How predictable is intraspecific variation? Insights from guppies and stickleback

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Abstract

Variation within species ("intraspecific variation") has strong effects on individual fitness and dictates how organisms interact with their environments. Intraspecific variation thus can shape ecological and evolutionary processes in nature and can also have important practical applications. However, the extent to which the causes and consequences of intraspecific variation are predictable (i.e., can be explained by statistical models) remains unknown. Addressing this knowledge gap is critical, because understanding the causes and consequences, or manipulating variation to generate desired outcomes for applied purposes, is contingent on how predictable that variation is. In this thesis, I seek to address this knowledge gap using two freshwater fish natural model organisms.

In Part One, I leverage Trinidad as a natural laboratory to investigate the extent to which the causes of intraspecific variation are predictable in guppies (*Poecilia reticulata*). Investigating the causes of variation in guppies is important because work on this species has contributed substantially to our foundational knowledge of evolutionary biology. In Chapter 1, I first quantify the extent to which predation regime can explain variance in guppy population-level trait means and assess various factors that could decrease parallelism (i.e., reduce predictability). I show that the majority (~75%) of the variance in guppy population-level trait means is not explained by a simplified predation regime dichotomy, that parallelism seems to be especially weak for colour traits, and that increasing the complexity of evolutionary history decreases estimates of parallelism. In Chapter 2, I focus within a single level of the regime (low predation) to ask how an overlooked aspect of the environment – its abiotic features – can shape two behavioural phenotypes. I show that abiotic environmental features, even on small spatial scales,

can be strongly associated with behaviour, and could therefore reduce overall predictability of the predation regime dichotomy.

In Part Two, I conduct experimental manipulations in nature to investigate the extent to which the consequences of intraspecific variation are predictable with stickleback (*Gasterosteus aculeatus)*. I specifically focus on the predictability of non-random movement (dispersal and assortment), because stickleback are often introduced into novel environments and non-random movement could have important ecological and evolutionary effects following introductions. In Chapter 3, I ask how experimentally induced variation in fibrosis – an inflammation and tissue repair response – affects dispersal, an ecologically important process. I find that the effects of fibrosis on dispersal are not predictable, although some compelling, but not statistically significant trends, suggest that early-stage inflammation may affect dispersal. For the last two chapters, I leverage conservation translocations where stickleback from multiple 'source' populations were experimentally introduced into lakes from which stickleback had been previously extirpated. In Chapter 4, I assess how stickleback from different source populations spatially assort in a novel environment, yielding insight into how evolution might unfold following introductions. I find that stickleback assortment is not predictable because stickleback seemingly assort randomly. In Chapter 5, I finally investigate the extent to which source population can be used to predict dispersal following a conservation translocation, providing insight into the practical applications of variation in dispersal. Although ancestry could not predict dispersal, less exploratory individuals were more likely to be captured far from the introduction point which suggests that less exploratory individuals could 'lead' the dispersal front following translocation.

Across all Chapters, the majority of variance was not explained by statistical models. My work therefore converges on the conclusion that the causes and consequences of intraspecific variation are typically only weakly predictable and are often not predictable. This conclusion shows that much of our foundational understanding of what causes variation in guppies lacks nuance surrounding the complexities of natural systems, and that other consequences, beyond movement, should be prioritized to understand the ecological and evolutionary consequences of stickleback introductions. Finer-scale sampling of aspects of the environment beyond 'key' sources of selection can improve predictability on the causes, and focusing on other traits that might be more predictable could improve predictability on the consequences. Although I found weak predictability overall, predictability will only continue to improve as we increase our knowledge of natural systems.

Résumé

La variation au sein d'une espèce (« variation intraspécifique ») a des effets considérables sur la valeur adaptative (*fitness*) individuelle et dicte la manière dont les organismes interagissent avec leur environnement. La variation intraspécifique peut ainsi influencer les processus écologiques et évolutifs en nature, tout en ayant des applications pratiques importantes. Toutefois, on ignore encore dans quelle mesure les causes et les conséquences de la variation intraspécifique sont prévisibles (c'est-à-dire, qu'elles peuvent être expliquées par des modèles statistiques). Il est essentiel de combler cette lacune de connaissances, car la compréhension des causes et des conséquences, ou la manipulation de variation pour générer des résultats souhaités à des fins appliquées, dépendent de la prédictibilité de cette variation. Dans cette thèse, je cherche à combler ce manque de connaissances en utilisant deux poissons d'eau douce comme modèles naturels.

Dans la première partie, j'utilise Trinidad comme laboratoire naturel pour étudier dans quelle mesure nous pouvons prédire les causes de la variation intraspécifique chez les guppys (*Poecilia reticulata*). L'étude des causes de la variation chez les guppys est importante, car les travaux sur cette espèce ont grandement contribué à notre connaissance fondamentale de la biologie évolutive. Dans le chapitre 1, je quantifie d'abord dans quelle mesure le régime de prédation peut expliquer la variance des moyennes des traits au niveau des populations de guppys et j'évalue divers facteurs qui pourraient réduire le parallélisme (c'est-à-dire la prédictibilité). Je montre que la majorité (~75 %) de la variance dans les moyennes des traits des populations de guppys n'est pas expliquée par une dichotomie simplifiée du régime de prédation, que le niveau de parallélisme semble particulièrement faible pour les traits de couleur, et que

l'augmentation de la complexité de l'histoire évolutive diminue les estimations de parallélisme. Dans le chapitre 2, je me concentre sur un seul niveau du régime de prédation (faible prédation) pour étudier comment un aspect négligé de l'environnement – ses caractéristiques abiotiques – peut influencer deux phénotypes comportementaux. Je montre que les caractéristiques abiotiques de l'environnement, même à petite échelle spatiale, peuvent être fortement associées au comportement et pourraient donc réduire la prédictibilité globale de la dichotomie du régime de prédation.

Dans la deuxième partie, j'effectue des manipulations expérimentales dans la nature pour étudier dans quelle mesure les conséquences de la variation intraspécifique sont prévisibles chez l'épinoche (*Gasterosteus aculeatus*). Je me concentre spécifiquement sur la prédictibilité des mouvements non aléatoires (dispersion et assortiment), car les épinoches sont souvent introduites dans de nouveaux environnements et les mouvements non aléatoires pourraient avoir des effets écologiques et évolutifs importants à la suite des introductions. Dans le chapitre 3, je demande comment une induction expérimentale de la variation de la fibrose – une réponse d'inflammation et de réparation des tissus – affecte la dispersion, un processus écologiquement important. Je constate que les effets de la fibrose sur la dispersion ne sont pas prévisibles, bien que certaines tendances intéressantes, mais non significatives, suggèrent que l'inflammation précoce pourrait affecter la dispersion. Pour les deux derniers chapitres, j'utilise des translocations avec un objectif de conservation où des épinoches de plusieurs populations « sources » ont été introduites expérimentalement dans des lacs d'où les épinoches avaient été précédemment extirpées. Dans le chapitre 4, j'évalue comment les épinoches de différentes populations sources s'assortissent spatialement dans un nouvel environnement, fournissant un aperçu sur la manière dont l'évolution pourrait se dérouler après les introductions. Je découvre que l'assortiment des

épinoches n'est pas prévisible, car les épinoches semblent s'assortir de manière aléatoire. Finalement, dans le chapitre 5, j'étudie dans quelle mesure la population source peut être utilisée pour prédire la dispersion suite à une translocation à but de conservation. Ce chapitre donne un aperçu des applications pratiques en considérant la variation associée à la dispersion. Bien que la population source n'ait pas permis de prédire la dispersion, les individus moins explorateurs étaient plus susceptibles d'être capturés loin du point d'introduction, ce qui suggère que ces individus pourraient « mener » le front de dispersion après la translocation.

Dans l'ensemble des chapitres, la majorité de la variance n'a pas été expliquée par des modèles statistiques. Mon travail suggère donc que les causes et les conséquences de la variation intraspécifique sont généralement faiblement prévisibles et sont souvent non prévisibles. Cette conclusion montre qu'une grande partie de notre compréhension fondamentale des causes de la variation chez les guppys manque de nuances concernant les complexités des systèmes naturels, et que d'autres conséquences, au-delà des mouvements, devraient être priorisées pour comprendre les conséquences écologiques et évolutives des introductions d'épinoches. Un échantillonnage plus détaillé des aspects de l'environnement, au-delà des « principales » sources de sélection, peut améliorer la prévisibilité des causes, et se concentrer sur d'autres traits qui pourraient être plus prévisibles pourrait améliorer la prévisibilité des conséquences. Bien que j'aie trouvé une faible prédictibilité dans l'ensemble, celle-ci continuera à s'améliorer à mesure que nos connaissances des systèmes naturels s'accroissent.

To my nephew.

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I'd like to start by thanking my supervisors, Andrew Hendry and Kiyoko Gotanda. Thank you for being excellent mentors. You've been incredibly supportive of my diverse research interests and have always encouraged me to pursue exciting opportunities. In doing so, you've pushed me to become far more confident and ambitious as a scientist.

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To mom, your support is endless. Thanks for your patience, your help – in almost every way imaginable – and for making sure I always knew you were here if I needed you. You've been the sounding board for most of my ideas. You've driven back-and-forth across the country with me a million times. I can't think of a better way to express how much your constant support has meant to me, except to say: mom, you are my Samwise Gamgee. To Lauren, thank you for not doing a PhD so that I can fool people into thinking I might be the smarter sibling. On a serious note, I learn so much from you. Thank you for always encouraging me to trust myself, and, of course, for Rex and Minke who add so much joy to my life. To dad, when I think about how you've inspired me, I remember cranking 'eye of the tiger' on the way to biathlon races and following colourful trails of jujubes up mountainsides. You've always demonstrated the value in pushing yourself and shown that hard work can be fun – but also that it's okay to just sit back sometimes and enjoy the view. To my extended family, my Saari aunts and cousins, the Jenkins, and grandma, you've all supported me in different ways, whether it was helping celebrate my wins or providing a place for me to stay (or a basement to store all my belongings!).

Finally, I must wrap up the acknowledgements by thanking Mr. Flea (and, in a big way, I am again thanking my colleagues who helped get a street dog back to Canada at the start of a global pandemic). It's hard to imagine what this experience would have been like if we didn't spot Mr. Flea on our drive to Las Cuevas, because this adventure has been so much better with a tiny Trini dog as my best friend and field assistant extraordinaire.

Thank you all so, so much.

Contribution to original knowledge

All five chapters in my thesis constitute original scholarship.

Chapter 1: Trinidadian guppies provide some of the most famous examples of parallel evolution in nature. I compiled data from over 40 years of literature on guppies to quantify the extent of predator-driven parallelism among guppy population-level trait means. Our results show that only about a quarter of the variance in population-level trait means could be explained by the predation regime dichotomy. Colour, despite being a very famous example of parallel evolution in guppies, seemed to be especially weakly parallel, and pooling populations with different evolutionary histories decreased parallelism. The findings from this chapter challenge our current understanding of how phenotypes are shaped in nature, and our study highlights several opportunities for future work that could improve our understanding of the contributors to variation in the extent of phenotypic parallelism. This work was published in *Journal of Evolutionary Biology*.

Chapter 2: Most work investigating the causes of variation in bold and exploratory behaviours focuses on biotic factors, and the few studies that investigate abiotic environmental features are typically conducted on large spatial scales (e.g., among populations). Despite being overlooked relative to biotic factors, the work presented in this chapter shows that abiotic environmental features can have important effects on guppy behaviour – even on very fine spatial scales (e.g., pools separated by less than a few metres). To my knowledge, this is the first study to investigate how the abiotic environment can shape these behaviours across more than one guppy population, and our results emphasize a need for finer-scale spatial sampling to understand how environments can shape behaviour. This manuscript is in revision at *Journal of Fish Biology.*

Chapter 3: This chapter presents results from a mark-recapture experiment where I investigated how the development of fibrosis (an inflammation and tissue repair response that can develop in response to parasite infection) affects dispersal. Although past studies have shown that fibrosis can affect aspects of stickleback locomotion in laboratory settings, we found that it does not affect stickleback dispersal in a lake. In showing that fibrosis does not affect dispersal in the wild, our study provides novel insight into the ecological effects of immune responses in natural settings. Our study also identifies interesting trends that suggest the timing of fibrosis may affect dispersal – although these trends lack statistical significance and could be scrutinized with additional work. This work was published in *Ecology and Evolution.*

Chapter 4: Individuals are often introduced from multiple populations during invasions or translocations, and how individuals from those populations spatially assort could shape the extent of admixture in novel environments. By showing that stickleback mostly assort randomly following introduction in novel environments, thereby seemingly maximizing admixture and evolutionary potential, this work provides novel insight into how stickleback evolution can unfold in recently founded populations. The resolution of this work is, to my knowledge, unprecedented among studies investigating variation in recently founded wild populations (we set 100 traps around the entire perimeter of a lake 3 years after we introduced stickleback into that lake, and we genotyped individuals from nearly every trap). This manuscript is in preparation to be submitted to *Evolution.*

Chapter 5: Predicting how dispersal unfolds following introductions into novel environments could be useful for facilitating conservation translocations or mitigating the effects of invasions. Our results show that the population from which stickleback are introduced does not influence

dispersal. However, we also found an interesting and surprising result – that is, individuals that moved less in an artificial arena (were seemingly more exploratory) were more likely to be captured near the introduction point (were seemingly weaker dispersers). 'Exploration' could thus be considered as a possible tool to predict dispersal in new environments. This work provides novel insight into how stickleback colonize new environments and the practical applications of intraspecific variation. This manuscript is in preparation to be submitted to *Evolutionary Applications*.

Contribution of authors

Chapter 1: APH conceived the initial idea for this project. AMH collected the data, performed data analysis and visualization, and wrote the first draft of the manuscript. AEP and KBO assisted with data analysis and visualization. KBO and APH supervised the work. All authors provided input on the analyses and provided edits and comments on the manuscript.

Chapter 2: APH, POM, and AMH conceptualized this project. AMH collected the data, performed data analysis and visualization, and wrote the first draft of the manuscript. SS and JAF assisted with data collection. POM and APH supervised the work. All authors provided input on the analyses and provided edits and comments on the manuscript.

Chapter 3*:* DIB, APH, NCS, and AMH conceptualized this project. AMH collected the data, performed data analysis and visualization, and wrote the first draft of the manuscript. FD assisted with data collection. DIB and NCS supervised the work. All authors provided input on the analyses and provided edits and comments on the manuscript.

Chapter 4: DIB, APH, and AMR conceptualized this project. AMH performed data analysis and visualization and wrote the first draft of the manuscript (except for the first draft of the genotyping methods, which was written by LE). DIB, RDHB, CLP and LE developed the single nucleotide polymorphism array used to genotype individuals. AMH did DNA extractions. KV and EA assisted AMR with field data collection. AMR and APH supervised the work. All authors provided input on the analyses and provided edits and comments on the manuscript.

Chapter 5: APH, KMG, and AMH conceptualized this project. AMH collected the data, performed data analysis and visualization, and wrote the first draft of the manuscript (except for

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General introduction

Intraspecific variation – that is, variation within a species – plays a critical role in evolution and can have strong ecological effects. Indeed, intraspecific variation is the raw material on which selection acts, and investigating the factors that give rise to this variation can provide insight into evolutionary processes such as adaptation and natural selection (Darwin 1859). Intraspecific variation can also dictate how organisms interact with their environments, and therefore can have consequences for population dynamics, community structure, and ecosystem function (Des Roches et al. 2017, Hendry 2017, Raffard et al. 2019). For instance, variation in Soay sheep (*Ovis arie*) body size can affect population growth (Pelletier et al. 2007), variation in the trophic traits of alewife (*Alosa pseudoharengus*) can shape the community structure of their zooplankton prey (Post et al. 2008), and variation in guppies (*Poecilia reticulata)* adapted to different predation regimes can have differing effects on primary production and leaf decomposition rates (Bassar et al. 2010). Owing to these evolutionary and ecological effects, intraspecific variation can also have important applied implications (Des Roches et al. 2021, Stange et al. 2021). For instance, intraspecific variation can buffer against the impacts of climate change (e.g., Reusch et al. 2005, Oney et al. 2013), along with other environmental disturbances (e.g., Hughes and Stachowicz 2004), and researchers are increasingly investigating how variation within species can be leveraged to benefit human societies, such as in medicine and agricultural practices (Des Roches et al. 2021). Yet, although it is established that intraspecific variation can be important, what remains unresolved is the extent to which it's causes and consequences are predictable. Identifying the extent to which the causes and consequences of intraspecific variation are predictable is critical, because understanding these causes and consequences, or manipulating

variation to generate desired consequences for applied purposes, is contingent on how predictable that variation is.

Predictability can have many meanings in ecology and evolution (Hendry 2023). When referring to predictability, I specifically mean the extent to which biological relationships can be explained by statistical models (e.g., Møller and Jennions 2002, Peek et al. 2003). A highly predictable association is one in which a high degree of variance in a response variable can be explained by the predictors. Predictability is shaped by many factors, some which can be ecological. For instance, biotic or abiotic environmental factors that differ within and among populations over space and time can confound associations between variables of interest. Other factors can be evolutionary. For instance, different evolutionary histories can lead to among-population variation in phenotypes and genotypes (Langerhans and DeWitt 2004, Weese et al. 2012), reducing the predictability of an association across multiple populations. Predictability will also be shaped by how reliably traits of interest can be measured (Møller and Jennions 2002). All these complexities can decrease the extent to which the causes and consequences of intraspecific variation are predictable. One might therefore be tempted to think that intraspecific variation must not be very predictable. Yet, in spite of the complexities that paint a grim picture for the predictability of intraspecific variation, examples of parallel evolution, where populations evolve similar phenotypes in similar environments, illustrate that some biological associations are strong, and thus challenge the notion that intraspecific variation must only be weakly predictable (Reiskind et al. 2021).

In this thesis, I aim to answer one central question: how predictable are the causes and consequences of intraspecific variation? In Part One (*The Causes of Intraspecific Variation*), I

assess the extent to which the causes of intraspecific variation are predictable given biotic (Chapter 1) and abiotic (Chapter 2) sources of environmental variation. In Part Two (*The Consequences of Intraspecific Variation*), I investigate the extent to which the ecological (Chapter 3), evolutionary (Chapter 4), and applied (Chapter 5) consequences of intraspecific variation are predictable. To conduct this work, I leverage two freshwater fish systems: Trinidadian guppies (*Poecilia reticulata*; hereafter 'guppies') and threespine stickleback (*Gasterosteus aculeatus*; hereafter 'stickleback'). Both species are exceptionally well-suited for studying intraspecific variation because they can be highly variable within and among populations (Reznick et al. 1996, Hendry et al. 2009, Haines 2023). This variation is likely driven in part by their short generation times, their ability to tolerate diverse environmental conditions, and the strong selection exerted by the environments they inhabit – factors that can promote rapid adaptation (e.g., Bell et al. 2004, Gordon et al. 2009). To investigate the causes and consequences of intraspecific variation with these species in nature, I take two approaches: with guppies, I leverage Trinidad as a natural laboratory, and with stickleback, I conduct experimental manipulations in nature.

Part One: The Causes of Intraspecific Variation

In Part One of this thesis, I specifically focus on the causes of intraspecific variation in phenotypes (rather than genotypes). Many factors can contribute to phenotypic variation, and phenotypic variation can be considered at three levels: among different populations, among individuals within a population, and within individuals. Among populations, environments can vary in the types, strengths, or sources of selection generating evolutionary change (Kingsolver

et al. 2001). Past evolutionary histories will shape the genetic material that current evolutionary processes can act upon, which can sometimes lead to phenotypic divergence (e.g., Weese et al. 2012). Within populations, genetic variation can arise owing to stochastic processes such as mutation or recombination. This genetic variation can contribute to among-individual phenotypic variation that will be acted on by selection (Hendry 2017). Within individuals, phenotypes can shift throughout ontogeny (Mazer and Damuth 2001), and traits that are repeatedly expressed (e.g., behaviour or physiology) can exhibit plastic shifts that affect trait expression on very short to longer, within-lifetime, timescales (Westneat et al. 2015). Because phenotypes are not only a product of genetics, but rather how genotypes interact with the environment, plastic responses to biotic and abiotic environmental factors will not only affect the expression of phenotypes within individuals but also at the other two levels (among populations, among individuals within populations) (West-Eberhard 2003). These factors, among other factors, all can affect the extent to which any causal association of phenotypic variation is predictable.

To investigate the causes of intraspecific variation in natural settings, researchers often leverage habitat contrasts, where one or a few key sources of selection are known to differ between environments (e.g., cave vs surface, urban vs non-urban, sulfidic vs non-sulfidic). Work leveraging these contrasts has allowed for the general consensus that similar environments often favour similar phenotypes (Bolnick et al. 2018). For example, Mexican tetra (*Astyanax mexicanus*) are small fish where some populations inhabit caves and others inhabit surface habitats. Cave populations, having evolved in complete darkness, have repeatedly and independently lost eyes, and they also differ from surface populations in pigmentation and a suite of metabolic traits (Jeffery 2020). Even within habitat contrasts, however, a substantial amount of variation often remains unexplained by the ‘focal’ source of selection (Oke et al. 2017,

Bolnick et al. 2018). It has been suggested that coarsely categorizing organisms into habitat 'types' might oversimplify environments that are otherwise seemingly similar, resulting in important sources of variation being overlooked (Oke et al. 2017). Aligning with this suggestion, one analysis that quantified effects of predation on trait divergence in Bahamas mosquitofish (*Gambusia hubbsi)* found that interspecific competition affected divergence between predation regimes for over a quarter of measured traits (Langerhans 2018). In this case, variation that is typically not considered when focusing on predation dichotomies (i.e., interspecific competition) was found to be an important factor in shaping phenotypes.

To investigate the extent to which the causes of intraspecific variation are predictable, I leverage a highly-studied habitat contrast, providing some of the most robust examples of parallel evolution in nature – yet where the environments within a single habitat 'type' (i.e., one level of the contrast) are also known to be highly variable: high vs low predation in Trinidadian guppies. In Chapter 1, I quantify the extent to which this predation dichotomy drives parallel evolution in guppy population-level trait means. In Chapter 2, I investigate how abiotic variation contributes to guppy behavioural phenotypes within a single predation regime, thus affecting predictability of the causes of intraspecific variation.

An overview of guppies

Guppies are a small freshwater fish native to Trinidad and nearby South American countries (Houde 1997). This species is sexually dimorphic, where males are smaller than females, and are brightly coloured. Male guppies can display bright orange and black colouration, among a mosaic of other possible colours, in dramatic patterns with spots and stripes (Haskins and

Haskins 1950, Houde 1997, Magurran 2005). Female guppies, by contrast, are larger, and are a beige or tan. Guppies are a member of the Poecilidae family, which encompasses many species that are known as 'livebearing' fishes. As the name suggests, members of this family have internal fertilization, and give birth to live young (Houde 1997). Female guppies can reach sexual maturity as early as two months and reproduce every month (Houde 1997); depending on the population, individuals can produce an average of 15-28 litters across their lifespan (Reznick et al. 2005).

Most wild guppy research takes place in Trinidad, where the environment has been referred to as a 'natural laboratory' (Magurran 2005). Many populations of guppies in Trinidad inhabit rivers that flow along the Northern and Southern Slopes of the Northern Mountain Range. These rivers are punctuated with waterfalls that prevent the upward movement of guppy predators to above waterfalls, whereas guppies live both above and below the waterfalls. As such, guppies above the waterfalls experience relatively low predation pressure (and these are typically referred to as "low predation" environments), whereas guppies below the waterfalls experience high predation pressure ("high predation" environments) (Magurran 2005). Some predation does occur in low predation environments, as guppies co-exist with Hart's rivulus (*Anablepsoides hartii*, more commonly known as *Rivulus hartii*) (Seghers 1973, Endler 1978). However, rivulus are gape-limited, and primarily consume only juvenile guppies, and they exert much weaker selection than the predators in high predation sites which can include the bigmouth sleeper, *Gobiomorus dormitor,* and the pike cichlid, *Crenicichla alta.* These predators consume both juvenile and adult guppies and at much higher frequencies than rivulus (Seghers 1973, Endler 1978).

High-predation and low-predation guppies differ in a multitude of traits, most notably in morphology, life history, and behaviour (Endler 1995). High-predation guppies are less colourful than their low-predation counterparts, and they are also smaller (Haskins and Haskins 1951, Endler 1978, 1980). They exhibit faster life histories characterized by earlier maturation and the production of more, but smaller, offspring (Reznick and Endler 1982, Reznick et al. 2001). Behaviours that are known to confer anti-predator benefits also show reliable shifts between high-predation and low-predation guppies. For instance, high-predation guppies shoal more and avoid predators at greater distances (Seghers 1973, Magurran and Seghers 1994). Other risk-associated behaviours, such as boldness and exploration, also can differ between high and low predation environments (e.g., Harris et al. 2010, Burns et al. 2016). Many of these phenotypic patterns have not only been observed in the wild but have also been experimentally tested with transplant experiments in nature or reinforced with mesocosm and laboratory studies (e.g., Seghers 1973, Reznick et al. 1990, Magurran et al. 1992, Gordon et al. 2009).

The high extent of similarities observed in guppy phenotypes between the predation regimes might suggest that guppy phenotypes are highly predictable. However, guppy phenotypes are not always highly predictable. For instance, even when considering famous examples like guppy colour and body size, these traits can still be extremely variable among populations within a given level of the predation regime (e.g., Millar et al. 2006, Gotanda et al. 2013). Many sources of variation beyond predation that can shape guppy phenotypes could contribute to decreased predictability. For instance, variation in mate choice (e.g., Endler and Houde 1995), parasitism (e.g., Jacquin et al. 2016), and canopy cover (e.g., Grether et al. 2001) have all been found to contribute to variation in guppy phenotypes. Other aspects of the ecology of high and low predation sites differ as well. High predation sites, being farther down the

mountains, are generally wider and deeper, with more open canopies, and higher primary productivity (Reznick and Endler 1982, Grether et al. 2001). Further, predation risks – even within a given predation regime – are highly variable and it has been emphasized that predation thus should be considered as a gradient of risk rather than a binary risk category (Deacon et al. 2018). Understanding the extent to which the causes of guppy phenotypes are predictable could have important implications because guppies provide many 'textbook' examples of parallel evolution, and guppy research has been foundational for our understanding of the role that natural selection can play in shaping phenotypes (Endler 1978).

