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**Titre de la thèse: Biological invasion risk assessment,
considering adaptation at multiple scales: the case of
topmouth gudgeon *Pseudorasbora parva*.**

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RÉSUMÉ

Afin de quantifier les risques d'invasion chez le goujon Asiatique *Pseudorasbora parva*, un petit cyprinidae d'eau douce, j'ai tout d'abord testé une approche corrélative de modélisation de la niche climatique dans laquelle j'ai intégré un proxy relatif à la probabilité d'introduction. Cette approche s'appuie sur des assomptions relatives à l'adaptation des organismes aux conditions environnementales locales ou régionales. J'ai ensuite comparé la niche climatique mesurée de deux lignées génétiques majeures à la fois dans les aires natives et envahies. Puis dans un second temps, par une approche expérimentale j'ai comparé la réponse aux variations de température, des traits d'histoire de vie de populations vivant dans des zones climatiques contrastées. Finalement, je me suis intéressé à la dispersion des goujons Asiatiques afin de mieux comprendre si les populations situées sur les fronts d'invasions avaient des capacités de colonisation plus importante. J'ai donc quantifié et comparé le long d'un gradient d'invasion, le potentiel de dispersion des individus ainsi que des traits pouvant y être liés (activité et morphologie).

L'étude des risques d'invasions a montré que de nombreuses zones – au delà des zones déjà envahies - étaient climatiquement favorables au goujon Asiatique. C'était le cas en particulier pour certaines zones comme l'Australie, l'Amérique du Sud et du Nord, indiquant que l'invasion de cette espèce pourrait encore s'étendre. Après avoir pris en compte les probabilités d'introduction, cette étude a montré que l'Amérique du Nord était la zone la plus à risques. Par contre, la niche climatique entre les différentes lignées dans les zones géographiques envahies est très similaire, ce qui laisse à penser qu'il n'y a pas de patrons d'adaptation locale chez cette espèce. Pour autant, la niche climatique observée dans la zone envahie est très différente de

celle observée dans la zone native, ce qui suggère un shift climatique important au cours de l'invasion. Les réponses thermiques des traits d'histoire de vie du goujon Asiatique testés expérimentalement n'ont pas varié significativement entre les populations originaires de conditions climatiques continentales et maritime-tempérées. Par exemple, l'effort reproductif global des femelles n'a pas varié entre les températures testées (15-25°C) mais la stratégie temporelle de reproduction a beaucoup varié. L'effort reproductif était plus court et plus intense à forte température, alors qu'il était plus étalé et avec des pics reproductifs moins forts à faible température. Pour ailleurs, il semble qu'il existe un gradient morphologique fort entre les populations situées à différentes distances du front d'invasion ce qui suggère une forte plasticité morphologique mais qui ne serait pas liée à la capacité de dispersion de ces populations. En effet, cette dernière ne variant pas significativement le long du gradient d'invasion. La capacité de dispersion serait principalement liée à la taille du corps de goujon Asiatique, les individus les plus grands ayant une probabilité plus élevée de disperser.

Bien que les prédictions générales du modèle de niche puissent être affectées par de potentielles adaptations à l'échelle de la population ou de la lignée évolutive, les résultats suggèrent qu'une certaine incertitude liée à ces prédictions persiste puisque la distribution native ne prédisait que très mal la distribution actuelle dans les zones envahies. Par ailleurs, mes travaux expérimentaux à plus fine échelle suggèrent que cette espèce est extrêmement adaptable et tolère une large gamme environnementale, ce qui pourrait expliquer son caractère invasif. Les connaissances produites au cours de cette thèse constituent donc des ressources extrêmement pertinentes pour

développer des stratégies de gestion visant à contrôler les invasions futures du goujon Asiatique.

Mots-clés: Invasion biologique, poisson, niche écologique, modèles spatiaux, *Pseudorasbora parva*.

ABSTRACT

In this thesis I set out to quantify the risk of invasion from the invasive freshwater fish, *Pseudorasbora parva*, at a global extent, using traditional correlative ecological niche modelling approaches with the integration of surrogate data representing introduction likelihood (Chapter I). These correlative approaches rely upon key assumptions relating to the presence or absence of local or regional adaptations, and so I subsequently tested for evidence of such adaptations in genetic lineages and in individual populations. This was achieved through analyzing climatic niche differentiation of key genetic lineages in the native and invasive ranges (Chapter II) and by conducting lab experiments comparing thermal responses of important life history traits in populations from contrasting climates (Chapter III). The initial risk assessment did not account for a key factor in invasions; namely, natural dispersal. Natural dispersal has been observed to be subject to selection in vanguard populations of invasive species, and adaptation of dispersal traits can infer additional invasive vigor, allowing the species to spread across the landscape quicker. For this reason, I quantified dispersal, activity and morphological differences, often associated with differential dispersal ability, in populations along a distance-gradient from an invasion front, in order to identify if *P. parva* is capable of such adaptations.

The initial risk mapping study showed that large areas, beyond the current distribution of the species, are climatically suitable. These areas are mainly in North and South America, Australia and New Zealand, and constitute significant scope for spread and impact of this species. When introduction likelihood was included, N. America appears most at risk. I found no evidence to suggest that native genetic lineages represented local adaptations to their respective native climates - there was little or no

differentiation of the lineages' climatic niches in the invasive range. It was also apparent, from the niche comparisons, that the climatic niche in the invaded range constituted a significant shift, compared to the native range. The thermal responses of *P. parva* life history traits did not differ significantly between populations from a strongly seasonal continental climate and a mild temperate maritime climate. The overall reproductive output of females did not vary according to breeding season temperature, however, temporal reproductive strategy showed a strong response, with lower temperatures inducing a protracted breeding season and higher temperatures inducing rapid and intense reproductive output. The dispersal and morphology-related study identified a strong gradient of morphological change, corresponding with distance from invasion front. This demonstrates a high degree of plasticity in *P. parva*'s morphology in an invasion context, however this was not linked to either dispersal or activity levels, neither of which was significantly linked to distance from invasion front. Dispersal was best explained by body size, with larger fish more likely to disperse further.

Whilst I found no evidence to suggest that the model predictions (Chapter I) were hampered by differentiation at either lineage or population levels, the findings of Chapter II do highlight the uncertainties surrounding the degree of conservatism in such predictions, mainly owing to the fact that past, native, distribution did not accurately predict the current invaded distribution. The results of Chapters II-IV show broad tolerances and great plasticity in *P. parva*, which likely underpin this species success as a pan-continental invader. The knowledge produced in this thesis provides a useful new resource for the development of management strategies for *P. parva* and

could be usefully enhanced by the additional of analogous studies on native populations, which could help elucidate the source of the observed plasticity.

Keywords: Biological invasion, fish, ecological niche, spatial modelling,

Pseudorasbora parva.

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RÉSUMÉ EXÉCUTIF EN FRANÇAIS

INTRODUCTION

Les invasions biologiques posent de sérieux problèmes pour la biodiversité et le fonctionnement des écosystèmes. La dispersion des espèces au delà de leur aire native est une des causes majeures du changement global. La globalisation du commerce et des transports a largement contribué à la translocation et la dispersion des espèces non-natives, que ce soit de façon intentionnelle ou non (Perrings *et al.*, 2005; Meyerson & Mooney 2007; Crowl *et al.*, 2008). Les voies d'introduction d'espèces non-natives sont nombreuses et au delà des introductions intentionnelles, elles peuvent être la conséquence de la contamination des biens, des gens ou des animaux d'élevages par des pathogènes, de la dispersion naturelle des organismes ou des changements environnementaux majeurs (par ex. construction de ponts, de tunnels, de canaux...).

Les impacts écologiques générés par l'introduction d'espèces non-natives peuvent se manifester de différentes façons. Une espèce introduite peut rentrer en compétition avec les espèces natives pour une ressource limitée (territoires, nourritures...) et conduire à un déplacement des espèces natives, voir à une extinction locale ou régionale. Par ailleurs, les espèces non-natives peuvent agir comme nouveaux vecteurs de maladies émergentes ou amplificateurs de maladies locales. Les impacts des espèces non-natives peuvent se révéler à l'échelle des individus, mais aussi à l'échelle de l'écosystème dans sa globalité. Par exemple, certaines plantes invasives peuvent affecter les concentrations en carbone ou en azote du sol (Liao *et al.*, 2008) et d'autres organismes non-natifs peuvent modifier complètement l'habitat physique en ayant un rôle « d'ingénieur » (Davis, 2009).

L'introduction d'espèces non-natives est responsable de pertes économiques majeures (équivalente à 5% du Produit Interieur Brut global) et conduit à des pertes de l'ordre de 20% de la production alimentaire globale (Nentwig 2007). Clavero & Garcia-Berthou (2005) ont par ailleurs démontrés en se basant sur la liste rouge IUCN que 54% des espèces listées comme étant en cours d'extinction (et pour lesquelles les causes étaient connues) étaient concernées par les invasions biologiques. Les invasions biologiques sont reconnues comme une cause majeure des problèmes environnementaux contemporains, comme en atteste le nombre florissant d'études sur le sujet (Hulme, 2009; Gurevitch *et al.*, 2011).

Les impacts écologiques et économiques causés par l'introduction d'espèces peuvent rapidement augmentés avec le temps depuis la première introduction (Norton 2005). Le plus souvent, il devient très rapidement impossible d'éradiquer les populations non-natives établies sans mettre en oeuvre des politiques extrêmement longues et coûteuses (Leung *et al.*, 2002). Cela montre l'importance d'intervenir très tôt au cours du processus d'invasion afin de contrôler les potentielles conséquences négatives (Kolar & Lodge 2001). Etant donné le consensus général sur les risques économiques et écologiques associés aux invasions biologiques, ce n'est pas surprenant qu'une part importante des ressources humaines et financières soient investies vers des outils permettant d'améliorer notre compréhension des facteurs déterminants la probabilité pour une espèce de devenir invasive (Kolar & Lodge 2001; Copp *et al.*, 2005b; 2009).

Prédire les régions à forts risques d'introduction d'espèces non-natives et d'invasions subséquentes est fondamental pour développer des politiques de gestion basées sur

des actions préventives visant en particulier ces zones. Le goujon asiatique est un poisson d'eau douce (Cyprinidés, *Pseudorasbora parva*) originaire d'Asie et qui a été introduit en Europe dans les années 1960 avant de proliférer et de devenir une espèce invasive (Gozlan *et al.*, 2010; Gozlan, 2012). Cette espèce a étendue son aire de distribution non-native à un taux d'environ cinq nouveaux pays par décennie pour couvrir à l'heure actuelle une région s'étendant de la Turquie à l'Est de l'Angleterre et le Pays de Galles. On le retrouve même en Afrique du Nord (Algérie et Maroc) et au Moyen Orient (Iran) (Gozlan *et al.*, 2010; Gozlan 2012). Bien que de petite taille corporelle (souvent moins de 120 mm de long), son introduction a causé de très forts impacts du fait de sa capacité à rapidement former des populations de très grandes tailles (fortes densités dans le milieu, Britton & Brazier 2006; Britton *et al.*, 2010; Britton & Gozlan 2013). Ceci est particulièrement le cas dans les milieux dégradés (Rosecchi *et al.*, 2001), ce qui conduit entre autre à une compétition pour les ressources avec les espèces natives (Jackson & Britton 2013). En plus de ces effets directs, le goujon asiatique est porteur sain d'un agent infectieux émergent (*Sphaerothecum destruens*) qui est connu pour causer des mortalités importantes chez de nombreuses autres espèces de poissons, parmi lesquelles certains salmonidés à fortes valeurs commerciales (Gozlan *et al.*, 2005; Spikmans *et al.*, 2013; Ercan *et al.*, 2015). Malgré le fait que cette espèce pose de sérieux risques écologiques et économiques et qu'elle ait été définie comme l'espèce de poissons d'eau douce la plus invasive en Europe (Gozlan *et al.*, 2010), il n'y a pas eu de quantification de ses risques d'invasion à l'échelle globale. Ceci constitue un des manques de connaissances les plus criants pour cette espèce et cela pourrait fortement limiter les efforts pouvant être fait pour limiter davantage une dispersion future et les impacts en résultants.

Au cours de cette thèse, mon objectif général était de quantifier les risques d'invasion de *Pseudorasbora parva* à l'échelle globale en utilisant des approches corrélatives de modélisation de la niche écologique (Chapitre I). Ces approches corrélatives dépendent d'hypothèses de bases liées à la présence ou l'absence d'adaptation aux échelles locales et régionales. J'ai donc testé par la suite les évidences pour des adaptations de ce type au sein des principales lignées génétiques de *P. parva* et de certaines de ses populations en analysant d'une part la différenciation en terme de niche climatique entre les aires natives et envahies (Chapitre II) et d'autre part en menant des expériences en laboratoires sur la réponse thermique de traits d'histoire de vie majeurs (Chapitre III). L'analyse du risque d'invasion proposée dans le premier chapitre ne prend pas en compte un facteur clef des invasions ; à savoir la capacité de dispersion naturelle. La capacité de dispersion a souvent été montré comme étant sujet à la sélection naturelle lors des phénomènes d'invasions et l'adaptation de certains traits liés à la dispersion pourrait permettre à certaines espèces (ou populations) d'améliorer leur pouvoir de propagation. Par exemple, lors de l'invasion du crapaud buffle en Australie, il a été observé que le taux de dispersion dans le paysage a été multiplié par cinq au cours des 70 dernières années du fait de l'adaptation génétique de certains traits liés à la dispersion (la longueur des jambes) le long du front d'invasion (Phillips *et al.*, 2010). Au cours du Chapitre IV, j'ai donc quantifié et comparé la capacité de dispersion, l'activité et la morphologie générale (deux traits pouvant être liés à la capacité de dispersion) chez six populations de *P. parva* le long d'un gradient d'invasion afin de tester si de telles adaptations existaient chez cette espèce.

SYNTHESE DU CHAPITRE I

Pouvoir prédire les régions à fort risque d'introductions d'espèces non-natives est un enjeu fondamental pour mettre en place des actions de prévention efficaces et des mesures de gestion des risques appropriées. *P. parva* est rapidement devenu une espèce très invasive depuis son introduction en Europe dans les années 1960. En plus des impacts négatifs directs que cette espèce peut avoir sur les populations natives de poissons, *P. parva* peut transmettre une maladie infectieuse émergente qui peut provoquer la mort de nombreuses autres espèces. Afin de quantifier les risques d'invasion de cette espèce dans les régions où l'espèce pourrait être introduite, j'ai développé des modèles de niches qui m'ont permis de construire un modèle d'ensemble prédisant l'adéquation de la niche de chaque région étudiée et un proxy pour la probabilité d'introduction. Ces modèles ont montré que *P. parva* pourrait envahir de nombreuses régions actuellement non concernées par son introduction. Cela inclut des régions dans plusieurs continents, avec en particulier la mise en évidence de points chauds d'adéquation climatique et de risque d'introduction. Ces modèles sont facilement adaptables pour de nombreuses autres espèces envahissantes et les cartes de risques en résultant peuvent être utilisées par les gestionnaires et les décideurs pour accroître les surveillances dans les régions concernées et mettre en place des systèmes de « early-warning » permettant d'éviter des introductions et invasions futures.

SYNTHESE CHAPITRE II

Les adaptations intraspécifiques aux conditions locales et régionales peuvent affecter les prédictions d'adéquation de la niche climatique issues des modèles de niche, que ce soit dans le contexte des invasions biologiques et plus largement dans le contexte

du changement global. Ces adaptations sont souvent concordantes avec la structure génétique des populations. J'ai dans un premier temps testé l'effet de la structure génétique des populations sur le shift potentiel de la niche climatique en utilisant les invasions biologiques comme cadre général. J'ai utilisé une Analyse en Composante Principale pour quantifier les niches bioclimatiques de deux lignées génétiques distinctes chez *P. parva* à partir de 57 points d'échantillonnage dans son aire native et invasive. A l'aide de tests de niche overlap (Schoener's D), d'équivalence et de similarité, j'ai comparé les niches entre les deux lignées et les étendues spatiales. Enfin, en utilisant de la modélisation réciproque de niche, les niches climatiques réalisées dans les zones natives et envahies prédites ont été comparées.

La distribution native des deux lignées correspondait à deux niches climatiques distinctes, mais cette ségrégation climatique disparaissait au sein de la zone envahie. En fait, les niches climatiques natives et envahies se chevauchaient assez peu. La perte de la différenciation de la niche climatique dans les zones envahies suggère que la différenciation génétique entre les deux lignées observée au sein de l'aire native était due à une ségrégation physique et à la dérive génétique plutôt qu'à une isolation par l'environnement. Ceci suggère que la plasticité phénotypique, plutôt que l'adaptation climatique par sélection naturelle, est le mécanisme principal ayant permis à *P. parva* de coloniser de nouveaux environnements avec autant de succès. Les barrières physiques contraignant la distribution des espèces limitait la relation entre l'occurrence de l'espèce et les conditions climatiques telle que caractérisée par les modèles de niche traditionnels. Ces approches corrélatives pourrait donc sous-estimer le potentiel adaptatif des espèces, surtout pour celle ayant un fort potentiel plastique. Dans ces cas précis il est essentiel d'obtenir des données précises

caractérisant les besoins environnementaux des espèces à l'aide d'approche contrôlées.

SYNTHESE DU CHAPITRE III

La gestion des espèces non-native basée sur l'analyse des risques dépend de notre compréhension de leur potentiel d'invasion, qui est lui même largement déterminée par l'expression des traits d'histoire de vie (THV) dans leur milieu d'accueil. Comprendre comment les THV varient avec les conditions environnementales permet de mieux prédire le potentiel invasif des espèces. Après un phénomène d'introduction, un prérequis pour que les individus fondateurs survivent (puis s'établissent) est que les conditions environnementales locales permettent l'expression de THV facilitant l'établissement (Moyle & Light 1996; Fletcher *et al.*, 2016). Pour les poissons d'eau douce, ces conditions dépendent beaucoup du climat et –dans les habitats lenticules– en particulier de la température (Crozier & Hutchings 2014). Ainsi, là où de nombreuses populations introduites d'espèces non-natives se sont adaptées aux conditions climatiques du milieu d'accueil (e.g. régime de température, Kavanagh *et al.*, 2010), cela indiquerait que la provenance des populations pourrait fortement influencer le potentiel invasif dans n'importe quelle autre localité secondaire d'introduction ou de translocation (e.g. Rey *et al.*, 2012). Dans ce chapitre, j'ai réalisé une expérience en laboratoire afin de quantifier les réponses liées à la capacité reproductrice d'adultes et à la croissance de leurs rejetons à trois températures (15 °C, 20 °C and 25 °C) chez *P. parva*. Afin de tester l'adaptation locale, j'ai comparé la réponse thermique des adultes et de leur rejetons provenant de trois populations invasives de deux pays et représentatives de deux climats très contrastés ; climat continental froid de Pologne et climat tempéré maritime du Royaume-Uni. Ces

expériences n'ont pas permis de mettre en évidence de différenciation significative pour ce qui est de la réponse thermique entre les différentes populations, suggérant une absence d'adaptation au climat local. Nos résultats suggèrent que la taille du corps, pas la température, détermine le rendement reproducteur brut des femelles sur l'ensemble de la saison de reproduction, avec les individus les plus grands qui étaient les plus féconds. Cependant, la température affectait la stratégie reproductive temporelle, avec des rendements reproductifs prolongés et moins intenses à des températures basses (et au contraire plus rapides et avec des pics plus élevés à des fortes températures). Le têt de croissance précoce des rejetons était plus élevé à des températures élevées et en particulier significativement différent en les températures de 25 °C et de 15 °C. Ces résultats ont d'importantes implications pour la gestion des espèces de poissons introduites, particulièrement d'un point de vue de la saisonnalité des mesures d'interventions. Ceci suggère que les études futures combinent des facteurs liés à la température et à la disponibilité en nourriture pour développer nos connaissances relatives quant à la façon dont ces facteurs interagissent pour influencer le succès d'invasion.

SYNTHESE DU CHAPITRE IV

Le processus d'invasion biologique est caractérisé par un certain nombre d'étapes, chacune d'elle est liée à une barrière pouvant limiter le succès d'invasion. Les caractéristiques (i.e. les traits) liées à la dispersion sont un des facteurs pouvant déterminer la capacité des espèces à franchir ces barrières à l'invasion. Il y a en effet de plus en plus d'évidence que les populations situées sur les fronts d'invasion sont phénotypiquement différentes par rapport à des populations établies depuis longtemps et une partie de ces différences impliquent des traits liés à la dispersion. La dispersion

est souvent liée à des traits morphologiques, mais peut aussi être dépendants de traits comportementaux. Ces traits peuvent former des ensembles cohérents comme par exemple des « phénotypes dispersants » dont la prévalence devrait diminuer avec l'éloignement au front d'invasion. La présence de ces phénotypes sur les fronts d'invasions pourrait être un déterminant majeur du succès d'expansions et donc de l'invasibilité d'une espèce. Dans ce chapitre j'ai échantillonné six populations de *P. parva* le long d'un gradient d'invasion (de 0 à 210 km depuis le front d'invasion) le long de la Garonne en France. J'ai utilisé une approche expérimentale en mésocosme pour quantifier la propension à disperser, le niveau d'activité ainsi que la morphologie générale de chaque population. J'ai testé si des covariations existaient entre ces traits, si elles variaient entre les populations et par rapport à la distance au front d'invasion. Au cours de ces expériences, j'ai montré que le seul prédicteur significatif de la propension à disperser était la taille corporelle des poissons qui cependant ne variait pas entre les populations. Bien que nous ayons pu mettre en évidence une cline morphologie le long du gradient d'invasion, nous n'avons trouvé aucun lien significatif entre ces variations morphologiques et la propension à disperser. Les différences morphologiques étaient principalement liées à la taille relative de la tête et au diamètre des yeux, ce qui pourrait indiquer un shift dans la diète ou le mode de vie le long du gradient d'invasion. Nos résultats ont montré que les individus les plus gros étaient ceux qui étaient le plus apte à disperser. Les individus les plus gros sont aussi les plus féconds, ce qui pourrait permettre à *P. parva* d'étendre rapidement son aire de répartition et à coloniser de nouveaux habitats plus rapidement qu'au hasard. Cependant, d'autres facteurs pourraient influencer la dispersion, comme par exemple la sociabilité et les effets densité-dépendances.

CONCLUSIONS

Cette thèse a permis de générer une carte mondiale des risques d'invasion de *P. parva*, ce qui a démontré que l'aire potentielle d'invasion de cette espèce est extrêmement grande, en particulier sur les continents Sud et Nord Américains. Bien que les résultats de cette thèse ne suggèrent pas que ces prédictions soient particulièrement affectées par les différenciations observées aux échelles des lignées ou des populations, j'ai pu démontrer qu'un certain degré d'incertitudes dans ces prédictions persistait, principalement du fait que la distribution native ne prédisait pas correctement la distribution actuelle. Néanmoins, en combinant les données de distributions connues dans les aires natives envahies, ces incertitudes étaient plus faibles qu'en utilisant uniquement les données disponibles dans l'aire native. L'absence d'évidence pour de l'adaptation locale (Chapitres II et III) devraient être complétés par des expériences impliquant des populations de l'aire native, ce qui devrait confirmer ou non que le shift climatique observé dans l'aire envahie a une base génétique. Si aucune base scientifique n'est démontrée pour ce shift climatique, l'invasion rapide de *P. parva* constituerait un cadre empirique pertinent pour identifier d'autres facteurs important pouvant expliquer la distribution de cette espèce (par ex. facteurs biotiques, facteurs abiotiques à l'échelle locale). Cette thèse a également permis de montrer de très fortes variations morphologiques liées à la distance au front d'invasion, ce qui suggère un fort degré de plasticité phénotypique par *P. parva* dans la zone envahie. Bien que ces variations n'étaient pas liées significativement à la dispersion des individus, cette plasticité pourrait avoir une influence sur le potentiel invasif de cette espèce. Davantage d'études devraient être menées car ces variations morphologiques pourraient être liées aux comportements de nutrition et ainsi avoir des effets sur la faune locale via un chevauchement

trophique. De plus, cela indiquerait que ces mesures morphologiques pourraient être utilisées pour différencier les populations en cours d'expansion des populations plus âgées, ce qui pourrait être utile afin de proposer des plans de gestions et d'éradication pertinents.

GENERAL INTRODUCTION

BIOLOGICAL INVASIONS

Biological invasions pose a severe threat to biodiversity and ecosystem functioning. Dispersal of species beyond their native range underpins this pervasive driver of global change. Dispersal is a natural process and is critical to the global distribution of species, to the functioning of demographic processes (i.e. metapopulations theory) and is even a major driver of species evolution (Nentwig 2007). The advent of globalisation and the proliferation of international trade and travel have led to the artificial removal of dispersal barriers, and we have seen rapid increases in the translocation of organisms, both intentionally and unintentionally (Perrings *et al.*, 2005; Meyerson & Mooney 2007; Crowl *et al.*, 2008). Species have been intentionally introduced for a variety of reasons, with many seen as assets due to their aesthetic properties (i.e. ornamental fish and plants), their desirability as pets, and their commercial value as a food or material (i.e. crops, livestock, trees for timber). Introduction pathways are manifold and aside from intentional introductions, they may also occur as a result of the contamination of goods, vessels, livestock or people (e.g. parasites or disease), or individuals may themselves disperse naturally, but aided by changes to the environment (e.g. opening up of new waterways, construction of bridges and tunnels).

It's not just human facilitated movements of biological organisms that pose the threat of invasions. Climate change has the potential for playing a significant role in determining invasion success, both directly, by influencing survivorship of transportation and initial introduction (Hellmann *et al.*, 2008; Pyke *et al.*, 2008), and

indirectly, by destabilising or disturbing ecosystems, making them more susceptible to invasion (Byers, 2002). Furthermore, climate change may lead to range expansions and/or range shifts of organisms, particularly at range edges, where conditions were previously of marginal suitability and may now rapidly become suitable (Dukes & Mooney, 1999). Similarly, historically introduced populations of seemingly benign species, may outperform native species and become invasive under novel climatic regimes (e.g. Stachowicz *et al.*, 2002). Such changes in species distributions may lead to novel biotic interactions, with unpredictable outcomes, some of which may mean loss of biodiversity or ecosystem services (Schweiger *et al.*, 2010).

Nonetheless, not all introduced non-native species go on to become invasive (Blackburn *et al.*, 2011). In fact many introduced species fail to survive initial introductions and, of those that do, many do not go on to become invasive (Jeschke & Strayer 2005). However, a subset of those species introduced go on to establish populations, spread rapidly and present a risk to the native recipient ecosystems (Williamson & Fitter 1996; Clavero & Garcia-Berthou 2005; Hejda *et al.*, 2009), and these are termed 'Invasive'. Although there is no single universal definition for the term "invasive species", common components of definitions typically include that it is an introduced species that poses a threat, or harm, to the environment, the economy or to human health (but see Copp *et al.*, 2005a).

IMPACTS AND COSTS

The negative ecological impacts generated by the introduction of non-native species can manifest in a number of ways. An introduced non-native species may compete directly with native species for finite resources (e.g. food, habitat) and territory,

leading to displacement of the natives and even local or regional extinctions. For instance, several species of Erythrean penaeid prawn, including *Marsupenaeus japonicus*, were introduced into the Mediterranean through the colonisation of the Suez Canal and increased numerically to the point where they outcompeted native prawn species, *Melicertus kerathurus* for habitat, leading to the extirpation of this species (Galil, 2007). Some ecosystems may have particular vulnerabilities, having evolved without the presence of certain taxonomic groups that are present in other systems. If members of these taxonomic groups are then artificially introduced, some native species may have no suitable defence mechanism to prevent being predated, or may be outcompeted for food resources by the introduced species. For example, the terrestrial faunal assemblages of New Zealand evolved in the absence of terrestrial mammalian predators. Since human colonisation and the associated introductions of cats, rats and mustelids, the avifauna of these naïve systems has been severely depleted through predation (Dowding & Murphy 2001). In addition to direct competition and predation, non-native species act as novel vectors for existing diseases or as carriers of novel pathogens. In fact, one of the earliest documented biological invasions, of unintentionally introduced Asian rats, is thought to be responsible for the spread of the bubonic plague throughout Europe in the mid-14th century (Morand *et al.*, 2015). Biological invasions can have impacts at the level of individual taxa, but also at the whole ecosystem level, with, for example, some invasive plants affecting the above and below ground carbon and nitrogen pool sizes (Liao *et al.*, 2008) and various invasive species impacting ecosystems by means of physical habitat alteration (Davis, 2009).

Biological invasion is a process operating at the global scale (McGeoch *et al.*, 2010). More than 120,000 non-native plants, animals and microbes have invaded the US, UK, Australia, South Africa, India and Brazil, causing an estimated annual USD \$314 billion worth of damage (Pimentel *et al.*, 2001). The introduction of non-native species is thought to be responsible for the loss of more than 5% of global Gross National Product (GNP) and the loss of more than 20% of global food production (Nentwig 2007). Clavero & Garcia-Berthou (2005) analysed records of extinction in the IUCN Red List database and found that 54% of the records, for which causes were noted, included the effects of invasive species. Recognition of the importance of the subject of biological invasions is increasing, reflected in the exponential increase in published studies on the subject (Hulme, 2009; Gurevitch *et al.*, 2011).

It is also important to recognise that the cost of biological invasions may sometimes be dependent upon perspective. As previously mentioned, many non-native species have been introduced intentionally, for commercial exploitation, and even some of those introduced unintentionally can be of some benefit. For instance, when the Erythrean penaeid prawns, mentioned above, invaded parts of the Mediterranean, they were considered a boon for the local fishermen, whilst at the same time threatening native biodiversity (Galil, 2007).

ADDRESSING THE ISSUE

Both the impact and the associated costs can increase sharply in magnitude with time since initial introduction (Norton 2005). Often, it rapidly becomes unfeasible to eradicate established non-native populations, necessitating expensive long-term management (Leung *et al.*, 2002). For example, since the discovery of the invasive

zebra mussel, *Dreissena polymorpha*, in the North American Great Lakes, in 1986, at low abundances (Hebert *et al.*, 1989), it quickly spread (inhabiting waterways from Minnesota to Louisiana and from Oklahoma to West Virginia, by the end of 1993 - Johnson & Padilla, 1996) and caused pervasive changes to the Great Lakes ecosystem. Now the problem is sufficiently severe that there is little prospect for eradication or control in open waters (Strayer 2009). This exemplifies why it is most practical to intervene early in the invasion process (Kolar & Lodge 2001), and is one of many cases that can be cited in support of the call for improved prediction and prevention. Given the general consensus regarding the economic and ecological risks posed by biological invasions, it is no surprise that there has been significant mobilisation of resources towards improving our understanding of what determines the likelihood that a species will invade (Kolar & Lodge 2001; Copp *et al.*, 2005b; 2009).

According to Blackburn *et al.* (2011) the invasion process can be characterised as having four key stages: (i) Transportation; (ii) Introduction; (iii) Establishment; (iv) Spread. Introduced species must negotiate each of these stages in order to be considered a successful invader. Ultimately a species' ability to overcome the barriers within each of these stages will be context-specific, depending on a variety of factors, including the biotic and abiotic conditions at the site of introduction. For instance, an organism is unlikely to be able to establish and reproduce if the climatic conditions exceed the limits of its physiological tolerances. Multiple studies have sought to identify life history traits indicative of invasiveness (Sakai *et al.*, 2001), and have observed a number of characteristics common amongst known invaders: the ability to reproduce sexually and asexually, rapid growth and early maturity, association with

humans, high fecundity, eurytopy, phenotypic plasticity, rapid dispersal, aggressive behaviour and competitiveness, polyphagy (Moyle 1986; Lodge 1993; Sakai *et al.*, 2001; Blackburn *et al.*, 2009; Chapple *et al.*, 2012). However, forecasts of invasion success are difficult, as some traits that aid the successful negotiation of one stage of the invasion may hinder the negotiation of another (see Kolar & Lodge 2001) and the quantification of some traits require data that may not yet exist, or may exist but not be applicable in a novel system (e.g. behavioural traits and competitiveness). The result is that in a great number of cases, these generic checklists of invasive traits may not be useful predictive tools, particularly for species that have no prior history of invasiveness.

Propagule pressure, which indicates both the number of individuals introduced and the number of introduction events (see Britton & Gozlan 2013) is thought to be strong predictor of the establishment and spread of introduced species (Levine 2000; Colautti *et al.*, 2006; Copp *et al.*, 2007), with greater establishment success associated with increased propagule pressure in a number of studies (Levine 2000; Kolar & Lodge 2001; Britton & Gozlan 2013). However establishment success may also vary, dependant on the number of source populations contributing to the introduction (Ahlroth *et al.*, 2003), with greater numbers of sources possibly increasing success via the mechanism of heterosis, also known as hybrid vigor (Drake 2006; Verhoeven *et al.*, 2011), or simply by the increasing the likelihood that pre-adapted individuals are introduced. Further, altering abiotic conditions can affect the minimum propagule pressure required for successful establishment (Thomsen *et al.*, 2006), so even the role of propagule pressure is context-dependant. Regardless of how many individuals are introduced and with what frequency, if the conditions are not suitable, then they

will not establish self-sustaining populations. For instance, many agricultural crops are planted in areas with climates that fail to support non-cultivated populations of them, despite high propagule pressure, yet some biological invasions originated from very low propagule pressure (Simberloff 2009). Therefore, identifying generic invasive traits or quantifying propagule pressure is not always sufficient to predict introduction outcomes, as the success of non-native species introductions is highly context-dependant. Necessarily, then, predicting possible invasion risks involves determining the presence or absence of certain prerequisites for survival and establishment in the potential introduction area.

