnement donné. Les phénotypes sélectionnés sont donc fortement dépendants des conditions biotiques et abiotiques locales, et seraient moins performants dans d'autres conditions environnementales.

⁴ Les dynamiques éco-évolutives traduisent les relations réciproques entre les processus écologiques et les processus évolutifs, aussi appelées boucles de rétroaction éco-évolutives. Elles correspondent à l'effet de l'évolution des individus sur la population, la communauté et l'écosystème mais aussi à l'effet de l'environnement qui modifie en retour l'évolution des individus (Hendry, 2016).

Les contraintes environnementales comme l'isolement géographique de la population (éloignement, ou barrière de dispersion d'individus) peuvent limiter les flux de gènes et façonner la variabilité génétique des populations (Manel et al., 2003). Les contraintes environnementales peuvent également entraîner un changement de phénotype sans changement de génotype appelé plasticité phénotypique, qui confère une capacité adaptative aux individus (Bradshaw, 1965). La plasticité phénotypique offre donc une rapidité de réponse (e.g. comportementale, physiologique, alimentaire, morphologique) des individus, ce qui la rend essentielle à court terme, en particulier dans le contexte de changement global (Aitken et al., 2008; Matesanz et al., 2010; Caruso et al., 2014). Il est important de souligner que s'il est généralement reconnu que la plasticité phénotypique confère une capacité d'adaptation aux individus, elle ne favorise pas toujours une augmentation de la valeur sélective, par exemple dans le cas de gros écarts fréquents de conditions environnementales (e.g. plasticité neutre ou mal-adaptative ; Ghalambor et al., 2007). La plasticité phénotypique peut également résulter de l'interaction gènes x environnement, c'est-à-dire que la différence entre deux phénotypes produits par deux génotypes différents varie en fonction des conditions environnementales. Des mécanismes relevant du contrôle endocrinien ou encore de l'épigénétique peuvent expliquer cette interaction (Nijhout, 2003; Vogt, 2017).

Les déterminants des covariations de traits restent à ce jour peu compris (Peiman & Robinson, 2017; Diaz Pauli et al., 2020). Une intégration génétique codant pour les traits (Via & Hawthorne, 2005; Jones et al., 2014), des réponses plastiques similaires ou différenciées de certains traits (Schlichting, 1989) ou encore des pressions de sélection favorisant certaines combinaisons de traits (Donovan et al., 2011; Taff et al., 2012) vont définir le nombre et l'intensité des covariations de traits d'un individu. Peu d'études se sont attachées à élucider la part de ces différents mécanismes dans la formation et le maintien des covariations de traits. Ces covariations procurent une capacité adaptative aux organismes, puisqu'elles leur confèrent la capacité de changer de phénotype de manière cohérente grâce à l'interdépendance des traits. En revanche, un fort syndrome fonctionnel peut traduire une faible plasticité phénotypique, et par conséquent limiter les individus à des conditions environnementales restreintes (Gianoli & Palacio-López, 2009).

Les patrons de variabilité génétique et phénotypique résultent donc à la fois de processus neutres impliquant le hasard (dérive, mutation, migration) et de processus adaptatifs (sélection, plasticité phénotypique) au regard de l'évolution. Il est complexe de mesurer l'héritabilité de nombreux traits dans les populations en milieu naturel, c'est-à-dire leur part

de variation due à des effets génétiques. En conséquence la variabilité génétique « neutre » est souvent utilisée pour estimer le potentiel adaptatif des populations ou des individus (Frankham et al., 2002). La comparaison des différences génétiques neutres (mesurées avec des marqueurs génétiques de type microsatellites⁵ par exemple) et des différences phénotypiques (pour des traits quantitatifs comme la taille du corps ou la position trophique par exemple) permet donc d'estimer les importances relatives des processus neutres et des processus adaptatifs dans la différenciation des individus ou des populations, sans différencier la sélection et la plasticité (Leinonen et al., 2006; Brommer, 2011; Merilä & Hendry, 2014).

Variabilité intraspécifique et fonctionnement des écosystèmes

Les conséquences de la variabilité intraspécifique sur la structure des communautés et le fonctionnement des écosystèmes sont de plus en plus reconnues et étudiées (Des Roches et al., 2017; Raffard et al., 2019b). D'une manière générale la variabilité génétique a surtout été considérée pour son importance dans les processus évolutifs. Cependant, la variabilité génétique au sein des espèces est également reconnue pour avoir des conséquences au niveau des populations mais aussi à des échelles supérieures (e.g. communautés et écosystèmes) qui sont appelées *phénotypes étendus* en opposition au phénotype 'traditionnel' dont nous avons déjà parlé dans cette introduction (*extended phenotypes* en anglais; Dawkins, 1982; Whitham et al., 2003). Les potentielles conséquences de la variabilité génétique peuvent être directes ou indirectes (Figure I.1). Par exemple, des génotypes différents au sein d'une espèce de plantes peuvent avoir des conséquences différentes sur les cycles biogéochimiques du carbone et de l'azote, et par extension sur les processus de décomposition de la litière (Madritch & Hunter, 2002; Schweitzer et al., 2005, 2011; Rudman et al., 2015). Intuitivement, la variabilité génétique a principalement des effets indirects sur le fonctionnement des écosystèmes, à travers les phénotypes qu'elle façonne. La variabilité génétique des traits écologiquement importants chez une espèce peut donc avoir un rôle notable sur le fonctionnement de l'écosystème. Hughes et al. (2008) soulignent également que « *la variabilité intraspécifique peut être équivalente à la variabilité interspécifique dans des écosystèmes dominés par une ou très peu d'espèces, ou encore si la variabilité génétique de l'espèce considérée affecte la distribution et l'abondance d'une espèce dite « clef de voûte », c'est-à-dire une espèce qui a un effet disproportionné sur son environnement par rapport à son effectif ou sa biomasse* » (Paine, 1995; Crawford et al., 2007).

⁵ Séquences courtes d'ADN (motifs de nucléotides) répétées en tandem qui sont parmi les marqueurs neutres les plus utilisés en génétique des populations (Balloux & Lugon-Moulin, 2002; Selkoe & Toonen, 2006).

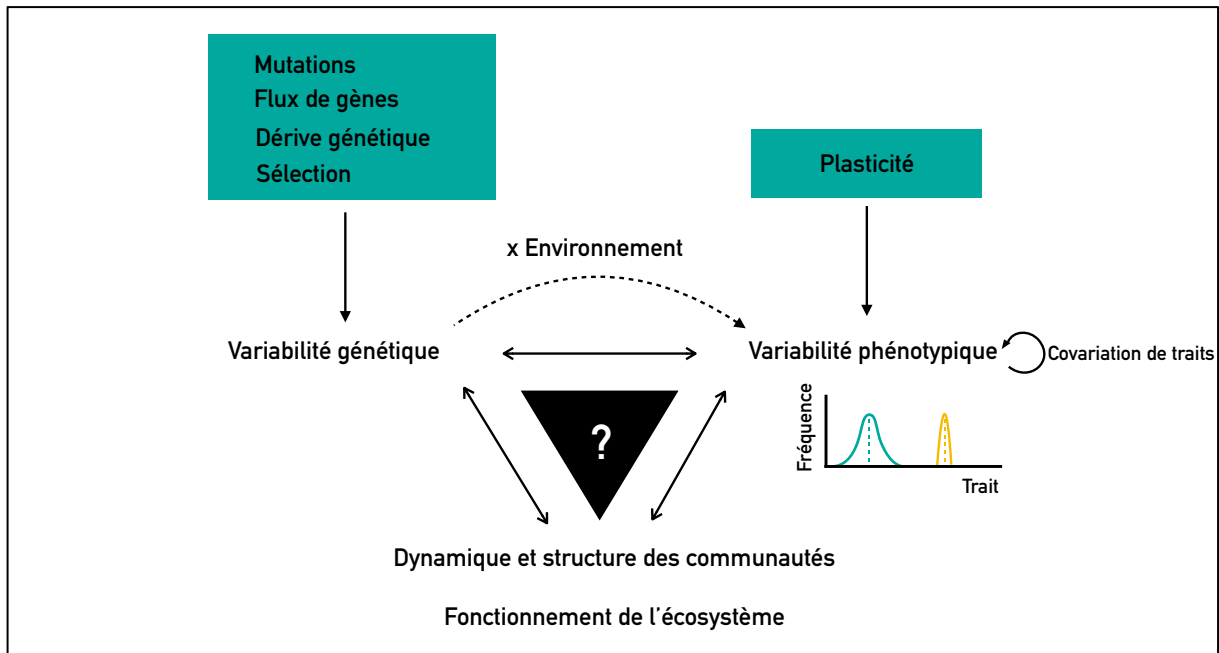


Figure I.1 Relations entre variabilités génétique et phénotypique, environnement et fonctionnement de l'écosystème.

Les approches écologiques basées sur les traits sont de plus en plus utilisées pour étudier les liens entre biodiversité et fonctionnement des écosystèmes (Bolnick et al., 2011; Cadotte et al., 2011; Matthews et al., 2014; Violle et al., 2014), et sont d'autant plus pertinentes lorsqu'elles se concentrent sur des traits d'effet. Les effets de la variabilité intraspécifique peuvent être tout aussi forts que l'effet de l'ajout ou le retrait d'une espèce dans l'écosystème, d'autant plus si les variations interindividuelles considérées relèvent de traits particulièrement importants au regard des processus écosystémiques (Des Roches et al., 2017). Il est généralement accepté que l'intensité des effets des individus sur les processus écosystémiques est fortement dépendante de la taille des organismes (Woodward et al., 2005) et de leur métabolisme (Brown et al., 2004). Si les différences fonctionnelles entre individus sont faibles, alors c'est plutôt l'abondance ou la biomasse des organismes qui prédira les impacts des individus sur le fonctionnement de l'écosystème que la variabilité intraspécifique (Wong et al., 2019). Les traits décrivant la niche trophique des individus permettent d'appréhender leur implication dans les flux de nutriments et d'énergie au sein de la chaîne alimentaire. La dynamique de cette chaîne alimentaire est également régulée par des mécanismes descendants et ascendants qui constituent des processus clés de l'écosystème. Les cascades trophiques (effets descendants, *top-down* en anglais) traduisent l'impact des individus de niveau trophique supérieur sur les niveaux inférieurs (Shurin et al., 2006). Il existe aussi des effets ascendants (*bottom-up* en anglais) qui résultent de la

disponibilité en ressources et/ou nutriments qui affectent la biomasse des niveaux trophiques supérieurs (Shurin et al., 2006). Les traits stœchiométriques de feuilles de peuplier peuvent par exemple affecter les interactions entre plantes, insectes herbivores et oiseaux insectivores : le taux de consommation et la préférence des oiseaux pour certains individus dépend de la stœchiométrie des feuilles consommées par les insectes (Müller et al., 2006). D'une manière générale, la stœchiométrie des organismes peut impacter les cycles biogéochimiques du carbone, du phosphore et de l'azote (Leal et al., 2017; Welti et al., 2017). Une étude a montré que la stœchiométrie des consommateurs primaires peut affecter les taux de décomposition de la litière à travers les communautés microbiennes (Hawlena et al., 2012) et par extension, affecter la biodisponibilité des nutriments dans le milieu. Souvent, les processus écologiques de l'écosystème sont prédits par l'association de différentes valeurs de traits.

Nous avons vu plus haut que les traits de réponse, au travers de corrélations avec les traits d'effets, peuvent également affecter l'intensité et la nature des processus écosystémiques induits par les organismes. A l'aide d'une modélisation bioénergétique, Raffard et al. (2017) ont montré que des variations de syndrome réponse chez l'écrevisse de Louisiane, *Procambarus clarkii*, pouvaient modifier les taux de décomposition de litière par les écrevisses avec la même ampleur que des variations importantes de taille de population. D'avantage d'études incluant des approches multi-traits sont nécessaires à l'avenir pour mieux comprendre les implications des covariations de traits sur le fonctionnement des écosystèmes. En particulier, une meilleure connaissance des syndromes fonctionnels entre populations de même espèce (Raffard et al., 2019a) mais également entre espèces qui coexistent permettrait de prévoir les conséquences de la variabilité intraspécifique sur les structures de communautés et le fonctionnement des écosystèmes.

Invasions biologiques

Multiplication des invasions biologiques dans le contexte de changement global

Les invasions biologiques constituent une des principales causes d'érosion de la biodiversité mondiale, avec la destruction et la perte d'habitat (Vitousek et al., 1997; Sala et al., 2000; Brook et al., 2008). Le processus d'invasion, qui conduit à considérer une espèce non native comme invasive⁶, se compose d'une succession d'étapes au cours desquelles les individus exotiques sont filtrés au travers de barrières géographique, environnementale, reproductive et de dispersion (Richardson et al., 2000; Blackburn et al., 2011)(Figure I.2). En premier lieu l'espèce doit être transportée et introduite hors de son aire de répartition naturelle. La seconde étape est la naturalisation de l'espèce dans son nouveau milieu, ce qui correspond au maintien de la capacité de survie et de reproduction. Troisièmement, l'espèce doit pouvoir étendre sa nouvelle aire de répartition géographique à partir du point d'introduction par dispersion spontanée. Enfin, la définition d'une espèce invasive implique que, d'un point de vue anthropocentré, elle a des impacts négatifs d'ordre économique, écologique et/ou sanitaire (Lockwood et al., 2007; Blackburn et al., 2011). L'introduction d'espèces peut être délibérée (e.g. plantes ou animaux d'ornementation, cultures) ou accidentelle (e.g. échappements, eaux de ballast, plantes obsidionales). Dans le contexte de globalisation des échanges mondiaux, les introductions d'espèces hors de leur aire de répartition naturelle se sont multipliées et accélérées au cours des dernières années (Lockwood et al., 2007; Gozlan, 2008; Seebens et al., 2017).

⁶ Dans la législation française le terme utilisé est « espèce exotique envahissante (EEE) ».

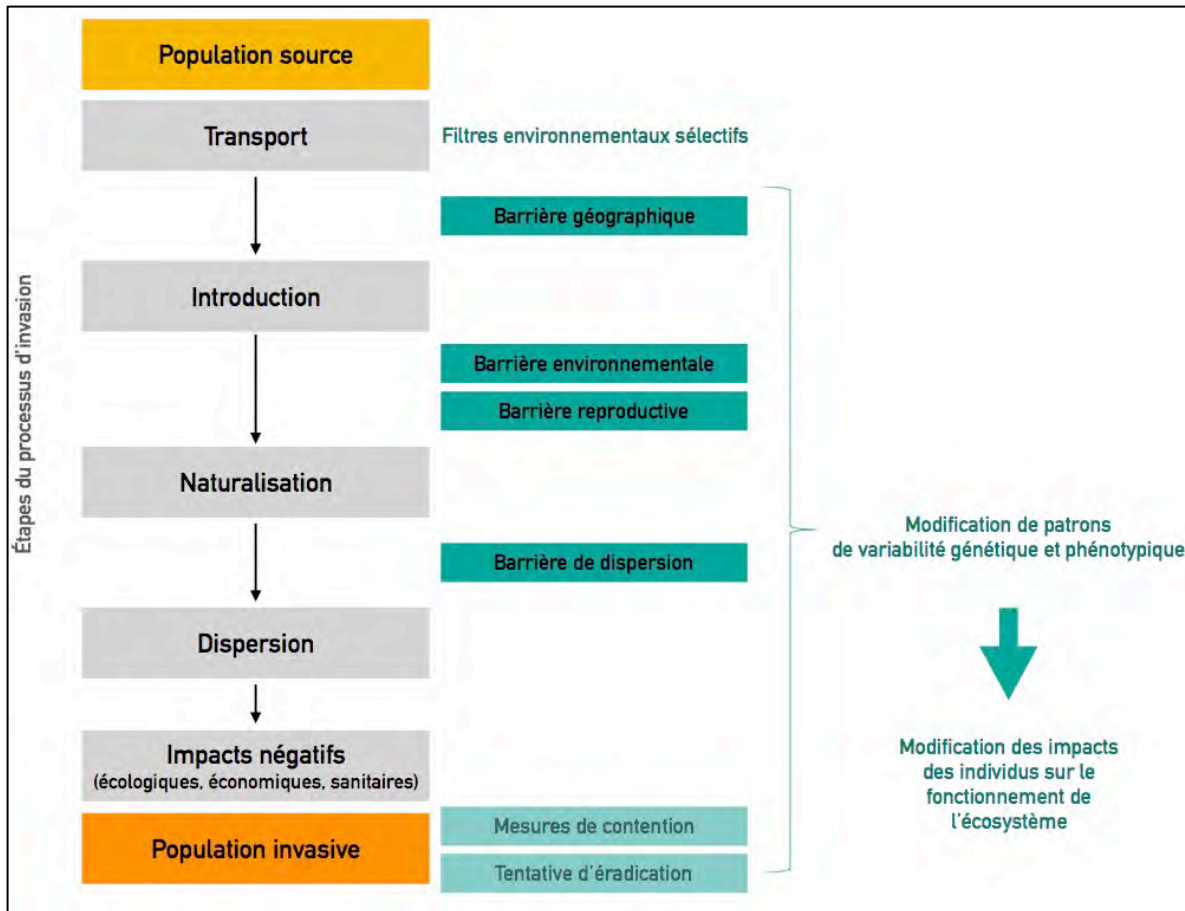


Figure I.2 Représentation schématique du processus d'invasion biologique et des différents types de barrière à travers lesquels les individus sont filtrés. Au cours du processus d'invasion les patrons de variabilité génétique et phénotypique de la population non native sont donc modifiés par rapport à la population native, ce qui peut résulter en des effets différents des individus sur les processus écosystémiques. Les individus non natifs peuvent échouer à chaque étape du processus d'invasion. Adaptée de Lockwood et al. (2007) et Blackburn et al. (2011).

Vulnérabilité des écosystèmes dulçaquicoles face aux invasions biologiques

Les milieux aquatiques sont particulièrement vulnérables et sujets aux invasions biologiques (Sala et al., 2000; García-Berthou et al., 2005). Ceci est en partie attribuable à la grande capacité de dispersion des organismes aquatiques par rapport aux organismes terrestres (Beisel, 2001), mais également à l'urbanisation et aux activités humaines qui sont souvent liées à la proximité des étendues d'eau, maximisant les perturbations d'origine anthropique des milieux aquatiques (Carpio et al., 2019). Or, dans les écosystèmes perturbés, les opportunités de contamination des milieux par des espèces non natives sont décuplées (Ricciardi, 2001; Ross et al., 2001). De plus, une grande partie des espèces invasives aquatiques a été introduite délibérément pour des activités commerciales (aquaculture) et de loisir (pêche et aquariophilie) principalement. Pimentel et al. (2005) ont estimé que les coûts liés aux espèces invasives aquatiques aux États-Unis, prenant en compte les dégâts et pertes qu'elles engendrent et le coût de leur contrôle, dépassaient les 5.4 milliards de dollars

annuels⁷. En Europe, les espèces invasives aquatiques coûtent près de 2.2 milliards d'euros par an (Keller et al., 2011). Malgré une prise de conscience progressive au niveau mondial (Conventions de Rome 1952, Ramsar 1971, Washington 1973, Berne 1979, Rio 1992, Aichi 2010), à l'heure actuelle il n'existe que très peu de mesures de gestion des espèces invasives coordonnées entre les territoires, ce qui limite l'efficacité de la lutte contre les espèces invasives. Cependant, en réponse à la constante augmentation des invasions biologiques ces dernières années, une stratégie européenne a été établie en 2014 (règlement UE 1143/2014), suivie d'une stratégie nationale pour la France en 2017 qui commence à être déclinée plus localement, au niveau régional par exemple.

La variabilité intraspécifique dans les invasions biologiques

Facteurs de succès d'invasion

Le succès des espèces invasives s'explique par de nombreux facteurs pouvant être externes (e.g. écosystèmes perturbés, ou communautés faiblement diversifiées), ou inhérents aux individus invasifs (e.g. forte plasticité phénotypique). La pression de propagules, à savoir le nombre initial d'individus introduits dans un environnement non natif, est un premier facteur déterminant du succès des espèces invasives (Colautti et al., 2006; Jeschke & Strayer, 2006). Intuitivement, plus la pression de propagules est grande plus les chances de succès d'invasion sont élevées. La pression de propagules est souvent associée aux traits des individus invasifs, que l'introduction soit volontaire (i.e. traits sélectionnés) ou non (i.e. traits traduisant la propension des individus introduits pour la survie (Ricciardi & Maclsaac, 2000; Cassey et al., 2004; Juette et al., 2014). Il est reconnu que les organismes invasifs partagent un certain nombre de traits qui les rendent plus compétitifs vis-à-vis des organismes indigènes, pouvant ainsi expliquer leur succès de colonisation. Ces traits sont souvent associés à la dispersion, à la reproduction (Deacon et al., 2011), ou au comportement des individus invasifs (Sol et al., 2002; Suarez et al., 2005; Juette et al., 2014). Les individus invasifs sont également souvent caractérisés par une meilleure acquisition des ressources (Polo-Cavia et al., 2011; Mathakutha et al., 2019), ainsi qu'un régime alimentaire plutôt généraliste et une plus grande tolérance aux conditions abiotiques (Moyle & Marchetti, 2006). Une grande variabilité phénotypique peut donc faciliter la naturalisation d'une espèce nouvellement introduite (Forsman, 2014).

⁷ Les chiffres présentés ici sont déjà compensés par les bénéfices annuels attribuables aux activités de pêches impliquant les espèces invasives (e.g. 69 milliards de dollars annuels aux États-Unis ; Pimentel et al., 2005). Idem pour les coûts européens.

La plasticité phénotypique confère une capacité d'adaptation et ainsi une plus forte probabilité de succès aux espèces invasives qui font souvent face à des contraintes environnementales différentes de celles présentes dans leur aire d'origine. Les traits des individus introduits/invasifs sont donc en général rapidement soumis à de fortes pressions de sélection. En conséquence, les phénotypes des individus invasifs peuvent évoluer peu de temps après leur introduction (Kinnison et al., 1998; Huey, 2000; Gibert et al., 2016). Même si une grande plasticité phénotypique caractérise certaines espèces invasives, ce n'est pas un facteur attribuable au succès d'invasion de toutes les espèces (Richardson & Pyšek, 2006; Davidson et al., 2011). Lorsque la plasticité phénotypique est associée à un fort potentiel adaptatif, les chances de succès d'invasion sont augmentées (Lee, 2002). Une variabilité génétique élevée de l'espèce invasive constitue de ce fait un autre facteur de succès dans le contexte des invasions biologiques (Lavergne & Molofsky, 2007; Rius & Darling, 2014; R. Garnas et al., 2016). Les mélanges génétiques (*admixture*) permettent d'augmenter la variance génétique des populations et potentiellement d'améliorer la valeur sélective des individus dans les environnements nouvellement colonisés (Keller & Taylor, 2008; Verhoeven et al., 2011; Dufresnes et al., 2017). En revanche, dans certaines situations les mélanges génétiques entraînent plutôt une homogénéisation des populations non favorable au succès d'invasion car elle réduit alors la capacité des individus à s'adapter à de nouveaux environnements (Spielman et al., 2004). Une grande variabilité génétique peut notamment s'expliquer par de multiples introductions et/ou la dispersion secondaire d'individus invasifs. La variabilité génétique des populations invasives (inter- ou intrapopulationnelle) est donc particulièrement intéressante à étudier puisqu'elle est porteuse d'informations concernant l'histoire d'invasion de l'espèce considérée.

La variabilité intraspécifique porteuse de l'histoire d'invasion

Tout au long des invasions biologiques, les différentes forces évolutives présentées dans la première partie de cette introduction agissent pour façonner la variabilité génétique et la variabilité phénotypique des populations invasives (Lee, 2002; Keller & Taylor, 2008). Les espèces invasives constituent donc une opportunité pour l'étude des processus écologiques et évolutifs contemporains (Huey et al., 2005; Sax et al., 2007; Moran & Alexander, 2014; Hendry, 2016). En général, une fraction restreinte d'individus (et par conséquent une représentation partielle de la variabilité génétique d'origine) est introduite, provoquant un type de goulot d'étranglement génétique appelé effet fondateur (*genetic bottleneck* et *founder effect* en anglais). Ces goulots d'étranglement correspondent à une forte réduction de la

variabilité génétique (i.e. pertes d'allèles) de l'espèce invasive par rapport à son aire d'origine (Excoffier et al., 2009; Bock et al., 2015). La dérive génétique peut alors entraîner une réduction supplémentaire de l'hétérozygotie dans cette petite fraction d'individus. Cette diminution de variabilité génétique inhérente à l'introduction d'un petit nombre d'individus peut augmenter la consanguinité (*inbreeding depression* en anglais), c'est-à-dire la réduction de la valeur sélective de la population due à la reproduction d'individus apparentés (Frankham et al., 2002). En conséquence, la fréquence des allèles délétères augmente et le risque d'extinction de la population est accru. Le paradoxe des invasions provient donc de la capacité des populations invasives à se maintenir malgré une faible diversité génétique *a priori* (Sax & Brown, 2000; Dlugosch & Parker, 2008). Il est admis que de multiples événements d'introductions, ainsi que des mécanismes épigénétiques héréditaires associés à l'apparition rapide de mutations suite à l'introduction d'individus dans un nouvel environnement, permettent en fait aux populations invasives de présenter rapidement une importante variabilité génétique (Richards et al., 2012; Zhang et al., 2016). Certaines structurations génétiques de populations permettent d'établir des scénarii d'invasion (e.g. déterminer le nombre d'évènements d'introduction ou encore les routes de dispersion). Par exemple, rechercher des patrons d'isolement par la distance (*Isolation By Distance* en anglais, IBD) peut révéler des déplacements secondaires des individus invasifs dans l'aire colonisée, ou au contraire l'absence flux génétique (Hutchison & Templeton, 1999; Bélouard et al., 2019).

Il existe souvent un temps de latence important (quelques dizaines d'années à quelques centaines d'années) entre l'introduction d'une espèce non native et les impacts écologiques qu'elle engendre (Pyšek & Prach, 1995). Ceci peut notamment s'expliquer par le temps inhérent à la croissance des populations et par le temps nécessaire à l'adaptation des organismes aux nouvelles contraintes environnementales. En conséquence, les études sur les processus d'invasion biologique en milieu naturel portent en général sur des invasions datant d'une soixantaine d'années et plus (Hendry et al., 2000; Lankau, 2012). Afin de mieux comprendre les mécanismes éco-évolutifs impliqués dans les invasions biologiques, il est essentiel d'étudier les populations invasives et leur variabilité intraspécifique le plus tôt possible au cours du processus d'invasion. Si les analyses génétiques sont utilisées de manière répandue pour identifier les routes d'invasion à l'échelle mondiale (Cristescu, 2015; Sherpa et al., 2019), peu d'études ont cherché à comprendre les routes d'invasion à une échelle plus locale.

Conséquences fonctionnelles de la variabilité intraspécifique des espèces invasives

Les espèces invasives peuvent induire des impacts écologiques à tous les niveaux d'organisation biologique (Cucherousset & Olden, 2011). Leurs impacts peuvent être directs ou indirects à travers de nombreuses interactions biotiques avec les espèces natives telles que la compétition, la prédation (Phillips & Shine, 2006; Walsh et al., 2016), la transmission de pathogènes (Tompkins et al., 2003), l'hybridation avec des espèces natives (McGinnity et al., 2003), ou encore abiotiques, comme la modification de l'habitat (Anderson & Rosemond, 2007). Généralement les invasions biologiques résultent en des écosystèmes perturbés dans leur fonctionnement, et des structures de communauté changées (Simberloff et al., 2013). Certaines études récentes ont montré que les effets des individus invasifs pouvaient varier entre populations (Yonekura et al., 2007; Evangelista et al., 2019). Pourtant, la variabilité intraspécifique reste peu considérée dans les études d'impacts des individus invasifs sur le fonctionnement des écosystèmes. Une meilleure compréhension des impacts des individus invasifs permettrait notamment de mettre en place des mesures de contrôle efficaces pour pallier les perturbations des processus écosystémiques qu'ils engendrent. Ces mesures de gestion, qui consistent souvent à éradiquer les individus invasifs naturalisés, peuvent provoquer un prélèvement non aléatoire d'individus et de traits phénotypiques particuliers, dans les écosystèmes. Les traits des individus invasifs sont donc filtrés par les pratiques de gestion, ce qui continue de façonner la variabilité intraspécifique des espèces invasives. En conséquence, les impacts des individus invasifs sur le fonctionnement de l'écosystème peuvent être modifiés par les pratiques de gestion, ce qui peut rendre ces dernières inutiles, voire contre-productives (Coltman, 2008; Mimura et al., 2017; Závorka et al., 2018, 2020; Box 2).

Box 2

Importance des changements de traits induits par la gestion des espèces invasives

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Les différences intraspécifiques constituent un moteur majeur des dynamiques écologiques (Des Roches et al., 2018). Pourtant, les études visant à intégrer la variabilité phénotypique et ses déterminants pour améliorer les pratiques de gestion sont encore limitées. En réponse à ce vide scientifique, Palkovacs et al. (2018) ont proposé un cadre théorique dans le but de clarifier les implications écologiques des changements phénotypiques liés au prélèvement d'individus dans le cadre de pratiques de gestion (e.g. chasse, pêche).

Dans le contexte des invasions biologiques, les pratiques de gestion consistent le plus souvent à contrôler, voire éradiquer les populations invasives. Il en résulte un prélèvement non aléatoire d'individus dans les populations gérées (Myers et al., 2000; Britton et al., 2011), ce qui peut avoir de fortes implications écologiques et évolutives. Le cadre conceptuel proposé par Palkovacs et al. (2018) peut parfaitement être appliqué à la gestion des espèces invasives car des modifications de traits induites par le prélèvement d'individus invasifs pourraient moduler leurs impacts écologiques, à travers des modifications de comportement par exemple (e.g. évitement et changement de phénologie ; Côté et al., 2014). Une tentative d'éradication pourrait donc induire des résultats complètement inattendus, voire contre-productifs, qui n'ont peut-être pas été pris en compte par les gestionnaires jusqu'à présent (Závorka et al., 2020). D'autant plus que les organismes invasifs soumis à des méthodes de contrôle sont ciblés en raison de leurs forts impacts écologiques négatifs (Kopf et al., 2017), ce qui implique que le prélèvement d'individus pourrait se traduire par de fortes réponses écosystémiques.

Les coûts des programmes de lutte et de contrôle des espèces invasives étant élevés (Myers et al., 2000), les efforts mis en place sont souvent intenses, mais sur des périodes

de temps relativement courtes. Par conséquent, de nombreux programmes d'éradication ne parviennent pas à réduire ou à éradiquer de manière durable les populations cibles (Britton et al., 2011). Les individus survivants permettent alors à la population de se rétablir, voire même à coloniser de nouveaux habitats à partir d'un groupe d'individus présentant des caractères phénotypiques fortement biaisés par la pression d'éradication.

Le contrôle des espèces invasives reste un outil de gestion essentiel qui permet parfois l'élimination complète de populations invasives (Britton et al., 2011; Kopf et al., 2017). Cependant, lorsque l'éradication n'est pas complète malgré les efforts de lutte, les populations rétablies peuvent comporter des individus avec des traits différents de ceux présents dans la population avant la tentative d'éradication. Il serait pertinent d'appliquer le cadre conceptuel proposé par Palkovacs et al. (2018) à la gestion des espèces invasives pour prendre en compte les impacts écologiques liés aux changements de traits induits par la pression d'éradication. Lors de l'évaluation nette des techniques de contrôle des invasions biologiques, cela permettrait de comparer les avantages d'une réduction de taille de population et les risques liés aux modifications phénotypiques induites par la pression d'éradication.

Il apparaît essentiel de prendre en compte explicitement la variabilité génétique et phénotypique des espèces invasives, et de décrire les covariations de traits pour ces espèces afin d'améliorer notre capacité à comprendre leur potentiel d'invasion dans différents environnements, les dynamiques éco-évolutives impliquées dans les processus d'invasion et leurs conséquences sur le fonctionnement des écosystèmes.

Objectifs

L'objectif général de cette thèse était de décrire les patrons de variabilité intraspécifique au sein de deux métapopulations, d'identifier les déterminants de la variabilité intraspécifique d'espèces invasives, et de mieux comprendre son implication sur le fonctionnement d'écosystèmes dulçaquicoles. Ce travail est fondé sur l'étude de populations invasives d'écrevisses de Louisiane (*Procambarus clarkii*) et d'écrevisses américaines (*Faxonius limosus*) établies en Haute-Garonne, ainsi que sur une approche expérimentale pour évaluer les syndromes fonctionnels de chaque espèce.

La thèse est structurée en deux parties comprenant un total de 4 chapitres. La première partie concerne l'étude la variabilité intraspécifique à l'échelle inter-populationnelle (*Chapitres III, IV*) et la seconde partie de la thèse se focalise sur la variabilité intra-populationnelle (*Chapitres V, VI* ; Figure I.3). Le premier objectif était d'identifier les déterminants environnementaux de la variabilité génétique des deux espèces invasives. Dans ce premier chapitre nous avons également montré l'intérêt et les limites de la génétique des populations pour la reconstitution d'histoires locales d'invasion, et la déduction précise des routes et vecteurs d'invasion à l'échelle de la métapopulation (*Chapitre III*). Le deuxième objectif était de quantifier la variabilité phénotypique à différentes échelles d'organisation chez les deux espèces invasives et d'identifier les facteurs environnementaux et les processus évolutifs (neutres ou adaptatifs) à l'origine de ces variations pour différents groupes de traits (morphologiques, trophiques et stœchiométriques ; *Chapitre IV*). Le troisième objectif était d'étudier l'existence d'un polymorphisme de ressource chez une des espèces invasives (*Procambarus clarkii*) établie récemment, en quantifiant les différenciations morphologiques et trophiques le long de l'axe littoral-pélagique benthique (*Chapitre V*). Enfin, nous avons comparé les structures de covariation de traits chez les deux espèces d'écrevisse dans un contexte de coexistence, afin de mieux comprendre leurs rôles écologiques respectifs sur le fonctionnement de l'écosystème. Pour cela, nous avons utilisé une approche expérimentale de façon à quantifier de multiples traits de réponse et traits d'effet chez des individus invasifs, puis d'estimer leurs impacts sur le fonctionnement de l'écosystème en utilisant le cadre conceptuel du *syndrome fonctionnel* (*Chapitre VI*).

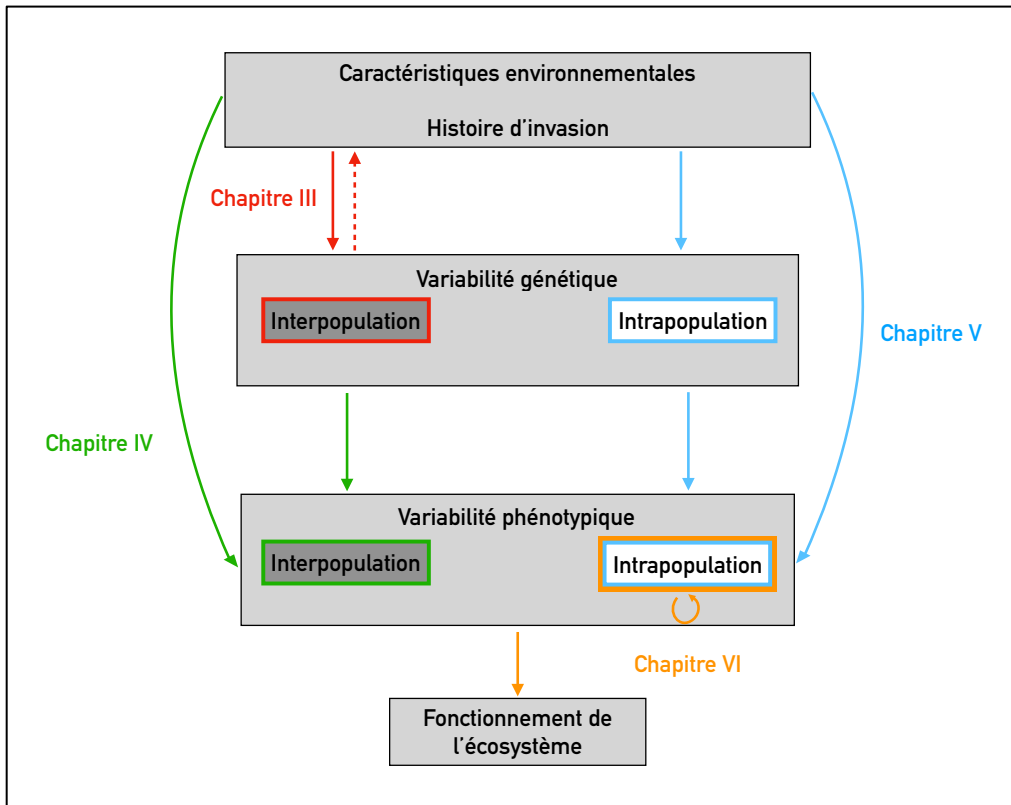


Figure I.3 : Représentation schématique des différentes parties de la thèse. Les chapitres III et IV traitent de la variabilité interpopulationnelle et les chapitres V et VI se focalisent sur la variabilité intrapopulationnelle des espèces invasives.

Chapitre II

Espèces modèles et sites d'étude

Espèce modèles

L'écrevisse américaine, *Faxonius limosus*

L'écrevisse américaine (*spiny cheek crayfish* en anglais) est originaire d'Amérique du Nord. Elle a été introduite avec succès pour la première fois en Europe vers 1890 en Pologne, où 90 individus ont été relâchés délibérément dans un étang dans le cadre d'une mesure prise par la *United States Commission of Fish and Fisheries* (McDonald, 1983; Petrussek et al., 2006). De multiples introductions ont ensuite eu lieu jusqu'à la fin du XX^{ème} siècle et l'espèce s'est naturellement dispersée par le réseau hydrographique européen, en France et en Allemagne dans un premier temps. Aujourd'hui, *F. limosus* est présente dans une vingtaine de pays européens (Souty-Grosset et al., 2006).

F. limosus peut occuper une grande variété d'habitats dulçaquicoles (cours d'eau de tailles et débits variés, mares, lacs) voire même les eaux saumâtres et possède une forte tolérance aux eaux polluées (Holdich & Black, 2007). L'espèce possède un cycle de vie rapide et une forte fécondité avec deux périodes de reproduction sur l'année permettant aux populations de croître rapidement, ce qui peut en partie expliquer son succès invasif (Kozák et al., 2006; Chybowski, 2007). *F. limosus* est également capable de se reproduire par parthénogénèse, ce qui pourrait lui conférer un avantage non négligeable sur les autres espèces s'il s'avérait qu'elle utilise ce mode de reproduction en milieu naturel (Buřič et al., 2011, 2013). Son régime alimentaire omnivore est principalement composé de végétaux et de détritux (Vojkovská et al., 2014). L'écrevisse américaine possède une grande capacité de dispersion aquatique par les réseaux hydrographiques, mais une occurrence de dispersion terrestre pour cette espèce a déjà été reportée (Puky, 2014).

L'écrevisse de Louisiane, *Procambarus clarkii*

L'écrevisse de Louisiane (*red swamp crayfish* en anglais) est originaire du sud des États-Unis et du nord-ouest du Mexique (Gherardi, 2006). C'est l'une des espèces d'écrevisse invasive la plus répandue dans le monde (plus de 40 pays), suite à de multiples introductions à des fins commerciales et de loisir (aquaculture, activités de pêche, consommation humaine... ; Souty-Grosset et al., 2006; Oficialdegui et al., 2019). En Europe, elle a d'abord été introduite en Espagne depuis la Louisiane en 1973, puis elle s'est rapidement dispersée dans le sud de la France ainsi que dans toute l'Europe depuis ce point d'introduction et diverses autres sources (Souty-Grosset et al., 2006; Oficialdegui et al., 2020).

Tout comme *F. limosus*, *P. clarkii* possède une croissance rapide, une grande fécondité et une à deux périodes de reproduction par an (Gherardi, 2006; Alcorlo et al., 2008). Elle se caractérise également par une grande tolérance thermique ainsi qu'à la dessiccation, et peut également occuper une grande diversité d'habitats dulçaquicoles et salins (Gherardi, 2006). *P. clarkii* est une espèce omnivore opportuniste qui consomme principalement des végétaux et débris au stade adulte, mais peut également se nourrir de larves d'insectes ou d'œufs de poissons (Correia, 2003; Alcorlo et al., 2004). L'écrevisse de Louisiane possède non seulement une grande capacité de dispersion par voie aquatique, mais elle peut également se déplacer par voie terrestre (Gherardi & Barbaresi, 2000; Thomas et al., 2019).



Figure II.1 Photo d'une écrevisse américaine *Faxonius limosus* (à gauche) et d'une écrevisse de Louisiane *Procambarus clarkii* (à droite). © Rémy Lassus

Impacts des écrevisses invasives

F. limosus et *P. clarkii* font partie des 66 espèces exotiques envahissantes (EEE) listées dans les règlements européens (UE) n°1143/2014 et n°2019/1262. Ces deux espèces ont en effet de nombreux impacts dans les écosystèmes colonisés (Twardochleb et al., 2013). Elles érodent les berges des cours d'eau ou des lacs en creusant des terriers, et ont par la même occasion une activité de bioturbation qui modifie la biodisponibilité des nutriments pour les autres organismes de l'écosystème et qui peut impacter à son tour la production de macrophytes (Statzner et al., 2000; Gherardi, 2007; Holdich & Black, 2007; Souty-Grosset et

al., 2016). Globalement, elles provoquent un déclin de biodiversité dans les écosystèmes natifs (e.g. macrophytes, macroinvertébrés, œufs et larves de poissons et d'amphibiens ; Souty-Grosset et al., 2016), et par leur régime omnivore généraliste elles peuvent impacter les chaînes trophiques à de multiples niveaux et modifier les dynamiques des communautés (Renai & Gherardi, 2004). Par leur forte voracité, les écrevisses invasives peuvent affecter la décomposition de la litière, un processus écosystémique fondamental qui intervient notamment dans le recyclage du carbone (Duffy, 2002; Dunoyer et al., 2014; Carvalho et al., 2016). Ces deux espèces ont également une agressivité plus importante que la plupart des écrevisses natives, ce qui leur confère un avantage lors d'interactions de compétition interspécifique (Gherardi & Cioni, 2004). Leurs caractéristiques morphologiques (taille de corps et des pinces) sont aussi déterminantes dans les interactions de compétition et de défense vis-à-vis de potentiels prédateurs (Butler IV & Stein, 1985; Garvey et al., 2003; Pintor et al., 2008; Graham & Angilletta, 2020). Enfin, *F. limosus* et *P. clarkii* sont toutes deux porteuses saines du champignon responsable de la peste des écrevisses, *Aphanomyces astaci*, et constituent donc une menace pour les populations d'écrevisses indigènes (Holdich et al., 2009).

Sites d'étude

Ce travail de thèse a été réalisé dans le sud-ouest de la France, dans des lacs artificiels situés dans la plaine alluviale de la Garonne, au sud de Toulouse. Ces lacs sont creusés pour l'extraction de granulats, et sont formés par la remontée naturelle de l'eau de la nappe phréatique. Pour la plupart, ils ne sont pas reliés directement au réseau hydrographique de la région. Pourtant, les lacs de gravière sont rapidement colonisés par de nombreux organismes aquatiques, en grande partie en raison des activités de pêche et aux déversements volontaires et/ou accidentels de poissons et autres espèces aquatiques, natives, non natives ou invasives (Zhao et al., 2016). Quand l'exploitation de granulats est terminée, les lacs sont rebouchés ou bien rétrocedés à diverses structures comme les fédérations de pêches, les AAPPMA⁸, les communautés de communes, les mairies, à des particuliers. Il existe un gradient d'ancienneté des plans d'eau associé à un gradient géographique et environnemental (e.g. productivité) et au type de gestion des lacs (i.e. pression anthropique): globalement les lacs les plus proches de la ville de Toulouse sont également les plus anciens et sont gérés par des associations de pêche, tandis que les lacs

⁸ Association Agréée pour la Pêche et la Protection du Milieu Aquatique.

les plus éloignés et les plus récents sont gérés par des sociétés privées (e.g exploitations de granulats; Jorigné, 2018; Figure II.2).

F. limosus et *P. clarkii* ont été respectivement détectées en 1988 (Magnier & Petit, 2016) et 1995 (Changeux, 2003) dans la zone étudiée. Les 47 plans d'eau étudiés dans le cadre de cette thèse sont situés dans un rayon de 50 x 70 km à proximité de Toulouse et ont été creusés entre 1963 et 2008. Les lacs de gravière offrent donc la possibilité d'étudier la variabilité intraspécifique de deux métapopulations d'espèces invasives à une échelle locale, et de comprendre leurs effets sur le fonctionnement de l'écosystème dans une multitude d'environnements hétérogènes (Figure II.2).