Part Two: The Consequences of Intraspecific Variation

Many different "consequences" could be considered in the context of intraspecific variation (e.g., individual fitness, population growth, population resilience; Hendry 2017). In this thesis, I specifically focus on how variation in dispersal (which I define as any movement with consequences for gene flow; Ronce 2007) and variation in spatial assortment can arise as a consequence of intraspecific variation. For brevity, I will refer to dispersal and spatial assortment collectively as "movement". My focus on movement is largely motivated by the fact that non-random movement can have important ecological and evolutionary effects (Edelaar and Bolnick 2012, Richardson et al. 2014). For instance, if individuals differ in the locations to which they move, then ecological effects, such as resource consumption and nutrient deposition, could also be spatially structured (Ferraro et al. 2022). Because phenotypic and genotypic variation can affect population dynamics, community structure, and ecosystem processes (e.g., Pelletier et al. 2007, Post et al. 2008, Bassar et al. 2010), phenotype-biased or genotype-biased movement could

also have spatially structured effects at population, community or ecosystem levels. Non-random movement can also have evolutionary implications through effects on gene flow, local adaptation, and assortative mating that could alter evolutionary trajectories over time (Edelaar and Bolnick 2012). If non-random movement is highly predictable, then these ecological and evolutionary consequences could be leveraged for practical applications. For instance, conservationists or biologists could attempt to select organisms for conservation translocations, in attempt to 'control' the outcomes of non-random movement.

Many factors that vary among individuals can influence movement, and thus likely reduce the extent to which movement is predictable when assessed in association with one or a few variables. For instance, individual-level variation in performance, dispersal capacity, or behavioural traits can generate non-random movement patterns (among other factors; Shine et al. 2011, Edelaar and Bolnick 2012). However, aspects of the external environment can also affect movement, including biotic factors, such as predation, parasitism, or the social environment (Weinstein et al. 2018, Gaynor et al. 2019, Webber et al. 2024) and abiotic factors, such as landscape structure, temperature, or water flow (Taylor and Cooke 2012, McLeod and Leroux 2021). The predictability of movement therefore depends not only on causal associations with phenotypes or genotypes but also on how those phenotypes respond to the external environment.

To investigate the extent to which the ecological, evolutionary, and applied consequences of intraspecific variation are predictable, I conduct experimental manipulations in nature using threespine stickleback. In Chapter 3, I experimentally induce variation in fibrosis – an inflammation and tissue repair response - to assess how variation in fibrosis affects dispersal, an ecologically important process. For Chapters 4 and 5, I leverage conservation translocations

where stickleback from up to eight ‘source’ populations were introduced into lakes with no other stickleback. In Chapter 4, I assess how individuals from the source populations spatially assort in the novel environment, providing insight into how evolution might unfold. In Chapter 5, I assess the extent to which source population identity and behaviour can be used to predict dispersal for use in conservation translocations, providing insight into its practical applications.

An overview on stickleback

Threespine stickleback are a small fish distributed throughout the northern hemisphere in both marine and coastal freshwater environments. With some exceptions (e.g., Reimchen 1989, Reimchen and Nosil 2004), stickleback are typically only visibly dimorphic during the reproductive season over which time the males, which are otherwise cryptically coloured, develop deep red throats and bright blue eyes (Wootton 1984). Stickleback can live for several years (although lifespan varies among populations) and females can produce up to 1000 eggs throughout their lives (Wootton 1984, Baker et al. 2015). Unlike guppies, where parents provide no care after birth, stickleback males provide care that involves establishing territories, constructing nests, and then oxygenating the eggs and defending the nests (Tinbergen 1952).

Whereas the natural settings that made guppies famous for evolutionary biology research are mostly limited to a small island, the settings that make stickleback an excellent evolutionary model system are distributed throughout much of the northern hemisphere. All stickleback populations are ancestrally marine – but, as glaciers receded over ten thousand years ago, stickleback became landlocked in freshwater environments ranging from small streams to large lakes (Reynolds et al. 1995). Each of these environments acts as an evolutionary replicate,

allowing investigation into how the transition from marine to freshwater shapes phenotypes, and how differences among freshwater environments can shape population-level variation (Reynolds et al. 1995). In addition to these 'historical' introduction events, modern introductions of stickleback into novel environments are common, facilitated both by natural and human-mediated processes (Makhrov et al. 2024).

Although some human-mediated introductions are accidental, many are deliberate with stickleback being introduced into environments from which they were previously extirpated (i.e., to restore the stickleback population) or in attempt to restore whole ecosystems (i.e., to facilitate cascading beneficial effects that stickleback might have on their environments) (Bell et al. 2016, Hendry et al. 2024, Makhrov et al. 2024). The value of stickleback for this latter objective arises because stickleback can have strong ecological effects. For instance, mesocosm studies have shown that stickleback that primarily eat macroinvertebrates (benthic stickleback) and stickleback that primarily eat zooplankton (limnetic stickleback) can have differing effects on prey community composition, primary production, and aspects of the light environment (Harmon et al. 2009, Des Roches et al. 2013). Similar effects have been observed for stickleback adapted to lake vs stream environments (Matthews et al. 2016).

Because stickleback can have such strong ecological effects, identifying the extent to which non-random movement is predictable could be highly important, both from the perspective of understanding their ecological effects and for potential conservation and restoration implications. Work in experimental streams has demonstrated that freshwater stickleback can move over 6 km in less than eight hours with sustained movement, suggesting high potential for movement throughout lakes (Whoriskey and Wootton 1987). Yet stickleback

in natural populations often exhibit strong site fidelity (Taylor and McPhail 1986), and evidence suggests that stickleback do sometimes exhibit non-random movement (e.g., Bolnick et al. 2009, Jiang et al. 2017). Even when only separated by a few metres, individual stickleback can show a high degree of morphological and genetic differentiation, indicating heritable microhabitat preferences at very small spatial scales (Maciejewski et al. 2020). In possibly the most direct test of non-random movement, when stickleback collected from either lacustrine environments or stream environments were released at a junction between a lake and a stream, 90% of stickleback returned to the habitat type from which they were collected (Bolnick et al. 2009).

Non-random movement might also be expected for benthic and limnetic stickleback, not only because they are adapted to living in different environments, and thus might select environments that best match their phenotypes (i.e., matching habitat choice; Edelaar et al. 2008), but also because morphological divergence could correspond to variation in movement capacity over larger spatial scales. For instance, benthic stickleback have deeper bodies, facilitating manoeuverability in shallow water, whereas limnetic stickleback have more slender bodies, enabling streamlined movement over longer distances in open water (Willacker et al. 2010). These implications for larger-scale movements might be especially relevant following introductions into novel environments, where individuals have yet to establish territories and thus might not show strong site fidelity.

Summary

Over the next five chapters my work seeks to better understand the extent to which the causes and consequences of intraspecific variation are predictable. I conduct this work with two

freshwater fish natural model organisms that show high extents of intraspecific variation, and where variation in the extent of predictability could be impactful. With guppies, this potential impact lies in the fact that research on the guppy system has been so foundational to the field of evolutionary biology. Given that there are many famous examples of parallel evolution in guppies, it is possible that high predictability is a pervasive assumption in guppy work and thus warrants being challenged. For threespine stickleback, the possible impact of variation in predictability emerges because stickleback are so commonly introduced into novel environments. The extent to which non-random movement is predictable in stickleback could therefore help us better understand how ecological and evolutionary processes play out during these introduction events, and even the extent to which we can manipulate their outcomes. Taken together, my thesis therefore provides insight into our current ability to understand, manipulate, and benefit from ecological and evolutionary processes that arise owing to variation within species.

References

Baker JA, Wund MA, Heins DC, King RW, Reyes ML, Foster SA. 2015. Life-history plasticity in female threespine stickleback. Heredity 115: 322–334.

Bassar RD, Marshall MC, López-Sepulcre A, Zandonà E, Auer SK, Travis J, Pringle CM, Flecker AS, Thomas SA, Fraser DF, Reznick DN. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. Proceedings of the National Academy of Sciences 107: 3616–3621.

Bell MA, Aguirre WE, Buck NJ. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution 58: 814–824.

Bell MA, Heins DC, Wund MA, Von Hippel FA, Massengill R, Dunker K, Bristow GA, Windsor EA. 2016. Reintroduction of threespine stickleback into Cheney and Scout Lakes, Alaska. Evolutionary Ecology Research 17: 157–178.

Bolnick DI, Barrett RDH, Oke KB, Rennison DJ, Stuart YE. 2018. (Non)parallel evolution. Annual Review of Ecology, Evolution, and Systematics 49: 303–330.

Bolnick DI, Snowberg LK, Patenia C, Stutz WE, Ingram T, Lau OL. 2009. Phenotype-dependent native habitat preference facilitates divergence between parapatric and stream stickleback. Evolution 63: 2004–2016.

Burns JG, Price AC, Thomson JD, Hughes KA, Rodd FH. 2016. Environmental and genetic effects on exploratory behavior of high- and low-predation guppies (*Poecilia reticulata*). Behavioral Ecology and Sociobiology 70: 1187–1196.

Darwin C. 1859. On the origin of species. John Murray.

Deacon AE, Jones FAM, Magurran AE. 2018. Gradients in predation risk in a tropical river system. Current Zoology 64: 213–221.

Des Roches S, Pendleton LH, Shapiro B, Palkovacs EP. 2021. Conserving intraspecific variation for nature's contributions to people. Nature Ecology & Evolution 5: 574–582.

Des Roches S, Post DM, Turley NE, Bailey JK, Hendry AP, Kinnison MT, Schweitzer JA, Palkovacs EP. 2017. The ecological importance of intraspecific variation. Nature Ecology & Evolution 2: 57–64.

Des Roches S, Shurin JB, Schluter D, Harmon LJ. 2013. Ecological and evolutionary effects of stickleback on community structure. PLoS ONE 8: e59644.

Edelaar P, Bolnick DI. 2012. Non-random gene flow: an underappreciated force in evolution and ecology. Trends in Ecology & Evolution 27: 659–665.

Edelaar P, Siepielski AM, Clobert J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. Evolution 62: 2462–2472.

Endler JA. 1978. A predator's view of animal color patterns. Pages 319–364 in Hecht MK, Steere WC, and Wallace B, eds. Evolutionary Biology. Springer US.

Endler JA. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34: 76.

Endler JA. 1995. Multiple-trait coevolution and environmental gradients in guppies. Trends in Ecology & Evolution 10: 22–29.

Endler JA, Houde AE. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. Evolution 49: 456–468.

Ferraro KM, Schmitz OJ, McCary MA. 2022. Effects of ungulate density and sociality on landscape heterogeneity: a mechanistic modeling approach. Ecography 2022: ecog.06039.

Gaynor KM, Brown JS, Middleton AD, Power ME, Brashares JS. 2019. Landscapes of fear: spatial patterns of risk perception and response. Trends in Ecology & Evolution 34: 355–368.

Gordon SP, Reznick DN, Kinnison MT, Bryant MJ, Weese DJ, Räsänen K, Millar NP, Hendry AP. 2009. Adaptive changes in life history and survival following a new guppy introduction. The American Naturalist 174: 34–45.

Gotanda KM, Delaire LC, Raeymaekers JAM, Pérez-Jvostov F, Dargent F, Bentzen P, Scott ME, Fussmann GF, Hendry AP. 2013. Adding parasites to the guppy-predation story: insights from field surveys. Oecologia 172: 155–166.

Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. Ecology 82: 1546–1559.

Haines GE. 2023. Intraspecific diversity of threespine stickleback (*Gasterosteus aculeatus*) populations in eastern Canada. Environmental Biology of Fishes 106: 1177–1194.

Harmon LJ, Matthews B, Des Roches S, Chase JM, Shurin JB, Schluter D. 2009. Evolutionary diversification in stickleback affects ecosystem functioning. Nature 458: 1167–1170.

Harris S, Ramnarine IW, Smith HG, Pettersson LB. 2010. Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. Oikos 119: 1711–1718.

Haskins CP, Haskins EF. 1950. Factors governing sexual selection as an usolating mechanism in the Poeciliid fish *Lebistes reticulatus*. Proceedings of the National Academy of Sciences 36: 464–476.

Haskins CP, Haskins EF. 1951. The inheritance of certain color patterns in wild populations of *Lebistes reticulatus* in Trinidad. Evolution 5: 216–225.

Hendry AP. 2017. Eco-evolutionary dynamics. Princeton University Press.

Hendry AP. 2023. Prediction in ecology and evolution. BioScience 73: 785–799.

Hendry AP, Barrett RDH, Bell AM, Bell MA, Bolnick DI, Gotanda KM, Haines GE, Lind ÅJ, Paccard M, Peichel CL, Peterson CR, Poore HA, Massengill RL, Milligan-McClellan K, Steinel NC, Sanderson S, Walsh MR, Weber JN, Derry AM. 2024. Designing eco-evolutionary experiments for restoration projects: opportunities and constraints revealed during stickleback introductions. Ecology and Evolution 14: e11503.

Hendry AP, Bolnick DI, Berner D, Peichel CL. 2009. Along the speciation continuum in sticklebacks. Journal of Fish Biology 75: 2000–2036.

Houde AE. 1997. Sex, color, and mate choice in guppies. Princeton University Press.

Hughes AR, Stachowicz JJ. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proceedings of the National Academy of Sciences 101: 8998–9002.

Jacquin L, Reader SM, Boniface A, Mateluna J, Patalas I, Pérez-Jvostov F, Hendry AP. 2016. Parallel and nonparallel behavioural evolution in response to parasitism and predation in Trinidadian guppies. Journal of Evolutionary Biology 29: 1406–1422.

Jeffery WR. 2020. *Astyanax* surface and cave fish morphs. EvoDevo 11: 14.

Jiang Y, Peichel CL, Torrance L, Rizvi Z, Thompson S, Palivela VV, Pham H, Ling F, Bolnick DI. 2017. Sensory trait variation contributes to biased dispersal of threespine stickleback in flowing water. Journal of Evolutionary Biology 30: 681–695.

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001. The strength of phenotypic selection in natural populations. The American Naturalist 157: 245–261.

Langerhans, DeWitt TJ. 2004. Shared and unique features of evolutionary diversification. The American Naturalist 164: 335–349.

Langerhans RB. 2018. Predictability and parallelism of multitrait adaptation. Journal of Heredity 109: 59–70.

Maciejewski MF, Jiang C, Stuart YE, Bolnick DI. 2020. Microhabitat contributes to microgeographic divergence in threespine stickleback. Evolution 74: 749–763.

Magurran AE. 2005. Evolutionary ecology: the Trinidadian guppy. Oxford University Press.

Magurran AE, Seghers BH. 1994. Predator inspection behaviour covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. Behaviour 128: 121–134.

Magurran AE, Seghers BH, Carvalho GR, Shaw PW. 1992. Behavioural consequences of an artificial introduction of guppies *(Poecilia reticulata)* in N. Trinidad: evidence for the evolution of anti-predator behaviour in the wild. Proceedings of the Royal Society of London. Series B: Biological Sciences 248: 117–122.

Makhrov AA, Houle EYK, Hendry AP, Derry AM, Lajus DL. 2024. Widespread evidence for rapid recent changes in global range and abundance of threespine stickleback (*Gasterosteus aculeatus*). Fish and Fisheries 1–18.

Matthews B, Aebischer T, Sullam KE, Lundsgaard-Hansen B, Seehausen O. 2016. Experimental evidence of an eco-evolutionary feedback during adaptive divergence. Current Biology 26: 483–489.

Mazer SJ, Damuth J. 2001. Nature and causes of variation. Evolutionary Ecology. Oxford University Press.

McLeod AM, Leroux SJ. 2021. Incorporating abiotic controls on animal movements in metacommunities. Ecology 102: e03365.

Millar N, Reznick D, Kinnison M, Hendry A. 2006. Disentangling the selective factors that act on male colour in wild guppies. Oikos 113: 1–12.

Møller A, Jennions MD. 2002. How much variance can be explained by ecologists and evolutionary biologists? Oecologia 132: 492–500.

Oke KB, Rolshausen G, LeBlond C, Hendry AP. 2017. How parallel is parallel evolution? A comparative analysis in fishes. The American Naturalist 190: 1–16.

Oney B, Reineking B, O'Neill G, Kreyling J. 2013. Intraspecific variation buffers projected climate change impacts on *Pinus contorta*. Ecology and Evolution 3: 437–449.

Peek MS, Leffler AJ, Flint SD, Ryel RJ. 2003. How much variance is explained by ecologists? Additional perspectives. Oecologia 137: 161–170.

Pelletier F, Clutton-Brock T, Pemberton J, Tuljapurkar S, Coulson T. 2007. The evolutionary demography of ecological change: linking trait variation and population growth. Science 315: 1571–1574.

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

Raffard A, Santoul F, Cucherousset J, Blanchet S. 2019. The community and ecosystem consequences of intraspecific diversity: a meta-analysis. Biological Reviews 94: 648–661.

Reimchen TE. 1989. Loss of nuptial color in threespine sticklebacks (*Gasterosteus aculeatus*). Evolution 43: 450–460.

Reimchen TE, Nosil P. 2004. Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine stickleback. Evolution 58: 1274–1281.

Reiskind MOB, Moody ML, Bolnick DI, Hanifin CT, Farrior CE. 2021. Nothing in evolution makes sense except in the light of biology. BioScience 71: 370–382.

Reusch TBH, Ehlers A, Hämmerli A, Worm B. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences 102: 2826–2831.

Reynolds JD, Bell MA, Foster SA. 1995. The evolutionary biology of the threespine stickleback. The Journal of Animal Ecology 64: 418.

Reznick D, Bryant M, Holmes D. 2005. The evolution of senescence and post-reproductive lifespan in guppies (*Poecilia reticulata*). PLoS Biology 4: e7.

Reznick D, Buckwalter G, Groff J, Elder D. 2001. The evolution of senescence in natural populations of guppies (*Poecilia reticulata*): a comparative approach. Experimental Gerontology 36: 791–812.

Reznick D, Endler JA. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36: 160.

Reznick DA, Bryga H, Endler JA. 1990. Experimentally induced life-history evolution in a natural population. Nature 346: 357–359.

Reznick DN, Rodd FH, Cardenas M. 1996. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. The American Naturalist 147: 319–338.

Richardson JL, Urban MC, Bolnick DI, Skelly DK. 2014. Microgeographic adaptation and the spatial scale of evolution. Trends in Ecology & Evolution 29: 165–176.

Ronce O. 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. Annual Review of Ecology, Evolution, and Systematics 38: 231–253.

Seghers, B. H. 1973. An analysis of geographic variation in the antipredator adaptations of the guppy *Poecilia reticulata*. PhD thesis, Department of Zoology, University of British Columbia, Vancouver, BC, Canada.

Shine R, Brown GP, Phillips BL. 2011. An evolutionary process that assembles phenotypes through space rather than through time. Proceedings of the National Academy of Sciences 108: 5708–5711.

Stange M, Barrett RDH, Hendry AP. 2021. The importance of genomic variation for biodiversity, ecosystems and people. Nature Reviews Genetics 22: 89–105.

Taylor EB, McPhail JD. 1986. Prolonged and burst swimming in anadromous and freshwater threespine stickleback, *Gasterosteus aculeatus*. Canadian Journal of Zoology 64: 416–420.

Taylor MK, Cooke SJ. 2012. Meta-analyses of the effects of river flow on fish movement and activity. Environmental Reviews 20: 211–219.

Tinbergen N. 1952. The curious behavior of the stickleback. Scientific American 187: 22–26.

Webber Q, Prokopenko C, Kingdon K, Turner J, Vander Wal E. 2024. Effects of the social environment on movement-integrated habitat selection. Movement Ecology 12: 61.

Weese DJ, Ferguson MM, Robinson BW. 2012. Contemporary and historical evolutionary processes interact to shape patterns of within-lake phenotypic divergences in polyphenic pumpkinseed sunfish, *Lepomis gibbosus*. Ecology and Evolution 2: 574–592.

Weinstein SB, Buck JC, Young HS. 2018. A landscape of disgust. Science 359: 1213–1214.

West-Eberhard MJ. 2003. Developmental plasticity and evolution. Oxford University Press.

Westneat DF, Wright J, Dingemanse NJ. 2015. The biology hidden inside residual within-individual phenotypic variation. Biological Reviews 90: 729–743.

Whoriskey FG, Wootton RJ. 1987. The swimming endurance of threespine sticklebacks, *Gasterosteus aculeatus* L., from the Afon Rheidol, Wales. Journal of Fish Biology 30: 335–339.

Willacker JJ, Von Hippel FA, Wilton PR, Walton KM. 2010. Classification of threespine stickleback along the benthic-limnetic axis. Biological Journal of the Linnean Society 101: 595–608.

Wootton RJ. 1984. A functional biology of sticklebacks. Springer US.

Chapter 1: Compiling forty years of guppy research to investigate the factors contributing to (non)parallel evolution

Heckley, A. M., Pearce, A. E., Gotanda, K. M., Hendry, A. P., & Oke, K. B. (2022). Compiling forty years of guppy research to investigate the factors contributing to (non) parallel evolution. *Journal of Evolutionary Biology*, 35(11), 1414-1431. https://doi.org/10.1111/jeb.14086.

Abstract

Examples of parallel evolution have been crucial for our understanding of adaptation via natural selection. However, strong parallelism is not always observed even in seemingly similar environments where natural selection is expected to favour similar phenotypes. Leveraging this variation in parallelism within well-researched study systems can provide insight into the factors that contribute to variation in adaptive responses. Here we analyse the results of 36 studies reporting 446 average trait values in Trinidadian guppies, *Poecilia reticulata*, from different predation regimes. We examine how the extent of predator-driven phenotypic parallelism is influenced by six factors: sex, trait type, rearing environment, ecological complexity, evolutionary history, and time since colonization. Analyses show that parallel evolution in guppies is highly variable and weak on average, with only 24.7% of the variation among populations being explained by predation regime. Levels of parallelism appeared to be especially weak for colour traits, and parallelism decreased with increasing complexity of evolutionary history (i.e., when estimates of parallelism from populations within a single drainage were compared to estimates of parallelism from populations pooled between two major drainages). Suggestive – but not significant – trends that warrant further research include interactions

between the sexes and different trait categories. Quantifying and accounting for these and other sources of variation among evolutionary 'replicates' can be leveraged to better understand the extent to which seemingly similar environments drive parallel and nonparallel aspects of phenotypic divergence.

Introduction

Independent populations that experience similar selective pressures often evolve similar phenotypes (Arendt & Reznick, 2008; Clarke, 1975; Langerhans & DeWitt, 2004; Losos, 2011). Notable examples include the repeated reduction of eyes in the cave dwelling amphipod *Gammarus minus* (Jones et al., 1992), the repeated reduction of body armour in marine threespine stickleback (*Gasterosteus aculeatus*) that colonize fresh water (e.g., Colosimo et al., 2005), and the repeated divergence of benthic and limnetic ecomorphs in Nicaraguan cichlid fishes (Elmer et al., 2014). These examples, and many others, of populations seemingly evolving in parallel have been crucial for our understanding of adaptation and the deterministic role of natural selection (Bolnick et al., 2018). Yet even in classic study systems, parallelism is often imperfect. That is, even when environments are similar – and so natural selection is expected to favour similar traits – the resulting extent of phenotypic parallelism can be weak or highly variable (Bolnick et al., 2018; Fitzpatrick et al., 2014; Kaeuffer et al., 2012; Langerhans, 2018; Oke et al., 2017; Stuart et al., 2017). Langerhans and Riesch (2013) highlight several such examples of 'nonparallel' responses in classic systems (see Table 1 in Langerhans & Riesch, 2013); examples such as these highlight the need to quantify the extent to which various factors contribute to deviations from parallelism. (Throughout, we use the term '(non)parallelism' as

defined by Bolnick et al. (2018): 'the distribution of outcomes across populations and traits forming a continuum from parallel to orthogonal, or even antiparallel, evolution'. This term is not to be confused with 'nonparallelism', which specifically refers to evolutionary outcomes that are not parallel). Potential reasons for weakly parallel outcomes include unrecognized (i.e., cryptic) – or simply unappreciated – environmental differences (e.g., variation in selection), different evolutionary histories (e.g., leading to different genetic backgrounds), evolutionary constraints (e.g., not enough time, excessive gene flow, drift), sexual selection (e.g., among-population variation in mate preferences), and many-to-one trait-to-function mapping (Bolnick et al., 2018). These various contributors to variation in the extent of parallelism can be assessed via extensive novel sampling (e.g., Stuart et al., 2017) or through meta-analysis of particularly well researched and appropriate study systems (e.g., Langerhans, 2018).

In a previous analysis where we quantified parallelism for 23 fish species in 92 studies, we found that the extent of parallelism was highly variable and often weak (Oke et al., 2017). That analysis included several study systems considered to be classics for studying parallel evolution, including the Trinidadian guppy (*Poecilia reticulata* - hereafter just 'guppy' or 'guppies'). Indeed, for over 40 years, guppies have provided one of the most profitable systems for revealing parallel evolution, particularly in the context of differences in predation intensity driving predictable evolutionary changes (e.g., Endler, 1978, 1980, 1995; Magurran, 2005; Reznick & Endler, 1982). The value of the guppy system for this endeavour is most apparent in Trinidad's Northern Mountain Range, where waterfalls sometimes allow guppies, but often not dangerous predatory fishes, to travel upstream. These natural barriers thus establish evolutionary replicates of low- and high-predation environments within many watersheds (that are independent across watersheds) (Reznick, Butler, et al., 1996). This low-predation versus high-

predation dichotomy replicated across many streams has facilitated extensive research and, accordingly, many traits have been described as evolving in parallel between the two predation regimes, including life history (e.g., Reznick, Butler, et al., 1996), behaviour (e.g., Magurran & Seghers, 1994), and colour traits (e.g., Endler, 1980).