A NICHE-BASED APPROACH

In simple terms, the distribution of a species is constrained by factors that can be categorized into three broad groups: (i) physical barriers to dispersal; (ii) the spatial distribution of environmental conditions within which the species can survive, reproduce and maintain a population; (iii) the biotic element of the environment and associated interactions, including competitors, predators including parasites and pathogens, as well as the dynamics and availability of food resources (Guisan & Thuiller 2005; Soberón & Peterson 2005; Soberón 2007; Sillero 2011). The combination of these three categories of constraints determines a species' ecological 'niche'.

This concept can be illustrated by a heuristic diagram (Fig. 1), depicting each of the constraints and visually demonstrating how they can interact, or overlap, to identify the set of conditions and locations that are available and suitable. The entirety of the area constrained by abiotic factors is referred to as the 'fundamental niche'. The

portion in the centre, where all three constraints interact, is typically referred to as the 'realised niche' and the portion constituting the intersection between the abiotic and biotic constraints is referred to as the 'potential niche' (Soberón & Peterson, 2005; Soberón, 2007).

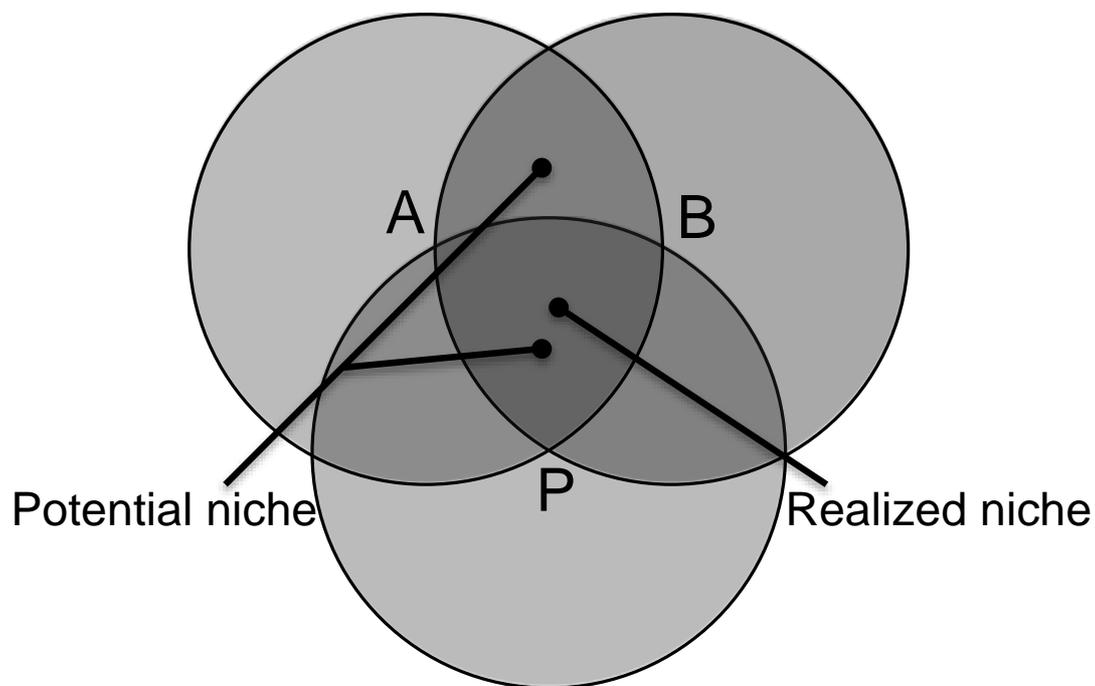


Figure 1 Heuristic diagram, adapted from Soberón & Peterson (2005), depicting visual representations of the three categories of constraints that determine a species distribution; Abiotic constraints (A); Biotic constraints (B); Physical barriers to dispersal (P).

Biotic interactions occur within complex food webs, which include multiple trophic levels (Van der Putten *et al.*, 2010). Furthermore, as environmental conditions can influence outcomes of biotic interactions (e.g. interspecific competition - Davis *et al.*, 1998), this means that such interaction outcomes can vary radically across entire distributions (Sagarin *et al.*, 2006), particularly for species with large geographical distributions that encompass a wide range of environmental conditions. Thus, quantitative or descriptive data that characterise such interactions

typically require lengthy detailed studies and, as a consequence, are often lacking (Soberón, 2010). However, other types of environmental data, covering large, sometimes global, spatial extents, have become increasingly available (see Table 1 in Turner 2003 for a list of data-types and sources).

CLIMATIC NICHE

Of the environmental data-types available, at the broad continental scales synonymous with the definition of species distributions (Soberón, 2007), climate is the single most important in determining suitability for organisms' survival (Willis & Whittaker, 2002; Pearson & Dawson, 2003). With the availability of high resolution temperature and precipitation-derived datasets, at a near global extent (e.g. Hijmans *et al.*, 2005; Karger *et al.*, 2017) and based on the lack of other suitable biological data on a large scale basis, climatic niche modelling is often the only feasible means of assessing niche and thus predicting potential distributions (Baker *et al.*, 2000). In fact, this form of niche modelling is now a well-established method of predicting the climatic suitability of sites for a particular species, based on their current or historical distribution (Guisan & Zimmerman, 2000). It has been used in many studies, including assessing biological invasion dynamics and risks (Soberón *et al.*, 2001; Peterson 2003; Thuiller *et al.*, 2005; Ficetola *et al.*, 2007; Jeschke & Strayer, 2008, Fletcher *et al.*, 2016).

The approach involves the characterisation of the ecological niche, using climatic datasets, based on locations of known species occurrences. Whilst correlational, there is significant utility in the application of these methods for the prediction of biological invasions, as, once characterised, the niche can be projected in multi-dimensional

climate-space, representing large geographical areas (Araújo & Peterson, 2012). This, in theory, allows the assessment of climatic suitability of a site for a particular species at any location for which these climatic data are available.

Using the current realised distribution to characterise the niche means that there is an implied assumption that this current distribution is representative of the modelled species' climatic limits (i.e. the distribution is not instead predominantly limited by physical or biotic factors, at the scale of the study). This assumption that the species is in equilibrium with its environment can be problematic for predictions based on an expanding or contracting distribution, or for a distribution significantly constrained by physical barriers (e.g. geographically isolated island, or peninsular, dwelling species). In the case of biological invasion predictions, the modelled species are typically already widespread (Colautti & MacIsaac, 2004), so less likely to possess a distribution significantly limited by physical barriers. However, there are examples of biological invasions by island-dwelling species, whose historic distribution was ostensibly limited by their geographic isolation (e.g. the New Zealand mudsnail, Loo *et al.*, 2007), so this assumption of equilibrium requires caution. Equally, abiotic factors are thought to be the most influential determinant of species distributions at large (i.e. continental or global) scales, however biotic interactions can also have a significant influence, albeit typically at a more local scale (Boulangéat *et al.*, 2012).

The niche modelling approach also relies on the key assumption of niche conservatism, which is “the tendency of a species to retain ancestral ecological characteristics” (Wiens & Graham, 2005) – essentially maintaining the characteristics of the potential niche (see Fig. 1, above) unchanged over time. Studies of pairs of

congeneric species between geographically separated ranges have found that they tend to share climatic niches, implying conservatism (Peterson *et al.*, 1999; Prinzig *et al.*, 2001). However, other studies have found that, for some species, the niche can shift, or evolve, even over relatively short time periods, e.g. decades (Moran & Alexander, 2014). This exemplifies that this important assumption of ecological niche modelling is not always met, although this could ultimately depend on the demographic level at which the niche is defined (i.e. genus, species, lineage, population).

INTRASPECIFIC ADAPTATION

Many species exhibit local adaptations, particularly across large and varied geographical ranges (Leimu & Fischer, 2008; Hereford 2009). For instance, many species of insects (Hoffmann *et al.*, 2002; Aardema *et al.*, 2011) and plants (McKay *et al.*, 2001; Savolainen *et al.*, 2007; Fournier-Level *et al.*, 2011) display intraspecific local adaptations to temperature. Such adaptations, where a new phenotype is produced through a shift in allele frequencies, due to natural selection, tend to involve trade-offs and so increased performance at local conditions often comes at the detriment of wider tolerances (Hereford 2009). Where such adaptations occur, high levels of gene flow from outside the local area tends to dilute them; hence their tendency to be concordant with intraspecific genetic structure (Aitken & Whitlock, 2013). The validity of the assumption of niche conservatism will tend to rely upon the identification of the correct demographic level at which to characterise the niche, i.e. to account for any local or regional adaptations. This means that, for accurate and representative modelling, the correct demographic unit should ideally be used as the basis for niche characterisation and, if necessary, more than one model may be

required in order to account for identified local adaptations, or particular ecotypes, within the species. Further, where this is the case, in the context of predicting biological invasions, this means that there may exist differential invasion risk, based on the location or genetic identity of the introduced propagule.

CONTEMPORARY ADAPTATION

Whilst evolutionary adaptations are classically associated with extended periods of time, i.e. thousands or even millions of years, contemporary micro-evolutionary adaptation is increasingly being observed to have occurred over shorter periods. Whilst typical examples of this micro-evolutionary adaptation occur over 50-150 generations, it can happen over fewer than 25 (Moran & Alexander, 2014). There are a number of studies where this contemporary adaptation has been observed to occur and some of the best-documented examples involve biological invasions (Quinn *et al.*, 2000; Ellstrand & Schierenbeck, 2006; Colautti & Lau, 2015). Introduced species can be subject to very different and rapid selective regimes, from biotic and abiotic conditions, in the introduced ecosystems, which can lead to these rapid adaptive evolutionary changes taking place (Mooney & Cleland, 2001; Sakai *et al.*, 2001; Savolainen *et al.*, 2007).

Such adaptations can also sometimes be facilitated by propagule pressure, as a low or moderate inflow of genetic diversity from the native range could increase the genetic variability within the introduced population, providing greater chance of recombination producing new genotypes upon which the novel selective pressures can act to produce locally adapted individuals (e.g. Lavergne & Molofsky, 2007). Lag phase, commonly observed in invasions, could sometimes be a result of this

phenomenon (Sakai *et al.*, 2001). For instance, the European green crab (*Carcinus maenas*) was introduced in the US, around New York, in the early 19th century and spread slowly northward over a period of approximately 100 years, stalling in its progress around Halifax, Nova Scotia. A sudden explosive range expansion since the 1980s has been linked to the augmentation of genetic diversity by multiple propagules, seemingly from a number of different genetic lineages (Roman 2006).

Adaptations may not always have a clearly heritable genetic basis (i.e. altered genotype). Instead, they can be the product of epigenetic effects – altered gene expression induced by developmental or environmental stimuli (Chown *et al.*, 2015). Such mechanisms, where environmentally induced epigenetic variation has shifted phenotypic means, plasticities and environmental tolerances, have been implicated in biological invasion success (e.g. Herrera *et al.*, 2012; Zhang *et al.*, 2013; Schrieber & Lachmuth, 2017), however they can be difficult to identify and quantify without strictly controlled experimental designs (Verhoeven *et al.*, 2016). Further, whether or not – and to what extent, if so – environmentally induced epigenetic variants are actually heritable is a source of much debate (Heard & Martienssen, 2014), all of which means that predicting responses in novel environments, under strong selective regimes (i.e. in the context of a typical invasion scenario), is likely unfeasible at present. Nonetheless, identification and quantification of phenotypic plasticity, regardless of its basis, has merit, as phenotypic plasticity in itself is a documented characteristic of successful invaders (Sakai *et al.*, 2001; Chapple *et al.*, 2012).

Failure to account for adaptations, or adaptability in invasion predictions could lead to significant under-predictions of the risk associated with introducing a particular

species, or individuals from a particular population (if that population is locally adapted, or shows increased adaptability). Such adaptations could influence transportation survival and establishment success, as well as determining the overall geographical extent of the area within which any introduction may spread.

DISPERSAL

Although genetic adaptation to climatic conditions may influence the size of the geographic area suitable for the introduced population, the speed with which that spread-potential is realised is largely determined by individuals' dispersal propensity and dispersal ability, which may be facilitated by the presence of dispersal corridors (i.e. suitable habitat linking habitable areas) and/or by association with human activities (e.g. horticulture, aquaculture, sport). As already mentioned, efficient dispersal is a common characteristic of successful invaders (Chapple *et al.*, 2012). Whilst it directly impacts the rate of spread, it is also a pivotal mechanism in a metapopulation context, providing an asymmetric flow of individuals between populations, allowing the persistence of populations in conditions that would otherwise not support net positive growth rates and the colonisation of new locations (Hanski & Gyllenberg, 1993; Hanski, 1998). This sort of asymmetric gene flow, typical of range expansions, can also provide ideal opportunities for contemporary adaptations, due to local novel selective forces and the supply of new genetic material (Savolainen *et al.*, 2007; Aitken & Whitlock, 2013). These types of contemporary adaptations can even concern the species dispersal ability, particularly due to spatial sorting, which would tend to mean that individuals more prone to dispersal, or with greater dispersal ability, would show higher prevalence in populations located on the dispersal frontline (Shine *et al.*, 2011). Evidence in the literature supports this

assertion, with examples of recently colonized populations phenotypically different from more established populations – particularly with reference to traits linked to dispersal (Olivieri *et al.*, 1995; Hanski *et al.*, 2004; Duckworth & Badyaev, 2007).

As adaptability and dispersal are both critical to invasion success, an improved understanding of these aspects in introduced non-native species could be key to predicting both likelihood and potential scope of invasion success. During the initial introduction phase, understanding the demographic scale at which niche is conserved (e.g. population, lineage, whole species) is critical for correct predictions of climatic suitability, whereas scope for spread may be strongly influenced by an organism's ability to adapt quickly and strength of introduction/dispersal pathways. This not only highlights the importance of preventing initial introductions of known invasive species, but it also highlights that the extent of the spread in such scenarios can be unpredictable. Further, knowledge of whether an invasive species is prone to such adaptations may influence the prioritisation of management and policy relative to that species. For instance, existing invasion risk assessment methods may use or suggest use of climate matching as part of overall evaluation (e.g. the Fish Invasiveness Screening Kit – FISK, Copp *et al.*, 2005c), however, these methods do not typically account for potential adaptability, whether it is heritable, or not.

OBJECTIVES OF THE THESIS

The main objective of this thesis is to address the prediction of invasion risk for a major invasive species, by using a multi-tiered approach, combining different tools to consider the implications of adaptations at varying spatial and temporal scales. The process was broken down into four stages, each stage addressing one of the following specific questions: (i) What is the global scope for invasion risk under the null model that there is not intraspecific adaptation? (ii) Is there evidence for intraspecific adaptation at the level of lineages? (iii) Is there evidence for intraspecific contemporary adaptation, at the population level? (iv) Is there evidence of intraspecific adaptation at the invasion front?

FOCAL SPECIES

My thesis focuses on the topmouth gudgeon, *Pseudorasbora parva* (Temminck & Schlegel), a small freshwater cyprinid fish (Fig. 2), native to East Asia, where it spans an enormous geographical and climatic range from Siberia, in the north of its range, to Southern China and Taiwan in the South (Kottelat & Freyhof, 2007; Gozlan *et al.*, 2010; Gozlan 2012).



Figure 2 Photograph of female (top) and male (bottom) Pseudorasbora parva.

The maximum length of *P. parva* is 120 mm, with females rarely exceeding 50 mm (Gozlan *et al.*, 2010). This sexual size dimorphism is thought to reflect their reproductive strategies, whereby large dominant males build nests and guard eggs against predation, and females allocate more energy toward egg production, as they are batch-spawners, spawning 1-14 times per season (Maekawa *et al.*, 1996; Katano & Maekawa, 1997; Kottelat & Freyhof, 2007). Although *P. parva* can produce populations in lotic conditions (Sunardi *et al.*, 2005, 2007; Asaeda *et al.*, 2007), they usually breed in areas of low or no flow (Kottelat & Freyhof, 2007). Individuals can

reach sexual maturity in their first year of life and have been recorded to live up to 5 years, although typical maximum ages are 3-4 years (Gozlan *et al.*, 2010).

The diet of *P. parva* is varied, usually consisting of assorted small zooplankton, including cladocerans, chironomids, copepods and aquatic insects (Xie *et al.*, 2005; Didenko & Kruzhylina, 2015), but it is also known to eat the eggs of other fish (Xie *et al.*, 2000) and macrophytes (Xie *et al.*, 2005). It occurs in a wide range of habitats, from reservoirs, lakes, ponds and irrigation ditches to the faster flowing waters of streams and rivers (Pinder & Gozlan, 2003; Ekmekçi & Kirankaya, 2006; Beyer *et al.*, 2007; Onikura & Nakajima, 2011). Individuals usually inhabit the low to mid water column (Xie *et al.*, 2005) and have been observed to show preference for areas containing submerged vegetation (Kapusta *et al.*, 2008).

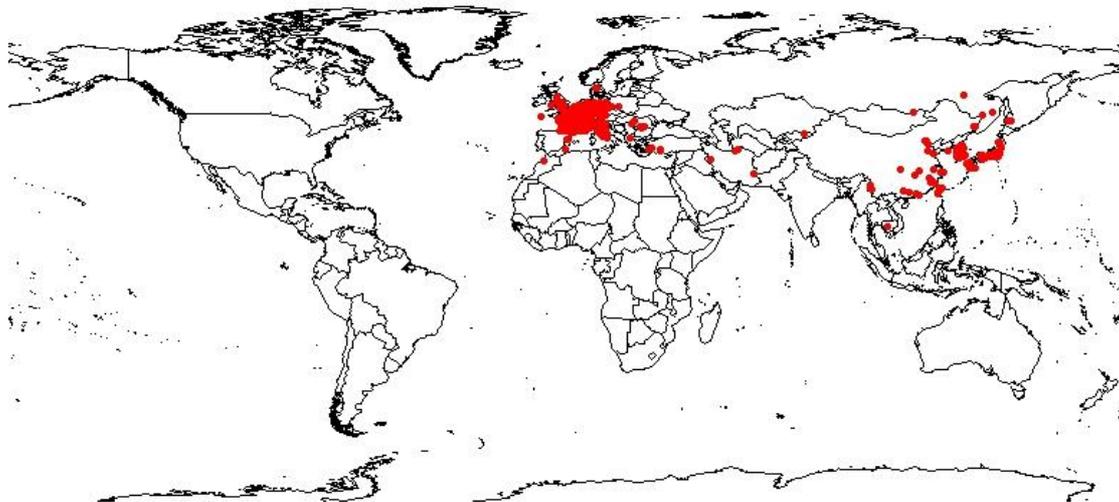


Figure 3 Current global distribution of *Pseudorasbora parva* (GBIF.org (9th March 2018) GBIF Occurrence Download <https://doi.org/10.15468/dl.smijch>)).

Since its introduction to Europe, in the 1960s (Gozlan *et al.*, 2010; Gozlan, 2012), *P. parva* has spread at a rate of approximately five new countries per decade and its invasive range (see Fig. 3) now spans Europe, from Turkey in the east to England and

Wales in the west and even occurs in North Africa (i.e. Algeria, Morocco) and parts of the Middle East (i.e. Iran) (Gozlan *et al.*, 2010; Gozlan 2012).

Although physically small in form, its introduction can cause severe impacts through its ability to rapidly form high-density populations (Britton & Brazier, 2006; Britton *et al.*, 2010; Britton & Gozlan, 2013), which thrive in degraded habitats (Rosecchi *et al.*, 2001) and compete with native fish for resources due to trophic niche overlap (Jackson & Britton, 2013). In addition to its direct impacts, it is also a healthy carrier of an emergent infectious disease (*Sphaerothecum destruens*) known to cause mortality in a number of other fish species, including commercially valuable salmonids (Gozlan *et al.*, 2005; Spikmans *et al.*, 2013; Ercan *et al.*, 2015). Although it poses significant risk and has been referred to as the Europe's most invasive freshwater fish (Gozlan *et al.*, 2010), there has been no global assessment of invasion risk to date. This constitutes an important knowledge gap, which could hamper efforts to prevent further spread and impact.

CHAPTER I

Predicting global invasion risks: a management tool to prevent future introductions.

ABSTRACT

Predicting regions at risk from introductions of non-native species and the subsequent invasions is a fundamental aspect of horizon scanning activities that enable the development of more effective preventative actions and planning of management measures. The Asian cyprinid fish topmouth gudgeon *Pseudorasbora parva* has proved highly invasive across Europe since its introduction in the 1960s. In addition to direct negative impacts on native fish populations, *P. parva* has potential for further damage through transmission of an emergent infectious disease, known to cause mortality in other species. To quantify its invasion risk, in regions where it has yet to be introduced, we trained 900 ecological niche models and constructed an Ensemble Model predicting suitability, then integrated a proxy for introduction likelihood. This revealed high potential for *P. parva* to invade regions well beyond its current invasive range. These included areas in all modelled continents, with several hotspots of climatic suitability and risk of introduction. We believe that these methods are easily adapted for a variety of other invasive species and that such risk maps could be used by policy-makers and managers in hotspots to formulate increased surveillance and early-warning systems that aim to prevent introductions and subsequent invasions.

INTRODUCTION

The introduction and establishment of species into novel ecosystems can result in rapid dispersal rates (Stohlgren & Schnase, 2006; Vitousek *et al.*, 2008), with the magnitude of subsequent impacts and associated management costs increasing sharply with the time since the initial introduction (Norton, 2005). When eradication is no longer a feasible management option, long-term strategies aimed at control and containment are generally adopted (Fernald *et al.*, 2007; Britton *et al.*, 2011a). Such approaches tend to be expensive and only prevent further damage rather than restoring the invaded systems to their former status (Myers *et al.*, 2000). Consequently, more effective management strategies focus more on preventing introductions rather than managing subsequent invasions (Vander Zanden & Olden, 2008).

Through increased global connectivity and trade, opportunities for the introduction of non-native species have increased (Crowl *et al.*, 2008), with resources at border control stretched and ineffective at preventing the entry of non-native species into new regions (Keith & Jaarsveld, 2002). Whilst risk assessment frameworks can be a strong mechanism to help allocate finite resources between preventing, detecting and managing introductions of non-native species (Leung *et al.*, 2002; Copp *et al.*, 2009), they sometimes fail to highlight specific areas within regions where risks of introduction and subsequent invasions are higher (Jiménez-Valverde *et al.*, 2011), although a notable exception is the recent application of the FISK risk assessment tool to a single catchment in Hungary (Ferincz *et al.*, 2016). Risk maps summarize landscape suitability for non-native species within a region by incorporating multiple factors, such as climatic or habitat suitability and potential introduction pathways, and

thus can highlight smaller spatial areas within the region, or continent, where the risk is greatest (Hulme, 2009).

Freshwater ecosystems provide societies with valuable goods and services, yet are disproportionately vulnerable to biological invasions, compared to terrestrial systems, due to their high degree of isolation and endemism (Gozlan *et al.*, 2010a; Moorhouse & McDonald, 2015). Introductions of non-native fishes represent a considerable threat to global freshwater fish conservation (Villéger *et al.*, 2015) and can cause considerable economic damage (Gozlan *et al.*, 2010a). For example, in North America the annual cost of invasive non-native fish species is estimated as at least US\$ 5.4 billion (Pimentel *et al.*, 2005). In Europe, at least 650 fish species have been introduced into aquatic ecosystems, yet only a relatively small proportion of these are considered invasive and cause detrimental impacts (Savini *et al.*, 2010), such as the topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel). This Asian cyprinid fish has been described as Europe's most invasive freshwater fish (e.g. Gozlan *et al.*, 2002), having achieved a pan-continental distribution in less than forty years, following its initial accidental introduction into Romania in the 1960s (Gozlan *et al.*, 2010b). Its ability to invade new waters stems from traits that include high tolerance to degraded ecosystems, high reproductive effort and early sexual maturity alongside batch spawning and paternal nest guarding (Gozlan *et al.*, 2013). Moreover, it can rapidly colonize new waters (Britton & Gozlan, 2013), with their larval stages particularly likely to disperse into the wider environment (Pinder *et al.*, 2005). It is also a healthy carrier of an emergent infectious fungal disease *Sphaerothecum destruens* that has been implicated in declines in native European fishes (Andreou *et al.*, 2012; Gozlan *et al.*, 2014). Given its rapid rate of invasion in Europe and its

disease risk, it is thus important to develop tools to predict additional locations at risk, outside of its current invasive range. Such tools would enable countries that contain potentially suitable habitats but have not yet been invaded to set up the necessary infrastructure to prevent an introduction and subsequent invasion.

Invasion risk is a combination of the likelihood of the species being introduced (e.g. the presence of an introduction pathway) and the suitability of the environment in the new region (e.g. climate suitability for the species). Consequently, the aim of this study was to predict the risk of *P. parva* invasion at the global scale in relation to its climate suitability and likely introduction pathways. Objectives were to (i) use climatic and other relevant abiotic data in both *P. parva*'s native and European invasive ranges to determine its realized niche; (ii) assess its potential global introduction pathways in relation to its known introduction pathways in Europe; and (iii) develop a niche model that characterizes its realized niche and combine this with data on introduction pathways to develop a global risk map of *P. parva* invasion. The implications of the risk map are then discussed in a global context.

MATERIALS & METHODS

Data on presence and absence of P. parva

Previous niche modelling studies suggest that presence location data from the invasive range can sometimes provide a significantly better approximation of the actual species niche breadth, thus producing more accurate suitability predictions beyond the native range (Fitzpatrick *et al.*, 2007; Guisan *et al.*, 2014). Consequently, our presence dataset incorporated data from the European invasive range, compiled from European national monitoring schemes with consistent recording over the last 30+ years, as well as data from its native Asian range. When the European national

monitoring data were combined with literature review and native and invasive presence records from the Global Biodiversity Information Facility (GBIF, www.gbif.org), this provided 2,882 confirmed *P. parva* observations from unique locations, across both native and invasive ranges, the latest of which was recorded in 2014. In order to minimize bias caused by multiple presence points in grid cells, i.e. spatial clustering of records, we applied a form of spatial filtering similar to that used by Boria *et al.* (2014), whereby we removed all but one record from grid cells, essentially leaving us with 2,048 “presence cells” in our dataset.

Absence data were generated on the principle of implied absence, as *P. parva*'s European distribution, after 40 years since introduction, suggested the species had already reached saturation point (cf. Gozlan *et al.*, 2010b) and we also assumed equilibrium in the native range. To avoid spatial autocorrelation with presence cells, random points were generated outside buffers of 50 km around presence cell centroids. These random points were limited to a maximum distance of 100 km from presence cell centroids to prevent the characterization of areas far outside the species' core areas, which can result in poor model performance (Mainali *et al.*, 2015). These methods also maintained a degree of parity with regard to the ratio of presence to absence points across the two distributional aggregations (Asian and European). A total of 10,000 absence points were generated in 10 absence datasets, and weighted to give equal prevalence for the overall response variable dataset, which was coded in a binary (1/0, presence/absence) fashion. Due to the relatively high recording effort achieved in this monitoring, it was assumed these locations represented genuine absences, rather than just characterizing the background environmental variation present within the invasive range.

Predictor variable data

We used a suite of abiotic variables as a basis for the description of *P. parva*'s niche. We used the 19 Bioclimatic datasets (Bio1 - Bio19, Hijmans *et al.*, 2005, see Table 1.1 for individual descriptions); Mean Potential Incoming Solar Radiation (INMSR); Topsoil pH (TpH); and Topographic Wetness Index (TWIS) as our initial suite of variables (see Table 1.1 for details of datasets).

Table 1.1 Description of abiotic descriptor datasets used in analysis along with source URLs. Variables in the column entitled code marked with an asterisk were retained for modelling procedures.

GIS data layer	Code	Source URL
Annual Mean Temperature	Bio1*	www.worldclim.org
Mean Diurnal Range (Mean of monthly (max temp - min temp))	Bio2*	www.worldclim.org
Isothermality (BIO2/BIO7) (*100)	Bio3*	www.worldclim.org
Temperature Seasonality (standard deviation *100)	Bio4	www.worldclim.org
Max Temperature of Warmest Month	Bio5	www.worldclim.org
Min Temperature of Coldest Month	Bio6	www.worldclim.org
Temperature Annual Range (BIO5-BIO6)	Bio7	www.worldclim.org
Mean Temperature of Wettest Quarter	Bio8*	www.worldclim.org
Mean Temperature of Driest Quarter	Bio9	www.worldclim.org
Mean Temperature of Warmest Quarter	Bio10	www.worldclim.org
Mean Temperature of Coldest Quarter	Bio11	www.worldclim.org
Annual Precipitation	Bio12	www.worldclim.org
Precipitation of Wettest Month	Bio13	www.worldclim.org
Precipitation of Driest Month	Bio14*	www.worldclim.org
Precipitation Seasonality (Coefficient of Variation)	Bio15	www.worldclim.org
Precipitation of Wettest Quarter	Bio16	www.worldclim.org
Precipitation of Driest Quarter	Bio17	www.worldclim.org
Precipitation of Warmest Quarter	Bio18*	www.worldclim.org
Precipitation of Coldest Quarter	Bio19*	www.worldclim.org
Mean potential incoming solar radiation (8-day average)	INMSR*	www.worldgrids.org
Topsoil pH (H2O) based on the Harmonized Worlds Soil Database	TpH*	www.worldgrids.org
SAGA GIS Topographic wetness index	TWIS*	www.worldgrids.org

So as to promote parsimony and minimize over-fitting in models, we refined the list of variables used in the final modelling procedure, using Variance Inflation Factor to

identify and remove collinearity in the final suite of variables used. Stepwise selection of variables was carried out using the R package ‘VIF’ (Lin *et al.*, 2012) and left us with a refined suite of ten abiotic variables (see variables marked with asterisk in Table 1.1) to use in our models. All datasets were used at a spatial resolution of 10 km and a spatial reference of GCS WGS 1984. Conforming to accepted protocols in ecological niche modelling (Guisan & Zimmerman, 2000), all of the remaining ten variables were ecologically relevant to *P. parva*.

Additional data

In order to produce a risk map accurately representing both the likelihood of introduction and climatic suitability, we incorporated a dataset representing the known major introduction pathways. Given that the spread of *P. parva* throughout Europe was strongly related to aquaculture-related introductions (Gozlan *et al.*, 2010b), and because there is a strong correlation between non-native introductions and international trade (Bradley *et al.*, 2012), the “Aquaculture Pressure” dataset from the “Global Threats to Human Water Security and River Biodiversity” database (Vörösmarty *et al.*, 2010) was used as a surrogate for introduction likelihood. This dataset was derived using national annual average aquaculture harvests from inland and diadromous fishes from 1997–2006 from the UN Food and Agriculture Organization’s FishStat Plus database (www.fao.org/fishery), which were then distributed proportionately to the grid-cell specific discharge for each nation. The original dataset was re-sampled using bilinear interpolation to match the resolution of all other datasets (10 km). All spatial datasets shared the same spatial reference (GCS WGS 1984).

Modelling methods

The first phase of modelling used nine modelling techniques: Artificial Neural Networks (ANN), Classification Tree Analysis (CTA), Flexible Discriminant Analysis (FDA, Hastie *et al.*, 1994), Generalized Additive Models (GAM), Generalized Boosted Models (GBM), Generalized Linear Models (GLM), Multivariate Adaptive Regression Splines (MARS), Random Forests (RF) and Surface Range Envelope (SRE, Busby 1991). The use of GLM, GAM, CTA and ANN is described and discussed in Thuiller *et al.* (2003). GBM incorporates interactive relationships between predictor variables, as well as being able to discern complex response curves (Wisz *et al.*, 2008). MARS and ANN are good at deciphering complex relationships (Moisen & Frescino, 2002) and RF models have been found to perform consistently well when evaluated against various other well established methods (Prasad *et al.*, 2006). The models were implemented in the 'R' software (Ihaka & Gentleman, 1996), using the 'biomod2' R package (Thuiller *et al.*, 2014). Modelling parameters were kept at default values, in the interest of repeatability. Model predictions were output as probability of presence (continuous values between 0 and 1).

Evaluation of model accuracy (predictive power) was carried out using the Area Under Curve (AUC) of the Receiver Operating Characteristic (ROC). A random 70% selection of the data was used to train each model before the remaining 30% of data were used to evaluate accuracy. To compensate for any possible spatial autocorrelation between training and evaluation data (Thuiller *et al.*, 2009), this cross-validation process was repeated ten times for each model, with the mean calculated to provide a value for a version of the model trained using all of the data. The final

ensemble forecast output was produced in ‘biomod2’ using a weighted average technique, based on the AUC values of each model. Models with higher AUC values were more influential in deciding final cell values. Evaluation of final ensemble model accuracy was conducted using the same cross-validation approach as that used for individual models. Variable importance for each individual model was assessed through the use of biomod2’s randomization function, where each variable’s values are randomized in turn. Pearson’s correlations of model predictions before and after randomization were used to infer relative importance of the variable being randomized (Thuiller *et al.*, 2009). Where the correlation is high, the relative importance of the variable to the prediction outcome is low. Final Ensemble Model (EMmw) variable importance was calculated by applying the weightings used for the ensemble forecasting to the variable importance scores for individual models, summing values by variable then dividing by the number of models used.