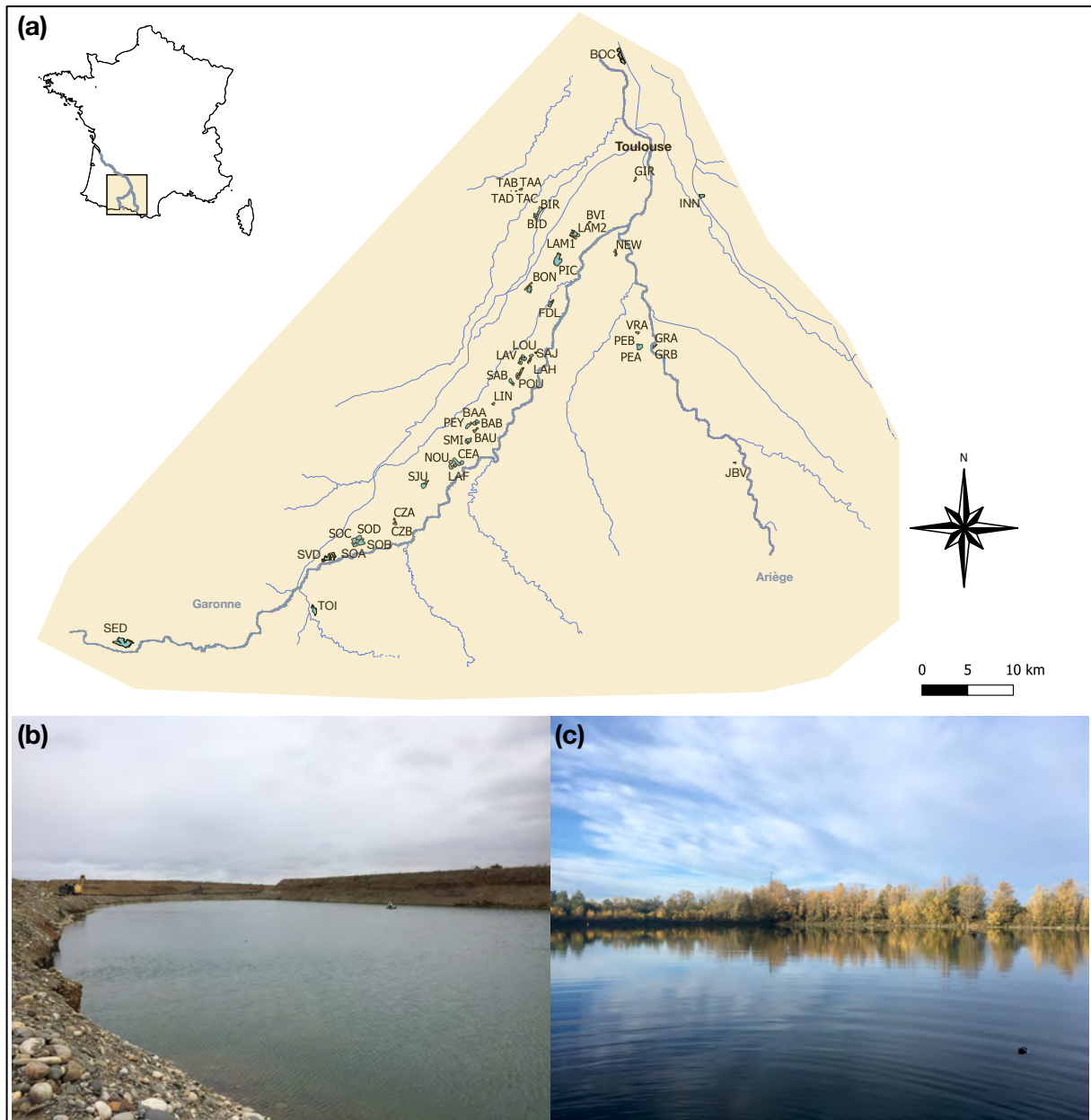


Figure II.2 (a) Cartographie des différents sites utilisés dans le cadre de cette thèse. Les plans d'eau sont légèrement grossis pour être tous distinguables. Photos d'un **(b)** lac jeune en exploitation d'extraction de granulats et d'un **(c)** lac mature géré par une fédération de pêche. © Iris Lang

Chapitre III

Inférence de routes locales d'invasion chez deux espèces d'écrevisses invasives aux patrons de variabilité génétique contrastés

Résumé

1- Les invasions biologiques sont une des principales causes de la crise que connaît la biodiversité actuellement. Il est donc nécessaire de développer des mesures de gestion efficaces des espèces invasives. Pour cela, il est essentiel d'identifier les principales routes d'introduction des espèces invasives : introduction délibérée à des fins commerciales, fuite de captivité, introduction involontaire comme contaminants d'autres produits, transport non intentionnel, dispersion spontanée non aidée ou suivant des corridors d'origine anthropique. Les études génétiques ont souvent permis de retracer les routes d'introduction d'espèces invasives à large échelle (nationale ou mondiale), pourtant elles n'ont été que rarement utilisées pour identifier les routes d'invasion à une échelle plus locale.

2- Nous avons réalisé des analyses génétiques pour identifier les routes locales d'invasion de deux espèces invasives (*Faxonius limosus* et *Procambarus clarkii*) dans un réseau de lacs dont dépendent de multiples activités socio-économiques. Dans un premier temps nous avons décrits les patrons spatiaux de variabilité génétique neutre pour chaque espèce à l'aide de microsatellites. Ensuite, nous avons identifié les déterminants environnementaux de cette structuration génétique, et nous avons inféré les potentielles routes locales d'invasion dans le site étudié pour chacune des espèces.

3- Nous avons mis en évidence des patrons de variabilité génétique contrastés entre les deux espèces : *F. limosus* avait une très faible variabilité génétique très peu structurée, tandis que *P. clarkii* possédait une variabilité génétique plus importante et plus structurée spatialement. Nous avons aussi montré une forte contexte-dépendance de différentes variables environnementales expliquant les patrons observés, avec une importance notable du type de gestion des lacs dans la structuration génétique des deux espèces.

4- Nous avons montré que *F. limosus* a probablement été introduite à la fois délibérément et de façon non intentionnelle, sans possibilité de conclure précisément. L'impossibilité d'identifier clairement ses routes locales d'invasion est sûrement due à cette très faible variabilité génétique. En revanche, nous avons mis en avant l'existence de multiples routes d'invasion pour *P. clarkii* à l'échelle locale de notre étude (introduction délibérée, contaminant stocks, dispersion aidée ou non, et passager clandestin).

5- Synthèse : Nous avons illustré à la fois les apports et les limites des analyses de variation génétique neutre dans le but d'inférer les routes locales d'invasion dans des environnements complexes. Cette approche est particulièrement informative pour des espèces invasives qui ont une histoire complexe d'invasion et une variabilité génétique importante. En revanche, il peut être difficile d'identifier les routes locales d'invasion des espèces invasives ayant une faible variabilité génétique.

Inference of local invasion pathways in two invasive crayfish species displaying contrasting genetic patterns

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Abstract

1- Biological invasions are a main driver of the current biodiversity crisis and developing efficient measures for managing Invasive Alien Species (IAS) is crucial. Such measures require identifying their major introduction pathways: deliberate *release* as commodities, *escape* from captivity, *contaminants* of specific commodities, *stowaways* on transport vectors, and spread through *unaided* dispersal or following anthropogenic *corridors*. Genetic assessments have proven useful to inform global invasion pathways at large (national to worldwide) scales. However, genetic assessments aimed at identifying invasion pathways at local scales remain scarce, despite their importance for guiding biological invasions management.

2- We used genetic analyses to identify local invasion pathways used by two invasive crayfish species (*Faxonius limosus* and *Procambarus clarkii*) to invade a dense network of artificial lakes displaying high spatio-temporal dynamics and hosting multiple socio-economic activities. We first characterized spatial patterns of genetic variation for each species using neutral microsatellite markers. We then identified their environmental determinants, and inferred the potential local invasion pathways they might have used for invading the study area.

3- We revealed contrasting patterns of genetic variability for both species: *F. limosus* displayed very low levels of genetic diversity and spatial structuring, while *P. clarkii* displayed much higher genetic diversities and spatial genetic structuring levels. We also highlighted context-dependent effects of different environmental variables, with notably a promoting effect of fishery management practices on both species' genetic diversities.

4- We found little evidence for the deliberate *release* and *contaminant* pathways for *F. limosus*, probably because its low levels of genetic variability precluded a clear identification of its local invasion pathways. We found however clear evidence of the co-occurrence of multiple invasion pathways (*release*, *contaminant*, *unaided/corridor* spread and *stowaway*) at the local scale for *P. clarkii*.

5- Synthesis and applications: We illustrate both the usefulness and limitations of neutral genetic variation assessments for inferring local invasion pathways in complex environments. This approach is particularly useful for species with complex invasion histories that display sufficient levels of genetic diversity, but may fail identifying all local invasion pathways in IAS displaying very low levels of genetic variability.

Introduction

Rates of introduction, establishment and subsequent range expansion of Invasive Alien Species (hereafter, IAS) are increasing, promoting a global rise in the number of biological invasions (Seebens et al., 2017, 2018). Invasive species exert negative economic and ecological effects (Simberloff et al., 2013; Jeschke et al., 2014) and act across levels of biological organization, ranging from changes in the genetic composition of native species (e.g. hybridization) to species extirpation and changes in ecosystem functioning (Cucherousset & Olden, 2011; Simberloff et al., 2013; Jeschke et al., 2014). Improving our ability to characterize and predict biological invasions is thus urgently needed for engaging efficient IAS management.

Invasion genetics allow reconstructing complex invasion histories through the inferential and correlative analysis of the genetic footprints left by different processes during invasions (Barrett, 2015; Cristescu, 2015). Indeed, founder effects (Dlugosch & Parker, 2008b), population size changes (Beaumont, 1999), admixture (Dlugosch et al., 2015), or interspecific hybridization (e.g. Bay et al., 2019) shape intraspecific genetic characteristics of invasive populations. Genetic analyses reveal patterns of variation across invasive populations, facilitating hypotheses-driven reconstruction of invasion histories (e.g. Rey et al., 2015) and informing global invasion pathways at large -national, continental or worldwide- spatial scales (Oficialdegui et al., 2019; Sherpa et al., 2019). Yet, genetic assessments identifying local invasion pathways are scarce despite their importance for the management of biological invasions.

The identification of invasion pathways and spread vectors is crucial for (i) containing ongoing invasions by reducing propagule pressure (Simberloff, 2009; Pergl et al., 2017), (ii) preventing invasive populations to act as bridgehead populations (Lombaert et al., 2010; Bertelsmeier & Keller, 2018), and (iii) hindering recolonization after successful eradication (Britton et al., 2011). Six major introduction pathways have been identified (Hulme et al., 2008) : deliberate *release* as a commodity, *escape* from captivity, *contaminant* of a specific commodity, *stowaway* on a transport vector, and *spread* through *unaided* dispersal from an invaded area, or through dispersal following anthropogenic *corridors* (Hulme et al., 2008; Essl et al., 2015; Hulme, 2015). When an invasion history is relatively simple (e.g. few introductions from identified sources; Simon et al., 2011), pathway identification is usually robust. However, biological invasions are often the result of complex socio-ecological interactions involving multiple and

often unreported introductions, propagules *per* introduction event and source populations (Blackburn et al., 2015; Rey et al., 2015). The information contained in the genetic footprints of a given pathway depend on multiple intrinsic factors such as species traits (e.g. dispersal capacities, demography, invasion history) and extrinsic factors such as distances between populations, physical configuration of ecosystems, or the socio-economic activities occurring in the study area (Washburn et al., 2020). Consequently, the knowledge gained from population genetics analyses can be highly variable and may modulate our ability to identify local invasion pathways.

Here, we aim to identify local invasion pathways of two global invasive crayfish (spiny cheek crayfish *Faxonius limosus* and red swamp crayfish *Procambarus clarkii*) exhibiting contrasting ecology and invasion histories in Europe (Filipová et al., 2011; Oficialdegui et al., 2019) using genetic analyses. We used as model ecosystems a dense network of artificial gravel pit lakes displaying high spatio-temporal dynamics and hosting a myriad of socio-economic activities within a restricted spatial scale (Evangelista et al., 2015; Zhao et al., 2016). We first quantified the spatial distribution of genetic variation for each species using neutral microsatellite markers. We then identified the potential environmental determinants of genetic variation patterns. We finally interpreted our empirical results in the light of Hulme's et al. invasion pathways classification to (i) identify potential local invasion pathways having been used by the two IAS for invading the study area and to (ii) discuss the assets and limitations of our approach with respect to the contrasting patterns of genetic variation we observed for the two species.

Material & methods

Model organisms

Our model organisms were two invasive crayfish species (listed in the European Union Regulation EU 1143/2014 and EU 2019/1262) displaying contrasting life-history traits and invasion histories at the European scale. *Faxonius limosus* is native from the Eastern Coast of North America (Filipová et al., 2011). Its only known successful introduction in Europe is from 1890 when 90 individuals from the US Commission of Fish and Fisheries were introduced in western Poland (Filipová et al., 2011). The species subsequently spread across Europe and arrived in central France between 1911 and 1913, where 2,000 individuals from Germany were

deliberately released (Buffault, 1925; Laurent, 1997). It invaded the Garonne river basin in the early 1960s (Laurent & Suscillon, 1962; Laurent, 1997) and its presence in the study area was first documented in 1988 (Magnier & Petit, 2016). *Procambarus clarkii* is native from Southern U.S.A. and North-eastern Mexico and has been widely introduced worldwide, colonizing almost all continents (Oficialdegui et al., 2019). It has been first introduced in Europe in 1973 from Louisiana to Spain, but the species rapidly spread across Europe (Oficialdegui et al., 2019). In France, the first introduction is reported in South-western France in 1976 with individuals from Spain (Laurent, 1997). Besides this introduction event, many individuals were imported from Spain and Kenya for aquaculture from the late 1970s to the early 1980s (Holdich, 1993; Oficialdegui et al., 2019, 2020). The presence of *P. clarkii* in our study area was first mentioned in 1995 (Changeux, 2003).

Study area and sampling design

The study area was composed of a network of artificial gravel pit lakes, isolated and disconnected from the hydrographic network, of different ages (from 10 to 60 years) located within a 70 x 75 km area located within the Garonne floodplain (South-western France; Figure III.1, Tables III.S1-2), representing a set of isolated aquatic islands within a terrestrial landscape matrix (Hortal et al., 2014; Zhao et al., 2016). They are also distributed along a decreasing North-to-South urbanisation gradient (i.e. Toulouse metropolitan area on the North, and Pyrenees Mountains' piedmont on the South) and host multiple socio-economic and recreational activities including angling, leisure and water sports (Santoul et al., 2004; Evangelista et al., 2015). Their fishery management in term of angling practices and fish stocking can be categorized as *high level* when managed by public and private angling clubs, and as *low level* when managed by municipalities or private owners (Zhao et al., 2016).

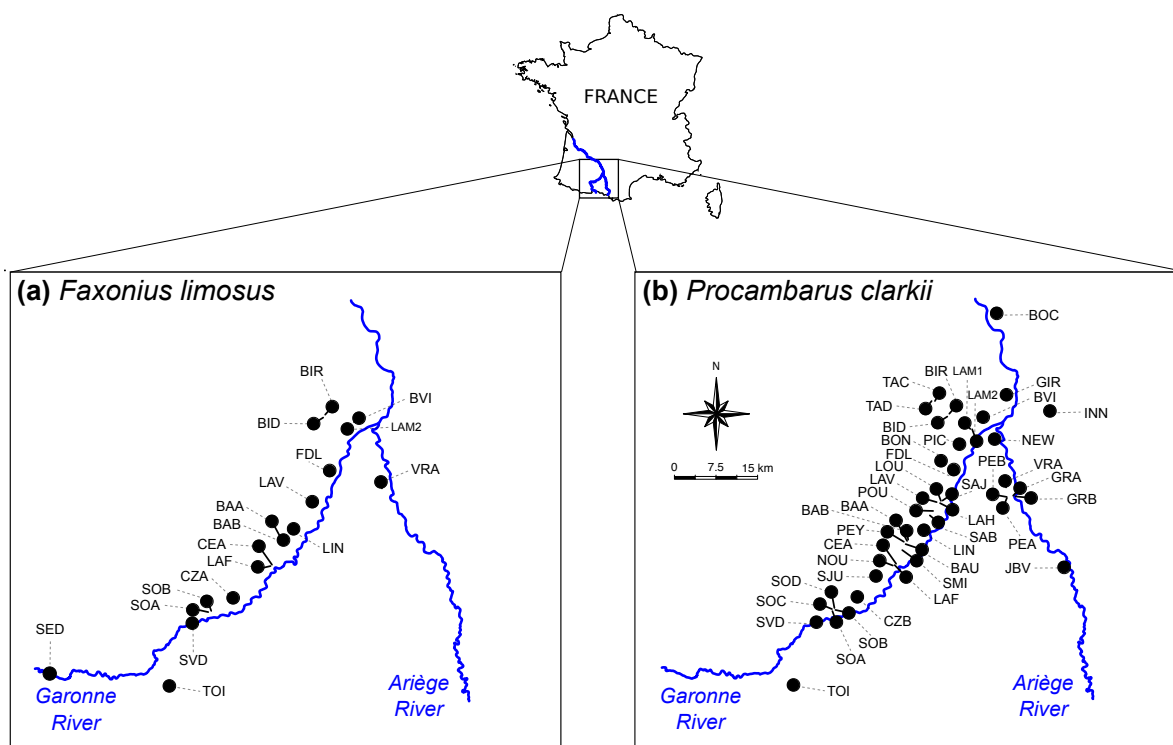


Figure III.1 Study area with the location (black dots) of all studied lakes for **(a) *Faxonius limosus*** and **(b) *Procambarus clarkii***.

This area is particularly interesting for studying local invasion pathways because many major pathways identified by Hulme et al. (2008) can potentially (co-)occur here for *F. limosus* and *P. clarkii*: deliberate releases in specific lakes for human consumption, contaminants of commodities (e.g. during fish stocking events), stowaways on transport vectors (e.g. dispersed from one lake to another by humans or aquatic birds; Anastácio et al., 2014; Coughlan et al., 2017), and unaided/corridor spread, by dispersing overland, through roads, or through the riverine network (Puky, 2014; Thomas et al., 2019).

The sampling was conducted from mid-September to mid-October 2016-2019 primarily using pairs of baited traps (a cylindrical trap of 62 x 34 x 34 cm, with a mesh size of 10 mm, and a rectangular trap of 95 x 20 x 20 cm with a mesh size of 4 mm) set both overnight ($n = 12$ traps) and during the day ($n = 8$; Alp et al., 2016; Závorka et al., 2020). We also performed active sampling using dip nets when needed. Additional samples were provided by local anglers and angling agencies. We successfully sampled 18 populations of *F. limosus* (514 genotyped individuals) and 43 populations of *P. clarkii* (1,182 genotyped individuals; Figure III.1). We targeted at least $N = 28$ sampled individuals per population per species for subsequent genetic analyses, although this number was not always reached (77.78% of *F. limosus*

populations with $N \geq 20$, mean N across populations = 29, SD = 13, Table III.S1 and 83.72% of *P. clarkii* populations with $N \geq 20$, mean N across populations = 27, SD = 7, Table III.S2).

Environmental variables

A set of environmental variables was collected to perform subsequent landscape genetics analyses. Lake *surface area* (km²) was calculated using aerial pictures (IGN 2019). We further determined both *Euclidean* and shortest *topological* (i.e. riverine) *distances* between (i) each pair of lakes and (ii) between each lake and Toulouse city using the R package 'riverdist' (Tyers, 2017). Distances between lakes and Toulouse can be viewed as a proxy of an *urbanisation gradient*, with decreasing urbanisation pressure with increasing distance. Given that both Euclidean and topological distances were highly correlated (Pearson's correlation coefficient r between both distances = 0.997 and 0.993 for *F. limosus* and *P. clarkii* respectively), we only used Euclidean distances for subsequent analyses. Finally, we quantified the *level of fishery management* as previously described (*high/low level management*).

Genotyping

Genomic DNA was extracted from abdominal muscle tissue using a modified salt-extraction protocol (Aljanabi & Martinez, 1997). We co-amplified 9 and 14 microsatellite loci for *F. limosus* and *P. clarkii*, respectively (Hulák et al., 2010; Jiang et al., 2015), using two (*F. limosus*) or three (*P. clarkii*) multiplexed PCRs, 5-20 ng of genomic DNA and QIAGEN® Multiplex PCR Kits (Qiagen, Valencia, CA, USA). Details on loci, primer concentrations, PCR conditions and multiplex sets are available elsewhere (Hulák et al., 2010; Jiang et al., 2015; Lang et al., 2020). Genotyping was conducted on an ABI PRISM™ 3730 Automated Capillary Sequencer (Applied Biosystems, Foster City, CA, USA), and allele size scoring using GENEMAPPER® v.4.0 (Applied Biosystems, Waltham, USA).

Genotyping quality controls

For each species, we assessed (i) null alleles and potential scoring errors incidence with MICROCHECKER 2.3 (Van Oosterhout et al., 2004), (ii) linkage disequilibria among loci within populations with FSTAT v2.9.3.2 (Goudet, 1995) and (iii) departures from Hardy-Weinberg (HW) equilibrium with GENEPOP v4.0 (Rousset, 2008). Levels of significance for these multiple tests were adjusted using the false discovery rate (FDR) procedure of Benjamini & Hochberg (1995). Finally, we used BAYESCAN v.2.1 (Foll & Gaggiotti, 2008) to test the

neutrality of the microsatellite datasets. We specifically ran four MCMC chains considering 100 prior odds for neutral model, sample sizes of 10,000 (with thinning intervals of 50), burn-in periods of 50,000 and 20 pilot runs with lengths of 5,000 *per* chain. The convergence of the four chains *per* species was checked through a Gelman and Rubin analysis (Gelman & Rubin, 1992) using a modified script from Paz-Vinas et al. (2013). We considered that chains reached convergence when values lower than 1.1 were obtained (Gelman & Hill, 2007).

Genetic diversity and structure

We assessed for each species and populations their genetic diversity levels by calculating (over all loci and for each population and species) both allelic richness (*AR*) and private allelic richness (*PA*) indices using the rarefaction procedures implemented in ADZE v.1.0 (Szpiech et al., 2008). We assumed minimum sample sizes for rarefaction of $N = 11$ and $N = 12$ for *F. limosus* and *P. clarkii* respectively (i.e. minimum sample size for both species; Tables III.S1-2). We also estimated (across loci for each species and populations) expected heterozygosities (*Hexp*) using GENETIX v.4.05 (Belkhir et al., 1996) and the Garza & Williamson (2001) statistic (*GW*) using the R package 'strataG' (Archer et al., 2017). Values of *GW* lower than 0.68 are indicative of recent genetic bottlenecks (i.e. due to a recent population collapse or a founder event following an introduction; Garza & Williamson, 2001). Finally, we calculated, for each species, pairwise genetic differentiation values (i.e. *Fst*) with the program FSTAT v2.9.3.2 (Goudet, 1995). We then calculated for each population and species the average of all pairwise *Fst* values estimated between one given population and all the remaining to obtain a within-population *genetic uniqueness* value (i.e. *Fst_{UNI}*; Paz-Vinas et al., 2018).

Spatial distribution of genetic diversity and spatial patterns of genetic differentiation

We first mapped *AR*, *PA*, and *Fst_{UNI}* to visually assess the spatial structure of genetic diversity and uniqueness. As *Hexp* were highly correlated with *AR* (Pearson's $r=0.958$ and 0.950 for *F. limosus* and *P. clarkii* respectively), they were not mapped. We then tested whether Isolation-By-Distance (IBD) patterns exist by exploring the relationship between pairwise Euclidean distances and pairwise *Fst* values by conducting single Mantel tests with 1,000 permutations with the R package 'vegan' (Oksanen et al., 2013). We finally used the R package 'segmented' (Muggeo, 2008) to identify breakpoints on the IBD relationship using piecewise regressions based on generalized linear models (Prunier et al., 2017). These breakpoints indicate

significant changes in the relative strength of genetic drift vs. gene flow on pairwise genetic differentiation.

Genetic clustering analyses

Genetically homogenous groups of individuals (i.e. clusters) were identified using the R package 'rmaverick' (Verity & Nichols, 2016) which uses thermodynamic integration (TI) procedures to estimate the best K number of clusters (Verity & Nichols, 2016). We explored values of K ranging from 1 to 18 for *F. limosus* and from 1 to 20 for *P. clarkii*, considering two different evolutionary models (i.e. with and without admixture). We ran MCMC chains considering burn-in periods of 10,000 iterations, 2,000 sampling iterations, and rung parameter equal to 10. We then determined for each species and evolutionary model (i.e. with/without admixture) the best K value according to the obtained TI posterior probabilities. Finally, we compared the evidences of the two tested evolutionary models to determine which one better fits our genotypic data.

Environmental determinants of genetic diversity and structure

To identify the environmental determinants of genetic variability, we build linear models using AR , PA and Fst_{UNI} as dependent variables, and *surface*, *distance* and *management* as explanatory variables. All full models were run with two-way interactions and the best models were selected using a backward-selection procedure using R v.2.6.2. Marginal effects of significant interactions were plotted using the R package 'sjPlot' (Lüdecke, 2018).

Results

Genotyping quality controls

We removed one locus (PCSH0042) for *F. limosus* that was monomorphic for all populations, and two loci for *P. clarkii* (PCLG27: presence of null alleles; PCSH0089: signals of purifying or balancing selection; see details in Appendix III.1).

Genetic diversity and structure

Faxonius limosus displayed very low levels of genetic diversity (Figure III.2; Table III.S1), with AR averaging 1.470 (SD = 0.217) and $Hexp$ averaging 0.131 (SD = 0.056) across populations. Mean within-population PA values ranged between <0.001 and 0.333 (mean PA across

populations of 0.054, SD = 0.08) and mean Fst_{UNI} values ranged between 0.088 and 0.235 (mean Fst_{UNI} across populations of 0.150, SD = 0.04). Contrastingly, *P. clarkii* populations displayed higher levels of genetic diversity (Figure III.2; Table III.S2), with AR averaging 3.586 (SD = 0.621) and $Hexp$ averaging 0.596 (SD = 0.082) across populations. Mean PA values ranged between <0.001 and 0.224 (mean PA across populations of 0.04, SD = 0.05), and Fst_{UNI} ranged from 0.120 to 0.316 (mean Fst_{UNI} value of 0.189, SD = 0.049). The GW statistic was lower than 0.68 for all populations and both species (mean GW across populations of 0.350, SD = 0.076 for *F. limosus*, and of 0.450, SD = 0.041 for *P. clarkii*; Tables III.S1-2), suggesting the occurrence of recent genetic bottlenecks in all lakes.

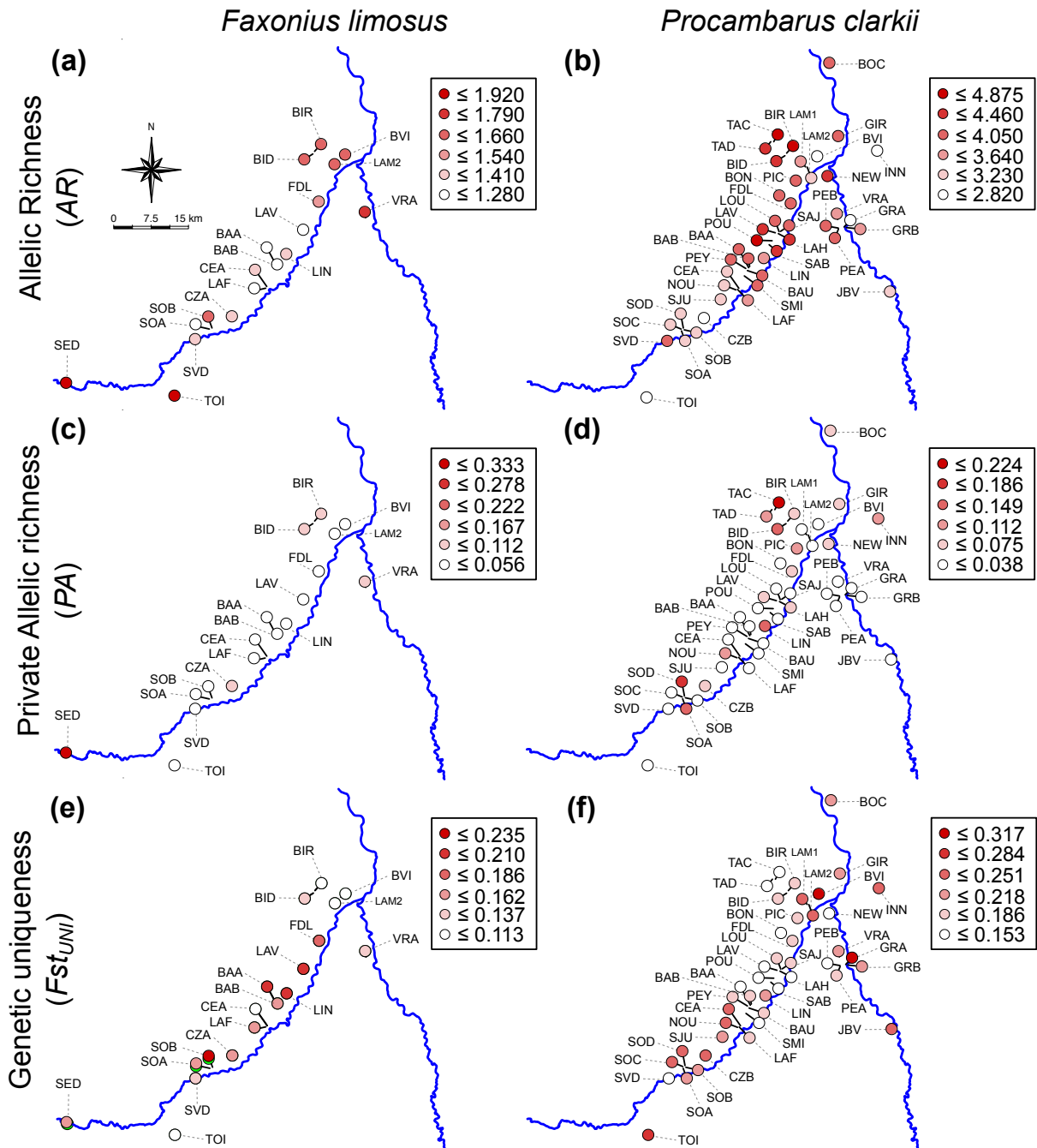


Figure III.2 Spatial distribution of allelic richness (AR; **a-b**), private allelic richness (PA; **c-d**) and genetic uniqueness ($F_{st_{UNI}}$; **e-f**) for *Faxonius limosus* (**a, c** and **e**) and *Procambarus clarkii* (**b, d** and **f**).

Spatial patterns of genetic diversity and structure

No significant IBD pattern was observed for *F. limosus* (Mantel $r = -0.127$, $p = 0.905$, Figure III.3a), but there was a significant IBD pattern for *P. clarkii* (Mantel $r = 0.371$, $p < 0.001$, Figure III.3b). Piecewise regressions revealed breakpoints of the positive relationship between genetic differentiation and distance at the very short distance of 1.166 km (CI95% = [0.887–

1.445 km], Figure III.3b), suggesting a strong effect of genetic drift and a low homogenizing effect of gene flow on genetic differentiation at short distances.

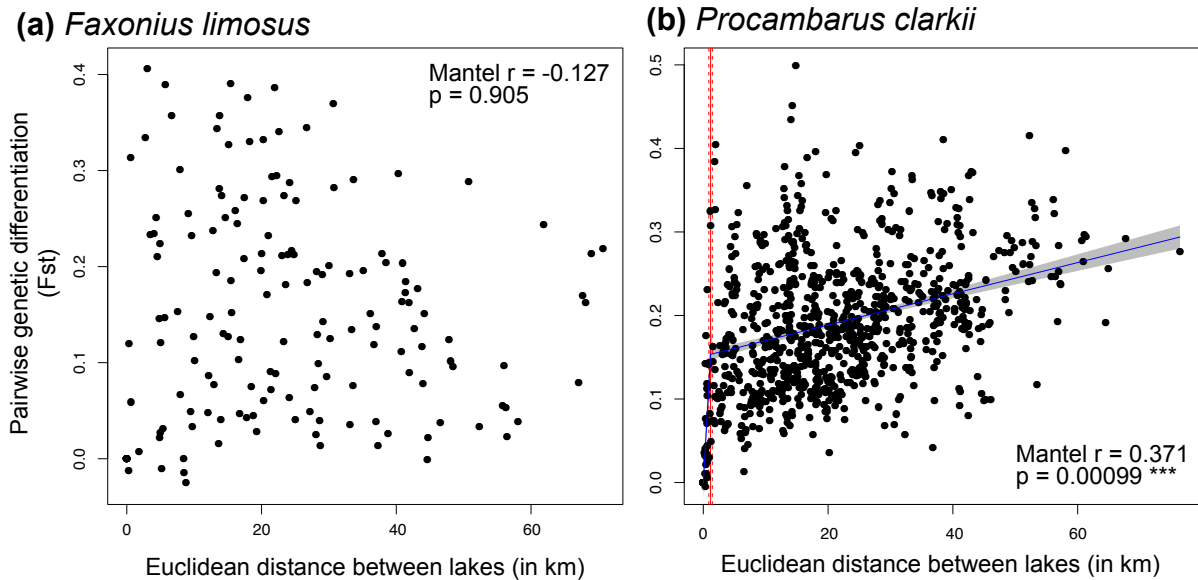


Figure III.3 Pairwise *Fst* values among populations against Euclidean distances between lakes for **(a)** *Faxonius limosus* and **(b)** *Procambarus clarkii*. A piecewise regression line **(b)** is represented in blue (CI95% in grey) as the Mantel test detected a significant IBD pattern for this species. Vertical red dotted line represents the distance at which a breakpoint between two different linear relationships occurs.

Genetic clustering

For *F. limosus*, the two evolutionary models were similarly supported (Figure III.S1a) and generated qualitatively similar results; we thus only report results obtained under the most parsimonious model (i.e. “without admixture”). We found strong evidence for $K=2$ for this species (Figure III.S1b). Overall, there was no clear spatial distribution of these two clusters, nor were populations exclusively belonging to one of these two clusters (Figure III.4a). For *P. clarkii*, there was a high support for the “without admixture” model (Figure III.S1c) and we found strong evidence for the occurrence of 15 different genetic clusters (Figure III.S1d). These clusters were highly spatially-structured, with many single lake populations (e.g. INN, CEA, VRA, or JBV, Figure III.4b) or groups of neighbouring populations (e.g. SOD, SOC, SOB and SOA for cluster 7; BIR and BID for cluster 4; Figure III.4b) belonging almost-exclusively to specific clusters. There were also some distant populations composed of individuals assigned almost-exclusively to a given cluster (e.g. LIN and BON for cluster 10; Figure III.4b) and some populations belonging principally to a specific cluster displaying introgression from individuals belonging to a different cluster (e.g. introgression of cluster 4, mainly represented

in BIR and BID, in TAC and TAD, two populations mainly represented by individuals assigned to cluster 1; Figure III.4b).

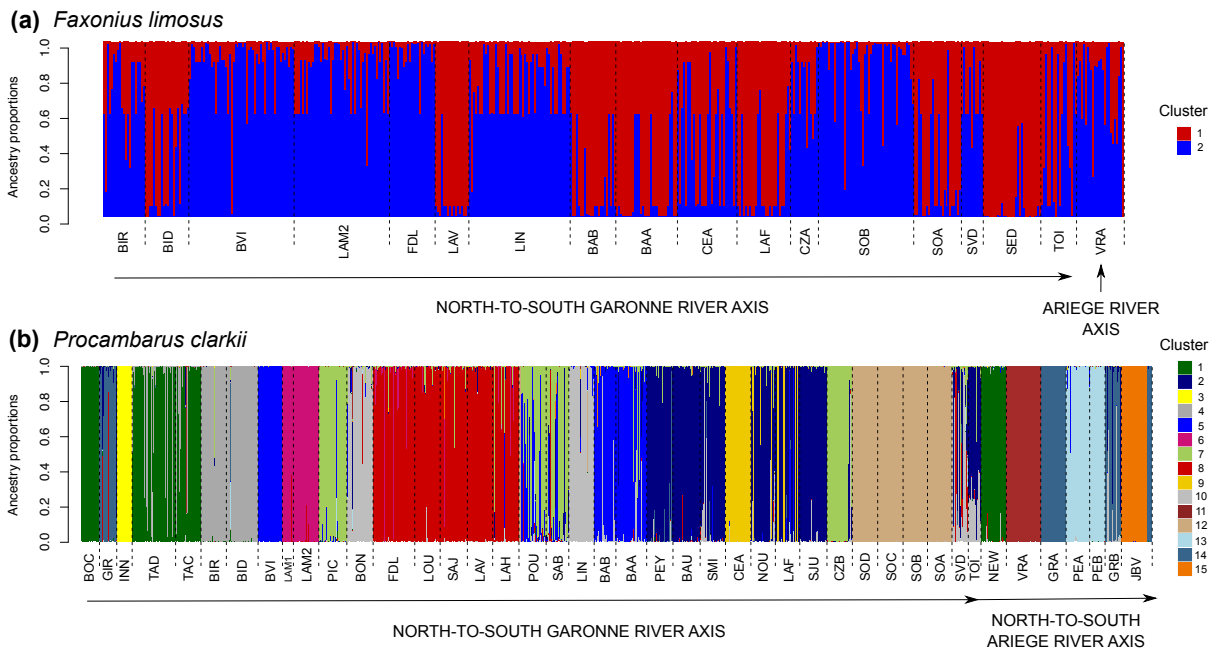


Figure III.4 Proportion of ancestry of individuals to each cluster identified using 'rmaverick' procedure under a model assuming no admixture: (a) *F. limosus* (2 clusters) and (b) *P. clarkii* (15 clusters).

Factors explaining observed patterns of genetic diversity and structure

We found a significant interaction term (Distance x Surface: $F_{1,13}=8.0014$, $p=0.014$) for *AR* of *F. limosus* (Table III.S3). Specifically, *AR* decreases with increasing lake surface in lakes close to Toulouse (Figure III.S2a), while *AR* increases with increasing lake surface in lakes farther from Toulouse (Figure III.S2a). We also found a significant interaction term (Management x Surface: $F_{1,12}=4.9458$, $p=0.046$) for *PA* of *F. limosus* (Table III.S3). *PA* increases with increasing lake surface in lakes with a *high-level* management (Figure III.S2b), but *PA* decreases with increasing lake surface in lakes with a *low-level* management (Figure III.S2b). We did not find significant effects of the environmental variables on $F_{st_{UNI}}$ of *F. limosus* (Table III.S3).

For *AR* of *P. clarkii*, all interaction terms were significant (Distance x Surface: $F_{1,36}=6.7022$, $p=0.014$; Distance x Management: $F_{1,36}=19.9144$, $p<0.001$; Management x Surface: $F_{1,36}=18.7135$, $p<0.001$; Table III.S3). Specifically, *AR* increases with increasing lake surface irrespective of the distance of lakes from Toulouse (Figure III.S3a). *AR* steadily decreases with increasing distance from Toulouse in lakes with *low level* management, and inversely

increases with increasing distance in lakes with *high level* management (Figure III.S3b). Surface of lakes did not affect *AR* in lakes with *low level* management, although it had a strong positive effect on *AR* in lakes experiencing *high level* management (Figure III.S3a). All three interaction terms were also significant for $F_{st_{UNI}}$ of *P. clarkii* (Distance x Surface: $F_{1,36}=5.2435$, $p=0.028$; Distance x Management: $F_{1,36}=31.4851$, $p<0.001$; Management x Surface: $F_{1,36}=5.6676$, $p=0.023$; Table III.S3). Specifically, $F_{st_{UNI}}$ decreases with increasing lake surface irrespective of the distance of lakes from Toulouse (Figure III.S4a). Conversely, $F_{st_{UNI}}$ increases with increasing distance from Toulouse in lakes experiencing *low level* management, but decreases with increasing distance in lakes experiencing *high level* management (Figure III.S4b). $F_{st_{UNI}}$ decreases with increasing lake surface irrespective of the level of management, although the decrease is steeper for lakes experiencing *high level* management (Figure III.S4c).

Discussion

The present study revealed very contrasting patterns of genetic variability for two invasive alien crayfish species co-occurring in a dense network of artificial lakes. One species (*Faxonius limosus*) displayed very low levels of genetic diversity and unclear spatial patterns of genetic structure, preventing a clear identification of its potential local invasion pathways. Contrastingly, the second crayfish species (*Procambarus clarkii*) displayed higher genetic diversity and spatial genetic structuring, allowing identifying specific genetic footprints that suggest that the invasion of the network of artificial lakes by this species has been fuelled by the co-occurrence of multiple local invasion pathways. Our analyses also indicate a context-dependent effect of fishery management practices on genetic diversity that may promote genetic diversity due to deliberate (*release pathway*) or undeliberate introductions as *contaminants* (during fish stocking events) of genetically-distinct individuals. Overall, our results illustrate both the usefulness and limitations of neutral genetic variation assessments for inferring local invasion pathways in complex environments.

Low levels of genetic variability in *F. limosus* preclude the identification of local invasion pathways

The *Faxonius limosus* meta-population was composed of populations characterized by very low genetic diversities. The *GW* statistic revealed evidence of genetic bottlenecks in all populations, suggesting that the observed low levels of genetic diversity are probably due to

founder effects having occurred during the species spread and the local colonisation of lakes. Additionally, facultative parthenogenesis has been reported under controlled conditions in females of this species (Buřič et al., 2011, 2013). Although this life-history trait may partly explain the observed low levels of genetic variability, its occurrence in natural conditions (and in the studied ecosystems specifically) remains unknown. Our analyses of genetic variability (using AR , PA and Fst_{UNI}) only provided moderate evidence of local invasion pathways. For instance, very high PA and high AR values found for the south-western-most population (SED; Figure III.2a-2c) suggest that it may originate from an independent introduction event, probably due to a deliberate *release* or as a *contaminant* of a fish stocking.

We failed to detect a significant IBD pattern for this species, which prevented us to identify whether *unaided/corridor spread* pathways may have occurred. Pairwise Fst values were highly idiosyncratic, with pairs of populations separated by comparable distances displaying highly variable genetic differentiation. Although this idiosyncrasy may suggest the co-occurrence of multiple invasion pathways different than the *unaided/corridor spread* pathways, the very low levels of genetic diversity observed for *F. limosus* have probably blurred our ability to infer any local invasion pathway. The presence of two genetic clusters in the study area may suggest the introduction and subsequent admixture of two genetically-distinct groups, although this hypothesis probably does not hold true. Alternatively, the lack of clear genetic structuring may be a consequence of the very low levels of genetic diversity. With this reduced genetic pool, the differential effects of genetic drift in populations may be driving a first subset of populations to fix a similar set of alleles by chance (e.g. cluster 1; Figure III.4a), while driving a second subset of populations to fix a second subset of alleles (e.g. cluster 2; Figure III.4a).

Finally, we found that PA increases with lake surface in lakes that are experiencing strong fishery management, suggesting that populations from larger managed lakes which likely received more stocking (quantity and frequency) are more prone to receive distinct (and unique) alleles from different sources through the *contaminant* pathway.

Local invasion pathways for *P. clarkii*

Values of AR , $Hexp$ and Fst_{UNI} for *P. clarkii* were overall higher than for *F. limosus*, and PA values were diverse across populations. As for *F. limosus*, all *P. clarkii* populations displayed evidence of recent genetic bottlenecks. Given that the invasion of this species is relatively

recent, and given the high densities of *P. clarkii* in almost all sampled lakes, these bottleneck footprints are probably due to recent founder events. These genetic patterns provided us with a robust playground to identify genetic footprints potentially linked to local invasion pathways. For instance, the TAC population, which experiences low level fishery management practices, displayed the highest values for *AR*, *PA* and *Hexp*, suggesting that it may originate from an independent introduction, probably due to a deliberate *release*. The SOD population, for instance, also displayed a high *PA* and a moderate $F_{st_{UNI}}$, suggesting that it can be the result of an independent deliberate *release* event instead of a colonisation by individuals from other bridgehead populations through the *unaided/corridor spread* pathways.

The sharp increase of pairwise *Fst* values in *P. clarkii* at short distances suggests that (i) neighboring populations are strongly isolated from each other, (ii) populations exchange very low numbers of migrants *per* generation, or that (iii) differential effects of genetic drift are fuelling population differentiation in lakes. These results agree with a recent study (Bélouard et al., 2019b) and suggest that a stepwise invasion process with a colonization front progressing through lakes (through *unaided/corridor spread*) may have occurred, with subsequent isolation and differentiation of small lake populations due to differential genetic drift effects. We found nonetheless high variability in pairwise *Fst* values for couples of populations separated by similar distances, suggesting that other invasion pathways (e.g. *contaminant*, *release*, *stowaway*) might co-occur.

The occurrence of multiple local invasion pathways for *P. clarkii* was confirmed by the genetic clustering analyses. Indeed, we detected a very high number of genetic clusters (15) despite the small spatial scale (~5,000 km²). Some of these clusters were only represented by individuals from specific populations (e.g. for INN, CEA or VRA; Figure III.4b), suggesting that these populations (i) may have been the produce of distinct introduction events (through the *release* or *contaminant* pathways) involving genetically-distinct source populations, and/or (ii) have differentiated from those from other lakes due to genetic drift and isolation. Groups of neighbouring populations belonging to the same private owner or experiencing similar management practices (e.g. SOD, SOC, SOB and SOA; BIR and BID; Figure III.4b) were also represented by individuals assigned to specific genetic clusters, hence suggesting that each group originated from specific introduction events, probably through deliberate *releases* or as *contaminants* of other commodities. Surprisingly, individuals from PIC and CZB lakes, two lakes belonging to the same private owner, were assigned to the same genetic cluster (cluster

7; Figure III.4b), despite being separated by 33 km, suggesting that deliberate *releases* (or releases as *contaminants*) of individuals from the same genetic pool have occurred in these lakes. We further identified individuals that may have been moved as *stowaways*, or through *unaided* long-distance dispersal between distant lakes (e.g. 4 individuals from JBV might have moved from GRA/GRB, located 16 km away; Figure III.4b).

Finally, we found that management practices may promote genetic diversity of *P. clarkii* and reduce among-lakes genetic differentiation, notably through an increased occurrence of releases as *contaminants*. This may be exacerbated in larger lakes with higher carrying capacities such as for *F. limosus*, but also in lakes that are distant from the main city, as they might be more attractive for anglers that want getaways from urbanised areas for leisure angling.

Conclusions

Overall, the patterns of genetic variation of *F. limosus* are congruent with the mainstream hypothesis of Europe's invasion history by this species: a probable strong genetic bottleneck due to a strong founder event in 1890, followed by successive founder events during subsequent introductions and colonization events across Europe. Although our analyses suggest that the *release* and *contaminant* pathways may have played an important role, the very low levels of genetic variability observed for this IAS may have precluded a clear identification of all the local invasion pathways used by this IAS to invade the studied ecosystem. Contrarily, the patterns of genetic variation we observed for *Procambarus clarkii* suggest that this species is able to use many different pathways (mainly *release* and *contaminant*, but also *stowaway* and *unaided/corridor* spread pathways) at a local scale to invade a given area, hence confirming the co-occurrence of multiple invasion pathways observed for this species at the global scale (Oficialdegui et al., 2019, 2020).

We also highlight that fishery management activities may have played an important role on the dissemination of these two IAS across the network of artificial gravel pit lakes we studied, by having promoted genetic diversities in lakes experiencing high level fishery management practices, probably through deliberate *releases* or undeliberate releases as *contaminants* of genetically-distinct individuals. Although further work is still needed to better assess the effects that fisheries and angling practices may have had on the genetic diversity of these IAS (e.g. by compiling information on the number, intensity and origin of crayfish/fish releases in

lakes), we argue that fish stocking must be conducted very carefully to impede the reinforcement of crayfish populations with new genetic variants in already-colonised lakes, and to avoid the colonization of new lakes or of lakes having experienced removal programs.