Despite being a classic system for illustrating parallel evolution (Endler, 1995), guppy traits often show substantial nonparallel components. For example, male guppies tend to be brightly coloured and can manifest complex colour patterns due to sexual selection based on female choice (Brooks & Endler, 2007; Houde, 1987; Houde & Endler, 1990). However, although males in 'high-predation' sites are classically described as having fewer, smaller, and duller colour spots resulting from predation-based selection against conspicuous males, this caricature does not do justice to a much more complex reality (Endler, 1978, 1980; Fuller, 2022). In particular, many studies have reported substantial variation in male colour among populations within a given predation regime, leading to substantial variation in the extent of parallelism between predation regimes among replicates (Kemp et al., 2009; Millar et al., 2006; Weese et al., 2010). Shoaling behaviour provides another example of a mixture of parallel and nonparallel patterns. Guppies in high-predation sites have been found to shoal more than those in low-predation sites, likely due to predation selecting for increased anti-predator behaviour (Magurran et al., 1992; Seghers, 1974). However, a recent study of six guppy populations found that predation regime explained only a small amount of the variance in shoaling behaviour (Jacquin et al., 2016). Further, traits such as brain size (Mitchell et al., 2020) and some traits related to life history (e.g., age at maturity) (Fitzpatrick et al., 2014) have been described as sometimes evolving along nonparallel trajectories. We suggest that this extensive variation in the extent of

parallelism in guppies provides an excellent substrate for insights into the factors shaping patterns of phenotypic divergence across seemingly similar environments.

Several factors could contribute to variation in the extent of parallelism in response to the classic low-predation versus high-predation dichotomy for guppies. Here, we investigate a non-exhaustive set of factors that could contribute to the extent of (non)parallelism using published studies on guppies. We start with factors that can stem from variable responses within populations: that is, among traits, and between sexes or rearing environments. First, different types of traits might be expected to show different patterns, such as when more complex traits (e.g., male colour patterns) show 'many-to-one' solutions (Bolnick et al., 2018; Thompson et al., 2017; Wainwright, 2005) to the same problem of conspicuousness versus crypsis (Millar & Hendry, 2012). For example, many different male colour patterns can be attractive to female guppies (Kodric-Brown, 1985). The extent of parallelism could appear low when attempting to quantify individual traits, even though they might show high extents of parallelism at the functional level. Additionally, different traits can have different genetic constraints (Blows & Hoffmann, 2005), or could experience different strengths or directions of selection (e.g., Kingsolver et al., 2001; Siepielski et al., 2013). Second, males and females could show different evolutionary trajectories and extents of parallelism owing to their different trait values (e.g., colour, behaviour, life history, physiology, and/or morphology) that cause differences in selection or genetic constraints (Butler et al., 2007; Oke, Motivans, et al., 2019). Indeed, some traits in guppies diverge between predation regimes in similar ways for males and females, whereas other traits are more variable in their responses, including aspects of morphology (Hendry et al., 2006), and parasite resistance/tolerance (Dargent et al., 2016; Stephenson et al., 2015). Third, different rearing environments (e.g., wild caught versus common-garden) could

generate different outcomes due to the differential effects of plasticity – as has been show for other fish species (Oke et al., 2016).

We now turn to factors contributing to (non)parallelism that stem from variable responses among populations.

1. Substantial ecological variation exists within and among watersheds even within a predation regime (Grether et al., 2001; McKellar et al., 2009; Reznick et al., 2001), but especially between the different slopes of Trinidad's Northern Mountain Range. Most notably, the predator community differs, with cichlids and characins dominating in high-predation sites on the southern slope, and gobies dominating in high-predation sites on the northern slope (Phillip & Ramnarine, 2001; Reznick, Rodd, et al., 1996). In low-predation sites, the killifish *Rivulus hartii* is found on both slopes, whereas freshwater prawns (*Machrobrachium* spp.) are much more common on the northern slope (McKellar et al., 2009; Millar et al., 2006; Phillip & Ramnarine, 2001; Reznick, Rodd, et al., 1996). These and other ecological differences between the two slopes could impose divergent selection between populations despite the same (high vs. low) predation regime – similar to effects documented for other contexts in fish (Stuart et al., 2017).

2. Guppy populations can show large genetic differences among river drainages (Fraser et al., 2015; Shaw et al., 1991; Suk & Neff, 2009) as a result of their different evolutionary histories. In particular, guppies in the west-draining (Caroni) versus east-draining (Oropouche) watersheds have been separated for an estimated 1.2 million years (Fajen & Breden, 1992) – to the point that some authors have suggested they are different species

(Schories et al., 2009). These different genetic backgrounds could generate different responses to a given predator regime – similar to effects documented for other contexts in fish (e.g., lateral plate evolution in stickleback fish colonizing freshwater environments; Leinonen et al., 2012).

3. Many experimental introductions have taken place in Trinidad, where guppies from one predation regime have been transplanted to the other predation regime, typically in the direction of high predation to low predation. Thus, time since colonization could influence the extent of current (non)parallelism, as introduced populations could differ from long-established natural populations in their extent of divergence from ancestral phenotypes – again, similar to effects documented in other contexts for fish (e.g., loci that repeatedly diverge as stickleback colonize freshwater environments; Roberts Kingman et al., 2021).

To investigate the extent to which the above factors contribute to parallelism in guppies, we first quantify phenotypic (non)parallelism using studies that have measured guppy traits from both low-predation and high-predation sites in Trinidad (Figure 1). We next investigate how sex, trait type, and rearing environment influence the extent of parallelism. Finally, we consider how the extent of parallelism varies with differing levels of ecological complexity, evolutionary history, and time since colonization. For ecological complexity, we compare estimates of parallelism from populations within only the southern slope to estimates of parallelism from populations pooled between the northern and southern slopes of the Northern Mountain Range, where the ecological contexts (e.g., predator communities) differ. For evolutionary history, we

compare estimates of parallelism from populations within only the Caroni to estimates of parallelism when populations are pooled between the Caroni and the Oropouche drainages, between which the populations have been separated for about 1.2 million years. For time since colonization, we compare parallelism within studies that have been conducted exclusively on natural populations to estimates of parallelism where natural and introduced populations are pooled. If increasing ecological complexity, differing evolutionary history, or decreasing time since colonization result in nonparallel outcomes (see above), then we predict traits measured within a single group will show higher extents of parallelism than traits pooled among groups. Stated another way, estimates measured within a single slope or drainage, or only within natural populations will be more parallel than estimates between pooled slopes or drainages, or when considering both natural and introduced populations.

Methods

Search and inclusion criteria

To find studies where guppy traits were measured in both low-predation and high-predation sites, we searched Web of Science using terms related to guppies (gupp*, *Poecilia reticulata*), and to predation regime (predat*, high, low) – note that the asterisk indicates that the search should include the exact letters before the asterisk, but any combination of letters after the asterisk (e.g., gupp* could include both guppy and guppies). The last search was conducted on February 1, 2021, resulting in 630 studies. From this full list, we included only studies that measured guppy traits from at least one low-predation and one high-predation site in each of two or more rivers in Trinidad. We included studies that measured ‘wild-caught’ guppies and ‘common-garden’

guppies raised in a laboratory for up to two generations (F2). Guppies that were raised in captivity for longer than two generations were excluded. This cut-off was selected because our focus was on (non)parallelism in natural guppy populations, and two generations are likely to include studies attempting to account for plasticity while still working with guppies that are genetically and phenotypically similar to wild populations.

We categorized sites as either low-predation or high-predation using the classifications described by Reznick, Rodd, et al. (1996) based on the predatory fishes documented (or expected) to be present by a study's authors, by other investigators working at the same sites, and based on our own extensive experience at many of the sites. On the northern slope of the Northern Mountain Range, low-predation sites included those primarily with (as potential non-avian and non-mammalian guppy predators) *Rivulus hartii or Macrobrachium* spp; and high-predation sites also variously included *Eleotris pisonis, Gobiomorus dormitor, Dormitator maculatus*, or *Agnostamus monticola*. (The latter species is not necessarily a guppy predator but is a good indicator for areas with access to the other species listed before it.) On the southern slope of the Northern Mountain Range, low-predation sites included those with primarily *Rivulus hartii* (historically, some sites also contained *Macrobrachium*) and high-predation sites also included *Crenicichla* sp. Other species are variously present at these southern slope sites (Phillip & Ramnarine, 2001), but *Crenicichla* presence/absence is a good indicator of the overall predation regime (e.g., Endler, 1978, 1980; Magurran & Seghers, 1994; Reznick, Rodd, et al., 1996). In one study, the authors classified some sites with *Aequidens pulcher* but without *Crenicichla* as medium risk (Ioannou et al., 2017), and those sites were excluded from our analysis.

Thirty-six studies had sampling designs (see above) that allowed their inclusion in our analyses (see Table S1). For each study, we extracted site-level trait means (including proportions, for example, the proportion of broods that had multiple sires) from the text, the within-text figures (using webplotdigitizer: Rohatgi, 2020), online data repositories, or by e-mailing the authors directly. Additionally, we recorded the following details when available: (1) the sex of the guppies sampled; (2) whether the guppies were wild caught or common garden; (3) the site where the guppies were collected (including ancestors of common-garden fish); (4) the start year, end year, and total duration of the study in years; (5) the standard deviation or standard error of the trait mean; and (6) the sample size (the number of guppies from which a given site mean was estimated). We also recorded whether the populations at a site were natural or the result of an introduction experiment. For the introduction sites, we additionally recorded when available the original source population, the introduction year, and how many years had passed after the introduction event until the guppies were sampled. Finally, we assigned each trait to a grouping category derived from Kingsolver and Diamond (2011): life history (n = 48), size (n = 47), other morphology (n = 42), behaviour (n = 133), colour (n = 109), physiology (n = 64), and other (n = 3). In all, we extracted 5176 population trait means for 446 different traits, of which 274 were measured in males and 172 in females.

Statistical analysis

Starting from the methodology of Oke et al. (2017) and Langerhans (2018), we first quantified the effects of predation regime (i.e., low-predation or high-predation) on estimates of phenotypic variance among the site-level trait means within each study. This quantification was done using

an ANOVA on the site means with predation regime as a fixed effect. The resulting R^2 values thus provide estimates of the extent of parallelism for each trait from each study (Figure 2).

We next used these R^2 values as the response variable in three binomial generalized linear mixed models (GLMM) that combined traits and studies to explore several basic potential determinants of parallelism (Table 1). These data were modelled with binomial error distributions because R^2 values are bounded between zero and one. The first model (the 'trait type model') was used to investigate whether the extent of parallelism differed across different trait types, and thus had only trait type as a fixed effect. 'Other' traits were excluded from the trait model due to small sample size. The second model (the 'sex model') was used to investigate whether the extent of parallelism differed between the sexes, and thus had only sex as a fixed effect. We ran this sex model with colour traits included and then with colour traits excluded, to ensure that colour was not driving any potential sex effects. We made this decision because colour was only reported for male guppies and shows low extents of parallelism (Yong et al., 2022). Traits from the 'other' category – 'testosterone effect on melanophores', 'testosterone effect on xanthophores' (Gordon et al., 2012), and the 'probability of recapture of marked female and immature fish' (Reznick & Bryant, 2007) – were exclusively female, but given that we did not have a priori expectations about the direction or magnitude of the effect of including 'other' traits, these traits were not excluded from the model (i.e., we did not include and then exclude, as we did for male colour). The third model (the 'rearing model') was used to investigate whether the extent of parallelism differed between rearing environments (i.e., wild caught or common garden), and thus had 'rearing environment' as the only fixed effect. First generation (F1) common garden fish were excluded from the rearing model because the sample size was too small; we thus only considered wild caught versus second generation (F2) common garden fish.

Because the effects of sex, trait type, and rearing environment are not mutually exclusive, we also ran one model with sex and trait type as fixed effects (the 'sex and traits' model), and another with sex and rearing environment as fixed effects (the 'sex and rearing' model). Unfortunately, efforts to include all three terms in the same model, or to include interaction terms, were unsuccessful due to low sample size. Study was included as a random intercept term in all models to account for non-independence of the estimates from a given study.

Next, we asked whether the extent of parallelism differed when sites from multiple slopes (or drainages or introduction histories) were included in the same model, compared to a model that included sites from only a single slope (or drainage or introduction history). For each trait in each study where sufficient data were available, we created two subsets of data for each of our questions (ecological complexity, evolutionary history, and time since colonization). In other words, data were only used to calculate R^2 values if a single study sampled across both slopes or drainages, or with both natural and introduced populations. The first subset was a 'within' group (slope, drainage, or introduction history) subset, which included sites from only a single group, and the second was a 'pooled' group subset. For example, for a trait measured in sites from both northern and southern slopes, we created a south only subset and a north and south (pooled between slopes) subset. For each of our questions, we compared these within-group to pooled-group subsets; we were unable to compare only within-group categories, due to limited sample sizes (e.g., because there were an insufficient number of sites sampled on the northern slope, we could not compare sites only on the northern slope to sites on the southern slope). With these subsets of sites, identical ANOVAs to the ones above were then conducted to calculate distinct 'within-group' or 'pooled-group' R^2 values for each trait for the ecological complexity, evolutionary history, and time since colonization questions. We will now discuss how we

specifically subset the sites from each of the studies in the data set to calculate each of these R^2 values.

To investigate the potential effect of ecological complexity on parallelism, we selected studies that measured traits in populations on both the northern and southern slopes. We ran ANOVAs for each trait within each study on only populations from the southern slope ('within one slope') and calculated R^2 values. We then repeated these ANOVAs on the same traits with populations sampled from both the southern and northern slopes (i.e., 'pooled between the slopes'). The 'northern and southern' and 'only southern' R^2 values were then compared. If differences in ecological complexity between the slopes (notably, the different predator communities) contribute to nonparallelism, then we expect that traits measured on a single slope will have higher R^2 values than when traits measured across both slopes are pooled.

To investigate the potential effect of evolutionary history on parallelism, we selected studies that measured traits in populations from both the Caroni and Oropouche drainages. We ran ANOVAs for each trait within each study using only populations in the Caroni ('within one drainage') and calculated R^2 values. We then repeated these ANOVAs on the same traits with populations sampled from both the Caroni and Oropouche drainages (i.e., 'pooled between the drainages'). Note that we did not include the Northern drainage as well, owing to the potential confounding effects of the differing predator communities. These 'Caroni and Oropouche' and 'only Caroni' R^2 values were then compared. If evolutionary history contributes to nonparallelism in guppies, then we expect traits measured in a single drainage will have higher R^2 values and thus greater extents of parallelism than when traits measured across both drainages that have distinct evolutionary histories are pooled.

To investigate the potential effect of time since colonization on parallelism, we selected studies where traits were measured from both natural and introduced populations. We ran ANOVAs for each trait within each study using only natural populations ('only natural') and calculated R^2 values. We repeated these ANOVAs on the same traits where guppies were sampled from both natural populations and introduced populations ('natural and introduced'). These 'natural only' and 'natural and introduced' R^2 values were then compared. Because some studies did not say whether the sites were natural or introduced, and some reported sites as natural that were reported in other studies or known to us to be introductions, we calculated R^2 values based on whether we knew the sites to be natural or introduced (based on other literature – see Table S2). If the extent of parallelism differs depending on the amount of time that guppies have been evolving in a particular environment, then we expect traits measured from sites with only natural populations will have higher R^2 values than when traits measured with both natural and introduced populations are pooled.

The R^2 values obtained from ANOVAs run for each question-specific among-site factor (ecological complexity, evolutionary history, time since colonization) were then used as response variables in three binomial GLMMs. Each model had a different fixed effect, depending on the question. For the 'ecological complexity model', the fixed effect variable was slope ('northern and southern' or 'only southern'), and we included an interaction between slope and sex. For the 'evolutionary history model', the fixed effect variable was drainage ('Caroni and Oropouche' or 'only Caroni'), and we included an interaction between drainage and sex. Because our sample size was small, we also compared the results from these GLMMs to those from a similarly structured generalized linear model (GLM - which had the same fixed effects and distribution as the GLMM, but had no random effect) and linear mixed effects model (LMM - which had the

same fixed and random effects as the GLMM, but did not have a binomial distribution) to confirm that the conclusions were consistent across all models. For the 'time since colonization model', the fixed effect variable was introduction history ('natural and introduced' or 'only natural'). We were unable to include an interaction between introduction history and sex due to the small sample size, so introduction history was the only fixed effect term in these models.

All of our models were constructed in the R environment with version 4.0.2 (R Development Core Team, 2021) using the lme4 package (Bates et al., 2015), and model validation was done using the DHARMA package (Hartig, 2022) and following recommendations outlined in Zuur et al. (2011). For all models where interactions were not significant, models were re-run without interactions and only results with the interaction dropped are presented.

Finally, we conducted additional tests to account for sample size, given that sample size can affect R^2 estimates. We first conducted a simple linear regression with sample size (fixed effect) against the overall R^2 values (without subsetting the data in any way; dependent variable). Next, we re-ran the six aforementioned models (trait type, sex, rearing environment, ecological complexity, evolutionary history, time since colonization) with mean sample size among populations for each trait as an additional fixed effect, when available. Sample size was not available for all traits so the models included fewer traits than our original models (n = 372). Due to insufficient sample sizes, these models were LMMs (rather than GLMMs; i.e., they were not modelled with binomial error distributions). We then conducted a permutation test where each trait within each predation regime was permuted 100 times without replacement, and reran the above ANOVA with these permutated values to generate R^2 values that might be observed if

estimations of parallelism were due to chance alone (Oke et al., 2017). Finally, we constructed a linear model with the R^2 values from the observed data against the mean R^2 from the permutation data and extracted the residuals, and re-ran all of the six GLMMs with these residual R^2 values as the dependent variable.

Results

Although some guppy traits did show high levels of parallelism, the majority showed only low-to-moderate parallelism, as indicated by the high proportion (82%) of R^2 values less than 0.5 (Figure 3a). At the extremes, 43% of R^2 values were <0.1, and only 1% were over 0.9. In the overall dataset (including all traits, sites, and studies), predation regime explained an average of 24.7% of the variance among sites in mean trait values (Table 2A), and variation in these R^2 values was high (SD = 0.261).

The trait type, sex, and rearing environment models revealed only weak or non-significant effects on the extent of parallelism (trait type: $\chi_5^2 = 10.23$, $p = 0.0689$; sex (with colour): $\chi_1^2 = 0.472$, $p = 0.492$; sex (without colour): $\chi_1^2 = 0.08$, $p = 0.780$; rearing: $\chi_1^2 = 1.14$, $p = 0.285$). The effects of these factors are not likely to be mutually exclusive – and they would ideally be analysed together. Although low sample size meant that models with all three factors were not possible, GLMMs combining two of the three factors provided some nuance. Specifically, an effect of trait type was revealed when accounting for sex (trait type: $\chi_6^2 = 55.82$, $p = 3.167 \times 10{-}10$), though the sex effect itself was not significant (sex: $\chi_1^2 = 0.022$, $p = 0.883$). This result appears to be driven by colour traits, which were measured only in males and showed lower extents of parallelism than other trait types (Figures 4 and 6, Table 2). Including rearing

environment and sex in the same model did not change our conclusions as neither had a significant effect (rearing: χ_1^2=1.06, p = 0.302; sex: χ_1^2=0.212, p = 0.646).

Trait-level ANOVAs that pooled populations from different slopes (northern and southern) of the Northern Mountain Range, and thus had increased ecological complexity owing to different major predators, did not result in significantly lower R^2 values than when considering populations from only a single slope (south). When sites were pooled between the slopes, the mean R^2 was 0.153, and when sites were analysed for the southern slope only, the mean R^2 was 0.197 (Table 2E, Figures 5a/d and 6). Nevertheless, the slope term was not significant in the ecological complexity GLMM (slope: χ_1^2=0.587, p = 0.444), and nor was sex (sex: χ_1^2=2.31, p = 0.129).

Trait-level ANOVAs that pooled populations from different major drainages (Caroni and Oropouche), and thus different evolutionary histories, resulted in R^2 values that were lower than when considering populations from a single drainage (Caroni). When populations from the two drainages were pooled, the mean R^2 was 0.181, but when populations were from the Caroni only, the mean R^2 was 0.250 (Table 2F, Figures 5b/e and and 6). In the corresponding evolutionary history GLMM, the drainage term was significant (χ_1^2=7.93, p = 0.00487) whereas sex (sex: χ_1^2=2.48, p = 0.116) was not. When the model was run without the random effect (as a GLM), the effect of drainage was only marginally significant (drainage: χ_1^2=3.64, p = 0.0563), whereas LMM results were similar to GLMM (drainage: χ_1^2=14.87, p = 0.000115).

Trait-level ANOVAs that considered populations from both natural and introduced populations did not result in significantly lower R^2 values than ANOVAs on natural populations only. When only natural populations were included the mean R^2 was 0.186, compared to 0.151

when both natural and introduced populations were included (Table 2G, Figures 5c/f and 6). These values were not significantly different (introduction history: χ_1^2=1.48, p = 0.224) in the time since colonization GLMM.

Finally, R^2 was higher for traits with lower sample sizes (F_1 = 9.16, p = 0.00259) (Figure 7). When mean sample size for each trait was included in the model the results were consistent for some models (the sex, rearing, sex and traits, and sex and rearing models), although for other models some terms that were not significant in the original models were significant once sample size was added. For the trait-type model, the trait-type term, which was previously marginal, became significant (χ_5^2=12.598, p = 0.0275, previously p = 0.0689). For the ecological complexity and time since colonization models, the slope/introduction history terms were significant when sample size was included (ecological complexity (slope): χ_1^2=7.153, p = 0.00749; time since colonization (introduction history): χ_1^2 = 4.721, p = 0.0298), although they previously were not (ecological complexity (slope): p = 0.444; time since colonization (introduction history) p = 0.224). We present the full GLMM results in Table S4. Note that these models included fewer traits than the original models because only 83% of studies reported sample sizes. As such, we do not interpret the results from these models beyond discussing the possible effects of sample size on R^2. The permutation test results suggest that our observed R^2 estimates were higher than expected by chance (Figure S1) and re-running our six GLMMs with the residual R^2 values provided equivalent results as the original models (Table S5). Taken together, these results suggest that although our results are not driven by statistical artefacts arising from estimating R^2 on traits measured in different numbers of populations, there does seem to be an effect of sample size on our estimates of parallelism, such that highly parallel results are more likely to occur in traits measured on relatively few individuals.

Discussion

We compiled data on (non)parallelism in Trinidadian guppies (by quantifying the variance among populations in mean trait values explained by predation regime) to explore possible contributors to the extent of parallelism. Overall, we found that about a quarter (24.7% on average) of phenotypic variation among populations was explained by predation regime. This result supports over 40 years of literature highlighting the value of the guppy system for investigating parallel evolution in nature. At the same time, our result makes clear that many other factors must be shaping mean trait values in this system. Thus, the important question to address now is: how do factors other than predation contribute to variation in mean trait values?

Previous studies have shown that trait type, sex, and rearing environment can influence (non)parallelism (e.g., Hendry et al., 2006; Langerhans, 2018; Oke et al., 2016, 2017; Vinterstare et al., 2021). In the current study, only trait type had a significant influence on the extent of parallelism, and only when sex was included in the model. In addition, we investigated several factors that might be expected to contribute to high-low predation (non)parallelism among evolutionary replicates: comparing populations from different ecological backgrounds (different mountain slopes with different predator communities), different evolutionary histories (different drainages), and different times since colonization (natural versus introduced). At face value, pooling populations from different ecological backgrounds, different evolutionary histories, and different times since colonization resulted in lower estimated mean R^2 values, as would be expected if these complexities increase nonparallelism. Of these three factors, however, only evolutionary history had a statistically significant effect – and the level of this significance depended on the specific structure of the various models used.

The frequent lack of statistical significance was likely due to modest sample sizes (36 studies maximum) and the highly variable level of parallelism within each 'category' (e.g., the male and female categories encompassed by the sex term, or the within one slope vs. pooled between both slope categories encompassed by the slope term). That is, even if the estimated change between two models (e.g., with and without introduced populations) in mean R^2 was substantial in response to many of the above variables, the heterogeneity was so high within each category that statistical support was weak. For these reasons, we thus interpret the specific output from these models with caution, and instead focus on suggestive trends. Indeed, even some non-significant trends warrant discussion given shifts in the expected direction. Although, we do note that even if we had the power to detect effects of the non-significant trends, they are unlikely to be very strong. Stated another way, although the effects of ecological complexity and time since colonization might contribute to (non)parallelism, and evolutionary history likely does, the effects are probably minor (at least on average) in comparison to other sources of variation.