Creation of the *P. parva* risk map

The final risk map was created by multiplying the values of the EMmw layer, which represents suitability of conditions according to modelled realized niche, by those of the “Aquaculture pressure” layer, which represents likelihood of introduction. Both layers’ values range from 0–1, so the final Risk Map represents equal weightings of the two factors in assessment of the risk of successful invasion, with potential values ranging from 0 (no risk) to 1 (high risk).

RESULTS

Predictive accuracy in models

Predictive accuracy across the nine individual modelling methods was consistently

high and the mean of each modelling method always exceeded 0.847 in cross-validation runs, except for SRE (Fig. 1.1). SRE models were dropped from the final EM, as they were consistently lower scoring than the others.

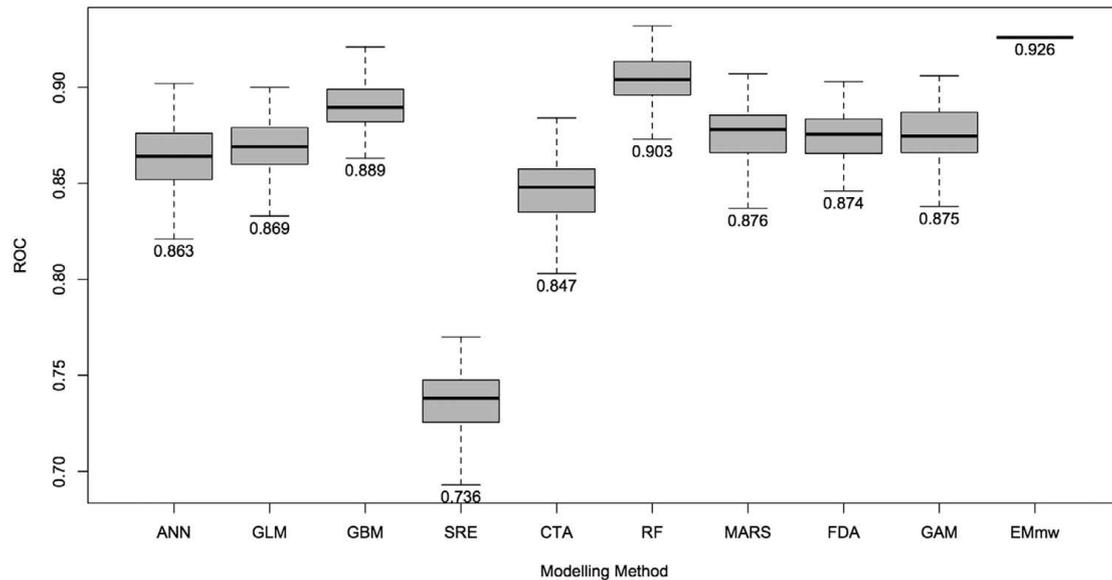


Figure 1.1 Box-plots displaying the Area Under Curve (AUC), Receiver Operating Characteristic (ROC) evaluation scores for all models, grouped by modelling method. Components of box-plots represent minimum, lower quartile, mean upper quartile and maximum values for each modelling method. For each group $n = 100$, except for EMmw, where $n = 1$.

AUC scores indicated that all of the retained individual models were acceptable (Hosmer & Lemeshow, 2000) for predicting the climatic suitability of locations for *P. parva*. However, the weighted method of ensemble forecasting provided a significant increase in model accuracy (Welch's t-test $p \leq 0.001$, testing results of all retained individual models against ensemble model). Variability of predictive accuracy across the different absence datasets and the randomized subsets of data (i.e. partitioning for training and testing) was very similar across all modelling methods, indicating that the data for training were representative of the total dataset in each instance (i.e. for each absence dataset and for each randomization run, of which there were 100 in total for

each modelling method; 900 in total).

Predictor variable importance

The three most important predictor variables, Bio14, Bio3 and TWIS (in that order) accounted for 66% of the sum of all importance values (Table 1.2). The density plot curve for Bio14, precipitation of driest month, (Fig. 1.2) suggested that *P. parva* prefer areas with values between 25 and 60 mm. The density plot for Bio3, isothermality, suggested that *P. parva* prefers areas with a mean monthly temperature range that is approximately 33% of the total annual range. Topographic wetness index was the most important of the non-‘bioclim’ variables used in the final modelling, ranking 3rd with a mean score of 11.64% in the randomization tests.

Table 1.2 Mean variable importance by modelling method, as a percentage. Variable importance scores, as measured by randomization technique, calculated for individual models as 1— Pearson’s correlation between predictions, before and after randomization. Scores were then converted into a % of the sum of all variable importance scores for each modelling method. The three most important variables in the final EMmw model are highlighted in bold.

Model	Bio1	Bio2	Bio3	Bio8	Bio14	Bio18	Bio19	TpH	INMSR	TWIS
ANN	12.50	4.33	8.96	6.79	30.11	12.43	16.46	0.42	2.54	5.44
GLM	3.05	2.89	22.36	6.63	41.50	1.17	5.00	0.79	4.37	12.24
GBM	4.36	2.42	25.89	1.65	46.73	1.63	1.08	0.03	2.05	14.16
SRE	10.08	10.12	10.11	10.45	7.28	9.60	9.37	10.52	11.25	11.22
CTA	9.87	5.64	20.10	4.67	34.33	5.24	3.60	0.60	3.52	12.43
RF	12.62	5.62	24.48	3.98	18.69	6.21	6.15	2.51	7.86	11.88
MARS	7.50	5.48	17.18	5.25	41.93	1.26	6.96	0.10	3.10	11.24
FDA	7.48	3.50	22.83	5.42	35.04	1.42	4.70	0.38	6.70	12.54
GAM	7.85	4.01	30.64	6.74	15.49	3.75	11.60	1.27	5.61	13.05
EMmw	8.15	4.23	21.60	5.13	32.94	4.13	6.93	0.77	4.48	11.64

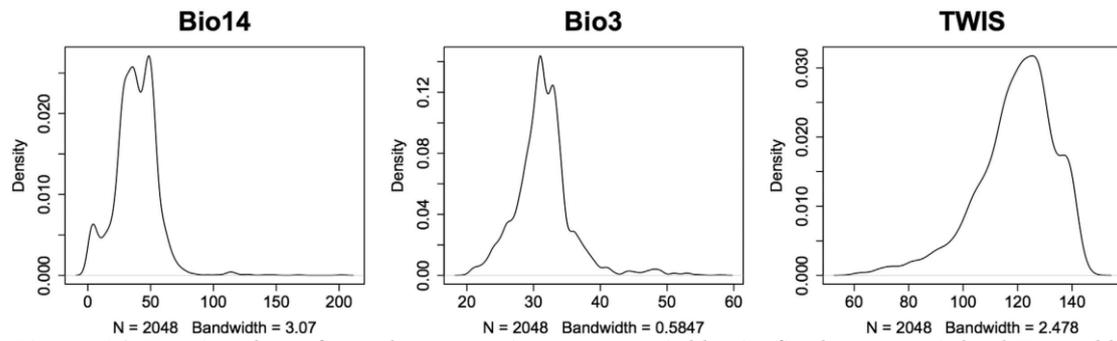


Figure 1.2 Density plots of top three most important variables in final mean-weighted Ensemble Model (EMmw), in order of importance; (a) Bio14 – Precipitation of driest month (mm); (b) Bio3 – Isothermality (mean diurnal temperature range divided by annual temperature range); (c) TWIS – Topographic Wetness Index.

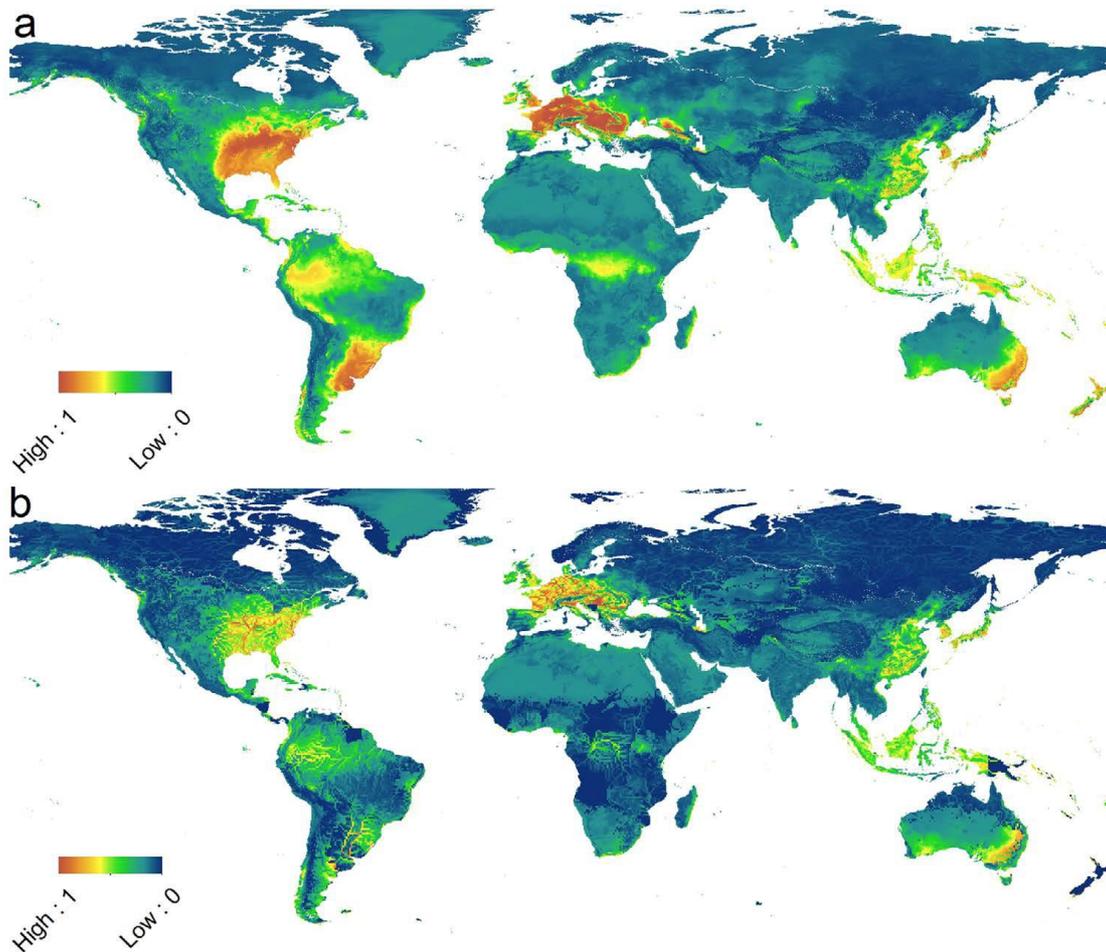


Figure 1.3 Suitability (a) and Risk (b) maps. Both maps use the same colour scale, from low value of 0 to high value of 1, indicating niche suitability and risk of successful invasion, respectively. Final layout of map panels was generated using Esri ArcMap (Version 10.0, Build 2414. [url: https://www.arcgis.com/](https://www.arcgis.com/)).

Suitability predictions

Current known ranges, both invasive and native, were generally well represented by the model output (Fig. 1.3a). However there were some areas within the native range where the suitability score was lower than expected, such as Taiwan. In addition to the known current invasive range, a number of other areas displayed medium to high suitability values. These areas included parts of central and southern Africa, Malaysia, Indonesia, New Guinea, New Zealand, Australia, North and South America. The highest values outside the current known range were in the USA (mainly in the western and southern states), South America (focused around southern Brazil, Eastern Paraguay and Argentina, and Uruguay), in Eastern Australia and in New Zealand.

Risk predictions

The process of incorporating introduction likelihood to the map refined the suitability map by producing a prediction of relative likelihood of a successful invasion (Fig. 1.3b). Areas with predicted high suitability and high levels of aquaculture pressure, a surrogate for introduction likelihood, were fairly widespread. The most significant areas of high and medium-high risk values included the USA, Brazil, Paraguay, Uruguay, Argentina, coastal South Africa, Malaysia, Indonesia and Australia. Although New Zealand possessed areas with high suitability values, the low values of aquaculture pressure meant that the risk levels were very low throughout the country. Similarly, the Island of New Guinea possessed medium-high suitability values, but aquaculture values in the country of Papua New Guinea meant that the eastern part of the island received generally low risk values, whilst the western part of the island, a region of Indonesia with higher levels of aquaculture, received a medium-high risk of successful invasion.

DISCUSSION

The risk maps indicated that *P. parva* has a substantial scope to invade areas and regions beyond its current invasive range, and these cover all continents. There were several hotspots for climatic suitability and risk of introduction in each continent that could represent a potential future point of entrance. In North America for example, the key states at risk are Mississippi, Louisiana, Arkansas, N/S Carolina, Georgia, Alabama, and Tennessee. In South America, two geographically separated hotspots were identified, which included medium-high values in upper parts of the Amazon basin in Peru and Columbia and Brazil. Further south, high values were observed in the south of Brazil, Uruguay and north-east parts of Argentina. In Africa there were narrow fringes of medium-high risk in coastal areas of South Africa, Madagascar and in Liberia. However, these were surrounded by more diffuse areas of medium risk, generally following the coast but also present in central countries such as the Democratic Republic of the Congo and Uganda, where the upper part of the Congo River is most at risk. Although already present in East Asia, our outputs indicated that areas in northern India would be suitable for *P. parva*, particularly the foot hills of the Himalayas, including the Indus River basin as far west as Srinagar in the Jammu and Kashmir state of India. Of particular note is the recent discovery of *P. parva* in the Brahmaputra River in India (Makhrov *et al.*, 2013), an area predicted to be climatically suitable and at medium-high risk by our analysis, thus providing confidence in the performance of the risk maps, particularly given that our training dataset contained only one presence cell in the entirety of India. Finally, in Oceania, predicted areas at medium-high to high risk were in Indonesia, Malaysia and parts of eastern Australia.

Evaluation of the model via cross-validation techniques indicated that the modelling method used to predict climatic suitability achieved a high degree of accuracy. However, there were areas where high suitability values were expected, due to known presence of *P. parva*, but were not predicted by the model. For instance, there were locations in Belarus and southern Turkey that received low suitability scores, but contained *P. parva* records – although they are only present in relatively low abundances (Ekmekçi & Kirankaya, 2006; Semenchenko *et al.*, 2011). Equally, in the native range, Taiwan received generally low suitability scores, despite being known to host widespread *P. parva* populations (Gozlan *et al.*, 2010b). This disparity between predicted and observed suitability in Taiwan may have been due to underrepresentation of distinct environmental conditions present there, owing to low numbers of presence points in this location. Such an issue can lead to underrepresentation of these conditions in the modelled niche. Because niche conservatism is a fundamental assumption of the ecological modelling techniques, the projected niche may not have included the full range of suitable conditions in Taiwan.

In time, introduced species will adapt and evolve within their new range, potentially shifting their climatic preferences and their ecological niche. Simon *et al.* (2011, 2015) revealed *P. parva* populations of Japan and Taiwan have been isolated from those found in continental China for some time (5–6 MYA and 1–1.5 MYA, respectively), allowing the adaptation of the Taiwanese populations to the more tropical climate there. Neither the Japanese, nor the Taiwanese populations have been introduced to the European invasive range, which could explain why the climatic niche of the invasive range may have not yet included the distinct part of the niche occupied only by the Taiwanese populations, which would compound the

underrepresentation of this part of the niche in the data used to train our models. One implication of this is that the risk of invasion may be heavily dependent upon the source population for the introduction, particularly when the native ecological niche is distinct compared to that of other populations. In the case of the Taiwanese populations, this may mean that scope for successful invasion into areas with more tropical climates is greater, and for the species as a whole, this may mean that current risk predictions are underestimated for tropical regions.

Despite some of the invasion predictions in India already being realized, the risk level of individual countries will also be determined by extant policies on managing non-native species regarding aspects such as risk assessments and surveillance. For example, the high level of awareness in Australia regarding non-native species introductions (Pyšek & Richardson, 2010) and the calls for more consistent use of appropriate risk assessment tools, as long ago as the 1990s (e.g. Pheloung *et al.*, 1999), should have by now resulted in adoption of such practices for all major groups of taxa. However, particularly with regard to non-native fishes, this has not been the case; moreover the only application of a generic risk assessment scheme for non-native fishes, to date, has been that of Vilizzi and Copp (2013). With this in mind, the addition of species-specific risk maps, such as the one produced in our study, present an important additional tool that can identify the specific regions of a country that are most at risk from an introduction and thus enable increased preventative measures such as import bans and additional screening at entry points to be implemented (Britton *et al.*, 2011b; Davies *et al.*, 2013). This increased prevention is preferable, as it should reduce the long-term management costs (Norton 2005). Currently, screening points for non-native species are often at ports or national/state borders (Vander

Zanden & Olden, 2008), locations which might not necessarily be identified as having a high risk of introduction and invasion but are entry points for species in transit and so can serve as useful points of inspection.

Failing to prevent future invasions of *P. parva* would greatly increase the associated threat to fish biodiversity. This species is a healthy carrier of a severe emerging fungal pathogen, *S. destruens* (Gozlan *et al.*, 2005), that is non species-specific (i.e. generalist) and has so far led to disease in all tested species including salmonids, cyprinids and percids (Arkush *et al.*, 2003; Andeou *et al.*, 2012; Ercan *et al.*, 2015). Because of the life history traits that make *P. parva* invasive in so many locations (e.g. broad tolerances, high fecundity, etc.) and because it is a healthy carrier of the disease (i.e. it doesn't suffer mortality from infection), it constitutes a significant threat, as it can rapidly build dense populations, which can act as persistent disease reservoirs for an outbreak. A recent study has highlighted the particular significance of the threat posed by such species (Al-Shorbaji *et al.*, 2016). Given the long distance of natural salmon migrations, the potential impact of an outbreak of *S. destruens* among North American salmonid populations, which are highly sensitive to the disease (Arkush *et al.*, 1998; Arkush *et al.*, 2003), could have far reaching consequences. *P. parva*'s invasion could thus be the potential stepping stone to a much wider transmission of the disease. We now know that the rate of spread after initial introduction in Europe was about 0.5 countries per year (Gozlan *et al.*, 2010b), and that invasion in a region comprising largely suitable areas is likely to result in an array of negative impacts.

Given the potential impacts of *P. parva* of disease transmission and the formation of

highly abundant populations that can lead to increased inter-specific competition (Pinder *et al.*, 2006), there are strong ecological drivers for the avoidance of introduction. Whilst simple climate-matching approaches can be useful, more representative models such as ours, accounting for critical factors such as introduction likelihood/ propagule pressure (cf. Colautti *et al.*, 2006; Britton & Gozlan, 2013) should be used in order to provide more accurate risk representations. Consequently, the outputs in this paper can be used to formulate stronger preventative measures and ensure the invasion of *P. parva* is limited to Europe and does not develop more globally.

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CHAPTER II

The role of genetic lineages in predicting climate suitability for invasive species.

ABSTRACT

Intraspecific adaptations to local or regional conditions can have implications for predictions of climatic suitability, both in the context biological invasions and in the wider context of global climate change. Such adaptations are often concordant with patterns in genetic structure. We aimed to test the effect of genetic population structure on potential climatic niche shifts, using a global biological invasion as a framework. We used Principal Component Analysis to quantify the bioclimatic niches of two distinct genetic lineages of one of the most invasive fish species in the world, the topmouth gudgeon *Pseudorasbora parva*, from 57 populations across its native and invasive ranges. In addition, the climatic niche of non-lineage-specific occurrences in the native and invasive ranges were characterised in the same way. Using tests of niche overlap (Schoener's D), equivalency and similarity we compared niches between both lineages and ranges. Finally, using reciprocal ecological niche modelling, realised climatic niches of the native and invasive predicted distributions were compared.

The native distribution of the two lineages corresponded to two relatively distinct climatic niches, but this climatic segregation disappeared within the invasive range. In fact, the native and invasive climatic niches displayed little overlap regardless of whether we considered the lineages or the non-differentiated data. The loss of climatic niche differentiation in the invasive range suggests genetic differentiation observed in the native range is a product of physical segregation and neutral drift rather than climate driven selection. We suggest this implies that phenotypic plasticity, rather than evolved climatic adaptation, is the main mechanism by which *P. parva* has successfully colonised novel environments. The constraint of species distributions by

physical barriers obfuscates the relationship between presence and environmental conditions characterised in traditional Ecological Niche Model (ENM) correlative approaches, potentially leading to significant underestimation of adaptive potential, particularly for species exhibiting a high degree of phenotypic plasticity. In such cases, it is essential to obtain data characterising species' relationships with environmental factors through controlled experimental trials.

INTRODUCTION

Understanding how species and ecosystems react to climatic shifts is critical to protecting biodiversity and has become a major focus of ecology and conservation biology (McMahon *et al.*, 2011). Rapid, sustained climate change is expected to impose strong selective pressures on life history traits likely to be important for fitness (Gienapp *et al.*, 2008). For those organisms unable to track their preferred environment in space or time (through an inability to migrate or other constraints) the only option is to adapt to the changing environmental regime *in situ*.

The main source of adaptation on earth is evolution through natural selection (Berteaux *et al.*, 2004). Although evolution is generally considered to occur over considerable periods of time, rapid evolutionary adjustments have been documented to occur over relatively short time-spans, e.g. <20 generations (Kremer 2016), with some studies purporting to have identified these ‘micro-evolutionary’ adaptations in response to the currently observed directional climate change (Bradshaw & Holzapfel, 2001; Réale *et al.*, 2003). Nonetheless, despite the seeming ubiquity of micro-evolutionary adaptations in the literature (see Hendry & Kinnison, 2001), there is still very little empirical genetic evidence to reinforce the view that the observed adaptations are directly attributable to changes in gene frequencies, as opposed to being a product of phenotypic plasticity (Gienapp *et al.*, 2008).

Phenotypic Plasticity (PP) is commonly implicated in the success of species in novel climatic conditions (Przybylo *et al.*, 2000). Some assert that PP acts to shield populations from the selective pressures of environmental change, essentially acting as a buffer to evolutionary adaptations (Futuyma, 2009). However, others assert that

PP can play a role in driving diversification and speciation (Pfennig *et al.*, 2010). Indeed, it is increasingly accepted that various forms of phenotypic plasticity can help facilitate evolutionary adaptation in populations subjected to novel environments (Chevin *et al.*, 2010). Nonetheless, PP has limitations, which in the long-term can only be overcome by evolutionary, genetic adaptations (De Witt, 1998; Pigliucci, 2005; Auld *et al.*, 2010). Understanding the dynamics and limits of evolutionary adaptation and adaptation by phenotypic plasticity, including the interplay between the two mechanisms, is key to the development of effective conservation strategies in the face of sustained climate change.

Biological invasions can closely match the fundamental process of climate change; in that introduction of an organism to a new location with novel climatic conditions can constitute a rapid change in experienced climate - from native range to invasive range (Losos *et al.*, 2001). As such, biological invasions can be studied to elucidate the mechanisms by which organisms can adapt to climate change. A central theme to the research on biological invasions is the prediction of future invasions (Vander Zanden & Olden, 2008; Moran & Alexander, 2014).

The use of bioclimatic models is well established as a means of predicting distributions of species and they are regularly used to predict biological invasions (e.g. Soberón *et al.*, 2001; Peterson, 2003; Thuiller *et al.*, 2005; Ficetola *et al.*, 2007; Soberón & Peterson, 2011), however these methods are underpinned by a number of assumptions which are not always validated. Two relevant critical assumptions are: i) assumed niche conservatism, ii) genetic and phenotypic uniformity over space and time (see Jeschke & Strayer, 2008 for a more exhaustive list of assumptions). One

direct consequence of these assumptions is that such models often underestimate the size of the non-native range (Fitzpatrick *et al.*, 2007; Loo *et al.*, 2007; Broennimann & Guisan, 2008; Medley, 2010; Hill *et al.*, 2013), as the projected realised niche (only a sub-set of the fundamental niche) is not always conserved in the introduced range (Rödder & Lötters, 2009; Guisan *et al.*, 2014). The realised niche in the introduced range can represent an addition to and/or shift in the climate-space defining the realised niche in the native range. However, these modelling methods necessarily use the realised niche, based on presence location data, as there is insufficient data to model the fundamental niche.

Species with a large native range across a range of habitats and climates often include a high degree of genetic and/or phenotypic variation that corresponds to regional or historical adaptations (Oberdorff *et al.*, 2011). Where this is the case, introductions from a limited number of native source locations will result in an invasive founder population capturing only a small subset of the total variation. This founder effect could lead to genetically and/or phenotypically distinct populations across the invasive range, with direct implications on the observed climatic niche shifts (Lauzeral *et al.*, 2011; Barbosa *et al.*, 2013). Whilst patterns of genetic differentiation highlight important population processes, they also echo historical refuges and recolonization events (Hewitt, 1999; Habel *et al.*, 2005; Michaux *et al.*, 2005; Reimann *et al.*, 2009). For the development of bioclimatic models, it is important to fully understand whether the clines observed across a species' native range are the result of adaptation to climatic conditions (Sezgin *et al.*, 2004; Huestis & Marshall, 2006) or of neutral evolutionary processes (Kontula & Väinölä, 2004; Sotka *et al.*, 2004; Macholán *et al.*, 2007).

In order to investigate the possible implications of the genetic population structure of a species on potential climatic niche shifts, we focussed on the invasion patterns of the topmouth gudgeon *Pseudorasbora parva*, a small freshwater fish species widely introduced to Europe from Asia (Gozlan, 2012b). This species is a major vector of the rosette agent *Sphaerothecum destruens*, a growing fungal threat to freshwater biodiversity in Europe (Gozlan *et al.*, 2005; Andreou *et al.*, 2009; Gozlan *et al.*, 2009; Andreou *et al.*, 2011; Andreou *et al.*, 2012; Rowley *et al.*, 2013) and thus predictions of future invasion risks would be useful to conservationists. Its native distribution spans Japan, Korea and in China from the Amur to the Pearl river basin, covering a wide range of climatic conditions from harsh continental in the north to tropical in the south. As a species of no commercial value, its introduction in Europe is presumed to have been accidental, along with the intentional introductions of various Chinese carp species. It has become one of the most rapidly invasive freshwater fish in the world and represents a major environmental risk for the spread of fungal *S. destruens*, similar to that observed in amphibians with chytrid fungus (Gozlan, 2012a).

Systematic sampling across both the native and invasive ranges has identified genetically distinct mitochondrial lineages across the native range of *P. parva*, of which two have been introduced into the invasive European range (Simon *et al.*, 2001; Simon *et al.*, 2015; Hardouin *et al.*, 2018) This provides a framework to test whether the genetic lineages (i.e. prevalence within populations) have a bearing on invasion success. Specifically, we ask the questions: i) Do the two lineages introduced in Europe occupy distinct climatic niches in the native distribution? ii) Is there a climatic niche shift from native to invasive range of these two lineages' populations,

i.e. does the species conserve its climatic niche? iii) Do the introduced lineages occupy distinct climatic niches in the invasive distribution, i.e. are genetic lineages implicated in the invasive distribution of *P. parva*?

MATERIALS & METHODS

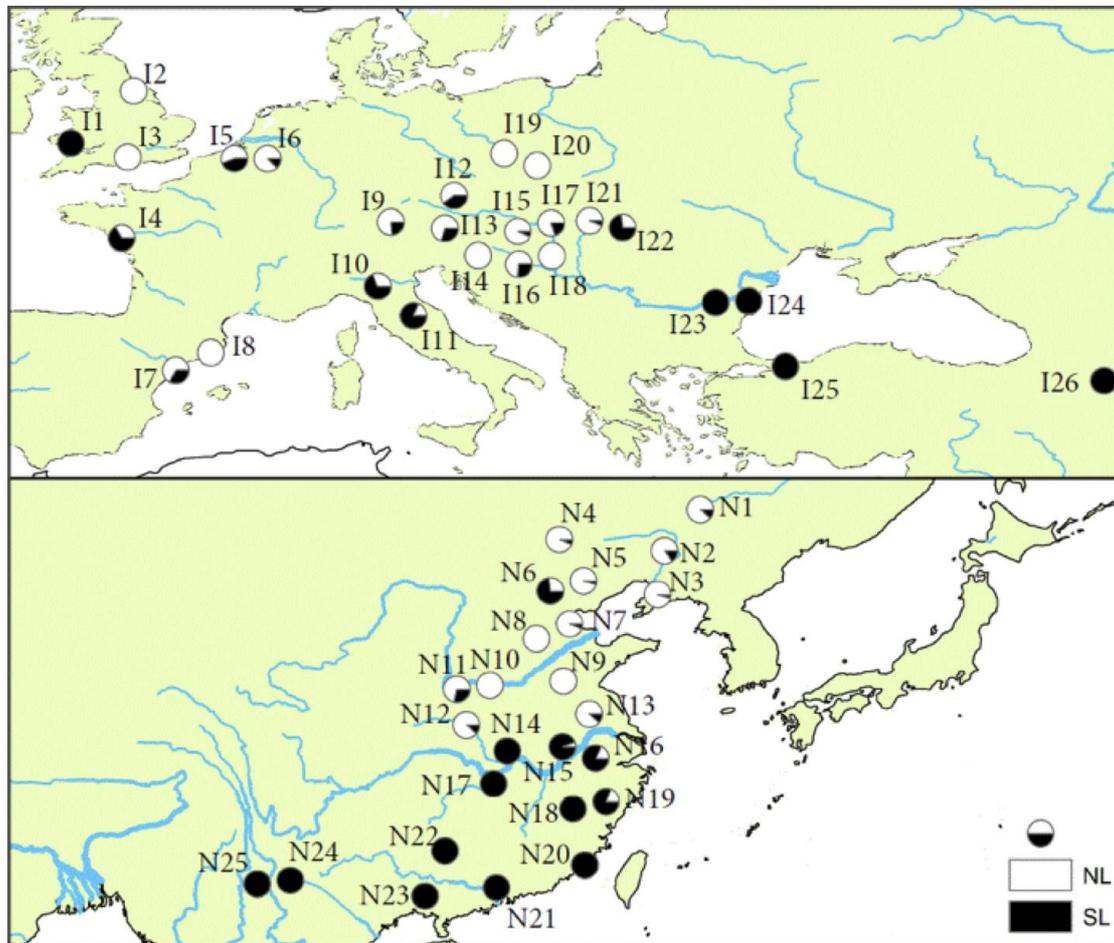
Occurrence data

Data on the locations of 2557 populations across both the native and invasive ranges were collected through a combination of annual national stock-assessments, as per Gozlan *et al.* (2010a), supplemented with data from the Global Biodiversity Information Facility (GBIF, 2013). Any points that were obviously incorrect (e.g. at sea) were removed from the final dataset. Finally, data were spatially harmonised to the same spatial resolution as the predictor variable layers (i.e. 10 km), leaving cell centre coordinates for cells within which presence records occurred. A total of 1262 10 x 10 km cells contained records of *P. parva* presence.

Lineage data

An expedition was undertaken to sample genetic material for 30 populations spread across China, Taiwan and Japan, covering the major catchments of the Amur River, Yellow River, Yangtze River, Pearl River and the majority of smaller intermediary catchments. Similarly, genetic material for 27 populations spread across the invasive range, from Iran to England, was obtained (see Gozlan *et al.*, 2010a). The relative proportions of lineages at each site (see Fig. 2.1) are based on mitochondrial DNA analysis conducted on our tissue sample collection (Simon *et al.*, 2001; Simon *et al.*, 2015; Hardouin *et al.*, 2018). As only two of the native Asian mainland lineages were found to have been introduced to the invasive range these were the only two lineages

used in these analyses and are referred to as “Northern Lineage” (NL) and “Southern Lineage” (SL). These names allude to the North/South distribution of the two lineages observed specifically in the native range on both side of the Yangtze River (see Fig. 2.1).



*Figure 2.1 Map displaying the locations of populations sampled and the identified lineages, including relative proportions depicted in pie charts, in both the native and invasive ranges identified by Simon *et al.*, 2011, 2015 and Hardouin *et al.*, 2018).*

Environmental Data

We included Bioclim 1950 – 2000 variables (Bio1 – Bio19, Hijmans *et al.*, 2005), topsoil pH (TpH), mean potential incoming solar radiation (INMSR), topographic wetness index (TWIS) as well as gross domestic product (GDP, Ghosh *et al.*, 2010),

in our initial predictive variables dataset (see Table 2.1). These variable datasets were chosen for their balance of biological relevance, coverage (coincident with occurrence records), and for a measure of likely sampling bias (i.e. GDP as a surrogate of human population density). We then used Variance Inflation Factor (VIF) analysis (Graham, 2003) to refine our final dataset, removing those variables displaying multi-collinearity. Our final refined dataset comprised 11 variables, which were given short codes (X1 – X11), for ease of labelling (see Table 2.1 for codes). All variables were used at a spatial resolution of 10 x 10 km.

Table 2.1 GIS layer datasets, used in analysis. Those with a short ‘code’ were retained for final analysis.