Here, we illustrate both the usefulness and limitations of neutral genetic variation assessments for inferring local invasion pathways in complex environments. This approach is particularly useful in species having experienced complex invasion histories such as *P. clarkii*, as these species will tend to display high levels of genetic diversity, providing a robust playground to identify the genetic signals of many concomitant local invasion pathways. However, this approach may only provide limited information on local invasion pathways for IAS displaying very low levels of genetic variability due to strong genetic bottlenecks, to particularities of their reproductive mode (clonal and/or parthenogenetic reproduction modes), or a potential combination of both, such as for *Faxonius limosus*. Combining genetic assessments with other complementary approaches such as among-populations phenotypic variability assessments (e.g. based on morphological or stoichiometric traits), or surveys aimed at capturing how socio-economic activities and perceptions might be fuelling the spread of IAS in a given ecosystem may be thus necessary to adequately reveal pathways that might remain obscure when solely using genetic approaches.

Supplementary material

Table III.S1 Populations of *Faxonius limosus* with information about lakes (*Code*, date of *Creation*, *Latitude*, *Longitude* and their level of fishery *Management*) and genetic analyses (sampling sizes (*N*), mean allelic richness (*AR*), expected heterozygosity (*Hexp*), mean private allelic richness (*PA*), mean genetic uniqueness (*Fst_{UNI}*) and mean Garza-Williamson statistic (*GW*)). A minimum sample size of 11 was considered for rarefaction procedures to obtain *AR* and *PA* estimates. The number of microsatellite loci (from a total of 8 polymorphic loci at the meta-population scale) monomorphic at the population level is also reported (*N_{MONO}*).

Code	Creation	Latitude	Longitude	Management	N	AR	Hexp	PA	Fst_{UNI}	GW	N_{MONO}
BAA	1984	43.1920456	1.1207434	High level	31	1.231	0.067	0.029	0.210	0.290	5
BAB	1998	43.1927746	1.1216025	Low level	23	1.267	0.079	0.023	0.162	0.427	5
BID	1993	43.3132063	1.1707778	High level	22	1.568	0.163	0.101	0.120	0.262	3
BIR	1992	43.3149184	1.1723150	High level	21	1.550	0.137	0.095	0.106	0.262	4
BVI	1963	43.3109127	1.2116333	High level	53	1.605	0.164	0.027	0.113	0.301	2
CEA	1970	43.1705748	1.1043968	Low level	30	1.392	0.112	<0.001	0.110	0.407	5
CZA	1973	43.1336084	1.0536924	Low level	14	1.321	0.083	0.069	0.140	0.377	5
FDL	1980	43.2623928	1.1809382	High level	23	1.448	0.157	<0.001	0.181	0.504	5
LAF	1982	43.1657828	1.1032664	Low level	27	1.154	0.067	<0.001	0.158	0.343	6
LAM2	1987	43.3021334	1.2028925	High level	48	1.604	0.149	0.055	0.113	0.304	3
LAV	1992	43.2311011	1.1557208	High level	17	1.245	0.073	0.001	0.190	0.167	4
LIN	1987	43.2035470	1.1337380	High level	51	1.400	0.094	0.022	0.193	0.357	4
SED	1976	43.0606415	0.4150075	High level	29	1.920	0.260	0.333	0.158	0.375	2
SOA	1986	43.1220218	1.0224061	Low level	24	1.272	0.106	<0.001	0.155	0.391	6
SOB	1992	43.1227472	1.0247659	Low level	48	1.600	0.186	<0.001	0.235	0.412	4
SVD	1958	43.1113178	1.0018878	High level	11	1.340	0.078	0.029	0.137	0.367	5
TOI	1997	43.0503217	0.5728948	Low level	18	1.795	0.194	0.049	0.088	0.388	2
VRA	1982	43.2445421	1.2510805	Low level	24	1.755	0.196	0.127	0.135	0.359	1
Mean					29	1.470	0.131	0.054	0.150	0.350	4
SD					13	0.217	0.056	0.080	0.040	0.076	2

Table III.S2 Populations of *Procambarus clarkii* with information about lakes (Code, date of Creation, Latitude, Longitude and their level of fishery Management) and genetic analyses (sampling sizes (N), mean allelic richness (AR), expected heterozygosity (Hexp), mean private allelic richness (PA), mean genetic uniqueness (FstUNI) and mean Garza-Williamson statistic (GW)). A minimum sample size of 12 was considered for rarefaction procedures to obtain AR and PA estimates.

Site	Creation	Latitude	Longitude	Management	N	AR	Hexp	PA	Fst _{UNI}	GW
BAA	1984	43.1920456	1.1207434	High level	34	3.937	0.649	0.018	0.151	0.469
BAB	1998	43.1927948	1.1216776	Low level	24	3.692	0.626	0.006	0.158	0.458
BAU	2000	43.1901757	1.1156223	Low level	30	3.650	0.616	0.0026	0.167	0.373
BID	1993	43.3132063	1.1707778	High level	35	4.445	0.713	0.122	0.168	0.467
BIR	1992	43.3149184	1.1723150	High level	28	4.562	0.714	0.067	0.165	0.473
BOC	1964	43.696586	1.391384	High level	20	3.976	0.634	0.048	0.211	0.380
BON	1990	43.2713507	1.1625212	Low level	29	3.769	0.632	0.018	0.142	0.458
BVI	1963	43.3109127	1.2116333	High level	27	2.649	0.412	<0.001	0.313	0.575
CEA	1970	43.1705748	1.1043968	Low level	28	2.850	0.525	0.005	0.239	0.414
CZB	2008	43.1347129	1.0540092	Low level	28	2.775	0.512	0.054	0.248	0.473
FDL	1980	43.2623928	1.1809382	High level	46	4.046	0.633	0.066	0.154	0.500
GIR	1954	43.3347052	1.2459544	High level	19	3.713	0.624	0.050	0.190	0.412
GRA	1979	43.235802	1.2608448	High level	28	2.406	0.399	0.008	0.316	0.430
GRB	1992	43.2353916	1.2628752	High level	18	3.327	0.567	<0.001	0.187	0.401
INN	1990	43.321721	1.3050378	High level	17	2.712	0.544	0.077	0.249	0.399
JBV	2003	43.170465	1.3307898	Low level	34	3.062	0.512	0.006	0.237	0.441
LAF	1982	43.1657828	1.1032664	Low level	27	3.368	0.599	<0.001	0.171	0.424
LAH	1972	43.2305593	1.1624622	Low level	29	4.189	0.652	0.054	0.139	0.491
LAM1	1970	43.3022554	1.2014410	High level	12	3.270	0.527	0.021	0.240	0.407
LAM2	1987	43.3021334	1.2028925	High level	28	3.107	0.538	0.013	0.240	0.431
LAV	1992	43.2311011	1.1557208	High level	28	4.378	0.699	0.073	0.131	0.471
LIN	1987	43.203547	1.1337380	High level	28	3.325	0.578	0.125	0.197	0.457
LOU	1982	43.2318786	1.1630981	High level	28	3.960	0.614	0.025	0.166	0.485
NEW	1966	43.2926411	1.2331285	Low level	28	4.086	0.688	0.068	0.144	0.447
NOU	1982	43.1654923	1.1018183	Low level	27	3.021	0.482	0.079	0.238	0.453
PEA	1990	43.2343908	1.2518984	Low level	26	3.876	0.662	0.025	0.157	0.461
PEB	1990	43.2349956	1.2517832	Low level	17	3.948	0.671	0.001	0.150	0.475
PEY	2007	43.1915169	1.1141565	Low level	29	3.681	0.641	0.007	0.163	0.416
PIC	1980	43.285232	1.1850026	Low level	31	4.008	0.667	0.102	0.159	0.504
POU	1996	43.2218941	1.1530503	High level	30	4.482	0.711	0.031	0.120	0.499
SAB	1997	43.2152621	1.1504806	Low level	25	4.106	0.673	0.001	0.135	0.459
SAJ	1983	43.2320905	1.1644074	Low level	30	3.778	0.604	0.037	0.172	0.507
SJU	1993	43.1551368	1.0805472	Low level	30	3.024	0.540	0.015	0.206	0.422
SMI	1976	43.1826343	1.1130333	Low level	28	3.974	0.660	0.013	0.131	0.410
SOA	1986	43.1220218	1.0224061	Low level	27	3.044	0.518	0.133	0.215	0.472
SOB	1992	43.1227472	1.0247659	Low level	27	3.053	0.560	<0.001	0.204	0.408
SOC	1998	43.1232123	1.0220173	Low level	28	2.824	0.508	0.005	0.228	0.507
SOD	2007	43.1238749	1.0239614	Low level	28	3.142	0.543	0.159	0.237	0.443
SVD	1958	43.1113178	1.0018878	High level	18	3.960	0.631	<0.001	0.133	0.445
TAC	1980	43.3301296	1.1525397	Low level	28	4.875	0.717	0.224	0.149	0.473

TAD	1990	43.3300972	1.1458884	Low level	48	4.435	0.690	0.088	0.152	0.491
TOI	1997	43.0503217	0.5728948	Low level	14	2.448	0.452	<0.001	0.269	0.388
VRA	1982	43.2445421	1.2510805	Low level	38	3.285	0.601	0.010	0.205	0.401
				Mean	27	3.586	0.598	0.043	0.189	0.450
				SD	7	0.621	0.082	0.050	0.049	0.041

Table III.S3 Selected linear models explaining *AR* and *PA* values observed in *Faxonius limosus*. No models with significant sources of variations were found for *Fst_{UNI}* for this species. Non-italic values correspond to p-values obtained through F-tests using the 'anova' function in R, while p-values reported in italic correspond to p-values obtained through T-tests using the 'summary.lm' function in R (only reported for the factor variable Management and for the Intercept of models). Significant p-values are in bold.

Dependent variable	Explanatory variable	Df	Estimate (SE)	F value	p-value
AR	Distance	1	-9.242x10 ⁻⁶ (<0.0001)	0.4854	0.066
	Management	1		0.713	0.414
	<i>Low level</i>		-6.626 x10 ⁻² (0.0951)		0.498
	Surface	1	-2.565x10 ⁻⁶ (<0.0001)	0.9324	0.352
	Distance x Surface	1	8.645x10 ⁻¹¹ (<0.0001)	8.0014	0.014*
	Intercept		1.756 (0.1786)		<0.001***
	Residuals (Error)	13			
PA	Distance	1	-2.922x10 ⁻⁶ (<0.0001)	0.5154	0.487
	Management	1		2.6667	0.128
	<i>Low level</i>		0.1167 (0.1218)		0.357
	Surface	1	-1.065x10 ⁻⁷ (<0.0001)	0.1136	0.742
	Distance x Surface	1	4.388x10 ⁻¹¹ (<0.0001)	2.5117	0.139
	Management x Surface	1		4.9458	0.046*
	<i>S x Low level</i>		-2.152x10 ⁻⁶ (<0.0001)		0.046*
Intercept		0.2649 (0.1282)		0.061	
Residuals (Error)	12				
Fst_{Uni}	Distance	1	3.266x10 ⁻⁷ (<0.0001)	0.3214	0.580
	Management	1		0.1979	0.663
	<i>Low level</i>		-0.0086 (0.0021)		0.701
	Surface	1	8.162x10 ⁻⁸ (<0.0001)	0.311	0.586
	Intercept		0.1326 (0.0257)		<0.001***
Residuals (Error)	14				

Table III.S4 Best linear models (after a backward-selection procedure) explaining AR and Fst_{Uni} values observed in *Procambarus clarkii*. No models with significant sources of variations were found for PA for this species. Non-italic values correspond to p-values obtained through F-tests using the 'anova' function in R, while p-values reported in italic correspond to p-values obtained through T-tests using the 'summary.lm' function in R (only reported for the factor variable Management and for the Intercept of models). Significant p-values are in bold.

Dependent variable	Explanatory variable	Df	Estimate (SE)	F value	p-value
AR	Distance	1	2.608 (0.2689)	16.3893	<0.001***
	Management	1		0.753	0.391
	<i>Low level</i>		2.390 (0.3856)		<0.001***
	Surface	1	4.857x10 ⁻⁶ (<0.0001)	1.7039	0.200
	Distance x Surface	1	7.127x10 ⁻¹¹ (<0.0001)	6.7022	0.014*
	Distance x Management	1		19.9144	<0.001***
	<i>D x Low level</i>		-6.278x10 ⁻⁵ (<0.0001)		<0.001***
	Management x Surface	1		18.7135	<0.001***
	<i>S x Low level</i>		-6.833x10 ⁻⁶ (<0.0001)		<0.001***
Intercept		2.608 (0.2689)		<0.001***	
Residuals (Error)	36				
PA	Distance	1	-2.347x10 ⁻⁶ (<0.0001)	3.186	0.082
	Management	1		0.1278	0.723
	<i>Low level</i>		0.0125 (0.0429)		0.773
	Surface	1	1.05x10 ⁻⁷ (<0.0001)	0.2596	0.613
	Intercept		0.2203 (0.0476)		<0.001***
	Residuals (Error)	39			
Fst_{Uni}	Distance	1	7.449x10 ⁻⁵ (<0.0001)	1.3740	0.249
	Management	1		1.2683	0.2675
	<i>Low level</i>		5.418 (0.9104)		<0.001***
	Surface	1	4.582x10 ⁻⁶ (<0.0001)	1.2152	0.2776
	Distance x Surface	1	2.308x10 ⁻¹⁰ (<0.0001)	5.2435	0.028*
	Distance x Management	1		31.4851	<0.001***
	<i>D x Low level</i>		-1.641x10 ⁻⁴ (<0.0001)		<0.001***
	Management x Surface	1		5.6676	0.0227*
	<i>S x Low level</i>		-8.879x10 ⁻⁶ (<0.0001)		0.023*
Intercept		2.979 (0.6349)		<0.001***	
Residuals (Error)	36				

Figure III.S1 Posterior probabilities of the two different evolutionary models (with/without admixture; **a** and **c**) and of the number of *K* clusters (**b** and **d**) tested with 'rmaverick' for *Faxonius limosus* (**a-b**) and *Procambarus clarkii* (**c-d**).

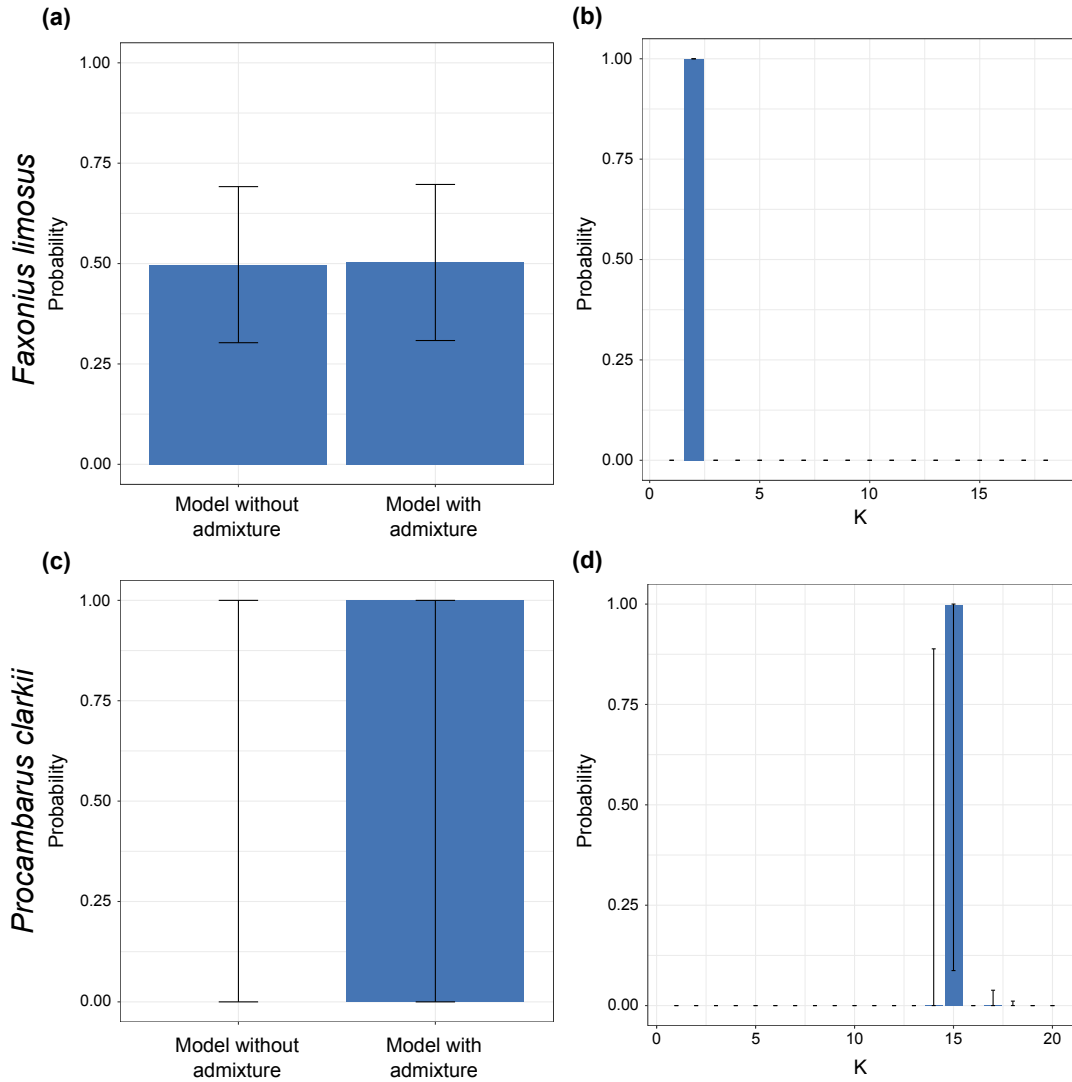


Figure III.S2 Significant interaction terms from the best linear models determined through a backward-selection procedure to explain values of **(a)** allelic richness (*AR*) and **(b)** private allelic richness (*PA*) observed in *Faxonius limosus* populations. **(a)** corresponds to the interactive effects of the *Surface* of lakes and their *Distance* to the main city (Toulouse) on *AR*. Distance is used here as a moderator term, with three values corresponding to the mean Distance value (38.235 km) and to the mean value \pm its standard deviation. **(b)** corresponds to the interactive effects of the *Surface* of lakes and the strength of fishing *Management* practices of lakes on *PA* (grouping levels of the moderator term = *Low* or *High* level).

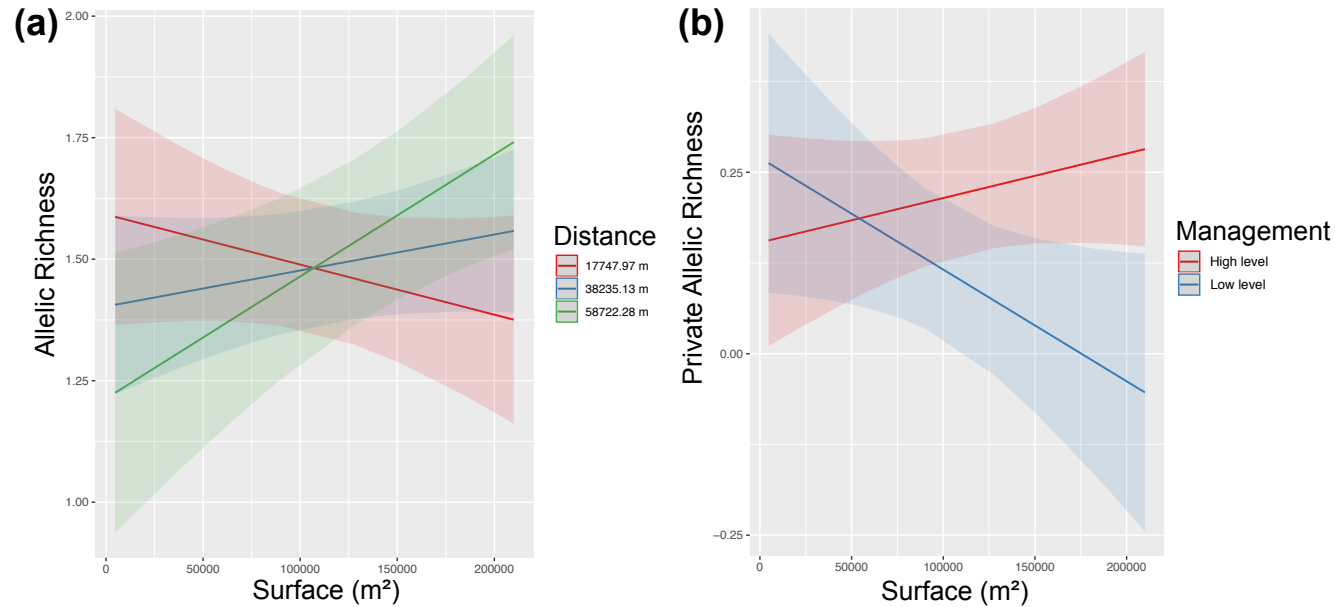


Figure III.S3 Significant interaction terms from the best linear model determined through a backward-selection procedure to explain allelic richness (*AR*) values observed in *Procambarus clarkii* populations. **(a)** corresponds to the interaction between the *Surface* of lakes and their *Distance* to the main city (Toulouse). Distance is used here as a moderator term, with three values corresponding the mean Distance value (30.509 km) and to the mean value \pm its standard deviation. **(b)** corresponds to the interaction between *Distance* and the strength of fishing *Management* practices of lakes (grouping levels of the moderator term = *Low* or *High* level). **(c)** corresponds to the interaction between *Surface* and the type of fishing *Management* practice of lakes.

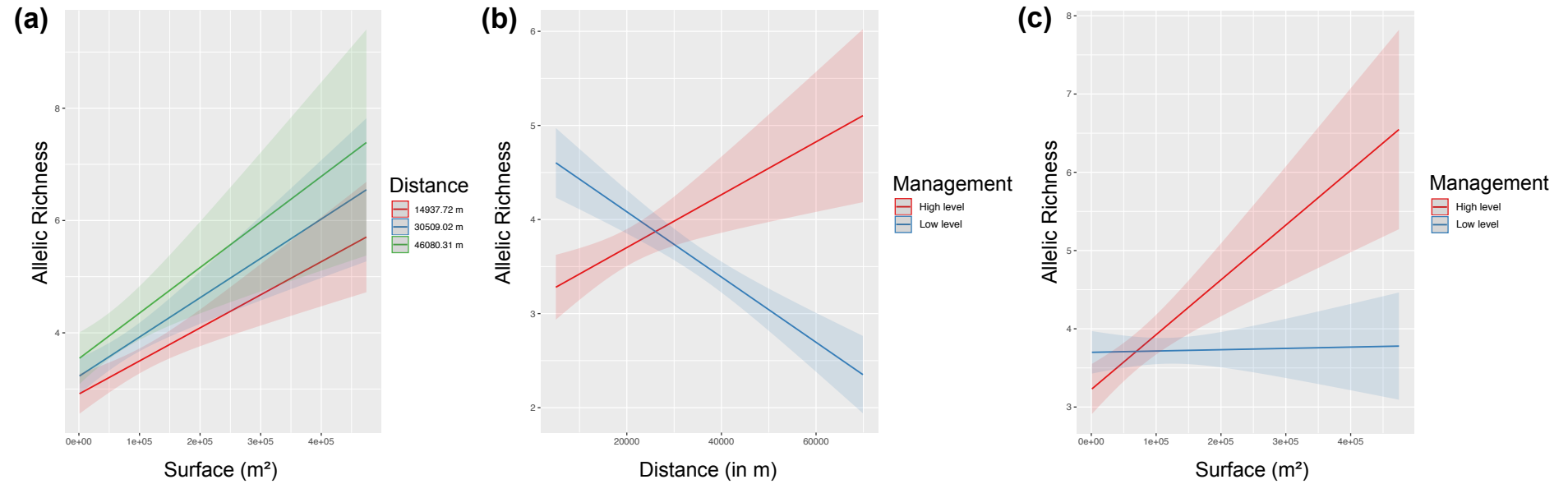
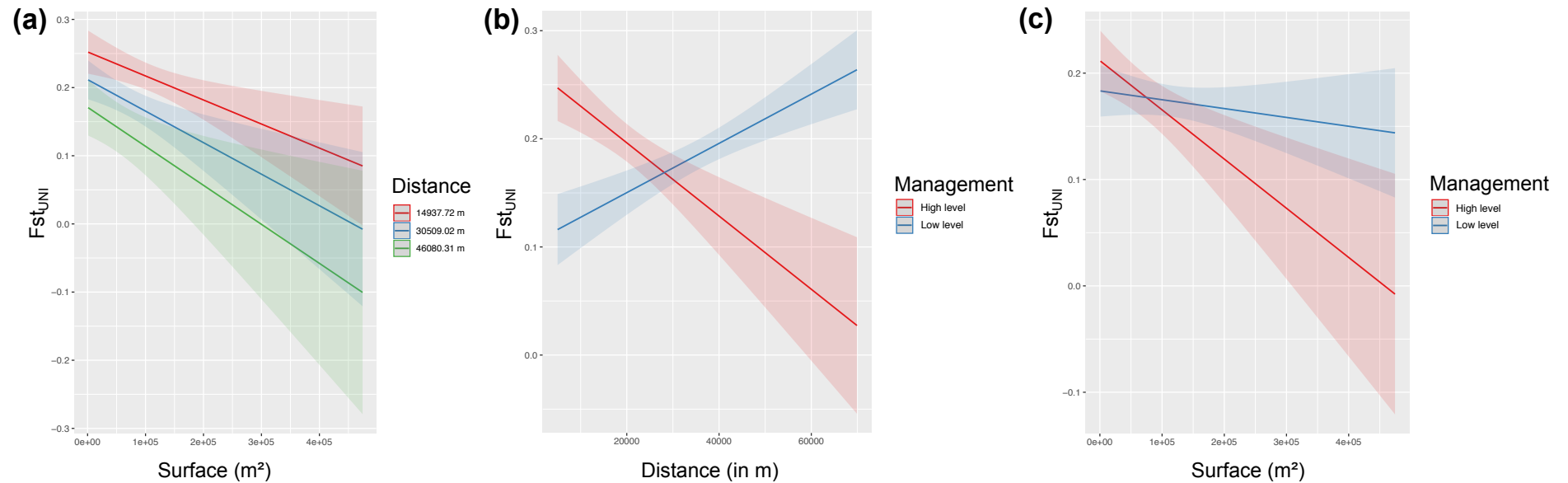


Figure III.S4 Significant interaction terms from the best linear model determined through a backward-selection procedure to explain genetic uniqueness ($F_{st_{UNI}}$) values observed in *Procambarus clarkii* populations. **(a)** corresponds to the interaction between the *Surface* of lakes and their *Distance* to the main city (Toulouse). Distance is used here as a moderator term, with three values corresponding the mean Distance value (30.509 km) and to the mean value \pm its standard deviation. **(b)** corresponds to the interaction between *Distance* and the type of fishing *Management* practice of lakes (grouping levels of the moderator term = *Low* or *High* level). **(c)** corresponds to the interaction between *Surface* and the type of fishing *Management* practice of lakes.



Appendix

Appendix III.1 Genotyping quality controls

A preliminary check of the overall levels of genetic diversity of *F. limosus* at the locus scale revealed that one locus (PCSH0042) was monomorphic for all populations and it was removed from subsequent analyses. We detected significant homozygote excesses for 10 locus/population pairs out of 144 possible pairs, although no clear intra-population or intra-locus pattern was detected. Significant linkage disequilibrium was detected for 1 out of 504 locus per locus per population combinations, and significant deviations from HW (after FDR correction) were only detected for 3 out of 144 locus/population pairs. Neutrality tests performed with Bayescan did not identify any outlier locus (Figure Appendix III.1-1).

For *P. clarkii*, we detected significant homozygote excesses for 27 locus/population pairs out of 602 possible combinations: loci PCLG48, PCSH0089, PCSH0005, PCLG15 and PCLG48 were involved in one pair each one; PCSH0006 was involved in two pairs, PCLG17, PCLG32, PCL29 and PCLG28 were involved in three pairs; and PCLG27 was involved in nine pairs. Given the moderate propensity of significant homozygote excesses (indicative of null alleles presence) observed for PCLG27 across populations (i.e. in 9/43 populations), it was removed for further analyses. We found one significant deviation from HW (after FDR correction) for all 597 locus/population pairs. Neither locus nor population was removed from the database following this analysis. We further detected significant linkage disequilibria between 141 out of 3,913 locus per locus per population combinations. Many of these significant disequilibria implied three specific populations (TAD, LAF, and VRA, with 62/91, 35/91 and 16/91 significant disequilibria respectively), accumulating 80% of all significant disequilibria, suggesting that the linkage disequilibria we detected for *P. clarkii* were mainly related to population-specific processes rather than to locus-specific problems. Neither locus nor population was therefore removed from the database following this analysis. Neutrality tests performed with Bayescan identified an outlier locus (PCSH0089) displaying significant negative q values at the 95% confidence level (Figure Appendix III.1-2). Negative q values are indicative of purifying or balancing selection. We then removed this locus from the genetic database for subsequent analyses.

Figure Appendix III.1-1 BayeScan outlier detection analysis plot for 8 polymorphic microsatellite loci from *Faxonius limosus*. The four chains (run 1 to 4) were convergent according to the Gelman-Rubin test (Gelman-Rubin statistic = 1).

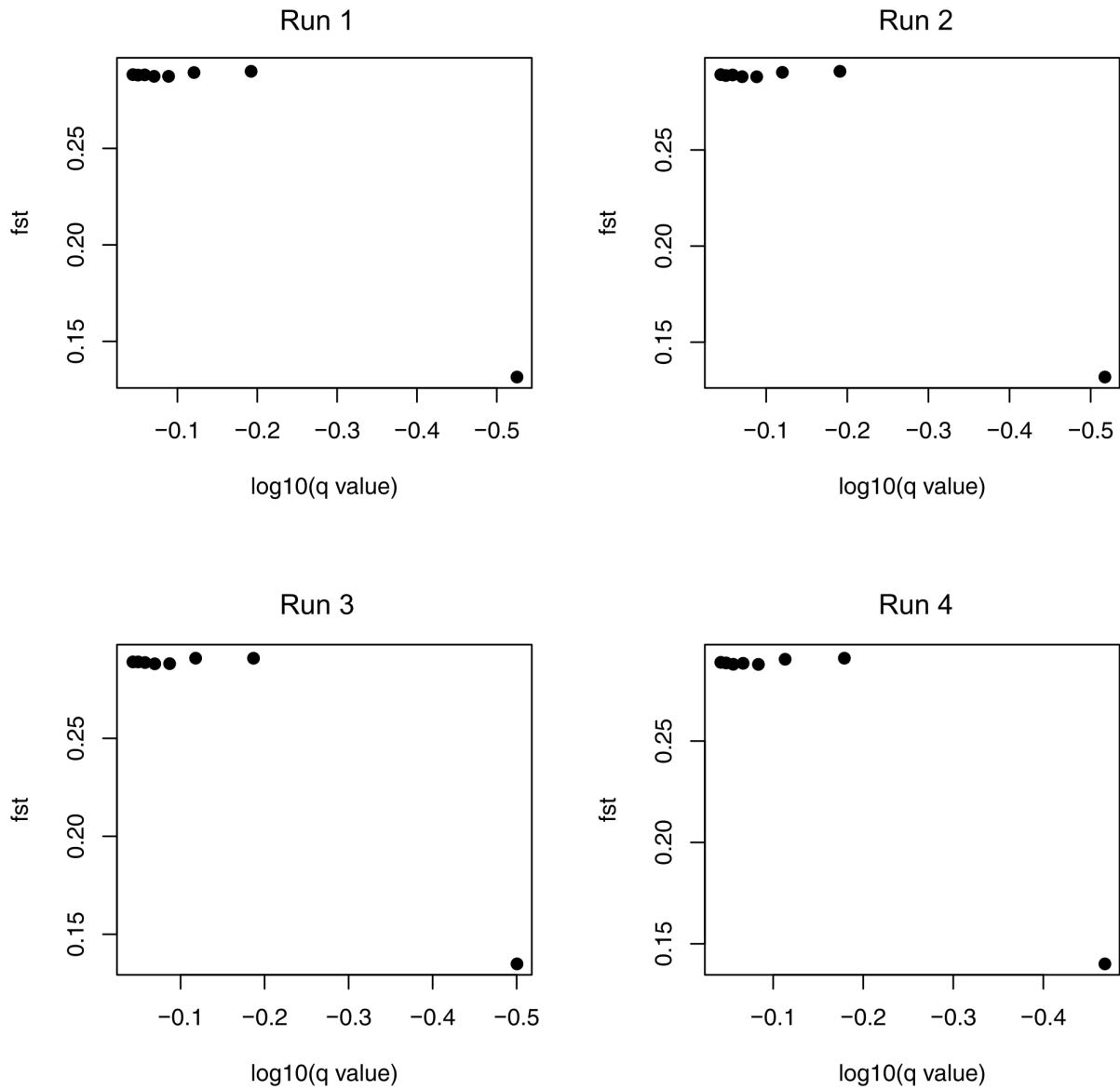
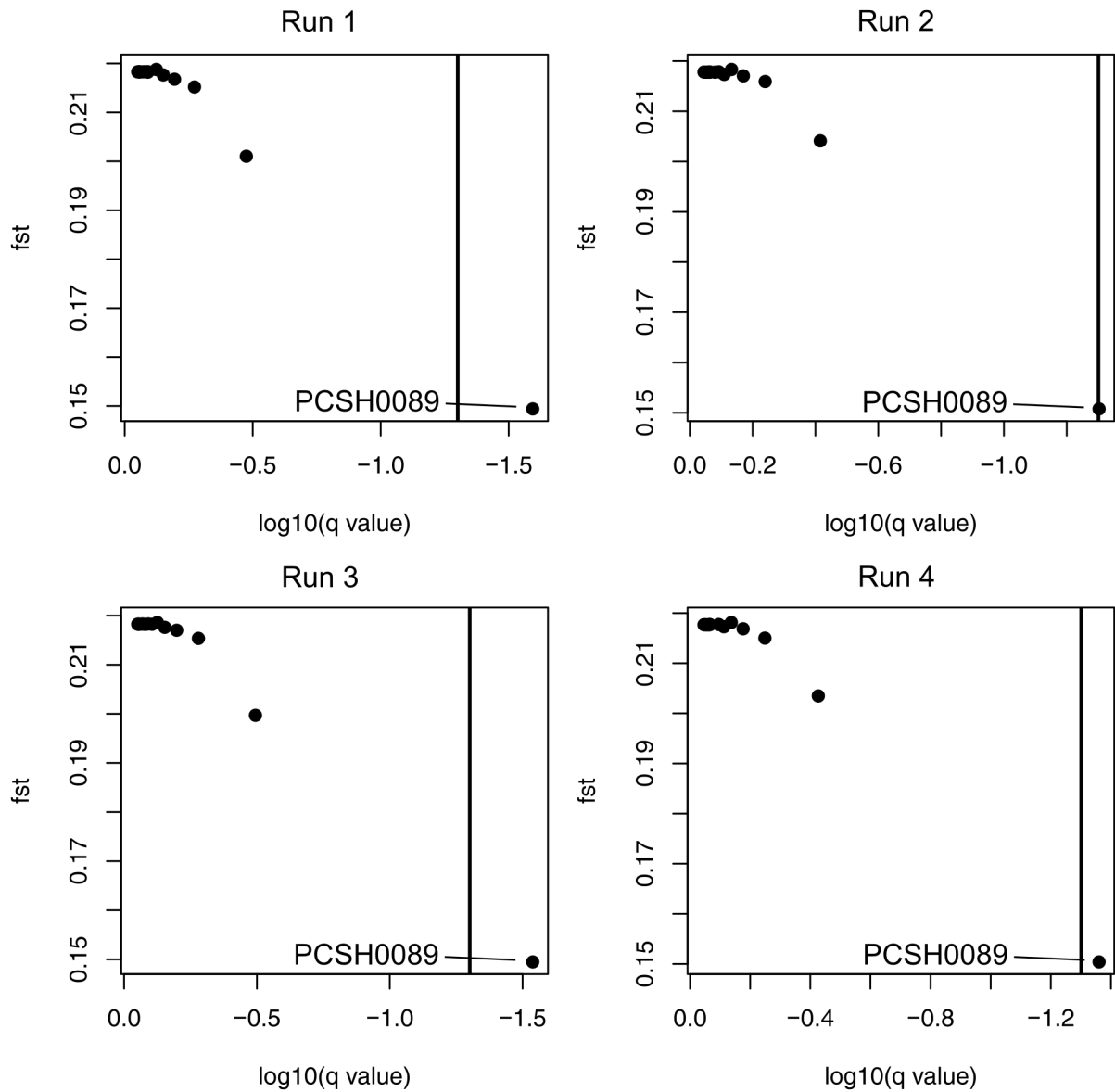


Figure Appendix III.1-2 BayeScan outlier detection analysis plot for 15 polymorphic microsatellite loci from *Procambarus clarkii*. The vertical lines show the critical values of the $\log_{10}(q)$ value for identifying outlier loci at $p = 0.05$. The four chains (run 1 to 4) were convergent according to the Gelman-Rubin test (Gelman-Rubin statistic = 1).



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Chapitre IV

Patrons et déterminants de la variabilité phénotypique au sein de deux espèces d'écrevisses invasives

Résumé

Il est indispensable d'améliorer nos connaissances des déterminants écologiques et des processus évolutifs qui façonnent la variabilité intraspécifique des espèces envahissantes, pour mieux comprendre leurs impacts sur le fonctionnement des écosystèmes natifs. Dans cette étude nous avons quantifié la variabilité phénotypique (traits morphologiques, trophiques et stœchiométriques) au sein de populations de deux espèces d'écrevisses envahissantes (*Procambarus clarkii* et *Faxonius limosus*) aux histoires d'invasion différentes. Nous avons mis en évidence que, pour chaque type de trait, *P. clarkii* et *F. limosus* présentaient des patrons de distribution de variance contrastés entre trois échelles écologiques (population, sexe, individu). Ensuite, nous avons montré que les variations morphologiques et stœchiométriques de *P. clarkii* étaient associées à des déterminants écologiques (i.e. pression de prédation et compétition intraspécifique) et à l'âge d'invasion, tandis que les traits morphologiques chez *F. limosus* variaient uniquement avec les facteurs écologiques (i.e. pression de prédation et compétition interspécifique). Enfin, nous avons mis en évidence que les processus neutres et adaptatifs, en différentes proportions, avaient façonné la variabilité phénotypique des deux espèces, avec une part plus importante des processus adaptatifs pour *F. limosus*. Globalement, nos résultats suggèrent que *F. limosus* a déjà été soumise à une adaptation locale au sein de la métapopulation tandis que *P. clarkii*, qui a été introduite plus tard, n'a pas encore subi ce processus d'adaptation. Nos résultats ont également mis en évidence que ces deux espèces envahissantes ont sûrement des impacts différents suivant l'échelle écologique considérée.

Patterns and determinants of phenotypic variability within two invasive crayfish species

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Abstract

Knowledge on the ecological determinants and evolutionary processes shaping intraspecific variability in invasive species is needed to fully understand their consequences on the functioning of recipient ecosystems. Here, we quantified phenotypic variability (morphological, trophic and stoichiometric traits) among invasive populations of two crayfish species (*Procambarus clarkii* and *Faxonius limosus*) with distinct invasion history. We demonstrated that, for each group of traits, *P. clarkii* and *F. limosus* displayed contrasting patterns of variance distribution across three ecological scales (population, sex, individual). Then, we demonstrated that *P. clarkii* trait variations in body morphology and stoichiometry were associated with both ecological and historical determinants (i.e. predation pressure, intraspecific invasion and invasion age), and morphological traits in *F. limosus* varied with ecological factors only (i.e. predation pressure and interspecific competition). Finally, we highlighted 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*. Overall, these results indicated 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 highlighted that these two invasive species might have contrasting effects across ecological scales.

Introduction

Intraspecific variability is increasingly studied within a multidisciplinary approach combining both population genetics and functional ecology, hence fostering our capacity to understand patterns of biodiversity (Vellend et al., 2014; Mims et al., 2017). Such multi-faceted integrative studies highlight the eco-evolutionary processes underlying intraspecific variability patterns at multiple organizational 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 (McGill, 2008; Messier et al., 2010; Evangelista et al., 2019a). These patterns generally result from (i) adaptive processes (e.g. selection, plasticity) under environmental gradients (i.e. linked to spatio-temporal dynamics of organisms and ecosystems; Holt & Gaines, 1992; Ackerly, 2003; Araújo et al., 2011; Prunier et al., 2018), and from (ii) 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; Klopstein et al., 2006; Excoffier & Ray, 2008; Bélouard et al., 2019). Because intraspecific variability can impact ecosystem functioning (Des Roches et al., 2018; Raffard et al., 2019b; Blanchet et al., 2020), through different contributions of individuals to trophic interactions, ecosystem productivity, nutrient cycling and/or through ecosystem engineering (Harmon et al., 2009; Bassar et al., 2012), there is a need to understand how it may vary across spatial and temporal scales.

In the context of biological invasions, intraspecific variability characterization may be helpful for predicting the ecosystem consequences of invasive individuals, which can intrinsically have important ecological effects (Lockwood et al., 2007). For example, ecological effects induced by sexual dimorphism can be modulate by sex-ratio variations (Fryxell et al., 2015). Biological invasions can be viewed as filtering processes during which invasive individuals pass through a succession of stages and of environmental filters (Lockwood et al., 2007; 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 colonization gradient (Huey, 2000; Strubbe et al., 2013; Juette et al., 2014). 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; 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 natural enemies and/or competitors (Mack et al., 2000; Shea & Chesson, 2002; Kinnison et al., 2008). Finally, the management of invaders might also lead to a change in their phenotypic traits, 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 exhibiting contrasting invasion histories and life-history traits that recently colonized our regional study area: the spiny-cheek crayfish, *Faxonius limosus*, which first occurred in 1988, and the red-swamp crayfish, *Procambarus clarkii*, which 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 organization (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 the spiny-cheek crayfish will display lower morphological variability at the individual level (i.e. within populations) compared to the population level (i.e. among populations). On the contrary, we expected a higher morphological variability in *P. clarkii* at the individual level compared to the population level, as it has colonized the study area more recently. 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., 2019b), 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 colonization 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 at unravelling 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.

Material & methods

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 to 55 km (Euclidean distance) away from Toulouse city along the Garonne River, in Southwestern France (Figure IV.1; see also Alp et al., 2016; Zhao et al., 2016). These lakes are disconnected from the hydrographic network unless during exceptional flood events, 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). The invasion process is relatively recent in those lakes, since the presence of *F. limosus* and *P. clarkii* in the studied area were first documented in 1988 and in 1995, respectively (Changeux, 2003; Magnier & Petit, 2016). Our field observations revealed that gravel pit lakes colonization 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 IV.1).

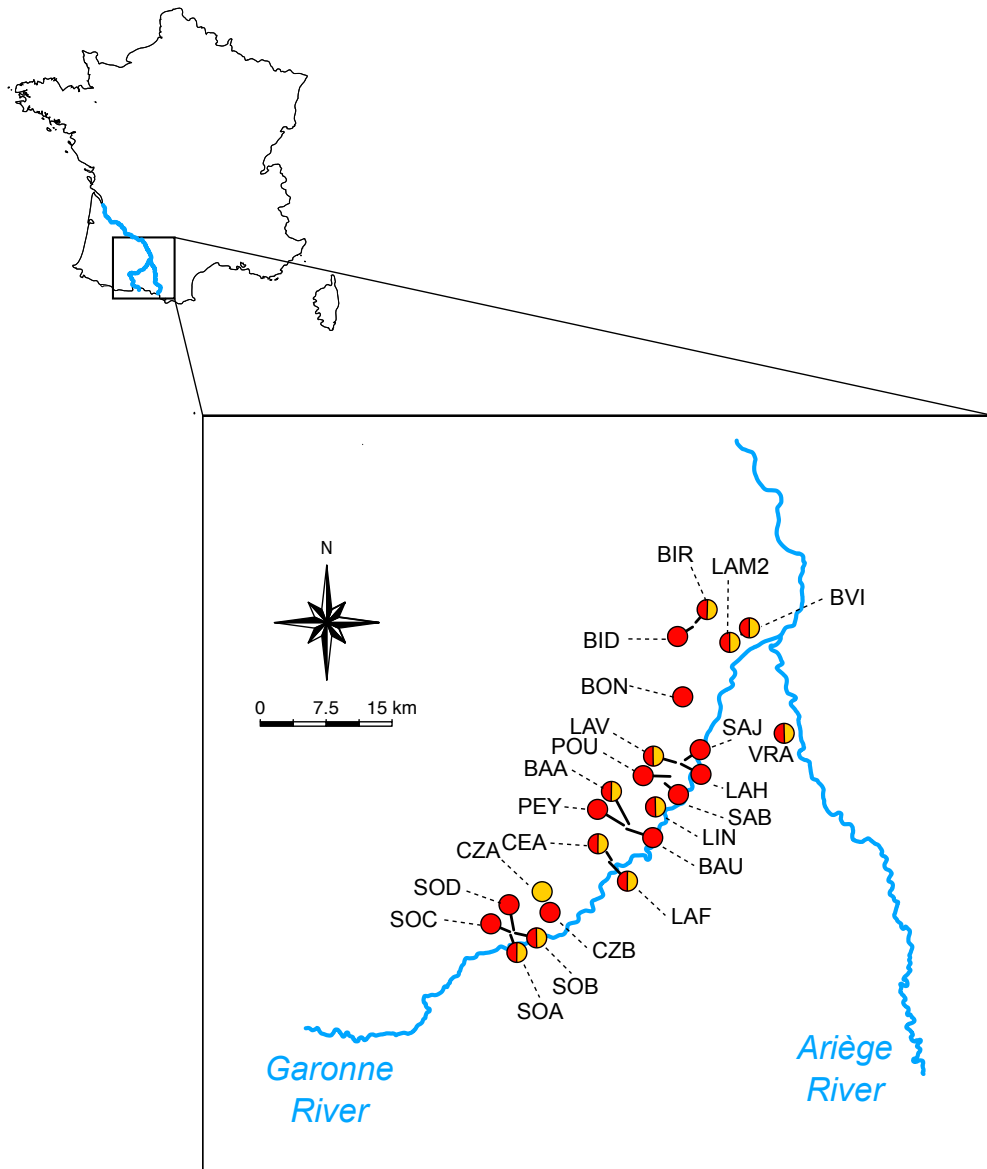


Figure IV.1 Map of the 23 studied gravel pit lakes, southwestern Toulouse, France. Red circles represent *Procambarus clarkii* populations, yellow circles represent *Faxonius limosus* populations, and coexisting populations are represented with red and yellow circles.