Our results suggest that estimates of parallelism are higher at low sample sizes, as has previously been shown in broadscale meta-analyses of effect sizes in ecology and evolution (Jennions & Møller, 2002; Low-Décarie et al., 2014). Most guppy traits were measured at low sample sizes, which had both higher average R^2 values and a greater spread of R^2 values than did traits measured at high samples sizes. The combination of publication bias and highly variable estimates of R^2 at small sample sizes has been proposed as an explanation for decreasing explanatory power through time in the field of ecology (Low-Décarie et al., 2014). Both factors could contribute to our results. High estimates ($R^2 > 0.8$) of R^2 were not observed at high sample sizes ($n > 100$), which, in combination with the large spread in R^2 values at low samples sizes, could suggest that some of the R^2 values might be artificially high due to sampling error at small

sample sizes (Jennions & Møller, 2002; Low-Décarie et al., 2014). Additionally, publication biases that typically favour strong and significant effects (Button et al., 2013; Jennions & Møller, 2002) could be contributing. In our case, guppies are often considered in the context of predation regimes, so the literature could be biased towards earlier reporting of strongly parallel traits that are 'low hanging fruit' and can be detected at smaller sample sizes (Low-Décarie et al., 2014). Finally, traits are not equally easy to quantify. Complex traits like life history traits demonstrated generally higher parallelism (Figures 4 and 6),but are likely often more time consuming to measure than traits like body size that can easily be included in studies focused on other traits, even if they are not expected to be highly parallel. Relatively few guppy traits included in our study were measured at high sample sizes, so generalization of our results awaits more studies with high sample sizes, which should be prioritized.

(Non)parallel evolution overall

Generally, our findings align with those from previous analyses of fishes (Langerhans, 2018; Oke et al., 2017; Stuart et al., 2017). In that, the extent of (non)parallel evolution is highly variable and many traits exhibit low extents of parallelism, although some traits in some populations do show remarkably high extents of parallelism. Surprisingly, our focused analysis on only guppies generated lower estimates of parallelism than did the broader multi-species survey of Oke et al. (2017). In that study of 23 fish species, the variance among population means (R^2) that could be attributed to 'ecotype' (e.g., high predation vs. low predation, marine vs. freshwater, benthic vs. limnetic) was 0.460 across all species, including a mean of 0.493 for guppy studies that used the low-predation versus high-predation dichotomy. Our new estimate of

the mean extent of parallelism is – by contrast – about half ($R^2 = 0.247$) of those earlier estimates.

What could explain the generally low extent of parallelism observed in studies on guppies, given that the vast majority of the factors we expected might contribute to variation among replicated environments appeared to have nonsignificant effects? To start, although we were unable to consider them here, other factors such as gene flow, drift, genetic covariances, and variation in heritability or in selection could also contribute to (non)parallelism. For example, gene flow has been reported (or suggested) to occur in several ways, including floods flushing upstream guppies into downstream environments as well as human-mediated movements (Blondel et al., 2019), such as for introduction experiments (e.g., Shaw et al., 1991). In cases where the amount of gene flow between predation regimes differs among replicates, the extent of parallelism could be reduced if gene flow from one predation regime (the immigrant population) limits adaptation towards the optimal phenotype in the other predation regime (the resident population) – as has been demonstrated in other systems (Bolnick & Nosil, 2007; Hendry & Taylor, 2004). Conversely, extents of parallelism could decrease if some populations (particularly small populations; Szendro et al., 2013) are more susceptible to drift or if selection on a given trait acts indirectly through other traits (i.e., genetic covariances) (Bolnick et al., 2018). Other sources of variation beyond predation could also contribute to the extent of parallelism. In the guppy system, in particular, substantial research has highlighted effects of guppy density (e.g., Bassar et al., 2013), predator biomass (e.g., Barbosa et al., 2018), resource availability (e.g., Grether et al., 2001), and parasite infection (e.g., Gotanda et al., 2013) on guppy phenotypes. Although we were unable to consider these (and other) additional factors in the present analysis, they almost certainly contribute to aspects of nonparallelism.

Within-population factors that contribute to (non)parallel evolution

One might imagine that, by focusing on a single ecological context (predation regime) in a single system (guppies) that is a model for parallel evolution (Magurran, 2005; Reznick, Rodd, et al., 1996), we would generate higher estimates of parallelism than found in broader surveys or less well-studied systems. However, as discussed above, our mean estimate of parallelism in the present study is about half of what was found in the multi-species survey conducted by Oke et al. (2017). One explanation for the lower extent of parallelism in our new study could be that the search terms used by Oke et al. (2017) were specifically related to parallel or convergent evolution, whereas we here considered all guppy papers – regardless of whether or not they emphasized parallelism. Our approach generated a much larger sample of estimates for guppies (n = 443) than did the earlier study (n = 29) and our result is therefore presumably less biased towards high extents of parallelism. The study closest to ours in approach is that of Langerhans (2018), who examined effects of predation regime in Bahamas mosquitofish (*Gambusia affinis*) and generated parallelism estimates of $R^2 = 0.37$ for males and $R^2 = 0.44$ for females. Here, our overall estimates of parallelism were again much lower than what Langerhans (2018) found: $R^2 = 0.206$ for males and $R^2 = 0.310$ for females (Table 1). That study, however, had a smaller geographical scope and did not explicitly consider some other layers of complexity that we assessed here, such as different predator communities, different evolutionary lineages, and experimental introductions. A more appropriate comparison between our study and the Langerhans (2018) study would be to assess sex differences with only natural sites within a single drainage. Among only natural populations in the Caroni drainage, our estimates of parallelism were $R^2 = 0.158$ for males and $R^2 = 0.279$ for females – still lower than those

generated by Langerhans (2018). Overall, then, guppies are no exception to the emerging consensus that the extent of parallelism within a given system is variable and often not high (e.g., Langerhans, 2018; Oke et al., 2017; Stuart et al., 2017). This variation is an asset because it facilitates the assessment of factors contributing to the extent of parallelism – which was the goal of our current analyses.

Colour traits appeared to be the least parallel of all the trait types that we examined (mean $R^2 = 0.102$), and the removal of colour traits increased parallelism estimates in males from $R^2 = 0.206$ to $R^2 = 0.275$ (these values were calculated across the full dataset, including both slopes, drainages, and with introduced populations). Recent studies have similarly found especially low extents of parallelism for guppy colour across the low-predation versus high-predation dichotomy (Yong et al., 2022). There are several possible reasons for why colour traits showed such low extents of parallelism. To start, colour represents an obvious situation of many-to-one mapping of traits-to-performance (Wainwright, 2005) – that is, many different colour patterns can be attractive to females (Kodric-Brown, 1985). Indeed, female guppy preferences for male colour patterns vary dramatically among populations, and female guppies often base their preference on a combination of male colour aspects (Endler & Houde, 1995; Schwartz & Hendry, 2007). Of course, colour traits are not the only traits that can be subject to many-to-one mapping – however, owing to the high dimensionality of colour traits and the many ways that colour can generate high conspicuousness for attracting females, we expect that many-to-one mapping would be stronger for colour traits than those in the other categories. Nevertheless, other factors likely contribute to the especially low extent of parallelism for colour traits. For instance, whether a colour pattern is cryptic to predators can depend on the specific predators present, as well as water clarity, forest cover, and other variables (Endler, 1980; Millar &

Hendry, 2012; Weese et al., 2010). The colour patterns that are most attractive to females and the least conspicuous to predators also can vary through time at a given site due to negative frequency dependent selection (Hughes et al., 2013; Olendorf et al., 2006). However, temporal variation has been found to be less important than spatial variation in selection for generating variation in guppy colour (Gotanda & Hendry, 2014). Finally, male guppies sometimes engage in sneaky mating attempts and the decision to sneak or not can be impacted by aspects of the external environment, such as the ambient light spectrum (Gamble et al., 2003) or food availability (Kolluru & Grether, 2005).

Males and females did not differ in their extents of parallelism when colour traits were excluded (Figure 6). This non-significant finding contrasts with other studies with other live-bearing fishes where males and females have been found to differ in their extents of parallelism. For example, Langerhans (2018) found that female *G. affinis* exhibit significantly higher extents of parallelism than males. One explanation for why we did not observe sex differences here could be that they vary by trait. In other words, males could show higher extents of parallelism for some traits, and females for others. In the present analysis, we had inadequate sample sizes to make this comparison. However, visualizing the raw data (Figure S2) highlights the value of future work investigating this interaction between sex and trait type, because males appear to show higher extents of parallelism for behaviour, morphology, and size, and females for life history and physiology. That such patterns of (non)parallelism are trait-dependent has been emphasized in past work on guppies; for example, in response to predation, males and females show higher extents of parallelism for body size than body shape (Hendry et al., 2006). Similarly, when considering another selective agent (parasitism), some aspects of resistance show high extents of parallelism within, but not between, the sexes (Dargent et al., 2016). With

the studies conducted to date, however, we were unable to provide an unequivocal assessment of such effects on average.

We also assessed whether rearing environment (wild caught or lab reared) influenced estimates of parallelism based on the recognition that phenotypic plasticity could increase or decrease the extent of parallelism (Oke et al., 2016). Guppies reared in common-garden environments seemed to exhibit a higher extent of parallelism ($R^2 = 0.351$) than those reared in the wild ($R^2 = 0.227$), as would be expected if plastic responses to environmental variation resulted in nonparallelism among replicates, but this difference was not significant. Oke et al. (2017) also did not detect a significant effect of rearing environment on the extent of parallelism. Taken together, these results suggest that plasticity might not be playing an important role in shaping the extent of parallelism among replicates. However, greater insights will likely be gained from studies that explicitly test for an effect of parallelism by comparing the same traits and populations in the wild and in common gardens, such as Torres Dowdall et al. (2012) or Oke et al. (2016).

Ecological complexity

Many previous studies have suggested that increasing environmental heterogeneity should be associated with a decreasing extent of trait parallelism (e.g., Landry et al., 2007; Morales et al., 2019; Stuart et al., 2017). The basic idea is that combining different locations into a single 'type' (here high predation or low predation) that is expected to impose parallel selection on a given trait obscures meaningful variation in other environmental variables that impose nonparallel selection on that same trait. This idea was first formally tested for guppies by examining life history traits across the northern and southern slopes of the Northern Mountain Range – because the predator faunas within each 'regime' are different between the slopes (Reznick, Rodd, et al., 1996). Many other factors also differ on average between the two slopes and even among sites on each slope, including within a predator regime. Examples include parasites (Gotanda et al., 2013), canopy cover (Grether et al., 2001), guppy density (Reznick et al., 2012), water turbidity (Ehlman et al., 2018), and human disturbance (Deacon et al., 2015).

We expected that these and other factors might lower estimates of parallelism when pooling data between the two slopes rather than only on a single slope. Although this anticipated trend was observed (within-slope $R^2 = 0.197$; pooled-slope $R^2 = 0.153$), the difference in these R^2 values from the ANOVAs was not significant. A possible explanation is that categorizing populations into binary northern slope or southern slope categories collapses too much environmental variation into two coarse categories to meaningfully address ecological complexity, much as has been described for binary ecotype categories (e.g., benthic versus limnetic: Boughman et al., 2005; cave versus surface: Tobler et al., 2006). However, including specific environmental variables was not possible in our analysis. Studies that formally account

for environmental variation among populations are often able to attribute phenotype variation to environmental variation (e.g., Santi et al., 2020; Stuart et al., 2017).

Evolutionary history

Many studies have argued that lineages experiencing longer periods (relative to shorter periods) of evolutionary isolation should show lower parallelism when subsequently colonizing similar environments (e.g., Conte et al., 2012; Liu et al., 2018). The basic idea is that longer periods of separation will lead to more divergent genetic backgrounds, which then will generate different responses to similar selective pressures. Past studies in guppies have often included samples from two major drainages (Caroni and Oropouche – that have been separated for over a million years; Fajen & Breden, 1992), which could result in lower estimates of parallelism if differences in evolutionary history among the drainages are not accounted for. We here leveraged those cross-drainage studies into a test of the effects of evolutionary isolation on parallel responses to a similar environmental contrast (predation regime).

Estimates of average parallelism were higher for models that included populations from the Caroni drainage only ($R^2 = 0.250$) compared to those that included populations from both the Caroni and Oropouche drainages ($R^2 = 0.181$). However, given that our various alternative models disagreed on the importance of evolutionary history, we must continue to equivocate on just how important this effect really is – an uncertainty that will surely be resolved when more studies accumulate. Regardless, it seems unlikely that any effect of evolutionary history will be especially strong given our provisional results. We do note, however, that this effect of evolutionary history (a decrease in mean R^2 from 0.250 in one drainage to 0.181 between

drainages) was estimated to be larger than the above effect of ecological complexity (a decrease in mean R^2 from 0.197 to 0.153). In short, evolutionary history does seem to be an obvious target for future work on parallelism in this system. Further, it is certainly possible that the Caroni and Oropouche drainages differ in other ways beyond evolutionary history (e.g., in environmental conditions or selective factors), which could confound the effect of evolutionary history – although, we expect that these differences will be minimal compared to the differences between the slopes (the ecological complexities question) which have difference predator communities. Quantifying other sources of environmental variation or selective factors between the two drainages is necessary to tease apart the effects of evolutionary history with these other possible effects.

As a subsidiary point, human influences have generated new mixing between the Caroni and Oropouche drainages (Becher & Magurran, 2000; Blondel et al., 2020; Suk & Neff, 2009) as well as among other watersheds (Blondel et al., 2019). These mixing events can complicate future analyses of evolutionary history; but they also provide opportunities. For example, trajectories of evolution of two lineages could now be compared in the Turure watershed (where mixing has occurred), akin to the similar analyses conducted for odd-year versus even-year lineages of pink salmon (Oke, Cunningham, et al., 2019).

Time since colonization

Evolution is not instantaneous – and so we generally expect that populations introduced to new environments will take some time for their phenotypes to fully adapt to those conditions (Clegg et al., 2002). Further, evolutionary trajectories might initially be ‘curved’ – taking phenotypes in

unexpected directions as a result of correlated shifts in selection or particular genetic correlations (Arnold et al., 2001) – as has been argued for parasite resistance traits in guppies (Dargent et al., 2016). Thus, recently introduced populations might show lower parallelism than long-standing populations, a comparison facilitated by the many experimental introductions of guppies. Moreover, if populations from natural and introduced populations are compared, the extent of parallelism might appear to be lower because natural populations are much farther along on their trajectories. However, although the trend in R^2 was observed in the expected direction (only natural = 0.186; natural and introduced = 0.151), our study did not reveal a significant effect of time since colonization on the extent of parallelism.

The apparent lack of influence of time since colonization in our study might be due to either – or both – of two important points. First, the average amount of time that guppies had been evolving in their new environment (after an introduction experiment) prior to data collection in the studies we compiled was 20 years (min = 8, max = 36). Twenty years – even the minimum of 8 years – is likely sufficient for guppies to evolve traits consistent with those in natural populations – especially given the fact that guppies can have up to three generations per year. That is, considerable evolution on even shorter time scales has been observed for many traits in guppies, including male gonopodium length within 2–3 years (Broder et al., 2020), colour traits within 6 years (Endler, 1980; Kemp et al., 2018), and female life history traits (e.g., embryo number) within 8–9 years (Gordon et al., 2009). Therefore, the introduced populations of guppies might now be locally adapted and perhaps introductions at shorter time scales need to be investigated to see if there is an effect of introductions. Second, some of the traits measured in wild-caught guppies could have shown rapid plastic responses in the expected direction, thus enhancing parallelism at the phenotypic level in introduced populations even if genetic changes

are limited. Certainly, rapid adaptive plastic responses have been observed in many taxa introduced to new environments (Ghalambor et al., 2007). In the guppy system, rapid changes are evident even for common-garden studies of recently introduced populations, where the differences are expected to be genetic; and plastic responses can sometimes be in the maladaptive direction (Ghalambor et al., 2015), which must then be counteracted by subsequent evolution. In short, guppies generally show exceptionally fast adaptation to new environments – as is the case in many other systems (Hendry, 2017).

Conclusions

Even in study systems considered to be classics for studying parallel evolution, variable and relatively low extents of parallelism are often observed. Thus, an opportunity – and a need – exists to quantify the extent to which various factors contribute to parallelism. In this study, we assessed six sources of nonparallelism in the classic Trinidadian guppy system. Overall, we found that the extent of predator-driven trait parallelism is not as high as that reported in other reviews (Langerhans, 2018; Oke et al., 2017). Indeed, an average of only 25% of the among-population trait variation was explained by predation regime, and that the extent of parallelism was highly variable among traits and populations and studies. For the six factors, we investigated (trait type, sex, rearing environment, ecological complexity, differing evolutionary history, time since colonization), the effects were generally weak and often non-significant, and we suggest that even if we had the power to detect effects, they would be relatively small. Nevertheless, the trends point to several potentially promising avenues for future research – especially the particularly low parallelism for colour traits and the likely effect of evolutionary history on

reducing parallelism. We further suggest the value of examining other factors that were beyond the scope of our study but that could contribute to the large amount of unexplained variance – such as other selective agents or sources of variation; or the effects of gene flow, genetic drift, or genetic constraints. We hope that our analyses and suggestions elicit new hypotheses for more empirical work on the factors that might contribute to (non)parallelism in well-researched and emerging study systems. Indeed, leveraging variation among evolutionary replicates within such systems will help provide substantial insight into the extent to which seemingly similar environments drive parallel patterns of phenotypic divergence.

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References

Arendt, J., & Reznick, D. (2008). Convergence and parallelism reconsidered: What have we learned about the genetics of adaptation? *Trends in Ecology & Evolution*, **23**, 26–32.

Arnold, S. J., Pfrender, M. E., & Jones, A. G. (2001). The adaptive landscape as a conceptual bridge between micro and macroevolution. *Genetica*, **112/113**, 9–32.

Barbosa, M., Deacon, A. E., Janeiro, M. J., Ramnarine, I., Morrissey, M. B., & Magurran, A. E. (2018). Individual variation in reproductive behaviour is linked to temporal heterogeneity in predation risk. *Proceedings of the Royal Society B*, **285**, 20171499.

Bassar, R. D., Lopez-Sepulcre, A., Reznick, D. N., & Travis, J. (2013). Experimental evidence for density-dependent regulation and selection on Trinidadian guppy life histories. *The American Naturalist*, **181**, 25–38.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.

Becher, S. A., & Magurran, A. E. (2000). Gene flow in Trinidadian guppies. *Journal of Fish Biology*, **56**, 241–249.

Blondel, L., Baillie, L., Quinton, J., Alemu, J. B., Paterson, I., Hendry, A. P., & Bentzen, P. (2019). Evidence for contemporary and historical gene flow between guppy populations in different watersheds, with a test for associations with adaptive traits. *Ecology and Evolution*, **9**, 4504–4517.

Blondel, L., Klemet-N'Guessan, S., Scott, M. E., & Hendry, A. P. (2020). Asymmetric isolation and the evolution of behaviors influencing dispersal: Rheotaxis of guppies above waterfalls. *Genes*, **11**, 180.

Blows, M. W., & Hoffmann, A. A. (2005). A reassessment of genetic limits to evolutionary change. *Ecology*, **86**, 1371–1384.

Bolnick, D. I., Barrett, R. D. H., Oke, K. B., Rennison, D. J., & Stuart, Y. E. (2018). (Non)parallel evolution. *Annual Review of Ecology, Evolution, and Systematics*, **49**, 303–330.

Bolnick, D. I., & Nosil, P. (2007). Natural selection in populations subject to a migration load. *Evolution*, **61**, 2229–2243.

Boughman, J. W., Rundle, H. D., & Schluter, D. (2005). Parallel evolution of sexual isolation in sticklebacks. *Evolution*, **59**, 361–373.

Broder, E. D., Ghalambor, C. K., Handelsman, C. A., Ruell, E. W., Reznick, D. N., & Angeloni, L. M. (2020). Rapid evolution and plasticity of genitalia. *Journal of Evolutionary Biology*, **33**, 1361–1370.

Brooks, R., & Endler, J. A. (2007). Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution*, **55**, 1002–1015.

Butler, M. A., Sawyer, S. A., & Losos, J. B. (2007). Sexual dimorphism and adaptive radiation in Anolis lizards. *Nature*, **447**, 202–205.

Button, K. S., Ioannidis, J. P. A., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. J., & Munafò, M. R. (2013). Power failure: Why small sample size undermines the reliability of neuroscience. *Nature Reviews Neuroscience*, **14**, 365–376.

Clarke, B. B. (1975). Contribution of ecological genetics to evolutionary theory: Detecting direct effects of natural-selection on particular polymorphic loci. *Genetics*, **79**, 101–113.

Clegg, S. M., Degnan, S. M., Kikkawa, J., Moritz, C., Estoup, A., & Owens, I. P. F. (2002). Genetic consequences of sequential founder events by an Island-colonizing bird. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 8127–8132.

Colosimo, P. F., Hosemann, K. E., Balabhadra, S., Villarreal, G., Jr., Grimwood, J., Schmutz, J., Myers, R. M., Schluter, D., & Kingsley, D. M. (2005). Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*, **307**, 1928–1933.

Conte, G. L., Arnegard, M. E., Peichel, C. L., & Schluter, D. (2012). The probability of genetic parallelism and convergence in natural populations. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 5039–5047.

Dargent, F., Rolshausen, G., Hendry, A. P., Scott, M. E., & Fussmann, G. F. (2016). Parting ways: Parasite release in nature leads to sex-specific evolution of defence. *Journal of Evolutionary Biology*, **29**, 23–34.

Deacon, A. E., Shimadzu, H., Dornelas, M., Ramnarine, I. W., & Magurran, A. E. (2015). From species to communities: The signature of recreational use on a tropical river ecosystem. *Ecology and Evolution*, **5**, 5561–5572.

Easty, L. K., Schwartz, A. K., Gordon, S. P., & Hendry, A. P. (2011). Does sexual selection evolve following introduction to new environments?. *Animal Behaviour*, **82**, 1085–1095.

Ehlman, S. M., Martinez, D., & Sih, A. (2018). Male guppies compensate for lost time when mating in turbid water. *Behavioral Ecology and Sociobiology*, **72**, 46.

Elmer, K. R., Fan, S., Kusche, H., Luise Spreitzer, M., Kautt, A. F., Franchini, P., & Meyer, A. (2014). Parallel evolution of Nicaraguan crater lake cichlid fishes via non-parallel routes. *Nature Communications*, **5**, 5168.

Endler, J. A. (1978). A predator's view of animal color patterns. In M. K. Hecht, W. C. Steere, & B. Wallace (Eds.), *Evolutionary biology*. Springer.

Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, **34**, 76–91.

Endler, J. A. (1995). Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution*, **10**, 22–29.

Endler, J. A., & Houde, A. E. (1995). Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, **49**, 456–468.

Fajen, A., & Breden, F. (1992). Mitochondrial DNA sequence variation among natural populations of the Trinidadian guppy, *Poecilia reticulata*. *Evolution*, **46**, 1457–1465.

Fitzpatrick, S. W., Torres-Dowdall, J., Reznick, D. N., Ghalambor, C. K., & Chris Funk, W. (2014). Parallelism isn't perfect: Could disease and flooding drive a life-history anomaly in Trinidadian guppies? *The American Naturalist*, **183**, 290–300.

Fraser, B. A., Künstner, A., Reznick, D. N., Dreyer, C., & Weigel, D. (2015). Population genomics of natural and experimental populations of guppies (*Poecilia reticulata*). *Molecular Ecology*, **24**, 389–408.

Fuller, R. C. (2022). Revisiting old truths: The evolution of male coloration in guppies as a function of predation. *Molecular Ecology*, **31**, 1333–1336.

Gamble, S., Lindholm, A. K., Endler, J. A., & Brooks, R. (2003). Environmental variation and the maintenance of polymorphism: The effect of ambient light spectrum on mating behaviour and sexual selection in guppies: Environmental variation and sexual selection. *Ecology Letters*, **6**, 463–472.

Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, **525**, 372–375.

Ghalambor, C. K., McKAY, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**, 394–407.

Gordon, S. P., López-Sepulcre, A., & Reznick, D. N. (2012). Predation-associated differences in sex linkage of wild guppy coloration. *Evolution*, **66**, 912–918.

Gordon, S. P., Reznick, D. N., Kinnison, M. T., Bryant, M. J., Weese, D. J., Räsänen, K., Millar, N. P., & Hendry, A. P. (2009). Adaptive changes in life history and survival following a new guppy introduction. *The American Naturalist*, **174**, 34–45.

Gotanda, K. M., Delaire, L. C., Raeymaekers, J. A. M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., Scott, M. E., Fussmann, G. F., & Hendry, A. P. (2013). Adding parasites to the guppy-predation story: Insights from field surveys. *Oecologia*, **172**, 155–166.

Gotanda, K. M., & Hendry, A. P. (2014). Using adaptive traits to consider potential consequences of temporal variation in selection: Male guppy colour through time and space: Spatiotemporal variation in guppy colour. *Biological Journal of the Linnean Society*, **112**, 108–122.

Grether, G. F., Millie, D. F., Bryant, M. J., Reznick, D. N., & Mayea, W. (2001). Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*, **82**, 1546–1559.

Hartig, F. (2022). *DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models*. https://cran.r-project.org/

Hendry, A. P. (2017). *Eco-evolutionary dynamics*. Princeton University Press.

Hendry, A. P., Kelly, M. L., Kinnison, M. T., & Reznick, D. N. (2006). Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *Journal of Evolutionary Biology*, **19**, 741–754.

Hendry, A. P., & Taylor, E. B. (2004). How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. *Evolution*, **58**, 2319–2331.

Houde, A. E. (1987). Mate choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution*, **41**, 1–10.

Houde, A. E., & Endler, J. A. (1990). Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science*, **248**, 1405–1408.

Hughes, K. A., Houde, A. E., Price, A. C., & Rodd, F. H. (2013). Mating advantage for rare males in wild guppy populations. *Nature*, **503**, 108–110.

Ioannou, C. C., Ramnarine, I. W., & Torney, C. J. (2017). High-predation habitats affect the social dynamics of collective exploration in a shoaling fish. *Science Advances*, **3**, e1602682.

Jacquin, L., Reader, S. M., Boniface, A., Mateluna, J., Patalas, I., Pérez-Jvostov, F., & Hendry, A. P. (2016). Parallel and nonparallel behavioural evolution in response to parasitism and predation in Trinidadian guppies. *Journal of Evolutionary Biology*, **29**, 1406–1422.

Jennions, M. D., & Møller, A. P. (2002). Relationships fade with time: A meta-analysis of temporal trends in publication in ecology and evolution. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, **269**, 43–48.