GIS data layer	Code	Source URL
Annual Mean Temperature	X1	www.worldclim.org/bioclimate
Mean Diurnal Range (Mean of monthly (max temp - min temp))	X2	www.worldclim.org/bioclimate
Isothermality (BIO2/BIO7) (* 100)	X3	www.worldclim.org/bioclimate
Temperature Seasonality (standard deviation *100)	-	www.worldclim.org/bioclimate
Max Temperature of Warmest Month	-	www.worldclim.org/bioclimate
Min Temperature of Coldest Month	-	www.worldclim.org/bioclimate
Temperature Annual Range (BIO5-BIO6)	-	www.worldclim.org/bioclimate
Mean Temperature of Wettest Quarter	X4	www.worldclim.org/bioclimate
Mean Temperature of Driest Quarter	X5	www.worldclim.org/bioclimate
Mean Temperature of Warmest Quarter	-	www.worldclim.org/bioclimate
Mean Temperature of Coldest Quarter	-	www.worldclim.org/bioclimate
Annual Precipitation	X6	www.worldclim.org/bioclimate
Precipitation of Wettest Month	-	www.worldclim.org/bioclimate
Precipitation of Driest Month	-	www.worldclim.org/bioclimate
Precipitation Seasonality (Coefficient of Variation)	X7	www.worldclim.org/bioclimate
Precipitation of Wettest Quarter	-	www.worldclim.org/bioclimate
Precipitation of Driest Quarter	-	www.worldclim.org/bioclimate
Precipitation of Warmest Quarter	-	www.worldclim.org/bioclimate
Precipitation of Coldest Quarter	-	www.worldclim.org/bioclimate
Topsoil pH (H2O) based on the Harmonized Worlds Soil Database	X8	www.worldgrids.org
Mean potential incoming solar radiation (8-day average) derived in SAGA GIS	X9	www.worldgrids.org
SAGA GIS Topographic wetness index	X10	www.worldgrids.org
GDP - derived from night-time lights (Ghosh <i>et al.</i> 2010)	X11	https://ngdc.noaa.gov

Ordination techniques – niche overlap, equivalency and similarity tests

Lineage data (i.e. NL or SL), along with range data (i.e. Native or Invasive) were used to define the groups: i) Native (Nat), ii) Invasive (Inv), iii) Native Northern Lineage (NNL), iv) Native Southern Lineage (NSL), v) Invasive Northern Lineage (INL), vi) Invasive Southern Lineage (ISL). These groups, henceforth referred to as entities, were analysed in a pair-wise manner (see Table 2.2 for list of entity pairs and respective PCA calibration data).

Table 2.2 List of entities compared and respective data used to calibrate each of the PCA-Env runs.

Entity a	Entity b	Calibration data
NNL	NSL	Native
INL	ISL	Invaded
NNL	INL	Native + Invaded
NSL	ISL	Native + Invaded
Nat	Inv	Native + Invaded

We calculated three commonly used metrics of niche comparison - overlap (Schoener's D, Schoener, 1968; Warren *et al.*, 2008), equivalency and similarity - following the Kernel Smoother ('KS') methodology of Petitpierre *et al.* (2012) and Broennimann *et al.* (2012). This methodology relies initially on calibrating a PCA on the entire environmental data for the region, or regions, of interest (i.e. 'PCA-Env' in Broennimann *et al.*, 2012). The regions of interest (native and invasive ranges) were delineated as all cells within a 1000 km buffer around occurrence data. The first two principal components (PCs) were then divided into a 100 by 100 grid, each cell representing a unique set of environmental conditions. 'Smoothed' occurrence densities (entities), in reduced-dimensionality environment-space (i.e. PC1 and PC2, from PCA-Env), were calculated by applying a kernel density function, which corrected for availability of environmental conditions in the study regions

(Broennimann *et al.*, 2012; Early & Sax, 2014) and occurrence densities were weighted, where applicable (i.e. lineage data), according to relative abundance of the lineage in question (e.g. at a location where NNL makes up 90% of the sampled population, the NNL occurrence is weighted at 0.9). All three niche comparison metrics were calculated on the ‘kernel smoothed’ niches, so as to give results that were unbiased by the arbitrarily selected resolution of the gridded environment-space (see Warren *et al.*, 2008). Equivalency and similarity were calculated using the same methodology and parameters as Broennimann *et al.* (2012), including the 100 randomization iterations, allowing the production of p-values for hypothesis testing. All statistical analysis was conducted using R (R Core Team, 2014).

Ecological Niche Models (ENMs)

We used reciprocal Ecological Niche Models (ENMs) to compare *P. parva*’s niche between the native and invasive ranges. We used an ensemble approach with seven modelling techniques; Artificial Neural Networks (ANN); Classification Tree Analysis (CTA); Flexible Discriminant Analysis (FDA); Generalised Boosting Model (GBM); Generalised Linear Model (GLM); Multiple Adaptive Regression Splines (MARS); Random Forests (RF), implemented in the R package ‘biomod2’ (Thuiller *et al.*, 2014). The use of GLM, CTA and ANN are described and discussed in (Thuiller, 2003). GBM is a modelling method that incorporates interactive relationships between predictor variables, as well as being able to discern complex response curves (Wisz *et al.*, 2008). MARS, along with ANN are very good at deciphering complex relationships (Moisen & Frescina, 2002) and RF models have been found to perform consistently well when evaluated against various other established methods (Prasad *et al.*, 2006).

As our data consisted only of occurrences, pseudo-absence data were generated for use in the ENMs. The pseudo-absences were generated using a random selection of locations within a ring-buffer around occurrence points (minimum distance = 100 km, maximum distance = 500 km). This ensured that only locations within a region that had definitely been surveyed were used, which increases the likelihood that the assumption of absence is correct and reduces possible bias from uneven sampling densities (Phillips *et al.*, 2009). Five of these random pseudo-absence datasets, each consisting of 1000 points, were generated for each range in order to increase the total climatic variability captured. So as to remove any bias due to differences in presence locations in each group, the pseudo-absence datasets were weighted to give equal prevalence in each replicate model run.

Evaluation of model accuracy (predictive power) was performed using the Area Under Curve (AUC) of the Relative Operating Characteristic (ROC) procedure, with a 75:25 split between training and evaluation data. To compensate for any spatial autocorrelation between training and evaluation data (Thuiller *et al.*, 2009), this cross-validation process was carried out five times for each individual model, trained on each of the five random pseudo-absence datasets, then averaged to provide a value for a version of the model trained using 100% of the data. Final predictions were produced using a weighted average of the seven algorithms, with the AUC evaluation scores as the weights. In order to compare the models across ranges (N / I) we used the occurrence and pseudo-absence data from the Native-trained models to evaluate the Invasive-trained models and *vice versa*, thus providing reciprocal evaluation of each model.

RESULTS

Niche comparisons of lineages in native range

Analysis of the ‘smoothed’ niche entities in the native range (Fig. 2.2) showed that the niches of the two lineages within the native range (NNL and NSL), exhibit relatively low overlap (Schoener’s $D = 0.196$). 63.8% of the variation in environmental variables was captured in the first two principal components (PCs). The first PC captured 42.5% and was also the axis on which the most marked separation of the lineages occurred. PC1 is dominated by three key variables; X5 – mean temperature of driest quarter, X1 – annual mean temperature, X6 – annual precipitation, in order of diminishing contribution. The NSL’s niche is characterized by higher values of these three variables (i.e. hotter and wetter). Randomization tests of niche equivalency and similarity showed that the niches of the NNL and NSL are not equivalent ($p < 0.05$), but they are significantly more similar to one another than randomly selected locations from within the native range ($p < 0.05$).

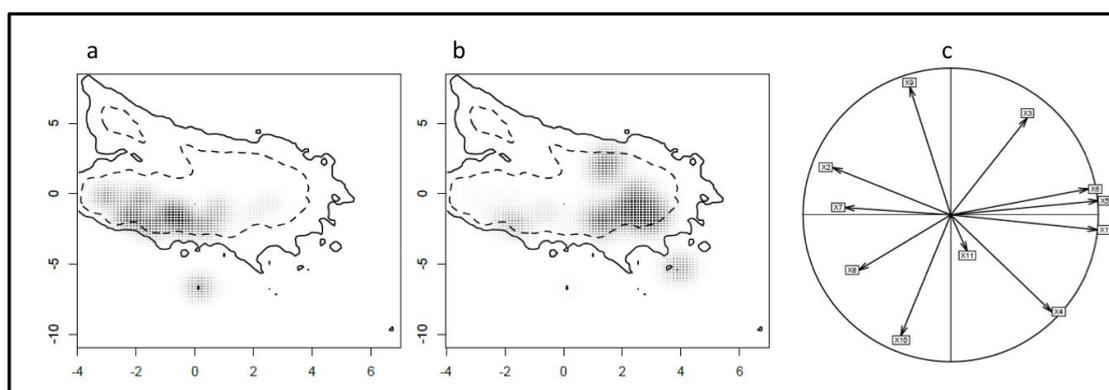


Figure 2.2 Kernel-smoothed output of PCA trained on data from native range, depicting bioclimatic niche of Native Northern lineage (a), Native Southern Lineage (b) and correlation circle of the PCA (c). Solid and dashed contours represent 100% and 50% of background environmental data.

Reciprocal ENMs

It is apparent from visual inspection of the prediction outputs from the Ensemble Models (EMs, Fig. 2.3 a, b, d & e) that models trained on native occurrence data produce underestimates of the invasive range, particularly in the western parts, and the models trained on invasive range data produce underestimates of the native range, particularly in the southern areas. For the EM calibrated using native data, the predicted suitability values for most of the invasive range (Fig. 2.3 a) are higher than expected (i.e. widespread values of 3-6, out of a possible 10), giving a higher chance of false positives (i.e. low specificity). Low predictions (< 3) are not widespread and are largely confined to mountainous regions (e.g. the Alps, Carpathians and Balkan mountain ranges). Although the eastern parts of the invasive range receive underestimates, higher values in western parts are largely in the correct places – notably in coastal areas Italy and Spain, the River Seine catchment in France, England, particularly the south-east, and western Hungary. However, there are only a handful of very small areas receiving predictions greater than seven, demonstrating that predictions of suitability from this model seem compressed (i.e. low variation) in the invasive range.

Possible areas of overlap exhibited in the output from the EM calibrated using invasive data (i.e. higher suitability areas in Fig. 2.3 e) are much more pronounced, although many areas in the native range where the species is known to occur are omitted, particularly in the south of China, but also around Beijing and Liaoning provinces. Central regions, notably Henan province, receive higher predictions of suitability (hence environmental similarity) from the EM trained on data from the invasive range, when compared to the output from the EM trained on native data.

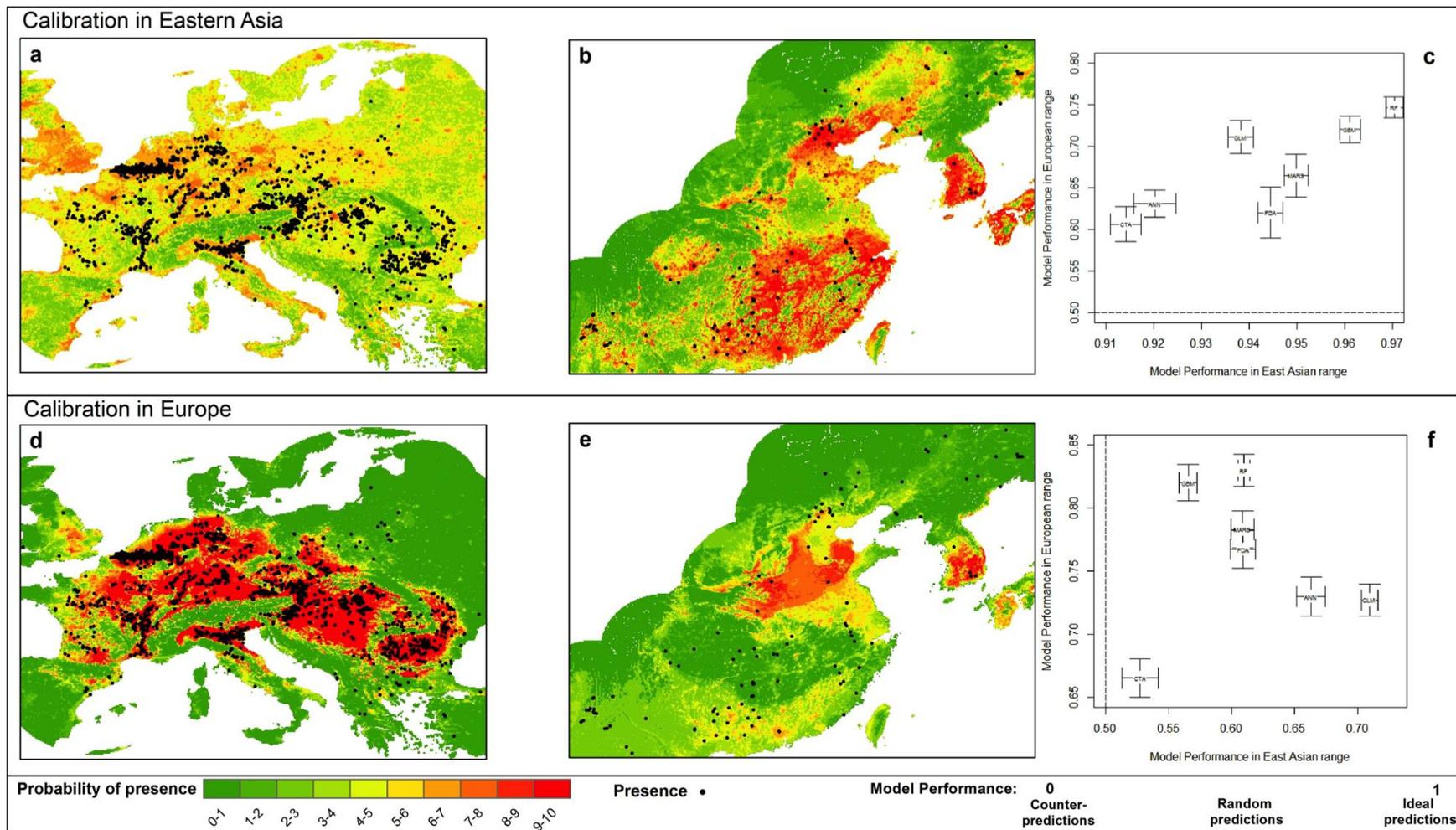


Figure 2.3 Reciprocal ecological niche model prediction maps and model evaluations. The upper and lower panes show, respectively, the prediction outputs from the Ecological Niche Models (ENMs) calibrated using native (upper) and invasive (lower) range data, projected in the native (boxes b and e) and invasive (boxes a and d) ranges. The graphs show model performance (Area Under Curve of the Receiver Operating Characteristic) of the individual models making up the Ensemble ENMs trained using native (box c) and invasive (box f) data. Error bars represent standard deviations, based on nine repetitions on random re-sampling of data.

EMs calibrated using native data scored higher on average in reciprocal evaluations using AUC than EMs calibrated using invasive data (Fig. 2.3 c & f). Variation in performance was greater in EMs calibrated using invasive data than those calibrated using native data. However, of all the methods used, only GLM trained on invasive data achieved an AUC score higher than 0.7 (widely considered the threshold for good predictions). Whilst it scored consistently higher than this threshold (0.71 ± 0.006), it also consistently failed to predict *P. parva* presence in the central part of the native range. When trained on native data, three of the modelling methods (GLM, GBM and RF) scored over 0.7 in reciprocal evaluations, although none of the scores were considerably higher than the threshold (0.71 ± 0.02 , 0.72 ± 0.02 and 0.74 ± 0.01 respectively) and all models under-predicted in the eastern parts of the invasive range. The ENMs generally highlight the low level of overlap between the native and invasive niches, with mean reciprocal evaluation scores of 0.67 ± 0.02 and 0.61 ± 0.01 for native-trained and invasive-trained ENMs respectively.

Niche comparisons between native and invasive ranges

Analysis of the ‘smoothed’ niche entities of the lineages and undifferentiated data (NNL vs INL – Fig. 2.4, panel 1; NSL vs ISL – Fig. 2.4, panel 2; Native vs Invasive – Fig. 2.4, panel 3) showed that there was low overlap ($D = 0.167$, 0.145 and 0.09 , respectively). 47.62% of the environmental variation was captured by the first two PCs. The first PC captured 28.32% of environmental variability and was also the axis on which the most marked separation occurred, in both northern and southern lineages as well as for undifferentiated data. PC1 is dominated by three key variables; X5 – mean temperature of driest quarter, X1 – annual mean temperature, X6 – annual precipitation, in order of diminishing contribution. The INL’s niche is characterized

by higher values of these three variables (i.e. hotter and wetter), when compared to that of NNL. The ISL's niche is characterized by somewhat lower values of these three variables, when compared to that of NSL. The invasive, undifferentiated, niche is characterized by having medium values for these three variables, falling between values characteristic of the NNL and NSL niche entities (Fig. 2.4, panels 1a and 2a).

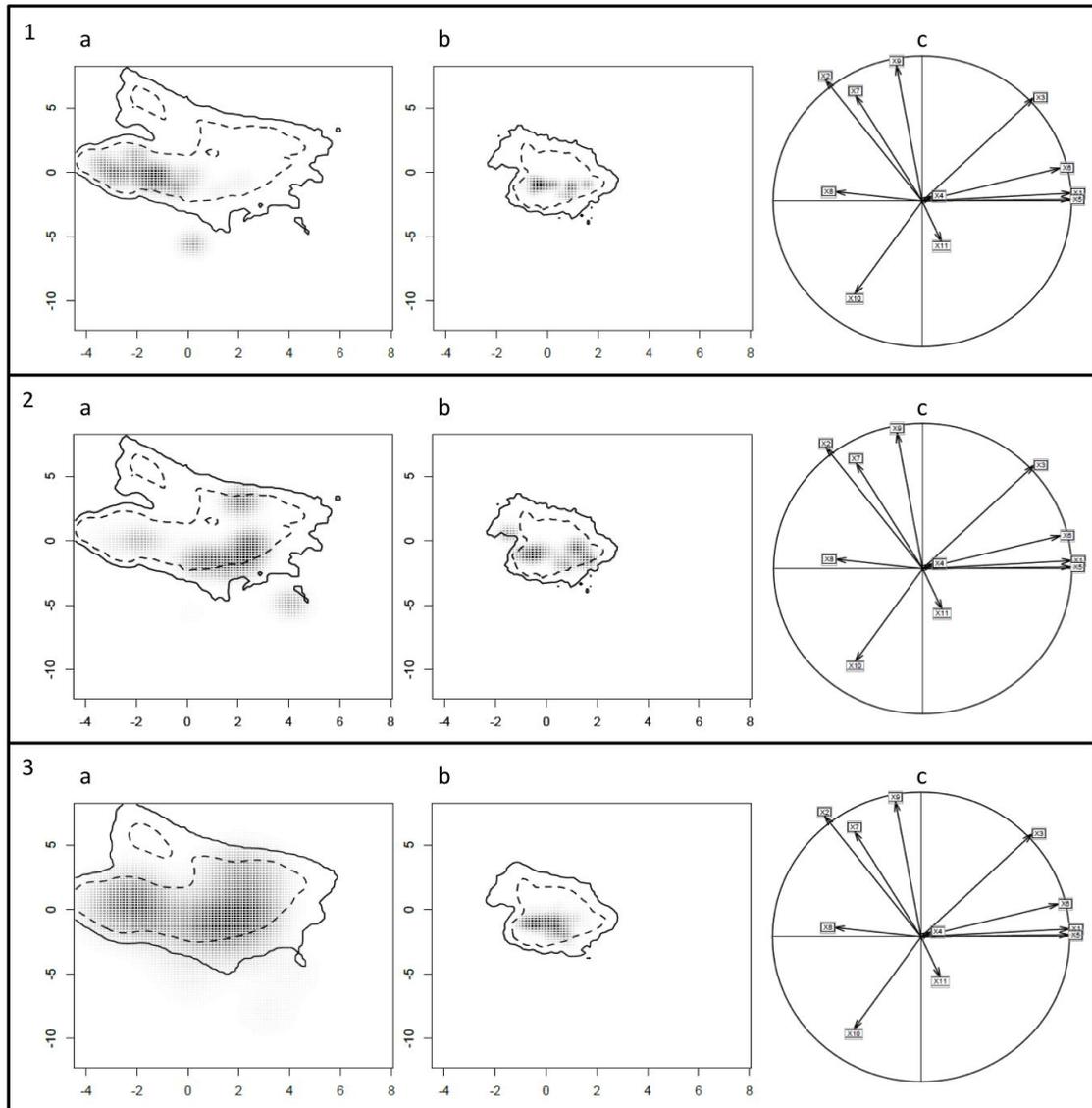


Figure 2.4 Kernel-smoothed output of PCA trained on data from native and invaded ranges, depicting bioclimatic niche of Native Northern lineage (panel 1, a), Invasive Northern Lineage (panel 1, b), Native Southern Lineage (panel 2, a), Invasive Southern Lineage (panel 2, b), undifferentiated Native niche (panel 3, a), undifferentiated Invasive niche (panel 3, b) and corresponding correlation circles (1 c, 2 c & 3 c). Solid and dashed contours represent 100% and 50% of background environmental data.

Randomization tests of niche equivalency showed that none of the niches were equivalent ($p = 0.02$ for all comparisons). Test of niche similarity showed that the niche of NNL was not significantly more similar to that of INL than random, nor INL to that of NNL, whilst NSL was not more significantly similar to ISL than random, but ISL was significantly more similar to NSL ($p = 0.02$) than random. Similarly, for the smoothed niche entities of the undifferentiated whole species data, native vs invasive similarity was not significantly greater than by random, however invasive vs native was significantly ($p = 0.04$) more similar than random. Looking at the PCA outputs, there appears to be a region in environment-space that is not occupied in the native range and falls between the two lineages' smoothed niche entities. This region corresponds, broadly, to the combined smoothed niche entities in the invasive range.

Niche comparisons of lineages in the invasive range

Analysis of the 'smoothed' niche entities in the invasive range (Fig. 2.5) showed that the niches of the two lineages within the invasive range (NNL and NSL), exhibit relatively high overlap (Schoener's $D = 0.645$). 50.72% of the variation in environmental variables was captured in the first two principal components (PCs). The first PC captured 30.08% and is dominated by five key variables; X5 – mean temperature of driest quarter, X2 – mean diurnal temperature range; X3 – isothermality; X8 - Topsoil pH, X1 – annual mean temperature, in order of diminishing contribution. Randomization tests of niche equivalency and similarity showed that the niches of the INL and ISL are not equivalent ($p < 0.05$), but they are significantly more similar to one another than to randomly selected locations from within the invasive range ($p < 0.05$ for both directional comparisons).

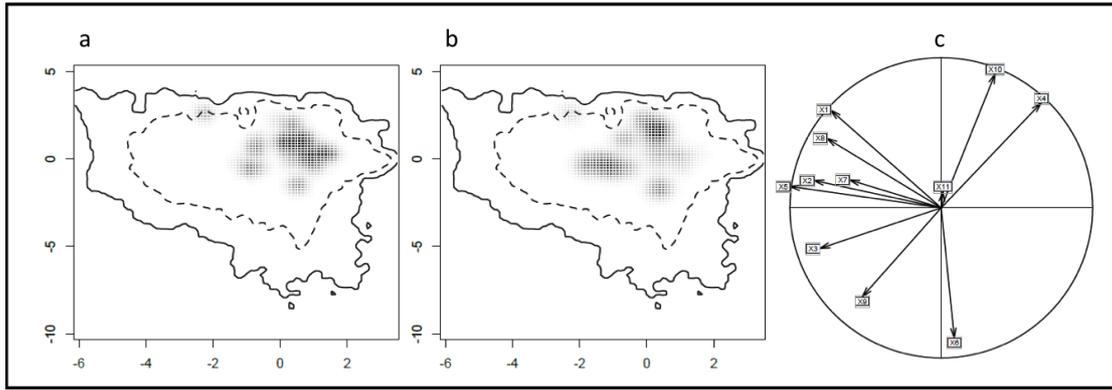


Figure 2.5 Kernel-smoothed output of PCA trained on data from invasive range, depicting bioclimatic niche of Invasive Northern lineage (a), Invasive Southern Lineage (b) and correlation circle of the PCA (c). Solid and dashed contours represent 100% and 50% of background environmental data.

DISCUSSION

The northern and southern lineages used in our study were identified by Hardouin *et al.* (2018) and their separation dated at approximately 2.5 MYA. Regardless of whether these lineages adapted to their respective differentiated climatic niches ($D = 0.196$) subsequent to separation, they both appear equally capable of surviving a rapid shift in experienced climatic conditions, i.e. the initial introduction to Europe. Initial introductions of these two lineages from the native Chinese range are thought to have been into Romania (1961), Hungary (1963), Lithuania (1963), and the Ukraine (1962) (Gozlan, 2012b). Hungary plays host to both northern and southern lineage dominated populations as well as a pure northern lineage population (see Fig. 2.1). The native trained ENM typically predicts low suitability values for these locations (Fig. 2.3a), which suggests that the environmental conditions here are dissimilar to the conditions found in the native range. Either individuals from both lineages were predisposed to tolerate the novel conditions of the sites where they were initially introduced (i.e. these conditions constituted part of their fundamental climatic niche), or some

mechanism enabled the individuals, upon their introduction, to survive these novel conditions, thus expanding or shifting their niche. In addition, neither lineage exhibits any apparent sign of a competitive advantage over the other in the range of climatic conditions they inhabit in the invaded range, as evidenced through the high level of niche overlap ($D = 0.645$) of the two lineages' invasive climatic niches.

Any reduction in stress associated with the removal of, or change in, biotic interactions could enable individuals or populations of an introduced species to inhabit climatic conditions that might otherwise be too physiologically costly to occupy. A number of well-established hypotheses concerning the success or failure of biological invasions relate to biotic interactions, i.e. interactions between the introduced species and the recipient community. For instance, the 'enemy release' hypothesis posits that introduced non-native species become invasive because of the lack of consumer control in the recipient system (Darwin, 1859; Elton, 1958). However, as non-native introduced species may have no shared evolutionary history with the species of the recipient system, they may lack adequate defensive strategies or adaptations, i.e. the 'new associations' or 'increased susceptibility' hypotheses (Hokkanen & Pimentel, 1989; Colautti *et al.*, 2004). The 'biotic resistance' hypothesis suggests that higher species diversity in the recipient community offers a greater degree of resistance against successful establishment of a non-native introduction (Elton, 1958). Conversely, the 'biotic acceptance' hypothesis works on the basis that native and non-native species diversity are positively correlated with one another (Stohlgren *et al.*, 2006).

Because of the potential complexity of biotic interactions and also because suitable data, of the scale and coverage necessary, were not available, it was beyond the scope

of this study to investigate possible causal links between niche shift and biotic interactions. However, biotic interactions may not always be as important to the success of biological invasions in aquatic systems as in terrestrial systems, particularly freshwater systems. For instance, a number of examples from aquatic invasions in the North American Great Lakes suggest that success of such invasions is dictated more by dispersal opportunities and abiotic conditions than by characteristics of the recipient community (Ricciardi & MacIsaac, 2000), which is reinforced by more recent work by (Stohlgren *et al.*, 2006). Further, a global study of fish invasions in 1,055 river basins covering more than 80% of the Earth's surface, found no support for either 'biotic acceptance' or 'biotic resistance' in explaining patterns of aquatic invasions, instead finding that non-native invasions are most closely related to human activity indicators (Leprieur *et al.*, 2008). As our study was conducted at a similar spatial scale, we see no reason why the omission of biotic data would significantly prejudice the results.

Other possible explanations for the apparent niche shifts of the two lineages in the invasive range are Rapid Evolutionary Adaptation (REA) and Phenotypic Plasticity (PP). REA has been implicated in a number of biological invasions, which represent some of the best-documented examples of the phenomenon, e.g., Ellstrand & Schierenbeck (2000) and Quinn *et al.* (2000). However, evidence from the invasion literature suggests that REA in response to geographic clines, e.g. climate, generally occurs 50-150 generations subsequent to initial introduction, and only occasionally in <25 generations (Moran & Alexander, 2014). The invasion of Europe is the product of multiple initial long-distance translocations from the native range (Gozlan *et al.*, 2010a) into novel environmental conditions, and populations quickly became established in many areas, with individuals surviving large, rapid shifts in experienced

climatic conditions. Because of the short timeframe of establishment, they must have utilised some mechanism other than REA, which would not be feasible over such a short time period.

Whilst we can neither prove nor disprove that there may have been some degree of REA subsequent to the European introductions, we suggest that it is unlikely that the initial survival and establishment of these populations was facilitated by such a mechanism, and instead assert that PP is a far more feasible and parsimonious explanation than REA. Invasive species, such as *P. parva*, are often characterised by a high degree of phenotypic plasticity (Davidson *et al.*, 2011) and this could be implicated in the species' broad climatic tolerances, including those observed in niche shifts exhibited during the process of invasions.

Further to the lack of relative niche differentiation in the invaded range of the two lineages, we show that the realised climatic niche of the native range constitutes only a subset of the fundamental niche, distinct from that of the realised niche in the invaded range ($D = 0.09$). This highlights the significant potential of some species to survive beyond the native realised niche, even when these are extremely broad, rendering ENMs based on the native range unsuitable for predicting the fate of this type of species in a climate change scenario because of the assumption of niche conservatism typically implicit in these methods. It is also an important point to note because, for some species, current predictions of fate under climate change may be inaccurate if this is not taken into account.

Our results also highlight that observed spatially and climatically distinct lineages are not necessarily a product of climate-based selection but could instead be the product

of physical segregation, which through neutral genetic drift has led to allopatric divergence. In such instances, it could be the case that current climatic conditions severely under-represent the potential of the species in question, which could in fact include in its fundamental niche conditions to which it has not been subjected for many generations. Here, the fact that both lineages, when introduced to the invasive range, occupied essentially the same climatic niche space shows that despite being separated 2.5 MYA (see Hardouin *et al.*, 2018) and subsequently occupying relatively distinct climatic niches in the native range, both lineages now apparently possess the same capability to survive in climatic conditions outside those found in their native niches. It could be that the invasive potential of *P. parva*, as a species, is a result of retained ancestral physiological tolerances to conditions that were inhabited prior to the separation of the two key invasive lineages 2.5 MYA (see Hardouin *et al.*, 2018).

Whilst long-term retention of physiological tolerances could be a characteristic of some species, others exhibit contrasting qualities, where adaptation to localised conditions is firmly conserved, to the detriment of broader tolerances. For instance, a number of experimental studies have investigated intraspecific adaptations of animal and plant populations to localised climatic conditions (see Hereford, 2009) and some reciprocal transplant studies have observed marked local adaptations to climatic conditions in studied tree species (Rehfeldt *et al.*, 1999; Savolainen *et al.*, 2007). For instance, Savolainen *et al.* (2007) observed that survival in northern populations of *Pinus sylvestris* transplanted $> 4^{\circ}$ northwards dropped to zero, even though populations of local provenance persisted in the same conditions. These kinds of adaptation are not restricted to plants, with Kavanagh *et al.* (2010) identifying intraspecific adaptations of individual growth and developmental rates in populations of grayling (*Thymallus thymallus*), a Nordic freshwater fish, between shaded, colder

streams and non-shaded, warmer streams, all connected to the same lake. For species displaying a narrow, tightly conserved climatic niche, using ENMs trained on data for the whole species, rather than smaller demographic units, might give a distorted picture in the context of invasion potential, as this potential would depend strongly upon the source of the introduction. Similarly, modelling such an organism at a whole-species level in order to predict its fate under climate change could considerably under-predict resultant mortality.

For such species displaying population-level, discrete and conserved climatic niches, the relevant biological units upon which to base predictive ENMs are the populations. It is thus important to be able to differentiate between those species with populations exhibiting discrete, tightly conserved narrow climatic niches and those species that display high phenotypic plasticity and broad climate niche flexibility, as it dictates the relevant demographic scale at which to base predictive ENMs (i.e. the relevant demographic/biological unit) and also because high phenotypic plasticity is thought to be a likely indicator of invasiveness (Gozlan *et al.*, 2010b).

A major step in support of this differentiation is to be able to test whether the observed genetic differentiation at a population level is a product of climatic selective forces, or merely a by-product of allopatric genetic drift. Developments in quantitative genetics and molecular biology have made it possible but some of these techniques (Whitehead & Crawford, 2006) can be restrictive in terms of the data required and are often not robust to demographic factors (Nielsen, 2005). Nonetheless, there are still challenges beyond that of identifying the appropriate biological units for niche modelling, as our study has highlighted.

The problem of ‘range under-filling’ (i.e. the lack of niche realisation caused by dispersal constraints) can still potentially remain and confound ENMs even if the relevant biological unit has been considered (Petitpierre *et al.*, 2012). For instance, if we identified that the two mainland Chinese lineages of *P. parva* are the likely product of neutral drift and thus model their potential distribution as one single homogeneous biological unit (i.e. the whole species), we still severely under-predict the species’ potential in the novel climatic conditions of Europe (see Fig. 2.3a). Consequently, there are a number of potential factors explaining the lack of realisation of the fundamental niche within the native range. For instance, parts of the fundamental niche are likely to be unrealised in the native range, either because physical barriers or biotic interactions prevent occupancy within areas where suitable climatic conditions exist. This is particularly true for freshwater fishes that are constrained by their catchments. Furthermore, as previously mentioned, it could be possible that some species retain physiological tolerances over long periods of time, in a cryptic manner (e.g. Schulte *et al.*, 2012). The inclusion of experimental data and mechanistic models in predictions of organisms’ potential to survive beyond their current realized niche is therefore necessary. A number of recent studies have shown that traditional ENM and correlative approaches, augmented with mechanistic models based on experimental data relating to organisms’ physiological tolerances, can improve prediction accuracy (Guisan *et al.*, 2006; Morin & Thuiller, 2009) and thus provide more realistic projections for the fate of species or populations under climate change scenarios.