Native from Northern America, *P. clarkii* and *F. limosus* are among the most invasive crayfish species worldwide (Holdich et al., 2009; Filipová et al., 2011; Oficialdegui et al., 2019). Both species strongly impact ecosystem functioning and native organisms through consumption (e.g. macroinvertebrates, fish, macrophytes; Correia & Anastácio, 2008; Vojtkovská et al., 2014), disease transmission (Changeux, 2003) and ecological engineering (e.g. burrowing activity, bioturbation; Correia & Ferreira, 1995; Holdich & Black, 2007). The high invasiveness potential of *P. clarkii* might be partly attributable to its ability to disperse overland (Kerby et al., 2005; Cruz & Rebelo, 2007; 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 has been reported overland on extremely rare occasions (Puky, 2014). To maximize their reproductive success, both species are suspected to combine sexual and asexual modes of reproduction (i.e. parthenogenesis; Yue et al., 2008; Buřič et al., 2011, 2013). In the studied system, previous studies highlighted the existence of substantial phenotypic variability (morphology, trophic ecology) among (Jackson et al., 2017; Evangelista et al., 2019a) and within populations of *P. clarkii* (Raffard et al., 2017; Lang et al., 2020). This suggests contrasted impacts of invasive individuals on ecosystem functioning among-populations of *P. clarkii* (Alp et al., 2016; Evangelista et al., 2019b). No study has yet focused on *F. limosus* intraspecific variability within our sampling area.

Sampling and environmental characteristics

Faxonius limosus and *P. clarkii* were sampled from mid-September to mid-October 2016-18 using pairs of baited traps (one cylindrical trap: 62cm x 34cm x 34cm, mesh size: 10mm; one rectangular trap: 95cm x 20cm x 20cm, mesh size: 4mm) set overnight ($n = 12$ traps) and during the day ($n = 8$ traps) in the littoral habitat. When needed, additional trapping, electrofishing (Deka 7000; Deka, Marsberg, Germany) 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 (e.g. Fourtune et al., 2018; Evangelista et al., 2019a). Following capture, crayfish were sexed, measured for carapace length (± 0.01 mm) and were euthanized 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 h).

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 poplar leaves (i.e. resource from terrestrial origin) were collected from the littoral since both species have a preference for vegetable diet at the adult stage (Vojkovská et al., 2014; Jackson et al., 2017). Periphyton and poplar leaves samples were freeze-dried (-50°C for 5 days) and oven-dried (60°C for 48h), respectively (further details available in Jackson et al. 2017).

The same day, lake productivity was assessed by measuring the chlorophyll a concentration ($\mu\text{g}\cdot\text{L}^{-1}$) (with an AlgaeTorch device, BBE moldaenke GmbH, Germany) at three locations

within each lake. These three values were averaged to account for within-lake variability in environmental conditions (Table IV.1). For each species, the abundance of crayfish was estimated in each lake as the number of individuals trapped over a 24-hour period (catch per unit effort (CPUE) expressed in ind. trap⁻¹.hr⁻¹; Table IV.1). The predation pressure in each lake was assessed as the biomass of predator fish trapped in a set of gillnets randomly distributed, over a 1-hour period (biomass per unit effort (BPUE) expressed in g. gillnet⁻¹.hr⁻¹; Table IV.1; see further details in Zhao et al., 2016 and Lang et al., 2020).

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 IV.1). *Faxonius limosus* and *P. clarkii* coexisted in eleven lakes (i.e. sympatric populations) and were isolated in one lake for *F. limosus* and eleven lakes for *P. clarkii* (i.e. allopatric populations; Table IV.1).

Table IV.1 Environmental, historical and management characteristics of the 23 sampled lakes.

Population	Year of lake creation	Productivity $\mu\text{g.L}^{-1}$	CPUE <i>P. clarkii</i> ind. trap \cdot hr $^{-1}$	CPUE <i>F. limosus</i> ind. trap \cdot hr $^{-1}$	BPUE predation g.gillnet \cdot hr $^{-1}$	Management type	Anthropogenic pressure	Coexistence of <i>P. clarkii</i> and <i>F. limosus</i>
BAA	1990	21.78	0.44	0.61	184.228	Federal	3	Yes
BAU	2000	2.03	4.28	0.00	18183.510	Private	1	No
BID	1992	10.90	0.67	0.00	11.878	Federal	3	No
BIR	1992	3.43	7.00	0.67	2156.477	Federal	3	Yes
BON	1990	5.00	0.33	0.00	581.871	Communal	2	No
BVI	1963	73.92	2.94	3.72	5258.807	Federal	3	Yes
CEA	1971	0.85	3.97	1.44	407.540	Private	1	Yes
CZA	1973	2.95	0.00	0.00	64.156	Private	1	No
CZB	2008	2.80	0.11	0.00	0	Private	1	No
LAF	1982	1.63	1.69	2.00	2106.577	Private	1	Yes
LAH	1972	1.32	14.72	0.06	596.239	Private	1	No
LAM2	1987	10.45	60.22	0.00	1323.978	Federal	3	Yes
LAV	1992	1.78	8.56	0.11	189.706	Communal	2	Yes
LIN	1990	43.80	0.75	0.83	263.392	Federal	3	Yes
PEY	2007	4.20	5.67	0.00	1376.866	Communal	2	No
POU	1996	2.68	3.33	0.00	360.520	Communal	2	No
SAB	2006	8.02	4.86	0.00	28.64739	Communal	2	No
SAJ	1983	10.18	3.28	0.00	0	Private	1	No
SOA	1993	36.90	5.11	3.00	1442.952	Private	1	Yes
SOB	1998	0.68	10.50	1.61	842.013	Private	1	Yes
SOC	2005	4.38	4.39	0.00	1468.924	Private	1	No
SOD	2007	2.53	3.11	0.00	399.820	Private	1	No
VRA	1982	38.05	0.39	0.56	1590.484	Private	1	Yes

Morphological, stable isotope and stoichiometric analyses

The morphological variation of crayfish body was analysed using a geometric morphometric technique (Zelditch et al., 2012) based on landmark analysis. Following Evangelista et al., (2019a) 19 homologous landmarks were digitized 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 further proceeding. We used a full-Procrustes fit (FPF) implemented in Morpho J v.1.06d to analyse the digitized 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 FPF (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 characterize individuals.

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

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

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

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

where baseline organisms are poplar leaves (base 1) and periphyton (base 2), $\text{TP}_{\text{baseline}} = 1$, and 3.4 is the fractionation coefficient between trophic levels (Post, 2002).

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 mineralized replicate subsample (121°C for 2 hours in sodium persulfate) was used to analyse dissolved Phosphorus contents (% P) using spectrophotometry (molybdate method; Parsons et al., 1984). All elemental ratios (C:N, C:P, N:P) are expressed as molar ratios.

Genetic analyses

DNA extraction, PCRs 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 optimized 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 IV.S1 for more details). PCR were performed following the procedure described in Lang et al., (2020) (see Figure IV.S1 for

the description of the multiplex used in this study). Amplified fragments were analyzed on an ABI PRISM 3730 capillary sequencer (Applied Biosystems, Foster City, USA) at the Génopole Toulouse Midi-Pyrénées.

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 (HW) 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 HW 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 lesser 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 (AR), mean number of alleles per locus (NA), and Wright fixation indices (Fis), computed with Fstat v2.9.3.2 (Goudet, 2002; see details in Supplementary Material SM1 and Appendix Table S1). The global genetic differentiation across lakes (*Fst*) and its 95% confidence interval were computed for each species, using Fstat v2.9.3.2 (Goudet, 2002).

Statistical analyses

Phenotypic variability

For each species, 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). Covariance matrix of the regression residuals was used to run two Principal Component Analyses (PCA), 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

characterize individuals' body shapes in further statistical analyses. PC1 and PC2 explained 33.1% and 21.6% of the body morphological variations in *P. clarkii*, and 54.9% and 11.5% of the variations in *F. limosus*, respectively (Figure IV.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 IV.2 a). 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 IV.2 b).

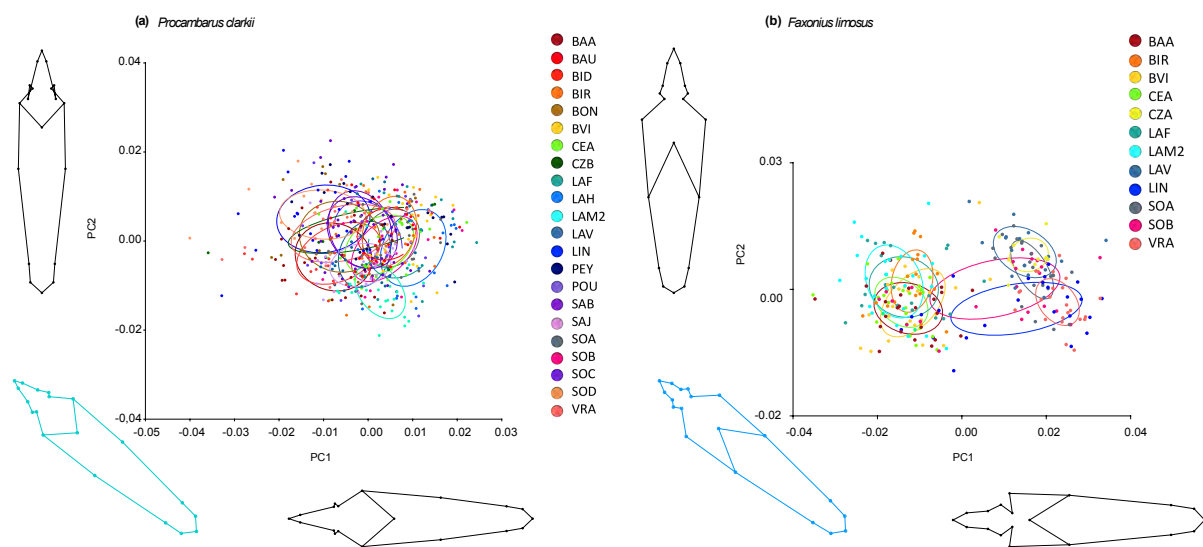


Figure IV.2 Principal Component Analysis (ACP) based on the covariance matrix of allometry regression residuals for **(a)** *Procambarus clarkii* and **(b)** *Faxonius limosus* showing the 22 populations and 12 populations, respectively. Body shapes variations (scaling factor: 0.1) along PC1 and PC2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

Linear models (LMs) 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.

Variance partitioning

As phenotypic traits may vary according to individuals, their sex and their respective population (Malavé et al., 2018; Evangelista et al., 2019a), 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 composition were computed using a bootstrap procedure based on 200 iterations.

Environmental determinants

To summarize lakes characteristics, a Multiple Factor Analysis (MFA) was performed on “productivity” (chlorophyll-a concentration in $\mu\text{g}\cdot\text{L}^{-1}$), “predation pressure” (BPUE in $\text{g}\cdot\text{gillnet}^{-1}\cdot\text{hr}^{-1}$), “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” (CPUE in $\text{ind. trap}^{-1}\cdot\text{hr}^{-1}$). The first and second MFA axes explained 36.8% and 27.7% of the total variance, respectively. Positive values on the first axis were associated with 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 IV.S2). These two axes were used as synthesizing explanatory variables (“environmental variables 1 and 2”) in the subsequent analyses.

LMMs 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 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.

Neutral vs. 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:

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

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 Pst (Lutz & Wolters, 1989; Leinonen et al., 2006). Traits evolve neutrally when Pst and Fst are equal, while different Pst and Fst imply adaptive processes (adaptive phenotype divergence for $Pst > Fst$, or a homogenizing adaptation if $Pst < Fst$). For each lake, the global phenotypic uniqueness was computed as the mean population-specific Pst 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).

Results

Phenotypic variability

We found high population- and sex-dependent morphological variations for both species (Figure IV.2; Table IV.S2). Concerning *P. clarkii*, morphological traits (PC1 and PC2 scores, carapace length) were significantly different among populations and between sexes (Appendix Table S2). More specifically, females had lower PC1 scores and higher PC2 scores than males, indicating that females had streamlined body compared to males, and they displayed higher carapace length 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 term_{PC2 score}: $F_{11,232} = 2.988$, $P < 0.001$; interaction term_{Carapace length}: $F_{11,232} = 2.359$, $P = 0.009$; Appendix 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 IV.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 IV.S2, Figure IV.4) and between sexes ($F_{1,478} = 11.367$, $P < 0.001$; Table IV.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).

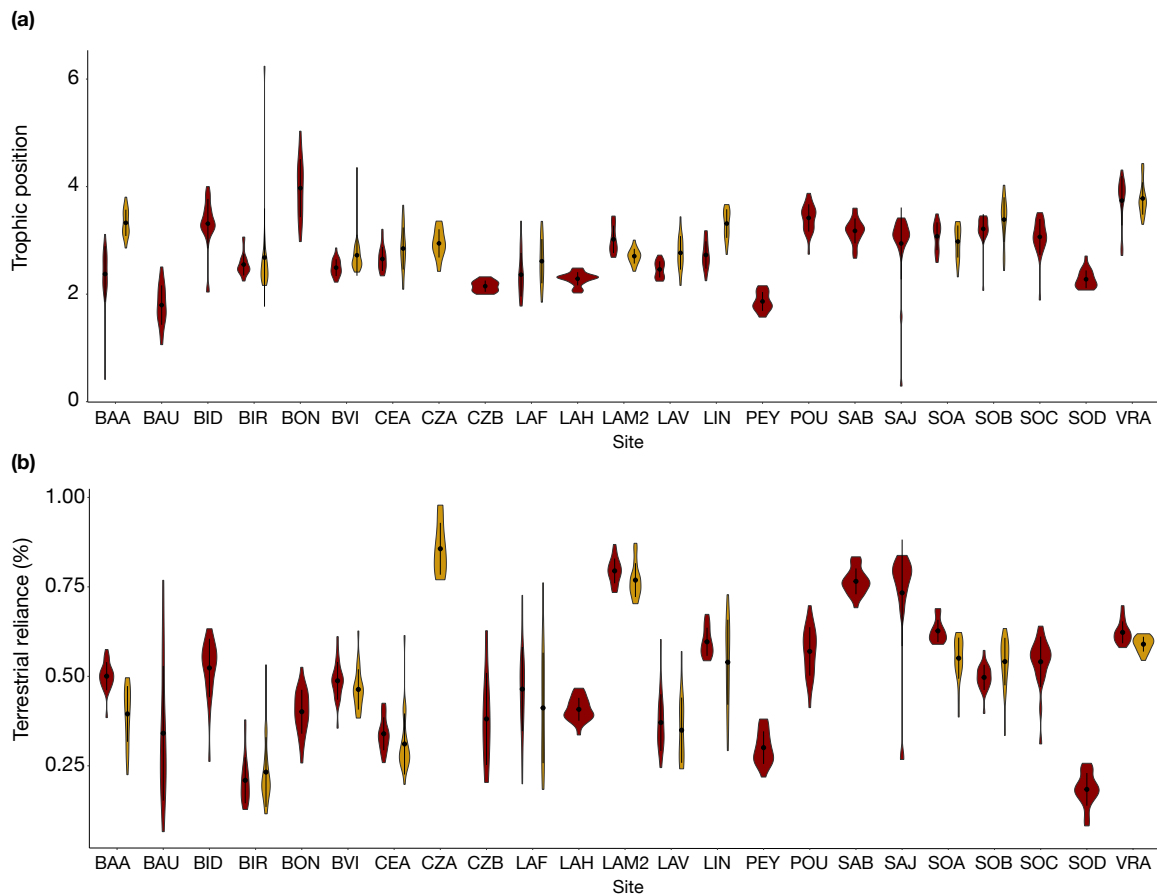


Figure IV.3 Violin plots representing (a) trophic position and (b) terrestrial reliance variations for *Procambarus clarkii* (red; n = 501) and *Faxonius limosus* (dark orange; n = 256). Mean trophic position = 2.77 ± 0.64 SE and mean = 3.03 ± 0.53 SE for *P. clarkii* and *F. limosus*, respectively.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

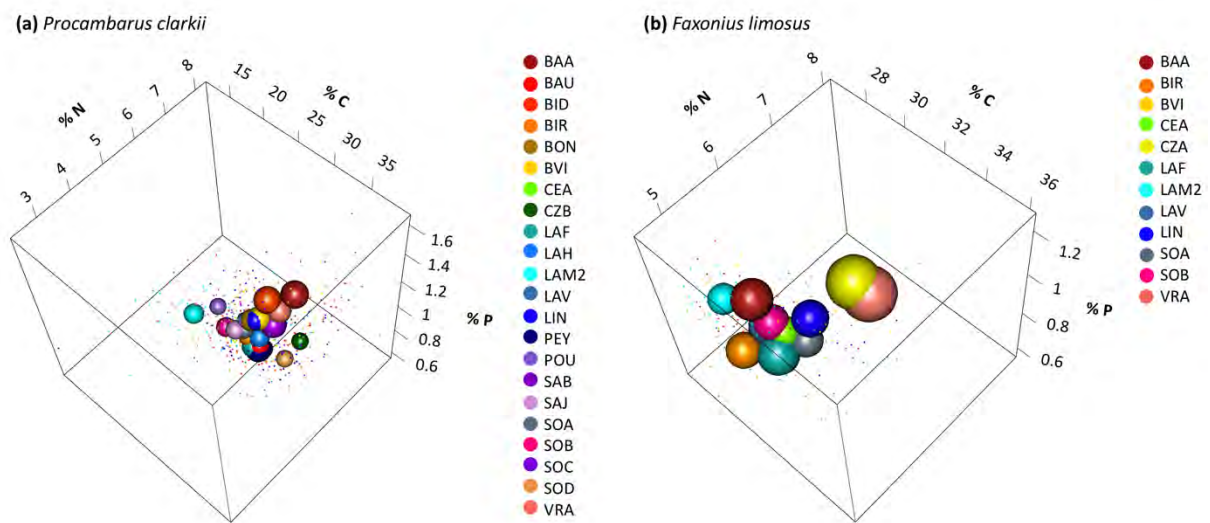


Figure IV.4 Stoichiometric niches for (a) *Procambarus clarkii* and (b) *Faxonius limosus*. Each sphere represents a population of crayfish, its position represents the mean elemental composition of the population and its volume corresponds to 40% of the variance.

Variance partitioning

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

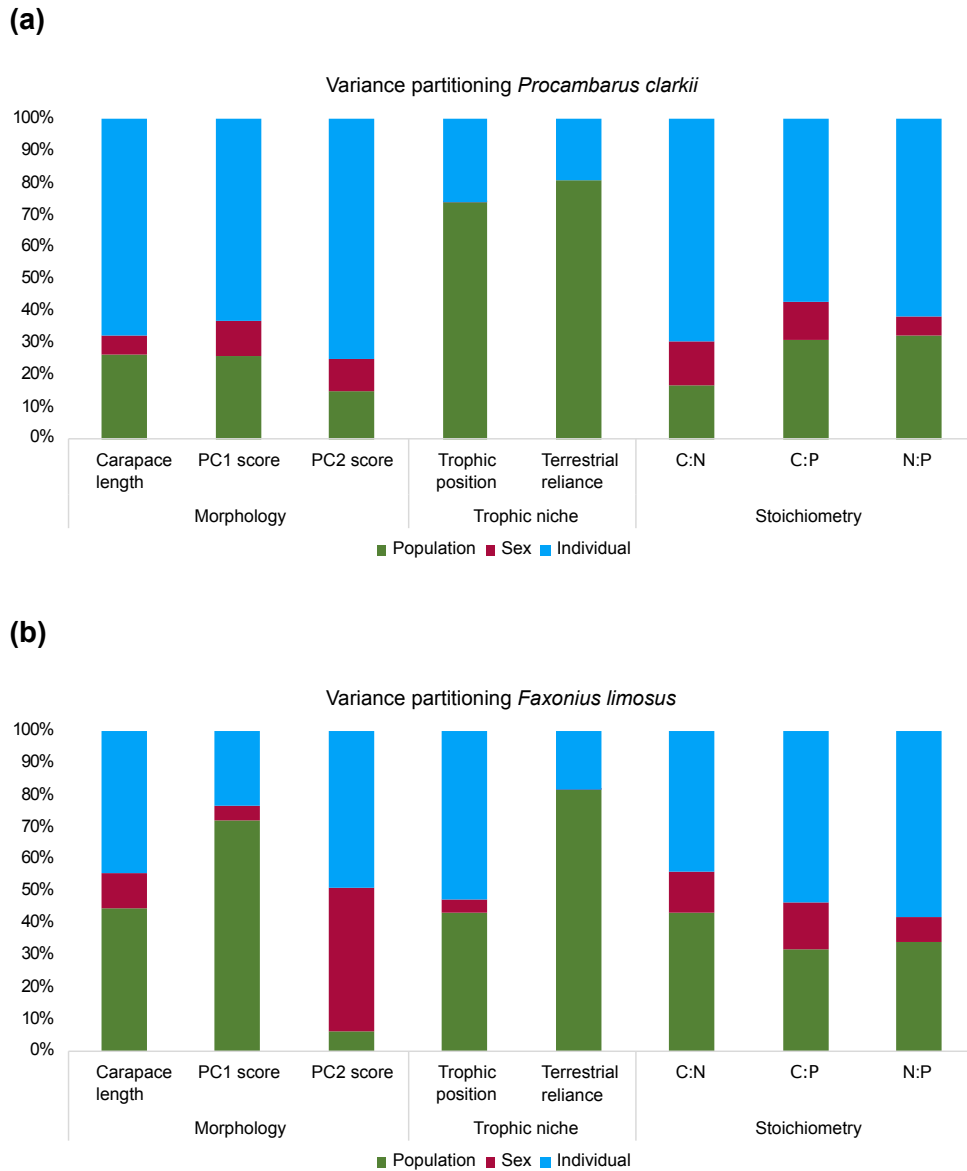


Figure IV.5 Partition of variance of the studied traits (PC1 and PC2 scores, carapace length, trophic position, terrestrial reliance, C:N:P ratios) for **(a)** *Procambarus clarkii* (n = 501) and **(b)** *Faxonius limosus* (n = 256).

Environmental determinants

For morphological traits in *P. clarkii*, we only found predictors for PC2 scores. PC2 scores divergences were 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 IV.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 IV.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 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 IV.S3). Specifically, C:P and N:P ratios of crayfish body 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 IV.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*.

Neutral vs. 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} ($P_{st_{trophic\ position}} = 0.652$ ($CI_{95\%}: 0.386 - 0.771$) and $P_{st_{terrestrial\ reliance}} = 0.882$ ($CI_{95\%}: 0.701 - 0.952$), respectively; Figure IV.6 a). 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 IV.6 b). Phenotypic differentiations in morphological and stoichiometric traits were 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 IV.7). However, the phenotypic differentiation in *P. clarkii* was higher for trophic position and terrestrial reliance, indicating that the relative importance of adaptive processes shaping these traits variations in *P. clarkii* was greater compared to *F. limosus* (Figure IV.7).

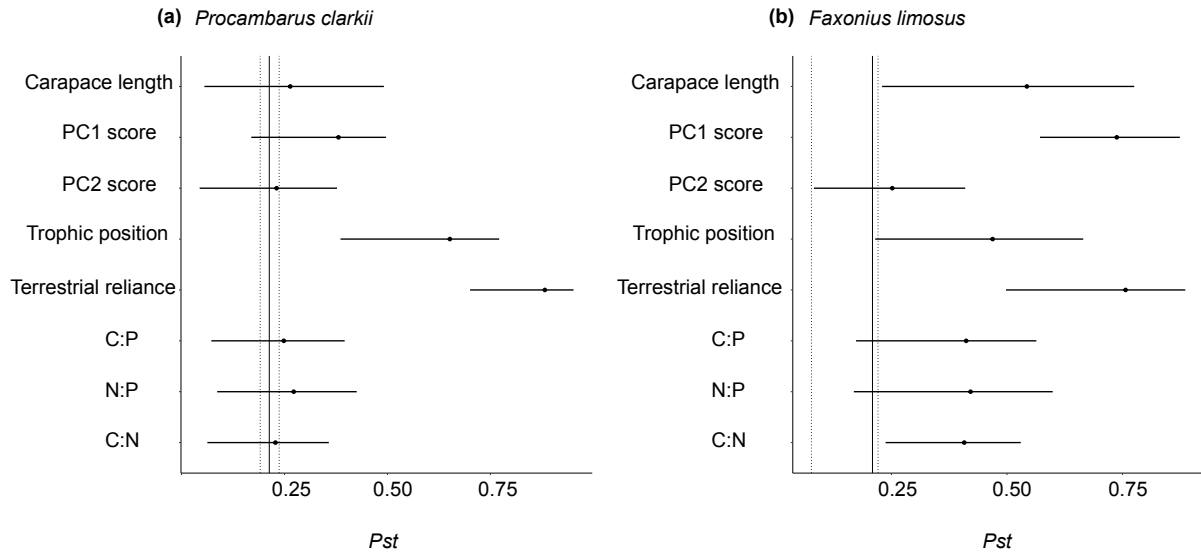


Figure IV.6 P_{st} estimates for each trait (carapace length, PC1 and PC2 scores, trophic position, terrestrial reliance, C:N, C:P, N:P ratios), and F_{st} (vertical straight line) on neutral microsatellite markers for (a) *Procambarus clarkii* (n = 257) and (b) *Faxonius limosus* (n = 246) from 11 sympatric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

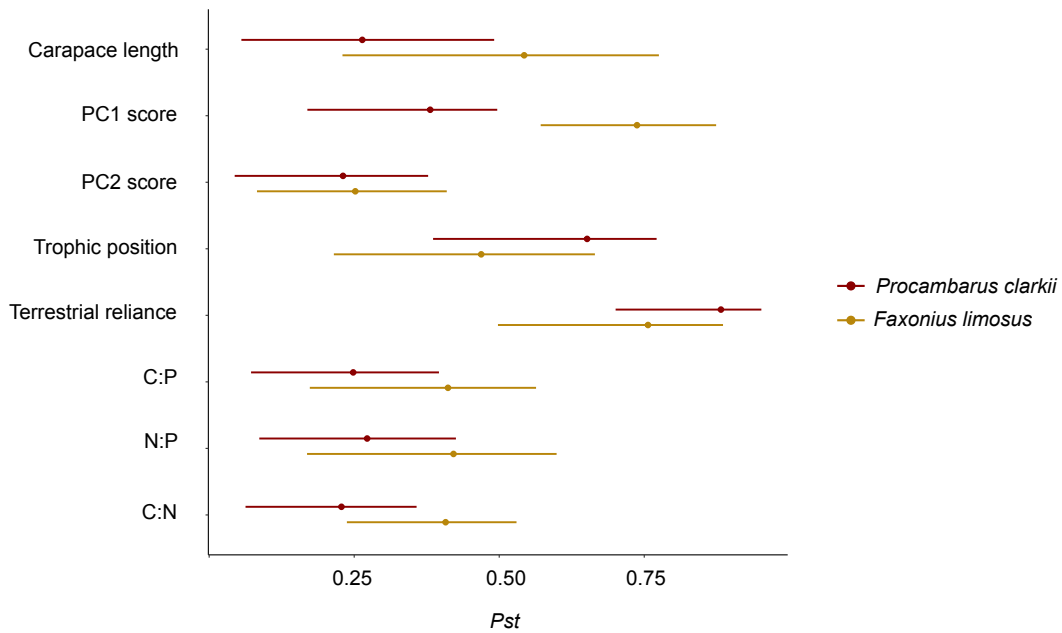


Figure IV.7 Comparison of P_{st} estimates for each trait (carapace length, PC1 and PC2 scores, trophic position, terrestrial reliance, C:N, C:P, N:P ratios), between *Procambarus clarkii* (red; n = 257) and *Faxonius limosus* (dark orange; n = 246) from 11 sympatric populations. Horizontal bars represent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

Discussion

This study stresses the importance to investigate intraspecific trait variations 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 genetic variability for *P. clarkii* and *F. limosus*, respectively. We also highlighted a 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*, morphology of individuals varied with environmental biotic conditions. Finally, we highlighted that different combinations of neutral and adaptive processes shaped the intraspecific variability of *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*.

Phenotypic variability and variance partitioning

As expected, morphological traits variations were mainly explained at the individual level (within population) and at the population level (among populations) for *P. clarkii* and *F. limosus*, respectively. 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 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 a differentiated elemental requirements in females for egg productions (Færøvig & Hessen, 2003).

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 great trophic traits variance at the population level highlighted the opportunistic omnivorous diet of *P. clarkii* and *F. limosus*, which rely on available trophic resources (Gutiérrez-Yurrita et al., 1998; Correia, 2003; Vojtkovská et al., 2014). However, although the variation of the stoichiometric traits at the population level was notable (i.e. due to differences in available nutrients; El-Sabaawi et al., 2012b, 2012a), 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). Heterotrophic organisms' capacity to maintain their elemental composition in various environments when feeding on different resources' 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)).

Environmental determinants

As expected, we found that morphological variations (carapace length) were explained by environmental characteristics for *F. limosus*, which might have undergone local adaptation. Body size adaptations generally maximize 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 interspecific competition (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 colonization history (invasion time-span) and environmental biotic determinants (predation pressure and abundance of *P. clarkii*) explained morphological variations, indicating that strong environmental pressure can actually rapidly lead to phenotypic variations in red-swamp crayfish, following its establishment (< 20 years; Evangelista et al., 2019a; Lang et al., 2020). Specifically, our results suggest that *P. clarkii* individuals with an elongated rostrum, a shortened cephalothorax and

a longer abdomen (i.e. deeper muscle involved in walking, Takahata et al., 1984) are likely to disperse easily overland, and to colonize new environments. This 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 traits variations were also explained by colonization 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 variations are likely due to P variations since C and N contents are relatively uniform in the different classes of molecules and cellular structures in organisms (Sterner & Elser, 2002). Elemental composition variations in crayfish might be due to ontogeny differences, or differences of resource quality within the ecosystem (e.g. high N:P ratio in prey could lead to higher N:P ratio in consumer; Salanon, 2019). Variations in C:P and N:P ratios in food resources between recent and old established populations might be due to lake eutrophication (%P increases with the invasion time span which is correlated to the age of lakes). However, we failed to detect the environmental and historical determinants of stoichiometric variability in *F. limosus*. This suggests that underlying determinants for this species are different.

Surprisingly, we failed to detect the determinants driving trophic traits variations for both crayfish species, and this is certainly because local resource availability in each lake was not considered in our models. A previous study on the same area found that body size, conspecifics abundance and lake size and productivity mediated the trophic ecology of *P. clarkii* (Jackson et al., 2017). The relationship between environmental conditions and trophic traits of crayfish might also have been not detected because we studied lakes large enough to reduce competition and predation pressures and increase resource availability (Stenroth et al., 2008; Jackson et al., 2017).

Neutral vs. 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 traits variations for *P. clarkii*, which has been established more recently in our study area. Trophic traits variations were due to adaptive processes, supporting our previous interpretation: this opportunistic generalist species relies on available trophic resources, constrained by the environment. Surprisingly, for *F. limosus*, adaptive and neutral processes contributed equally to the variations 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. But if there are some limitations to F_{st}/P_{st} comparisons (Hendry 2002, Edelaar et al. 2011), P_{st} for a given trait are comparable between both species. As we 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. It is worth noting that *P. clarkii* is an early colonizer in our study area but this has not been observed for *F. limosus* (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 overtime would be insightful for describing the succession of processes involved in phenotypic differentiation within and between species. Combined with the study of population dynamics, such approach would be relevant to develop local management of invasive species to counter their impacts across the meta-population.

Conclusion

We highlighted strong morphological, trophic and stoichiometric variations among and within populations from two co-occurring invasive species, and more importantly, we found 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 (Phillips & Shine, 2006b; Juette et al., 2014; Evangelista et al., 2019b), and that these impacts differ depending on the considered ecological scale and on the considered species. It is of high importance to improve our understanding of the ecological and evolutionary mechanisms that shape genetic and phenotypic variations 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

Supplementary information

Figure IV.S1 List of the 14 microsatellites amplified in three PCR multiplex for *Procambarus clarkii* and of the 9 microsatellites in two PCR multiplex for *Faxonius limosus*. All primers at 10 μ M each. Selection based on Belfiore & May (2000) and Jiang et al. (2015) for *Procambarus clarkii*, and Jiang et al. (2015) and Hulák et al. (2010) for *Faxonius limosus*.

Multiplex 1 *Procambarus clarkii*

Locus	GenBank accession number	Allele size range (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Fluorescent dye
PclG-15	AF290927	120-200	GGC GTG ACG CCA ACG TGT CTT	GGC TGG CCA CTT TGT TAG CCT GAG	ATTO 550
PclG-27	AF290932	100-130	AAT CTT AAG ATC ATG AAA AAG GTA	TTT AAG GAA CGT ATA AGA AAA GAC	FAM
PclG-16	AF290928	80-180	CTC GGA ATG TCC ACC TGA GA	TCA TTA TGG ATT TTG TCA ATC TAT	HEX
PclG-04	AF290921	170-255	TAT ATC AGT CAA TCT GTC CAG	TCA GTA AGT AGA TTG ATA GAA GG	FAM

PCR MIX	Volume (μ L) x1
H ₂ O	2.1
Qiagen multiplex PCR Master Mix	5
DNA	2
PclG-15 F	0.1
PclG-15 R	0.1

PclG-27 F	0.15
PclG-27 R	0.15
PclG-16 F	0.1
PclG-16 R	0.1
PclG-04 F	0.1
PclG-04 R	0.1

Multiplex 2 *Procambarus clarkii*

Locus	GenBank accession number	Allele size range (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Fluorescent dye
PclG-29	AF290934	159-210	GAA AGT CAT GGG TGT AGG TGT AAC	TTT TTG GGC TAT GTG ACG AG	ATTO 550
PclG-07	AF290922	112-124	CCT CCC ACC AGG GTT ATC TAT TCA	GTG GGT GTG GCG CTC TTG TT	FAM
PclG-28	AF290933	238-266	CTC GGC GAG TTT ACT GAA AT	AGA AGA AAG GGA TAT AAG GTA AAG	HEX
PclG-32	AF290935	173-221	CCC CCA CTC GTC TCT GTG TAT G	TGT GCT TGC GGG AGT GAG C	FAM
PCSH0038	KJ607979	150-190	CAG AGC ACT GTT TGC TAG TGT GT	GCT TCC TCT GTT ATT CAT CAT GC	HEX

PCR MIX	Volume (µL) x1
H ₂ O	2
Qiagen multiplex PCR Master Mix	5
DNA	2
PclG-29 F	0.1
PclG-29 R	0.1
PclG-07 F	0.1
PclG-07 R	0.1
PclG-28F	0.1

PclG-28 R	0.1
PclG-32 F	0.1
PclG-32 R	0.1
PCSH0038 F	0.1
PCSH0038 R	0.1

Multiplex 3 *Procambarus clarkii*

Locus	GenBank accession number	Allele size range (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Fluorescent dye
PclG-48	AF290941	145-175	CTG TTG GTG ATT TCC GTC AAT TTT	AGA TTC AAC GCT GTG TTC CTG ATC	ATTO 550
PclG-17	AF290929	159-184	GTC GGG AAC CTA TTT ACA GTG TAT	AAG AGC GAA GAA AGA GAT AAA GAT	HEX
PCSH0089	KJ607988	80-120	GTA TAC ACA GCT TTG GAA CTG GG	GCT TCC TCT GTT ATT CAT CAT GC	HEX
PCSH0006	KP675956	140-180	GGC CAA AAT GTG AAG AGT TGT TA	GAA CCA GAT CAG TGT CAT GTG AG	FAM
PCSH0005	KP675955	110-135	AAC AGA GTG GCA AGG TAC TTG AA	GGC TGT CAC TCG TGT CTT TAG TT	FAM

PCR MIX	Volume (µL) x1
H ₂ O	2
Qiagen multiplex PCR Master Mix	5
DNA	2
PclG-48 F	0.1
PclG-48 R	0.1
PclG-17 F	0.1
PclG-17 R	0.1

PCSH0089 F	0.1
PCSH0089 R	0.1
PCSH0006 F	0.1
PCSH0006 R	0.1
PCSH0005 F	0.1
PCSH0005 R	0.1

Multiplex 1 *Faxonius limosus*

Locus	GenBank accession number	Allele size range (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Fluorescent dye
PCSH0006	KP675956	125-185	GGC CAA AAT GTG AAG AGT TGT TA	GAA CCA GAT CAG TGT CAT GTG AG	FAM
PCSH0011	KP675961	141-201	CCT AGC AGC CTT GGT AGT AAC CT	TGA AAG ACG TGA TGT AAA GTT GC	ATTO565
PCSH0038	KJ607979	160-230	CAG AGC ACT GTT TGC TAG TGT GT	GCT TCC TCT GTT ATT CAT CAT GC	HEX
PCSH0077	KJ607986	125-185	GGG ATA GCA CAA TAC ACT CAT CC	GGG TGC TAT GCA TCA CAT TAA AA	ATTO 550

PCR MIX	Volume (µL) x1
H ₂ O	2.48
Qiagen multiplex PCR Master Mix	5
DNA	2
PCSH0006F	0.06
PCSH0006R	0.06
PCSH0011F	0.04
PCSH0011R	0.04
PCSH0038F	0.08
PCSH0038R	0.08

PCSH0077F 0.08
 PCSH0077R 0.08

Multiplex 2 *Faxonius limosus*

Locus	GenBank accession number	Allele size range (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Fluorescent dye
PCSH0005	KP675955	100-160	AAC AGA GTG GCA AGG TAC TTG AA	GGC TGT CAC TCG TGT CTT TAG TT	FAM
PCSH3.1	AY112995	284-374	TTC AGG GGC GAG AAA GTT GTG AC	GTG GGA AGG GGT AAG GGA GAG	FAM
PCSH0042	KJ607980	117-177	AGT GAC TCT TTA CTG ATC GCT CG	TTA GTG AGG AAG AGG AAG TGG TG	ATTO 550
PCSH0054	KJ607983	140-200	TTG TAA CAA ATG TTT TCT GTG TGC	TGG AAG CAC TAG CAC TAC CTT TC	ATTO565
PCSH0089	KJ607988	85-145	GTA TAC ACA GCT TTG GAA CTG GG	GCT TCC TCT GTT ATT CAT CAT GC	HEX

PCR MIX	Volume (µL) x1
H ₂ O	2.24
Qiagen multiplex PCR Master Mix	5
DNA	2
PCSH0005F	0.08
PCSH0005R	0.08
PCSH3.1F	0.1
PCSH3.1R	0.1
PCSH0042F	0.04
PCSH0042R	0.04
PCSH0054F	0.1
PCSH0054R	0.1
PCSH0089F	0.06
PCSH0089R	0.06

PCR cycling conditions	
95°C	15 min
94°C	30 s
56°C	90 s
72°C	60 s
60°C	45 min
10°C	∞

} 35 cycles

Figure IV.S2 MFA analysis of the environmental characteristics of the 23 gravel pit lakes: chlorophyll-a [$\mu\text{g}\cdot\text{L}^{-1}$], coexistence [Yes/No], anthropogenic pressure [1/2/3], predation pressure [gillnet biomass per unit effort (BPUE) expressed in $\text{g}\cdot\text{gillnet}^{-1}\cdot\text{hr}^{-1}$], *Faxonius limosus* and *Procambarus clarkii* abundances [catch per unit effort (CPUE) expressed in $\text{ind}\cdot\text{trap}^{-1}\cdot\text{hr}^{-1}$].

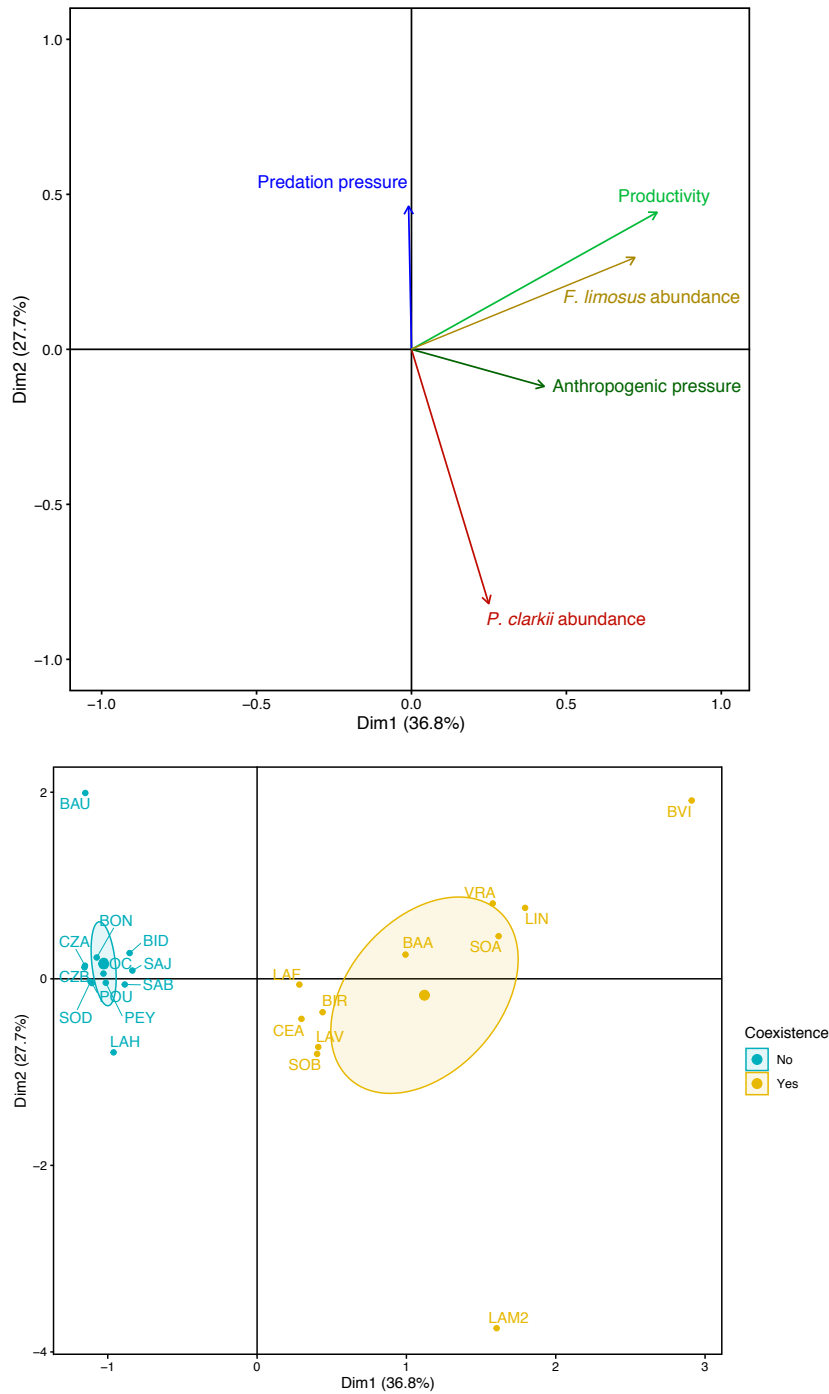


Table IV.S1 Genetic diversity of *Faxonius limosus* (based on 8 microsatellites) and *Procambarus clarkii* (based on 13 microsatellites) in the 11 lakes where the species co-occur. We quantified genetic diversity using allelic richness (A_R), the mean number of alleles per locus (NA), and the fixation index (Fis) computed with Fstat v2.9.3.2 (Goudet, 2002), and expected (Hexp) and observed (Hobs) heterozygosity, computed with Genetix v4.05 (Belkhir et al., 1996).

Lake	Species	A_R	NA	Hexp	Hobs	Fis
BAA	<i>F. limosus</i>	1.31	1.38	0.06	0.04	0.41
	<i>P. clarkii</i>	4.14	5.62	0.67	0.67	0.02
BIR	<i>F. limosus</i>	1.60	1.63	0.11	0.11	0.05
	<i>P. clarkii</i>	4.58	6.23	0.71	0.68	0.07
BVI	<i>F. limosus</i>	1.86	2.00	0.14	0.15	-0.03
	<i>P. clarkii</i>	2.82	4.46	0.43	0.45	-0.02
CEA	<i>F. limosus</i>	1.53	1.63	0.11	0.13	-0.14
	<i>P. clarkii</i>	2.93	3.85	0.53	0.56	-0.03
LAF	<i>F. limosus</i>	1.21	1.25	0.07	0.05	0.21
	<i>P. clarkii</i>	3.43	4.00	0.61	0.53	0.16
LAM	<i>F. limosus</i>	1.60	1.63	0.13	0.14	-0.06
	<i>P. clarkii</i>	3.13	5.46	0.54	0.56	-0.02
LAV	<i>F. limosus</i>	1.43	1.50	0.07	0.08	-0.07
	<i>P. clarkii</i>	4.48	6.54	0.71	0.73	-0.01
LIN	<i>F. limosus</i>	1.57	1.63	0.09	0.10	-0.15
	<i>P. clarkii</i>	3.43	6.46	0.58	0.52	0.12
SOA	<i>F. limosus</i>	1.32	1.38	0.11	0.11	-0.02
	<i>P. clarkii</i>	3.12	4.69	0.53	0.48	0.12
SOB	<i>F. limosus</i>	1.70	1.75	0.19	0.19	0.03
	<i>P. clarkii</i>	3.10	4.62	0.57	0.61	-0.06
VRA	<i>F. limosus</i>	2.16	2.50	0.20	0.21	-0.04
	<i>P. clarkii</i>	3.35	5.00	0.61	0.63	-0.02

Table IV.S2 Results of the linear models testing the effect of Population and Sex on each phenotypic trait (PC1 and PC2 scores, carapace length, trophic position, terrestrial reliance, C:N:P ratios) for *Procambarus clarkii* (n = 501) and *Faxonius limosus* (n = 256). The full model included an interaction term (Sex x Population) which was removed from the final model if non-significant. Significant P values are in bold.