Jones, R., Culver, D. C., & Kane, T. C. (1992). Are parallel morphologies of cave organisms the result of similar selection pressures? *Evolution*, **46**, 353–365.

Kaeuffer, R., Peichel, C. L., Bolnick, D. I., & Hendry, A. P. (2012). Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback: Parallel and non-parallel evolution. *Evolution*, **66**, 402–418.

Kemp, D. J., Batistic, F., & Reznick, D. N. (2018). Predictable adaptive trajectories of sexual coloration in the wild: Evidence from replicate experimental guppy populations*. *Evolution*, **72**, 2462–2477.

Kemp, D. J., Reznick, D. N., Grether, G. F., & Endler, J. A. (2009). Predicting the direction of ornament evolution in Trinidadian guppies (*Poecilia reticulata*). *Proceedings of the Royal Society B: Biological Sciences*, **276**, 4335–4343.

Kingsolver, J. G., & Diamond, S. E. (2011). Phenotypic selection in natural populations: What limits directional selection? *The American Naturalist*, **177**, 346–357.

Kingsolver, J. G., Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hill, C. E., Hoang, A., Gibert, P., & Beerli, P. (2001). The strength of phenotypic selection in natural populations. *The American Naturalist*, **157**, 245–261.

Kodric-Brown, A. (1985). Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, **17**, 199–205.

Kolluru, G. R., & Grether, G. F. (2005). The effects of resource availability on alternative mating tactics in guppies (*Poecilia reticulata*). *Behavioral Ecology*, **16**, 294–300.

Landry, L., Vincent, W. F., & Bernatchez, L. (2007). Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape. *Journal of Evolutionary Biology*, **20**, 971–984.

Langerhans, R. B. (2018). Predictability and parallelism of multitrait adaptation. *Journal of Heredity*, **109**, 59–70.

Langerhans, R. B., & DeWitt, T. J. (2004). Shared and unique features of evolutionary diversification. *The American Naturalist*, **164**, 335–349.

Langerhans, R. B., & Riesch, R. (2013). Speciation by selection: A framework for understanding ecology's role in speciation. *Current Zoology*, **59**, 31–52.

Leinonen, T., McCairns, R. J. S., Herczeg, G., & Merilä, J. (2012). Multiple evolutionary pathways to decreased lateral plate coverage in freshwater threespine sticklebacks: Functional convergence in sticklebacks. *Evolution*, **66**, 3866–3875.

Liu, S., Ferchaud, A.-L., Grønkjaer, P., Nygaard, R., & Hansen, M. M. (2018). Genomic parallelism and lack thereof in contrasting systems of three-spined sticklebacks. *Molecular Ecology*, **27**, 4725–4743.

Losos, J. B. (2011). Convergence, adaptation, and constraint. *Evolution*, **65**, 1827–1840.

Low-Décarie, E., Chivers, C., & Granados, M. (2014). Rising complexity and falling explanatory power in ecology. *Frontiers in Ecology and the Environment*, **12**, 412–418.

Magurran, A. E. (2005). *Evolutionary ecology: The Trinidadian guppy*. Oxford University Press.

Magurran, A. E., & Seghers, B. H. (1994). Predator inspection behaviour covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. *Behaviour*, **128**, 121–134.

Magurran, A. E., Seghers, B. H., Carvalho, G. R., & Shaw, P. W. (1992). Behavioural consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad: Evidence for the evolution of anti-predator behaviour in the wild. *Proceedings of the Royal Society of London B: Bological Sciences*, **248**, 117–122.

McKellar, A. E., Turcotte, M. M., & Hendry, A. P. (2009). Environmental factors influencing adult sex ratio in Trinidadian guppies. *Oecologia*, **159**, 735–745.

Millar, N. P., & Hendry, A. P. (2012). Population divergence of private and non-private signals in wild guppies. *Environmental Biology of Fishes*, **94**, 513–525.

Millar, N. P., Reznick, D. N., Kinnison, M. T., & Hendry, A. P. (2006). Disentangling the selective factors that act on male colour in wild guppies. *Oikos*, **113**, 1–12.

Mitchell, D. J., Vega-Trejo, R., & Kotrschal, A. (2020). Experimental translocations to low predation lead to non-parallel increases in relative brain size. *Biology Letters*, **16**, 20190654.

Morales, H. E., Faria, R., Johannesson, K., Larsson, T., Panova, M., Westram, A. M., & Butlin, R. K. (2019). Genomic architecture of parallel ecological divergence: Beyond a single environmental contrast. *Science Advances*, **5**, eaav9963.

Oke, K. B., Bukhari, M., Kaeuffer, R., Rolshausen, G., Räsänen, K., Bolnick, D. I., Peichel, C. L., & Hendry, A. P. (2016). Does plasticity enhance or dampen phenotypic parallelism? A test with three lake–stream stickleback pairs. *Journal of Evolutionary Biology*, **29**, 126–143.

Oke, K. B., Cunningham, C. J., Quinn, T. P., & Hendry, A. P. (2019). Independent lineages in a common environment: The roles of determinism and contingency in shaping the migration timing of even- versus odd-year pink salmon over broad spatial and temporal scales. *Ecology Letters*, **22**, 1547–1556.

Oke, K. B., Motivans, E., Quinn, T. P., & Hendry, A. P. (2019). Sexual dimorphism modifies habitat-associated divergence: Evidence from beach and creek breeding sockeye salmon. *Journal of Evolutionary Biology*, **32**, 227–242.

Oke, K. B., Rolshausen, G., LeBlond, C., & Hendry, A. P. (2017). How parallel is parallel evolution? A comparative analysis in fishes. *The American Naturalist*, **190**, 1–16.

Olendorf, R., Rodd, F. H., Punzalan, D., Houde, A. E., Hurt, C., Reznick, D. N., & Hughes, K. A. (2006). Frequency-dependent survival in natural guppy populations. *Nature*, **441**, 633–636.

Phillip, D. A. T., & Ramnarine, I. W. (2001). *An illustrated guide to the freshwater fishes of Trinidad and Tobago*. Department of Life Sciences, The University of the West Indies.

R Development Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Reddon, A. R., Chouinard-Thuly, L., Leris, I., & Reader, S. M. (2018). Wild and laboratory exposure to cues of predation risk increases relative brain mass in male guppies. *Functional Ecology*, **32**, 1847–1856.

Reznick, D., Butler, M. J., & Rodd, H. (2001). Life-history evolution in guppies. VII. The comparative ecology of high- and low- predation environments. *The American Naturalist*, **157**, 126–140.

Reznick, D. N., Bassar, R. D., Travis, J., & Helen Rodd, F. (2012). Life-history evolution in guppies VIII: The demographics of density regulation in guppies (*Poecilia reticulata*). *Evolution*, **66**, 2903–2915.

Reznick, D. N., & Bryant, M. J. (2007). Comparative long-term mark-recapture studies of guppies (*Poecilia reticulata*): Differences among high and low predation localities in growth and survival. *Annales Zoologici Fennici*, **44**, 152–160.

Reznick, D. N., Butler, M. J., Rodd, F. H., & Ross, P. (1996). Life-history evolution in guppies *(Poecilia reticulata)* 6. Differential mortality as a mechanism for natural selection. *Evolution*, **50**, 1651–1660.

Reznick, D. N., & Endler, J. A. (1982). The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, **36**, 160.

Reznick, D. N., Rodd, F. H., & Cardenas, M. (1996). Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. *The American Naturalist*, **147**, 319–338.

Roberts Kingman, G. A., Vyas, D. N., Jones, F. C., Brady, S. D., Chen, H. I., Reid, K., Milhaven, M., Bertino, T. S., Aguirre, W. E., Heins, D. C., von Hippel, F. A., Park, P. J., Kirch, M., Absher, D. M., Myers, R. M., di Palma, F., Bell, M. A., Kingsley, D. M., & Veeramah, K. R. (2021). Predicting future from past: The genomic basis of recurrent and rapid stickleback evolution. *Science Advances*, **7**, eabg5285.

Rohatgi, A. (2020). *WebPlotDigitizer*. http://arohatgi.info/WebPlotDigitizer

Santi, F., Petry, A. C., Plath, M., & Riesch, R. (2020). Phenotypic differentiation in a heterogeneous environment: Morphological and life-history responses to ecological gradients in a livebearing fish. *Journal of Zoology*, **310**, 10–23.

Schories, S., Meyer, M. K., & Schartl, M. (2009). Description of *Poecilia (Acanthophacelus) obscura* n. sp., (Teleostei: Poeciliidae), a new guppy species from western Trinidad, with remarks on P. wingei and the status of the Endler's guppy. *Zootaxa*, **2266**, 35–50.

Schwartz, A., & Hendry, A. P. (2007). A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies. *Evolutionary Ecology Research*, **9**, 71–90.

Seghers, B. H. (1974). Schooling behavior in the guppy (*Poecilia reticulata*): An evolutionary response to predation. *Evolution*, **28**, 486–489.

Shaw, P. W., Carvalho, G. R., Magurran, A. E., & Seghers, B. H. (1991). Population differentiation in Trinidadian guppies (*Poecilia reticulata*): Patterns and problems. *Journal of Fish Biology*, **39**, 203–209.

Siepielski, A. M., Gotanda, K. M., Morrissey, M. B., Diamond, S. E., DiBattista, J. D., & Carlson, S. M. (2013). The spatial patterns of directional phenotypic selection. *Ecology Letters*, **16**, 1382–1392.

Stephenson, J. F., van Oosterhout, C., Mohammed, R. S., & Cable, J. (2015). Parasites of Trinidadian guppies: Evidence for sex- and age-specific trait-mediated indirect effects of predators. *Ecology*, **96**, 489–498.

Stuart, Y. E., Veen, T., Weber, J. N., Hanson, D., Ravinet, M., Lohman, B. K., Thompson, C. J., Tasneem, T., Doggett, A., Izen, R., Ahmed, N., Barrett, R. D. H., Hendry, A. P., Peichel, C. L., & Bolnick, D. I. (2017). Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nature Ecology and Evolution*, **1**, e0158.

Suk, H. Y., & Neff, B. D. (2009). Microsatellite genetic differentiation among populations of the Trinidadian guppy. *Heredity*, **102**, 425–434.

Szendro, I. G., Franke, J., de Visser, J. A. G. M., & Krug, J. (2013). Predictability of evolution depends nonmonotonically on population size. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 571–576.

Thompson, C. J., Ahmed, N. I., Veen, T., Peichel, C. L., Hendry, A. P., Bolnick, D. I., & Stuart, Y. E. (2017). Many-to-one form-to-function mapping weakens parallel morphological evolution. *Evolution*, **71**, 2738–2749.

Tobler, M., Schlupp, I., Heubel, K. U., Riesch, R., de León, F. J. G., Giere, O., & Plath, M. (2006). Life on the edge: Hydrogen sulfide and the fish communities of a Mexican cave and surrounding waters. *Extremophiles*, **10**, 577–585.

Torres Dowdall, J., Handelsman, C. A., Ruell, E. W., Auer, S. K., Reznick, D. N., & Ghalambor, C. K. (2012). Fine-scale local adaptation in life histories along a continuous environmental gradient in Trinidadian guppies: Fine-scale local adaptation in guppies. *Functional Ecology*, **26**, 616–627.

Vinterstare, J., Ekelund Ugge, G. M. O., Hulthén, K., Hegg, A., Brönmark, C., Nilsson, P. A., Zellmer, U. R., Lee, M., Pärssinen, V., Sha, Y., Björnerås, C., Zhang, H., Gollnisch, R., Herzog, S. D., Hansson, L. A., Škerlep, M., Hu, N., Johansson, E., & Langerhans, R. B. (2021). Predation risk and the evolution of a vertebrate stress response: Parallel evolution of stress reactivity and sexual dimorphism. *Journal of Evolutionary Biology*, **34**, 1554–1567.

Wainwright, P. C. (2005). Many-to-one mapping of form to function: A general principle in organismal design? *Integrative and Comparative Biology*, **45**, 256–262.

Weese, D. J., Gordon, S. P., Hendry, A. P., & Kinnison, M. T. (2010). Spatiotemporal variation in linear natural selection on body color in wild guppies (*Poecilia reticulata*). *Evolution*, **64**, 1802–1815.

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis* (2nd ed.). Springer International Publishing.

Yong, L., Croft, D. P., Troscianko, J., Ramnarine, I. W., & Wilson, A. J. (2022). Sensory-based quantification of male colour patterns in Trinidadian guppies reveals no support for parallel phenotypic evolution in multivariate trait space. *Molecular Ecology*, **31**, 1337–1357.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2011). *Mixed effects models and extensions in ecology with R*. Springer.

Tables

Table 1. Structure of, and data that informed, each of the factor-specific question models.

Model name	Model (dependent variable ~ explanatory variable)	Categories excluded from dataset (term: category)	Reason for exclusion
Trait type	R^2 ~ Trait Type	Trait type: Other	Low sample size
Sex	1) R^2 ~ Sex	1) Trait type: Colour	1) Traits only reported for one sex, expected to impact R^2
	2) R^2 ~ Sex	2) NA	2) NA
Rearing environment	R^2 ~ Rearing environment	Rearing environment: Common Garden (F1)	Low sample size
Trait type and Sex	R^2 ~ Sex + Trait type	NA	NA
Sex and Rearing environment	R^2 ~ Sex + Rearing Environment	Rearing environment: Common Garden (F1)	Low sample size
Ecological complexity	R^2 ~ Slope*Sex	NA	NA

Evolutionary history	R^2 ~ Drainage*Sex	NA	NA
Time since colonization	R^2 ~ Introduction history	NA	NA

*All models include study as a random intercept term.

Table 2. Summary of parallelism (R^2 – the proportion of variance among site means explained by predation regime for a given trait in a given sex in a given study), (A) overall and for each of the (B-D) within-population and (E-G) among-population factors.

	Factor		Mean R^2	SD
A	Overall		0.246	0.261
B	Sex	Females	0.310	0.275
		Males	0.206 (with colour)	0.243 (with colour)
			0.275 (without colour)	0.269 (without colour)
C	Trait type	Colour	0.102	0.146
		Behaviour	0.284	0.284
		Life history	0.387	0.286
		Size	0.309	0.257
		Other morphology	0.335	0.288
		Physiology	0.179	0.178
		Other	0.760	0.967
D	Rearing environment	Common garden (F2)	0.351	0.280
		Wild-caught	0.227	0.253
E	Ecological complexity	Within one slope	0.197	0.210

		Between both slopes	0.153	0.196
F	Evolutionary history	Within one drainage	0.250	0.248
		Between both drainages	0.181	0.193
G	Time since colonization	Only natural	0.186	0.213
		Natural and introduced	0.151	0.191

Figures

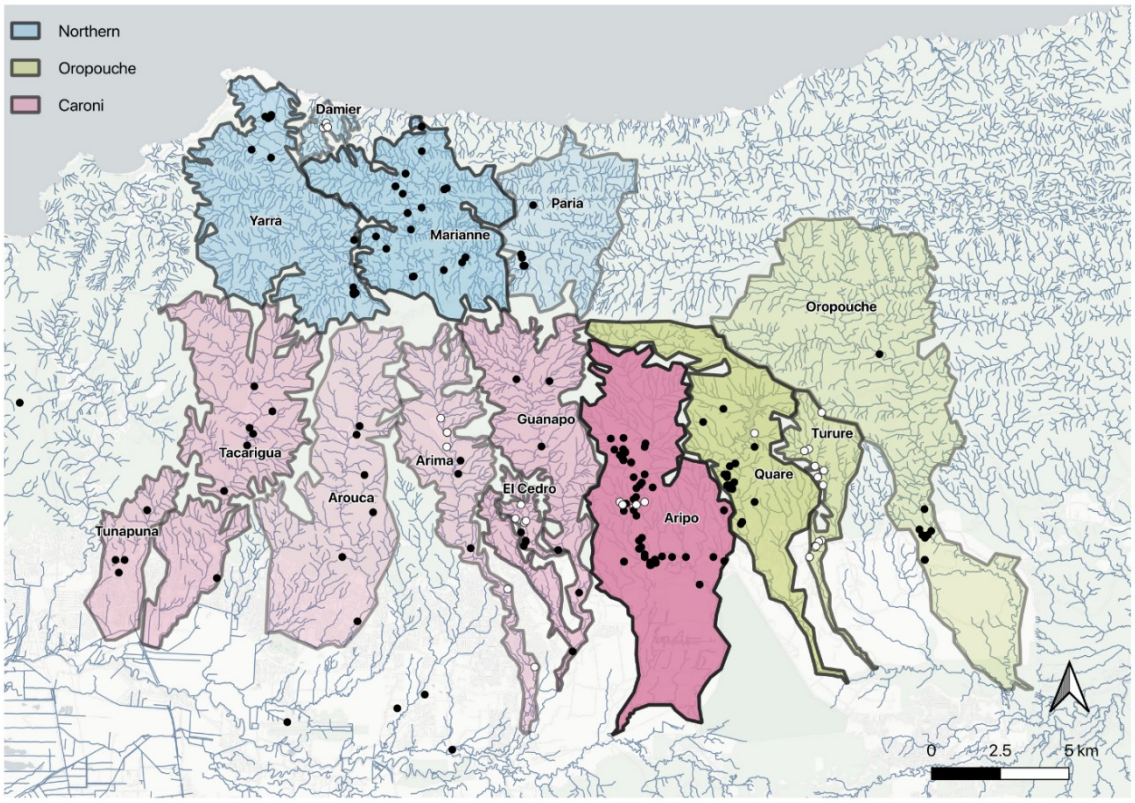

Figure 1. Map of the Northern Mountain Range of Trinidad, coded to illustrate how we considered ecological complexity (within one slope: southern; or between both slopes: southern and northern), evolutionary history (within one drainage: Caroni; or between both drainages: Caroni and Oropouche), and time since colonization (with only natural populations or with both natural and introduced populations). Data points (n = 205) represent the sites that were sampled where GPS coordinates were provided by the original authors; black circles are natural sites, and white circles are introduction sites. Individual rivers are outlined, and the opacity of the river colour represents the number of studies that were conducted in that river, with more highly studied rivers being darker. The river shading includes all of the sites in our dataset, including those where GPS coordinates were not provided.

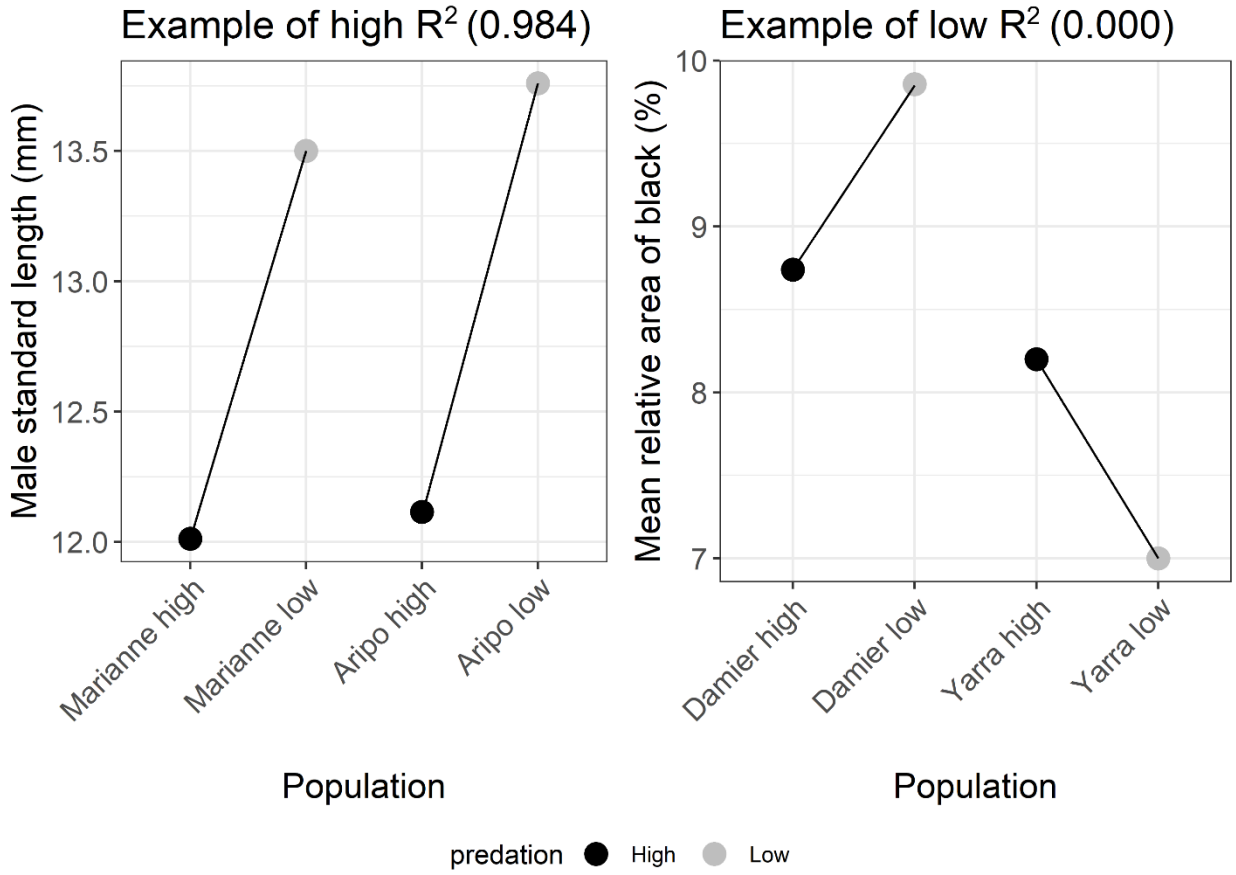

Figure 2. Example of traits from our dataset that are (A) highly parallel and (B) highly nonparallel. The data for this figure were extracted from (A) Reddon et al. (2018) and (B) Easty et al. (2011).

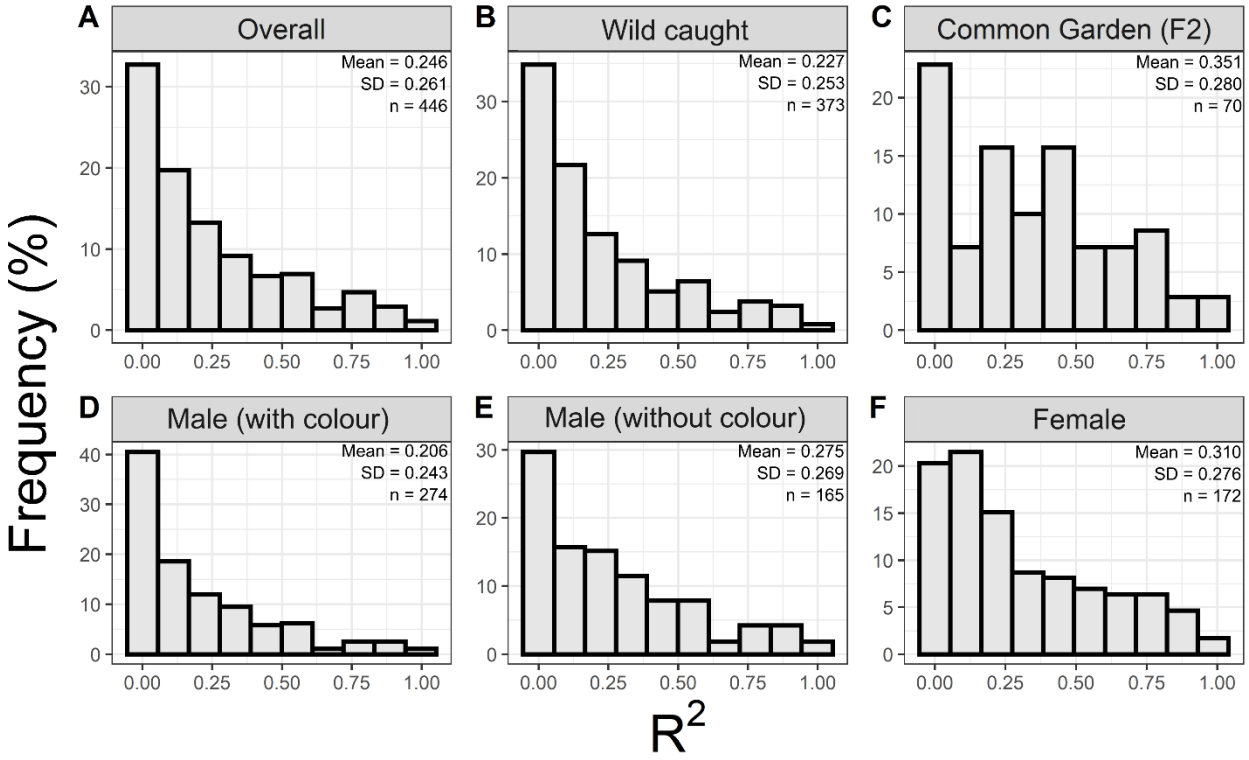

Figure 3. Frequency distributions (in %) of parallelism (R^2 – the proportion of variance among site means explained by predation regime for a given trait in a given sex in a given study) in guppies (A) overall, (B,C) in the two different rearing environments, and in the (D-F) two sexes. For males, distributions are shown with 'Colour' traits (D) included, and (E) excluded.