CONCLUSIONS

Our study highlights that a number of confounding factors may prevent us from obtaining accurate predictions of species current potential climatic niche through

traditional correlative ENM techniques. In particular: i) The existence of physical barriers to dispersal which are unaccounted for. ii) Potential cryptic niche conservatism i.e. retained physiological tolerances from historic exposure to different climates. iii) Tightly conserved localised adaptation to climatic conditions. In the context of physical barriers to dispersal, all too often the importance of the absence component of these modelling techniques is downplayed, with conventional wisdom suggesting that restricting pseudo-absence data to outside the species known climatic envelope is sufficient to obtain realistic predictions (Austin & Meyers, 1996; Le Maitre *et al.*, 2008). However, for those species whose distributions are severely restricted by their local geography (for instance, those occupying islands) we could never hope to obtain a realistic representation of the true potential of the species to survive in novel climates using these methods. The inclusion of mechanistic models based on physiological attributes is showing significant promise in increasing the realism and accuracy of methods providing predictions relating to species' potential to persist in novel climatic conditions (Guisan *et al.*, 2006; Morin & Thuiller, 2009) and may provide a significant contribution to addressing the three above confounding factors, as well as complementing studies of niche evolution (including REA). However, this development brings with it the need for more data and better understanding of physiological requirements and limitations.

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CHAPTER III

Thermal impact on life history traits of invasive fish populations: an experimental approach.

ABSTRACT

The risk-based management of non-native fishes is reliant on understanding their potential invasiveness, which is largely determined by the expression of key life history traits (LHTs) in the recipient environment. Understanding how these key LHTs vary with environmental conditions allows better predictions of invasive potential. Here, we ran a laboratory experiment to quantify responses of reproductive output of adults and early growth in their progeny at three temperatures (15 °C, 20 °C and 25 °C) for the invasive species *Pseudorasbora parva*. We found that body size, not temperature, determines gross reproductive output across the full breeding season, with larger individuals more fecund. However, temperature affects temporal reproductive strategy, with more protracted, lower peak, reproductive output at lower temperatures and more rapid, short-lived, higher peak reproductive output at higher temperatures. Early growth rates in progeny increased with temperature, and were significantly higher at 25 °C compared to 15 °C. These findings have strong implications for the management of introduced fish populations, particularly in terms of seasonality of management interventions, and we suggest that future studies combine the factors of temperature and food rations to further develop our understanding of how these factors interact to influence invasion success.

INTRODUCTION

Biological invasions are one of the most pervasive contemporary threats to global biodiversity (McGeoch *et al.*, 2010). Increased global connectivity and trading has led to the systematic introduction of species into naïve systems and communities (Hulme, 2009; Levine & D'Antonio, 2003), often with profound implications (e.g. Edelist *et al.*, 2013; Ricciardi & MacIsaac, 2000). Negative impacts can manifest through a variety of mechanisms (Pimental *et al.*, 2005; Gozlan *et al.*, 2010a), including competition and predation (Gallardo *et al.*, 2016), hybridization (Brennan *et al.*, 2015), and novel pathogen transmission (Gozlan *et al.*, 2005; Jussila *et al.*, 2015). The isolated nature and high endemism of freshwaters result in these ecosystems being at disproportionately high risk from invasions (Dudgeon *et al.*, 2006; Moorhouse & Macdonald, 2015). Whilst invasions in freshwaters can develop from introductions of non-native fish from a range of pathways, the global aquaculture trade is recognised as the principal pathway (Gozlan *et al.*, 2016).

The risk-based management of non-native fishes is reliant on understanding the ability of particular species to establish, impact and disperse in the novel environment, i.e. its invasiveness (Britton *et al.*, 2010b; Britton *et al.*, 2012). Fundamental to such understandings are analyses of life-history traits (LHTs), as these tend to be strong predictors of establishment success (Vila-Gispert *et al.*, 2005; Olden *et al.*, 2006), especially for species exhibiting high plasticity (Davies & Britton, 2015). Thus, understanding how the LHTs of introduced fishes vary over time and space, and across a range of environmental conditions, enables us to predict the invasion risk (Cucherousset *et al.*, 2009; Gozlan *et al.*, 2010a). Some studies have compared LHTs of introduced non-native fishes within regions (e.g. Iberia; Garcia-

Berthou, 2007), of species between their introduced and native ranges (e.g. *Micropterus salmoides*, Britton *et al.*, 2010c; *Cyprinus carpio*, Oyugi *et al.*, 2010), and of non-native fishes across their introduced range (e.g. *Gambusia holbrooki*, Benejam *et al.*, 2009; *Lepomis gibbosus*, Copp & Fox, 2007). However, most of these studies have been based on wild populations in field conditions, limiting precise inferences into the response of introduced founders from one region to another of similar or contrasting climate due to the lack of control and measurement of the environmental conditions.

Following an introduction, a pre-requisite for the founders to survive and then establish is the presence of suitable environmental conditions that enables LHT expression to facilitate establishment (Moyle & Light, 1996; Fletcher *et al.*, 2016). For freshwater fishes, these conditions are strongly influenced by climate and, in lentic habitats, this will be primarily temperature related (Crozier & Hutchings, 2014). General patterns are of faster growth and earlier maturity in more southerly latitudes where water temperatures tend to be higher (Benejam *et al.*, 2009; Cucherousset *et al.*, 2009; Oyugi *et al.*, 2010). Whilst most studies on the invasive potential of non-native species consider the climate pattern in their native range and transpose them on to non-native regions (e.g. Gallardo & Aldridge, 2013; Fletcher *et al.*, 2016), there has been less focus on secondary introductions within the invasive range. Yet, for many invasive species, especially those that are moved within global trade (such as global aquaculture and ornamental trades), new introductions often involve species being moved within their non-native range (Gozlan *et al.*, 2010b). These populations will already have adapted to new conditions following initial introductions, in an ‘invasive bridgehead effect’ (see Lombaert *et al.*, 2010) with, for example, shifts in their reaction norms that can represent climatic niche shifts (e.g. Broennimann *et al.*, 2007;

Tingley *et al.*, 2014; Medley, 2010). Thus, where numerous populations of a non-native species have adapted to the local novel climatic conditions of their recipient systems (e.g. temperature regime; Kavanagh *et al.*, 2010), these local adaptations would mean that provenance could strongly influence their invasive potential in any given new, secondary location of introduction/translocation (e.g. Rey *et al.*, 2012).

The topmouth gudgeon *Pseudorasbora parva* is a highly invasive freshwater cyprinid fish in Europe, having gained a pan-continental distribution since the 1960s (Gozlan *et al.*, 2010b; Gozlan, 2012). Its Asian native range spans much of China, parts of Russia, Japan, Taiwan and the Korean peninsula, with movement to and within Europe being strongly associated with the aquaculture trade (Gozlan *et al.*, 2010a). It now inhabits a wide range of climatic conditions across its invasive range, from highly seasonal continental European climates (e.g. Poland) to warmer Mediterranean regions (e.g. Spain and Morocco) and temperate maritime climates (e.g. Great Britain). These climates generally represent novel conditions when compared to those occupied in the Asian native range, where the species encounter tropical climatic conditions in south China and harsh continental ones in the North (see Chapter II). If these invasive populations of *P. parva* have adapted for optimum performance in their respective climates, particularly during the warmer months that cover their reproductive period and growth season, it would be expected that there will be differences in their performance response across temperature gradients. For example, fish adapted to more continental climates (e.g. Poland) would be predicted to be poorly adapted to cooler temperate climates (e.g. Great Britain).

The aim of this study was to test, under common garden conditions, the plastic responses and adaptive potential of *P. parva* over a temperature gradient. The

responses measured were the reproductive output (as fecundity) of adults and the growth performance of their progeny using a British population (temperate climate), and two populations from Poland (continental climate). It was predicted that reproductive outputs and early growth would be optimal at higher temperatures, with fish from continental climates showing low adaptation to temperatures that represent temperate climates (i.e. cooler breeding season).

MATERIALS & METHODS

Sources of adult fish

Adult fish were sourced from three geographical locations. Two populations were sampled from a continental climate in Poland: population 'PL1' (N: 51 42`54.45", E: 19 30`46.99") and 'PL2' (N: 52 16`3.9", E: 21 17`42.11"), and one population from a temperate climate in England, 'UK' (N: 51 00`00.1", W: 1 27`01.1"). For all populations but the Polish population, which arrived in the laboratory in June, sampling was conducted in late summer 2013, using a combination of electro-fishing and baited fish traps. Following their capture, adult *P. parva* (identified as fish > 35 mm) were transported from sampling locations in sealed large plastic bags, containing approximately 25 L of water, with 15 L of oxygen added, kept cool and transported to the aquarium facility.

Aquarium facility and initial holding conditions

The aquarium facility at Bournemouth University, Southern England, comprised of a discrete room where light: dark cycle and water temperatures could be controlled. The fish were initially held in 90 L tanks, arranged in a column of three tanks on a

recirculating flow through filtration system (2.5 L/min) for the maintenance of water quality. In-line refrigeration units and heaters were used to control and maintain water temperatures. Aquaria and pipes were insulated to minimise temperature drop across systems, which was maintained at a differential, from flow entry to exit, of 0.5 °C (see Fig. 3.1).



Figure 3.1 Photograph showing the experimental setup, with reservoirs and refrigeration units at the base and experimental units within aquaria, divided by green Corex™ sheets.

In order to be able to measure the subsequent reproductive output of the adult *P. parva*, they had to experience an initial ‘winter’ period for gonadal development. Thus, following initial conditions of 18 °C for 14 days when they were brought into the aquarium facility, their water temperature was continuously decreased at a rate of 0.5 °C every two days until reaching a consistent 5 °C. This temperature was then maintained for 100 days, at a 10:14 hour light:dark cycle, before commencing the

experimental procedures. These winter conditions were similar to those experienced by these populations in the wild.

Experimental design

Each experimental system comprised a stack of three 90 L tanks in a recirculation configuration, with in-line filtration and heater/chillers, to control temperature. Each 90 L tank was separated into five equal compartments, using Corex™ plastic sheeting, which defined reproductive units of two females and one male. Each of the compartments were kept bare, but included an upturned 120 mm section of plastic guttering, to act as a refuge/nest (Fig. 3.2). The top two tanks in each system (i.e. ten compartments) were available for reproductive output assessments and the bottom tanks (i.e. five compartments) were used for growth assessments. In total, nine systems were prepared, one at each of the three temperatures (15 °C, 20 °C and 25 °C) for each of the three populations. Due to limitations in the number of either males or females, depending on population, it was not possible to have a full ten replicates for each population at each temperature treatment (see Table 3.1 for details of replicates).



Figure 3.2 Photograph showing experimental unit, with the male guarding the nest, made from an upturned length of plastic guttering.

Table 3.1 *The number of replicates used per temperature treatment and population. Two populations from Poland (PL1 & PL2) and one from England*

Temperature treatment (°C)	Source population		
	PL1	PL2	UK
15	7	4	9
20	7	0	10
25	7	4	10

Fish were sexed, counted and placed into their experimental compartments at 5 °C, to match their holding conditions, and maintained for a minimum of 10 days to acclimate to the new surroundings. The lighting timer was set to a 12:12 light:dark cycle, at which point temperatures were increased by 1 °C per day, until the target temperatures were reached. Temperature increases were staggered so that target temperature attainment was synchronised for all systems. Fish were fed *ad libitum* on a daily basis, on ‘Skretting Nutra Plus Fry Feed’, and any uneaten food removed.

Data collection

Laid eggs were counted daily, and recorded for each of the active experimental units, for a total duration of 125 days from target temperature attainment. This was completed using digital photography (Nikon AW100 16.0 MP) and manual counts of individual eggs from the captured images. These egg count data were used to quantify the reproductive output of the fish through ‘clutch size’ (number of eggs within a clutch), ‘number of clutches’ (the number of clutches produced) and ‘total eggs laid’ (i.e. total fecundity). A ‘clutch’ was defined as a temporally and spatially discrete set of laid eggs. Correspondingly, if two separated spatial clusters were laid in an area on the same day, they were interpreted as two clutches. Similarly, if eggs were laid over the top of existing eggs, but on another day, then this was also considered a separate

clutch. At the end of the 125-day period, standard length was recorded for the female parent fish.

Monitored progeny were removed, in a single clutch, from parental compartments prior to hatching – fertilised eggs were identified by checking for developed eyes (black dots), then selected – and placed in net fish hatcheries (165 mm x 135 mm x 120 mm) in the bottom aquaria of the corresponding system, where they were maintained at the same temperature at which they were conceived. This procedure was carried out once for each population at each temperature treatment. Progeny, once hatched, were fed *ad libitum* on the same fry feed as the adult fish, but it was first crushed to a powdered form. As before, uneaten food was removed to prevent water quality issues.

Growth rates of progeny were recorded by measuring lengths of a sample of approximately 25 individuals from each population and each temperature treatment, using digital photography, with a Canon 600D DSLR camera attached to an AmScope© 7-45x dissecting stereo microscope. Individuals were placed in a petri dish containing a reference scale bar, with 0.1 mm increments in the frame, and Image-J software version 1.48 (Schneider *et al.*, 2012) was used to measure lengths from captured images. Individuals were returned to their corresponding hatcheries immediately after photographs were captured. The start of the spawning season for *P. parva*, as observed by Yan & Chen, (2009), typically coincided with water temperatures reaching 15 °C, around April/May and ceased approximately 10 weeks later, in July/August. In Britton *et al.* (2010a), it is noted that the growth season is typically from April to September, meaning a nominal growth period of approximately 10 weeks and a maximal growth period of approximately 20 weeks for

young-of-the-year. An initial sample measurement was conducted on the day of hatching, and then subsequent weekly measurements were taken for a period of 10 weeks, with a final measurement at 20 weeks, each time taking a sample of approximately 25 individuals to measure. As the fish were very small and were measured live, it was not possible to tag or identify individuals within each subset. Length measurements were made on the basis that analyses would be on mean growth of the sample, rather than a mean value of the growth rates of multiple individuals.

Data analyses

Reproductive effort was assessed using two-way analysis of covariance (ANCOVA) that tested differences in the three response variables (i.e. total clutches laid, mean clutch size and total eggs laid) across the treatments (i.e. temperatures, populations). Data on ‘total eggs laid’ were log-transformed, as they violated assumptions of normality in their raw state. The effect of fish size was controlled using the mean standard length (SL) of the female fish as a covariate (after Almeida *et al.*, 2016). These were followed by post hoc Tukey-Kramer honest significant difference (HSD) tests. Assumptions of normality and homogeneity of variance were verified using Shapiro-Wilk and Levene’s tests, respectively.

In order to assess temporal differences in reproductive effort across temperatures and populations, data were aggregated into 25-day laying periods (i.e. a total of five 25-day periods for the 125 day observed laying season). Aggregation by 25-day periods was chosen in order to smooth out the variation present at higher temporal resolutions, and because the total observation period, 125 days, divided into a whole and manageable number of periods, i.e. five, for robust statistical analyses.

Furthermore, studies of temporal gonadosomatic index (GSI) variation in *P. parva* (e.g. Yan & Chen, 2009; Zahorska & Kovac, 2013; Zahorska *et al.*, 2014) indicate that general shifts in GSI in the wild are captured at this level of temporal resolution. Before analysis, ‘number of eggs laid’ and ‘number of clutches’ were transformed into proportion of total laid (i.e. quantity laid in each 25-day period, expressed as a fraction of quantity laid over entire duration of trial) in order to allow the shape of the relationship with time (i.e. period) to be assessed. ‘Clutch size’ data were also aggregated into the 25-day periods, to give a total of four response variables; (i) Proportion of total eggs laid, (ii) Proportion of total clutches laid, (iii) Mean clutch size, (iv) Total eggs laid. Generalised Linear Models (GLMs) were used to compare the responses between temperatures and populations, with Period and SL as covariates and Temperature and Population as factors, as well as assessing the interactions between Period, Temperature and Population. *Post hoc* tests were carried out, where slopes of individual regression lines were compared in pairwise fashion, within-population groups and within-temperature groups, by testing the significance of the interaction terms in an ANOVA framework (i.e. F-tests of model *with* interaction term vs. model *without* interaction term). These tests were performed on corresponding pairwise subsets of the data (e.g. PL1 15 °C and PL1 20 °C for between-temperature test, or PL1 15 °C and UK 15°C for between-population test) and all p-values were adjusted using the Bonferroni method.

The growth of the progeny was assessed using two-way ANCOVAs that tested differences in growth responses between temperature treatments and populations. SL was used as the dependant variable, with Temperature and Population as factors, and Age (in days) as the covariate. *Post hoc* tests were carried out, where slopes of individual regression lines were compared in pairwise fashion, within-population

groups and within-temperature groups, by testing the significance of the interaction terms in an ANOVA framework (i.e. F-tests of model *with* interaction term vs. model *without* interaction term). These tests were performed on corresponding pairwise subsets of the data (e.g. PL1 15 °C and PL1 20 °C for between-temperature test, or PL1 15 °C and UK 15°C for between-population test) and all p-values were adjusted using Bonferroni corrections. All statistical tests were completed using R (R Core Team, 2016).

RESULTS

Reproductive effort

Across the three populations, there were significant differences in the size of clutches laid (ANCOVA: $F_{2, 49} = 5.56$, $P < 0.01$), but Temperature and SL were not significant predictors of clutch size ($F_{2, 49} = 0.17$, $p = 0.84$ and $F_{1, 49} = 1.15$, $p = 0.29$, respectively), including the interaction term between Population and Temperature ($F_{3, 49} = 0.30$, $p = 0.82$). Pairwise *post hoc* tests showed that mean clutch size (see Fig. 3.3) was significantly higher in PL2 and UK, compared to PL1 (ANOVA: $F_{1, 27} = 9.426$, $p < 0.01$ and $F_{1, 48} = 11.34$, $p < 0.01$, respectively).

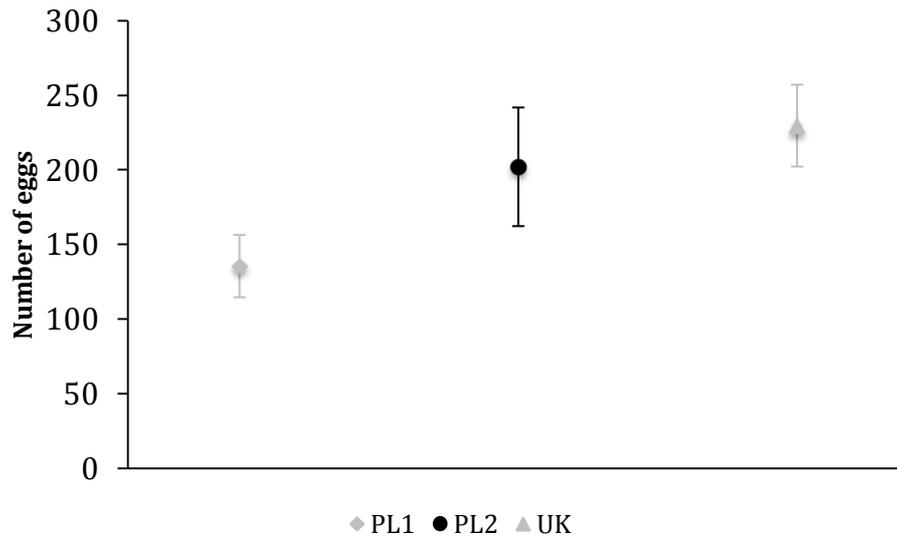


Figure 3.3 Fitted mean clutch size by Pseudorasbora parva population, with standard errors.

Tests on log-transformed data showed that total fecundity, over the entire duration of the trials, was not significantly different between temperature treatments, or populations (ANCOVA: $F_{2, 49} = 1.43$, $p = 0.25$ and $F_{2, 49} = 0.72$, $p = 0.49$, respectively). Nor was there a significant interaction between temperature and population (ANCOVA: $F_{3, 49} = 0.60$, $p = 0.62$). Fecundity was, however, significantly influenced by the size of the female fish (ANCOVA: $F_{1, 49} = 5.07$, $p < 0.01$), with females of 60 mm typically producing more than 1500 additional eggs compared to females of 50 mm (see Fig. 3.4).

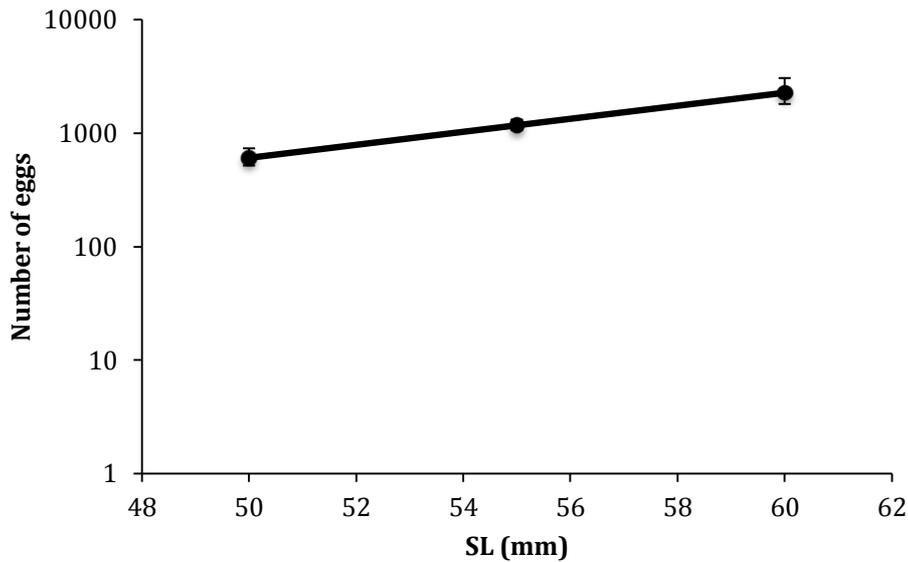


Figure 3.4 Fitted relationship of total fecundity (full duration of study) of Pseudorasbora parva with Standard Length (SL), with standard errors.

Within the populations, there were no significant differences in ‘number of clutches’ between temperature treatments (ANCOVA: $F_{2, 49} = 1.24$, $p = 0.30$). There were, however, significant differences between populations (ANCOVA: $F_{2, 49} = 10.65$, $p < 0.001$), linked to corresponding differences in SL ($F_{1, 49} = 11.33$, $p = 0.001$), with a mean 0.64 ± 0.18 additional clutches laid per 1 mm increase in body length. Pairwise *post hoc* tests showed a significant difference in ‘number of clutches’ (depicted in Fig. 3.5) between PL1 and UK85 (ANOVA: $F_{1, 47} = 26.50$, $p < 0.001$), a marginally significant difference between PL1 and PL2 (ANOVA: $F_{1, 26} = 3.09$, $p < 0.09$), but no significant difference between PL2 and UK (ANOVA: $F_{1, 34} = 1.48$, $p < 0.232$).

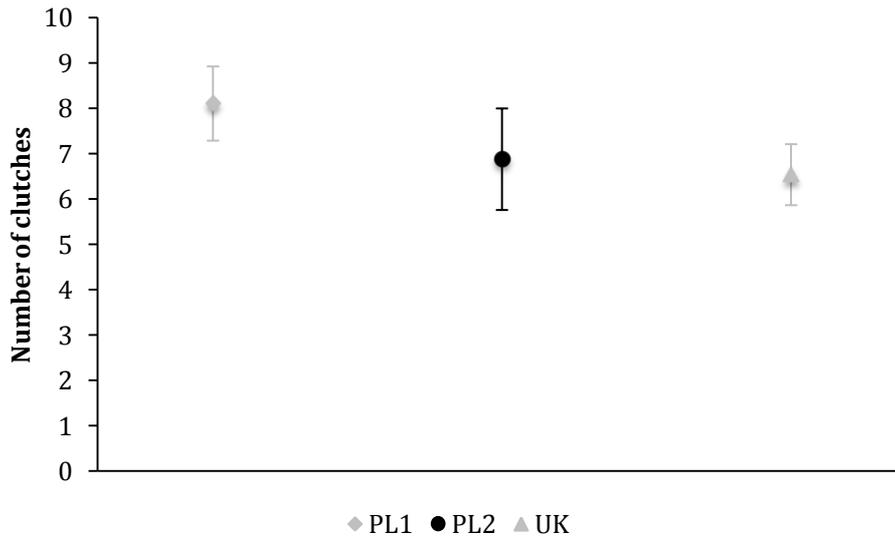


Figure 3.5 Fitted number of clutches by Pseudorasbora parva population, with standard errors.

Temporal pattern in reproductive effort

There was a significant, negative relationship between proportion of total eggs laid and time (GLM: $F_{1,288} = 361.65$, $p < 0.001$), with significant interactions between Period and Temperature (GLM: $F_{2,279} = 32.66$, $p < 0.001$), and Period and Population (GLM: $F_{2,281} = 7.16$, $p < 0.001$). The proportion of eggs produced per 25-day period diminished much more rapidly in the PL2 fish than either of the other two populations (GLM: $F_{1,141} = 17.67$, $p < 0.001$ and $F_{1,181} = 7.30$, $p = 0.008$, for PL1 vs PL2 and UK vs PL2 respectively), as can be seen in Fig. 3.6. The rate of egg production diminished more rapidly with increasing temperature (Fig. 3.7), with post hoc tests confirming a decreasing tendency from 15 °C to 20 °C (GLM: $F_{1,181} = 3.59$, $p = 0.060$) and highly significant decreasing tendencies from 15 °C to 25 °C and from 20 °C to 25 °C (GLM: $F_{1,201} = 60.56$, $p < 0.001$ and $F_{1,186} = 39.18$, $p < 0.001$, respectively).

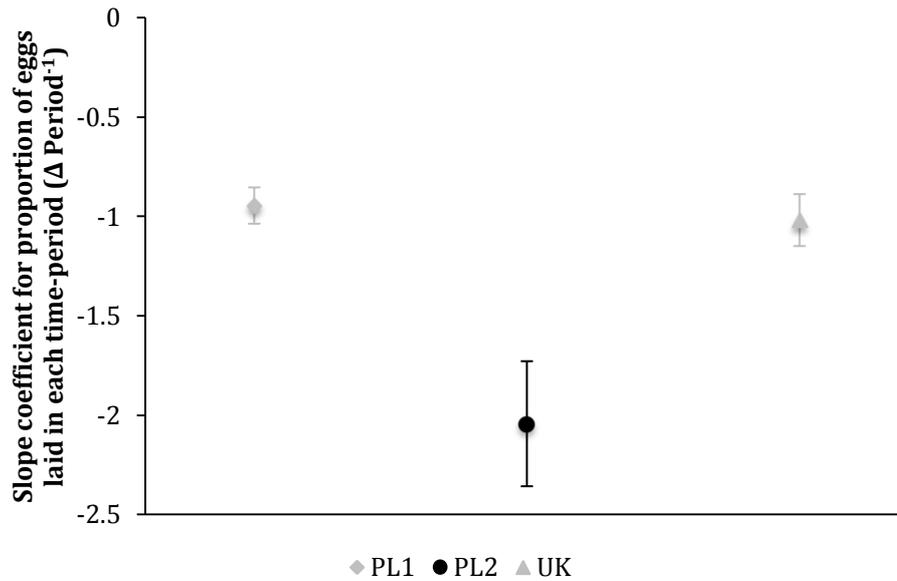


Figure 3.6 Slope coefficients, for proportion of eggs laid over time, by *Pseudorasbora parva* population, with standard errors.

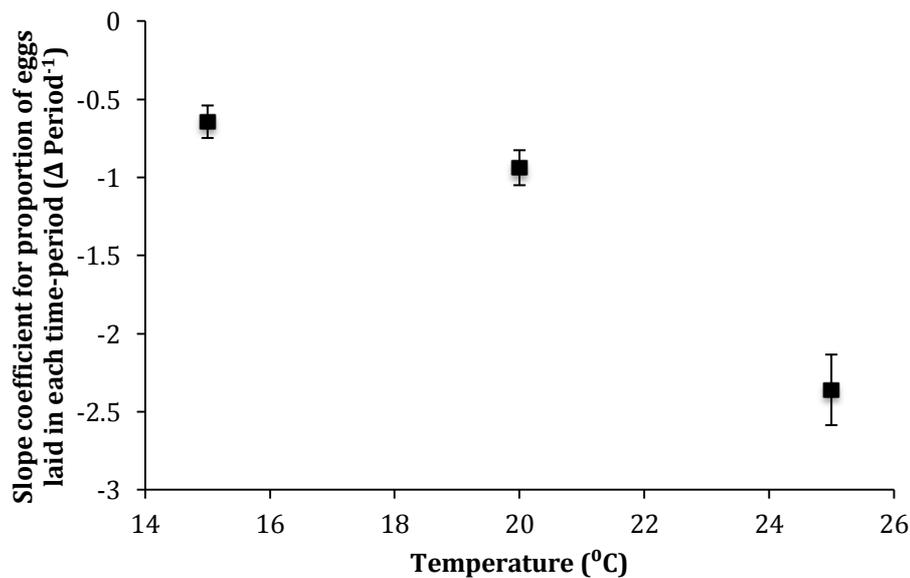


Figure 3.7 Slope coefficients, for proportion of *Pseudorasbora parva* eggs laid over time, by temperature treatment, with standard errors.

There was a significant negative relationship between proportion of total clutches laid and time (GLM: $F_{1, 288} = 234.66$, $p < 0.001$) and significant interactions between Period and Temperature (GLM: $F_{2, 281} = 28.92$, $p < 0.001$) and Period and Population (GLM: $F_{2, 279} = 5.88$, $p = 0.003$). There was also a significant three-way interaction

between Period, Temperature and Population (GLM: $F_{3,273} = 3.78$, $p = 0.011$). For all populations, the proportion of total clutches laid declined over time, with the steepness of these declines increasing with temperature (Fig. 3.8). *Post hoc* tests showed that increases in the steepness of these declines were not significant between 15 °C and 20 °C for either UK or PL1 populations (GLM: $F_{1,91} = 2.23$, $p = 0.14$ and $F_{1,66} = 2.60$, $p = 0.11$, respectively), however they were significant between 15 °C and 25 °C (GLM: $F_{1,91} = 51.26$, $p < 0.001$ and $F_{1,66} = 16.33$, $p < 0.001$, for UK and PL1 respectively) and between 20 °C and 25 °C (GLM: $F_{1,96} = 34.03$, $p < 0.001$ and $F_{1,66} = 7.01$, $p = 0.01$, for UK and PL1 respectively) for these same populations. The increase in steepness between 15 °C and 25 °C for PL2 was not significant (GLM: $F_{1,36} = 0.78$, $p = 0.38$). For PL2 fish, the proportion of total clutches per 25-day period declined significantly more rapidly at 15 °C than either of the other two populations (GLM: $F_{1,61} = 9.12$, $p = 0.004$ and $F_{1,51} = 7.95$, $p < 0.001$, for UK and PL1 respectively). It also declined significantly more rapidly in UK fish, compared to PL1 fish (GLM: $F_{1,81} = 14.15$, $p < 0.001$).

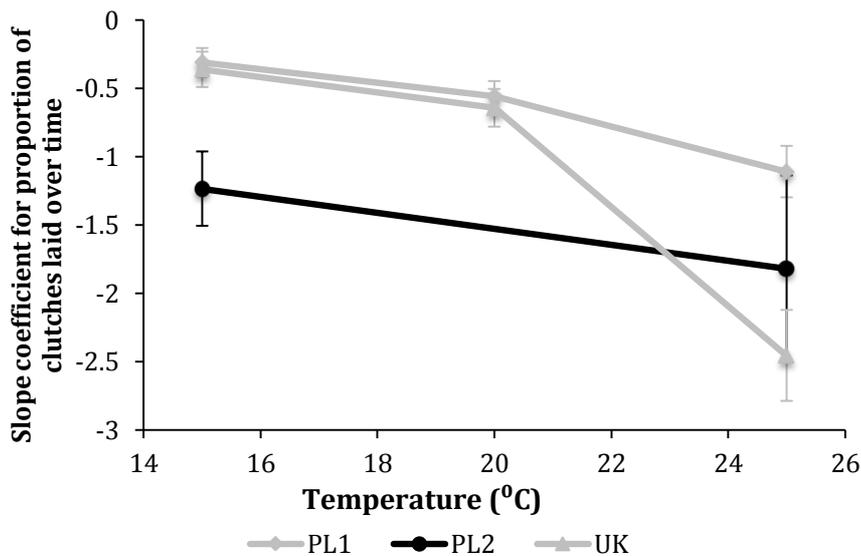


Figure 3.8 Slope coefficients, for proportion of *Pseudorasbora parva* eggs laid over time, by population and temperature treatments, with standard errors.