Species	Phenotypic trait	Predictor	SumSq	Df	F	P value	
<i>Procambarus clarkii</i>	PC1 score	Population	0.0157163	21	12.548	<2.2e-16	***
		Sex	0.0015995	1	26.819	3.299e-07	***
		Residuals	0.0285089	478			
	PC2 score	Population	0.0073204	21	6.9491	<2.2e-16	***
		Sex	0.0011634	1	23.1926	1.97e-06	***
		Residuals	0.0239780	478			
	Carapace length	Population	7480.1	21	11.537	<2.2e-16	***
		Sex	446.3	1	14.454	0.0001622	***
		Residuals	14758.6	478			
	Trophic position	Population	149.769	21	62.7568	<2e-16	***
		Sex	0.013	1	0.1123	0.7377	
		Residuals	54.321	478			
	Terrestrial reliance	Population	13.5258	21	97.6302	<2e-16	***
		Sex	0.0056	1	0.8459	0.3582	
		Residuals	3.1535	478			
	C :N	Population	29.285	21	8.9890	<2.2e-16	***
		Sex	0.842	1	5.4289	0.0202402	*
		Population:Sex	8.071	21	2.4773	0.0003335	***
Residuals		70.897	457				
C :P	Population	54431	21	15.8245	<2.2e-16	***	
	Sex	3416	1	20.8537	6.383e-06	***	
	Population:Sex	6011	21	1.7475	0.02173	*	
	Residuals	74854	457				
N :P	Population	1585.5	21	13.162	<2.2e-16	***	
	Sex	65.2	1	11.367	0.0008082	***	
	Residuals	2741.9	478				
<i>Faxonius limosus</i>	PC1 score	Population	0.055630	11	69.663	<2.2e-16	***
		Sex	0.001770	1	24.386	1.467e-06	***
		Residuals	0.017641	243			
	PC2 score	Population	0.0042398	11	12.4418	<2.2e-16	***
		Sex	0.0027145	1	87.6218	<2.2e-16	***
		Population:Sex	0.0010183	11	2.9883	0.0009767	***
		Residuals	0.0071872	232			
	Carapace length	Population	12096.0	11	26.6983	<2.2e-16	***
		Sex	264.5	1	6.4207	0.011939	*
		Population:Sex	1068.6	11	2.3587	0.008918	**
		Residuals	9555.5	232			
	Trophic position	Population	34.368	11	20.4096	<2,00E-16	***
		Sex	0.591	1	3.8606	0.05057	.
		Residuals	37.199	243			
	Terrestrial reliance	Population	6.3647	11	80.0690	<2,00E-16	***
		Sex	0.0217	1	2.9965	0.08472	.
		Residuals	1.7560	243			
	C :N	Population	12.6578	11	19.3507	<2.2e-16	***
Sex		1.7629	1	29.6458	1.318e-07	***	
Population:Sex		1.3414	11	2.0506	0.02485	*	
Residuals		13.7961	232				
C :P	Population	18748.2	11	17.949	<2.2e-16	***	

	Sex	1791.5	1	18.866	2.097e-05	***
	Population:Sex	2697.0	11	2.582	0.004131	**
	Residuals	22030.2	232			
<i>N :P</i>	Population	663.67	11	16.9097	<2,00E-16	***
	Sex	23.50	1	6.5865	0.01090	*
	Population:Sex	80.94	11	2.0623	0.02392	*
	Residuals	827.78	232			

Table IV.S3 Results of the linear mixed models testing the effect of environmental variables 1 and 2, and invaded time on each phenotypic trait (PC1 and PC2 scores, carapace length, trophic position, terrestrial reliance, C:N:P ratios) for *Procambarus clarkii* (n = 501) and *Faxonius limosus* (n = 256). The full model included double interaction terms which were removed using a backward procedure if non-significant. Significant P values are in bold. Increasing value for environmental variable 1 corresponds to increasing anthropogenic pressure, abundance of *F. limosus* and lake productivity and increasing value for environmental variable 2 corresponds to increasing predation pressure and decreasing abundance of *P. clarkii*.

Species	Phenotypic trait	Predictor	F	Df	Df.res	P value	
<i>Procambarus clarkii</i>	PC1 score	Invaded time	0.0170	1	18.366	0.8977	
		Environmental variable 1	0.1772	1	17.806	0.6788	
		Environmental variable 2	0.1635	1	17.781	0.6907	
	PC2 score	Invaded time	11.1815	1	18.796	0.0034499	**
		Environmental variable 1	0.3953	1	17.304	0.5377600	
		Environmental variable 2	20.0109	1	17.182	0.0003263	***
	Carapace length	Invaded time	0.0001	1	18.412	0.9942	
		Environmental variable 1	0.0084	1	17.778	0.9279	
		Environmental variable 2	0.8730	1	17.749	0.3627	
	Trophic position	Invaded time	2.4648	1	18.095	0.1337	
		Environmental variable 1	0.2303	1	17.953	0.6371	
		Environmental variable 2	0.3168	1	17.948	0.5805	
	Terrestrial reliance	Invaded time	0.1124	1	18.067	0.7413	
		Environmental variable 1	0.9849	1	17.968	0.3342	
		Environmental variable 2	1.1078	1	17.964	0.3065	
	C :N	Invaded time	1.0206	1	18.536	0.3254	
		Environmental variable 1	0.2079	1	17.694	0.6540	
		Environmental variable 2	1.6654	1	17.652	0.2135	
C :P	Invaded time	10.4327	1	18.551	0.004511	**	
	Environmental variable 1	0.8063	1	17.683	0.381294		
	Environmental variable 2	3.1506	1	17.639	0.093158	.	
N :P	Invaded time	8.6947	1	18.568	0.00839	**	
	Environmental variable 1	0.5184	1	17.669	0.48093		
	Environmental variable 2	4.6395	1	17.624	0.04535	*	
<i>Faxonius limosus</i>	PC1 score	Invaded time	0.2647	1	8.7212	0.6197	
		Environmental variable 1	0.3055	1	8.0855	0.5954	

	Environmental variable 2	1.2098	1	7.9948	0.3034	
<i>PC2 score</i>	Invaded time	0.1691	1	8.1334	0.6915	
	Environmental variable 1	2.6340	1	8.5838	0.1407	
	Environmental variable 2	0.1923	1	7.8799	0.6728	
<i>Carapace length</i>	Invaded time	0.0435	1	8.1836	0.83996	
	Environmental variable 1	0.1261	1	8.3624	0.73130	
	Environmental variable 2	8.8947	1	7.9293	0.01771	*
<i>Trophic position</i>	Invaded time	0.1817	1	8.2429	0.6808	0.1817
	Environmental variable 1	0.0369	1	8.2585	0.8523	0.0369
	Environmental variable 2	0.7224	1	7.9519	0.4202	0.7224
<i>Terrestrial reliance</i>	Invaded time	0.3761	1	9.1564	0.5546	0.3761
	Environmental variable 1	0.0033	1	8.0571	0.9558	0.0033
	Environmental variable 2	0.5745	1	8.0081	0.4702	0.5745
<i>C :N</i>	Invaded time	0.8654	1	8.2416	0.3787	0.8654
	Environmental variable 1	0.5590	1	8.2602	0.4754	0.5590
	Environmental variable 2	1.1141	1	7.9516	0.3222	1.1141
<i>C :P</i>	Invaded time	0.8502	1	8.1652	0.3829	0.8502
	Environmental variable 1	1.6135	1	8.4178	0.2380	1.6135
	Environmental variable 2	0.9244	1	7.9171	0.3648	0.9244
<i>N :P</i>	Invaded time	0.3736	1	8.1545	0.5577	0.3736
	Environmental variable 1	2.7297	1	8.4601	0.1350	2.7297
	Environmental variable 2	2.1500	1	7.9078	0.1812	2.1500

Appendix

Appendix IV.A1 Genotyping, quality control

Concerning *P. clarkii*, the average observed heterozygosity (Hobs) across sites ranged from 0.45 to 0.73, and the expected heterozygosity (Hexp) ranged from 0.43 to 0.71 (Appendix Table S1). We found significant homozygote excesses for only 15 out of 308 locus/populations pairs, significant HW deviations for 4 out of 308 locus/populations pairs, and significant linkage disequilibria for 2 out of 2002 within-population locus/locus combinations. No loci were removed following these analyses. Concerning neutrality tests, we detected one outlier locus (PCSH0089) that was probably affected by balancing or purifying selection (negative alpha) (Foll, 2012). We removed this locus from subsequent analyses. The allelic richness (A_R) ranged from 2.82 to 4.58 (see all genetic summary statistics in Appendix Table S1).

Concerning *F. limosus*, locus PCSH0042 was monomorphic in all populations and was removed for subsequent analyses. Hobs ranged from 0.04 to 0.21 while Hexp ranged from 0.06 to 0.20 (Appendix Table S1). We found significant homozygote excesses for only 4 out of 96 locus/populations pairs, no significant HW deviations and no linkage disequilibrium. A_R varied from 1.21 to 2.16 (see all genetic summary statistics in Appendix Table S1).

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Chapitre V

Polymorphisme de ressource stable chez
une écrevisse envahissante le long de l'axe
littoral-pélagique benthique

Résumé

Bien que la variabilité intraspécifique soit maintenant reconnue pour ses effets sur les processus évolutifs et écologiques, notre connaissance de l'importance de la variabilité intraspécifique des espèces envahissantes est encore limitée. Pourtant, la compréhension de l'association des divergences morphologiques et trophiques au sein des populations (i.e. le polymorphisme de ressource) peut nous aider à mieux appréhender les impacts écologiques des individus envahissants sur les écosystèmes natifs. Dans cette étude nous avons quantifié l'ampleur du polymorphisme de ressource dans 16 populations lacustres d'une espèce d'écrevisse envahissante, *Procambarus clarkii*, en comparant les traits trophiques (estimés à l'aide d'isotopes stables) et morphologiques des individus des habitats littoral et pélagique des lacs. Premièrement, nos résultats ont permis de montrer que les écrevisses occupaient à la fois les habitats littoral et pélagique dans 7 des lacs échantillonnés, et que l'utilisation de l'habitat pélagique était associée à une abondance plus importante des individus dans l'habitat littoral. Ensuite, nous avons montré l'existence de différences morphologiques (la forme du corps et des pinces) et trophiques (utilisation du carbone littoral) entre les individus des habitats littoral et pélagique, mettant ainsi en évidence l'existence d'un polymorphisme de ressource au sein de populations invasives. Il n'existait pas de différenciation génétique entre les individus des deux habitats, permettant donc de conclure à un polymorphisme de ressource stable (flux de gènes important entre les individus). Enfin, nous avons démontré que des processus adaptatifs divergents étaient à l'origine des différences morphologiques du corps et des pinces entre les deux habitats, tandis que les différences d'utilisation de la ressource ont évolué de manière neutre sous la dérive génétique. Ces résultats démontrent que l'écrevisse envahissante *P. clarkii* possède une très forte variabilité phénotypique dans des populations récemment établies, et ces différences pourraient conduire à des impacts écologiques contrastés entre les individus des habitats littoral et pélagique.

Stable resource polymorphism along the benthic littoral-pelagic axis in an invasive crayfish

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Adapted from an article published in *Ecology and Evolution*

Abstract

Although intraspecific variability is now widely recognized as affecting evolutionary and ecological processes, our knowledge on the importance of intraspecific variability within invasive species is still limited. This is despite the fact that understanding the linkage between within-population morphological divergences and the use of different trophic or spatial resources (i.e. resource polymorphism) can help to better predict their ecological impacts on recipient ecosystems. Here, we quantified the extent of resource polymorphism within populations of a worldwide invasive crayfish species, *Procambarus clarkii*, in 16 lake populations by comparing their trophic (estimated using stable isotope analyses) and morphological characteristics between individuals from the littoral and pelagic habitats. Our results first demonstrated that crayfish occur in both littoral and pelagic habitats of 7 lakes and that the use of pelagic habitat was associated with increased abundance of littoral crayfish. We then found morphological (i.e. body and chelae shapes) and trophic divergence (i.e. reliance on littoral carbon) among individuals from littoral and pelagic habitats, highlighting the existence of resource polymorphism in invasive populations. There was no genetic differentiation between individuals from the two habitats, implying that this resource polymorphism was stable (i.e. high gene flow between individuals). Finally, we demonstrated that a divergent adaptive process was responsible for the morphological divergence in body and chela shapes between habitats while difference in origin of the resource use neutrally evolved under genetic drift. These findings demonstrated that invasive *P. clarkii* can display strong within-population phenotypic variability in recent populations, and this could lead to contrasted ecological impacts between littoral and pelagic individuals.

Introduction

Intraspecific variability is now widely recognized as playing a crucial role in evolutionary and ecological processes (Violle et al., 2012; Read et al., 2016). Genetic and/or phenotypic differences among conspecific individuals can have important implications for community structure and ecosystem functioning by mediating the intensity of bottom-up or top-down processes (see review in Des Roches et al., 2017; Raffard et al., 2018). Biological invasions provide a unique opportunity to study intraspecific variability in recently established populations. Indeed, substantial trait and genetic variability among invasive individuals has been reported (Forsman, 2014; González-Suárez et al., 2015), indicating that a high level of intraspecific variability can occur following the introduction stage (60 - 100 years after establishment, e.g. Kinnison et al., 1998; Hendry et al., 2000; Lankau, 2012). Because intraspecific variability can modulate the ecological effects of invasive individuals on ecosystem processes (Evangelista et al., 2017), quantifying the extent of intraspecific variability in invasive species, notably within-populations and across the invasion landscape, is therefore relevant for both applied and theoretical perspectives.

Resource polymorphism refers to within-population morphological divergences due to differences in habitat and trophic resource use (Smith & Skúlason, 1996). It involves the use of an underexploited ecological niche by some individuals of the population, associated with changes in functional traits due to new environmental conditions (Sol et al., 2005; Komiya et al., 2011). Stable resource polymorphism is defined as the existence of discrete morphs with no genetic isolation, and is associated with high gene flow between morphs (Smith & Skúlason, 1996). When gene flow is limited, subsequent genetic isolation can occur among morphs, resulting in distinct subpopulations (Smith & Skúlason, 1996) (Figure V.1). In freshwater lentic ecosystems, resource polymorphism commonly occurs along the littoral-pelagic axis (Quevedo et al., 2009; Faulks et al., 2015). Littoral and pelagic habitats have distinct environmental characteristics (e.g. resource diversity, predation pressure, habitat structure, competition) and individuals using these distinct habitats often display significant morphological differences associated with trophic niche partitioning (Svanbäck et al., 2008; Bartels et al., 2012; Faulks et al., 2015; Marklund et al., 2019). Additionally, genetic differences between littoral and pelagic morphs can occur due to assortative mating (Robinson & Wilson, 1996). Studying resource polymorphism could therefore provide new insights into an underexplored aspect of phenotypic variability within freshwater invasive species (but see Yonekura et al., 2002; Huey et al., 2005; Davidson et al., 2011; Komiya et al., 2011), which can subsequently help understanding their ecological impacts.

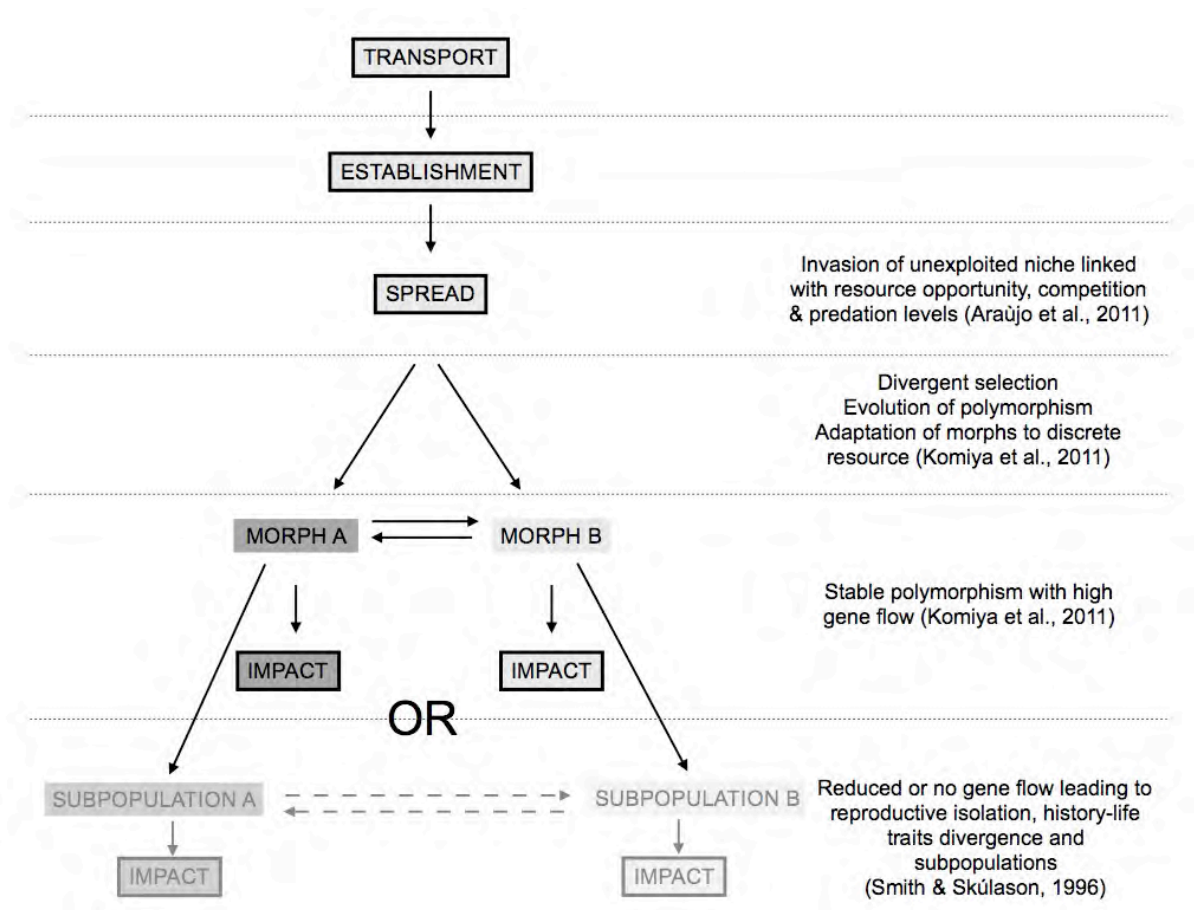


Figure V.1 Conceptual diagram of the establishment of resource polymorphism during the different stages of a biological invasion. Resource polymorphism might result in stable resource polymorphism or in the existence of distinct subpopulations. Adapted from Smith & Skúlason (1996) and Lockwood et al., (2007).

In the present study, we quantified the extent of resource polymorphism in populations of highly invasive red-swamp crayfish (*Procambarus clarkii*) across benthic-littoral and benthic-pelagic habitats (hereafter referred as littoral and pelagic habitats). In lakes, *P. clarkii* has been reported to preferentially occupy littoral habitats (Gherardi & Acquistapace, 2007), but has also been occasionally reported in the pelagic habitat (Foster & Harper, 2006). We first aimed at quantifying the existence of variability in habitat use (littoral vs. pelagic) and at identifying its associated ecological determinants. We predicted that crayfish abundance in the pelagic habitat would increase with increased abundance of crayfish in the littoral habitat, decreased habitat availability (proportion of littoral habitat compared to proportion of pelagic habitat), increased time of invasion and decreased predation pressure. Then, we quantified morphological and trophic traits of individuals from the littoral and pelagic habitats. We predicted the existence of differences in body and chelae morphology functionally associated with differences in habitat structure and resources consumed (trophic position and origin of

resource use). Finally, we quantified genetic differentiation between individuals from the littoral and pelagic habitats to determine the stability of resource polymorphism and its underlying mechanisms (i.e. adaptive or non-adaptive processes). We predicted that gene flow would be high (i.e. associated with stable polymorphism, Smith & Skúlason, 1996) in these recently colonized ecosystems, and that phenotypic variability would be mainly caused by an adaptive response to environmental conditions.

Material & methods

Study system and model species

The study was conducted in 16 gravel pit lakes ranging from 0.7 ha to 27.1 ha and located along the Garonne River in southwestern France (Alp et al., 2016; Jackson et al., 2017) (Table Appendix V.1). Created between 1963 and 2007, these lakes are characterized by different environmental conditions arising from various levels of maturity and management practices (Zhao et al., 2016). Native from Northern America, *P. clarkii* is one of the most invasive crayfish species worldwide (Oficialdegui et al., 2019). The species was introduced to France in 1976 and its presence in the studied area was first documented in 1995 (Changeux, 2003), indicating that the colonization process is relatively recent in those lakes. In the study area, *P. clarkii* are usually observed very rapidly once the lakes are created. Consequently, we assumed that lakes created before 1995 were colonized by *P. clarkii* in 1995, and that the lakes created afterwards were colonized during the first year of their creation (Table Appendix V.1). *P. clarkii* is known to induce strong negative impacts on native organisms and ecosystem processes due to predation, high competitiveness, disease transmission and ecological engineering (Gherardi & Acquistapace, 2007; Jackson et al., 2014). Chelae are important organs involved in multiple ecological functions of crayfish (e.g. predator-prey and competitor interactions, feeding behaviour, biological engineering; Gherardi et al., 2000; Matsuzaki et al., 2009) which are known to display intraspecific morphological variations (Claussen et al., 2008; Malavé et al., 2018). In the studied system, previous investigations have revealed the existence of intraspecific variability among *P. clarkii* populations in terms of body morphology (Evangelista et al., 2019a), trophic ecology (Jackson et al., 2017) and ecosystem impacts (Alp et al., 2016; Evangelista et al., 2019b), as well as the presence of within-population phenotypic variability (Raffard et al., 2017).

Sampling and environmental characteristics

P. clarkii were sampled in the littoral and pelagic habitats of each lake. The littoral habitat was shallow (< 3 m) and characterized by a high level of structural heterogeneity. The nearshore substrate was composed of a mixture of gravels and cobbles with vegetation debris (e.g. downed trees, branches, helophytes) which provided sheltering opportunities for crayfish to hide against predators. The pelagic habitat was deeper and structurally more homogeneous. The substrate was soft and exclusively composed of mud. Importantly, these lakes are not stratified.

Sampling was performed from mid-September to mid-October 2014 in the two habitats of each lake using pairs of baited traps (one cylindrical trap: 62cm x 34cm x 34cm, mesh size: 10 mm; one rectangular trap: 95cm x 20cm x 20cm, mesh size: 4 mm) set overnight ($n_{\text{littoral}} = 4.0 \text{ traps} \pm 0.0 \text{ SD}$; $n_{\text{pelagic}} = 3.9 \pm 0.5 \text{ SD}$) and during the day ($n_{\text{littoral}} = 6.0 \pm 0.0 \text{ SD}$; $n_{\text{pelagic}} = 4.9 \pm 2.4 \text{ SD}$). Littoral traps were located within the first 5 meters along the shoreline in a shallow part (depth mean = 1.44 m \pm 0.28 SD). Pelagic traps were located in the central (mean distance to shoreline = 71.01 m \pm 26.57 SD) and profundal (depth mean = 3.59 m \pm 1.24 SD) part of each lake (Table Appendix V.1). In each lake, we aimed at collecting 20 individuals from each habitat to capture intraspecific variability in the studied phenotypic traits (e.g. Weese et al., 2012; Faulks et al., 2015; Lostrom et al., 2015). When required, additional trapping in both habitats and hand netting along the shoreline (not feasible in the pelagic habitat) were performed to capture the targeted number of individuals. Crayfish were sexed, measured for carapace length (\pm 0.01 mm) and placed on ice for anesthesia. A small sample of muscle from the abdomen was subsequently collected on each specimen, stored in RNAlater© and frozen at the laboratory (-20°C) until subsequent genetic analyses. After collecting muscle tissue, each individual was placed in a labeled plastic bag and frozen in the laboratory. After defrosting, a sample of abdominal muscle was collected on each specimen, rinsed with distilled water and oven dried (60°C for 48 h) for stable isotope analyses.

On the same day as crayfish sampling, putative trophic resources of *P. clarkii* were collected in three different locations in each habitat of each lake to capture potential spatial heterogeneity in their stable isotope values. Specifically, periphyton and leaf litter were collected from the littoral, as they represent important components of *P. clarkii*'s diet (Alp et al., 2016; Jackson et al., 2017), while pelagic zooplankton was collected using a 200- μ m mesh net as we assume that pelagic individuals on muddy bottoms could consume detritus including zooplankton debris (Smart et al., 2002; Ruokonen et al., 2012). Periphyton and

zooplankton samples were freeze-dried (-50°C for 5 days) and oven dried (60°C for 48h), respectively (further details available in Jackson et al., 2017). Samples of crayfish and putative prey were collected in September-October (i.e. at the end of the growing season) to ensure that stable isotope analyses were representative of the trophic interactions occurring during this period.

Abundance of littoral and pelagic crayfish were calculated as the number of crayfish trapped over a 24-hour period in each habitat (Catch Per Unit Effort (CPUE) expressed in ind. trap⁻¹.hr⁻¹, Table Appendix V.1). On the same day, fish community was sampled to assess predation pressure. Gillnets were set in the littoral (length: 20 m, height: 2.4 m; mesh size: 12, 20, 30, 60 mm, n = 4 to 6 depending on the lake size) and pelagic habitats (length: 25 m, height: 3.1 m; mesh size: 20 and 50 mm respectively, n = 2) following Zhao et al., (2016). Fish species were determined and each specimen was measured for fork length (± 0.01 mm). For each fish species, the body mass of each fish was computed using length-weight relationships (Zhao et al., unpublished data). Predator biomass in each lake was then calculated as the biomass of predator fish trapped in gillnets over a 1-hour period biomass (Biomass Per Unit Effort (BPUE) expressed in g gillnet⁻¹ hr⁻¹; Appendix 1). Based on gape limitation and knowledge about trophic interactions in these studied lakes, potential crayfish predators were juveniles and adults of pike *Esox lucius* (> 275 mm FL), common carp *Cyprinus carpio* (all individuals), European perch *Perca fluviatilis* (> 110 mm FL), pikeperch *Sander lucioperca* (> 200 mm FL), largemouth bass *Micropterus salmoides* (> 105 mm FL – fork length), and European catfish *Silurus glanis* (> 200 mm FL). Because the studied lakes were relatively small and these predatory species are highly mobile (i.e. they feed on crayfish in both habitats; Garvey et al., 2003), a global predation pressure for each lake was calculated. In four lakes, *P. clarkii* coexisted with the invasive spiny-cheek crayfish (*Faxonius limosus*) which was present only in 25% of the studied lakes and in low density (ind.trap⁻¹.hr⁻¹ mean = 0.07 \pm 0.05 SE). As this species was rare, we did not consider potential interspecific competition with *P. clarkii* as a key driver of their habitat use in our analyses. The surfaces of littoral (< 3 m deep) and pelagic (> 3 m deep) habitats were measured for each lake using bathymetry data (Appendix 1). A depth threshold of 3.0 m was used to separate littoral from pelagic habitats following Garvey et al., (2003) and Ruokonen et al., (2012). The proportion of littoral habitat (%) was then calculated as the ratio of littoral habitat surface and total lake surface.

Morphological and stable isotope analyses

Each crayfish and its right chela were photographed dorsally directly after defrosting and before tissue samples were taken for stable isotope analyses. Pictures were analyzed for morphological variation using TpsDig2 v.2.17 (Rohlf, 2015). We used a geometric morphometric technique (Zelditch et al., 2012) based on landmark analysis that has been widely used to quantify shape variations of morphological structures along the littoral-pelagic axis (e.g. Quevedo et al., 2009; Bartels et al., 2012; Faulks et al., 2015). Here, we digitized 19 homologous landmarks on *P. clarkii* individual bodies (i.e. cephalothorax and abdomen) following Evangelista et al., (2019a) and 7 landmarks on their chela propodus (adapted from Malavé et al., 2018). For each morphological structure (i.e. body and chela), a Full Procrustes Fit (FPF) was then performed using Morpho J v.1.06d to obtain a global shape comparison by superimposing individual shapes and remove the bias due to different sizes, positions and orientations among individuals (Klingenberg, 2011). The deformation components (i.e. landmark coordinates) obtained with each FPF were projected into two separate matrices to characterize whole-body and whole-chela shape using partial warps (i.e. non-uniform variation localized to particular regions of geometry) and uniform scores (i.e. uniform variation throughout the body or the chela) (Zelditch et al., 2012). Using whole-body and whole-chela datasets, the centroid size of each individual morphological structure was also calculated as the square root of the summed squared distances of each landmark from their centroid and used as a proxy of individual body and chela size.

Stable isotope samples were ground to a fine powder and analyzed for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes at the Cornell Isotope Laboratory (COIL, New York). The trophic position ($\text{TP}_{\text{crayfish}}$) of each individual was computed following Vander Zanden et al., (1997):

$$\text{TP}_{\text{crayfish}} = \text{TP}_{\text{baseline}} + (\delta^{15}\text{N}_{\text{crayfish}} - \delta^{15}\text{N}_{\text{baseline}}) / 3.4$$

where baseline organisms are a mix of leaf litter (allochthonous primary producer) and periphyton (autochthonous primary producer) ($\text{TP}_{\text{baseline}} = 1$), $\delta^{15}\text{N}_{\text{baseline}}$ corresponds to the mean of $\delta^{15}\text{N}_{\text{periphyton}}$ and $\delta^{15}\text{N}_{\text{litter}}$ and 3.4 is the fractionation coefficient between trophic levels (Vander Zanden et al., 1997; Post, 2002). The origin of resource use was assessed by quantifying the littoral reliance (LR: relative dietary contribution of littoral resources to each individual), with periphyton and zooplankton as baselines for littoral and pelagic habitats respectively, and following Vander Zanden & Vadeboncoeur (2002):

$$LR_{\text{crayfish}} = (\delta^{13}\text{C}_{\text{crayfish}} - \delta^{13}\text{C}_{\text{zooplankton}}) / (\delta^{13}\text{C}_{\text{periphyton}} - \delta^{13}\text{C}_{\text{zooplankton}}).$$

Regarding littoral reliance, zooplankton is the only group of primary consumers that was consistently collected in all studied lakes, and which could contributed to the diet of crayfish (Correia, 2003; Alcorlo et al., 2004). We have considered that *P. clarkii* were not selective on zooplankton taxa, so the pooled samples have been analyzed even though zooplankton have varying trophic positions (Matthews & Mazumder, 2003).

Genetic analyses

Neutral genetic differentiations were assessed using 14 microsatellites. 10 microsatellites were selected from Belfiore & May (2000) (PclG04, PclG07, PclG15, PclG16, PclG17, PclG27, PclG28, PclG29, PclG32 and PclG48) and 4 additional microsatellites (PCSH0038, PCSH0005, PCSH0006, PCSH0089) were used, based on Jiang et al., (2015). DNA was extracted from the abdomen muscle of crayfish using a salt-extraction method (Aljanabi & Martinez, 1997). Loci were amplified by Polymerase Chain Reaction (PCR) in a final volume of 10 μL , containing 10-20 ng of genomic DNA, 5 μL of QIAGEN® Multiplex PCR and locus-specific optimized combination of primers (see Table Appendix V.2). PCR was performed in a Mastercycler (Eppendorf®) under the following conditions: 15 min at 95°C followed by 35 cycles of 0.5 min at 94°C, 1.5 min at 56°C and 1 min at 72°C and finally followed by a 45 min final elongation step at 60°C (see Appendix 2 for the description of the multiplex used in this study). Amplified fragments were analyzed on an ABI PRISM 3730 capillary sequencer (Applied Biosystems, Foster City, USA) in the Génopole Toulouse Midi-Pyrénées. Allele size results were scored using GENEMAPPER v.4.0 (Applied Biosystems). Then, deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between all pairs of loci were tested using FSTAT v.2.9.3.2 (Goudet, 2002) and null alleles were tested using MICROCHECKER v.2.2.3 (Van Oosterhout et al., 2004). Genetic diversity was quantified using observed (H_o) and expected (H_e) heterozygosity, Wright fixation indices F_{IS} and allelic richness (A_R), based on the minimum sampling size. The genetic differentiation (F_{st}) between littoral and pelagic individuals was calculated using FSTAT v.2.9.3.2 (Goudet, 2002). No null alleles were detected in the genotyped loci. The number of alleles per locus ranged from 2 to 11 (Table V.1). We found no linking disequilibrium between pairs of loci. There was no evidence for any significant heterozygous deficit for the considered loci after Bonferroni correction, suggesting that all populations were at Hardy-Weinberg equilibrium.

Table V.1 Genetic diversity of *Procambarus clarkii* in the littoral and pelagic habitats based on 14 microsatellites of 7 studied lakes. NA = mean number of alleles per locus, A_R = allelic richness, H_e = expected heterozygosity, H_o = observed heterozygosity, F_{IS} = fixation indices.

Lake	Habitat	NA	A_R	H_e	H_o	F_{IS}
A	Littoral	5.0714	4.8065	0.6585	0.6821	-0.010
	Pelagic	5.1429	4.9456	0.6709	0.7120	-0.033
B	Littoral	4.7143	4.3966	0.5921	0.5918	0.027
	Pelagic	4.6429	4.4206	0.6102	0.5523	0.122
G	Littoral	6.4286	5.9626	0.7014	0.7297	-0.014
	Pelagic	6.4286	6.0222	0.6898	0.6582	0.073
I	Littoral	4.8571	4.6441	0.6407	0.6172	0.064
	Pelagic	4.4286	4.1959	0.6181	0.6417	-0.013
J	Littoral	5.7857	5.4438	0.6897	0.6910	0.026
	Pelagic	6.0000	5.8495	0.7127	0.6964	0.056
K	Littoral	5.1429	4.7662	0.6480	0.6679	-0.005
	Pelagic	5.2143	4.7738	0.6461	0.6455	0.027
M	Littoral	3.4286	3.3727	0.5616	0.5438	0.058
	Pelagic	3.7143	3.6164	0.5882	0.6212	-0.030

Statistical analyses

To test the association between environmental conditions and the abundance of *P. clarkii* in the pelagic habitat of the 16 lakes, we performed a linear model (LM) using abundance of *P. clarkii* in littoral habitat, predation pressure (predators' biomass), habitat availability (proportion of littoral habitat), time of invasion (expected date of lake invasion) and their two-way interactions as fixed effects. The full model was built and interactions were removed when non-significant using a backward procedure. Variance inflation factor (VIF) did not detect multicollinearity between predictors ($VIF < 5$) (R package "car" v. 3.0-0; Fox et al., 2018).

Comparison of ecological and genetic characteristics between habitats were performed in 7 lakes where a sufficient number of individuals was collected in each habitat (mean number of individuals per habitat = 19.79 ± 0.8 SD; Table Appendix V.1). We used a linear mixed-model (LMM) with "carapace length" as response variable, "habitat" and "sex" as fixed effects, and "lake" as random effect to test for a significant difference of crayfish size between littoral and

pelagic individuals. To remove potential allometric component of shape variation, partial warps scores were regressed against centroid sizes, using a pooled within-habitat regression in Morpho J (Klingenberg, 2016). Regression residuals scores (for body and for chela) were analysed in two distinct Discriminant Function Analysis (DFA) implemented in Morpho J to determine if the morphology of individuals from the littoral and pelagic habitats differs significantly (Klingenberg, 2011). Each individual was thus characterized by a DFA morphological score for both body and chela along the littoral-pelagic axis. Sexual dimorphism is a potent agent of intraspecific morphological divergence (Malavé et al., 2018) and this effect was assessed using a LMM with “DFA score” as response variable, “sex” and “habitat” and their interaction as fixed effects, and “lake” as random effect. When significant, the interaction was further investigated with post-hoc pairwise comparison of the estimated marginal means using the “emmeans” function in “emmeans” R package v.1.4.3.01 (Lenth, 2019). Because trophic resources use has been reported not to differ between sex in crayfish (Gutiérrez-Yurrita et al., 1998; Pérez-Bote, 2004; Houghton et al., 2017), sex was not included in the subsequent trophic analyses. To test for the presence of resource polymorphism, we first used a LMM with “trophic position” as response variable and a generalized linear mixed-model (GLMM) with “littoral reliance” as response variable, with each model including “habitat” and “lake” as fixed and random effects, respectively. The LMM was run using “lme4” R package v. 1.1-21 (Bates et al., 2015). Littoral reliance was a continuous variable bounded between 0 and 1 and the GLMM was thus run using a beta distribution in the “glmmTMB” R package v. 0.2.3 (Magnusson et al., 2019). We then tested the association between individual morphology (body and chela DFA scores) and origin of resource use (i.e. littoral reliance) using a beta regression implemented in “betareg” R package v3.1-1 (Cribari-Neto & Zeileis, 2010). For all models, residuals normality and homoscedasticity were checked using Q-Q plot and Tukey-Anscombe plot, respectively. Abundance of *P. clarkii* in pelagic habitat was square-root transformed to conform with these assumptions.

Genetic and phenotypic differentiations were compared to determine the underlying process (neutral or adaptive process) explaining variability between littoral and pelagic individuals (Leinonen et al., 2006). We used a quantitative genetic approach based on *Fst* calculated using microsatellites as neutral genetic markers and the phenotypic equivalent *Pst* (i.e. used as a proxy of *Qst* for natural environments) was calculated for both morphology and diet as:

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

where σ^2 is the variance of the phenotypic trait X (i.e. DFA scores, trophic position or littoral reliance) and h^2 is the heritability of X defined as the proportion of phenotypic variance with a genetic origin (Leinonen et al., 2006). Because we had no information on trait heritability, h^2 was set to 0.5 to avoid overestimating Pst (e.g. heritability estimates for the studied traits are close to 0.3 - 0.5; Lutz & Wolters, 1989). When Pst and Fst are equal, considered traits evolve neutrally under genetic drift. A greater Pst than Fst implies an adaptive phenotype divergence while a higher Fst suggests a homogenizing adaptation. We estimated the between-habitats variance using LMMs (“lme4” R package v. 1.1-21) with the phenotypic traits as response variables, the intercept as a fixed effect and the “habitat” as a random effect. “Littoral reliance” was square-root transformed to improve the fit of the model. We computed 95% confidence interval ($CI_{95\%}$) for Pst , using bootstrapping procedure (Raffard et al., 2019a), while $CI_{95\%}$ for Fst was implemented in FSTAT. All statistical analyses were performed using R v.3.4.4 (R Core Team, 2018).

Results

P. clarkii occurred in all littoral habitats, and was detected in the pelagic habitat of 75% of the sampled lakes. *P. clarkii* abundances within littoral and pelagic habitats were highly variable among lakes, ranging from 0.00 to 4.79 ind. trap⁻¹ hr⁻¹ in the littoral habitat and from 0.00 to 10.63 ind. trap⁻¹ hr⁻¹ in the pelagic ones (Table Appendix V.1). In the latter, crayfish abundance was significantly and positively related to the abundance of *P. clarkii* in the littoral habitat (LM, $F_{1,10} = 38.90$, $P < 0.001$). There was no significant effect of the proportion of littoral habitat (LM, $F_{1,10} = 0.49$, $P = 0.500$), predators’ biomass (LM, $F_{1,10} = 0.04$, $P = 0.856$) and time of invasion (LM, $F_{1,10} = 2.87$, $P = 0.121$) on the abundance of *P. clarkii* in the pelagic habitat.

In the 7 lakes where *P. clarkii* was abundant in both littoral and pelagic habitats, sex ratio did not differ between littoral and pelagic habitats (t-test, $t = 2.23$, $df = 6$, $P = 0.07$; performed using all the crayfish captured). Carapace lengths differed significantly between females and males (LMM, $F_{1,268} = 8.89$, $P = 0.003$), with females displaying longer carapace lengths than males (mean \pm SE = 49.14 \pm 0.50 mm and 47.62 \pm 0.41 mm respectively). There was no significant difference in carapace length between individuals from littoral and pelagic habitats (LMM, $F_{1,268} = 0.65$, $P = 0.423$; Figure Appendix V.3). However, individuals from each habitat differed significantly in body morphology (DFA; T-square = 80.31; $P < 0.001$). Specifically, individuals from littoral habitat had lower DFA scores than those from pelagic habitat (mean = -0.06 \pm 0.11 SE and 0.62 \pm 0.08 SE, respectively), indicating that littoral crayfish were characterized by stocky body and rostrum, while pelagic crayfish had a more streamlined

body and rostrum (Figure V.2a). It is, however, interesting to note that the extent of morphological differences between littoral and pelagic individuals varied between lakes (Figure Appendix V.4). Even if females had higher DFA body scores than males (mean = 0.30 ± 0.09 SE and -0.24 ± 0.11 SE, respectively), the effect of sex alone did not affect body shape. However, DFA scores were significantly affected by the effect of habitat (LMM, $F_{1,268} = 79.16$, $P < 0.001$; as also seen with the DFA analysis) and this effect was sex-dependent (LMM, interaction term: $F_{1,270} = 18.54$, $P = 0.004$). Specifically, for both sexes, pelagic individuals had higher DFA body scores compared to littoral ones (Post-hoc tests, t ratio = -3.62 , $df = 268$, $P < 0.001$ and t ratio = -8.59 , $df = 269$, $P < 0.001$ for females and males respectively; Figure V.3a). Chela morphology also differed significantly between littoral and pelagic individuals (DFA; T-square = 23.7 ; $P = 0.014$). Individuals from littoral habitat displayed lower DFA scores than those from pelagic (mean = -0.19 ± 0.05 SE and 0.19 ± 0.06 SE, respectively), indicating that chelae from littoral individuals were thicker while those from pelagic individuals were more elongated (Figure V.2b). The effect of habitat on chela shape was not sex-dependent (LMM, interaction term: $F_{1,246} = 0.0035$, $P = 0.953$) but DFA chela scores also differed significantly between females and males (LMM, $F_{1,248} = 43.56$, $P < 0.001$), with females displaying higher DFA chela scores than males (mean = 0.39 ± 0.05 SE and -0.23 ± 0.05 SE, respectively; Figure V.3b).

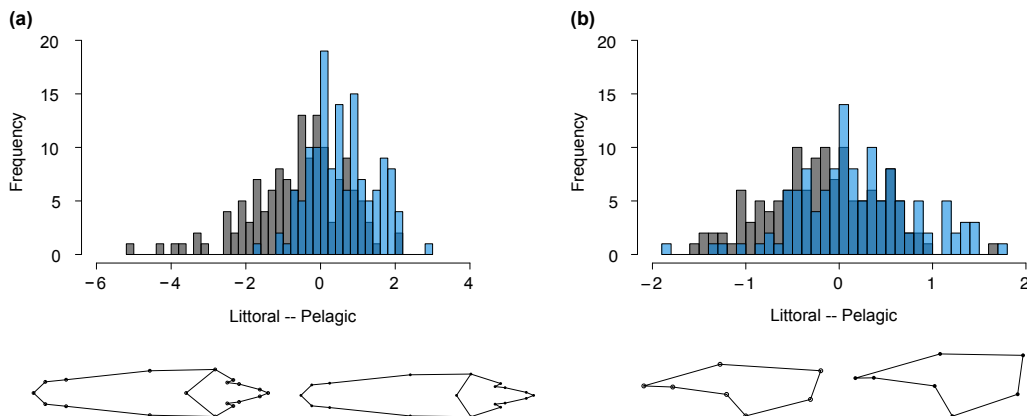


Figure V.2 Frequency distribution of the morphological scores of *Procambarus clarkii* obtained using a Discriminant Functional Analysis (DFA) between littoral (dark grey) and pelagic (blue) habitats for **(a)** bodies ($n = 140$ and $n = 137$, respectively) and **(b)** chelae ($n = 129$ and $n = 124$, respectively). Body and chela shapes of extreme landmark-based values are displayed (amplified ten times).

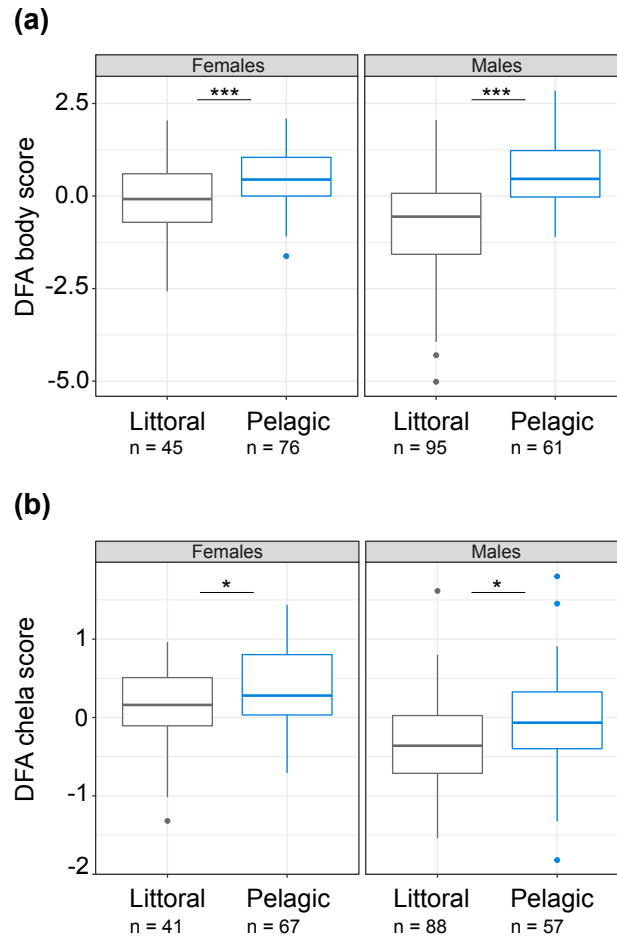


Figure V.3 Boxplot of DFA scores for **(a)** bodies ($n_{\text{females}} = 121$ and $n_{\text{males}} = 156$) and **(b)** chelae ($n_{\text{females}} = 108$ and $n_{\text{males}} = 145$) for female and male individuals captured in littoral (dark grey) and pelagic (blue) habitats.

Trophic position of *P. clarkii* did not significantly differ between littoral (mean = 3.00 ± 0.05 SE) and pelagic (mean = 3.05 ± 0.05 SE) individuals (LMM, $F_{1,268} = 2.96$, $P = 0.086$; Figures V.4a, Figure Appendix V.5). However, littoral reliance of littoral individuals (mean = 0.35 ± 0.02 SE) was significantly higher than for pelagic individuals (mean = 0.33 ± 0.02 SE), supporting the existence of a differential niche use (GLMM, $\chi^2 = 7.50$, $df = 1$, $P = 0.006$; Figures V.4b, Figure Appendix V.5).

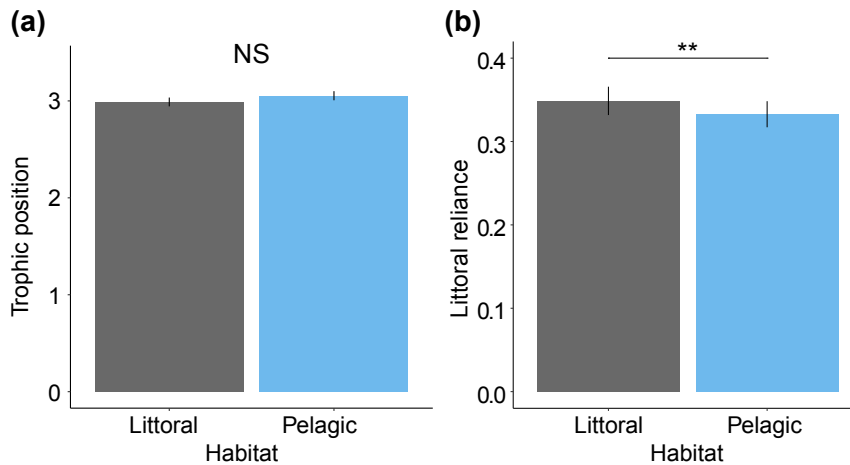


Figure V.4 Trophic position **(a)** and littoral reliance **(b)** of *Procambarus clarkii* in littoral (dark grey; $n = 139$) and pelagic (blue; $n = 137$) habitats. Predicted values obtained from the models are reported with their standard errors.