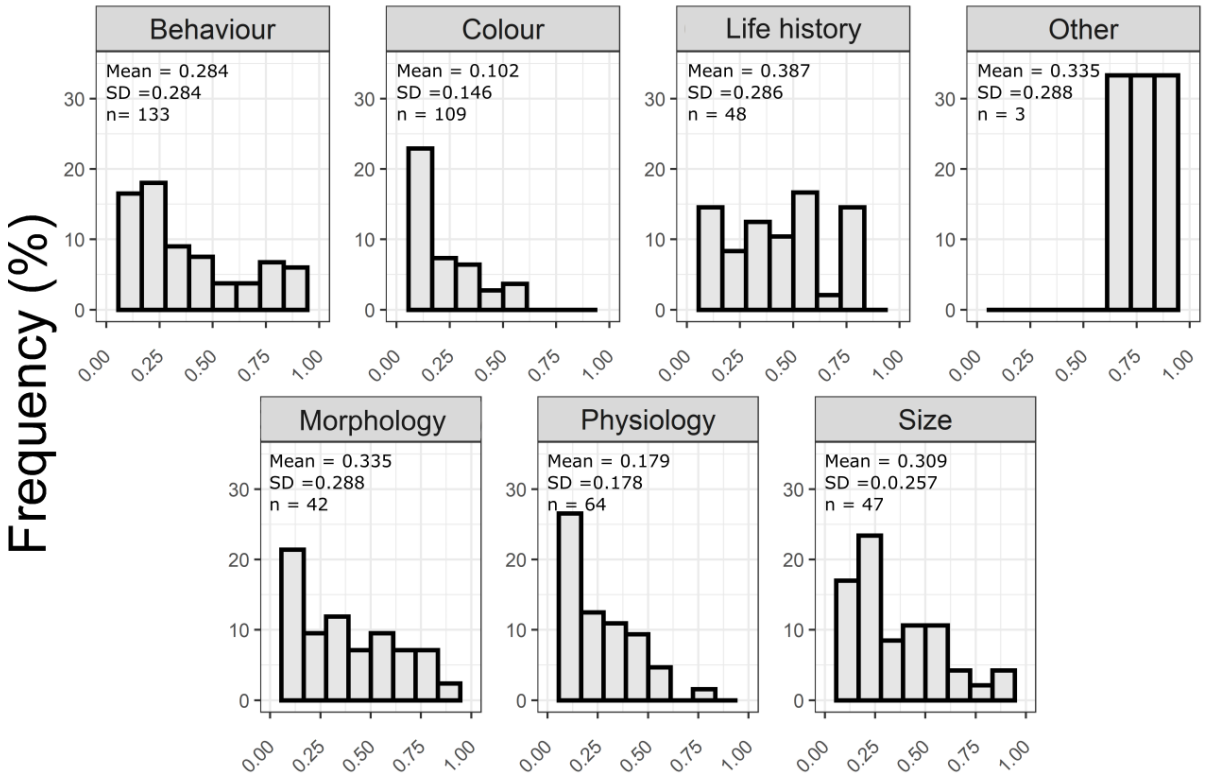

Figure 4. Frequency distributions (in %) of parallelism (R^2 – the proportion of variance among site means explained by predation regime for a given trait in a given sex in a given study) in guppies for each trait type.

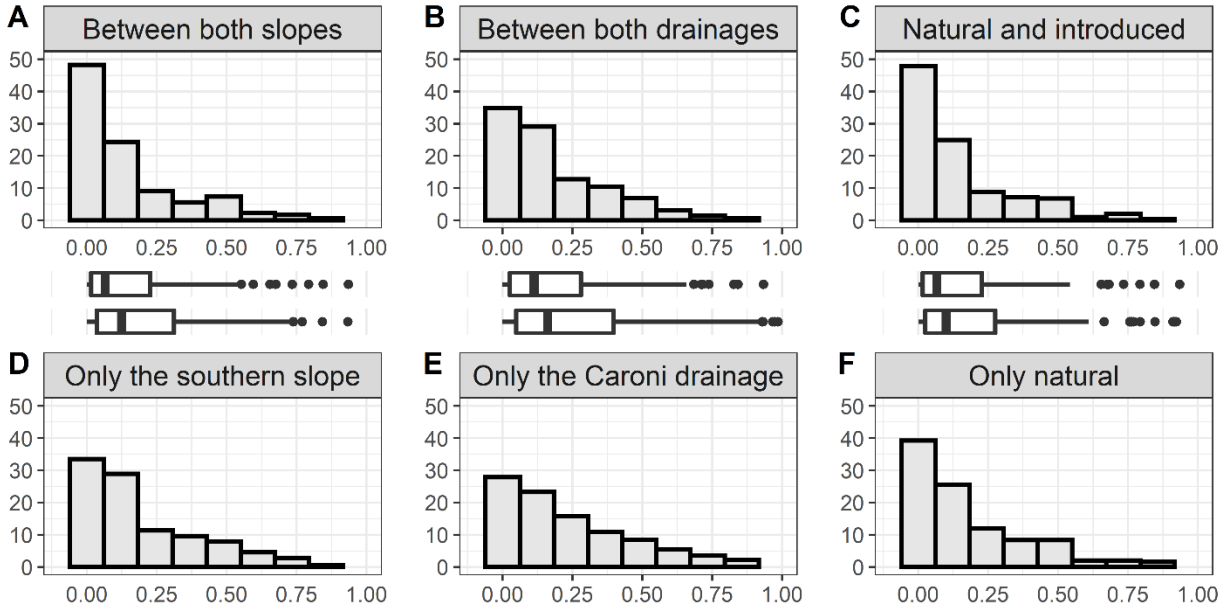

Figure 5. Frequency distributions (in %) of parallelism (R^2 – the proportion of variance among site means explained by predation regime for a given trait in a given sex in a given study) for (A, D) ecological complexity, (B, E) evolutionary history, and (C, F) time since colonization. For panels on the top row (A, B, C), the corresponding boxplot is directly beneath, and for panels on the bottom row (D, E, F) the corresponding box plot is above. The vertical line through the box plots denotes the median.

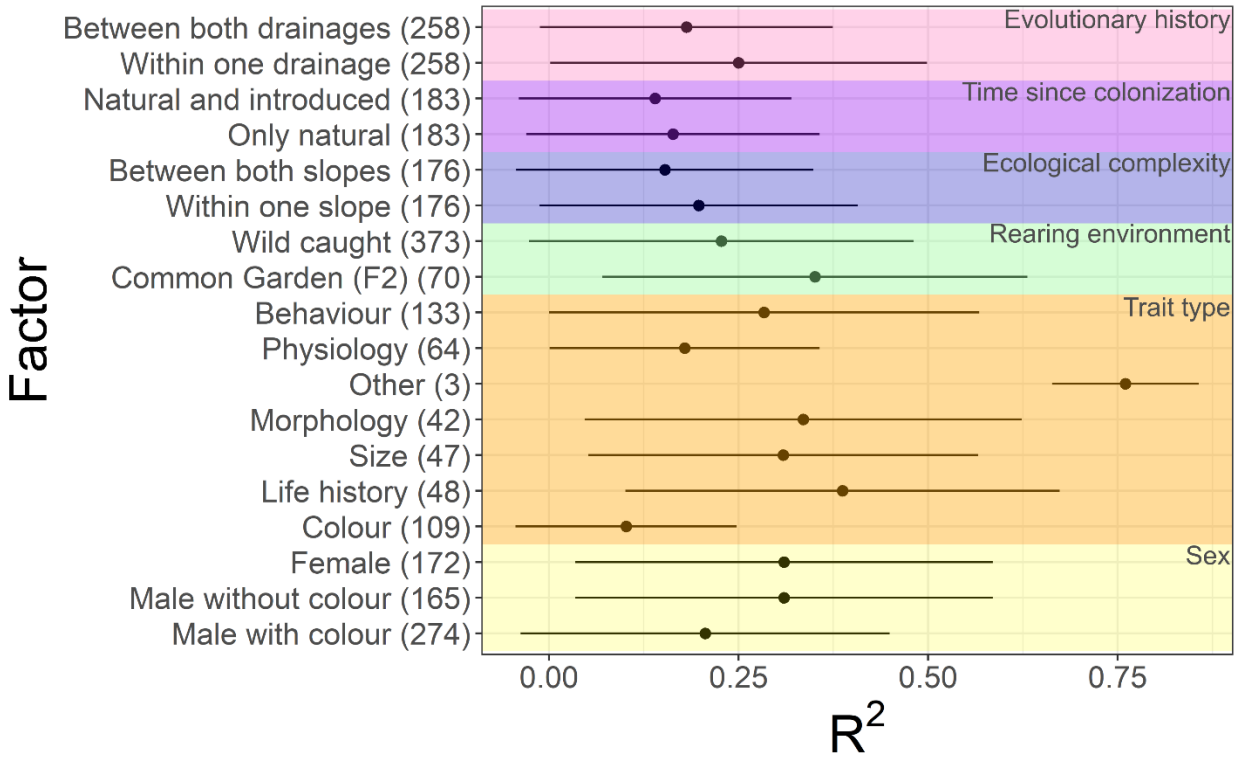

Figure 6. The mean R^2 value for each category within each of the factor-specific questions. Each question is shown in a different colour block. The values in parentheses denote the sample sizes for each group. The question being asked by these different factors is shown on the top right of each colour block.

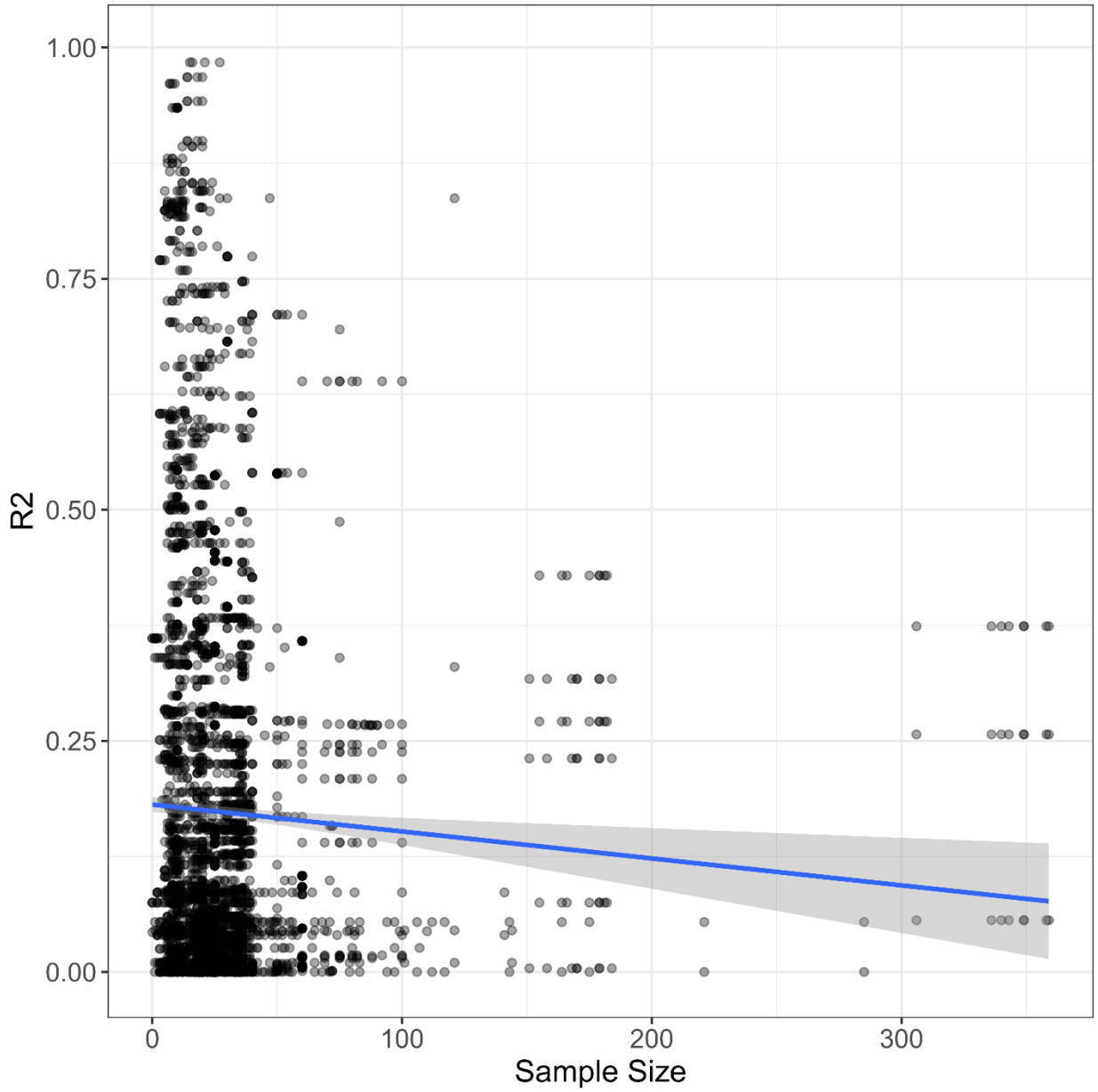

Figure 7. The relationship between sample size and R^2 value. Each point represents a trait in our dataset, and are semi-transparent so that overlapping points are visible. The line is a linear regression fit by ggplot2 (Wickham, 2016) and the grey shading is the standard error.

Bridging statement 1

In Chapter 1, I quantified the extent to which predation drives parallel evolution among guppy population-level trait means. I found that colour – despite being one of the most famous examples of parallel evolution in guppies – seemed to be especially weakly parallel, and that increasing complexity of evolutionary history decreased estimates of parallelism. I also notably found that, although 25% of the variance in guppy population-level trait means could be explained by predation overall, the vast majority of variance was **not** explained by predation regime. This finding raises the question of what other factors within the environment, beyond predation, are contributing to guppy phenotypes? This question served as the motivation for my second chapter.

In Chapter 2, I focus my efforts within a single level of the predation regime (only low predation sites), to investigate how other sources of environmental variation might be shaping guppy phenotypes, and thus could be reducing predictability of the predation regime dichotomy. Specifically, I focus on how aspects of the abiotic environment, which are overlooked relative to biotic factors in the environment, shape guppy behavioural phenotypes. Behavioural phenotypes represent a good candidate set of phenotypes for this line of inquiry, because my results from Chapter 1 show that parallelism for behavioural phenotypes is highly variable and is not exceptionally highly or weakly parallel.

Chapter 2: Abiotic environmental factors contribute to spatial variation in boldness and exploration in guppies (*Poecilia reticulata*)

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Abstract

Research with wild fish has generally overlooked the role of the abiotic environment in shaping bold and exploratory behaviours. This oversight could exist because it might be assumed that small-scale variations in the abiotic environment are unlikely to affect these behaviours. We challenged this assumption using the Trinidadian guppy system. Specifically, we investigated how bold behaviour (our measure of boldness was time spent under a shelter and seconds frozen in an open field) and exploratory behaviour (number of grid squares crossed in an open field) vary within and among guppies collected from 15 pools distributed across two streams, where, within a stream, all pools are within < 150 m. We next assessed how individual level attributes (sex, mass) and abiotic environmental factors (e.g., temperature, specific conductance) contribute to this variation. Bold and exploratory behaviours were mostly associated with the pool, rather than the stream, from which guppies were collected, highlighting that environments can shape these behaviours over fine spatial scales. Small guppies displayed slightly bolder behaviour, but small effect sizes indicate that individual-level attributes were not important contributors to this variation. Many abiotic factors had higher effect sizes, demonstrating their importance for shaping pool-level behaviour. Individuals from pools with higher specific conductance displayed bolder behaviour, and individuals from pools with less dissolved oxygen also displayed bolder behaviour and possibly explored more. Our results highlight the importance of abiotic

environmental factors, often-ignored aspects of the environment, for shaping these behaviours even at fine spatial scales, ultimately improving our understanding of how behavioural variation emerges in natural populations, with implications for other fishes and taxa more broadly.

Introduction

With the recognition that intraspecific phenotypic variation can have strong ecological and evolutionary effects (Des Roches *et al.*, 2018), researchers have been increasingly interested in understanding the causes and consequences of among-individual variation. A large proportion of research investigating intraspecific variation has focused on behaviour; with two behaviours, boldness (risk taking) and exploration (movement in a novel environment) (Réale *et al.*, 2007), having received particular attention (e.g., Brown *et al.*, 2007; Chapman *et al.*, 2011; Reader, 2015). These behaviours can affect ecological processes ranging from altered acquisition of information and resources (e.g., Patrick *et al.*, 2017) to success in mating interactions (e.g., Ariyomo & Watt, 2013; McCowan *et al.*, 2014) and survival (e.g., Ballew *et al.*, 2017; Rödel *et al.*, 2015). Among individuals, boldness and exploration can be highly variable, particularly among individuals from different populations (e.g., Magnhagen *et al.*, 2012; Rudh *et al.*, 2013). Investigating how bold and exploratory behaviours are associated with factors that differ within and among populations can provide insight into the causes of these ecologically and evolutionarily important behaviours.

Nearly all work investigating the causes of variation in bold and exploratory behaviours has focused on linking these behaviours to individual-level attributes (e.g., sex, size, or individual behavioural type - i.e., each individual's average behavioural trait value), or biotic

components of the environment experienced by individuals, such as the number and type of conspecifics or heterospecifics (e.g., Archard & Braithwaite, 2011; Brown & Braithwaite, 2004; Burns *et al.*, 2016; Harris *et al.*, 2010; Ingley *et al.*, 2014; Magnhagen *et al.*, 2012; Moran *et al.*, 2016; Piyapong *et al.*, 2010; Rasmussen & Belk, 2017; Ward *et al.*, 2007). The many studies of such effects suggest that individual-level attributes and biotic environmental factors can strongly shape bold and exploratory behaviours within and among natural populations, yet there usually remains considerable unexplained behavioural differences among populations in similar biotic environments (e.g., Bell *et al.*, 2010; Magurran & Seghers, 1994). For instance, Trinidadian guppies (*Poecilia reticulata*) collected from sites with similar predation and parasitism regimes show substantial between-river behavioural variation (including in boldness), indicating that other factors, beyond these biotic components, are important contributors to guppy behaviour (Jacquin *et al.*, 2016).

A few studies have analysed associations between abiotic factors and boldness and exploratory behaviours, and have found strong effects (e.g., temperature, sulfide, dissolved oxygen, water flow velocity; Culumber, 2020; Riesch *et al.*, 2009; Sommer-Trembo *et al.*, 2017; Tang & Fu, 2021). Abiotic environmental factors thus could contribute to unexplained variation within biotically similar environments. Such abiotic factors vary at small (e.g., two pools separated by a shallow riffle within a stream) and large spatial scales (e.g., two different streams), but studies in nature typically focus on the associations between abiotic factors and behaviours on larger scales only (e.g., among populations: Sommer-Trembo *et al.*, 2017; Tang & Fu, 2021), or deliberately exaggerate abiotic factors to exceed the conditions of typical, undisturbed environments (e.g., thermal or salinity stress; Culumber, 2020; Leite *et al.*, 2019, 2022). Understanding effects that abiotic variations at small spatial scales have on behaviour,

and therefore the extent to which these factors might contribute to unexplained behavioural variation within biotically similar environments, requires contrasting the effects of these 'thought-to-be-unimportant' sources of variation with those of 'known-to-be-important' sources of variation, such as individual-level attributes. This objective can be difficult to achieve in nature, because it requires linking abiotic and individual-level factors to individual behaviour measured at multiple spatial scales.

Our study

In the present study, we measure bold and exploratory behaviours with Trinidadian guppies *Poecilia reticulata* (Peters 1859) collected from fifteen pools (i.e., microhabitats with seasonally restricted gene flow) distributed across two streams. Within a given stream, the pools are separated by as little as 0.75 m and by no more than < 150 m. The two streams are within the same watershed, and so are geographically close but are genetically distinct and thus can be considered separate populations (Blondel *et al.*, 2019) (Figure 1).

We first establish how bold and exploratory behaviour vary at three spatial "scales". First, we investigate variation among individuals within a given pool. Second, we investigate variation among pools within a given stream. Third, we investigate variation between the two streams. We next examine different factors that can contribute to behavioural variation at these spatial scales. Within pools we focus on sex and size, two individual-level attributes that could contribute to behavioural variation among individuals. Among pools we focus on several abiotic environmental factors, such as dissolved oxygen (mg/L) and temperature (°C), that we expected could be associated with behaviour (e.g., Culumber, 2020; Sommer-Trembo *et al.*, 2017). To

focus on biotically similar environments, we selected only "low-predation" guppy populations, where the main predator is the gape-limited *Anablepsoides hartii* that only eats small guppies (Endler, 1978; Seghers, 1973), that also had very low levels of the most studied guppy parasite, *Gyrodactylus* spp.

To quantify variation in bold and exploratory behaviours, we used an open field test, a well-established assay for quantifying variation in these behaviours in guppies (Burns, 2008). Whereas many studies that investigate these behaviours do so with the intention of quantifying repeatable behavioural differences (aka 'personalities' or 'temperament'; Réale *et al.*, 2007), our goal is not to emphasize personality. Rather, our proxies of bold and exploratory behaviours likely include effects of among-individual behavioural differences, as well as within-individual plastic responses to the environment from which they were collected and the experimental process (e.g., stress and handling). Although repeatability of these behaviours is not an emphasis of our study, it is perhaps worthwhile noting they are often repeatable when measured at multiple times in other studies (Table S1).

Materials and methods

Pool selection and ecological data collection

Our study focused on a total of 15 pools selected across two streams ("Stream One": n = 7; "Stream Two": n = 8) in the Marianne River in Trinidad (Figure 1). These two streams represent distinct genetic clusters (Blondel *et al.*, 2019), although they are still part of the same river. Our work was conducted during low-water/dry-season (February 2020), which ensured that the pools were mostly or entirely isolated from each other. Before collecting guppies, we recorded pool-

level ecological variables that could affect their behaviour. First, for each pool we used a tape measure or marked PVC pipe to measure the maximum width (m), maximum length (m), maximum depth (m), and mean depth (m) – the last of these measures was obtained by taking depth measurements every 50 cm along the longest and widest sections of the pool. These linear dimensions were used to then estimate the surface area (length x width in m^2) and volume (surface area x mean depth in m^3) of each pool. Second, we used a Yellow Strings Instrument (YSI) probe (model 10,102,030; Yellow Springs Inc., Yellow Springs, Ohio, USA) to record pH, temperature (°C), and dissolved oxygen concentration (mg/L) of the water. We also recorded specific conductance (μS/cm) with the YSI, which provides conductivity standardized by temperature. Finally, we used a concave spherical densitometer to record canopy cover above each pool.

We returned to the pools 72 hours after the above physical and ecological variables were measured to collect guppies. Using butterfly-nets, we attempted to capture all guppies from each pool, which is usually possible because capture rates are extremely high in small pools in the dry season – as shown by numerous mark-recapture studies (e.g., Bryant & Reznick, 2004; Reznick *et al.*, 1996; Weese *et al.*, 2010). To ensure a high capture rate in our study, we waited for at least two minutes following the last captured guppy. If another guppy was spotted, it was captured and the two-minute timer was reset. This process was repeated until no more guppies were seen. Although it is possible that we failed to capture some resident guppies, we at least captured a very high proportion of them – yielding a total of 303 adult guppies (Stream One: n = 126; Stream Two: n = 177) (see Table S2 pool-specific sample sizes).

On the same day that guppies were collected (Stream One: February 17th, 2020; Stream two: February 18th, 2020), all the fish were transported in 2 L cleaned plastic bottles by car to the William Beebe Tropical Research Station in the Arima Valley, where they were housed in pool-specific aquaria. While in the laboratory, guppies were fed fish flakes (TetraMin Tropical Flakes) daily, the aquarium water was treated with API Stresscoat and API Quick Start, and water changes were conducted every few days. The guppies were held under these conditions for at least two days, with a maximum holding time of 18 days, prior to being used in the behavioural assays. The length of time varied in this manner owing to the total processing time needed for all fish before subsequent simultaneous release back into the stream.

Behavioural assays

The behavioural assays were conducted in 72 L aquaria (30.5 cm W, 38.5 cm D, 61 cm L) with a 5x5 cm grid drawn across the bottom. Red-brown and yellow aquarium gravel was lightly spread across the bottom to approximate some natural substrate colours in Trinidad streams. The water was maintained at 6 cm deep and was changed twice per day: before the first trial and after approximately half the trials were completed. Tanks were illuminated with both natural light from a large window and indoor lighting at the field station. An artificial plant was placed in the corner of each aquarium to provide an opportunity for the fish to seek refuge during the trial.

Behaviours (details below) were scored live by an observer. An opaque plastic blue sheet was placed between the tank and the body of the observer to minimize disturbance to the fish while allowing the observer to still see the fish from above the sheet. Assays were conducted between sunrise and sunset to correspond to the diurnal activity period.

For each trial, an individual fish was placed in the experimental arena inside a clear plastic holding container, which was constructed by gluing two clear mini food storage containers together. The holding container was placed in the corner of the aquarium on the same short side as the plant-refuge (the refuge was in one corner, and the holding container was placed in the other corner). The fish were left in the holding cylinder for a 3-minute acclimation period, after which the holding cylinder was gently lifted by hand by the observer and the trial immediately began. Each individual trial then ran for 5 minutes, during which the four behaviours were recorded. Our measures of bold behaviour were refuge use (time, in seconds, that the fish spent in an artificial plant refuge), freezing instances (number of instances where the fish was moving and then stopped), and total freezing time (time, in seconds, that the fish was not moving). Our measure of exploratory behaviour was number of squares crossed in a novel open field. These proxies were chosen because previous studies confirmed their use for studying variation in bold and exploratory behaviours (e.g., Carlson & Langkilde, 2013; Diaz Pauli *et al.*, 2019). We used the behaviour software BORIS v 7.9.7 (Friard & Gamba, 2016) to live-record the time spent in the refuge, the total freezing time, the number of freezing instances, and the number of grid squares crossed. Upon completing the behavioural trials, the fish were lightly anaesthetized using tricaine methanesulfonate (MS-222) and visually scanned for ectoparasites (we did not find any, although other studies have reported *Gyrodactylus* in the Marianne; Gotanda *et al.*, 2013) under a Zeiss dissecting microscope. Sex and mass (g) were recorded. Only data for adult guppies are included in the present manuscript.