There was a significant negative relationship between mean clutch size and time (GLM: $F_{1,288} = 159.2$, $p < 0.001$), with mean values diminishing from 227 eggs in the first 25-day period, to 24 in the final period (see Fig. 3.9). Significant differences in mean clutch size were observed between different temperature treatments (GLM: $F_{2,284} = 9.83$, $p < 0.001$) and between populations (GLM: $F_{2,286} = 3.95$, $p < 0.02$). Mean clutch size had a significantly positive relationship with SL (GLM: $F_{1,283} = 8.78$, $p < 0.003$), with over 50 eggs per clutch more in fish of 60 mm, compared to fish of 50 mm (see Fig. 3.10).

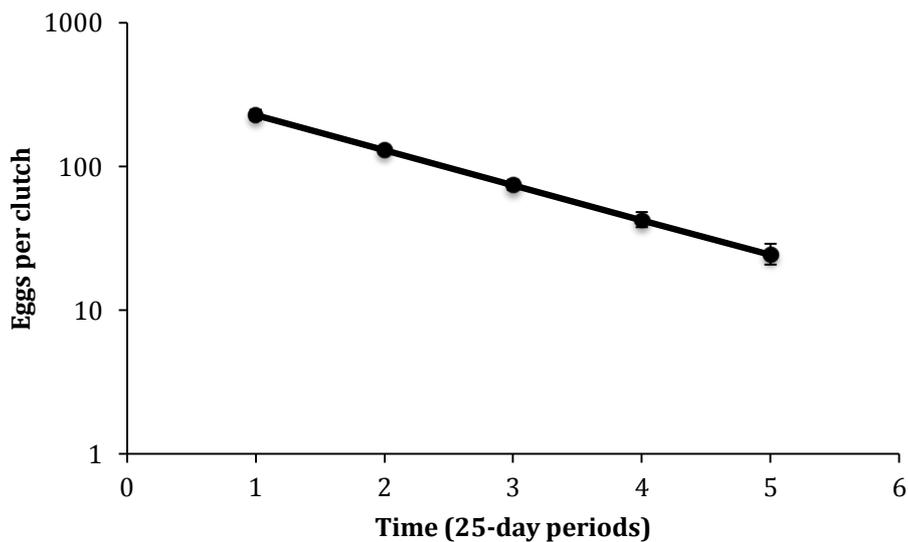


Figure 3.9 Fitted relationship between *Pseudorasbora parva* clutch size and time, with standard errors.

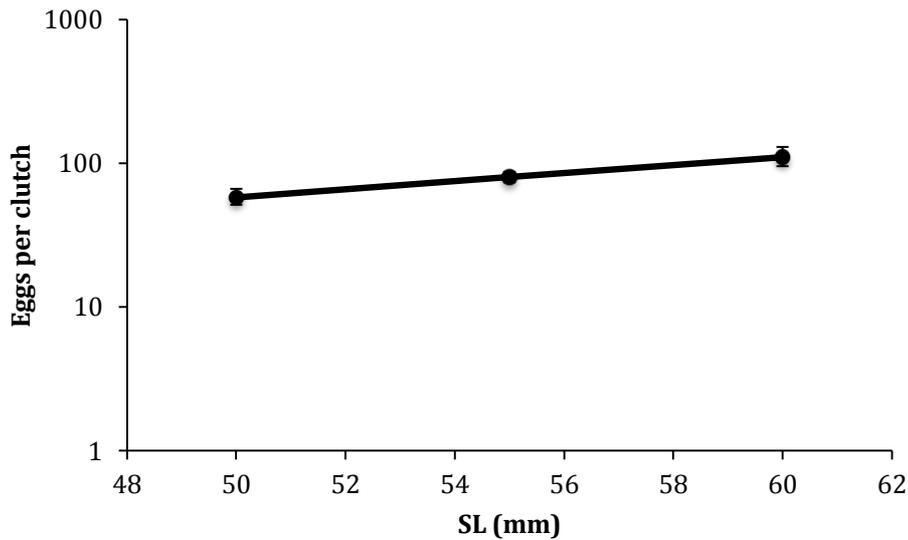


Figure 3.10 Fitted relationship between *Pseudorasbora parva* clutch size (25-day aggregated data) and Standard Length (SL), with standard errors.

There was a borderline significant interaction between Period and Population (GLM: $F_{2, 281} = 2.99$, $p = 0.052$) and a significant interaction between Period and Temperature (GLM: $F_{2, 279} = 13.24$, $p < 0.001$). *Post hoc* tests showed that clutch size declined with increasing temperature (Fig. 3.11), significantly so between 15 °C and 25 °C (GLM: $F_{1, 202} = 18.18$, $p < 0.001$) and between 20 °C and 25 °C (GLM: $F_{1, 187} = 11.20$, $p < 0.001$). Clutch sizes in UK fish (Fig. 3.12) were significantly higher than PL1 (GLM: $F_{1, 245} = 5.90$, $p = 0.016$), but not significantly higher than PL2 (GLM: $F_{1, 180} = 2.25$, $p = 0.14$).

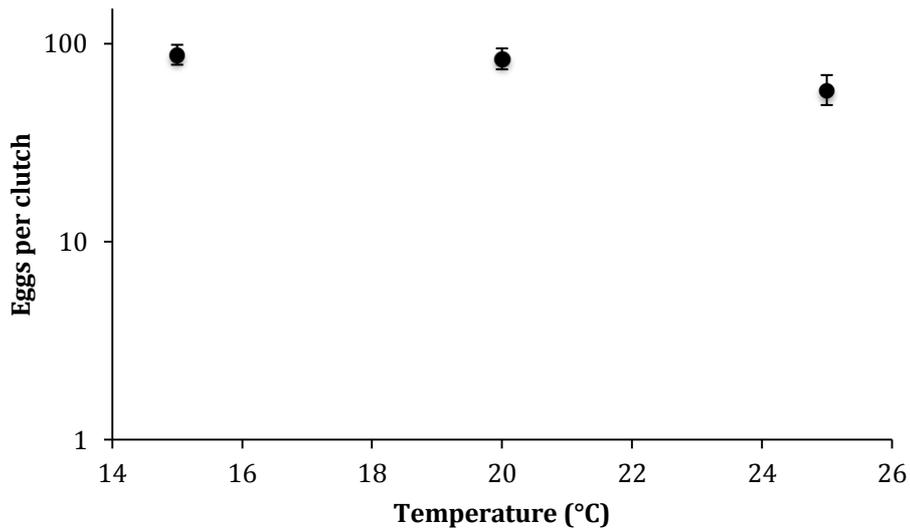


Figure 3.11 Fitted *Pseudorasbora parva* clutch size (25-day aggregated data) by temperature treatment, with standard errors.

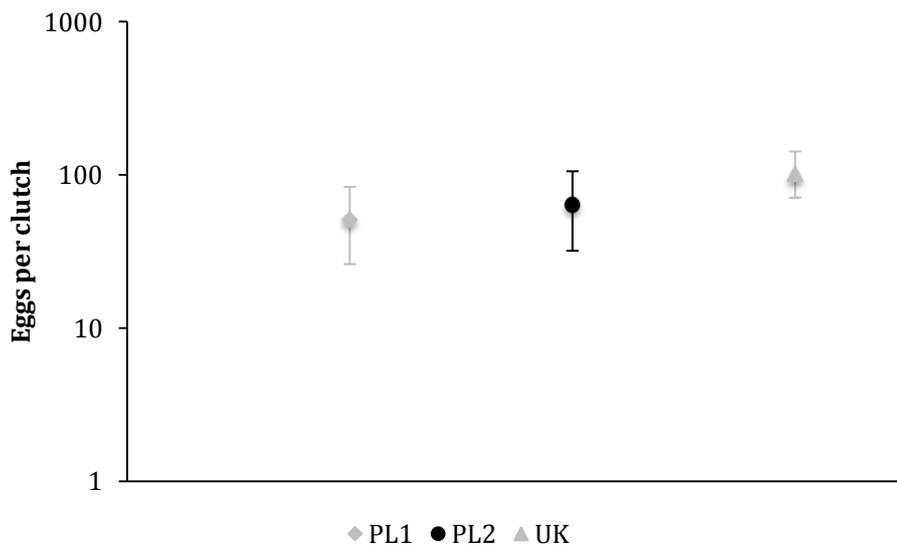


Figure 3.12 Fitted clutch size (25-day aggregated data) by *Pseudorasbora parva* population, with standard errors.

The rate at which clutch size diminished over time (i.e. steepness of slope – see Fig. 3.13) was most rapid in the PL2 population, which was significantly steeper than PL1 (GLM: $F_{1, 136} = 7.96$, $p = 0.006$). The most marked and consistent relationship was the interaction between Period and Temperature (Fig. 3.14), with the rate at which

clutch size diminished over time increasing with temperature. These shifts were borderline significant from 15 °C to 20 °C (GLM: $F_{1,178} = 3.34$, $p = 0.069$) and significant between 20 °C and 25 °C (GLM: $F_{1,183} = 13.33$, $p < 0.001$).

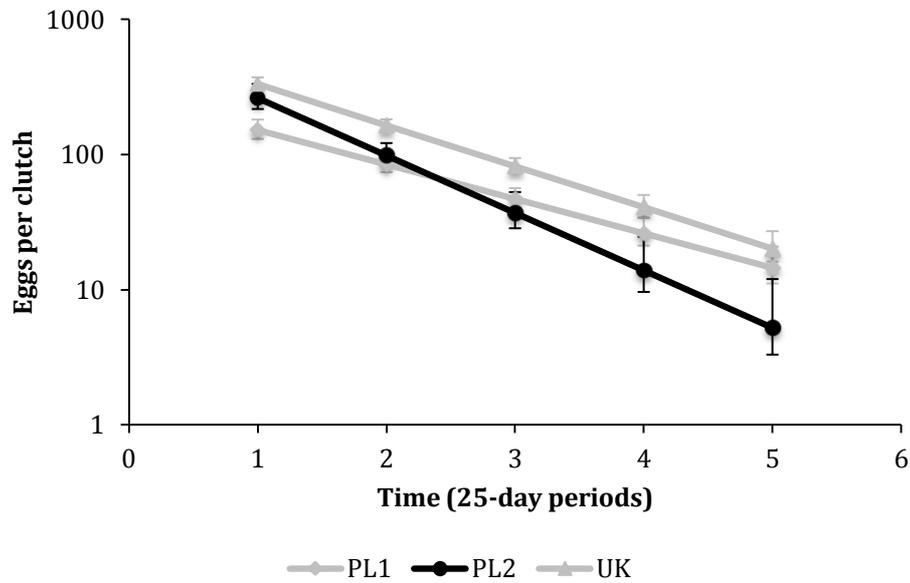


Figure 3.13 Fitted clutch size over time, by *Pseudorasbora parva* population, with standard errors.

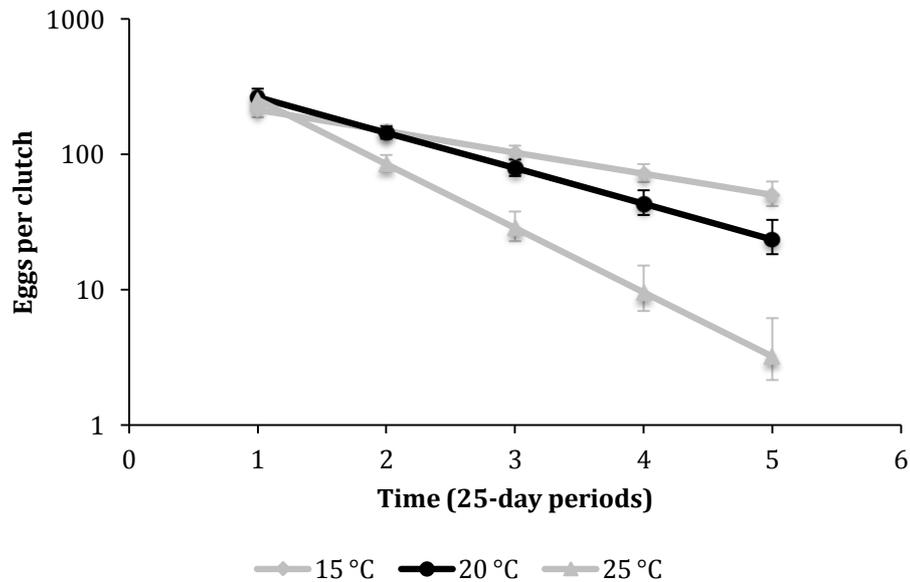


Figure 3.14 Fitted *Pseudorasbora parva* clutch size over time, by temperature treatment, with standard errors.

Fecundity diminished significantly over time (GLM: $F_{1,288} = 301.7$, $p < 0.001$) and there was a significant interaction between Period and Temperature (GLM: $F_{2,281} = 13.78$, $p < 0.001$). Female size (SL) had a significant positive relationship with fecundity (GLM: $F_{1,283} = 22.67$, $p < 0.001$), with mean values showing an increase of almost 80% from 50 mm to 60 mm (see Fig. 3.15). *Post hoc* tests showed that temperature increased the rate at which fecundity diminished over time (Fig. 3.16), with each increment in temperature showing a significant shift in this rate (GLM: $F_{1,180} = 5.42$, $p = 0.021$ and GLM: $F_{1,185} = 7.60$, $p = 0.006$, for 15 °C to 20 °C and 20 °C to 25 °C respectively).

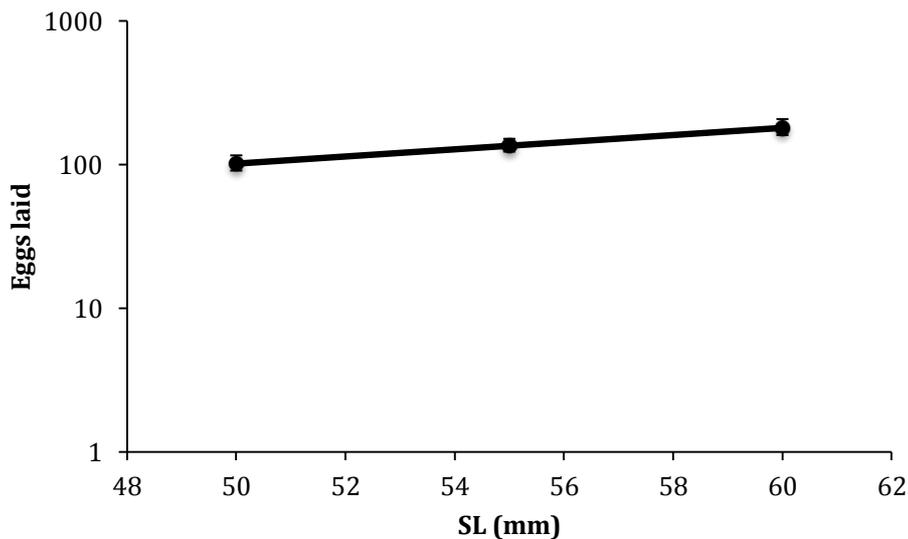


Figure 3.15 Fitted relationship between *Pseudorasbora parva* fecundity (25-day aggregated data) and Standard Length (SL), with standard errors.

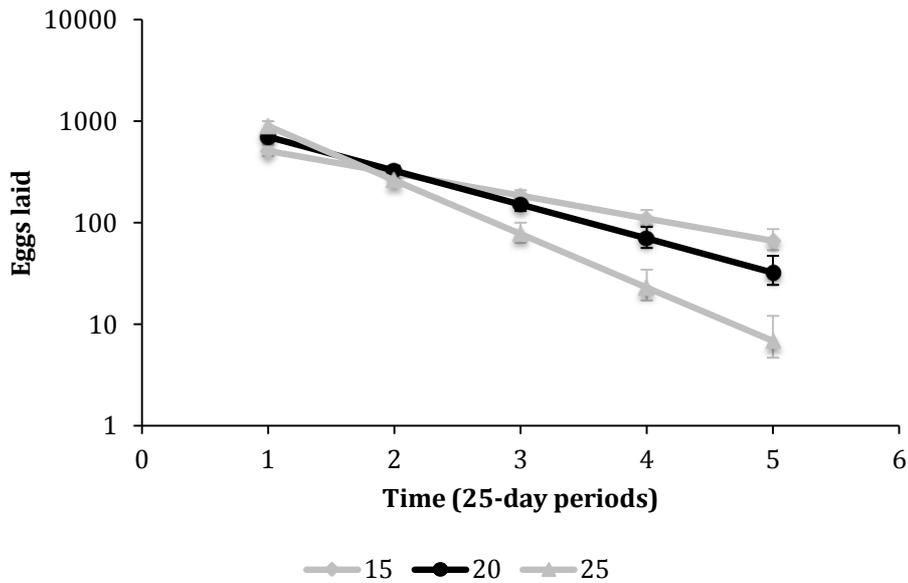


Figure 3.16 Fitted relationship between *Pseudorasbora parva* fecundity and time, by temperature treatment, with standard errors.

Growth of progeny

Growth rates of offspring increased significantly with temperature (ANCOVA: $F_{2, 1299} = 40.56$, $p < 0.001$). Whilst growth rates were not significantly different between populations (ANCOVA: $F_{2, 1299} = 1.60$, $p > 0.05$), there was a significant Temperature:Population interaction, affecting growth (ANCOVA: $F_{3, 1299} = 4.43$, $p = 0.004$). For all populations, growth rates increased as a function of increasing temperature (Fig. 3.17), with a significant increase in growth rate between 15 °C and 25 °C for all populations (PL1: $F_{1, 390} = 8.29$, $p = 0.029$, PL2: $F_{1, 263} = 40.65$, $p < 0.001$, UK: $F_{1, 279} = 29.67$, $p < 0.001$). Generally, the thermal responses were not significantly different between populations, but at 15 °C, PL1 larval growth was significantly higher than PL2 and UK ($p = 0.002$ for both comparisons). For combined data (i.e. without differentiating by population), growth rates (Fig. 3.18) increased significantly with each increment in temperature ($p < 0.005$ for all comparisons).

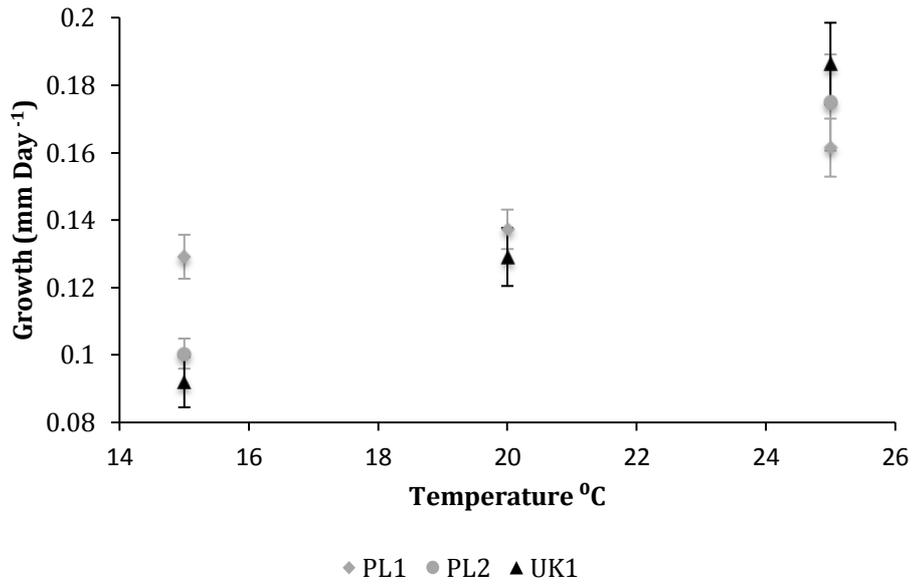


Figure 3.17 Fitted growth rates of fish progeny for all *Pseudorasbora parva* populations, at all temperature treatments, with standard errors.

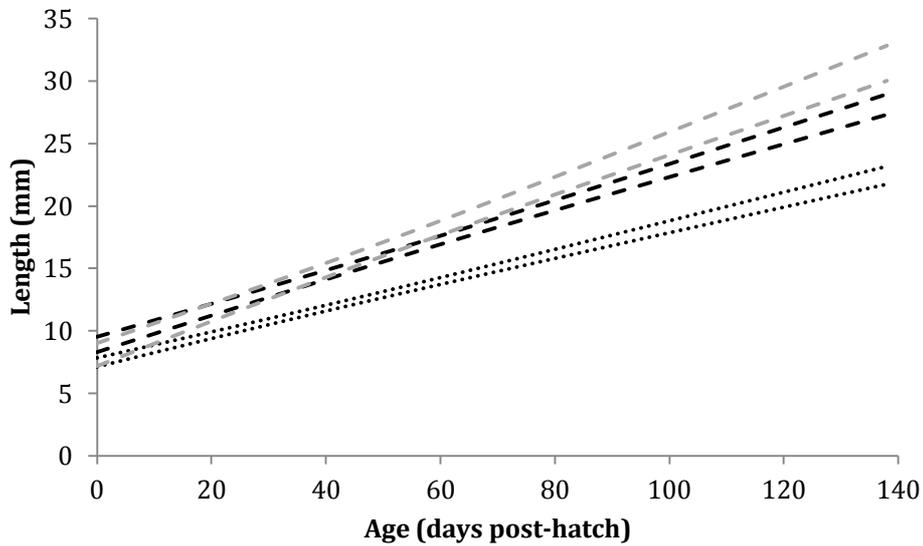


Figure 3.18 95% confidence intervals for linear regressions of early growth at each of the three temperatures using combined data from all three *Pseudorasbora parva* populations. 15 °C, 20 °C and 25 °C represented by black dotted, black dashed and grey dashed lines, respectively.

DISCUSSION

The study tested for the first time the plastic responses and adaptive potential of *P. parva* over a temperature gradient, using metrics of female fecundity and the growth performance of their progeny. Contrary to prediction, the results indicated there was no significant variability in total reproductive output across a temperature range of 15 °C to 25 °C. Although there were differences in numbers of clutches laid by individuals from different populations, these were associated with the variation in the size of individuals from those populations. Temperature did significantly impact the duration and intensity of reproductive output, with higher temperatures inducing rapid intensive output and lower temperatures inducing more protracted and lower intensity output, with this consistent with the prediction. Growth rates of progeny increased with temperature and were similar across populations, with the exception of fish from PL1, which had a significantly higher growth rate at 15 °C than those of the other two populations studied. Observed growth rates of all populations at all temperatures in the present study were consistent with offspring reaching body sizes and development stages that would enable reproduction in their first year of life (Zahorska *et al.*, 2013).

Water temperatures above physiological optima can act as a stressor in fishes, resulting in a suppressive influence on reproductive output through elevated cortisol levels (Pankhurst *et al.*, 2011). In temperate fishes, inhibitory effects on reproduction are typically observed above 24 °C (Pankhurst & Munday, 2011). In this study, however, there was no indication of reproductive output being suppressed at 25 °C when compared to the lower temperatures. This emphasizes the broad tolerances of *P. parva* to thermal conditions. Indeed, it can be argued that their invasion success at global levels, with colonisation of Europe and spread into North Africa and the

Middle East (Gozlan *et al.*, 2010b), is at least in part related to their very broad temperature tolerances, whereby they are able to form sustainable populations in all climates encountered between North Africa and Northern Europe (Gozlan *et al.*, 2010b). This then has considerable implications for their potential to invade other areas of the world, should introductions manifest, given that Fletcher *et al.*, (2016) predicted a number of high-risk areas for *P. parva* invasion, including southern states in the USA where summer water temperatures are likely to be in the range of 25 °C.

Responses to stressors, including altered reproductive output, can sometimes be hormetic, whereby slight increases in stress actually enhance reproductive performance; it is then only at more severe levels of stress that the suppressive effect becomes significant (Schreck, 2010). Correspondingly, it may be that these effects would only be measureable when the thermal tolerance limits of *P. parva* are neared, with this clearly above 25 °C in the tested populations, under controlled conditions. In addition, nutritional status may have a protective effect against thermal-related reproductive suppression (Donelson *et al.*, 2010). Given that the experimental fish were fed *ad libitum* then this might have acted to dampen any potential suppressive effect of the elevated temperature. Photoperiod was kept constant in the reproductive period of the experiment, with this factor often referred to as a critical factor in teleost reproductive cycling (e.g. Baggerman, 1980; Fraile *et al.*, 1993; Shimizu, 2003; Singh & Chaturvedi, 2010; Vlaming, 1972). However, the reproductive cycle of some cyprinid fishes are primarily regulated by temperature changes (Vlaming, 1972).

The lack of significant thermal response of total reproductive output across the experiment may be partly a function of the relatively moderate and controlled overwinter conditions (stable 5 °C water temperature) of all treatments. In the wild,

overwinter conditions and associated mortality are considered to be a strong selective force (Hurst, 2007), with survival positively related to fish body size (Wootton, 1990). Further, wild populations are present at latitudes in Europe, where harsh winter conditions are experienced (e.g. PL2 near Warsaw, Poland), implying that 5 °C is within comfortable lower limits for this species. Future studies of the thermal responses of reproductive output and early growth in invasive fish could therefore be significantly enhanced by including factors such as nutritional status and variation in simulated winter conditions in order to assess their relative importance versus the conditions in the reproductive period.

Despite the larger clutch sizes from the UK fish, they then laid a reduced number of clutches compared to the other populations and thus the overall reproductive outputs were similar across the populations. In addition, temperature also affected how the reproductive output was distributed over time, with distinct strategies detected between lowest and highest temperatures. At the highest temperature, there was rapid production of eggs over a relatively short period, whereas lower temperatures induced a more gradual and protracted production of eggs over a longer time period. In many fish species, protracted breeding seasons are thought to be a response to variable conditions, with the production of progeny over extended periods increasing the chance of survival of the cohort, such as from stochastic weather events like floods that can be deleterious to larval fishes (Harvey, 1987; Bolland *et al.*, 2007; Nunn *et al.*, 2002; Nunn *et al.*, 2007). Examples of protracted spawners include *L. macrochirus* (Garvey *et al.*, 2002), *L. gibbosus* and *Squalius cephalus* (Nunn *et al.*, 2002, 2007). However, as the water temperatures were tightly controlled in the experiment then these results suggest that the reduced temperature difference between the winter and spawning temperature induced this protracted reproductive strategy.

The general plasticity in reproductive strategy (i.e. shape of output over time) relative to temperature has been observed in the wild, by Yan & Chen (2009), who also associated this with environmental factors, including temperature. They observed a more “protracted”, less pronounced (lower peak GSI) breeding season in a lake with relatively cooler, more stable thermal regime. This corroborates our *ex situ* observations and potentially lends credence to the applicability of such observations in real world situations (i.e. management of wild populations).

In *P. parva*, sexual maturity in females has been observed at lengths as low as 24 mm SL (Zahorska *et al.*, 2013). Given a mean size at hatching of 6mm across temperatures of 15 °C, 20 °C and 25 °C and growth rates of 0.11 ± 0.004 , 0.14 ± 0.005 and 0.17 ± 0.007 mm d⁻¹ respectively, individuals could reach sexual maturity between 121 and 187 days (assuming a linear relationship and consistent temperature, with non-limiting food resources). This is consistent with field observations of individuals reaching sexual maturity within their first year (Rosecchi *et al.*, 1993; Rosecchi *et al.*, 2001; Zahorska *et al.*, 2013; Yan & Chen, 2009), although actual reproduction might not actually occur until the following spring (Britton *et al.*, 2007). This highlights the considerable influence that water temperatures have on *P. parva* growth, with individuals taking 1.5 times as long to reach potential maturity at 15 °C as at 25 °C.

The combined effects of both adult breeding strategy and early growth of progeny, determined by environmental factors, could have implications on the demographics of populations over time. Over longer periods of time (i.e. multiple generations), the combined effects of these two temperature-dependant life history traits could constitute divergent ontogenies leading, to morphologically differentiated

populations, as predicted by temperature-size theories. Temperature-size relationships in the natural world have long been of interest to biologists (Angilletta & Dunham, 2003) and a number of theories or rules have been formulated over the years. For instance, James' rule (James, 1970), concerning intraspecific variation of body size, states that populations in warmer environments tend to have smaller bodies at age than their conspecifics in cooler environments. Further, the temperature-size rule (TSR) asserts that the size of individuals' bodies, in ectotherms, is negatively related to temperature (Atkinson, 1994). A more recent synthesis, of these two rules and others, in relation to the effects of climate change on aquatic systems (Daufresne *et al.*, 2009), suggests that size-at-ages should decrease over time with increased temperatures, as the TSR predicts that a more rapid growth leads to lower final size in these conditions. Applying this to the case of *P. parva*, this would mean: northerly populations of less abundant, slower growing, later maturing individuals of larger body size and greater age, and southerly populations of more abundant, faster growing, early maturing individuals of smaller body size and lower age. However mean size of individuals could be significantly impacted by other factors, such as size-dependant predation, population density and resource availability. Furthermore, selective pressure on size, related to reproductive success being associated with larger size in males, could potentially lead to reverse temperature-size relationships (Ohlberger, 2013).

Where food is not limiting, we have identified that total reproductive output is a function of body size, whereas reproductive strategy and early growth are directly linked with water temperature. In the wild, these unquantified factors (e.g. food availability, predation pressure, competition) may combine with temperature to impact on reproductive output and, ultimately, recruitment. For instance, towards the

climatic extremes of *P. parva*'s mainland European distribution (e.g. Poland), a relatively small shift in any of these factors, might lead to populations reducing in numbers and dying out. One such population in Lithuania, where *P. parva* was numerically abundant when first introduced, but was absent in later surveys of the same lake (Copp *et al.*, 2005; Gozlan, 2012), implying that it had initially survived introduction, but had subsequently failed to recruit. Summer water temperatures at the location are likely to achieve approximately 15 °C in small isolated water bodies, suggesting that a protracted breeding season would likely be induced, a number of the offspring would hatch late in the season and have little time to grow and improve body-condition before the relatively harsh winter conditions. If resource availability is less than optimal, the situation could be compounded and lead to higher over winter mortality and poor condition of surviving individuals. Coupled with other factors, such as predation, competition, and background mortality (i.e. disease and age-related deaths), this could mean that mortality becomes greater than recruitment and the population dies out. Such a scenario could explain the disappearance of *P. parva* from Lithuania, particularly if the population was originally abundant and consumed resources much faster than could naturally replenish. As the species are relatively short-lived (3-4yrs), then it would not take very long for a population to die out if offspring were not surviving and reaching sexual maturity quickly enough to reproduce and offset mortality rates.

We feel that there would be considerable merit in further studies to investigate the interactive effects of temperature and food ration of *P. parva* adults on reproductive output and growth of progeny, particularly if a reliable protocol could be developed for a surrogate of nutritional status, such as those discussed in Congleton & Wagner (2006). Such a metric of nutritional status, or condition, could them potentially be

used in field studies to quantify the parameter for studies individuals or populations. Once these factors that are testable in a controlled environment are investigated and the corresponding relationships quantified, it will make *in situ* studies easier to interpret, as we will then know the combined effects of, for instance, temperature and food rations.

CONCLUSION

Whilst the present study did not identify the temperature at which reproductive output exhibited suppression, it did confirm the broad tolerances of *P. parva* and highlight its ability to maintain gross reproductive output across a wide range of simulated summer temperatures (i.e. 15-25 °C). The study also identified the strong link between temperature and temporal reproductive output strategy, which could have implications for the management of detected introductions with respect to both eradication and dispersal prevention, as protracted breeding seasons may increase the risks of fluvial larval dispersal to secondary locations. Furthermore, where temperatures are optimal for rapid growth – 25 °C in this study – reproductive output is short and intensive; meaning that utilization of limited warmer conditions is maximized, potentially leading to higher likelihood of winter survival and reaching sexual maturity in time for the following breeding season.

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CHAPTER IV

Biological drivers of fish dispersal during an invasion process.

ABSTRACT

The process of biological invasion has a number of distinct stages, each containing barriers to invasion success. Dispersal characteristics have been suggested as important factors in determining success at overcoming these barriers. There is increasing evidence that populations on expanding range fronts are phenotypically different from longer-established populations, with part of these differences being related to dispersal characteristics. Dispersal ability is often linked to morphological traits, but can also be connected to behavior and together these factors can combine to form a dispersing phenotype (dispersal-type), which will diminish in prevalence with distance from invasion front. The presence of such dispersal-types at range edges can be a strong determinant of range expansion success and hence invasiveness. Here we use six populations of an invasive fish, *Pseudorasbora parva*, spread along the River Garonne in France, at incremental distances from the invasion front (from 0 - 210 km). We experimentally quantified dispersal propensity in mesocosms and we measured activity levels and characterized morphology in all six populations. We tested for covariation patterns in measured traits, relative to one another and to distance from invasion front. The only significant predictor of dispersal propensity in our study was fish size and this did not vary significantly with distance from invasion front. Although we found a strong morphological cline, concordant with distance from invasion front, we found no significant link between this morphological variation and dispersal propensity or activity levels. The morphological differences were predominantly explained by relative head-size and eye diameter, which could indicate a shift in diet, or mode of life. Our results showed that larger individuals have a higher propensity to disperse. As larger individuals are generally more fecund, this could enable *P. parva* to expand its range and recolonize new areas more quickly than

if dispersal were random. However other factors may also significantly influence dispersal, including sociability and potential density-dependent effects.