There was no significant genetic differentiation between individuals from the littoral and pelagic habitats (littoral-pelagic global $F_{st} = 0.000$, $CI_{95\%}: 0.000 - 0.002$ and ANOVA – H_e : $P = 0.407$; A_R : $P = 0.561$) (Table V.1). P_{st} for body morphology and chela morphology were 0.364 ($CI_{95\%}: 0.269 - 0.457$) and 0.152 ($CI_{95\%}: 0.056 - 0.275$) respectively, and were significantly higher than F_{st} , indicating that morphological variations were due to adaptive divergent processes. P_{st} for trophic position and littoral reliance were 0.000 ($CI_{95\%}: 0.000 - 0.0448$) and 0.000 ($CI_{95\%}: 0.000 - 0.0542$), respectively. There was no significant difference between P_{st} for trophic position and littoral reliance and the global F_{st} value, indicating that both trophic traits evolved under non-adaptive processes (i.e. genetic drift).

Discussion

Our results supported the existence of resource polymorphism within invasive species. Indeed, we observed that morphological divergences between littoral and pelagic habitats were also associated with changes in the origin of the resource used by crayfish. This resource polymorphism might occur due to intraspecific competition since the abundance of pelagic crayfish was strongly and positively associated with the abundance of littoral crayfish. There was no genetic differentiation between individuals from the two habitats, indicating that the resource polymorphism was stable (Figure V.1). Finally, we demonstrated that morphological divergences in body and chela shapes between habitats were driven by a divergent adaptive process, while difference in the origin of the resource use neutrally evolved under genetic drift.

Although crayfish abundance was highly variable between lakes, the species occurred in the pelagic habitat of 75% of the studied lakes and was abundant in both littoral and pelagic habitats in 44% of the studied lakes. In addition, we found that increased pelagic abundance was associated with increased littoral abundance. This suggests that intraspecific competitive exclusion was a potential mechanism explaining the presence of crayfish in the pelagic habitat. Increased population density in the preferred littoral habitat of crayfish (Nyström et al., 2006) can favor aggressiveness between conspecifics (Gherardi & Cioni, 2004), limiting the access to shelters (e.g. under cobble, tree trunks, macrophytes, rocks) and forcing some weak competitors to migrate to the pelagic habitat. Competitive exclusion may also explain morphological divergences between littoral and pelagic crayfish for both sexes, with stockier-bodied and streamlined individuals occupying the littoral and pelagic habitats, respectively. Although this remains to be tested experimentally, individuals with a stocky cephalothorax and rostrum and with longer chelae might have a competitive advantage compared to more streamlined individuals to occupy littoral shelters (Stein, 1977).

Predation might also be a driver of morphological differences observed between the two habitats (Stein, 1977; Kershner & Lodge, 1995). In the littoral habitat, stockier-bodied individuals might have an advantage to face predation pressure from both aquatic (i.e. fish) and terrestrial (i.e. birds) predators (Davis & Huber, 2007) by hiding and defending their shelters. In the pelagic habitat, streamlined individuals with more elongated abdomen might be more efficient to move in the muddy substrate and escape from predators via tail flipping (Wine & Krasne, 1972; Patullo & MacMillan, 2004). Furthermore, streamlined bodies with thicker chelae might provide a defense against predators through gape limitation (Englund & Krupa, 2000; Garvey et al., 2003; Davis & Huber, 2007). As the extent of morphological differentiation between habitats differed between lakes (Figure Appendix V.4), it would be interesting to determine whether the extent of differences in environmental conditions between the littoral and the pelagic habitats drive the intensity of the morphological differentiation observed.

As expected, littoral individuals consumed more resources originating from the littoral habitat than pelagic individuals. Given the turnover rate of stable isotope values in crayfish muscle tissue (> 1 month; Carolan et al., 2012; Glon et al., 2016), these findings indicate that the feeding activity of individuals occurs within their respective habitats. There was, however, no significant difference in trophic position between littoral and pelagic individuals. Trophic positions of 3 indicated that *P. clarkii* feed on more than one trophic level and are thus

omnivorous, as observed previously in the studied ecosystems (Jackson et al., 2017). This omnivorous diet was likely composed of a mixture of primary producers, invertebrates and fish (eggs and larvae or carrion) in both habitats (Gutiérrez-Yurrita et al., 1998). Although it remains to be quantified, these results suggest that, in each habitat, *P. clarkii* display an opportunistic foraging strategy with a diet being primarily driven by resource availability rather than a form of trophic specialization that varies between habitats.

Phenotypic differentiation between littoral and pelagic individuals was not associated with genetic differentiation, highlighting the existence of non-assortative mating. The absence of significant genetic differentiation might be due to the recent colonization of lakes by *P. clarkii* (< 60 years, recent population bottleneck event; Dlugosch & Parker, 2008) and/or low reproductive isolation due to the relatively small size of the studied lakes (mean \pm SE = 13.90 \pm 1.78 ha). Thus, our results demonstrate that, within each lake, *P. clarkii* display a stable resource polymorphism with high gene flow between morphs and form a unique population (Smith & Skúlason, 1996). However, the temporal dynamic of this resource polymorphism remains to be quantified because gene flow between morphs could be reduced (e.g. philopatry behavior, breeding temporal segregation, emerging differences in mate choice), thus increasing the genetic differentiation along the littoral-pelagic gradient (Meyer, 1990). In general, littoral-pelagic divergences observed in fish species are explained by combination of both phenotypic plasticity and genetic differences (Smith & Skúlason, 1996; Komiya et al., 2011; Faulks et al., 2015). Here, trophic differentiation was not different from what was expected under the drift hypothesis ($P_{st} < F_{st}$), but P_{st} value for morphology was significantly higher than the F_{st} . Thus, adaptive process could explain the divergence of morphology, but this needs further investigations. Therefore, future studies should explore the relative importance of selection vs. phenotypic plasticity in driving phenotypic variation within invasive species.

In conclusion, we showed that stable resource polymorphism occurred between littoral and pelagic individuals of a recent biological invasion (Smith & Skúlason, 1996). The establishment of resource polymorphism within invasive populations can have important ecological and evolutionary implications, such as leading to different ecological impacts on the littoral and pelagic food chains (Vander Zanden & Vadeboncoeur, 2002; Ruokonen et al., 2012). Ecosystem impacts of invasive crayfish can vary among their populations (Evangelista et al., 2019b), and the present study suggests that they could also depend on within-population characteristics.

Appendix

Table Appendix V.1 Environmental characteristics of the 16 sampled lakes (Haute-Garonne, France). Resource polymorphism was studied in 7 lakes (highlighted in bold) where sufficient number of *Procambarus clarkii* were collected in both habitats (mean number of individuals per habitat = 19.79 ± 0.8 SD). Distance pelagic traps was assessed by measuring the distance (in meters) perpendicularly to the shore with GIS (accuracy of GPS ± 5 m). Predation pressure was not assessed in lake O this year.

Lake	Latitude (N)	Longitude (E)	Invasion date	Surface (m ²)	Proportion of littoral habitat (%) (depth <3 m)	Max depth (m)	Depth pelagic traps (m)	Pelagic traps distances to the shore (GPS accuracy ± 5 m)	Predation (BPUE fish g.gillnet ⁻¹ .hr ⁻¹)	Littoral abundance (CPUE <i>P. clarkii</i> ind.trap ⁻¹ .hr ⁻¹)	Pelagic abundance (CPUE <i>P. clarkii</i> ind.trap ⁻¹ .hr ⁻¹)
A	43.322349	1.202065	1995	91044	42	5.00	4.76	71.72	654.91	3.21	1.17
B	43.3171547	1.1989507	2000	92217	20	6.10	4.96	43.67	1109.721	2.96	3.92
C	43.530329	1.289764	1995	203628	100	2.90	1.82	89.83	318.26	1.38	0.17
D	43.453752	1.27367	1995	271400	48	5.60	1.93	42.10	18.43	0.00*	0.00
E	43.519202	1.354537	1995	18895	100	1.90	1.57	82.33	453.12	0.00*	0.00
F	43.506265	1.337336	1995	213016	84	3.50	2.91	77.50	2071.40	1.79	0.00
G	43.386392	1.265891	1995	182103	65	3.70	2.80	65.77	829.92	2.83	1.71
H	43.343186	1.22705	1995	43951	80	3.70	3.25	75.00	2719.99	2.25	1.00
I	43.3208803	1.1948792	2007	131679	34	5.00	4.23	80.40	1035.88	2.54	2.21
J	43.3719279	1.258473	1996	103909	44	4.80	3.58	53.31	0	2.17	1.08
K	43.364617	1.251335	1997	162864	55	5.10	3.56	72.60	617.45	7.13	10.63
L	43.205616	1.040017	1995	87037	34	5,90	5.16	85.77	1166.15	2.92	0.38
M	43.207631	1.046572	1995	210002	39	6.90	5.33	139.40	745.54	4.79	2.63
N	43.208923	1.038937	1998	145985	34	7.00	5.15	86.50	1958.70	0.00*	0.63
O	43.551857	1.261958	1995	6961	64	3.90	3.16	22.33	NA	0.00*	0.00

P	43.551353	1.259427	1995	28332	61	4.30	3.25	48.00	109.00	0.38	0.13
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* *P. clarkii* was present in the lake and collected using complementary sampling methods but not were sampled in the traps.

Table Appendix V.2 List of the 14 microsatellites amplified in three PCR multiplex. All primers at 10µM each. Selection based on Belfiore & May (2000) and Jiang et al., (2015).

Multiplex 1

Locus	GenBank accession number	Allele size range (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Fluorescent dye
PclG-15	AF290927	120-200	GGC GTG ACG CCA ACG TGT CTT	GGC TGG CCA CTT TGT TAG CCT GAG	ATTO 550
PclG-27	AF290932	100-130	AAT CTT AAG ATC ATG AAA AAG GTA	TTT AAG GAA CGT ATA AGA AAA GAC	FAM
PclG-16	AF290928	80-180	CTC GGA ATG TCC ACC TGA GA	TCA TTA TGG ATT TTG TCA ATC TAT	HEX
PclG-04	AF290921	170-255	TAT ATC AGT CAA TCT GTC CAG	TCA GTA AGT AGA TTG ATA GAA GG	FAM

PCR MIX	Volume (µL) x1
H ₂ O	2.1
Qiagen multiplex PCR Master Mix	5
DNA	2
PclG-15 F	0.1
PclG-15 R	0.1
PclG-27 F	0.15
PclG-27 R	0.15
PclG-16 F	0.1
PclG-16 R	0.1
PclG-04 F	0.1
PclG-04 R	0.1

Multiplex 2

Locus	GenBank accession number	Allele size range (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Fluorescent dye
PclG-29	AF290934	159-210	GAA AGT CAT GGG TGT AGG TGT AAC	TTT TTG GGC TAT GTG ACG AG	ATTO 550
PclG-07	AF290922	112-124	CCT CCC ACC AGG GTT ATC TAT TCA	GTG GGT GTG GCG CTC TTG TT	FAM
PclG-28	AF290933	238-266	CTC GGC GAG TTT ACT GAA AT	AGA AGA AAG GGA TAT AAG GTA AAG	HEX
PclG-32	AF290935	173-221	CCC CCA CTC GTC TCT GTG TAT G	TGT GCT TGC GGG AGT GAG C	FAM
PCSH0038	KJ607979	150-190	CAG AGC ACT GTT TGC TAG TGT GT	GCT TCC TCT GTT ATT CAT CAT GC	HEX

PCR MIX	Volume (µL) x1
H ₂ O	2
Qiagen multiplex PCR Master Mix	5
DNA	2
PclG-29 F	0.1
PclG-29 R	0.1
PclG-07 F	0.1
PclG-07 R	0.1
PclG-28F	0.1
PclG-28 R	0.1
PclG-32 F	0.1
PclG-32 R	0.1
PCSH0038 F	0.1
PCSH0038 R	0.1

Multiplex 3

Locus	GenBank accession number	Allele size range (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Fluorescent dye
PclG-48	AF290941	145-175	CTG TTG GTG ATT TCC GTC AAT TTT	AGA TTC AAC GCT GTG TTC CTG ATC	ATTO 550
PclG-17	AF290929	159-184	GTC GGG AAC CTA TTT ACA GTG TAT	AAG AGC GAA GAA AGA GAT AAA GAT	HEX
PCSH0089	KJ607988	80-120	GTA TAC ACA GCT TTG GAA CTG GG	GCT TCC TCT GTT ATT CAT CAT GC	HEX
PCSH0006	KP675956	140-180	GGC CAA AAT GTG AAG AGT TGT TA	GAA CCA GAT CAG TGT CAT GTG AG	FAM
PCSH0005	KP675955	110-135	AAC AGA GTG GCA AGG TAC TTG AA	GGC TGT CAC TCG TGT CTT TAG TT	FAM

PCR MIX	Volume (µL) x1
H ₂ O	2
Qiagen multiplex PCR Master Mix	5
DNA	2
PclG-48 F	0.1
PclG-48 R	0.1
PclG-17 F	0.1
PclG-17 R	0.1
PCSH0089 F	0.1
PCSH0089 R	0.1
PCSH0006 F	0.1
PCSH0006 R	0.1
PCSH0005 F	0.1
PCSH0005 R	0.1

PCR cycling conditions	
95°C	15 min
94°C	30 s
56°C	90 s
72°C	60 s
60°C	45 min
10°C	∞

} 35 cycles

Figure Appendix V.3 Carapace length (mm) of crayfish from littoral (dark grey) and pelagic (blue) habitats in the 7 gravel pit lakes. The boxplot indicates the median (horizontal bar), the first and third quartiles (box), and the maximum and the minimum values no further than 1.5 x inter-quartile range (whiskers). Dots represent outliers.

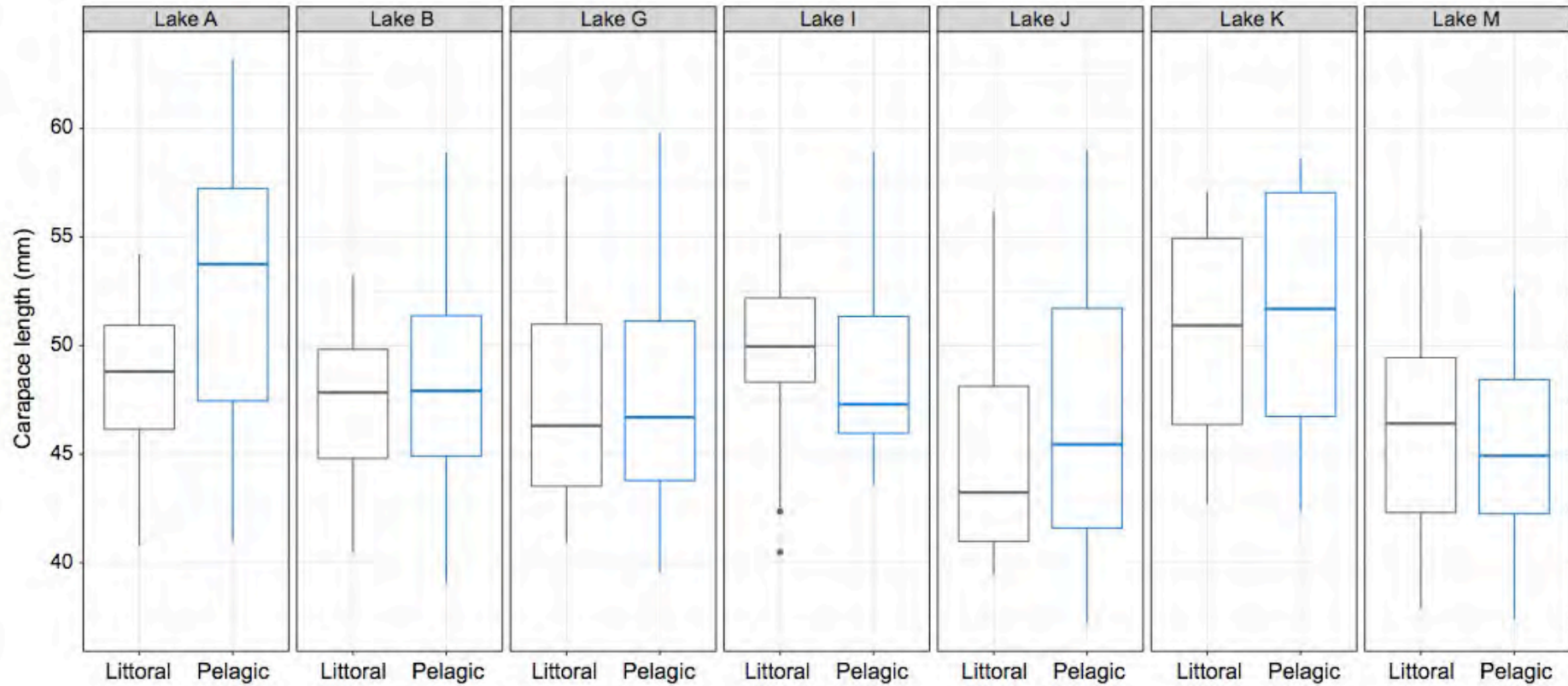


Figure Appendix V.4 Frequency distribution of *Procambarus clarkii* body morphological scores obtained using a Discriminant Functional Analysis along the littoral-pelagic axis ($n_{\text{littoral}} = 20$, dark grey; $n_{\text{pelagic}} = 20$ except for lake J where $n_{\text{pelagic}} = 17$, blue) of the 7 gravel pit lakes. Note that the X-axis scale is different for Lake K.

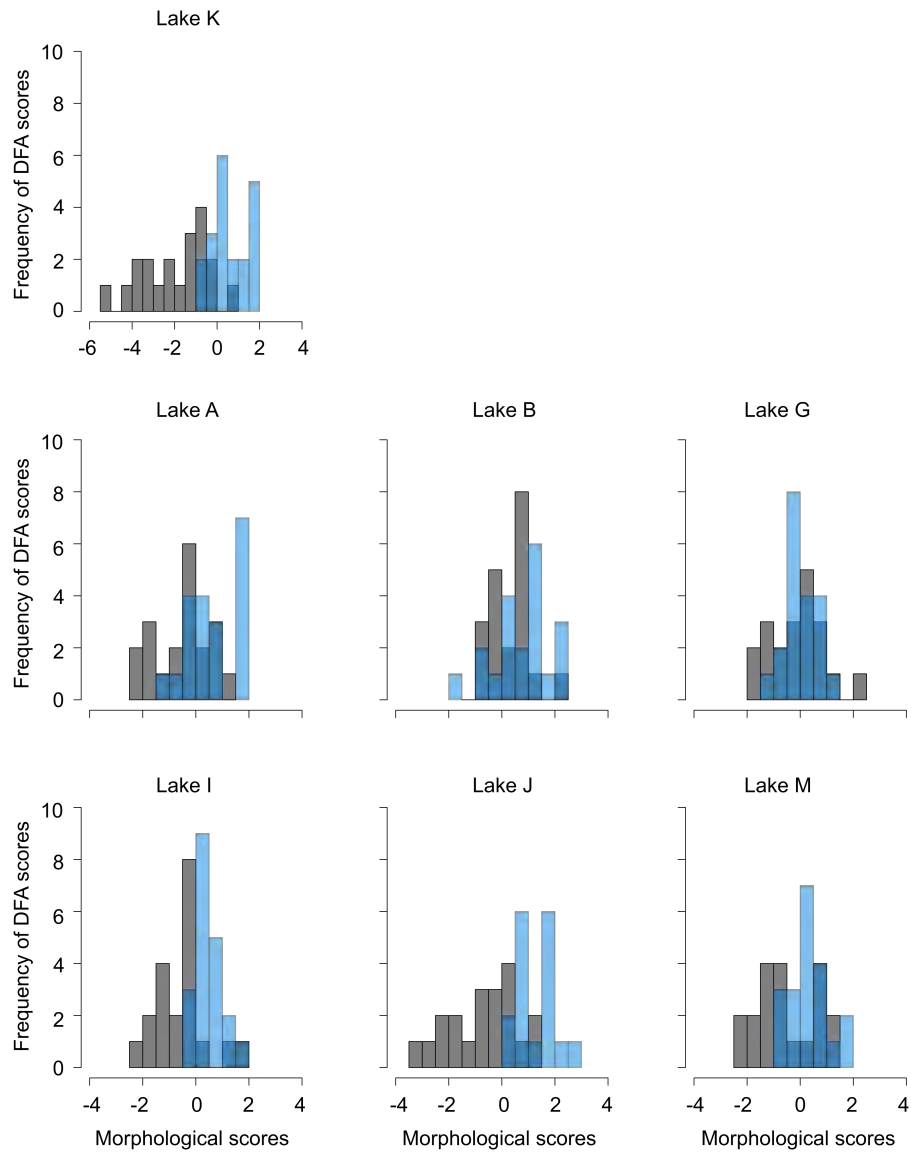
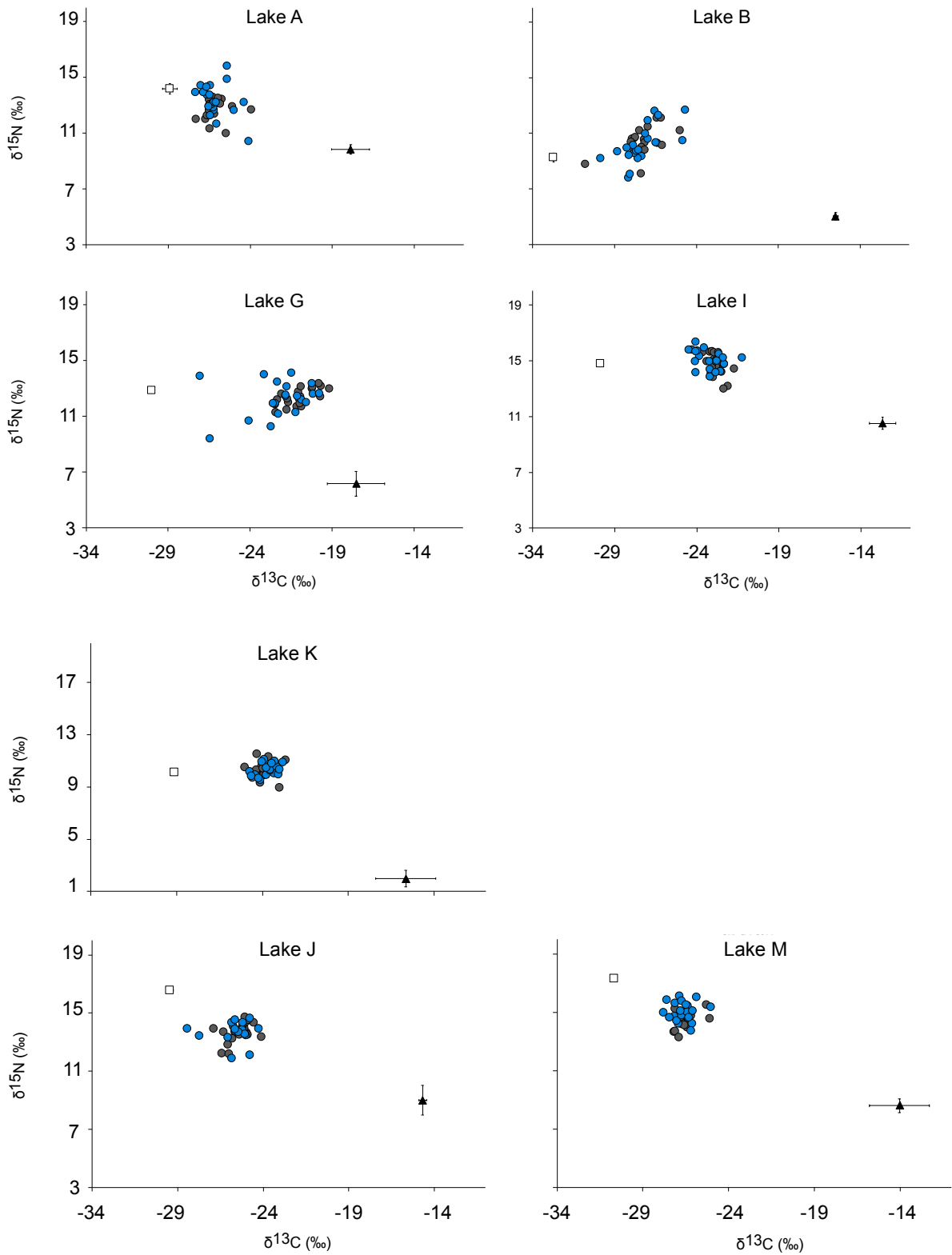


Figure Appendix V.5 Stable isotopes values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$; ‰) of individuals *Procambarus clarkii* (circles) from littoral (dark grey) and pelagic (blue) habitats in the 7 gravel pit lakes. Black triangles and white squares represent periphyton and zooplankton in each lake, respectively. Note that the Y-axis scale for Lake K differs from the others.



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Chapitre VI

Covariations de traits fonctionnels chez deux espèces d'écrevisses invasives coexistantes

Résumé

L'importance de la variabilité intraspécifique dans les processus écologiques et évolutifs est maintenant reconnue. Cependant, améliorer notre connaissance de la structure des variations et des covariations des traits de réponse et d'effet (i.e. *syndromes fonctionnels*) est nécessaire pour bien comprendre les relations intrinsèques entre les différentes fonctions des organismes et leurs effets sur le fonctionnement des écosystèmes. Ceci est particulièrement important dans le contexte des invasions biologiques pour pouvoir mieux appréhender les impacts des espèces invasives sur les écosystèmes natifs. A l'aide d'une expérimentation utilisant une approche multi-traits, nous avons comparé les structures de variations et de covariations de traits de réponse et d'effet de deux espèces d'écrevisse coexistantes, *Procambarus clarkii* et *Faxonius limosus*. Globalement, nous avons mis en évidence une forte variabilité intraspécifique chez les deux espèces ainsi qu'une forte variabilité interspécifique. Nous avons également montré que les *syndromes de réponse* et *d'effet* étaient différents entre les deux espèces, et qu'au sein de chaque espèce ils étaient corrélés pour former des *syndromes fonctionnels* contrastés. *P. clarkii* possédait une plus forte covariation de traits que *F. limosus*. Nos résultats illustrent une forte différence dans les relations existant entre les fonctions des individus des deux espèces, suggérant qu'elles avaient des impacts contrastés sur le fonctionnement de l'écosystème. Nous avons aussi montré que la capacité à prédire les impacts des individus invasifs peut être très variable selon l'espèce considérée.

Contrasting functional trait covariations in two coexisting invasive crayfish species

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In preparation

Abstract

While the importance of intraspecific variability on evolutionary and ecological processes is now widely recognized, knowledge on the structure of response and effect trait variations and covariations (i.e. *functional syndromes*) is needed to understand the intrinsic links between the functions of organisms and their effect on ecosystem functioning. This is particularly important in the context of biological invasions because it can enhance our ability to predict the impacts of invasive species on recipient ecosystems. Using an experimental and multi-trait approach, we compared the structure of response and effect traits variations and covariations of two co-occurring invasive crayfish, *Procambarus clarkii* and *Faxonius limosus*. Overall, we demonstrated the existence of a high level of trait variability among individuals within invasive populations and between species. We also demonstrated that the two species displayed contrasting *response* and *effect syndromes* that were correlated to form different *functional syndromes*. Globally, *P. clarkii* displayed stronger trait covariations. These findings highlighted the contrasting functional relations of coexisting *F. limosus* and *P. clarkii* individuals, indicating that they had sharply distinct impacts on ecosystem functioning. More importantly, our understanding and ability to predict these impacts can be highly variable between species.

Introduction

Biological invasions can induce impacts at multiple levels of biological organization (Cucherousset & Olden, 2011). Invaders interact with native organisms through competition, predation and hybridization (Hasegawa & Maekawa, 2006; Muhlfeld et al., 2017), and affect biotic processes (e.g. trophic cascades; Walsh et al., 2016) and abiotic conditions in recipient ecosystems through ecosystem engineering (e.g. habitat structure; Anderson & Rosemond, 2007). Recent investigations have revealed the ecological role of intraspecific variability that can have major effects on ecosystem functioning (see Des Roches et al., 2018; Raffard et al., 2019b for review). This is particularly important for biological invasions because intraspecific variability (i) may favor species establishment along environmental gradients (Moyle & Marchetti, 2006; González-Suárez et al., 2015) and (ii) can modulate the ecological impacts of invasive species on native organisms and recipient ecosystems (Evangelista et al., 2015; Závorka et al., 2018). Therefore, quantifying variability in invasive species across multiple functional traits is crucial to anticipate local, regional and global ecological consequences of invaders (Mimura et al., 2017).

Functional traits are informative on how individuals respond to environmental fluctuations (response traits), and how they affect their environment (effect traits, Violle et al., 2007; Díaz et al., 2013). For instance, morphological (e.g. body size, defense structures; Garvey & Stein, 1993; Smith & Knapp, 2014) or behavioral (e.g. aggressiveness; Pintor et al., 2008; Polocavia et al., 2011) response traits are major in defining reproduction success, predatory defense or dominance of invasive individuals in recipient ecosystems. Invasive generalist consumers can directly impact food webs at multiple trophic levels and affect community dynamics, in comparison with specialized individuals (Olden et al., 2004; Layman & Allgeier, 2012; Schmitt et al., 2019). They also have important nutrient-mediated impacts (nutrients dynamics and bioavailability ; Evans-White & Lamberti, 2005; Usio et al., 2006). Holistic approaches are appropriate to apprehend the implication of multiple trait covariations on the role of organisms in ecosystems, and particularly, to unravel how response traits can infer ecological processes in return (Raffard et al., 2017; Diaz Pauli et al., 2020). Correlations among and between response and effect traits, which form *syndromes*, are common (e.g. Reale et al., 2010; Dingemanse et al., 2012; Raffard et al., 2017). The most noticeable life-history trade-off relies on covariations of metabolism with life history traits and involves the cost of reproduction against survival (Reale et al., 2010), which are crucial for the establishment of invasive species (Kozák et al., 2007; Burton et al., 2011; Lagos et al., 2017).

Recently, Raffard et al. (2017) demonstrated that response and effect traits do covary in the highly invasive the red swamp crayfish *Procambarus clarkii* to form a *functional syndrome*. However, our understanding of the ecosystem implications of these syndromes is limited (but see Raffard et al., 2017; Diaz Pauli et al., 2020). Quantifying phenotypic syndrome (i.e. multi-trait covariations) in invasive species, would help to fully understand inherent links between traits and functions of invaders, and their invasiveness potential.

Crayfish are one of the most introduced taxa worldwide and their impacts are challenging to predict because they are highly species and context-dependent (Twardochleb et al., 2013; Jackson et al., 2014). On many occasions, invasive crayfish have been reported to co-occur in their introduced area (Chucholl et al., 2008; Larson & Olden, 2013; Anastácio et al., 2015). For instance, *P. clarkii* and the spiny cheek crayfish *Faxonius limosus*, which are among the most widespread invasive crayfish species (Filipová et al., 2011; Oficialdegui et al., 2019) coexist in gravel pit lakes in southwestern France (Alp et al., 2016; Jackson et al., 2017; Lang et al., 2020). These two species share similar features contributing to their invasion success: a high fecundity, a rapid life cycle, and a great dispersal ability, in comparison with native species, and an opportunistic diet (Renai & Gherardi, 2004; Souty-Grosset et al., 2006). Nevertheless, they also differ in some important aspects: *P. clarkii* is generally more aggressive than *F. limosus* and display larger burrowing ability and thermal tolerance, which provides prior access to shelter and trophic resources and therefore, an advantage on direct competition (Holdich & Black, 2007; Capinha et al., 2013; Gherardi et al., 2013). The functional roles of *P. clarkii* and *F. limosus* likely differ (Gherardi, 2007). These co-occurring species, which display a high intraspecific variability, are the perfect candidates to study the structure of trait variations and covariations and related implications on ecosystem functioning (Twardochleb et al., 2013).

In the present study we aimed at quantifying functional trait variation and covariations in co-occurring *P. clarkii* and *F. limosus* by measuring a series of functional traits on individuals in an experimental context. First, we quantified interspecific differences in a suite of response (morphology, chela strength, growth rate, behavior, and metabolism) and effect (excretion, elemental composition, trophic position, consumption rate, and bioturbation) traits. Because intraspecific variability plays a crucial role in community dynamics and can promote species coexistence through niche partitions, we expected significant trait differences between the two species (Shea & Chesson, 2002; Jackson & Britton, 2014). Moreover, we expected *P.*

clarkii to have greater impacts on ecosystem functioning because other studies reported that *F. limosus* can be disadvantaged when co-occurring with other crayfish invaders in terms of access to shelter and trophic resources (Hudina et al., 2011). Second, we quantified the covariations in response and effect traits within each species (i.e. *response and effect syndromes*) and we evaluated how they covaried together to impact the ecosystem functioning (i.e. *functional syndrome*; Raffard et al., 2017; Diaz Pauli et al., 2020). We predicted that coexisting *P. clarkii* and *F. limosus* would display contrasting structure of trait variations and covariations (i.e. different *functional syndromes*; Raffard et al., 2017) due to their intrinsic biological differences (developmental pathways and genetic influence), hence entailing distinct specific impacts on ecosystem functioning (Clark et al., 2010).

Material & methods

Model species

Native from Northern America, the invasive spiny-cheek crayfish *F. limosus*, was deliberately first introduced into Poland in 1890 and spread successfully across Europe (Filipová et al., 2011). As a vector of the crayfish plague (*Aphanomyces astaci*), *F. limosus* highly contributed to decimate indigenous crayfish populations (Filipová et al., 2013). The red-swamp crayfish of *P. clarkii* is a worldwide invasive species originated from north-eastern Mexico and southcentral USA (Gherardi, 2006; Oficialdegui et al., 2019). *P. clarkii* was introduced into Spain in the 1970s for aquaculture purpose and, from there, the species has successfully colonized many countries in Europe. The first recording of *P. clarkii* in the sampling area (i.e. southwestern France) dates back to 1995 (Changeux, 2003). *Procambarus clarkii* and *F. limosus* have been widely reported to induce various negative impacts resulting in a general loss of biodiversity in aquatic ecosystems (Gherardi, 2007; Twardochleb et al., 2013; Souty-Grosset et al., 2016). Both species have an opportunistic omnivorous diet, with a preference for aquatic vegetation, decomposing organic matter and detritus, and therefore, can alter aquatic food webs at multiple levels (Pavlović et al., 2006; Twardochleb et al., 2013; Jackson et al., 2014; Vojtkovská et al., 2014). *P. clarkii* and *F. limosus* are both known as bioturbators (Statzner et al., 2000; Gherardi, 2007). Particularly, *P. clarkii* highly damages river and lake bank and depletes water quality with its burrowing behavior (Correia & Ferreira, 1995; Gherardi, 2007). Both *P. clarkii* and *F. limosus* display high aggressiveness and

competitiveness (Gherardi & Cioni, 2004; Buřič, 2009), as well as a high level of plasticity (Gherardi, 2006), which can explain their invasive potential.

Despite these similarities, the two species display contrasted behavior: numerous studies suggest that *F. limosus* is disadvantaged in terms of shelter competition (Maiwald et al., 2006; Chucholl et al., 2008), and aggressiveness (Chucholl et al., 2008; Hudina & Hock, 2012; Gherardi et al., 2013) compared to *P. clarkii*, and differs in defence posture (claws opened and raised for *P. clarkii* and crossed locked claws in a “spinous ball” for *F. limosus*; Holdich & Black, 2007). Furthermore, *F. limosus* has already been reported as disadvantaged when co-occurring with other crayfish invaders, which suggests that *P. clarkii* would be favored in co-occurring populations, and could have greater implications on ecosystem functioning than *F. limosus* (Hudina et al., 2011).

Experimental design

In June 2018, 64 sympatric *P. clarkii* (carapace length range: 41.86 – 62.97 mm) and 64 *F. limosus* (carapace length range: 28.58 – 46.91 mm) individuals were collected on two occasions (14th and 29th June) from lake Lamartine, southwestern France (43°30'21.5" N, 1°20'32.7" E, max depth = 2.80 m, surface area = 3.85 ha). In this lake, *P. clarkii* and *F. limosus* have synchronized seasonal activity, which strengthens local interspecific competition throughout the year (Figure VI.S1). Individuals were sampled using 36 pairs of baited traps (one cylindrical trap: 62cm x 34cm x 34cm, mesh size: 10 mm; one rectangular trap: 95cm x 20cm x 20cm, mesh size: 4 mm) placed for one night along transect lines, so that the sampling encompassed the whole habitat variability, considering that phenotypic variability can be high across littoral and pelagic habitats in lakes (Lang et al., 2020). In laboratory, crayfish were individually sexed, weighed, measured for carapace length (± 0.01 mm), before being individually maintained in 50 l tanks, filled with 35L of dechlorinated water, and containing a bubble system. A pipe was provided as shelter only after the measurement of the first functional trait (i.e. voracity), to allow a visual inspection of crayfish. All individuals were under the same control photoperiod (9:15) and temperature (mean air temperature = 22.65 °C \pm 0.21 SE, measured daily). The experiment was divided in two rounds with each round lasting 13 days. The exact same equipment and protocols were used in each round. Prior to trait measurements, individuals were acclimatized for 72h in the laboratory conditions. No trait measurement was done on one *P. clarkii* individual and one *F. limosus* individual which passed out during the first round passed, so measurements were performed on 63 individuals

for each species. In fine, data were complete for 58 and 61 individuals for round 1 and round 2, respectively, due to mortality and/or technical issues during the experiment. More specifically, 33 *P. clarkii* (18 females and 15 males) and 25 *F. limosus* individuals (14 females and 11 males) were analysed during round 1, and 27 *P. clarkii* (14 females and 13 males) and 34 *F. limosus* individuals (14 females and 20 males) were used for the analysis. Sex ratio did not differ between species and round in our experimental design ($\chi^2 = 0.032$, $df = 1$, $p\text{-value} = 0.86$).

Functional response trait measurements

Morphology

Body morphology was assessed using a combination of linear traits measured to the nearest 0.01 mm with a digital caliper (body height/ carapace length, chela height/body height; Larson et al., 2012) and geometric morphometrics (Zelditch et al., 2012). To avoid any bias in morphological analyses, individuals were measured and pictured before any other sampling just after they have been euthanized. Nineteen landmarks were placed on crayfish bodies including cephalothorax and abdomen, and body shape variations were analysed using TpsDig2 v.2.17 (Rohlf, 2015) and Morpho J v.1.06d (Klingenberg, 2011), after the removal of allometry effect on body shape variation, using a regression of the Procrustes coordinates against log₁₀-transformed centroid sizes, following Evangelista et al. (2019) and Lang et al. (2020). A principal component analysis (PCA) implemented in Morpho J was performed on the regression residuals. The first axis explained 68.5% of the total variance and corresponded to the variability between species (Figure VI.S2). The scores on the second axis (PC2 morphoscores, which explained 10,2% of the total variance) corresponded to within species variability and were used in subsequent analyses to characterize each individual regardless of its species (i.e. without maximizing the effect of the species in further analyses). Increasing values on the second axis were associated with shorter rostrum and larger cephalothorax (Appendix 2).

To synthetize body morphology, a PCA was performed on “PC2 morphoscores”, “body height/ carapace length ratio” and “chela height/body height ratio”. Body morphology score was computed as the score on the first PCA axis, which explained 62.2% of the variance. Increasing values on the first axis were associated with increasing “PC2 morphoscores” (i.e. shorter and wider cephalothorax and rostrum) and increasing “chela height/body height” (i.e.

larger chelae), and decreasing “body height/ carapace length” (i.e. more elongated individuals; Figure VI.S3).

Chela strength was measured as pinching force of crayfish prior to placing individuals in their own tank, on one occasion in order to reduce the stress of individuals before subsequent measurements throughout the experiment. Individuals were induced to pinch a sensor (Magtrol SA, Switzerland), by holding them by the cephalothorax and inserting the device in their right chela following Malavé et al. (2018) and Závorka et al. (2020). Each individual was given sufficient time to produce maximum pinching force and this was recorded to nearest 0.001 N. The maximum pinching force was then corrected by the chela weight (g).

Growth rate

We estimated the specific growth rates (SGR, % d⁻¹) as:

$$SGR = \frac{\ln(X_f) - \ln(X_i)}{T} \times 100$$

where X_f and X_i were the final and initial body mass, and the initial and final carapace length, respectively, and T the time between the two measurements (13 days). Growth rate was computed as the score on the first PCA axis based on body weight growth rate and carapace length growth rate, which explained 84.4% of the variance. Increasing values on the first axis were associated with increasing growth rates (Figure VI.S3).

Behavior

Activity level was assessed using rectangular opaque containers (67,5 cm x 39 cm) filled with dechlorinated tap water. Individuals were put individually in stall buckets for 10 minutes and then acclimated for 10 minutes in the tanks, before being filmed for 10 minutes. The video was then analysed using LoliTrack software v.4 (Loligo@Systems) to get the active percentage (i.e. time proportion during which the individual is moving) of each individual (Raffard et al., 2017). Anxiety-like behavior was quantified by analysing video by eye during 10 extra minutes, as the proportion of time spent in dark zones created by the addition of a completely opaque lid on the half surface of the tank, at the end of activity measurement (Fossat et al., 2014). Behavioral was estimated as the “behavioral score” on the first PCA axis synthesizing activity and anxiety-like behavior, which explained 59.7% of the variance.

Increasing values on the first axis were associated with increasing activity and anxiety-like behavior levels (Figure VI.S3).

Metabolism

Individuals were inserted in airtight container connected to a closed circuit with circulating water and a measurement cell with a dioxygen sensor (optical probe WTW FDO®925 IDS and respirometer Multi 3620 IDS SET G WTW). The whole system was completely immersed in 50 L black and opaque tanks filled with dechlorinated water. Following 5 minutes of acclimation, dissolved dioxygen content was recorded every 30 seconds, during 20 minutes. Metabolism of individuals was estimated using their dioxygen consumption rate as a proxy (Brown et al., 2004), which was calculated as the slope opposite of the linear regression of the dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$) divided by crayfish body mass to get the metabolism per gram of crayfish.

Functional effect trait measurements

Excretion

Two hours prior to excretion measurement, crayfish were fed *ad libitum*. Nitrogen and phosphorus excretion were quantified by placing crayfish individually in plastic bags containing 500mL of mineral water, and measuring dissolved ammonium NH_4^+ and dissolved phosphate PO_4^{3-} after 2 hours, once individuals were removed and taken back to their own tank. We filtered 100mL of water through a glass microfiber filter (Whatman, GF/C, diameter 1/4 25 mm) and samples were frozen at -20°C . Excretion rates (NH_4^+ , PO_4^{3-} , $\text{mg l}^{-1} \text{h}^{-1}$) were assessed using a high-performance ionic chromatograph (Dionex DX-120), following Raffard et al. (2017) and were corrected for crayfish body mass (g). We performed a PCA on NH_4^+ and PO_4^{3-} excretion to synthesize nutrient excretion, using the score on the first axis which explained 81.2% of the variance. Increasing values on the first axis were associated with increasing excretion rates (Figure VI.S3).

Stoichiometry

At the end of the experiment, after other trait measurements, crayfish were frozen and stocked at the laboratory at -20°C to be subsequently lyophilized (Christ Alpha 1-4 LDPLUS). Individuals were then ground to powder with two successive grinders (Waring WSG30E, Retsch MM200) and Carbon and Nitrogen contents were measured using an elementary

analyser (Flash 2000 Thermofisher). Samples were mineralized by sodium persulfate oxidation at 121°C for 2 hours, and mineral phosphorus contents were obtained using spectrophotometric analyses, by reacting with ammonium molybdate (AFNOR 1990). C:N, C:P and N:P molar ratios were then computed using the molar masses of the three elements. For elemental composition, we used the score on the first PCA axis as “stoichiometric score”, synthesizing C:N:P molar ratios (explained variance = 79.3%). Increasing values on the first axis were associated with increasing C:N ratio and decreasing C:P and N:P ratios (Figure VI.S3).

Trophic position

Trophic position of individuals was estimated using stable isotope analyses. To this end, a sample of abdominal muscle was collected on each individual after they have been euthanized and photographed. The tissue sample was subsequently rinsed with distilled water, oven dried at 60°C for 48h, ground to a fine powder, and analysed for nitrogen ($\delta^{15}\text{N}$) stable isotopes at the Cornell Isotope Laboratory (COIL, New York).

Consumption rate

Voracity was quantified by introducing 30 freshly defrosted red maggots in individual tanks. We assessed the number of remaining pinkies after 15 minutes. Voracity was estimated as the number of red maggots consumed by gram of crayfish per minute.

Then, we quantified leaf consumption rate by introducing 4.0g of *Populus nigra* leaves in their home tank. In order to allow bacterial colonization and to improve appetite and digestibility of litter by crayfish, leaves were submerged in thin mesh bags (0.5mm; to prevent decomposition by invertebrates) for 10 days in water collected in the Canal du Midi (43° 33' 36.194'' N, 1° 28' 31.967'' E). Leaves were left for 72h in individuals' tanks, following Raffard et al. (2017). After the 72h, the remaining leaf litter was removed, rinsed carefully on a sieve (250µm mesh-size) and then oven-dried for 72h at 70°C, before being weighed twice at the nearest 0.01mg. The weight of remaining leaf litter was computed at the average of these two values. We estimated the leaf consumption rate as the weight of leaves consumed per day per gram of crayfish. The “consumption rate” was computed as the score on the first PCA summarizing voracity and litter consumption rate (explained variance = 66.1%). Increasing values on the first axis were associated with increasing litter consumption and increasing voracity (Figure VI.S3).

Bioturbation

Bioturbation was measured as a consequence of burrowing by placing crayfish individually in buckets containing 3l of dechlorinated water and a roll of natural white clay (diameter 3.4 cm; length 3.9 cm; weight = 179 g) for 30 minutes. We used a portable turbidimeter (EUTECH TN-100 IR) to measure the turbidity (± 0.01 NTU) at $t_{\text{initial}} = 0$, and $t_{\text{final}} = 30$ minutes. The bioturbation was calculated as the difference between final and initial turbidity and was divided by crayfish body mass to get the bioturbation per gram of crayfish.