To determine the validity of our behavioural proxies, we used the ‘factoextra’ package v 1.0.7 in the R environment (Kassambara & Mundt, 2020; R Core Team, 2024 v 4.3.0) to conduct a principal component analysis (PCA) on a correlation matrix of the behavioural data and the

four behavioural proxies (refuge use, number of freezing instances, total freezing time, and number of squares crossed). The first principal component (PC1) explained 41% of the total variance and was associated with refuge use (38% contribution to PC1), number of freezing instances (31% contribution to PC1), and total freezing time (31% contribution to PC1) indicating that this PC consisted of boldness behaviour. PC2 explained 35% of the total variance and was mostly associated with the number of squares crossed (64% contribution to PC2) (Figure S1) indicating that this PC consisted of exploratory behaviour.

In the following analyses, rather than use the two PCs, we instead used the raw behavioural scores as proxies for bold behaviour and exploratory behaviour. As a proxy for bold behaviour, we created a new variable that represented the sum of the time spent frozen in the open field and the time spent underneath the refuge. This new variable was necessary because these two behavioural measures are not independent. For instance, if a fish spent most of the trial under the refuge, indicative of a shyer behaviour, that same individual would likely have a lower time frozen score (because they are spending less time in the open field for that behaviour to be observed), indicative of a bolder behaviour. As a proxy for exploratory behaviour, we used number of squares crossed in the open field. We selected these behaviours as they were easily transformed to satisfy the assumptions of linear models (see below), whereas the PCs were not easily transformed to meet assumptions of linearity, and their use in the models could therefore risk inflating Type 1 errors. The Pearson correlation between these two behavioural proxies was $r = 0.67$.

Statistical analysis

We started our investigation of the spatial structure of bold behaviour (the sum of time spent frozen in the open field and under the plant refuge) and exploratory behaviour (number of squares crossed in the open field) by using nested ANOVA (SPSS v 29.0.0.0) to partition the total variation in bold and exploratory behaviours among stream and pool nested within stream. Estimates of the partial eta squared (proportion of the total variance; η_p^2) explained by each random effect allow us to answer how bold and exploratory behaviours are structured at these scales. We next investigated how the spatial structure of bold and exploratory behaviours might be explained by the individual-level and pool-level variables.

Individual-level and pool-level variables were analyzed with linear mixed-effects models (LMMs) using the 'lme4' R package (Bates *et al.*, 2015), with either bold or exploratory behaviours as the dependent variables. Fixed effects in these models included sex and mass, as well as the independent effects of several ecological variables (outlined below). We also included time of day (am/pm) of behavioural assay as a covariate to account for variation in behaviour that could emerge throughout the day – as time of day can affect some guppy behaviours (e.g., O'Neill *et al.*, 2019). We included random effects for pool nested within stream to account for non-independent spatial structuring. Initially, because male and female guppies differ in size, we included a sex-by-mass interaction; however, the bold behaviour model did not converge with this interaction included, and the interaction was not significant in the exploratory behaviour model ($\chi^2 = 0.76$, df = 1, $p = 0.38$) so it was removed from both models to improve model fit (we instead considered the independent effects of sex and mass) (Engqvist, 2005). The abiotic environmental variables in the models were dissolved oxygen (mg/L), specific conductance (μS/cm), surface area (m^2), temperature (°C), canopy cover (squares covered on

densiometer), and pool volume (m^3). We excluded mean depth (m) and pH owing to high correlations (i.e., $r > 0.8$) with volume ($r = 0.93$) and specific conductance ($r = 0.83$), respectively (Table S3). We also excluded maximum depth (m) because its inclusion resulted in an estimate of zero variance for the pool level random effect (i.e., effectively only considering the effect of stream). All continuous predictors (in these models and those described below) were standardized via conversion to z-scores prior to their inclusion in the models. Model diagnostics were assessed using the 'DHARMa' R package (Hartig, 2022). Bold behaviour was cube-root transformed and exploratory behaviour was square-root transformed to meet assumptions of linearity. No other assumptions were violated. Using the 'car' R package, we calculated p-values for each LMM with a type II sum of squares.

To calculate effect sizes for sex and mass, we used the 'r2glmm' package (Jaeger, 2017) to extract semi-partial R^2 values for each fixed effect from the LMMs. Much like partial eta-squares that come from ANOVAs or linear models (LMs), semi-partial R^2 estimates the variance explained for each predictor in LMMs (Jaeger *et al.*, 2017), providing insight into the amount of variation in the response variable (i.e., behaviour) that is explained by the fixed effects (i.e., individual-level attributes).

We used a different approach to calculate effect sizes for the ecological variables. Because the level at which we collected our ecological data (i.e., the pool level) differs from the level at which we collected our behavioural data (i.e., the individual level), calculating R^2 for ecological variables using the LMMs above generates very low effect sizes (see the supplementary materials for effect size estimates and plots built using the LMM results; figures S1-S2). Indeed, a single value as an independent variable cannot explain variation in the

dependent variable; to have assessed R^2 values from the LMMs in a biologically meaningful way would have required finer-scale sampling to collect micro-environmental data for each individual. Therefore, rather than assess how much of the variation in *individual* behaviour can be explained by the fixed effects (as above), we instead assess how much of the variation in *pool-level* behaviour can be explained by the fixed effects. To calculate these effect sizes, we built two linear models (LMs). The response variable in these models was the average score for bold or exploratory behaviour for a given pool (n = 15). The fixed effects were the independent effects of all abiotic environmental variables included in the above models. To control for between-stream variation, we also included stream as an additional co-variate in these models. We extracted partial eta-squared using the 'effectsize' R package with a type 2 sum of squares (Ben-Shachar *et al.*, 2020). Exploratory behaviour was log transformed to meet assumptions of linearity.

Although sex and mass are individual-level attributes, and estimating R^2 from the LMMs is therefore appropriate, we conducted a sensitivity analysis to ensure that the effect sizes calculated from LMs do not differ substantially from those estimated from the LMMs. Therefore, we also built two additional LMs, with bold or exploratory behaviours as the response variable (n = 303) where sex, mass, and stream were the only fixed effects. Bold behaviour was cube root transformed to meet assumptions of normality and linearity. We again extracted partial eta-squared using the 'effectsize' R package with as type II sum of squares. The effect sizes from these LMs do not qualitatively differ from those extracted from the LMMs above, and so only the effect sizes from the LMMs are presented in-text (Table S4).

Ethical statement

Permission to carry out this work came from McGill University Animal Care (AUP 8058).

Results

When examining the spatial structure of bold and exploratory behaviours, considerable variation was evident among pools within streams, but not between streams, for both behaviours (η_p^2 for pools within streams: bold = 9.0%, $p < 0.01$; exploratory = 12%, $p < 0.001$; η_p^2 for streams: bold = 2.0%, $p = 0.50$; exploratory = 0%, $p = 0.99$) (Table 2) (Figure 2).

For individual-level variables, sex explained 0% of variance in both behaviours (Table 3; Figure 3). Whereas mass also explained 0% of variance in exploratory behaviour, mass explained 3% (95% CI: 0.00 – 0.08) of variance in bold behaviour, and smaller individuals displayed bolder behaviours (χ^2_1: 9.3, $p < 0.01$) (Table 3; Figure 3).

For the abiotic pool-level variables, first concerning bold behaviour, individuals in pools with higher specific conductance (χ^2_1: 4.84, $p = 0.03$) and in pools with lower dissolved oxygen (χ^2_1: 4.73, $p = 0.03$) displayed bolder behaviour. We found 22% of the variation in pool-level mean bold behaviour was attributed to specific conductance, and 16% was attributed to dissolved oxygen (Figure 4). Although not statistically significant in the LMMs, volume (m^3) also had small effects on pool-level mean bold behaviour ($\eta_p^2 = 0.05$). Stream explained 11% of the variance in bold behaviour when included as a covariate in these models. Concerning exploratory behaviour, although only marginally significant, the trend suggests that individuals explore more in environments with less dissolved oxygen ($\chi^2_1 = 3.55$, $p = 0.06$); and, consistent with this

finding, dissolved oxygen explained 27% of the variance in pool-level mean exploratory behaviour (Figure 5). Temperature and specific conductance also had small effects on pool-level mean exploratory behaviour (η_p^2 = 0.08 and 0.06, respectively). Stream explained 14% of the variance in pool-level mean exploratory behaviour. All other ecological variables had small (η_p^2 < 0.02) effects on pool-level mean behaviour, and no other pool-level variable had a statistically significant effect on behaviour (Table 3). Among all pools in a stream, the mean +/- standard deviation for specific conductance (μS/cm) was 106.03 +/- 9.48 in Stream One and 84.61 +/- 18.30 in Stream Two; the mean dissolved oxygen (mg/L) was 4.16 +/- 0.82 in Stream One and 4.06 +/- 0.86 in Stream Two; the mean temperature (°C) was 22.52 +/- 0.17 in Stream One and 22.89 +/- 0.13 in Stream Two; and the mean volume (m^3) was 0.20 +/- 0.25 in Stream One, and 0.31 +/- 0.33 in Stream Two (Table 1). Finally, time of day was also significant in both models, confirming the value of including this term as a covariate, with individuals exhibiting bolder behaviour in the am and more exploratory behaviour in the pm.

Discussion

The role of the abiotic environment in shaping bold and exploratory behaviours has been overlooked relative to individual-level attributes and biotic factors, and it might be assumed that abiotic differences are unlikely to shape these behaviours. In the present study, we aimed to fill this knowledge gap and challenge this assumption. To do so, we started by establishing how guppy behaviour varies at three “scales”: among individuals, among pools within a stream, and between streams. We next examined how two individual-level attributes (sex, size) and many abiotic environmental factors contribute to this variation. We found that both bold and

exploratory behaviours are more highly explained by the pool than the stream from which guppies were collected. Although smaller guppies displayed bolder behaviour, this effect was small (only explaining 3% of variance in behaviour), and sex had no effect on behaviour. Abiotic pool-level variables were far more important for explaining behavioural variation. Individuals from pools with higher specific conductance displayed bolder behaviour, and individuals from pools with lower dissolved oxygen displayed bolder behaviour and possibly explored more. At the pool-level, 22% and 16% of the variance in mean bold behaviour could be attributed to specific conductance and dissolved oxygen, respectively, and 27% of the variance in mean exploratory behaviour could be attributed to dissolved oxygen. These results show the importance of local abiotic environmental factors in shaping bold and exploratory behaviours in nature.

The spatial structure of bold and exploratory behaviours

Pool and stream collectively explained 12% of variance in exploratory behaviour and 11% of variance in boldness. For both behaviours, pool within stream was an important contributor to this variance, highlighting the very small spatial scale over which environments can shape behaviour. These smaller spatial scales are often overlooked in many study systems, including in guppies where behavioural researchers often report only single coordinates from the river, and associated predation regime, from which they collected (e.g., Burns *et al.*, 2016; Kniel *et al.*, 2020). Our work here emphasizes that a high degree of variation exists within small stretches of streams that can shape these behaviours, and that behavioural studies in nature therefore require finer-scale sampling and reporting.

Although we collected guppies in the dry season, during the wet season gene flow is likely high among pools within our two streams as the pools are near to each other (<1 m – <50 m between adjacent pools) and are surely connected during high flow events. Indeed, previous work has shown that at this scale, if they are not separated by a barrier, guppies can – in essence – be considered a panmictic population (Blondel *et al.*, 2019; Crispo *et al.*, 2006). By contrast, the two streams in our study are genetically distinct (Blondel *et al.*, 2019). Behaviour primarily varying among pools rather than between streams therefore suggests that, at least in these populations, a higher degree of variation might be attributed to plastic responses than to genetic causes. For instance, if plasticity exhibited in the dry season in response to the local environment is mostly owing to contextual plasticity (i.e., "reversible" plasticity) then perhaps the behavioural signatures of those specific environmental conditions will erode once the local environment changes. Depending on the type of plasticity that contributes to behavioural variation at this scale, behavioural variation could, or could not, persist into the wet season or subsequent dry seasons. However, if developmental plasticity or transgenerational plasticity ("irreversible" plasticity) is at play, then perhaps behavioural variation owing to the local environment in one season will shape behaviour in subsequent seasons or years. Such "cumulative" plasticity could provide one mechanism to explain within-pool behavioural variation (Wright *et al.*, 2022). Indeed, guppy reproduction occurs year-round, and guppies have short generation times, with females giving birth as young as 10 weeks old (Reznick, 1997), and with an average of 1.74 generations per year in low predation localities (Reznick *et al.*, 1997). Although it therefore is possible that some of the guppies collected from a given pool were born in that pool, it is also likely that some of the guppies in our study (collected in the dry season) developed in a previous

wet or dry season, given that the mean lifespan of wild guppies is over 2 years (Reznick *et al.*, 2004).

Individual level factors

Many individual-level attributes have been investigated as possible contributors to variation in bold and exploratory behaviours. However, researchers often fail to find evidence demonstrating the importance of these individual-level attributes. For instance, whereas associations between size and bold behaviour are sometimes observed in fish (e.g., Brown *et al.*, 2005; Brown & Braithwaite, 2004), other studies have found no association between fish size and this behaviour (e.g., Archard & Braithwaite, 2011; Bell, 2005; Fraser *et al.*, 2001; Wilson *et al.*, 1993). Our results are consistent with the latter; in our study of wild-caught guppies, the two individual-level attributes we investigated (sex and mass) were of remarkably low importance for explaining variation in bold and exploratory behaviours. Indeed, the largest effect size for either of these variables was for body mass, which explained 3% of the variance in bold behaviour and, in all other cases, these variables explained 0% of behavioural variance.

Although the effect was weak, we did find that smaller guppies displayed bolder behaviour. This finding is somewhat surprising because, although body size sometimes differs between guppies for some behaviours (e.g., Anderson Berdal *et al.*, 2018), other work has found that bold and exploratory behaviours do not differ much between guppies of different sizes (e.g., Diaz Pauli *et al.*, 2015; Kemp *et al.*, 2022) – even for wild caught guppies in Trinidad (e.g., Harris *et al.*, 2010). One explanation for why smaller guppies displayed bolder behaviour in our study could be that smaller fish have larger metabolic requirements that require energy to be

allocated to resource acquisition at the cost of reduced anti-predator behaviours (as has been previously hypothesized for other fish species; Brown & Braithwaite, 2004). However, another study that collected wild guppies from both high-predation and low-predation sites did not find effects of body size on behaviour in any site, regardless of predation regime (Harris *et al.*, 2010). Additional work will be required to identify the context-dependencies associated with this variation in size-dependent behavioural variation, which could include differing predation risks (which exist to an extent even within 'low predation' environments), or correlations between bold and exploratory behaviours and other behaviours that can be size-dependent (e.g., mating, courtship).

Precedent also exists to suggest that sex can sometimes be an important source of behavioural variation in fish (e.g., Harris *et al.*, 2010; Ingley *et al.*, 2014; Kemp *et al.*, 2022). In the present study, we did not observe sex differences in behaviour. Perhaps, independent of other factors or contexts, sex alone is not important for generating behavioural variation in guppies. Indeed, a meta-analysis that included data from over 200 studies did not find sex differences in the mean or variance of animal personality across five taxonomic groups (Harrison *et al.*, 2022). In cases where sex differences in behaviours do emerge, these effects could be due to other factors or dynamics that generate context-dependent variation in behaviour. For instance, female guppies are bolder in male-biased social groups than female-biased social groups, possibly to reduce male harassment (Piyapong *et al.*, 2010). Such context-dependencies for the role of sex in contributing to bold behaviour highlights the probable importance of within-pool dynamics. Future work could investigate the extent to which within-pool dynamics or other interactions mediate variation in bold and exploratory behaviours between the sexes in guppies.

Pool level factors

Our study is the first to our knowledge to measure the contributions of *abiotic* environmental factors to variation in bold and exploratory behaviours across more than one wild guppy population – an effort that seems somewhat overdue. Indeed, we found that some abiotic environmental factors have strong effects on these behaviours. Our study therefore points to the value of additional studies conducting larger scale assessments of the abiotic environmental contributors to behaviour – in guppies and other fish species.

We first found that guppies displayed bolder behaviours in environments with higher specific conductance. Higher specific conductance here might be indicative of agricultural runoff (Drerup & Vadeboncoeur, 2016; Sohoulande *et al.*, 2022). Some support for this idea comes from the fact that one of our sites, Stream Two, is accessible via a small plantation. Although Stream One has higher mean specific conductance than Stream Two, and less obvious links to agriculture, there could still be runoff as low-intensity agriculture is increasing throughout Trinidad (Northern Range Assessment, 2005). Higher dissolved nutrients are associated with more nutritious epilithon in streams in Trinidad, and so fertiliser runoff could generate variation in food resource quality between pools (Kohler *et al.*, 2012). Such resource quality differences could affect boldness. For instance, our sites were all low predation, where the primary predator only eats small guppies (Endler, 1978; Seghers, 1973). It's possible that higher quality resources enable guppies to grow faster or larger, reducing overall risks and thus risk-aversive behaviours. Given that we did not directly test for these nutrients, concrete assertions on why specific conductance impacts guppy behaviours at these small scales will require future work.

We also found that individuals displayed bolder behaviour and possibly explored more in environments with lower dissolved oxygen (although the latter association lacked statistical significance). Associations between dissolved oxygen and exploratory behaviour echo past research on another species of poecilid fish (*P. vivipara)* where fish from lagoons with low dissolved oxygen moved more in an open field, which they speculated could be due to fish having to move greater distances to forage in low oxygen environments that have scarcer food resources (Sommer-Trembo *et al.*, 2017). These energetic demands could result in resource acquisition being favoured at the cost of decreased anti-predator behaviour. Nevertheless, similar hypotheses about primary productivity could be made about canopy cover or temperature, which are also associated with primary productivity (Grether *et al.*, 2001; Lewandowska *et al.*, 2012). However, although temperature had a small effect on exploratory behaviour (explaining 8% of variance), the effects of canopy cover and temperature on both behaviours were otherwise very weak ($\eta_p^2 < 0.02$). Because we did not assess causal associations between pool-level factors and behaviour, additional work will be required to better understand the mechanistic underpinning of these associations.

Future directions

The scarcity of research investigating contributors to variation in bold and exploratory behaviours across multiple wild populations, coupled with the importance of abiotic pool-level variables in the present study, underscores several opportunities for research into the causes of behavioural variation.

To start, other researchers could focus on sampling more ecologically diverse streams. Indeed, the two streams that we sampled here, although genetically distinct (Blondel *et al.*, 2019), are environmentally similar (Table 1; Table S2). We selected our streams specifically because they are both low predation with few *Gyrodactylus* parasites (we did not find any parasites on any of our fish). Further, they are in the same watershed and so guppies in the two streams are from the same lineage, and they are geographically close. These decisions help to minimize many factors that could have confounding effects on our results – allowing us to focus on abiotic variables within a given set of environments that are standardized for other factors. However, in establishing that bold and exploratory behaviours can be highly sensitive even to small differences in abiotic environmental factors, our work provides a foundation that could now be extended to include other factors, such as additional behaviours, abiotic environmental factors, or high predation environments.

In addition to increased spatial sampling, researchers could repeatedly collect guppies from the same pools at various time points to obtain an improved understanding of how ecological factors fluctuate over time, and how these fluctuations affect behaviour within and among generations. These time points could be on relatively short time scales (e.g., days to weeks) or could be over much longer time points (e.g., multiple seasons or years). Indeed, given the importance of small spatial scales in our study, perhaps small temporal scales would also be important contributors to guppy behaviour. Repeatedly sampling pools could also provide insight into how pool-level dynamics contribute to variation in behaviour, such as frequency dependence, which is hypothesized to contribute to variation in behavioural traits (Wolf & McNamara, 2012). Density dependence could also be considered (Travis *et al.*, 2023), yet density was quite highly correlated with volume in our dataset ($r = 0.73$) and, although a small

amount of variance in bold behaviour could be attributed to volume ($\eta_p^2 = 0.05$), volume had a no effect on exploratory behaviour ($\eta_p^2 = 0.00$), and neither of these associations were statistically significant.

Repeatably sampling the same sites also has the potential to generate insights into the role of plasticity in guppy behaviour. If bold or exploratory behaviours are highly associated with plasticity (as we postulate above), multiple sampling events or reciprocal transplant experiments between pools could provide insight into the extent to which different types of plasticity (e.g., contextual, developmental, transgenerational) are most highly associated with these behaviours in wild guppies (Fox *et al.*, 2024). For instance, guppies born during the wet season likely experience far differing ecological conditions to guppies born during the dry season; developmental plasticity could result in behavioural differences emerging and persisting among adult guppies. Further, future work that repeatably collects guppy behavioural and environmental data could provide interesting insight into spatial variation of plasticity in guppy behaviour (i.e., behavioural reaction norms).

Investigating how associations between guppy behaviour and the ecological variables we assessed vary over space and time would provide deeper insight into how these environmental factors shape behaviour. Although more behavioural variance was observed within pools rather than among streams in our study, most of the behavioural variance was left unexplained. It's therefore possible that the ecological variables we assessed were of weak importance relative to other unmeasured variables. With this in mind, a potentially insightful avenue of future work could be to measure the effects of other ecological variables on guppy behaviour, such as nutrients (phosphorus and nitrogen), dissolved organic carbon, periphyton biomass, or the

macroinvertebrate community. In a similar vein, although we attempted to "control" for the predation environment in the present study, as stated above some predation pressures still exist in low-predation sites. Therefore, a similar study could be conducted that directly quantifies predation-pressure to either consider this as an additional factor of interest, or to control for the effects of predation more effectively.

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References

Anderson Berdal, M., Rosenqvist, G., & Wright, J. (2018). Innovation as part of a wider behavioural syndrome in the guppy: The effect of sex and body size. *Ethology*, *124*, 760–772.

Archard, G. A., & Braithwaite, V. A. (2011). Increased exposure to predators increases both exploration and activity level in *Brachyrhaphis episcopi*. *Journal of Fish Biology*, *78*, 593–601.

Ariyomo, T. O., & Watt, P. J. (2013). Disassortative mating for boldness decreases reproductive success in the guppy. *Behavioral Ecology*, *24*, 1320–1326.

Ballew, N. G., Mittelbach, G. G., & Scribner, K. T. (2017). Fitness consequences of boldness in juvenile and adult largemouth bass. *The American Naturalist*, *189*, 396–406.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*.

Bell, A. M. (2005). Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology*, *18*, 464–473.

Bell, A. M., Henderson, L., & Huntingford, F. A. (2010). Behavioral and respiratory responses to stressors in multiple populations of three-spined sticklebacks that differ in predation pressure. *Journal of Comparative Physiology B*, *180*, 211–220.

Ben-Shachar, M., Lüdecke, D., & Makowski, D. (2020). effectsize: estimation of effect size indices and standardized parameters. *Journal of Open Source Software*, *5*, 2815.

Blondel, L., Baillie, L., Quinton, J., Alemu, J. B., Paterson, I., Hendry, A. P., & Bentzen, P. (2019). Evidence for contemporary and historical gene flow between guppy populations in different watersheds, with a test for associations with adaptive traits. *Ecology and Evolution*, *9*, 4504–4517.

Brown, C., Jones, F., & Braithwaite, V. (2005). In situ examination of boldness–shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Animal Behaviour*, *70*, 1003–1009.

Brown, C., & Braithwaite, V. A. (2004). Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour*, *68*, 1325–1329.

Brown, C., Burgess, F., & Braithwaite, V. A. (2007). Heritable and experiential effects on boldness in a tropical poeciliid. *Behavioral Ecology and Sociobiology*, *62*, 237–243.

Bryant, M. J., & Reznick, D. (2004). Comparative studies of senescence in natural populations of guppies. *The American Naturalist*, *163*, 55–68.

Burns, J. G. (2008). The validity of three tests of temperament in guppies (*Poecilia reticulata*). *Journal of Comparative Psychology*, *122*, 344–356.

Burns, J. G., Price, A. C., Thomson, J. D., Hughes, K. A., & Rodd, F. H. (2016). Environmental and genetic effects on exploratory behavior of high- and low-predation guppies (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, *70*, 1187–1196.

Carlson, B. E., & Langkilde, T. (2013). Personality traits are expressed in bullfrog tadpoles during open-field trials. *Journal of Herpetology*, *47*, 378–383.

Chapman, B. B., Hulthén, K., Blomqvist, D. R., Hansson, L., Nilsson, J., Brodersen, J., … Brönmark, C. (2011). To boldly go: individual differences in boldness influence migratory tendency. *Ecology Letters*, *14*, 871–876.

Crispo, E., Bentzen, P., Reznick, D. N., Kinnison, M. T., & Hendry, A. P. (2006). The relative influence of natural selection and geography on gene flow in guppies. *Molecular Ecology*, *15*, 49–62.

Culumber, Z. W. (2020). Thermal stress increases activity and risk-taking behavior but not anxiety in a livebearing fish. *Environmental Biology of Fishes*, *103*, 313–317.

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., … Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology & Evolution*, *2*, 57–64.

Diaz Pauli, B., Garric, S., Evangelista, C., Vøllestad, L. A., & Edeline, E. (2019). Selection for small body size favours contrasting sex-specific life histories, boldness and feeding in medaka, *Oryzias latipes*. *BMC Evolutionary Biology*, *19*, 127.

Diaz Pauli, B., Wiech, M., Heino, M., & Utne-Palm, A. C. (2015). Opposite selection on behavioural types by active and passive fishing gears in a simulated guppy *Poecilia reticulata* fishery: fishing gear selection on behavioural types. *Journal of Fish Biology*, *86*, 1030–1045.

Drerup, S. A., & Vadeboncoeur, Y. (2016). Elevated specific conductance enhances productivity and biomass of periphytic cyanobacteria from Lake Tahoe and Lake Tanganyika. *Phycologia*, *55*, 295–298.

Endler, J. A. (1978). A predator's view of animal color patterns. In M. K. Hecht, W. C. Steere, & B. Wallace (Eds.), *Evolutionary Biology* (pp. 319–364). Boston, MA: Springer US.