INTRODUCTION

Species are increasingly introduced to areas outside their historical native range, often facilitated by transportation and commerce (Meyerson *et al.*, 2007). Whilst numerous introduced species fail to survive (Blackburn *et al.*, 2011), a proportion of those introduced successfully establish, spread and have a negative impact on the recipient system (Williamson & Fitter, 1996; Clavero & Garcia-Berthou, 2005; Hejda *et al.*, 2009). These invasive species can have severe ecological consequences for the native ecosystem, in terms of biodiversity, as well as for economic interests (Mack *et al.*, 2000; Bax *et al.*, 2003). Eradication is not always feasible, either due to possible collateral damage (Thresher & Kuris, 2004), or because of the sheer scale of the problem and the associated cost (e.g. Feral goats in Australia, see Bomford & O'Brien, 1995; the zebra mussel in North America, see Strayer 2009). Prevention is the most effective form of biological invasion management (Britton *et al.*, 2010b), but this involves better understanding of how invasions happen and what characterises successful invaders, relative to unsuccessful introduced species.

The process of biological invasion has several distinct stages, each containing barriers to invasion success (see Blackburn *et al.*, 2011). Various studies suggest that dispersal characteristics may be important in determining the success, or otherwise, of organisms negotiating one or more of these barriers and ultimately becoming invasive (e.g. Weis, 2010; Cote *et al.*, 2010; Chapple *et al.*, 2012). Dispersal is also pivotal in a metapopulation context, as it determines the flow of individuals between local populations, enabling the persistence of populations experiencing otherwise net negative growth as well as the colonisation of new patches of suitable habitat (Hanski & Gyllenberg, 1993; Hanski, 1998). Dispersal can also facilitate local adaptations and

mitigate maladaptations in novel conditions, where gene flow is asymmetric due to non-random patterns in dispersal (Savolainen *et al.*, 2007; Aitken & Whitlock, 2013). Intraspecific variability in dispersal may have direct impacts on population dynamics and certainly will impact a species' ability to expand its range, as the ability of a species to expand or invade will be determined by the demographic structure of the populations at the edge of the current range and the presence of dispersal phenotypes (Clobert *et al.*, 2009). Dispersal has often been linked to physiology, particularly morphological traits that aid the dispersal process (Thomas *et al.*, 2001; Berwaerts *et al.*, 2006). However, variability in dispersal may be reflective of morphological and behavioural adaptations together (Hanski *et al.*, 2004) and so a more holistic approach considers a host of behavioural traits combined with physical ability, in the form of dispersal syndromes (Arjona *et al.*, 2017; Cote *et al.*, 2017). A number of specific behaviours are thought to influence the success of establishment and spread of introduced species, including boldness, activity, exploratory behaviour and dispersal tendency (Chapple *et al.*, 2012). Despite the importance of holistic approaches to dispersal, suggested by the literature, there are still very few such studies, particularly concerning freshwater vertebrates (with the exception of *Gambusia affinis*, e.g. Cote *et al.*, 2010).

There is increasing evidence in the literature to suggest that recently colonised populations differ phenotypically from older populations, particularly with respect to morphological traits linked to dispersal (Olivieri *et al.*, 1995; Hanski *et al.*, 2004; Duckworth & Badyaev, 2007), with persistence in an area sometimes leading to selection for reduced mobility (Zera & Denno, 1997). Therefore, we might expect to see morphologically distinct populations at an active invasion front, but we may also

see that this morphologically distinct phenotype is also more prone to disperse and more active.

Pseudorasbora parva is a highly invasive freshwater cyprinid fish, native to East Asia. In less than 40 years, it has achieved pan-continental invasion success, with an average of 5 new countries succumbing per decade (Gozlan *et al.*, 2010). *P. parva* possesses a number of life-history traits that facilitate its invasion success, including: high reproductive effort, early sexual maturity, batch spawning and paternal nest guarding (see Chapter III) as well as a high tolerance for degraded ecosystems (Gozlan, 2012). Aside from the negative impacts associated with its observed trophic niche overlap with native fishes (Britton *et al.*, 2010a), it is also known to be a healthy carrier of *Sphaerothecum destruens*, an emergent infectious disease known to cause mortality in a number of species (Gozlan *et al.*, 2005; Andreou & Gozlan, 2016). Specific information on dispersal in this species is largely restricted to human-mediated dispersal (Gozlan *et al.*, 2010), with a dearth of information quantifying natural dispersal behaviour.

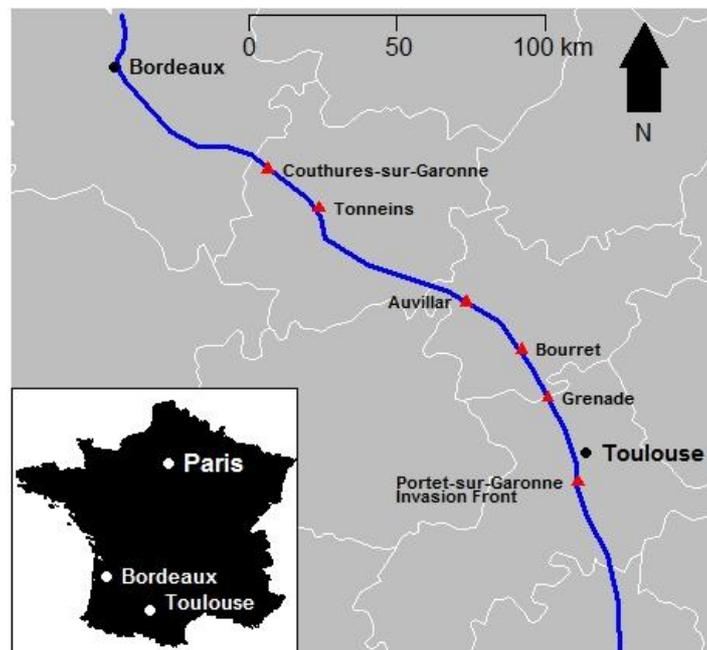
Here we take six *P. parva* populations in the River Garonne in the south of France and investigate the relationship between dispersal propensity, activity levels and morphological characteristics, with distance from invasion front. We tested the leading-edge effect along a distance-gradient with regards to population morphology and dispersal-related behaviour. Specifically, we expected that: (i) there would be morphological differences between populations, related to distance from the invasion front; (ii) there would be individual variation in activity levels, related to both morphological variation and to distance from the invasion front; (iii) there would be

variation in propensity to disperse and this would be related to morphological characteristics, activity levels and to distance from the invasion front.

MATERIALS & METHODS

Sampling methods and locations

Fish were sampled using electrofishing at a total of six sampling locations along the River Garonne (see Fig. 4.1), starting from the upstream invasion front (Portet-sur-Garonne, Blanc & Lair, 2017), to 210 km downstream at Couthures-sur-Garonne. Sampled individuals (between 50 and 100 for each location) were placed into polythene sacks with about 20 L of river water and 40 L of oxygen ($n \leq 25$ in each sack), then transported to Station d'Ecologie Théorique et Expérimentale (SETE), where they remained in the sacks to acclimate to holding-tank conditions for a minimum of 10 hours, before being released into the holding tanks.



*Figure 4.1 Map displaying the six sampled *Pseudorasbora parva* population locations on the River Garonne: Portet-sur-Garonne (POR); Grenade (GRE); Bourret (BOU); Auvillar (AUV); Tonneins (TON); Couthures-sur-Garonne (COU).*

Activity

Activity assays were carried out under controlled laboratory conditions, in white plastic buckets of 20 cm diameter. Each bucket contained 4 cm depth of dechlorinated water and one fish – fresh, clean buckets and water were used for each experiment, to avoid behavioural reactions to scent of previous fish. A total of eight individual buckets could be monitored simultaneously by the lab setup. Activity was filmed on four 12 MP webcams, mounted at a height of 40 cm above the buckets, and footage captured using iSpy™ software (<https://www.ispyconnect.com>) at a rate of 10 frames per second. Activity was recorded for a total of 20 minutes, but only the final 15 minutes was analysed, to allow 5 minutes at the beginning for fish to settle after the lids of the buckets were removed and the room vacated. Footage was analysed using idTracker™ software (Pérez-Escudero *et al.*, 2014) to track the position of individuals and quantify the total distance travelled. Distance travelled was converted, from pixels into km, using individual calibrations for each of the cameras used, to allow for any minor differences in field/angle of view and optical aberrations.

Morphology

Following the activity quantification assays, each group of eight fish was photographed on a gridded background (see Fig. 4.2) then individually tagged, using sub-dermal elastomer tags (northwest Marine Technologies, Shaw Island, WA, USA) under a low dose of anaesthetic (MS-222), so that individual fish could later be identified within each group during the subsequent stage (dispersal assays) of the trials. Each fish received a unique two-tag combination from a choice of four coloured elastomer tags – one on each side of the base of the dorsal fin – and each

group of eight fish were returned to a separate cage in holding pools, organised by source populations.

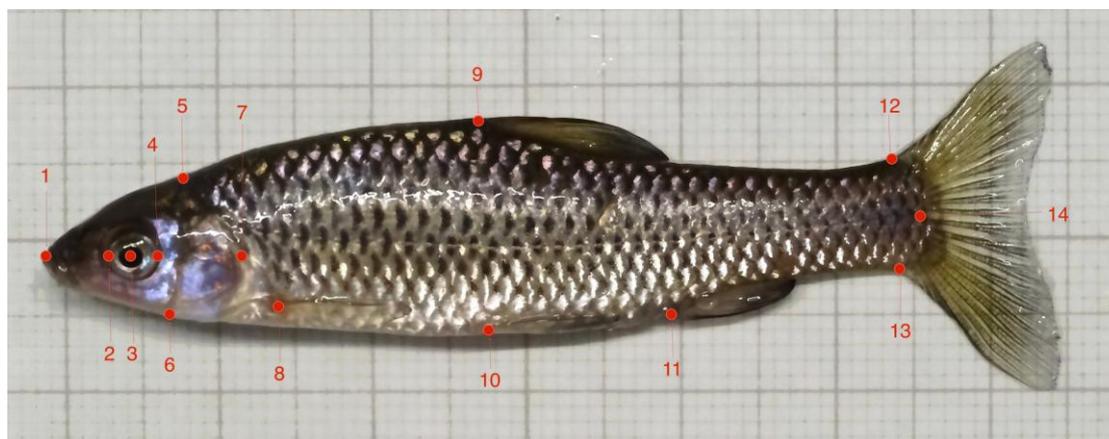


Figure 4.2 Photograph of *Pseudorasbora parva* sample, with digitised landmarks (LMs) indicated and numbered. Morphometrics calculated are: Preorbital distance (1-2); Head length (1-7); Eye diameter (2-4); Postorbital distance (4-7); Head depth (5-6); Body length (7-14); Pectoral fin to pelvic fin (8-10); Body depth (9-10); Pelvic fin to anal fin (10-11); Caudal peduncle length (11-13); Caudal peduncle depth (12-13).

Captured photographs were processed in tpsDig software (Rohlf, 2010), where 14 landmarks (see Fig. 4.2) were digitised, and in ImageJ software (Schneider *et al.*, 2012), to calibrate lengths against the gridded background. Digitisation was carried out on photographs in a randomised order, so as to avoid bias associated with the sequence of specimens. The tps file was imported into R v.3.3.2 (R development core team 2015), where the 11 morphometric characteristics (see Fig. 4.2) were measured from the landmarks and standardised using standard length (SL), to provide shape characteristics of individual fish.

Dispersal propensity

Dispersal assays were conducted in custom-built experimental systems. Each system comprised three 1100 L circular MDPE water tanks, linked in a linear configuration

(Fig. 4.3) by 1 m lengths of 110 mm diameter PVC pipe at 35 cm height. A total of 12 systems, arranged in a two by six array, were used. Systems were filled with a 45 cm depth of tap water and left for a minimum of 72 hours prior to use, to allow for dechlorination. Each system was drained, cleaned and refilled with clean water after each experimental trial.

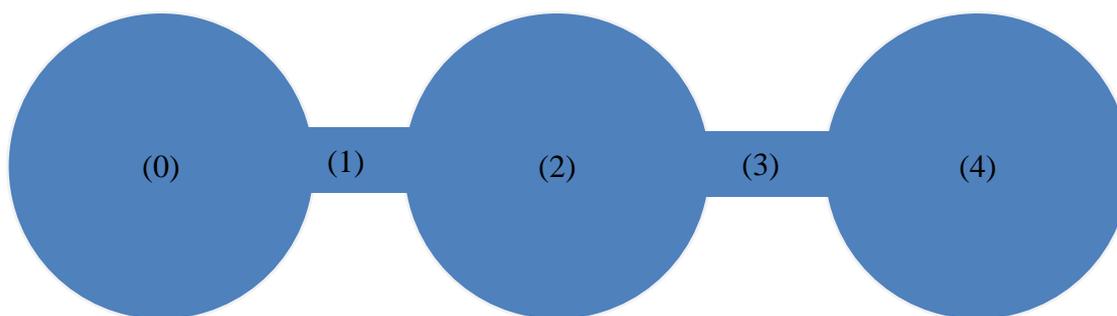


Figure 4.3 Tank configuration of experimental dispersal systems, with dispersal location number in parentheses.

The dispersal assays were conducted on the fish in groups of eight individuals (from the same population), as *P. parva* are a gregarious species that typically aggregate in groups and to isolate individuals might have led to abnormal behaviour, however the issue of pseudoreplication was addressed in the statistical methods used to analyse the collected data. Fish were introduced to the first tank (location 0 in Fig. 4.3) of randomly assigned systems, in their groups of eight, at 5 pm and left overnight to acclimate to the system. Connecting pipes were closed for the duration of the acclimation period, to confine fish to start tanks until the beginning of the dispersal period. Connections were opened at 9 am the following day and then the fish were left to disperse for 72 hours, after which time links were closed, the fish were identified and their final locations recorded (see Fig. 4.3). In some instances the elastomer tags were rejected by the fish during the trial. Where this happened and definitive identification was not possible, individuals were removed from the analysis. In total

data were collected for 188 fish, over five trial runs, giving between three and five replicates for each population (POR = 5, GRE = 4, BOU = 5, AUV = 5, TON = 3, COU = 3).

Morphology analysis

Linear discriminant analysis (LDA) was performed, using the implementation in the ‘Momocs’ R package (Bonhomme *et al.*, 2014), on the 11 measured morphometric characteristics, using source populations as the grouping factor, to produce dimensionally reduced measures of fish shape (five linear discriminants). Only the first two linear discriminants (LDs) were used for subsequent statistical analysis (For bi-plot, see Fig. 4.4), as these captured the majority of the between group variance - a combined 69.9%. The relationship between shape (i.e. LD1 and LD2) and distance from invasion front was tested by means of analysis of variance, using each of the LDs as the dependant variable in turn and using river channel distance, rather than straight-line distance, between population sample locations. We also tested for underlying patterns in SL between populations by using the same method, with SL as the dependant variable. Subsequent pairwise *post hoc* tests were carried out on relevant subsets of data, where appropriate, using population as the independent variable in an ANOVA framework.

Activity analysis

Because the activity data showed signs of overdispersion and because these data were non-integer, we used GLMs with a quasi-Poisson error term to estimate the relationship between activity (km travelled in 15 minutes), the first two LDs, and

standard length (SL) along with possible interactions of each of these variables with distance from invasion front.

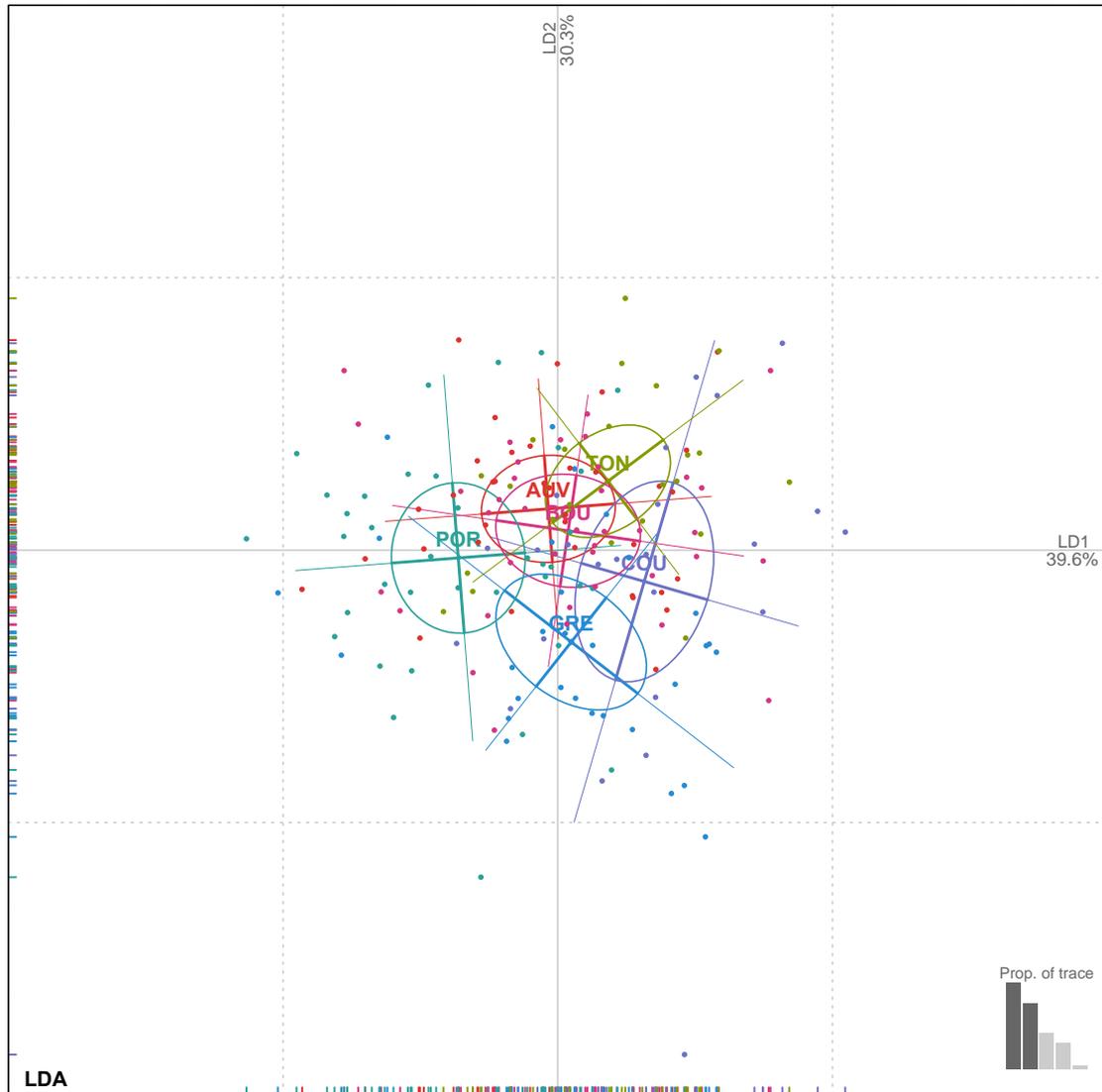


Figure 4.4 First two linear discriminants from linear discriminant analysis of 11 measured morphometric traits, with colours and ellipses identifying the population groups: Portet-sur-Garonne (POR); Grenade (GRE); Bourret (BOU); Auvillar (AUV); Tonneins (TON); Couthures-sur-Garonne (COU).

Dispersal analysis

We used generalized linear mixed effects models (GLMMs) and multi-model inference, through model averaging, in the R-package ‘MuMIn’, to quantify

relationships between propensity to disperse ('Disp') and our five predictor variables: 'Act_km' (Activity); 'log(SL)' (log of Standard Length); 'PropFront' (proportion of total distance from invasion front); 'LD1' and 'LD2' (first two linear discriminants). 'PropFront' and log(SL) were used in place of distance from invasion front (km) and SL in order to maintain similar range magnitudes for all the predictor variables, as this aids convergence of the models. We used the 'dredge' function, in MuMIn, to construct, fit and rank by AICc, models containing all combinations of our five predictor variables, including all two-way and three-way interactions. All models also contained a random intercept term, structured by experimental system used and experimental run (i.e. 1|Sys/Run), to account for pseudoreplication. We then discarded all those models that had a Δ AICc of > 2 , or failed to converge. We calculated estimates, standard errors, 95% confidence intervals and relative importance of the model parameters by full model averaging, based on this subset of 29 best models (i.e. Δ AICc of ≤ 2).

RESULTS

Morphometric analysis

LD1 and LD2 together explained 69.9% of the between-group variance (see Table 4.1), with LD1 dominated by traits relating to relative proportional size of head and other head-related metrics (eye diameter, head length, postorbital distance and preorbital distance cumulatively accounted for 94% of contributions to coefficients of linear discriminants) and LD2 was similarly dominated by head-related measures (see Table 4.2). Analysis of variance showed that LD1 was not significantly associated with SL (ANOVA: $F_{1, 186} = 3.3853$, $P > 0.05$), however LD2 was (ANOVA: $F_{1, 186} = 12.203$, $P < 0.001$), meaning that LD2 likely represents characteristics of shape that

are associated with overall size (i.e. allometry). LD1 and LD2 were both significantly associated with distance from invasion front (ANOVA: $F_{1,186} = 40.471$, $P < 0.01$ and $F_{1,186} = 4.0604$, $P < 0.05$, respectively), with LD1 more consistent in this trend (see Fig. 4.5), however there was no significant association between SL and distance from invasion front (ANOVA: $F_{1,186} = 2.4396$, $P = 0.12$).

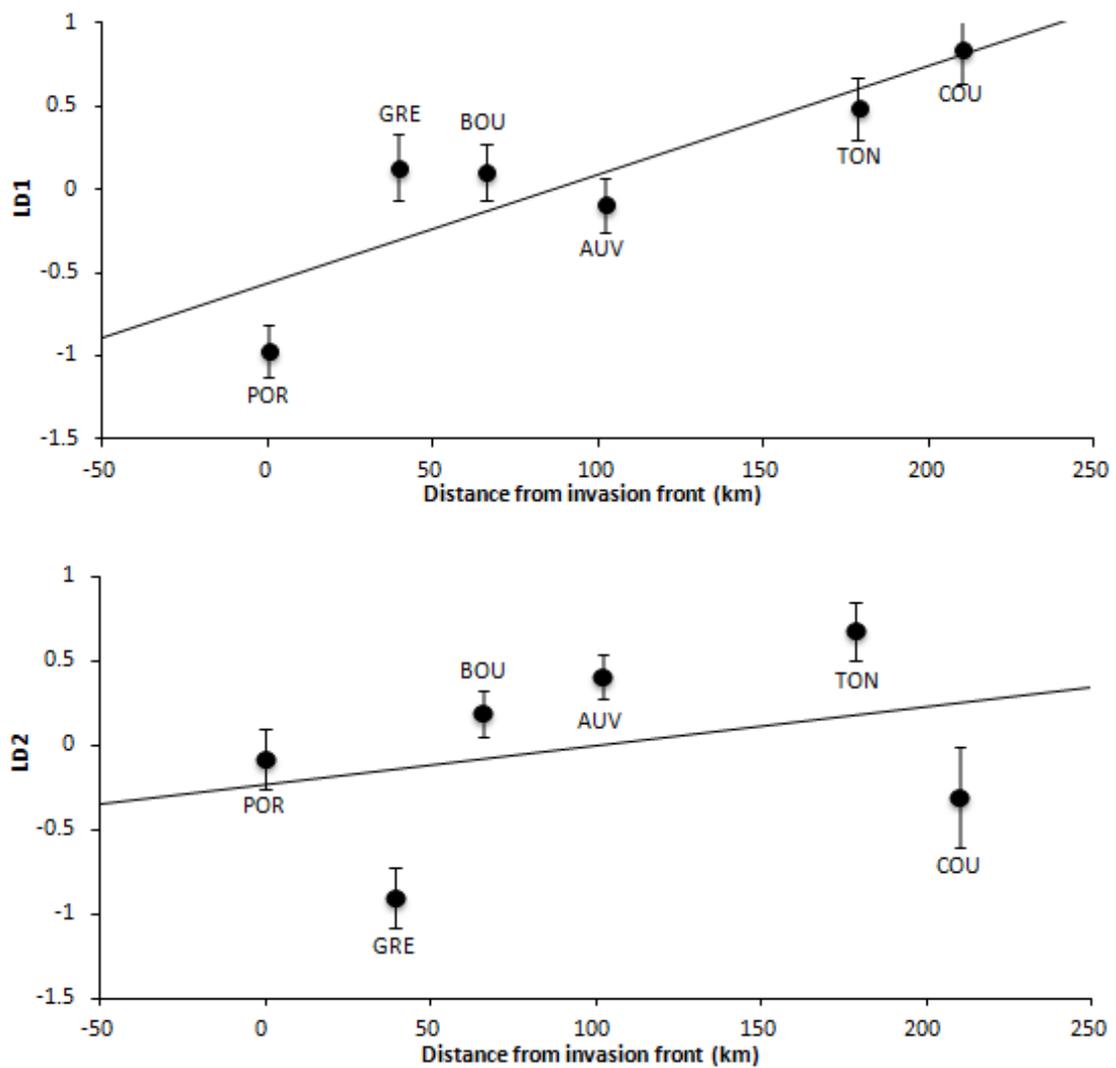
Table 4.1 Proportion of between-group variance explained by each of the five linear discriminants.

LD1	LD2	LD3	LD4	LD5
39.6%	30.3%	16.4%	12.0%	1.7%

Table 4.2 Coefficients of linear discriminants for LDA of 11 morphometric characteristics of

Pseudorasbora parva, using population location as grouping factor.

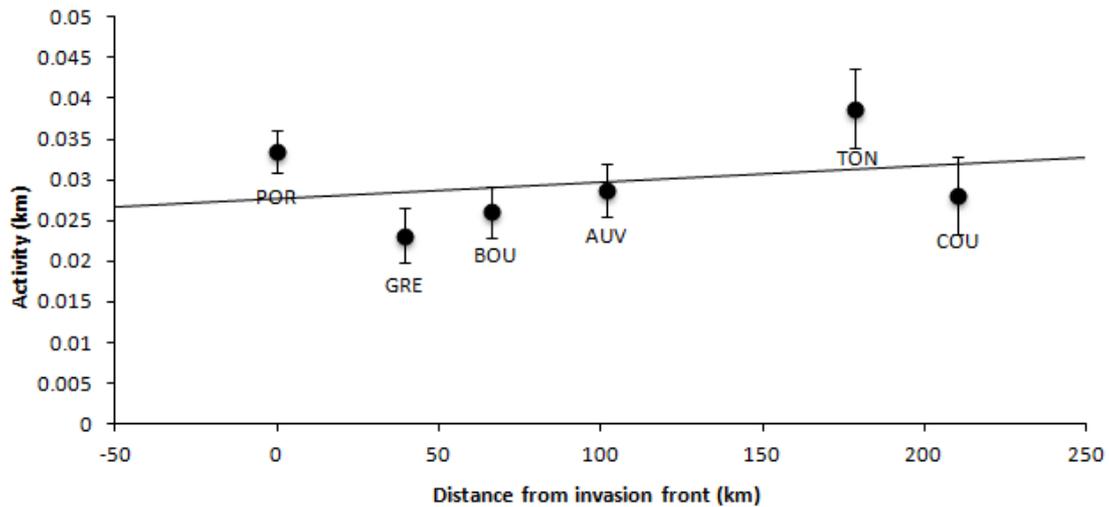
Description	LD1	LD2
SL minus head length	0.00	0.02
Preorbital distance	5.57	-4.12
Eye diameter	11.24	-3.34
Head depth	-0.58	0.17
Postorbital distance	6.32	-7.42
Pectoral fin to pelvic fin	0.14	0.15
Pelvic fin to anal fin	0.19	0.07
Depth of caudal peduncle	0.78	1.01
Body depth	0.18	-1.27
Head length	-7.99	5.90
Anal fin to lower caudal fin	0.08	-0.04



*Figure 4.5 LD1 (top) and LD2 (bottom) plotted against distance from *Pseudorasbora parva* invasion front, with standard errors and linear regression lines.*

Activity

Likelihood ratio tests on our GLMs showed that ‘Activity’ was not significantly associated with distance from invasion front ($\chi^2_1 = 0.49494$, $P = 0.4817$, see Fig. 4.6), nor with LD1 ($\chi^2_1 = 0.63903$, $P = 0.4241$), LD2 ($\chi^2_1 = 0.648$, $P = 0.4208$), or SL ($\chi^2_1 = 0.80796$, $P = 0.3687$).



*Figure 4.6 Activity (distance travelled in 15 minutes) plotted against distance from *Pseudorasbora parva* invasion front, with standard errors and linear regression line.*

Dispersal

Of the 367 models fitted, 29 had a Δ AICc of < 2 . Model averaging, based on these 29 models, showed that three of the 11 parameters had high relative importance ('log(SL)', 'LD1' and 'PropFront'), with the rest receiving medium to low values (see Fig. 4.7). Final parameter estimates (Fig. 4.8) showed that 'log(SL)' had a positive relationship with dispersal distance (i.e. larger individuals dispersed further) and the lower 95% confidence interval remained above zero. 'LD1' had a generally negative relationship with dispersal distance, however the upper 95% confidence interval crossed zero, meaning that the sign of the relationship could not be predicted with confidence. 'PropFront' (proportion of total distance from invasion front) had a slight positive relationship with dispersal distance, but contained a high degree of variability across individual models and the lower confidence interval crossed zero by some margin, meaning that the sign of the relationship could not be predicted with certainty. All other parameters, other than intercept, had 95% confidence intervals

straddling zero, meaning that the sign of the relationships could not be predicted with confidence.

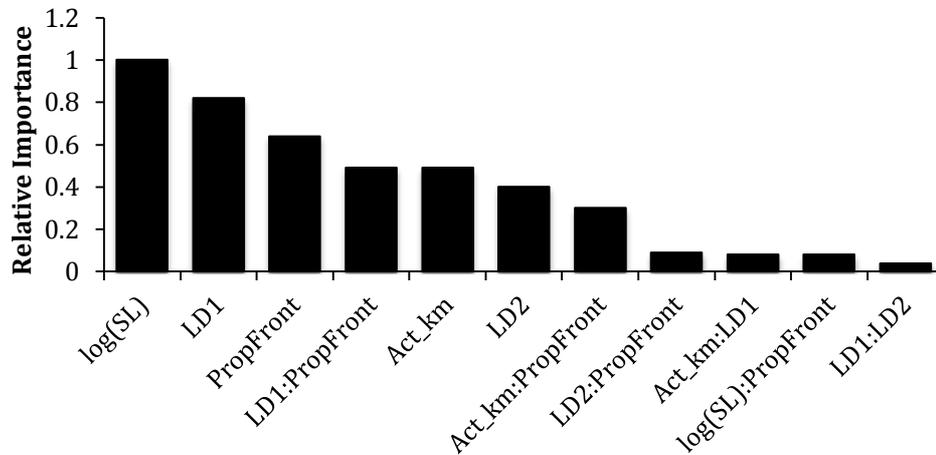


Figure 4.7 Relative importance of parameters in full-averaged model, composed of all fitted models where $\Delta AICc < 2$. Models terms are: log(SL) (1); LD1 (2); PropFront (3); LD1:PropFront (4); Act_km (5); LD2 (6); Act_km:PropFront (7); LD2:PropFront (8); Act_km:LD1 (9); log(SL):PropFront (10); LD1:LD2 (11).

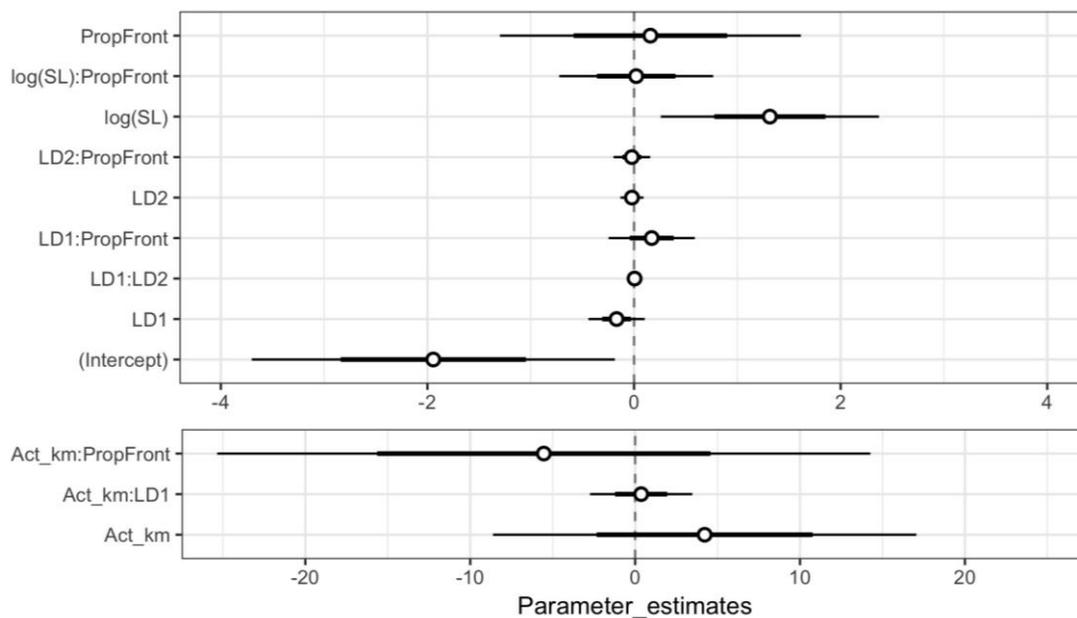


Figure 4.8 Full model averaged parameter coefficient estimates (unfilled circles), with adjusted standard errors (heavier lines) and 95% confidence intervals (lighter lines). Parameters containing the variable 'Act_km' are plotted on a separate axis, as the values are an order of magnitude greater than that of other parameters.