Statistical analyses

Quantification of intraspecific variability and species comparison

In the subsequent analyses we used 5 response traits (body morphology, chela strength, growth rate, behavior, and metabolism) and 5 effect traits (excretion, elemental composition, trophic position, consumption rate and bioturbation) to characterize *P. clarkii* (n = 60) and *F. limosus* (n = 59).

To test if each response and effect trait differed between species and sexes, linear mixed models (LMMs) were run on each response and effect traits, using “sex” and “species” as fixed effects and “round” as random factor. Interactions were removed when non-significant. Type II “Anova” implemented in the “car” package (v.3.0.5; Fox & Weisberg (2019)) was used to test the significance of each factor.

It is interesting to note that body morphology, chela strength and elemental composition differed significantly between sexes (Table VI.1; Figure VI.1). However, as the type of sexual dimorphism was similar in both species, the sex factor was not included in the following analyses.

Response and effect syndromes

For each species, correlations among response traits and among effect traits, respectively, were assessed using Spearman ranks correlations. Prior to further analyses, missing values were replaced using multivariate imputation with chain equations, using the R-package mice (Azur et al., 2011; van Buuren & Groothuis-Oudshoorn, 2011). Then, we assessed whether response and effect syndrome (i.e. covariance matrix of response and effect traits, respectively) differed between *P. clarkii* (n = 63) and *F. limosus* (n = 63) using structural

equation modelling (SEM) and run models with covariations between all pairs of traits. First, we fitted a null model where all covariances were constrained to be equal between the two species (i.e. no differences of trait covariation between species). Second, we fitted an alternative model in which all covariances were unconstrained between the two species (i.e. trait covariations differed between species). Finally, the models were compared using AIC and likelihood ratio test to assess whether the covariance matrices were globally different (i.e. alternative model with the lowest AIC) or not (i.e. null model with the lowest AIC) between *P. clarkii* and *F. limosus* (Raffard et al., 2020). This procedure was performed for response and effects traits separately. SEM were run using the R-package lavaan (Rosseel, 2012).

Functional syndromes

To assess the potential consequences of response traits on ecosystem functioning in the two species, the link between response and effect traits was tested using partial least-squares path modelling (Sanchez, 2013). Traits were first scaled to the mean within each species to facilitate comparison. We constructed models with response and effect traits summarized in latent variables representing response and effect syndromes, and a directional path was fitted between the response syndrome and the effect syndrome (Raffard et al., 2017). The significance of the path from response syndrome to effect syndrome, and the contribution (i.e. loadings) of each trait in the syndrome (i.e. latent variables) were estimated using 95% confidence intervals (CIs) based on 1000 bootstraps. We performed one model for each species, and loadings on latent variables and the link from response syndrome to effect syndrome were compared using 95% CIs. PLS-PM was run using the R-package plspm (Sanchez, 2013).

Results

Interspecific differences

Response traits

Overall, we found that body morphology, behavior and metabolism differed significantly between these two species (LMM, $F_{1,115.82} = 116.18$, $P < 0.001$; $F_{1,115.31} = 9.64$, $P = 0.002$; $F_{1,115.76} = 66.23$, $P = 0.008$, respectively; Table VI.1). Specifically, *F. limosus* had higher body morphology scores than *P. clarkii* individuals (mean = 0.94 ± 0.13 SE and mean = -0.93 ± 0.13 SE for *F. limosus* and *P. clarkii*, respectively),

indicating that they had overall shorter rostrum and wider cephalothorax than *P. clarkii* (Figure VI.1; Figure VI.S3a). Compared to *P. clarkii*, they also display higher “chela height/body height” ratio (mean = 0.40 ± 0.007 SE and mean = 0.36 ± 0.010 SE for *F. limosus* and *P. clarkii*, respectively; Appendix 3a) and smaller “body height/carapace length” ratio (mean = 0.39 ± 0.004 SE and mean = 0.44 ± 0.004 SE for *F. limosus* and *P. clarkii*, respectively; Figure VI.S3a), indicating that at similar body size, *F. limosus* individuals had bigger chelae and more elongated body morphology than *P. clarkii* (which is the deformation also observable along morphometric PC1 axis; Figure VI.S2). The behavioral score was higher in *F. limosus* than in *P. clarkii* (Figure VI.1; Figure VI.S3a), indicating that *F. limosus* individuals were more active than *P. clarkii* individuals (mean proportion of active time = $60.90 \% \pm 2.24$ SE and mean = $46.93 \% \pm 2.05$ SE for *F. limosus* and *P. clarkii*, respectively) and tended to have a higher anxiety-like behavior (mean proportion of time in the shadow = $62.85 \% \pm 2.74$ SE, and mean = $59.43 \% \pm 3.32$ SE for *F. limosus* and *P. clarkii*, respectively). Metabolic rate also differed between the two species: *F. limosus* individuals consumed more dioxygen than *P. clarkii* individuals (mean \pm SE = 3.11 ± 0.16 g(O₂). L⁻¹.h⁻¹.g⁻¹ and mean \pm SE = 1.73 ± 0.08 mg(O₂). L⁻¹.h⁻¹.g⁻¹ for *F. limosus* and *P. clarkii*, respectively; Figure VI.1). Finally, chela strength and growth rate did not differ significantly between the two species (Table VI.1; Figure VI.1).

Table VI.1 Results of the linear mixed models testing the effect of species and sex on response (body morphology, chela strength, growth rate, behavior, and metabolism) and effect trait (excretion, elemental composition, trophic position, consumption rate and bioturbation) traits. Significant P values are in bold. σ^2_{Round} corresponds to the variance between rounds of the experiment (intercept), and $\sigma^2_{\text{Residual}}$ corresponds to the variance within rounds (residual).

Response variable	Explanatory variable	F	Df	Df.res	Pr(>F)		σ^2_{Round}	$\sigma^2_{\text{Residual}}$
Body Morphology	species	116.182	1	115.82	<2.2e-16	***	0.003	0.797
	sex	30.835	1	115.92	1.81e-07	***		
Chela strength	species	2.2455	1	115.71	0.13673		0.324	6.988
	sex	6.7404	1	115.41	0.01065	*		
Growth rate	species	2.7631	1	115.94	0.09916	.	0.037	1.684
	sex	0.0227	1	115.61	0.88044			
Behavior	species	9.6417	1	115.31	0.002394	**	0.159	1.026
	sex	0.4465	1	115.16	0.505320			
Metabolism	species	66.2964	1	115.76	5.016e-13	***	0.034	0.845
	sex	3.5324	1	115.44	0.0627	.		
Elemental composition	species	95.7099	1	115.45	<2.00E-16	***	0.000	1.244
	sex	6.5835	1	115.98	0.01157	*		
Excretion	species	82.5427	1	115.74	3.346e-15	***	0.042	0.959
	sex	0.4355	1	115.43	0.5106			
Trophic position	species	23.4342	1	115.45	4.042e-06	***	0.000	0.156
	sex	0.4214	1	115.98	0.5175			
Consumption rate	species	0.2369	1	115.45	0.6274		0	1.346
	sex	0.7521	1	115.98	0.3876			
Bioturbation	species	1.4102	1	116.00	0.2374		0.047	3.633
	sex	0.3580	1	115.74	0.5508			

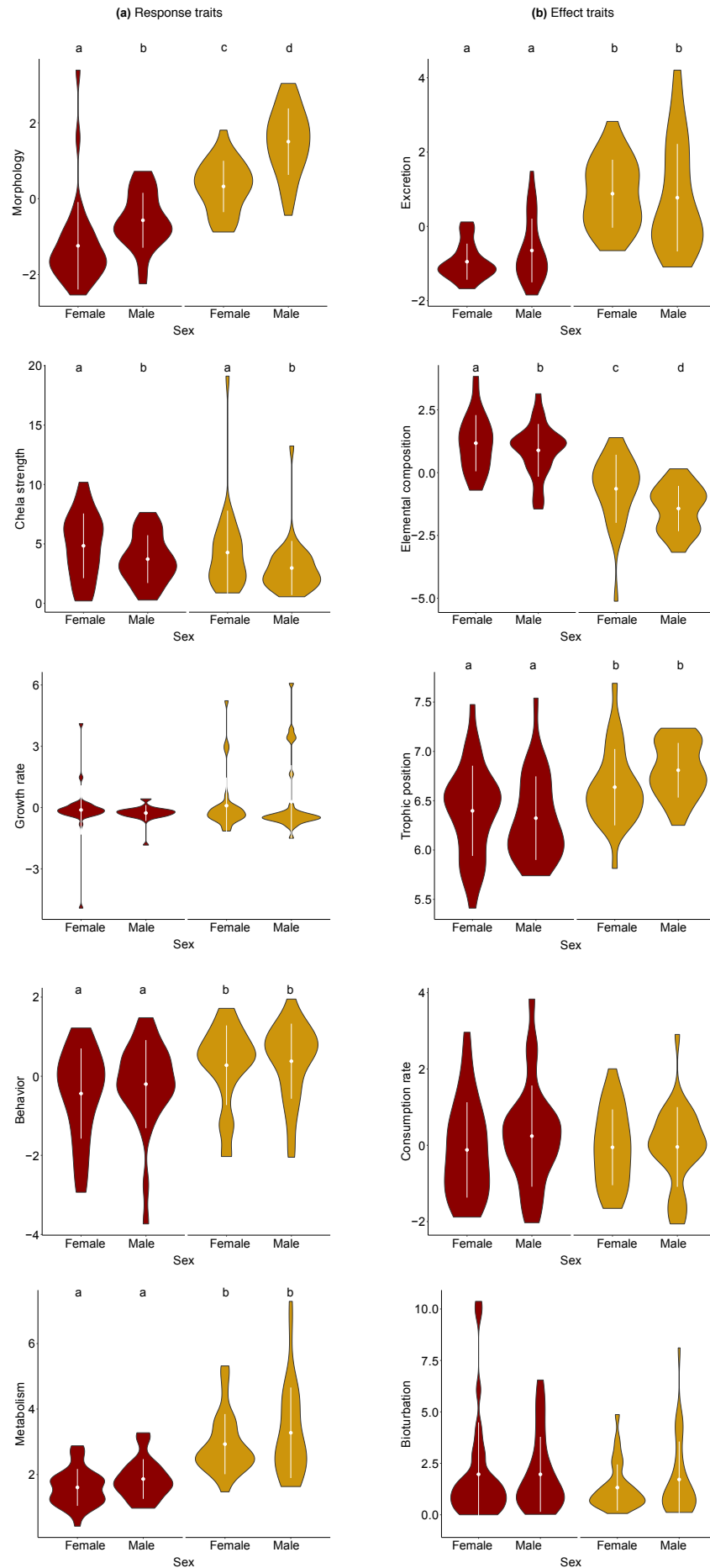


Figure VI.1 Comparison of intraspecific variability in **(a)** response and **(b)** effect traits (observed values) for *Procambarus clarkii* (red) females (n = 32) and males (n = 28) and *Faxonius limosus* (dark yellow) females (n = 28) and males (n = 31). White point and vertical lines represent trait mean and standard deviation, respectively.

Effect traits

Elemental composition, excretion and trophic position differed significantly between the two species (LMM, $F_{1,115.45} = 95.71$, $P < 0.001$; $F_{1,115.74} = 82.54$, $P < 0.001$; $F_{1,115.76} = 23.43$, $P < 0.001$, respectively; Table 1). Specifically, *P. clarkii* contained a higher C:N ratio, and lower N:P and C:P ratios than *F. limosus* (Figure VI.1; Figure VI.S3b). Additionally, NH_4^+ and PO_4^{3-} excretion rates were higher for *F. limosus* than for *P. clarkii* (Figure VI.1; Figure VI.S3b). *F. limosus* displayed higher trophic position than *P. clarkii* (mean = 6.73 ± 0.05 SE and mean = 6.36 ± 0.06 SE for *F. limosus* and *P. clarkii*, respectively; Figure VI.1). Finally, the consumption rate and bioturbation did not differ between the two species (Table VI.1; Figure VI.1).

Response and effect syndromes

Regarding response syndromes, behavior was significantly and positively correlated with metabolism in *P. clarkii* ($\rho = 0.43$, $P < 0.001$; Table VI.2) and negatively correlated with growth rate in *F. limosus* ($\rho = -0.35$, $P = 0.007$; Table VI.2). All other correlations among response traits were non-significant (Table VI.2). Regarding effect syndromes, elemental composition was significantly and negatively correlated with excretion and consumption rates, and bioturbation in *P. clarkii* ($\rho = -0.38$, $P = 0.003$; $\rho = -0.58$, $P < 0.001$; $\rho = -0.51$, $P < 0.001$; Table VI.3). Excretion and consumption rates, and bioturbation were all positively correlated among each other ($\rho_{\text{excretion} - \text{consumption}} = -0.54$, $P < 0.001$; $\rho_{\text{excretion} - \text{bioturbation}} = 0.33$, $P = 0.010$; $\rho_{\text{consumption} - \text{bioturbation}} = 0.44$, $P < 0.001$; Table VI.3). Trophic position was also significantly and positively correlated with excretion rate in *P. clarkii* ($\rho = 0.26$, $P = 0.046$), but was not correlated with any other traits (Table VI.3). In *F. limosus*, there was a unique significant and negative correlation between trophic position and body stoichiometry ($\rho = -0.34$, $P = 0.008$; Table VI.3). Overall, *F. limosus* and *P. clarkii* displayed contrasted patterns of covariations in both response and effect traits. Specifically, the comparisons of the null and the unconstrained models showed that covariations among response and effect traits significantly differed between species ($\chi^2 = 45.73$, $P < 0.001$ and $\chi^2 = 27.45$, $P = 0.002$ for response and effect trait covariations, respectively; Table VI.S1).

Table VI.2 Spearman correlations among response traits (body morphology, chela strength, growth rate, behavior, and metabolism) for *Procambarus clarkii* (n = 60) and *Faxonius limosus* (n = 59). Significant correlations are displayed in bold.

Species		Morphology	Chela strength	Growth rate	Behavior
<i>P. clarkii</i>	Chela strength	-0.066			
	Growth rate	-0.177	-0.079		
	Behavior	-0.178	-0.169	-0.024	
	Metabolism	-0.236	-0.0499	-0.152	0.432
<i>F. limosus</i>	Chela strength	-0.182			
	Growth rate	-0.008	-0.251		
	Behavior	0.0177	-0.019	-0.348	
	Metabolism	0.0314	-0.116	0.233	0.088

Table VI.3 Spearman correlations among effect traits (excretion, elemental composition, trophic position, consumption rate, and bioturbation) for *Procambarus clarkii* (n = 60) and *Faxonius limosus* (n = 59). Significant correlations are displayed in bold.

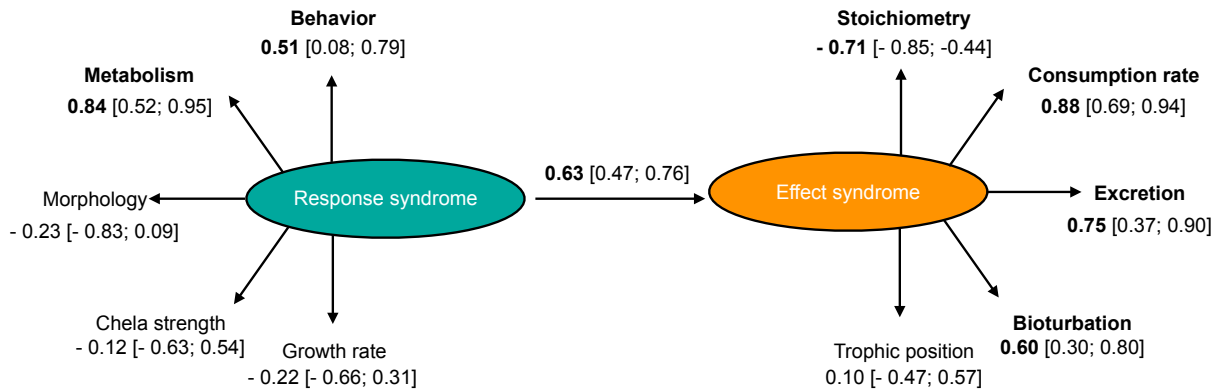
Species		Elemental composition	Excretion	Trophic position	Consumption rate
<i>P. clarkii</i>	Excretion	-0.378			
	Trophic position	-0.042	0.259		
	Consumption rate	-0.583	0.542	-0.023	
	Bioturbation	-0.514	0.331	0.059	0.438
<i>F. limosus</i>	Excretion	0.024			
	Trophic position	-0.340	0.022		
	Consumption rate	-0.131	0.051	0.057	
	Bioturbation	-0.032	0.207	-0.193	-0.144

Functional syndromes

Regarding *P. clarkii*, the latent variable related with the response syndrome was positively and significantly associated with behavior (activity and anxiety-like behavior) and metabolism. The latent variable related with the effect syndrome was negatively associated with stoichiometry, and positively associated with consumption and excretion rates and bioturbation (Figure VI.2). The response syndrome was positively linked to the effect syndrome (path coefficient = 0.63, $CI_{95\%} = [0.47; 0.76]$, $R^2 = 0.38$; Figure VI.2a). *F. limosus* displayed a different functional syndrome. The latent variable corresponding to the response syndrome was positively associated with growth rate and metabolism, while the latent variable corresponding to the

effect syndrome included bioturbation only (Figure VI.2). The response syndrome was positively associated with the effect syndrome (path coefficient = 0.68, $CI_{95\%} = [0.52; 0.81]$, $R^2 = 0.35$; Figure VI.2b).

(a) *Procambarus clarkii*



(b) *Faxonius limosus*

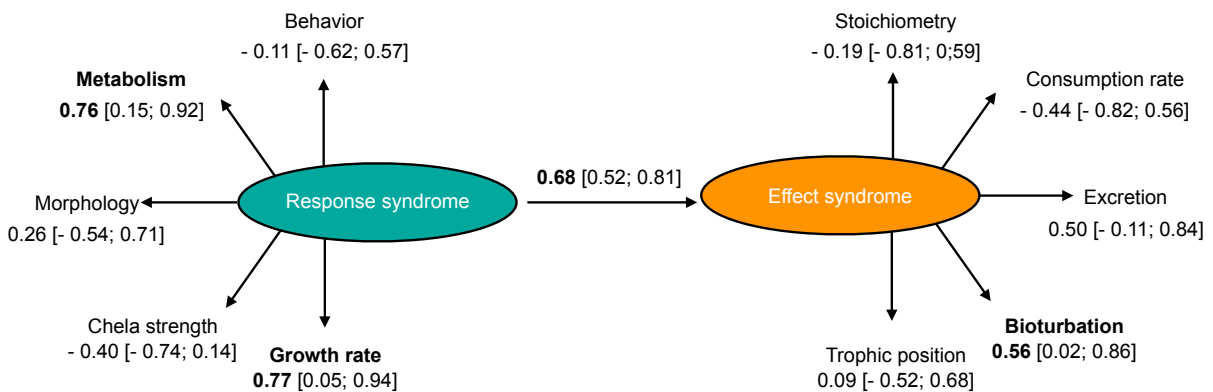


Figure VI.2 Representation of the functional syndrome for (a) *Procambarus clarkii* (goodness-of-fit of overall model = 0.38) and (b) *Faxonius limosus* (goodness-of-fit of overall model = 0.35), based on the PLS-PM model assessing the relationship between response and effect syndromes. The path coefficient is displayed on the arrow linking response and effect syndromes. Each trait contributes to response and effect syndromes according to the loading displayed on arrows. $CI_{95\%}$ based on 1000 bootstraps are displayed in brackets. Significant path coefficients and loadings are reported in bold.

Discussion

To our knowledge, this is the first study focusing on *functional syndromes* of co-existing invasive species. Our study demonstrated a high difference in functional traits, trait covariations, and *functional syndromes* between two co-occurring invasive crayfish species. First, we highlighted a high level of trait variability among individuals between *P. clarkii* and *F. limosus*. We also demonstrated a significant difference of response and effect trait covariations between crayfish species. Finally, we demonstrated that this heterogeneous structure of trait covariations resulted in different *functional syndromes*. This high level of intraspecific variability, which often characterizes invasive species, is crucial to respond to changing environmental conditions (Messenger & Olden, 2019). Our results suggest that co-occurring invasive crayfish species could develop differentiated strategies that entail contrasting impacts on ecosystem functioning.

Response traits and covariations

F. limosus and *P. clarkii* had contrasting response and effect traits and syndromes (i.e. heterogeneous intensities and direction of covariations), suggesting that they do not respond equally to the same environmental conditions. The coexistence of these two ecologically close crayfish species might be favored by their differentiation due to their respective high intraspecific variations (leading to niche partitions; Courbaud et al., 2012; Wong et al., 2019). Globally, *F. limosus* individuals had a stockier cephalothorax, larger chelae and more elongated abdomen than *P. clarkii*. Despite a significant difference in chelae morphology, the species did not differ in chelae strength, suggesting that *F. limosus* and *P. clarkii* had the same advantage in foraging, defense against predators, and reproductive success (Hudina et al., 2012). *F. limosus* also displayed a higher level of locomotor activity than *P. clarkii* and a higher metabolism, but there was no pronounced difference in anxiety-like behaviour between species which could suggest a mismatch during competitive encounters between the two species with an advantage for *P. clarkii*. Surprisingly, anxiety-like behaviour and activity were positively correlated suggesting that activity had reflected a stress level of crayfish in our study. This goes against the POLS concept (Reale et al., 2010) and previous studies on crayfish behavior that have highlighted a positive correlation between boldness (i.e. opposite to anxiety-like behavior), activity and aggressiveness (Pintor et al., 2008, 2009; Linzmaier & Jeschke, 2020). For both species, response traits were poorly correlated, and *response syndrome* involved the behavior of individuals. Concerning *P. clarkii*, the more active and

anxious individuals had the higher metabolism and in *F. limosus*, the more active and anxious individuals had slower growth rate, indicating that *P. clarkii* seemed to have a faster pace of life than its sympatric heterospecific congeners (Reale et al., 2010). It has been demonstrated that behavior can be associated with dispersal rate in invasive species (Fraser et al., 2001; Duckworth & Badyaev, 2007). Here, our results illustrated the importance of behavior in response trait covariations and in *functional syndromes* of invasive species which could lead to contrasting impacts of invaders on native prey communities (Pintor et al., 2009). Individuals, through different behavior, face various selection pressures that lead to a high variability in morphological or physiological traits (e.g. metabolism) which can result in differential impacts on ecosystem functioning (Duckworth, 2009; Rota et al., 2018).

Effect traits and covariations

Concerning effect traits, the consumption rate and the bioturbation due to the burrowing activity of *F. limosus* and *P. clarkii* did not differ, indicating that these traits cannot be the factors of differentiated nutrients bioavailability or recycling between these species in the ecosystem. However, *F. limosus* displayed a higher trophic position than *P. clarkii* while both crayfish species are omnivorous with a preference for detritus and vegetation. Hence, our results indicated that the diet of *P. clarkii* involved a higher proportion of plants and detritus, and suggested that *F. limosus* is disadvantaged in food acquisition in the study system and consumes more preys of higher trophic levels (e.g. macroinvertebrates, fish eggs or larvae). *F. limosus* also excreted more NH_4^+ and PO_4^{3-} than *P. clarkii*, and displayed lower C:N and higher N:P and C:P ratios) than *P. clarkii*. Phosphorous is essential for individual growth and has already been shown to be the most important source of stoichiometric variations for crayfish, while the two major molecules (proteins and nucleic acids) vary little in terms of C and N contents (Sterner & Elser, 2002; Færøvig & Hessen, 2003). Higher N:P ratios in *F. limosus* suggested that the allocation in chitin in spiny-cheek crayfish is more important, providing a stronger carapace as defence against predators compared to *P. clarkii* (Elser et al., 1996). Lower C:P and N:P ratios in *P. clarkii* (i.e. higher %P) could be the result of an increased allocation in growth rate, or greater storage of P in the organism's tissues, suggesting that *P. clarkii* had a rapid growth rate (i.e. predation defence through gape limitation) or would have a better tolerance to P limitation, compared to *F. limosus*. Hence, we suggest that differences of elemental composition are key to contrasting effects of *P. clarkii* and *F. limosus* on microbial respiration rates, producers dynamics and nutrients cycling in the environment due to different intake and contrasting excretion rates (Yamamichi et al.,

2015; Vanni & McIntyre, 2016; Welty et al., 2017), and dissimilar bottom-up consequences in the food web (Malzahn et al., 2007).

P. clarkii individuals with higher N:P and C:P ratios and lower C:N ratios had lower levels of NH_4^+ and PO_4^{3-} excretion, and lower consumption rate and bioturbation. This suggested that *P. clarkii* can better retain N and P in their tissues compared to *F. limosus* (i.e. better resistance to P limitations), and that they are globally more efficient in nutrients acquisition (i.e. less bioturbation maybe due to reduced foraging activity, as individuals with higher consumption rates make higher level of bioturbation). As expected, individuals with greater consumption rates had greater excretion rates. For both species, the *effect syndrome* involved trait correlations with the stoichiometry, suggesting that effects of individuals directly depend on their physiological characteristics (here, stoichiometric traits), and supporting the idea that stoichiometric traits should be considered as functional traits (e.g. C:N:P ratios and proportions, Leal et al., 2017b), to fully appreciate their intrinsic role in consumer-driven nutrient recycling and eco-evolutionary dynamics (Matthews et al., 2011; Hawlena et al., 2012; Welty et al., 2017). Effect traits were more strongly correlated in *P. clarkii*, suggesting that this invasive species might have more predictable impacts on ecosystem functioning compared to *F. limosus*.

Functional syndromes

In *P. clarkii* the *response syndrome* correlated with anxiety-like behavior and activity, while in *F. limosus* it was positively associated with growth rate. In both species, the metabolism was correlated with the *response syndrome*, which confirms that variations in metabolism could drive covariations between response and effect traits, as previously suggested (Biro & Stamps, 2010; Reale et al., 2010; Raffard et al., 2017). Our results are in line with the metabolic theory of ecology which states that the metabolism of organisms drive ecological processes through different levels of biological organization (Brown et al., 2004). Metabolism is a key trait that results from a fitness trade-off of individuals and constrains their contribution to biogeochemical processes through trophic fluxes, respiration and nutrients excretion (Vanni & McIntyre, 2016).

The *effect syndrome* in *P. clarkii* was based on a suite of effect traits (stoichiometry, excretion and consumption rates and bioturbation), while it was only associated with bioturbation in *F. limosus*. The *functional syndromes* of these two invasive crayfish species highlight their ability

to physically modify their environment, as biological engineers. Their burrowing activity is functionally important because it impacts the physical structure of habitat (e.g. water turbidity, reduced benthic vegetation), and the bioavailability of nutrients in the ecosystem (Statzner et al., 2000; Twardochleb et al., 2013). The numerous trait covariations in *P. clarkii*' *functional syndrome* suggested that the variation of response traits in this species would result in high intensity effects and/or a high range of effects on ecosystem functioning, compared to *F. limosus*.

Our study demonstrated that, in each species, the *response syndrome* was associated with the *effect syndrome*, resulting in a *functional syndrome*. This indicated that variations in response traits and response trait covariations in each species could result in differentiated impacts on ecosystem functioning. Raffard et al. (2019, 2020) demonstrated that covariations among functional traits are population-specific. Our study provides the evidence that ecologically close species that co-occur can display contrasting functional syndromes. This can be facilitated by the necessity for *P. clarkii* and *F. limosus* to use different life-history strategies to reduce the overlap of ecological niches, therefore, allowing their coexistence (Wong et al., 2019). Závorka et al. (2017) demonstrated that coexistence of an invader and a native species can lead to the breakdown of trait covariations in the native species which is outcompeted, reducing its fitness. As we predicted based on previous studies, *P. clarkii* seems to be more competitive than *F. limosus* which displayed a weaker *functional syndrome*. Indeed, this suggested that *P. clarkii* individuals had a greater adaptive capacity by changing their phenotype in a consistent way thanks to trait intercorrelations. It could be interesting to compare the *functional syndromes* of coexisting populations and allopatric populations (close to our study site (Lang et al. *in prep*)) within both invasive species to assess if trait covariations are stronger when they are allopatric and if coexistence does affect their traits (through competition, predation pressure or indirectly; Závorka et al., 2017).

Conclusion

To conclude, we demonstrated that co-occurring invasive crayfish species displayed contrasting *functional syndrome*. Our study supports that stoichiometric traits and metabolism play a pivotal role in *the functional syndrome*. In the context of biological invasions, quantifying trait covariations can help to fully apprehend invasive individuals' intrinsic functional strategies. Plus, it offers a better comprehension and prevision of invasive

species impacts by linking response trait measurements to ecosystem processes without measuring effect traits, which can be challenging in natural populations. This could also help predicting the potential shifts of trait covariations due to invasive species management, and the resulting effects on ecosystem functioning, that could be unexpectedly counterproductive otherwise. However, our study highlighted that these predictions can be intricate depending on the considered species in a context of multi-invasions.

Supplementary information

Table VI.S1 (a) Covariance matrix of response traits for *Procambarus clarkii* and *Faxonius limosus* and **(b)** covariance matrix of effect traits for *Procambarus clarkii* and *Faxonius limosus*. Significant covariations are displayed in bold.**(a)**

Species	Covariances		Estimate	Std.Err	z-value	P(> z)
<i>P. clarkii</i>	Morphology	Chela strength	-0,023	0,082	-0,278	0,781
	Morphology	Growth rate	-0,035	0,073	-0,476	0,634
	Morphology	Behavior	-0,077	0,095	-0,806	0,42
	Morphology	Metabolism	-0,079	0,049	-1,628	0,104
	Chela strength	Growth rate	-0,135	0,091	-1,495	0,135
	Chela strength	Behavior	-0,084	0,116	-0,73	0,466
	Chela strength	Metabolism	-0,031	0,058	-0,543	0,587
	Growth rate	Behavior	0,09	0,103	0,872	0,383
	Growth rate	Metabolism	-0,071	0,052	-1,354	0,176
	Behavior	Metabolism	0,213	0,072	2,962	0,003
<i>F. limosus</i>	Morphology	Chela strength	-0,256	0,118	-2,161	0,031
	Morphology	Growth rate	0,168	0,107	1,57	0,116
	Morphology	Behavior	-0,009	0,079	-0,116	0,908
	Morphology	Metabolism	0,079	0,09	0,88	0,379
	Chela strength	Growth rate	-0,341	0,194	-1,753	0,08
	Chela strength	Behavior	0,048	0,143	0,333	0,739
	Chela strength	Metabolism	-0,164	0,162	-1,011	0,312
	Growth rate	Behavior	-0,194	0,134	-1,447	0,148
	Growth rate	Metabolism	0,701	0,173	4,058	0
	Behavior	Metabolism	0,097	0,113	0,862	0,389

(b)

Species	Covariances		Estimate	Std.Err	z-value	P(> z)
<i>P. clarkii</i>	Elemental composition	Excretion	-0,112	0,05	-2,236	0,025
	Elemental composition	Trophic position	-0,101	0,092	-1,097	0,272
	Elemental composition	Consumption	-0,416	0,111	-3,741	0
	Elemental composition	Bioturbation	-0,376	0,114	-3,286	0,001
	Excretion	Trophic position	0,095	0,068	1,39	0,165
	Excretion	Consumption	0,345	0,085	4,073	0
	Excretion	Bioturbation	0,162	0,08	2,028	0,043
	Trophic position	Consumption	0,041	0,138	0,296	0,768
	Trophic position	Bioturbation	0,005	0,146	0,035	0,972
		Consumption	Bioturbation	0,606	0,175	3,465
<i>F. limosus</i>	Elemental composition	Excretion	-0,056	0,093	-0,606	0,544
	Elemental composition	Trophic position	-0,193	0,081	-2,391	0,017
	Elemental composition	Consumption	-0,069	0,084	-0,824	0,41
	Elemental composition	Bioturbation	-0,03	0,078	-0,382	0,702
	Excretion	Trophic position	0,086	0,094	0,92	0,358
	Excretion	Consumption	0,052	0,101	0,517	0,605
	Excretion	Bioturbation	0,099	0,095	1,046	0,295
	Trophic position	Consumption	0,063	0,084	0,743	0,458
	Trophic position	Bioturbation	-0,089	0,079	-1,125	0,26
	Consumption	Bioturbation	-0,13	0,087	-1,502	0,133

Figure VI.S1 Abundances of *Procambarus clarkii* (red) and *Faxonius limosus* (dark yellow) in lake Lamartine, calculated using catch per unit effort (CPUE) expressed in ind. trap⁻¹.hr⁻¹, on 9 occasions from June to December 2018. 36 baited traps (one cylindrical trap: 62cm x 34cm x 34cm, mesh size: 10 mm; one rectangular trap: 95cm x 20cm x 20cm, mesh size: 4 mm) were placed along transect lines, for one night (16h), and covered both littoral and pelagic habitats.

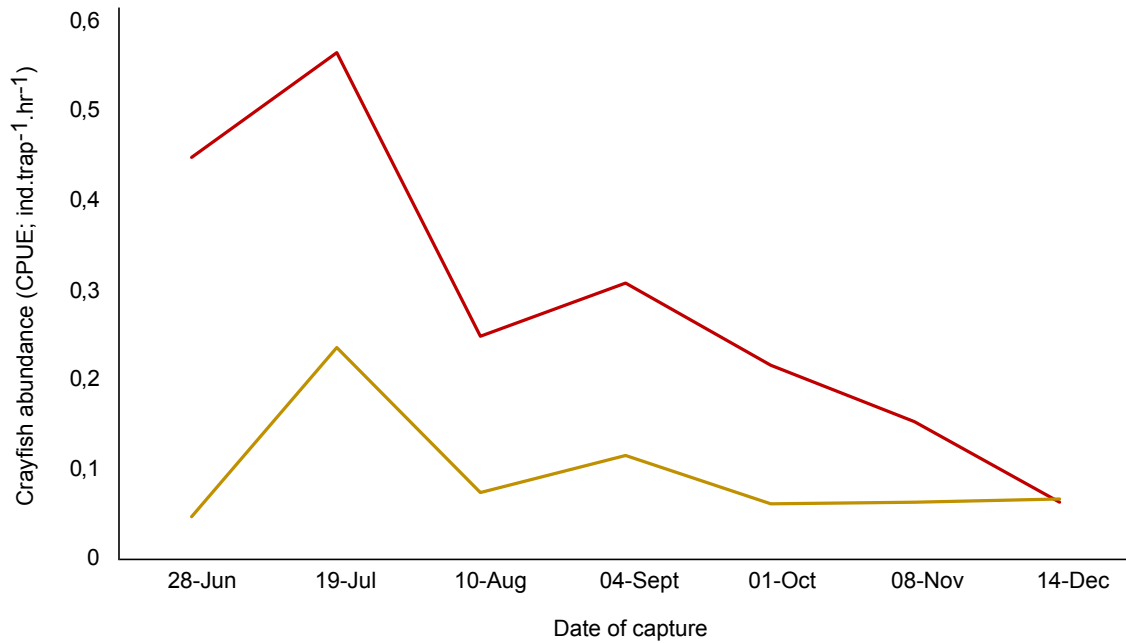


Figure VI.S2 Principal component analysis on residuals of the regression of the Procrustes coordinates against log₁₀-transformed centroid sizes, performed in Morpho J v.1.06d (Klingenberg, 2011) and associated body shape variations. *Procambarus clarkii* individuals are represented in red (n = 63) and *Faxonius limosus* individuals are in dark yellow (n = 63).

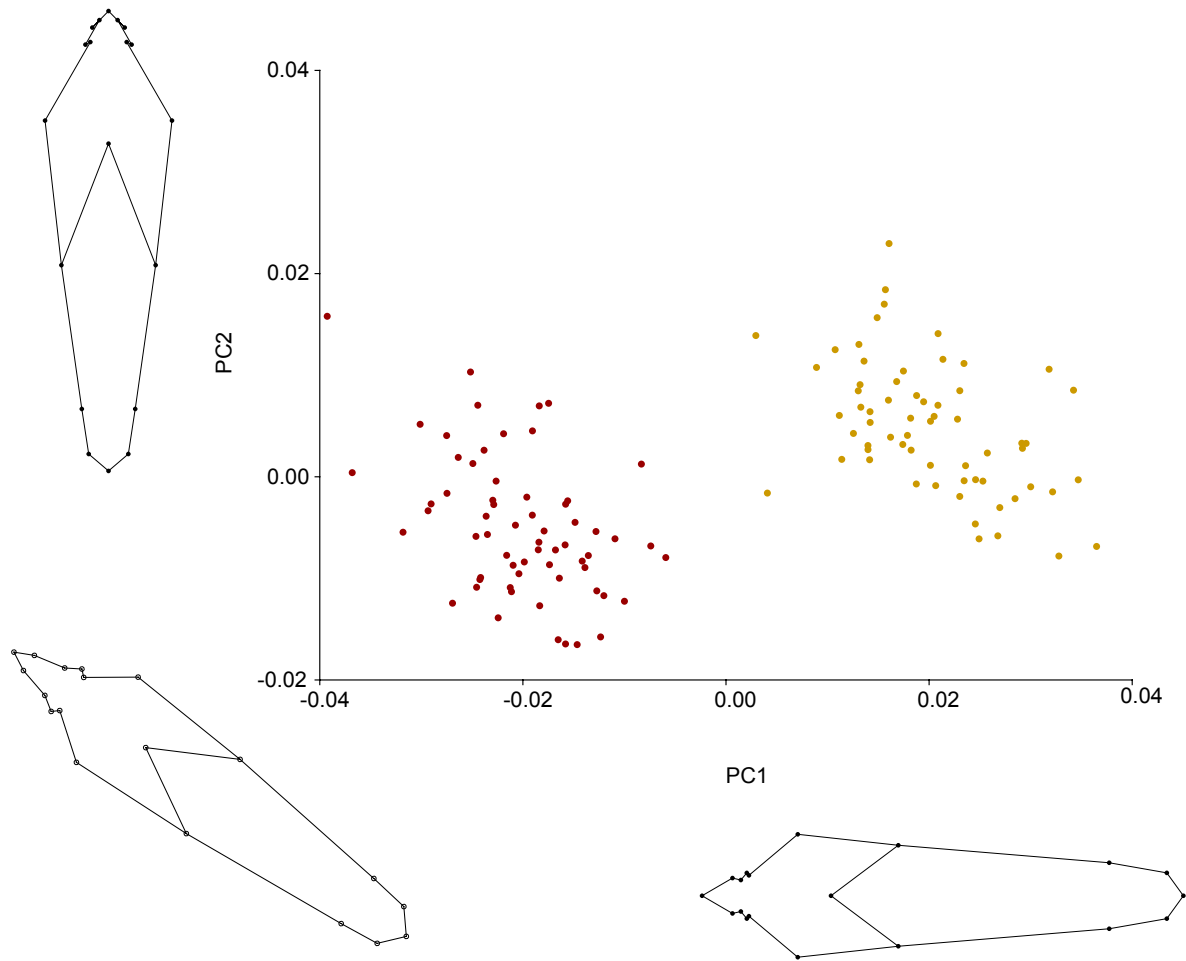
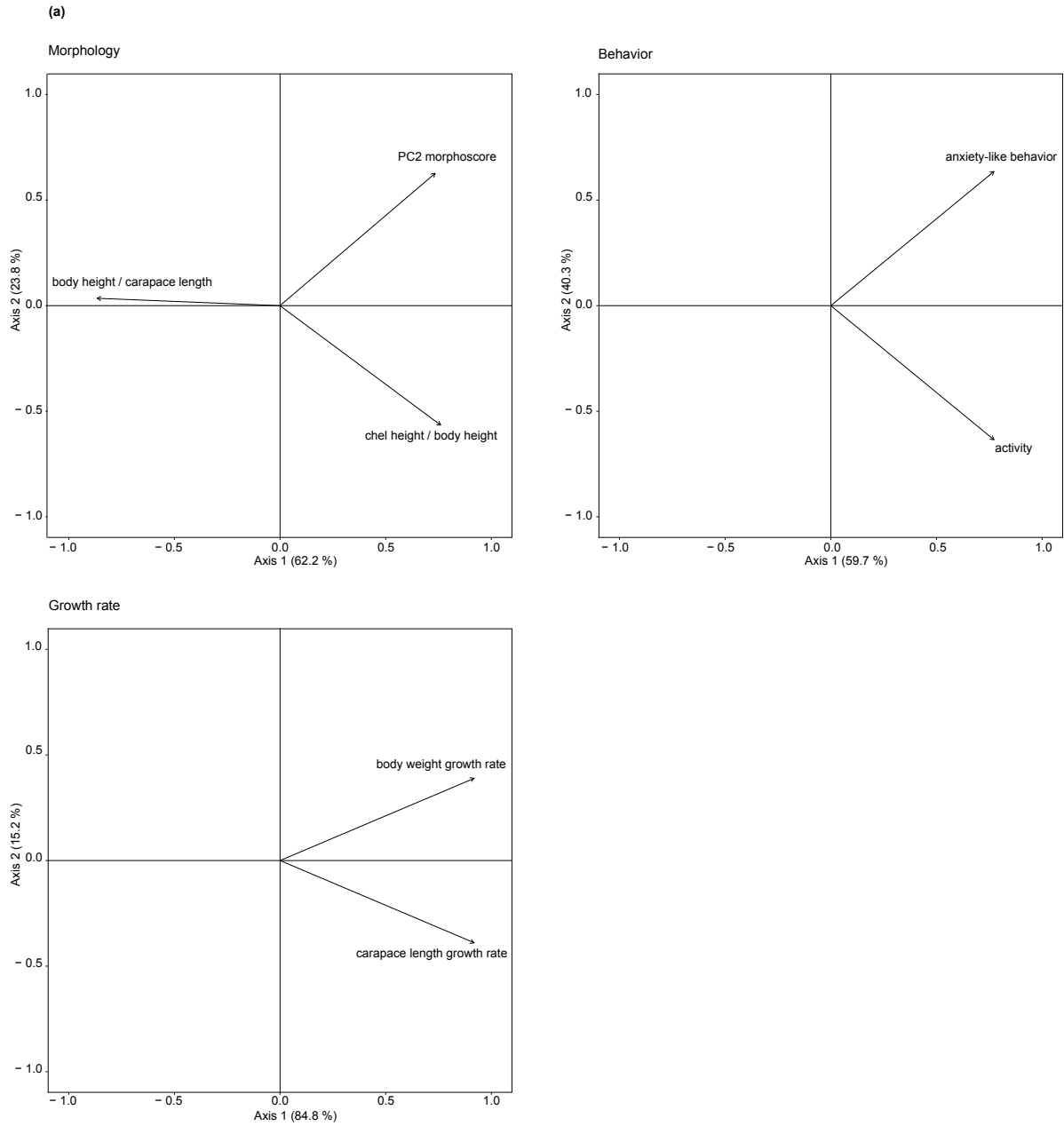
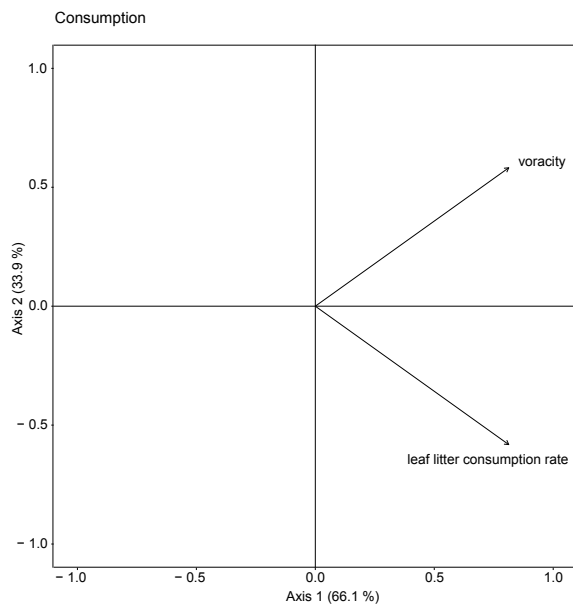
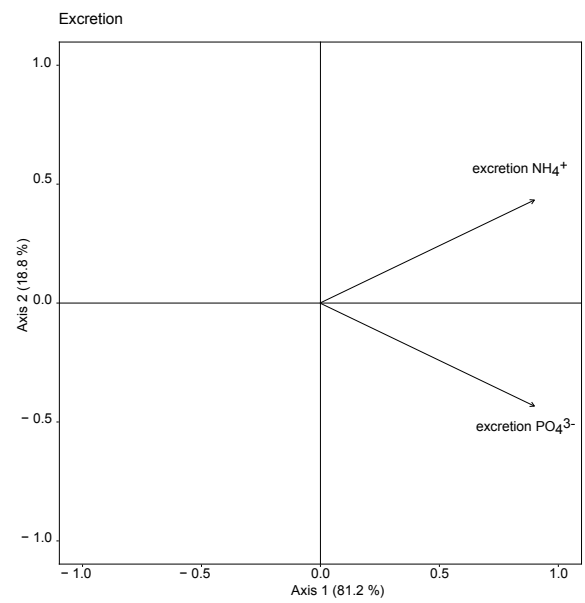
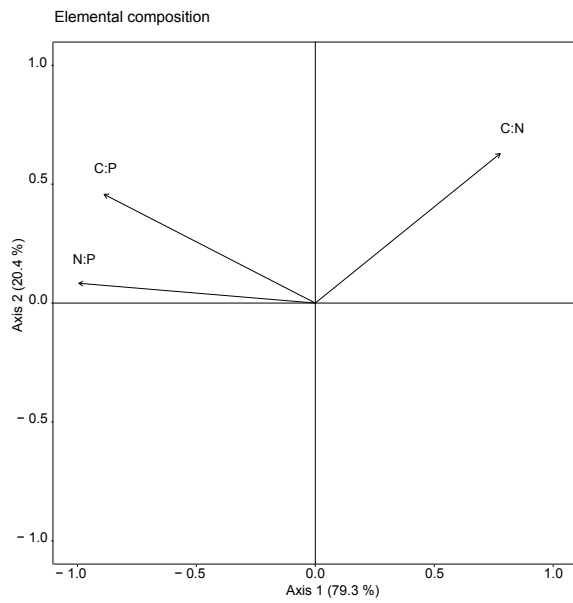


Figure VI.S3 Principal component analyses performed on response and effect traits to summarize **(a)** response traits (morphology, behavior, growth rate) and **(b)** effect traits (elemental composition, excretion rate, consumption rate).



(b)



Acknowledgements

We are grateful to Paul Millet, Gary Williams, Camille Mulatero, and Thibault Pivetta for their help during the fieldwork. We particularly thank Stéphanie Boulêtreau for her help during the setting up of the experimentation. This work was supported by the OFB (Office Français pour la Biodiversité) as part of the ERADINVA project and by CNRS as part of the EC2CO project STOECHIOINVA. IL was supported by a PhD grant ATP from the University Toulouse III – Paul Sabatier.

Chapitre VII

Discussion et perspectives

Ce travail de thèse a mis en évidence l'existence d'une forte variabilité intraspécifique au sein de deux espèces invasives aux histoires d'invasion différentes, et a montré que les déterminants de cette variabilité étaient différents entre les deux espèces. Globalement, la variabilité génétique de *F. limosus* était plus faible et moins structurée spatialement que celle de *P. clarkii* (Chapitre III). Pour les deux espèces, la variabilité phénotypique (morphologique, trophique et stœchiométrique) était très importante au sein, mais également entre, les populations (Chapitres IV, V, VI). Nous avons identifié le rôle notable du type de gestion des écosystèmes dans la dissémination des deux espèces et leur structuration génétique mais celui-ci était extrêmement contexte-dépendant (e.g. introduction délibérée ou non intentionnelle ; Chapitre III). La variabilité phénotypique interpopulationnelle était surtout façonnée par des processus adaptatifs chez *F. limosus*, présente depuis plus longtemps dans le site d'étude, tandis qu'une part plus importante de processus neutres ainsi que l'histoire d'invasion expliquaient la variabilité des traits morphologiques et stœchiométriques chez *P. clarkii* (Chapitres IV). Pour les deux espèces, les traits trophiques étaient très fortement dépendants des ressources disponibles dans les lacs. Au niveau intrapopulationnel, nous avons montré qu'une forte différenciation morphologique pouvait être associée à des différences d'utilisation des ressources trophiques entre les individus des habitats littoral et pélagique chez *P. clarkii* (Chapitre V). Nous avons également mis en évidence de fortes différences de syndrome fonctionnel entre les deux espèces dans un écosystème où elles coexistent (Chapitre VI). Ces résultats suggèrent que des individus d'espèces différentes mais aussi d'une même espèce pourraient impacter différemment les chaînes trophiques des habitats littoral et pélagique, et le fonctionnement de l'écosystème dans son ensemble.

Globalement, ce travail de thèse souligne l'importance de considérer conjointement la variabilité génétique et la variabilité phénotypique au niveau intraspécifique, de façon à mieux comprendre les processus façonnant la biodiversité ainsi que les effets des organismes sur le fonctionnement des écosystèmes. Ceci est d'intérêt majeur dans le contexte des invasions biologiques qui perturbent les dynamiques des communautés et le fonctionnement des écosystèmes.

Mieux comprendre les conséquences fonctionnelles de la variabilité intraspécifique

Les traits physiologiques au cœur du fonctionnement de l'écosystème

De nombreuses études ont montré l'importance d'étudier les traits morphologiques des organismes (e.g. masse ou taille corporelle, Hildrew et al., 2007) puisqu'ils traduisent à la fois l'adaptation des organismes à leur environnement et leur rôle écologique (e.g. tant le type que l'intensité des processus impactés ; Woodward et al., 2005). En particulier, la masse corporelle est souvent associée aux variations de consommation de ressources (taux et types de ressources consommées ; Woodward & Hildrew, 2002; Reuman & Cohen, 2005). Nos travaux ont permis de mettre en évidence une forte variabilité morphologique indépendante de la masse résultant d'une adaptation locale entre les populations des deux espèces invasives, mais également au sein de populations de *P. clarkii* entre individus occupant les habitats littoral et pélagique (Chapitres IV, V, VI). Cela suggère que les variations de forme de corps sont toutes aussi importantes à considérer que les variations de masse corporelle en général, puisqu'elles semblent mieux traduire la fonction écologique des individus dans l'écosystème (Parsons et al., 2003; Zelditch et al., 2012). D'autres travaux avaient déjà mis en évidence une très grande variabilité de morphologie chez les deux espèces, soulignant la nécessité de considérer la variabilité intraspécifique à une échelle géographique fine (Chybowski, 2007; Evangelista et al., 2019a). Cette forte variabilité morphologique suggère que chaque espèce invasive possède une grande diversité d'impacts sur les processus écosystémiques des lacs de gravière étudiés que ce soit au sein ou entre populations (Chapitres IV, V; Evangelista et al., 2019b). Des études récentes ont démontré que les traits physiologiques ou comportementaux pouvaient être de meilleurs prédicteurs des effets des individus sur le fonctionnement de l'écosystème que les traits morphologiques (Raffard et al., 2017; Rota et al., 2018). Les résultats des travaux présentés ici vont également dans ce sens puisque nous avons montré l'importance des traits stœchiométriques et comportementaux (*P. clarkii*) ainsi que métaboliques (*P. clarkii* et *F. limosus*) dans le syndrome fonctionnel, alors que les différences morphologiques (masse et forme de corps) n'étaient pas associées de manière significative au syndrome fonctionnel des deux espèces (Chapitre VI). Nous avons mesuré d'importantes variations stœchiométriques chez les deux espèces d'écrevisses en intra- et interpopulations (Chapitres IV, VI). Ceci supporte l'idée que les impacts entre individus peuvent être très variés au sein des espèces invasives, par exemple à travers des variations d'excrétion de nutriments induisant des différences dans les cycles biogéochimiques (Vanni & McIntyre, 2016).

Nous avons également montré que les impacts des organismes peuvent être prévisibles par l'étude des traits de réponse dans certaines populations invasives en raison de l'existence d'un fort syndrome fonctionnel (*P. clarkii*) tandis que d'autres sont plus cryptiques compte tenu d'un faible syndrome fonctionnel (*F. limosus* ; Chapitre VI). Plusieurs études ayant souligné la forte contexte-dépendance des covariations de traits (Peiman & Robinson, 2017; Raffard et al., 2019, 2020), il serait intéressant d'étendre cette étude à plusieurs populations afin de (i) déterminer si le patron que nous avons observé est généralisable à d'autres populations pour chacune des deux espèces, et (ii) dans quelle mesure le degré de coexistence des deux espèces détermine la structure de covariations de traits (Závorka et al., 2017). Nous avons vu que les traits physiologiques (stœchiométrie et métabolisme) semblent contraindre les covariations de traits chez les deux espèces étudiées (Chapitre VI ; Raffard et al., 2017). Il est intuitif de penser que les traits physiologiques peuvent affecter directement les processus écosystémiques tels que les flux de nutriments, la production primaire, ou encore la décomposition de la matière organique. Pourtant, la stœchiométrie, par exemple, reste relativement peu intégrée dans les approches fonctionnelles multi-traits permettant de comprendre les mécanismes sous-jacents des effets sur les processus écosystémiques ou les structures de communauté (e.g. des covariations entre les compositions élémentaires, l'assimilation ou l'excrétion de nutriments, pourraient impacter la biodisponibilité des nutriments, la productivité ou encore le métabolisme de l'écosystème ; Welti et al., 2017; *mais voir* Diaz Pauli et al., 2020 *pour un effet sur les communautés d'invertébrés*). Une meilleure compréhension des impacts des variations stœchiométrique (à la fois en intensité et direction) sur le fonctionnement de l'écosystème permettrait d'appréhender correctement les futurs impacts des espèces invasives dans le contexte de changement global, notamment dans les milieux dulçaquicoles où certains éléments comme le phosphore sont limitants et contraignent donc les processus écosystémiques.

Coexistence d'espèces invasives et conséquences sur le fonctionnement de l'écosystème

Plusieurs résultats dans ce travail de thèse suggèrent que, même si *P. clarkii* et *F. limosus* ont des impacts importants sur le fonctionnement des écosystèmes, il semblerait que (i) *P. clarkii* soit plus compétitrice que *F. limosus* dans les sites étudiés, et (ii) la nature et l'intensité des impacts diffèrent entre les deux espèces. Ces deux constats résultent de plusieurs observations. Premièrement, la variabilité génétique de *P. clarkii* est beaucoup plus importante que celle de *F. limosus* (Chapitre III), ce qui lui confère un avantage pour assurer le maintien de ses populations (Frankham et al., 2002). Il faut cependant noter que *F. limosus*

a l'avantage non négligeable d'être capable de parthénogenèse, du moins en milieu contrôlé (Buřič et al., 2011), ce qui pourrait expliquer le maintien de ses populations naturelles malgré une faible variabilité génétique. Ensuite, nous avons observé que les densités de population sont souvent beaucoup plus importantes chez *P. clarkii* à la fois dans les populations sympatriques et allopatriques ce qui suggère que l'espèce est plus compétitrice que *F. limosus* (Chapitres IV, V ; e.g. stratégie r plus marquée pour *P. clarkii*). Enfin, le faible syndrome fonctionnel de *F. limosus* suggère que les impacts de l'espèce sont moins diversifiés et/ou de plus faible intensité que ceux de *P. clarkii* (Chapitre VI). De plus, cette faible structuration de covariation pourrait être due à la colonisation plus tardive du lac par *P. clarkii* (lac creusé en 1987) qui, à son arrivée, aurait pu provoquer l'écroulement du syndrome fonctionnel de *F. limosus* (Závorka et al., 2017). Nos résultats vont dans le sens d'autres études qui ont montré que *P. clarkii* était plus agressive, plus compétitrice (dans l'acquisition des ressources et l'accès au terrier) et plus tolérante aux stress environnementaux que *F. limosus* (Holdich & Black, 2007; Capinha et al., 2013; Gherardi et al., 2013). Enfin, des analyses préliminaires sur l'ensemble de nos données ont mis en évidence des différences de trait trophique chez *F. limosus* entre populations allopatriques et sympatriques, mais pas chez *P. clarkii* (Chapitre IV et analyses préliminaires ; Figure VII.1). Bien que des analyses plus poussées sont nécessaires, ces nouveaux résultats tendent à confirmer la supériorité compétitive de *P. clarkii* pour l'accès à la ressource. Dans les écosystèmes où les deux espèces coexistent, il existe une différence significative de l'utilisation de la ressource de *F. limosus*, qui possède une plus faible proportion de carbone d'origine terrestre dans son régime alimentaire par rapport aux populations allopatriques (Figure VII.1). Cela suggère que *F. limosus* est contrainte de se nourrir dans une partie plus centrale des lacs, alors que *P. clarkii* se maintient dans une zone littorale plus favorable, où la disponibilité des ressources est plus grande et où l'habitat est plus structuré (voir Chapitre V). En revanche, la compétition avec *P. clarkii* n'entraîne pas de différence sur la position trophique de *F. limosus* par rapport aux populations allopatriques.

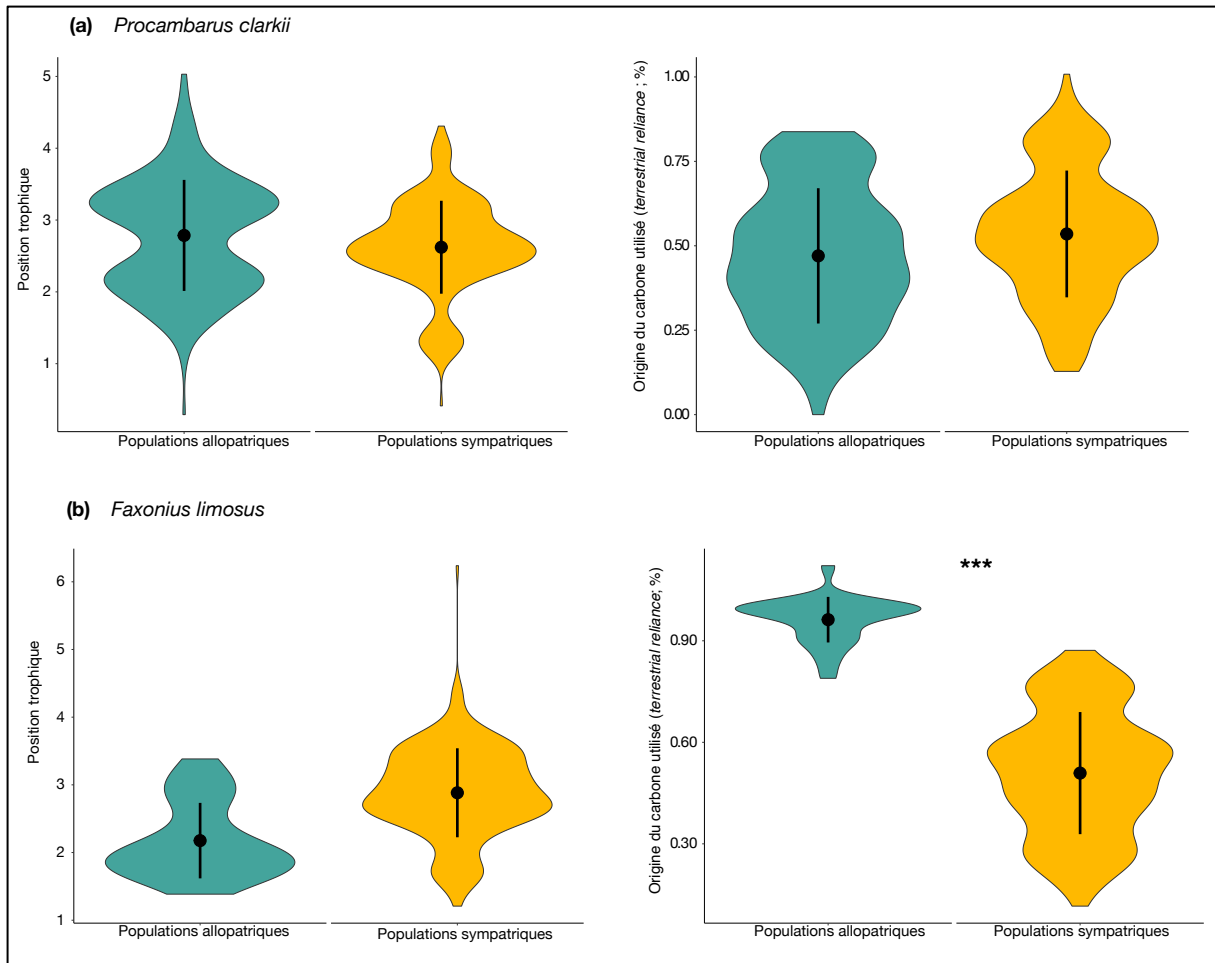


Figure VII.1 Comparaison des positions trophiques (trophic position) et de l'origine du carbone utilisé de **(a) *Procambarus clarkii*** et **(b) *Faxonius limosus*** entre populations allopatriques (une seule espèce d'écrevisse) et sympatriques (coexistence des deux espèces d'écrevisse) faite sur 28 lacs (13 populations sympatriques et 12 et 3 populations allopatriques pour *P. clarkii* et *F. limosus* respectivement).

Les écosystèmes perturbés seront plus susceptibles de connaître des invasions dans le futur. La variabilité intraspécifique est cruciale dans les dynamiques des communautés, et facilite notamment la coexistence des espèces (e.g. plasticité phénotypique ; Turcotte & Levine, 2016). Dans le cadre d'invasions multiples, il est nécessaire de pouvoir prédire les effets combinés de la variabilité intraspécifique de co-envahisseurs sur le fonctionnement des écosystèmes et les dynamiques de communautés (e.g. de proies). Il est possible d'envisager trois types de réponse de l'écosystème ; (i) les effets sont co-amplifiés par facilitation, (ii) les effets des individus de la première espèce invasive sont contrebalancés par les effets des individus de l'autre espèce invasive, ou (iii) les effets des individus des deux espèces invasives sont indépendants (Jackson et al., 2014).

Afin de tester ces trois hypothèses une expérimentation en mésocosmes devait être réalisée, mais n'a pas pu être mise en place pendant la période du confinement en raison du COVID

19. Le but de cette expérimentation était de quantifier les effets d'individus invasifs issus de populations où les deux espèces coexistent (que nous appellerons sympatriques) et où elles ne coexistent pas (que nous appellerons allopatriques). Le principe de l'expérimentation était de comparer les effets de ces individus aux effets d'individus replacés dans des populations théoriques : en séparant les individus en coexistence en milieu naturel, et en mettant en coexistence les individus des deux espèces isolés en milieu naturel. Il faudrait sélectionner des populations sympatriques et allopatriques de *P. clarkii* et *F. limosus* au sein du méta-écosystème de manière à ce que les différences phénotypiques entre les individus soient le moins attribuables à des différences environnementales. Pour cela, les lacs devraient être choisis selon leurs caractéristiques physico-chimiques (remarque : le choix serait vite contraint par les populations de *F. limosus* allopatriques qui sont rares sur la zone d'étude). Le design expérimental comporterait deux principaux traitements pour chaque espèce : la population d'origine (allopatrique ou sympatrique) et le traitement appliqué sur cette population (allopatrique ou sympatrique). Il y aurait deux sources pour chaque population d'origine (lacs A, B pour *P. clarkii* allopatrique, lacs C, D pour *F. limosus* allopatrique, lacs E, F pour *P. clarkii* et *F. limosus* sympatriques ; Figure VII.2). La combinaison des traitements consisterait à introduire 4 écrevisses par mésocosme, c'est-à-dire 4 individus de la même espèce pour simuler les populations allopatriques et 2 individus de chaque espèce pour simuler les populations sympatriques. Les sex-ratios seraient équilibrés (1:1) dans chaque mésocosme afin de pallier aux potentiels effets de sex-ratio sur les dynamiques écosystémiques (Fryxell et al., 2015). Enfin, pour comparer l'effet de l'introduction d'écrevisses et l'effet du traitement (allopatrique ou sympatrique), il y aurait un traitement sans écrevisse. Chaque traitement serait répliqué 3 fois comme présenté sur la figure VII.2 :

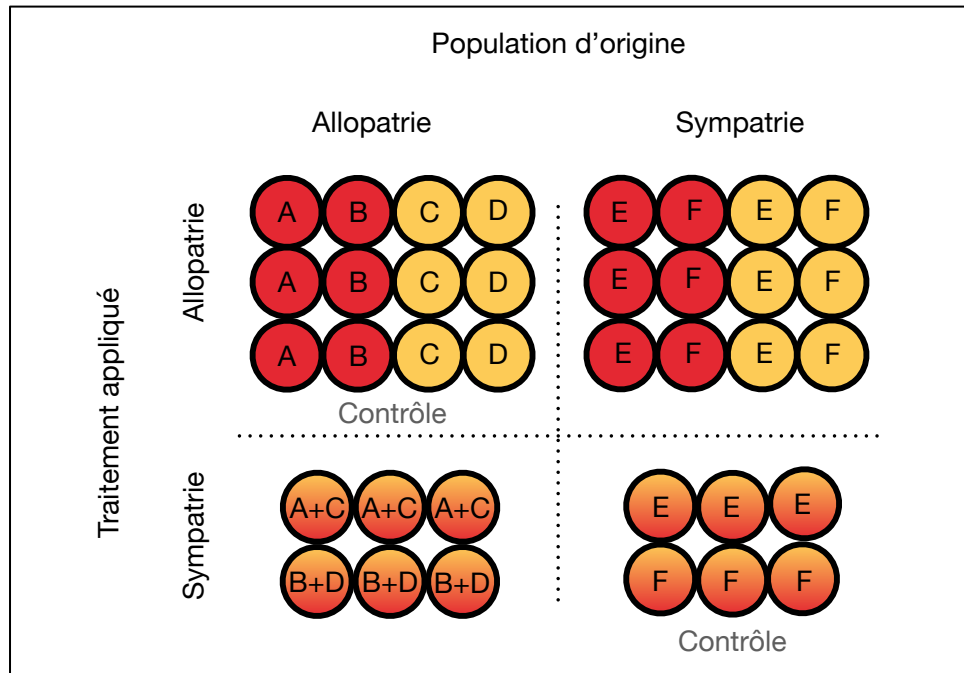


Figure VII.2 Design expérimental proposé pour évaluer les impacts sur les communautés d'invertébrés et le fonctionnement de l'écosystème d'individus des espèces *P. clarkii* (rouge) et *F. limosus* (jaune) provenant de populations allopatriques ou sympatriques. Le traitement sympatrique est représenté par le dégradé de couleur (orange), le traitement allopatrique est représenté par une couleur unie (rouge ou jaune). Chaque cercle représente un mésocosme contenant 4 individus avec un sex ratio équilibré. Les 3 mésocosmes sans écrevisse servant de contrôle pour la taille d'effet ne sont pas représentés ici.

Prendre en compte les dynamiques spatio-temporelles de la variabilité intraspécifique

L'adaptation locale est un mécanisme pouvant mener à des divergences entre, et au sein même des populations à des échelles géographiques très restreintes (Fraser et al., 2011; Weber et al., 2012). Elle est cruciale chez les espèces invasives puisqu'elle favorise leur capacité de dispersion, et nous avons montré qu'elle pouvait être très importante dans des populations récemment établies (Chapitres IV, V). Des études montrent qu'elle se développe en 6-30 générations (Fraser et al., 2011) et nos résultats vont dans ce sens puisque nous avons mis en évidence une forte adaptation locale aux ressources trophiques après moins de 30 ans de colonisation chez *F. limosus* et *P. clarkii*, qui peuvent toutes deux avoir jusqu'à deux générations par an (Chapitres IV, V ; Souty-Grosset et al., 2006). Bélouard et al. (2019) ont montré que la dispersion naturelle pouvait être limitée une fois que les individus sont établis dans des écosystèmes isolés. La limitation des flux de gènes pourrait donc être suffisante pour permettre l'adaptation locale, malgré les transferts « aidés » d'individus entre populations dues aux diverses activités humaines (Chapitre III). Il serait également intéressant de quantifier le rôle d'une potentielle adaptation épigénétique chez les deux espèces d'écrevisses, en particulier chez *F. limosus* qui, malgré sa faible diversité génétique, possède une forte capacité d'adaptation (Marin et al., 2019). Jusqu'à présent, peu d'études se sont intéressées à la variation intraspécifique des espèces invasives à une échelle locale. Améliorer notre connaissance à ce niveau est essentiel, tant d'un point de vue théorique que pour adapter les pratiques de gestion des espèces invasives à la meilleure échelle possible.

Le changement global implique des variations temporelles dans les dynamiques éco-évolutives (Bailey et al., 2009). Par exemple, sous l'effet de l'anthropisation continue des milieux (e.g. urbanisation), les traits phénotypiques des organismes sont soumis à de fortes pressions environnementales et sont amenés à changer rapidement (Alberti, 2015). Les inconnues sont encore nombreuses concernant les réponses adaptatives des espèces au changement climatique, qui peuvent impliquer de multiples traits (e.g. plasticité des traits comportementaux, physiologiques, morphologiques) ainsi que des mécanismes génétiques et épigénétiques (Catullo et al., 2019). Intégrer l'évolution temporelle dans l'étude de la variabilité intraspécifique est essentiel pour tirer des leçons du passé et mieux prévoir le futur. Ce type d'approche permettrait notamment d'étudier en synergie divers mécanismes agissant à différentes échelles de temps : par exemple cela aiderait à mieux comprendre les relations entre plasticité et adaptation (Hendry, 2016; Fox et al., 2019). Il serait également

pertinent d'étudier la stabilité des structures de covariation de traits dans le temps. Des études prenant en compte la temporalité des variations phénotypiques permettent de mieux estimer notre capacité à restaurer des populations d'animaux sauvages, et notamment leur variabilité intraspécifique et leurs dynamiques éco-évolutives d'origine (Thompson et al., 2019).

En conséquence de ces changements de variabilité génétique et phénotypique au cours du temps, les impacts des variations intraspécifiques sur les dynamiques éco-évolutives et le fonctionnement des écosystèmes sont susceptibles de varier dans le temps (Crutsinger et al., 2008). Les espèces invasives constituent un modèle d'étude pertinent pour étudier l'aspect temporel de la variabilité intraspécifique puisqu'elles ont des impacts fonctionnels importants et qu'au cours du processus d'invasion, les individus sont soumis très rapidement à une succession de pressions de sélection qui façonnent leurs patrons de variabilité intraspécifique (Pyšek & Hulme, 2005). Il en résulte que les processus écologiques et évolutifs peuvent évoluer à des échelles de temps comparables (Matthews et al., 2011; Figure VII.3). Un suivi des populations d'écrevisses permettrait d'évaluer l'évolution des parts respectives des processus neutres et adaptatifs (et les parts respectives de la sélection et de l'adaptation) au cours du temps dans les différentes populations. La variation temporelle des traits et donc de l'impact des organismes invasifs devrait être prise en compte par les gestionnaires qu'elle soit saisonnière ou, à une plus large échelle de temps, due à des variations climatiques ou à l'histoire de colonisation (Parker et al., 2003; Phillips & Shine, 2006).

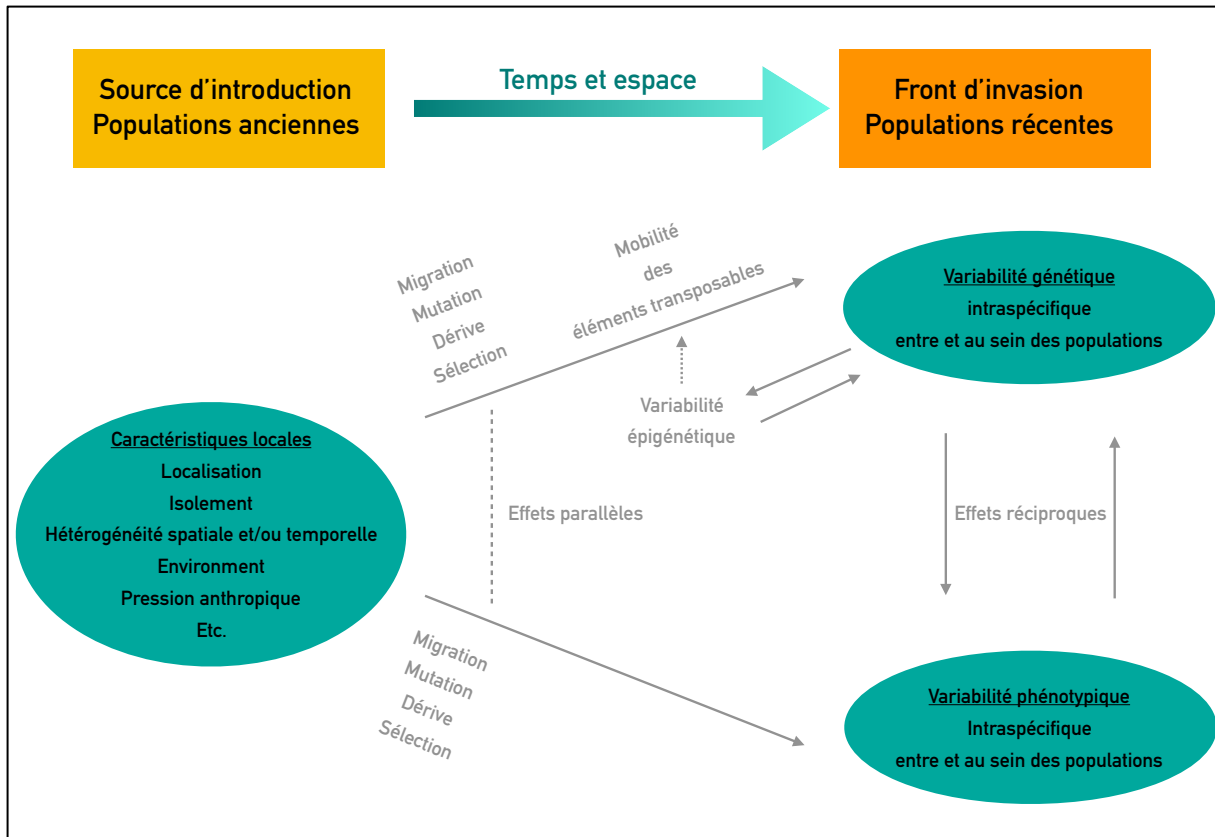


Figure VII.3 Relations entre variabilités génétique et phénotypique au cours du processus d'invasion. Adapté de Vellend & Geber (2005) et Marin et al. (2019).

La variabilité intraspécifique dépend aussi fortement de la dynamique spatio-temporelle des systèmes étudiés (réseau hydrographique, barrages, îles, lacs...). Dans le cas particulier des lacs de gravière, la variabilité intraspécifique connaît également une dynamique spatio-temporelle liée directement au devenir des lacs. Une fois creusés et peuplés, quand l'exploitation de granulats prend fin, les plans d'eau peuvent être rebouchés (i.e. suppression de population) ou rétrocedés à des communes, des AAPPMA, ou des particuliers (i.e. maintien de la population). La variabilité intraspécifique d'une population invasive au sein d'un écosystème maintenu dans le temps sera modulée par les pratiques de gestion appliquées sur cet écosystème. Dans un lac géré par une fédération de pêche ou une AAPPMA par exemple, les pratiques de repeuplement de poissons mises en place peuvent maintenir une population invasive (introduite volontairement pour la pêche ou de manière accidentelle comme contaminant des stocks de poissons) et moduler sa variabilité intraspécifique dans le temps selon : le contrôle ou non de la présence d'individus invasifs dans les stocks de poissons, la provenance des stocks de poissons, et la fréquence de repeuplements de poissons par exemple.

Considérer la sociologie de l'environnement dans le cadre des invasions biologiques

Les invasions biologiques, des processus socio-écologiques

Ce travail de thèse rend compte de la relation entre la société et son environnement : ici, l'Homme façonne son environnement et la biodiversité associée par les activités d'extraction de granulats (création de nouveaux habitats dulçaquicoles) puis par les différentes activités développées autour des lacs (e.g. la pêche ; Chapitre III, Annexe I). Les relations réciproques entre société et environnement sont étudiées par la discipline de la sociologie de l'environnement (Catton & Dunkap, 1978). D'une manière générale, l'étude des invasions biologiques s'inscrit parfaitement dans ce champs disciplinaire puisque l'Homme est souvent à l'origine du déplacement des individus hors de leur aire de répartition naturelle et les espèces invasives peuvent en retour impacter les services écosystémiques, et donc les sociétés (Simberloff et al., 2013). Bien que les changements culturels puissent être beaucoup plus fréquents et rapides que les variations de traits biologiques (Catton & Dunkap, 1978), il est important de prendre en compte les facteurs anthropiques sous-jacents aux invasions biologiques dans le but de mieux les prédire (e.g. quelles motivations, quels vecteurs), mais aussi pour mieux comprendre leurs impacts sur le fonctionnement des écosystèmes (e.g. quels traits sont sélectionnés par l'Homme ; Jeschke & Strayer, 2006). Nous l'avons évoqué en introduction, l'Homme peut également modifier les traits et donc les impacts des individus invasifs par les pratiques de gestion qu'il met en place (ou non) pour contrôler ou éradiquer les populations invasives (Annexe I), ou par le transport d'individus invasifs (volontaire ou accidentel) entre écosystèmes. Ces pratiques de gestion et déplacements d'individus invasifs sont susceptibles d'évoluer avec le contexte éco-sociologique selon la localisation de la population invasive, mais aussi au cours du temps à travers des perceptions changeantes de la part du grand public et des gestionnaires, ou encore des changements politiques (Verbrugge et al., 2013; Crowley et al., 2017; Kochalski et al., 2019). Dans le contexte de changement global, les impacts dus au changement climatique et aux activités humaines sur la variabilité intraspécifique sont souvent étudiés séparément. Une étude récente a mis en évidence la sélection synergique de la pêche et du changement climatique sur les traits phénotypiques des poissons marins (Morrongiello et al., 2019). Une compréhension plus fine des patrons de variabilité intraspécifique et des conséquences de cette variabilité sur le fonctionnement des écosystèmes nécessiterait plus d'études intégrant à la fois les déterminants environnementaux et anthropiques de la variabilité intraspécifique. Ce type

d'approche est notamment pertinent pour l'étude la variabilité intraspécifique des espèces invasives.

Un cadre d'étude : le méta-socio-écosystème

Dans ce travail, nous avons donc considéré deux métapopulations d'espèces invasives (Hanski & Gilpin, 1997). Les lacs de gravière étudiés dans cette thèse constituent en effet une véritable mosaïque d'îles aquatiques dans une matrice terrestre et forment un méta-écosystème commun aux deux espèces (Loreau et al., 2003). Par définition, le méta-écosystème implique l'existence de flux de matière, d'énergie et d'organismes entre les populations (Loreau et al., 2003; Gounand et al., 2018). Ces flux résultent de la nature même des gravières : les flux de minéraux sont rendus possibles entre lacs proches par la nappe souterraine commune (Mollema & Antonellini, 2016) ; les gravières constituent des habitats pour la faune aviaire qui participe à entretenir des flux entre les populations. Certaines études suggèrent que les oiseaux sont susceptibles de déplacer des individus invasifs (Anastácio et al., 2014; Lovas-Kiss et al., 2020). Les écrevisses ont également la capacité de se déplacer entre sites proches par voie terrestre (Puky, 2014; Thomas et al., 2019), mais une étude récente menée dans un méta-écosystème composé de mares pourtant très proches les unes des autres par rapport au site d'étude de cette thèse, a montré que ce type de déplacement est peu fréquent car très coûteux (Tréguier et al., 2018). Dans le cas des lacs de gravière, il est probable que les flux soient principalement assurés par les flux anthropiques liés aux différentes activités qu'hébergent les lacs (Chapitre III ; Rahel, 2005). La nature et l'intensité de ces activités peuvent être elles-mêmes impactées par l'accessibilité des lacs par l'Homme (Kaufman et al., 2009). La notion de méta-écosystème ne prend pas en compte les relations que nous avons illustrées dans le précédent paragraphe, entre la société et son environnement. Dans le but de mieux prédire les futures invasions et d'appréhender plus finement les conséquences des espèces invasives, il serait intéressant de considérer le système comme un *méta-socio-écosystème*, cadre conceptuel proposé par (Renaud et al., 2018). Intégrer cet aspect social est pertinent surtout dans le contexte des invasions biologiques puisque les hommes en sont le principal vecteur. Cela peut se faire par l'intégration d'approches cognitives (i.e. modèles mentaux) pour mieux comprendre l'impact des effets culturels et de la perception des espèces invasives par les gestionnaires la variabilité intraspécifique de celles-ci à travers les pratiques de gestion, et *in fine* sur leur rôle des individus invasifs dans les processus écosystémiques. La désirabilité ou l'intérêt humain pourrait être considérés comme des traits à part entière pouvant expliquer le potentiel invasif des espèces non natives (Marchetti et al., 2004). Il a été montré récemment que d'autres

espèces invasives aquatiques (la perche-soleil *Lepomis gibbosus* et le poisson-chat *Ameiurus melas*) du même site d'étude présentaient des variations trophiques et morphologiques respectivement, dépendant des pratiques de gestion appliquées sur les différentes populations, validant l'utilisation du cadre conceptuel du méta-socio-écosystème (Jorigné, 2018).

Des approches combinant les facteurs sociaux et environnementaux (biotiques et abiotiques) permettraient donc d'améliorer notre connaissance des mécanismes façonnant la variabilité intraspécifique des espèces invasives, et donc leurs effets sur les dynamiques des communautés et le fonctionnement des écosystèmes (Marr et al., 2010; Clancy & Bourret, 2020; Projet DISPERINVA). L'intérêt de telles approches intégratives réside également dans le développement et l'application de pratiques de gestion des espèces invasives adaptées (Caplat & Coutts, 2011).

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Annexes



Importance of harvest-driven trait changes for invasive species management

Although intraspecific differences between the phenotypes of organisms are an important driver of ecological dynamics (Des Roches *et al.* 2018), research to help integrate phenotypic variation and its drivers with ecosystem management has been limited. For this reason, the novel conceptual framework proposed by Palkovacs *et al.* (2018) – which helps to clarify the ecological implications of harvest-driven trait changes – is timely.

Biological invasions are a key component of the current biodiversity crisis and affect all levels of biological organization. From local to global scales, efforts to control or eradicate invasive species aim to reduce the negative ecological and economic impacts associated with invaders (Kopf *et al.* 2017). Invasive species management commonly relies on methods including harvest (eg hunting and angling), as well as chemical and biological control measures, and can result in non-random removals of individuals from targeted populations (Myers *et al.* 2000; Britton *et al.* 2011). The potential selectivity of these methods therefore has strong ecological and evolutionary implications. Consequently, we suggest that Palkovacs *et al.*'s (2018) framework could be applied to invasive species management. Indeed, harvest-driven trait changes in invasive species might induce unexpected and potentially counterproductive results that may not have been explicitly considered by ecosystem managers.

Recent studies have demonstrated how harvest modifies the traits of invaders and how these changes could modulate their ecological impacts (Figure 1). In populations of invasive lionfish (*Pterois volitans*) controlled by spearfishing, individuals have shifted their behavior to become more crepuscular, potentially increasing their encounter rates with native reef fishes at dawn and dusk (Côté *et al.* 2014). Behavioral changes were also observed in dingoes (*Canis lupus dingo*) controlled by

shooting and baiting, which reduced their activity at dusk and increased it at dawn, relaxing their top-down control of invasive feral cats *Felis catus* and increasing the likelihood of encounters between feral cats and native prey (Brook *et al.* 2012). There is also evidence to suggest that harvest-driven trait changes could affect ecosystem functioning. For example, in an experiment on the invasive red swamp crayfish (*Procambarus clarkii*), a modeling approach revealed that a substantial decrease in crayfish population size affected leaf-litter decomposition rate (a key ecosystem process) to a similar degree as did changes in crayfish behavioral, morphological, and life-history traits (Raffard *et al.* 2017). Harvest-driven trait changes might also modify the indirect effects of invasive species on recipient ecosystems. The removal of invasive pumpkinseed sunfish (*Lepomis gibbosus*) by angling leads to changes in sunfish population size distribution and size at maturity (Evangelista *et al.* 2015). As life-history traits affect fish diet composition (Zandonà *et al.* 2011), these harvest-mediated changes can then alter the nutrient-mediated effects of pumpkinseed sunfish on ecosystem processes (Evangelista *et al.* 2017).

The direction and magnitude of harvest-driven ecosystem effects might be expected to differ between native and invasive species. Although harvested native species usually have high economic, nutritional, and cultural values, these attributes are not necessarily linked to their ecosystem role. Conversely, invaders subjected to control methods are targeted due to their strong negative ecological impacts (Kopf *et al.* 2017) and are therefore likely to comprise a higher proportion of functionally important taxa (eg ecosystem engineers) than harvested native species. This leads us to predict that harvest-induced effects on invasive species should result more frequently in strong ecosystem responses. Moreover, invasion is a selective process where individuals pass through a sequence of filters that act on individual phenotypes to determine whether introduction, establishment, and spread are successful (Blackburn *et al.* 2011). Consequently, in

populations prior to harvest, the trait distributions of invaders might be limited as compared to the trait distributions of their native conspecifics (Juetter *et al.* 2014), modifying the potential for harvesting to subsequently affect trait distribution. In addition, given the high costs of invasion control programs (Myers *et al.* 2000), invaders are often harvested at high intensity but over relatively short time periods. As a result, many invasion control programs are unsuccessful at permanently reducing or eradicating target populations (Britton *et al.* 2011; Pless

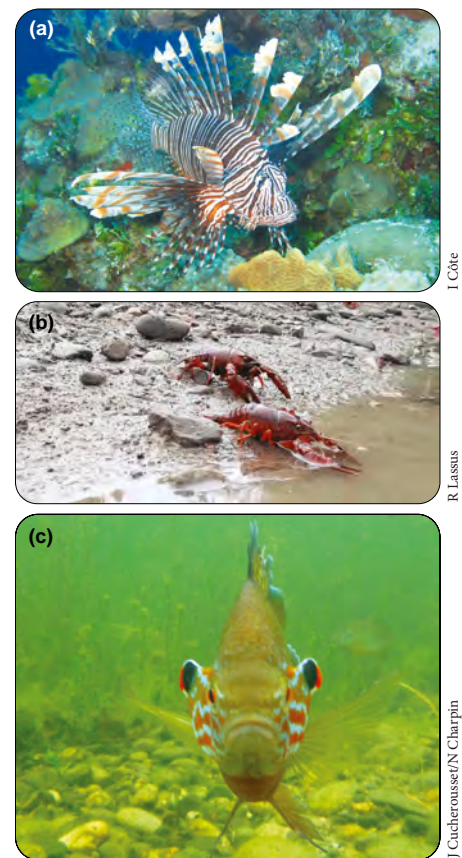


Figure 1. Examples of effects on community structure and ecosystem functions induced by changes in the traits of invasive species targeted by selective invasion control techniques. (a) Elevated crepuscular activity in lionfish (*Pterois volitans*) increases the chance of the predator to encounter native reef fishes. (b) Changes in behavioral, morphological, and life-history traits of red swamp crayfish (*Procambarus clarkii*) affect the leaf-litter decomposition rate in invaded lakes. (c) Changes in life history and diet of pumpkinseed sunfish (*Lepomis gibbosus*) alter nutrient-mediated effects on ecosystem processes.

et al. 2012), and the remaining individuals can re-establish populations (or colonize previously unoccupied habitats) from a pool of individuals with strongly harvest-biased phenotypic traits.

Invasive species control remains an essential management tool with reported successes (Britton *et al.* 2011; Kopf *et al.* 2017). However, when complete eradication is not achieved through control efforts, re-established populations may contain individuals with traits different from those observed during the pre-control period. By extending the framework of Palkovacs *et al.* (2018) to invasive species, practitioners of ecosystem-based management would be able to explicitly consider ecological impacts of harvest-driven trait changes when assessing the net efficiency of invasion control techniques, by comparing the benefits of population size reduction versus the risks of harvest-driven trait changes.

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Wind energy and wildlife: 20 years of translational ecology in action

Frontiers' Special Issue on translational ecology (TE) focused on the urgent need for “effective translation between good science and informed practice”. As scientists, practitioners, and conveners dedicated to facilitating wind energy development while protecting wildlife, we read that issue with the exciting ring of recognition. We commend such work that advances TE and would like to offer a few supporting observations, based on over 20 years of multi-stakeholder collaboration in the area of wind energy and wildlife.

Energy production presents a complex resource management challenge, given its association with habitat degradation, public-health impacts, and greenhouse-gas emissions. Energy production may now also be the largest driver of land-use change in the US, with biofuels – as well as mining and drilling for fossil fuels – having the heaviest footprints (Trainor *et al.* 2016).

Standing out in this energy landscape is the dedicated and potentially unique collaboration among stakeholders in the scientific community, conservation organizations, wildlife management agencies, and the wind industry. This collaboration began as wind energy expanded in the US from the early 1990s onward; first led to multi-stakeholder groups, such as the National Wind Coordinating Collaborative Wildlife Workgroup (www.nationalwind.org); and continued to expand and deepen over time. For example, in 2003, when a wind project in West Virginia was associated with large numbers of bat fatalities, it became apparent that research was needed to understand bat interactions with wind turbines. Bat Conservation International and stakeholders from the wind industry, scientific community, and government agencies subsequently created the Bats and Wind Energy Cooperative (BWEC; www.batsandwind.org). As a result, wind industry companies offered operational

Genetic and phenotypic variability within two invasive species: patterns, determinants and consequences on freshwater ecosystem functioning.

Abstract

Intraspecific variability is now recognized for its importance on ecosystem functioning. In the context of biological invasions, which can strongly impact ecological processes, it is of high importance to understand the determinants and the patterns of genetic and phenotypic variability to fully apprehend the consequences of invasive individuals on recipient ecosystems. We demonstrated a high variability among and within populations of two crayfish species, *Procambarus clarkii* and *Faxonius limosus*, with distinct life-history traits and colonization histories in a narrow-invaded area. We highlighted that colonization history and environmental conditions were the main drivers of the contrasting patterns of genetic and phenotypic variability between the two species. Genetic analyses provided a great inference of local invasion pathways for *P. clarkii*, which had a great genetic variability, compared to *F. limosus* for which the local invasion pathways were more cryptic. We found that neutral and adaptive processes shaped the phenotypic variability of the two species in differing proportions. Then, we demonstrated the existence of a stable resource polymorphism along the benthic littoral-pelagic axis within populations of *P. clarkii*, suggesting that invaders could have contrasting impacts on ecosystem functioning between littoral and pelagic trophic chains. Finally, in an experiment context using a multi-traits approach, we demonstrated that the structure of trait covariations differed between species in a sympatric population, suggesting that *P. clarkii* impacts would be more predictable than *F. limosus*, and that *P. clarkii* could affect a higher range of ecological processes or impact the ecosystem functioning with a greater intensity than *F. limosus*. Overall, our findings stress the need to integrate intraspecific variability in the context of biological invasions to better understand their impacts on ecosystem functioning.

Variabilité génétique et phénotypique de deux espèces invasives : patrons, déterminants et implications pour le fonctionnement des écosystèmes dulçaquicoles.

Résumé

L'importance de la variabilité intraspécifique dans le fonctionnement des écosystèmes est maintenant reconnue. Dans le contexte des invasions biologiques, qui impactent fortement les processus écologiques, il est donc important de décrire les patrons de variabilité génétique et phénotypique et d'en identifier les déterminants, afin de mieux comprendre les conséquences des individus invasifs sur les écosystèmes natifs. Nous avons mis en évidence une très grande variabilité intraspécifique au sein et entre populations de deux espèces d'écrevisses invasives aux traits d'histoires de vie et aux histoires de colonisation différentes, *Procambarus clarkii* et *Faxonius limosus*. Les deux espèces possédaient des patrons de variabilité génétique et phénotypique différents. Nous avons montré que les principaux déterminants de la variabilité inter populationnelle étaient la durée d'invasion et les conditions environnementales. Les analyses génétiques nous ont permis d'identifier les routes locales d'invasion pour *P. clarkii*, qui possédait une grande variabilité génétique, tandis que pour *F. limosus*, qui avait une faible variabilité génétique, il était plus complexe d'inférer les routes locales d'invasion. Nous avons montré que des processus neutres et adaptatif avaient façonné la variabilité phénotypique en différentes proportions pour chaque espèce. Ensuite, nous avons mis en évidence l'existence d'un polymorphisme de ressource stable le long de l'axe benthique littoral-pélagique au sein de populations de *P. clarkii*, ce qui suggérait que des individus avaient des impacts différents sur le fonctionnement de l'écosystème entre les chaînes trophiques littorales et pélagiques. Enfin, dans un contexte expérimental avec une approche multi-traits sur des individus provenant d'une population sympatrique, nous avons montré que la structure de covariation de traits différait entre les deux espèces. Nos résultats suggèrent que *P. clarkii* aurait des impacts plus prédictibles que *F. limosus*, et qu'elle pourrait affecter plus de processus écologiques ou impacter plus fortement le fonctionnement de l'écosystème par rapport à *F. limosus*. Globalement, les résultats du présent travail soulignent l'importance d'intégrer la variabilité intraspécifique dans le contexte des invasions biologiques, afin de mieux comprendre et évaluer leurs impacts sur le fonctionnement des écosystèmes.