DISCUSSION

This study showed that: (i) there were significant morphological differences between populations, synonymous with distance from invasion front; (ii) variation in individuals' activity levels was not significantly linked to either morphology or distance from invasion front; (iii) Propensity to disperse was not significantly related to activity levels, or distance from invasion front, however it was significantly related to morphology, but only with respect to individuals' size (SL) and not shape (LD1 & LD2).

LD1 and LD2 were significantly related to distance from invasion front, with LD1 highly significant and LD2 marginally significant. This supported our hypothesis that there would be a gradient of morphological change, relative to distance from the invasion front. Whilst LD2 was considered to capture typical shifts in morphology as size increases (i.e. scaling in growth), because of its association with SL, LD1 was considered to represent a phenotypic morphological plasticity associated with the 'leading edge' effect we predicted would be present. LD1 was dominated by 'eye diameter' and 'head length', with the former positively related to distance from invasion front and the latter negatively related to distance from invasion front. This means that individuals at the invasion front typically had smaller eyes and larger heads, and individuals further from the invasion front had larger eyes and smaller heads.

The observed relationship between eye diameter and head length corresponds with known trade-offs between eye size and mandibular arch musculature, seen in other fish (von Scheven *et al.*, 2006), including some cyprinids (Hulsey & Hollingsworth, 2011), however it is unclear whether the driver of this morphological variation is

directly related to the eye, or to the head and jaw, as both have been observed to be plastic in response to various factors. For instance, Franssen *et al.* (2013) found that individuals from low flow environments had shallower bodies and smaller heads than those from higher flow environments (in *Cyprinella venusta*) and variation in head size has been found to relate to gill size (i.e. larger heads have larger gills) and be influenced by water flow and dissolved oxygen levels in other cyprinid species (*Barbus neumayeri* - Langerhans *et al.*, 2007). In the context of a leading edge effect at the invasion front, larger heads and consequently larger gills could result in enhanced swimming endurance (Domenici, 2003), a phenomenon similar to that seen in cane toads, where individuals at the invasion front have greater endurance (Llewelyn *et al.*, 2010).

Intraspecific diet-related plasticity in jaw and head morphology has been observed in numerous fish, with divergent morphotypes apparent in individuals, based on the predominant food-type consumed (Grudzien & Turner, 1984; Wimberger, 1991). In *P. parva* there could be a shift in diet at the invasion front leading to larger jaw muscles and the trade-offs already mentioned leading to smaller eyes. Such a shift in dietary preference has been observed in the European catfish, between recently introduced populations (i.e. invasion front) and earlier introductions (Carol *et al.*, 2009).

Whilst greater eye size is typically synonymous with greater focal length and resolving power (Fernald, 1988), and has been linked with importance of vision in feeding (Evans, 1950; Otten, 1980), large eyes can make individuals more conspicuous to predators and so increase predation risk (Wickler, 1968). Kim *et al.*, (2015) observed, in three-spined sticklebacks, individuals with larger eyes were more

fearful when foraging under predation. If this were applied to *P. parva*, then individuals at the invasion front, with smaller eyes, may suffer less from predator related stress, which could be a particular benefit to individuals who are less social and more prone to disperse, as they would likely be subject to greater predation risk.

Activity is thought to be an important determinant of invasion success (see Chapple *et al.*, 2012) and is thought to be indicative of exploratory behaviour (Walsh & Cummins, 1976). However, contrary to our hypothesis, we found no link between activity and distance from invasion front, or morphological characteristics, which may mean that this is independent of body shape and size (i.e. a personality trait, such as that seen by Cote *et al.*, 2010 in *Gambusia affinis*) and does not appear to be more prevalent at the invasion front.

The only clear predictor of dispersal propensity that we found was fish size (SL), in that larger individuals dispersed further in our experimental systems. Whilst relative importance values of other parameters were moderate or high, in the full-averaged model (i.e. 'LD1', 0.82 and 'PropFront', 0.64 in Fig. 4.7), confidence intervals straddled zero, so the sign of the relationship was not consistently predicted. So, contrary to our hypothesis, dispersal was not linked to the measured morphological traits (beyond that of SL) and did not vary corresponding to distance from invasion front.

Movement and range size in animals typically increases with body size (Peters, 1986; Knight *et al.*, 2016) and this is certainly the case for a number of freshwater fish (Minns, 1995; Radinger & Wolter, 2014). Our results seem to support this general rule, and other studies too have similarly found a lack of differentiation in dispersal

between populations from the invasion front and from areas where they are well established (i.e. *Hemichromis bimaculatus* - Lopez *et al.*, 2012). However, it is also possible that our experimental dispersal assays did not measure dispersal in a fully realistic way. Whilst the *P. parva* populations in the River Garonne are subjected to flowing water when dispersing, our systems contained static water. This could mean that instead of quantifying dispersal, the assays merely quantified exploratory behaviour, which along with boldness, Cote *et al.*, (2010) found to be unrelated to dispersal in another invasive freshwater fish (*Gambusia affinis*). Studies of dispersal in *P. parva* relative to flow velocity, specifically the willingness of individuals to move into flowing water from still waters, could certainly help to provide a means of calibration for future studies of dispersal, to ensure that what is being measured is in fact a fair representation of dispersal, relevant to the natural environment.

Although Cote *et al.* (2010) found exploratory behaviour and boldness unrelated to dispersal, sociability was strongly linked to dispersal. In fact, sociability has been linked to dispersal in other taxa too (e.g. Cote & Clobert, 2007; Quinn *et al.*, 2011; Thorlacius *et al.*, 2015), suggesting that dispersal can be context dependant, relating to factors linked to social interactions. *P. parva* is a gregarious species and tend to form groups, which is why we conducted our dispersal assays on groups, rather than individuals. However, if dispersal is linked to social interactions, then population density, or group size, could be critical in triggering the expression of variation in this behaviour. For example, Broderon *et al.* (2008) found that the decision to make partial-migrations in *Rutilus rutilus* was context-dependent, determined by body condition and food availability. Competition and hence food availability will vary according to the population density (Bertram, 1978), potentially leading to density-dependent dispersal behaviour, which could also be dictated by body size (a

determinant of competitive ability, Hoare *et al.*, 2000). Increasing the group size of fish in our dispersal assays may have had a significant impact and future studies should consider the potential impact of population density on dispersal outcomes. It is possible that differences in dispersal may only manifest at higher population densities, particularly if social interactions are triggers for such behaviour.

CONCLUSIONS

At low densities, the dispersal propensity of *P. parva* in still waters relates to fish size, with larger fish dispersing further. Introductions of larger individuals may be more prone to actively spread and to do so more rapidly than smaller individuals. However, this pattern of dispersal may differ at higher population densities and could include a shift in diet, manifested physically in craniofacial morphological changes. Whilst in our study morphological traits measured were not implicated in dispersal, or activity levels, there was a strong gradient of change relative to distance from invasion front. This morphological shift shows that these *P. parva* possess the potential to be highly phenotypically plastic, even along a c. 210 km stretch of the same river; a relatively short distance. Such plasticity is thought to be a key indicator of invasive species (Davidson *et al.*, 2011) and our results provide evidence to reinforce this view. Whether this plasticity is related, directly or indirectly to dispersal, further research on social behaviour and possible density-dependent effects could be invaluable in elucidating mechanisms that determine successful spread and population dynamics in this species, including how it maintains a seemingly high potential for phenotypic plasticity in invasion scenarios, where we would often expect low genetic diversity.

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GENERAL DISCUSSION

APPROACH

After the initial summary section, I will approach this discussion by looking at the data chapters in sequence, but combining the Chapters II and III, as they both relate to climatic adaptations. I will first consider the relevance of the main findings and their practical implications, then opportunities for improvement and possible future research directions. I will finish by giving a brief personal perspective.

SUMMARY

In this thesis I set out to quantify the risk of invasion from the invasive freshwater fish, *Pseudorasbora parva*, at a global extent, using traditional correlative ecological niche modelling approaches. These correlative approaches rely upon key assumptions relating to the presence or absence of local or regional adaptations, and so I subsequently tested for evidence of such adaptations in genetic lineages and individual populations, by analysing climatic niche differentiation in the native and invasive ranges (Chapter II) and by conducting lab experiments comparing thermal responses of important life history traits (Chapter III). The initial risk assessment (Chapter I) carried out did not account for a key factor in invasions; namely natural dispersal. Natural dispersal has been observed to be subject to selection in vanguard populations of invasive species, and adaptation of dispersal traits can infer additional invasive vigour, allowing the species to spread across the landscape quicker. For example, in the Australian cane toad invasion, a fivefold increase in rate of spread over 70 years was attributed to genetic dispersal adaptations in individuals at the

expansion front (Phillips *et al.*, 2010). I quantified dispersal, activity and morphological differences, often associated with differential dispersal ability, in six populations along a distance-gradient from an invasion front, in order to identify if *P. parva* is capable of such adaptations (which could influence management and containment protocols to prevent secondary translocation of individuals from expansion front locations).

The projection of the niche model in the risk mapping study (Chapter I) showed that large areas, beyond the current distribution of the species, are climatically suitable. These areas are mainly in North and South America as well as Australia and New Zealand and constitute significant scope for spread and impact in this species. When introduction likelihood was added, N. America appears most at risk. In investigating the climatic niches of the lineages, in the native and invaded ranges (Chapter II), I found no evidence to suggest that the genetic lineages represented local adaptations to their respective native climates. There was little or no differentiation of the lineages' climatic niches in the invasive range. It was also apparent, from the niche comparisons, that the climatic niche in invaded range constituted a significant shift in realized niche, compared to the native range.

I found that thermal responses of important life history traits (Chapter III) were not significantly different between populations from two different climates in the invaded range; one strongly seasonal continental and the other mild temperate maritime. The overall reproductive output of females did not vary according to breeding season temperature, however, temporal reproductive strategy showed a strong response, with lower temperatures inducing a protracted breeding season and higher temperatures inducing rapid and intense reproductive output. In the dispersal and morphology-

related study (Chapter IV), I found a strong gradient of morphological change, corresponding with distance from invasion front, demonstrating a high degree of plasticity in morphology. However, this morphology did not determine propensity to disperse in my experiments and nor did activity levels. Size was the only significant explanatory factor in dispersal in my study.

RISK MAP

The fact that North America is predicted to be at high risk from invasion of *P. parva*, seems wholly reasonable given the history of this region with regard to aquatic invasions. A number of Ponto-Caspian species have invaded both European and North American inland waters (e.g. *Hemimysis anomala* – Audzijonyte *et al.*, 2008 and various others - see Leppäkoski & Olenin, 2001), demonstrating both the similarity in climatic conditions between these regions (Mills *et al.*, 1994) and the high incidence of introductions from Eurasia to N. America. Studies of biological invasions of N. American inland waters have shown the possible impacts of aquatic invasions are severe, both in terms of biodiversity and in economic terms (Vanderploeg *et al.*, 2002; Pimentel *et al.*, 2005). Aside from the direct impacts of invasions, the increased number of introduced species in the N. American Great Lakes region is possibly facilitating further invasions, making it increasingly easy for new introductions to establish (Ricciardi, 2001). Further, a successful invasion by *P. parva* would bring with it the associated issue of this fish being a healthy carrier of *Sphaerothecum destruens*, a pathogen previously observed to have had severe impacts on North American Salmonid populations (Gozlan *et al.*, 2005; Arkush *et al.*, 2003). Established *P. parva* populations could act as major reservoirs of the pathogen, increasing likelihood of outbreaks (Peeler *et al.*, 2011, Ercan *et al.*, 2015).

The US Great Lakes are already subject to a considerable burden from invasive species and there is concern that this may be facilitating further invasions (Ricciardi, 2001). However, the US Fish and Wildlife Service (USFWS) have already identified *P. parva* as a high risk invasive species with potential for high negative impact and in September 2016 it was added to their list of “injurious species”, effectively prohibiting its import into and movement within the US (USFWS, 2016). Whilst this is positive and will prevent intentional introductions, given that most introductions of this species throughout its invasive range were unintentional, through contaminated consignments of other species, these measures may not necessarily prevent such introductions in the US (Britton *et al.*, 2011). Although fines resulting from even accidental breaking of laws may give added incentive to those importing other species to more thoroughly check consignments for *P. parva*, a high level of vigilance is required in order to detect possible introductions early and deal with them before they escalate beyond the invasion-event-horizon for feasible eradication (Davies *et al.*, 2013).

In the Eastern regions of the USA, my model’s suitability predictions are largely concordant with those of the USFWS climate matching assessment (Section 6; USFWS, 2016), however their assessment predicts high levels of climatic match across much of Central and Western Mainland US, where my model predicts low suitability. The USFWS climate matching analysis, using CLIMATCH (Australian Bureau of Rural Sciences 2018), appears to be missing *P. parva* source locations in the invasive range. Two particular areas where *P. parva* is present but underrepresented in the USFWS training dataset are northern Central Europe (i.e. Germany and Poland) and parts of the Middle East. The omission of these locations,

where *P. parva* have successfully invaded, could mean that certain climatic conditions are missing from the characterised climatic niche, used to compare to conditions at weather stations in the target area (i.e. the US). Nonetheless, the CLIMATCH method uses Euclidean distances, based on 16 climate variables, as a means of characterising the climate at included weather stations, which essentially means that all 16 of these variables are given equal weighting and are treated as non-interacting in determining suitability for *P. parva*. Such a method will tend to consistently over-predict, as the Euclidean CLIMATCH metric is based on comparison of target sites with a mean value of each climatic variable. The authors of the software do acknowledge that this is the case (Crombie *et al.*, 2008) and this also fits with precautionary principal approach, which enjoys widespread support from conservationists (e.g. Simberloff, 2003). In the case of species such as *P. parva*, which are known to have strongly negative impacts and little or no commercial value, this seems eminently sensible. However, care should be taken to not adopt too conservative an approach for all taxa, as inappropriate rejection of species with considerable commercial value, due to overestimation of invasion risks, could have net negative effects (see Smith *et al.*, 1999). As an example of the difficulty that can be faced when trying to *a priori* differentiate potentially invasive species, from those that are benign. *Pseudorasbora elongata*, as a close relative of *P. parva* (Yang *et al.*, 2006), is similar in both biology and ecology, however is in decline in its native range and despite being associated with ornamental fish trade, is not recorded to be invasive (Chen, 2011). *Pseudorasbora pumila* is also very closely related to *P. parva*, but is endemic to Japan and, in some instances, may be threatened by introductions of *P. parva* (see Koga & Goto, 2005).

Parts of South America are identified as other possible locations at risk from *P. parva* invasion. The fact that South America is not typically closely linked with the topic of biological invasions perhaps owes much to the relative dearth of research and general interest on the subject in this part of the world (Speziale *et al.*, 2012). Nevertheless, invasive species were estimated to cost Brazil alone USD\$ 49.3 billion per year, to the turn of the century, through losses to the crops, pastures, forests and the environment at large (Pimentel *et al.*, 2001). In fact, 40 of the top 100 world's worst invasive species have been introduced to South America (IUCN-GISD, 2017) and most of the introduced species that went on to become invasive in S. America came from Eurasia (Speziale & Ezcurra, 2011; Speziale *et al.*, 2012). Various Asian carp species, closely associated with initial introductions of *P. parva* to Europe (Gozlan *et al.*, 2010), are invasive in S. America (IUCN - GISD, 2017). Despite this, *P. parva* are, as yet, not known to be present – perhaps due to historically lower propagule pressure, relative to introductions into Europe – although it is likely that introduced species here are under-recorded (Quiroz *et al.*, 2009). Nonetheless, we can expect to see an increase in the number of non-native introductions, analogous to the trends of increasing GDP in a number of S. American countries (Pyšek *et al.*, 2008; Hulme *et al.*, 2009), so the risks should be taken seriously, particularly because the region hosts a number of biodiversity hotspots (Myers *et al.*, 2000) and are lagging behind in terms of environmental policies and risk assessments.

In South America, the outlook for preventative action is less positive than that of N. America, in relation to *P. parva*. There has been a recent raft of proposed and implemented legislative change in Brazil, designed to nurture economic growth, particularly in the aquaculture sector, including for food production and the ornamental trade (Vitule, 2012; Pelicice *et al.*, 2014; Vitule *et al.*, 2014; Junior *et al.*,

2015; Azevedo-Santos *et al.*, 2017). However little thought has been given to the likely negative consequences of such activity, which will undoubtedly increase the number of non-native introductions (Vitule *et al.*, 2014) and also provide a system where spread is enabled. For instance, a number of non-native fish, notorious for their high invasive potential, are being exempted from import restrictions (Pelicice *et al.*, 2014; Junior *et al.*, 2015), so that they can be reared and traded. Further, rules on transporting live fish are being relaxed and inter-catchment connectivity is being increased by means of large-scale water diversion, man-made channels and the construction of hydroelectric dams (Pelicice *et al.*, 2014; Vitule *et al.*, 2015). Despite opposition from the scientific community, the Brazilian government have pushed ahead with such policies, seemingly unperturbed (Azevedo-Santos *et al.*, 2017). If the current trend of increased non-native aquatic species introductions continues, and it seems likely to, then parts of South America could be facing similar magnitudes of invasion and consequent impacts to those seen in the North American Great Lakes, one of the most heavily invaded ecosystems on the planet (Holeck *et al.*, 2004; Ricciardi *et al.*, 2006).

Makhrov *et al.* (2013) found evidence of a reproducing *P. parva* population in Tibet, at an approximate altitude of 3600 m above sea level. My model predictions generally show that mountainous regions are not suitable for *P. parva*, however this could be due to lack of propagule pressure, as these are unlikely locations for introductions, given that most introductions were associated with aquaculture. As Makhrov *et al.* (2013) point out, it is traditional to release live fish into water bodies, in Asian Buddhist culture, so this is a likely source of its introduction there. The presence of *P. parva* in this Tibetan location implies that perhaps there is still more scope for this species to spread than my model in Chapter I predicts.

My final risk map integrated the Vörösmarty *et al.* (2010) “Aquaculture Pressure” dataset, a surrogate for introduction likelihood, in order to provide an estimate of successful invasion likelihood. I used an equal weighting with bioclimatic suitability, implying that both factors are equally important to invasion success, however one could argue that suitability is more important, with suitable locations only needing as few as one propagule introduced to lead to an invasion. Whilst integration of introduction likelihood may well provide a refined risk assessment, able to differentiate between suitable locations with high and low likelihood of introduction, care should be taken to maintain a precautionary approach, whereby any suitable location is at potential risk. Furthermore, and directly related to the output of the risk map, these “Aquaculture Pressure” data are already out of date, with Brazil in particular likely to harbour much larger values now, due to the recent legislative-backed growth in the aquaculture sector.

CLIMATIC ADAPTATIONS

In niche comparisons of the two key genetic lineages, in both native and invaded ranges, I found no evidence to support the idea that the lineages represent local climatic adaptations in the native range, or that either lineage was differentially associated with any particular climate-type in the invaded range. Similarly, the thermal response experiments identified no significant differentiation between populations from two differing climate-types. The most parsimonious explanation for these results is that, instead of local genetic adaptations, *P. parva* has very broad tolerances and maintains fitness in a wide variety of conditions. Despite their seeming ubiquity in the literature, there are few empirically proven examples of contemporary genetic adaptations (Gienapp *et al.*, 2008). Biological invasions are often typified by

having small, genetically depauperate founder populations and generally speaking, the literature says that small populations, bottlenecks and generally low genetic diversity should preclude adaptation, or at least provide little opportunity for it to occur (Allendorf & Lundquist, 2003). Specialist, or local adaptations typically have trade-offs, so broader tolerances could suffer as a result for attenuated fitness in specific conditions (Hereford, 2009). If the conditions are tolerable without adaptation, then adaptation is unlikely to occur in the short-term, as there is a lack of selective pressure.

The fact that the native and invasive climatic niches did not overlap significantly implies that for some species the current range is not necessarily a good predictor of the potential niche (see General Introduction). Although this phenomenon is not in itself a particularly remarkable or rare observation (Fitzpatrick *et al.*, 2007; Loo *et al.*, 2007; Hill *et al.*, 2013), it does highlight that predicting invasive potential using such climatic niche-based approaches is prone to significant uncertainty. Further, species with large native ranges, which are not overtly constrained by extensive physical geography, are typically thought to realize much of their fundamental niche (Early & Sax, 2014), so predictions based on native distributions should be good. In the case of *P. parva*, this means that the lack of niche overlap between native and invasive ranges is perhaps uncharacteristic, given the sprawling nature of its native range, both in terms of spatial extent and also in terms of climatic breadth. This raises two important implications: (i) if the potential niche was greater than the realised niche, prior to invasion, then there is a possibility that the potential is yet greater than predicted in my risk maps, meaning that wider areas are potentially at risk; (ii) other factors, not captured in the predictor dataset, must be important in determining the distribution of *P. parva*. With this in mind, the data available on the invasion of *P. parva* provides a

good basis to investigate what these other factors may be. For instance, we know that *P. parva* is a healthy carrier for an infectious disease that causes mortality and morbidity in other fish species (Andreou *et al.*, 2012). The presence of this disease could, in theory, depress competitiveness of naïve communities where *P. parva* is introduced, thus facilitating invasion. It could be informative, then, to investigate if susceptible fish are more abundant at *P. parva* invaded locations representing higher levels of climatic stress (i.e. far from native range conditions). In this context, distance, measured on PCA axes, from native range niche centroid could be used as a measure of climatic stress. Other potential factors could also be tested in a similar way, using degree of differentiation from native niche-space as a response variable, representing potential climatic stress, in various models.

Although total reproductive output was not affected by temperature, in the thermal response experiments (Chapter III), *P. parva* did exhibit a strong response of temporal reproductive strategy, with intensity positively related to temperature. Variations in growth and reproductive strategies typically reflect trade-offs between juvenile mortality, adult fecundity and age at maturity, in order to maximise annual recruitment to the population (Garvey *et al.*, 2002). In the case of *P. parva*, this strong thermal response may enhance their ability to rapidly form dense populations in favourable warm conditions, maximising growth potential in progeny by concentrating reproductive effort at the beginning of the productive warm season, which will in turn enhance overwinter survival of juveniles and their subsequent fecundity, when sexually mature. At more poleward latitudes, where conditions are colder and the productive season is shorter, the observed shift in strategy may enhance medium-term persistence of adults by minimising wasteful reproductive effort (i.e. on producing offspring in climates where early growth would be minimal

and overwinter mortality severe), thus maintaining body condition and increasing adult overwinter survival. In an invasion context, this would still mean that this population had invasion potential, particularly if it was associated with the aquaculture trade, wherein it could contaminate outgoing consignments and flourish when introduced to locations with warmer conditions.

Whilst my study showed no significant difference between populations from Poland and England, there are populations in locations that differ from one another in climate more strongly than these two locations. Selection of populations for future tests could, in theory, be selected on an *a priori* basis of being climatically dissimilar and include populations from the native range too, in order to corroborate the implied lack of adaptation from native to invasive ranges observed in Chapter II. A large number of replicates would allow for other important factors to be investigated, which could shed light on important determinants of overall reproductive performance of invasive fish, such as overwinter conditions and food availability (as discussed in Chapter III). These factors were not varied in my experiments, as there were insufficient numbers of fish and also capacity for replication, without which statistical power in analyses would be unacceptably low.

DISPERSAL AND MORPHOLOGY

In my dispersal and morphology study (Chapter IV), I found a strong gradient of morphological change, corresponding to distance from the invasion front. Spatial sorting theory predicts that where there is differential dispersal between individuals in range-expanding populations, individuals exhibiting greater dispersal ability will tend to aggregate along the expansion fronts (Shine *et al.*, 2011). Dispersal ability is often

linked to morphological characteristics or traits and so dispersal-types, idiosyncratic by their morphology are often observed to be at higher prevalence on expanding range fronts (e.g. Phillips *et al.*, 2006; Hughes *et al.*, 2007; Forsman *et al.*, 2011). I found that the morphological gradient was not linked to dispersal, with only size a significant factor for dispersal distance in experimental systems. That size was the most significant predictor of dispersal in my experiments, was not especially remarkable – it is a common phenomenon across a number of different taxa, including fishes (Peters, 1986; Minns, 1995; Radinger & Wolter, 2014) – however, the lack of connection between the strong morphological change gradient and dispersal was unexpected, as was the similar lack of connection between dispersal propensity and distance from invasion front. Spatial sorting theory states that dispersal phenotypes will tend to have higher incidence in colonising populations (Shine *et al.*, 2011), so it seems atypical to not find a connection with either morphology (commonly associated with differential dispersal ability) or distance from invasion front.

Higher propensity to disperse in larger individuals could facilitate the invasion process, purely by the mechanism of more fecund individuals (see Chapter III) being more likely to disperse. It is, however, also possible, as mentioned in Chapter IV, that the experimental design used did not allow enough scope for the differentiation in dispersal amongst individuals to be expressed. For instance, whereas my experiments were conducted under still water conditions, other assessments of dispersal (e.g. Cote *et al.*, 2010; Ashenden *et al.*, 2017) used systems with a channel of flowing water. The additional effort and swimming ability necessary from individuals in order to classify as dispersers may refine the response and provide a clearer picture. Future studies could incorporate such a design and should also investigate the potentially important factor of population density (i.e. starting density), as this could be an

important link to social behaviour, which could express itself as density-dependant dispersal propensity.

CONCLUSIONS

This work provides a global scale invasion risk map for *P. parva*, which shows significant scope for further spread of the invasive range, particularly in areas of North and South America. Whilst I found no evidence to suggest that these predictions are hampered by differentiation at either lineage or population levels, the findings of Chapter II do highlight the uncertainties surrounding the degree of conservatism in such predictions, mainly owing to the fact that past distribution did not accurately predict the current invaded distribution. Nonetheless, using the invaded and native distributions, as in Chapter I, does mean that predictions are less conservative than those based purely on the native range. The lack of evidence for local adaptation (Chapters II & III) should be complemented by tests of populations of *P. parva* from the native range, which could help confirm or refute, with certainty, that the shift in realised niche has a genetic basis. If there is no genetic basis for this shift, then the *P. parva* invasion provides an ideal framework for investigation other important factors in the determination of species distributions (e.g. biotic factors, fine-scale abiotic factors).

The results of Chapter IV show a strong morphological shift with distance from a spreading invasion front, which emphasizes the high degree of phenotypic plasticity exhibited by *P. parva* in an invasion context. Although this was not significantly linked to dispersal in my experimental systems, this plasticity could have an impact on invasiveness. Further investigation of this strong pattern is warranted, as it could

relate to feeding behaviour, which may directly influence the impact on native taxa through trophic overlap. Moreover, it could mean that morphological measures could be used to differentiate vanguard populations from older populations, which would undoubtedly be useful when formulating plans for invasion management and eradication.

PERSPECTIVES

My global risk map shows scope for further spread of *P. parva*, which, if realized, will likely result in negative impacts on invaded ecosystems. There are also possible economic implications if these invasive *P. parva* populations increase commercial fisheries' exposure to disease (e.g. *S. destruens*). Whilst I found no evidence to suggest that there have been climatic adaptations in *P. parva*, at either the level of genetic lineages, or individual populations within the invasive range, my dispersal and morphology study did identify a strong morphological cline in the context of landscape scale metapopulations (i.e. populations along the same river channel).

Intraspecific morphological variability, particularly in head-related measurements, in fishes often relates to prey type, or diet (e.g. in *Lepomis gibbosus*, Mittelbach *et al.*, 1999; in *Archosargus probatocephalus*, Cutwa & Turingan, 2000; in *Perca fluviatilis* and *Rutilus rutilus*, Svanbäck *et al.*, 2008). Trophic plasticity is a trait exhibited by a number of other invasive freshwater cyprinids (Almeida *et al.*, 2009; Godard *et al.*, 2013) and flexibility in diet may facilitate the invasion of novel systems (Almeida *et al.*, 2016). Mittelbach *et al.* (1999) observed that another invasive fish species, *Lepomis gibbosus*, exhibit plasticity in jaw morphology, directly related to diet. Their study tested for phenotypic plasticity versus localized genetic adaptation, by using

common garden experiments to compare two morphologically divergent populations, exposing the lab-reared offspring from each of these populations to two differing diets. Such experimental methods could also be used to test for the same in *P. parva*, perhaps in addition to, or in conjunction with, gut content analysis on the fish from the locations I sampled along the River Garonne (Chapter IV).

Although my analysis in Chapter II found no evidence for climatic adaptations of the two native lineages to the conditions of their respective native distributions (i.e. northern and southern), the results did highlight the shift in occupied climatic niche from native to invasive range. Chapter III tested for differences in life history trait thermal response between fish from invasive populations, but I was unable to include fish from native populations, due to a combination of time constraints and difficulties in obtaining and shipping samples to the UK. The majority of the studies in the literature conducted on *P. parva* are based on observations in the invasive range (e.g. Rosecchi *et al.*, 1993; Pinder *et al.*, 2005; Britton *et al.*, 2008, 2010), with relatively few accessible examples of native population studies. Comparisons of native and invasive populations could prove essential to identifying what makes *P. parva* such a successful invader and how it is able to establish populations in novel conditions. For instance, Nolte *et al.* (2005) compared native populations and invasive populations of *Cottus gobio* and identified a morphological shift and a new ecological potential, which appeared to facilitate invasion, as a result of hybridization between two genetic lineages of *Cottus gobio*. They found that the new hybrid invaded locations with environmental conditions beyond the range of occupied conditions previously observed in either genetic lineage. It is feasible that the two native mainland Chinese lineages (see Chapter II) of *P. parva* have similarly hybridized and that hybrid vigor

manifests as phenotypic plasticity, with a broadening of climatic tolerances, as seen in the shift in occupied climatic niche between native and invasive ranges, in Chapter II.

The plasticity exhibited by *P. parva* in the invasive range (e.g. in life history traits, Britton *et al.*, 2008; in morphology, Chapter IV) may also be present in the native range, but it could equally be an evolutionary response to release from natural enemies in the introduced range. Invasive populations often evolve to be more plastic than their native conspecifics (e.g. Lavergne & Molofsky, 2007; Caño *et al.*, 2008) and this evolved plasticity can be a product of release from enemies, or stress in the native range (Huang *et al.*, 2015). Release from native range enemies may significantly shift the balance of costs and benefits of developing plasticity, which may be too costly in a strongly competitive or stressful native range, but feasible in naive ecosystems. *P. parva* could be a successful invader because it evolved in a highly competitive environment and consequently is able to outcompete many other species in new ecosystems. However, it is possible that evolved plasticity gives *P. parva* a degree of flexibility in behavior, morphology and life history traits that allows it to rapidly adapt to varying conditions. Further, it is possible that any adaptation to conditions associated with human activities (i.e. degraded, or disturbed conditions) could facilitate spread and augment their invasiveness, through increased exposure to possible accidental transportation to new locations.

Below, I make some suggestions for subsequent pertinent questions and give some brief ideas of how it may be possible to address these questions:

Q 1 - Is the morphological cline in *P. parva* populations along the River Garonne, associated with diet?

Parallel study of cranio-facial morphology (e.g. similar traits as those used by Martin & Wainwright, 2011) and gut content analysis (e.g. see Almeida *et al.*, 2016) testing for concordance with the between these factors and distance along transect, starting at the invasion front. Stable isotope analysis may also be informative in such a context.

Q 2 - Is the morphology cline observed in Chapter IV plasticity, or does it have a genetic basis?

Rear offspring of two strongly morphologically divergent populations and then use common garden experimental design similar to that of Mittlebach *et al.* (1999), basing the two diet types on the results of the morphology vs diet study (assuming that a relationship is identified).

Q 3 - The two key native lineages hybridized and led to an invasive phenotype, able to inhabit novel conditions?

The methods of Nolte *et al.* (2005), but with morphometric analysis methods of Záhorská *et al.* (2009), could test for such a scenario, however, it would involve an extensive sampling program across both native and invasive ranges in order to ensure that suitable populations of both native lineages and potential invasive hybrids were captured in the analysis.

Q 4 - Is phenotypic plasticity in the invasive range an evolved characteristic?

Using morphologically divergent native range populations (i.e. northern and southern) and two or more morphologically divergent invasive populations, conduct common garden experiments, like those of Mittlebach *et al.* (1999) on the lab reared offspring of the to compare plasticity in these populations. If the invasive populations exhibit plasticity, but the native populations exhibit a lack of plasticity, then this supports the

hypothesis that the plasticity is an evolved characteristic in the invasive range. However, if all populations show similar responses, then this implies that the plasticity is universal across both ranges.

n.b. *P. parva* are also documented to exhibit density-dependent plasticity of life history traits in the invasive range (Britton *et al.*, 2008) and the two lineages do show morphological differentiation in the native range (pers comm. R. Gozlan 2015), so it may also be useful to compare plasticity in these traits too.

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