Engqvist, L. (2005). The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*, *70*, 967–971.

Fox, J. A., Toure, M. W., Heckley, A., Fan, R., Reader, S. M., & Barrett, R. D. H. (2024). Insights into adaptive behavioural plasticity from the guppy model system. *Proceedings of the Royal Society B: Biological Sciences*, *291*, 20232625.

Fraser, D. F., Gilliam, J. F., Daley, M. J., Le, A. N., & Skalski, G. T. (2001). Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. *The American Naturalist*, *158*, 124–135.

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*, 1325–1330.

Gotanda, K. M., Delaire, L. C., Raeymaekers, J. A. M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., … Hendry, A. P. (2013). Adding parasites to the guppy-predation story: insights from field surveys. *Oecologia*, *172*, 155–166.

Grether, G. F., Millie, D. F., Bryant, M. J., Reznick, D. N., & Mayea, W. (2001). Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*, *82*, 1546–1559.

Harris, S., Ramnarine, I. W., Smith, H. G., & Pettersson, L. B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos*, *119*, 1711–1718.

Harrison, L. M., Noble, D. W. A., & Jennions, M. D. (2022). A meta-analysis of sex differences in animal personality: no evidence for the greater male variability hypothesis. *Biological Reviews*, *97*, 679–707.

Hartig, F. (2022). DHARMa: residual diagnostics for hierarchical (multi-level / mixed) regression models. R package version 0.4.6, https://CRAN.R-project.org/package=DHARMa.

Heckley, A. M., Pearce, A. E., Gotanda, K. M., Hendry, A. P., & Oke, K. B. (2022). Compiling forty years of guppy research to investigate the factors contributing to (non)parallel evolution. *Journal of Evolutionary Biology*, *35*, 1414–1431.

Ingley, S. J., Rehm, J., & Johnson, J. B. (2014). Size doesn't matter, sex does: a test for boldness in sister species of *Brachyrhaphis* fishes. *Ecology and Evolution*, *4*, 4361–4369.

Jacquin, L., Reader, S. M., Boniface, A., Mateluna, J., Patalas, I., Pérez-Jvostov, F., & Hendry, A. P. (2016). Parallel and nonparallel behavioural evolution in response to parasitism and predation in Trinidadian guppies. *Journal of Evolutionary Biology*, *29*, 1406–1422.

Jaeger, B. 2017. r2glmm: computes R squared for mixed (multilevel) models. R package version 0.1.2, https://CRAN.R-project.org/package=r2glmm.

Jaeger, B. C., Edwards, L. J., Das, K., & Sen, P. K. (2017). An R^2 statistic for fixed effects in the generalized linear mixed model. *Journal of Applied Statistics*, *44*, 1086–1105.

Kassambara A, Mundt, F. (2020). factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R package version 1.0.7, https://CRAN.R-project.org/package=factoextra.

Kemp, D. J., Lynch, K. E., & Samantha, St. J. (2022). Variation in boldness behaviour across individuals, sexes and strains of the guppy (*Poecilia reticulata*). *Marine and Freshwater Research*, *73*, 441–453.

Kniel, N., Guenther, A., & Godin, J.-G. J. (2020). Individual personality does not predict learning performance in a foraging context in female guppies, *Poecilia reticulata*. *Animal Behaviour*, *167*, 3–12.

Kohler, T. J., Heatherly, T. N., El-Sabaawi, R. W., Zandonà, E., Marshall, M. C., Flecker, A. S., … Thomas, S. A. (2012). Flow, nutrients, and light availability influence Neotropical epilithon biomass and stoichiometry. *Freshwater Science*, *31*, 1019–1034.

Leite, T., Santos, J. M., Ferreira, M. T., Canhoto, C., & Branco, P. (2019). Does short-term salinization of freshwater alter the behaviour of the Iberian barbel (*Luciobarbus bocagei*, Steindachner 1864)? *Science of The Total Environment*, *651*, 648–655.

Leite, T., Branco, P., Ferreira, M. T., & Santos, J. M. (2022). Activity, boldness and schooling in freshwater fish are affected by river salinization. *Science of The Total Environment*, *819*, 153046.

Lewandowska, A. M., Breithaupt, P., Hillebrand, H., Hoppe, H.-G., Jürgens, K., & Sommer, U. (2012). Responses of primary productivity to increased temperature and phytoplankton diversity. *Journal of Sea Research*, *72*, 87–93.

Magnhagen, C., Hellström, G., Borcherding, J., & Heynen, M. (2012). Boldness in two perch populations – long-term differences and the effect of predation pressure. *Journal of Animal Ecology*, *81*, 1311–1318.

Magurran, A. E., & Seghers, B. H. (1994). Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *255*, 31–36.

McCowan, L. S. C., Rollins, L. A., & Griffith, S. C. (2014). Personality in captivity: more exploratory males reproduce better in an aviary population. *Behavioural Processes*, *107*, 150–157.

Moran, N. P., Mossop, K. D., Thompson, R. M., & Wong, B. B. M. (2016). Boldness in extreme environments: temperament divergence in a desert-dwelling fish. *Animal Behaviour*, *122*, 125–133.

Northern Range Assessment 2005. Report of an assessment of the Northern Range, Trinidad and Tobago: people and the Northern Range. State of the Environment Report 2004. Environmental Management Authority of Trinidad and Tobago. 184pp.

O'Neill, S. J., White, T. E., Lynch, K. E., & Kemp, D. J. (2019). Male guppies differ in daily frequency but not diel pattern of display under daily light changes. *Behavioral Ecology and Sociobiology*, *73*, 157.

Patrick, S. C., Pinaud, D., & Weimerskirch, H. (2017). Boldness predicts an individual's position along an exploration–exploitation foraging trade-off. *Journal of Animal Ecology*, *86*, 1257–1268.

Piyapong, C., Krause, J., Chapman, B. B., Ramnarine, I. W., Louca, V., & Croft, D. P. (2010). Sex matters: a social context to boldness in guppies (*Poecilia reticulata*). *Behavioral Ecology*, *21*, 3–8.

R Core Team. (2024). *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rasmussen, J. E., & Belk, M. C. (2017). Predation environment affects boldness temperament of neotropical livebearers. *Ecology and Evolution*, *7*, 3059–3066.

Reader, S. M. (2015). Causes of individual differences in animal exploration and search. *Topics in Cognitive Science*, *7*, 451–468.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*, 291–318.

Reznick, D. N. (1997). Life history evolution in guppies (*Poecilia reticulata*): guppies as a model for studying the evolutionary biology of aging. *Experimental Gerontology*, *32*, 245–258.

Reznick, D. N., Bryant, M. J., Roff, D., Ghalambor, C. K., & Ghalambor, D. E. (2004). Effect of extrinsic mortality on the evolution of senescence in guppies. *Nature*, *431*, 1095–1099.

Reznick, D. N., Butler, M. J., Rodd, F. H., & Ross, P. (1996). Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. *Evolution*, *50*, 1651–1660.

Reznick, D. N., Shaw, F. H., Rodd, F. H., & Shaw, R. G. (1997). Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science*, *275*, 1934–1937.

Riesch, R., Duwe, V., Herrmann, N., Padur, L., Ramm, A., Scharnweber, K., … Plath, M. (2009). Variation along the shy–bold continuum in extremophile fishes (*Poecilia mexicana*, *Poecilia sulphuraria*). *Behavioral Ecology and Sociobiology*, *63*, 1515–1526.

Rödel, H. G., Zapka, M., Talke, S., Kornatz, T., Bruchner, B., & Hedler, C. (2015). Survival costs of fast exploration during juvenile life in a small mammal. *Behavioral Ecology and Sociobiology*, *69*, 205–217.

Rudh, A., Breed, M. F., & Qvarnström, A. (2013). Does aggression and explorative behaviour decrease with lost warning coloration?: Coloration and Aggression in Frogs. *Biological Journal of the Linnean Society*, *108*, 116–126.

Seghers, B. H. (1973). Analysis of geographic variation in the antipredator adaptations of the guppy : Poecilia reticulata.

Sohoulande, C. D. D., Szogi, A. A., Novak, J. M., Stone, K. C., Martin, J. H., & Watts, D. W. (2022). Long-term nitrogen and phosphorus outflow from an instream constructed wetland under precipitation variability. *Sustainability*, *14*, 16500.

Sommer-Trembo, C., Petry, A. C., Gomes Silva, G., Vurusic, S. M., Gismann, J., Baier, J., … Plath, M. (2017). Predation risk and abiotic habitat parameters affect personality traits in extremophile populations of a neotropical fish (*Poecilia vivipara*). *Ecology and Evolution*, *7*, 6570–6581.

Tang, Z., & Fu, S.-J. (2021). Effects of habitat conditions on the boldness and sociability of wild-caught fish (*Zacco platypus*) along a river. *Journal of Ethology*, *39*, 379–391.

Travis, J., Bassar, R. D., Coulson, T., Reznick, D., & Walsh, M. (2023). Density-dependent selection. *Annual Review of Ecology, Evolution, and Systematics*, *54*, 85–105.

Ward, Hart, & Webster. (2007). Boldness is influenced by social context in threespine sticklebacks (*Gasterosteus aculeatus*). *Behaviour*, *144*, 351–371.

Weese, D. J., Gordon, S. P., Hendry, A. P., & Kinnison, M. T. (2010). Spatiotemporal variation in linear selection on body color in wild guppies (*Poecilia reticulata*): selection on color in wild guppies. *Evolution*, *64*, 1802–1815.

Wilson, D. S., Clark, A. B., & Laurence, B. (1993). Shy-bold continuum in Pumpkinseen Sunfish (*Leopmis gibbosus*): an ecological study of a psychological trait. *Journal of Comparative Psychology*, *107*, 250–260.

Wolf, M., & McNamara, J. M. (2012). On the evolution of personalities via frequency-dependent selection. *The American Naturalist*, *179*, 679–692.

Wright, J., Haaland, T. R., Dingemanse, N. J., & Westneat, D. F. (2022). A reaction norm framework for the evolution of learning: how cumulative experience shapes phenotypic plasticity. *Biological Reviews*, *97*, 1999–2021.

Tables

Table 1. Mean +/- standard deviation for ecological variables from both streams. See Table S2 for pool-specific ecological measurements and sample sizes.

Ecological variable	**Stream One**	**Stream Two**
Temperature (°C)	22.52 +/- 0.17	22.89 +/- 0.13
Dissolved oxygen (mg/L)	4.16 +/- 0.82	4.06 +/- 0.86
Specific conductance (µS/cm)	106.03 +/- 9.48	84.61 +/- 18.30
pH	8.32 +/- 0.57	6.79 +/- 0.40
Surface area (m^2)	0.57 +/- 0.19	0.89 +/- 0.23
Volume (m^3)	0.20 +/- 0.25	0.31 +/- 0.33
Mean depth (m)	0.30 +/- 0.30	0.34 +/- 0.32
Max depth (m)	0.19 +/- 0.03	0.25 +/- 0.12
Canopy cover (squares covered on densiometer)	4.36 +/- 1.67	2.16 +/- 0.76

Table 2. Results from the univariate ANOVAs conducted in SPSS for bold behaviour (sum of time spent frozen in the open field and under the plant refuge) and exploratory behaviour (number of squares crossed in the open field). Pool nested in stream was included as a random effect. Significant terms ($p < 0.05$) are bolded.

			df	f	p	η_p^2
Bold behaviour	Intercept	Hypothesis	1.00	360.64	0.03	1.00
		Error	1.00			
	Stream	Hypothesis	1.00	0.48	0.50	0.02
		Error	21.16			
	Pool	Hypothesis	13.00	2.24	0.01	0.09
	(Stream**)**	Error	288.00			
Exploratory behaviour	Intercept	Hypothesis	1.00	186140.64	<0.001	1.00
		Error	2.44			
	Stream	Hypothesis	1.00	0.00	0.99	0.00
		Error	19.06			
	Pool	Hypothesis	13.00	2.94	<0.001	0.12
	(Stream**)**	Error	288.00			

Table 3. Results from the linear mixed models investigating the individual-level and pool-level factors. Pool nested in stream was included as a random effect. Bold behaviour (the sum of time spent frozen in the open field and under the plant refuge) was cube root transformed, and exploratory behaviour (number of squares crossed in the open field) was square root transformed to meet assumptions of linearity.

Behaviour		**Term**	**χ^2**	**df**	**p**
Bold	Individual-level	Sex	0.01	1	0.94
	factors	Mass	9.26	1	<0.01
	Pool-level factors	Dissolved oxygen (mg/L)	4.73	1	0.03
		Surface area (m^2)	0.44	1	0.51
		Temperature (°C)	0.79	1	0.37
		Specific conductance (µS/cm)	4.84	1	0.03
		Volume (m^3)	0.52	1	0.47
		Canopy cover (squares covered on densiometer)	0.65	1	0.42
	Co-variate	Time of day	4.76	1	0.03
Exploratory	Individual-level	Sex	0.43	1	0.51
	factors	Mass	0.64	1	0.42
	Pool-level factors	Dissolved oxygen (mg/L)	3.55	1	0.06
		Surface area (m^2)	0.56	1	0.45
		Temperature (°C)	0.31	1	0.58

	Specific conductance (μS/cm)	0.05	1	0.82
	Volume (m^3)	0.04	1	0.84
	Canopy cover (squares covered on densiometer)	0.00	1	0.95
Co-variate	Time of day	12.23	1	<0.001

Figures

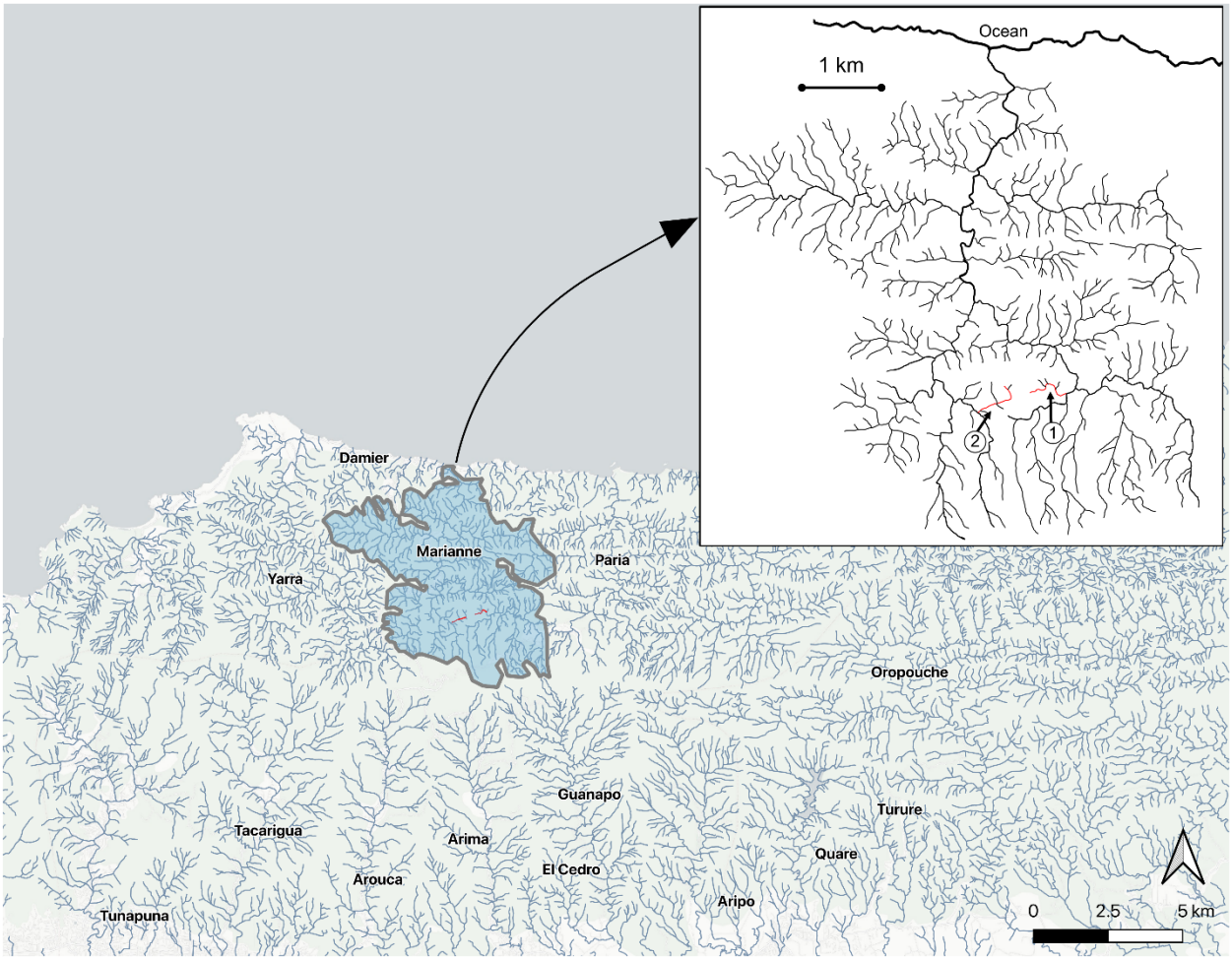

Figure 1. Map of the Northern Mountain Range in Trinidad. The Marianne River, where our study was conducted, is shaded in blue and shown "zoomed-in" with the inlay map. The specific streams that we studied are coloured red, and the approximate locations of Stream One and Stream Two are labelled on the inlay map (1 = Stream One; 2 = Stream 2). Other major rivers where guppy research is often conducted are also labelled. The map of the Northern Mountain Range was modified from Heckley et al. (2022).

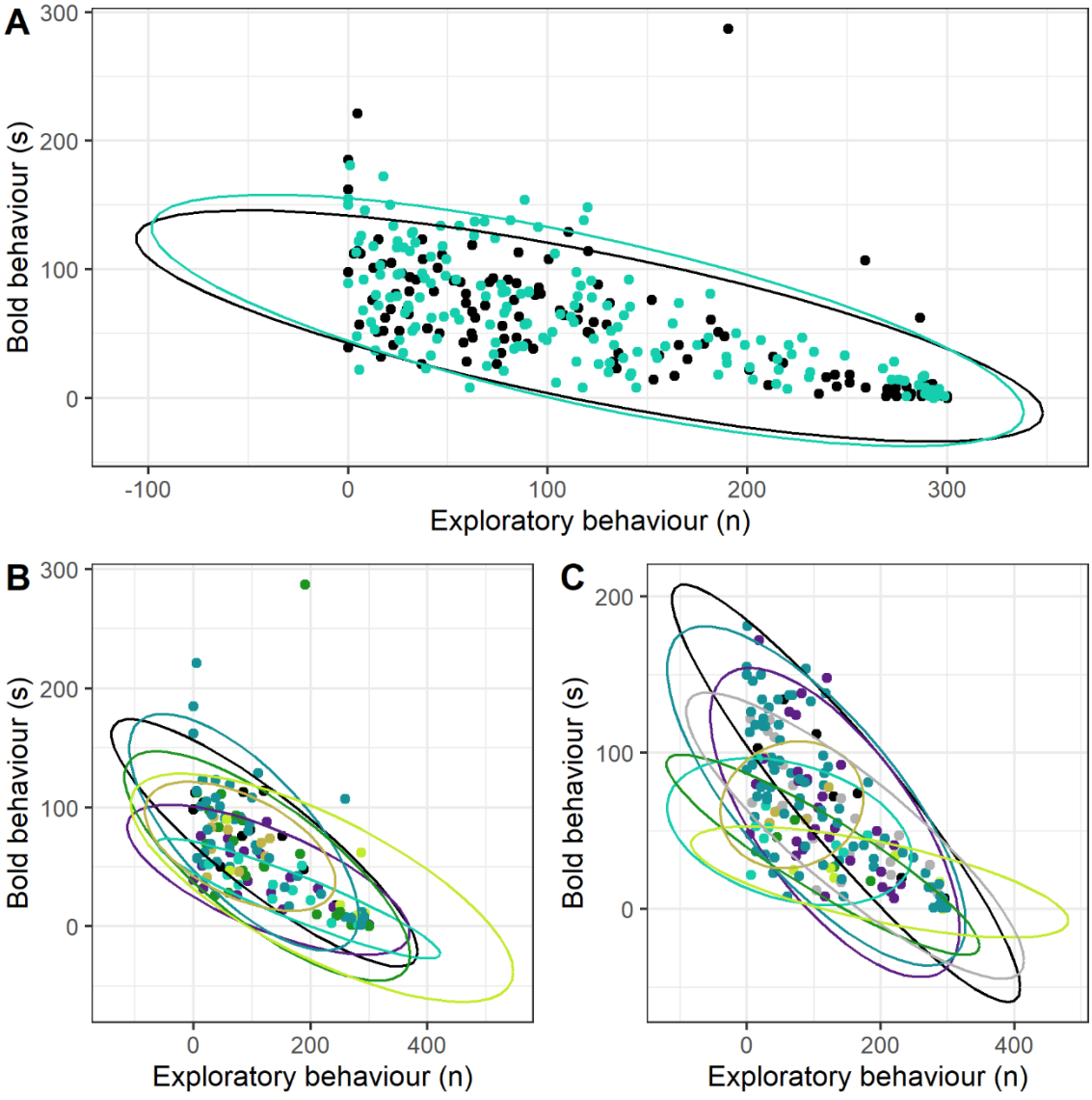

Figure 2. Variation in bold behaviour (the sum of time spent frozen in the open field and under the plant refuge) and exploratory behaviour (number of squares crossed in the open field). The panels show (A) all data, with the ellipses around each stream; (B) data for Stream One only, with the ellipses around each pool; and (C) data for Stream Two only, with ellipses around each pool. Panel A is coloured by stream and panels B and C are coloured by different pools within each stream.

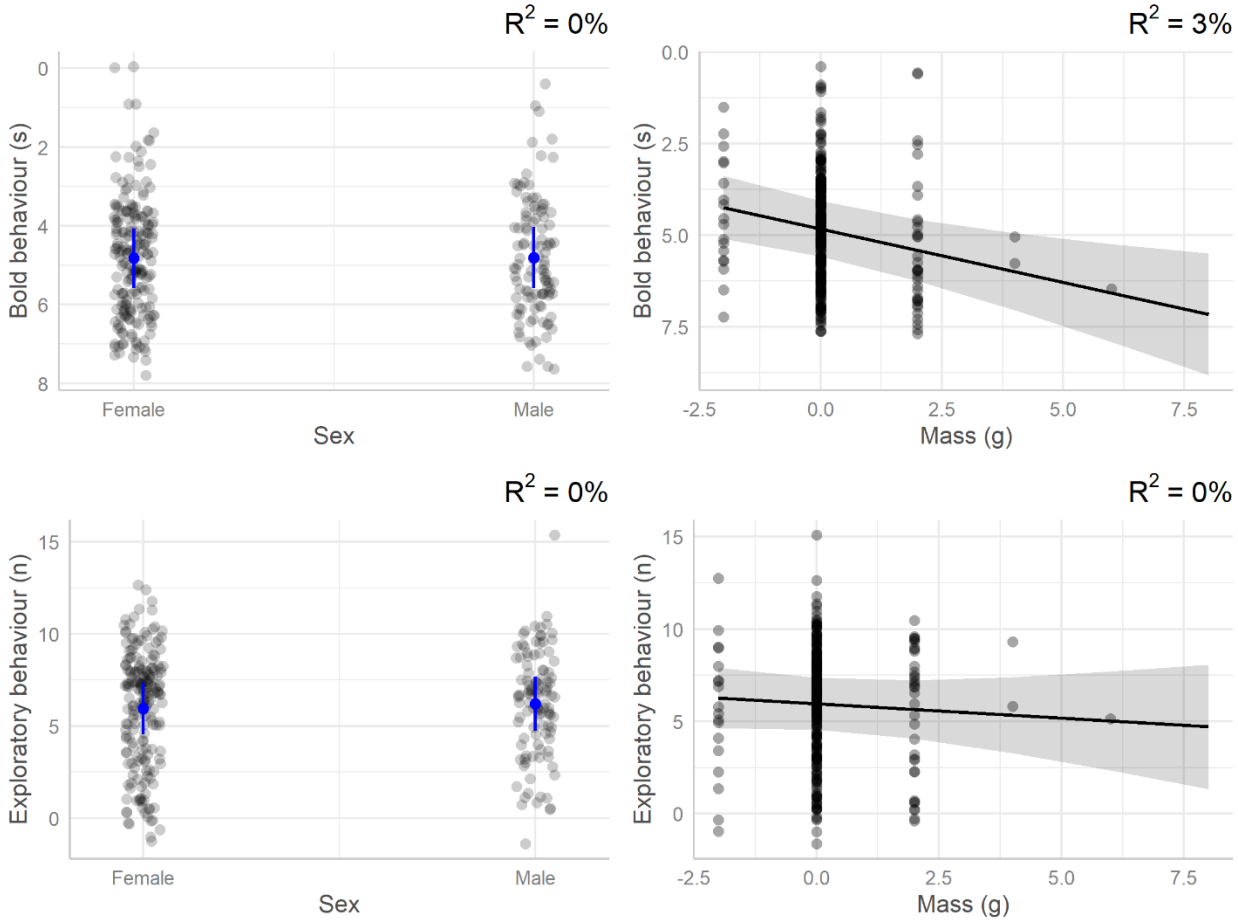

Figure 3. The effects of sex and mass on bold behaviour (the sum of time spent frozen in the open field and under the plant refuge) and exploratory behaviour (number of squares crossed in the open field). Data points are the partial model residuals and are alongside trend lines predicted by the linear mixed models. Mass is scaled and centered, so values on the x-axis do not correspond to the literal measured mass. Bold and exploratory behaviours were cube root and square root transformed, respectively, to meet assumptions of linearity. The y-axis is inversed in the bold behaviour plots (but not the exploratory behaviour plots) for more intuitive interpretation: bold behaviour increases as the y-axis approaches zero – towards the top of the plot.

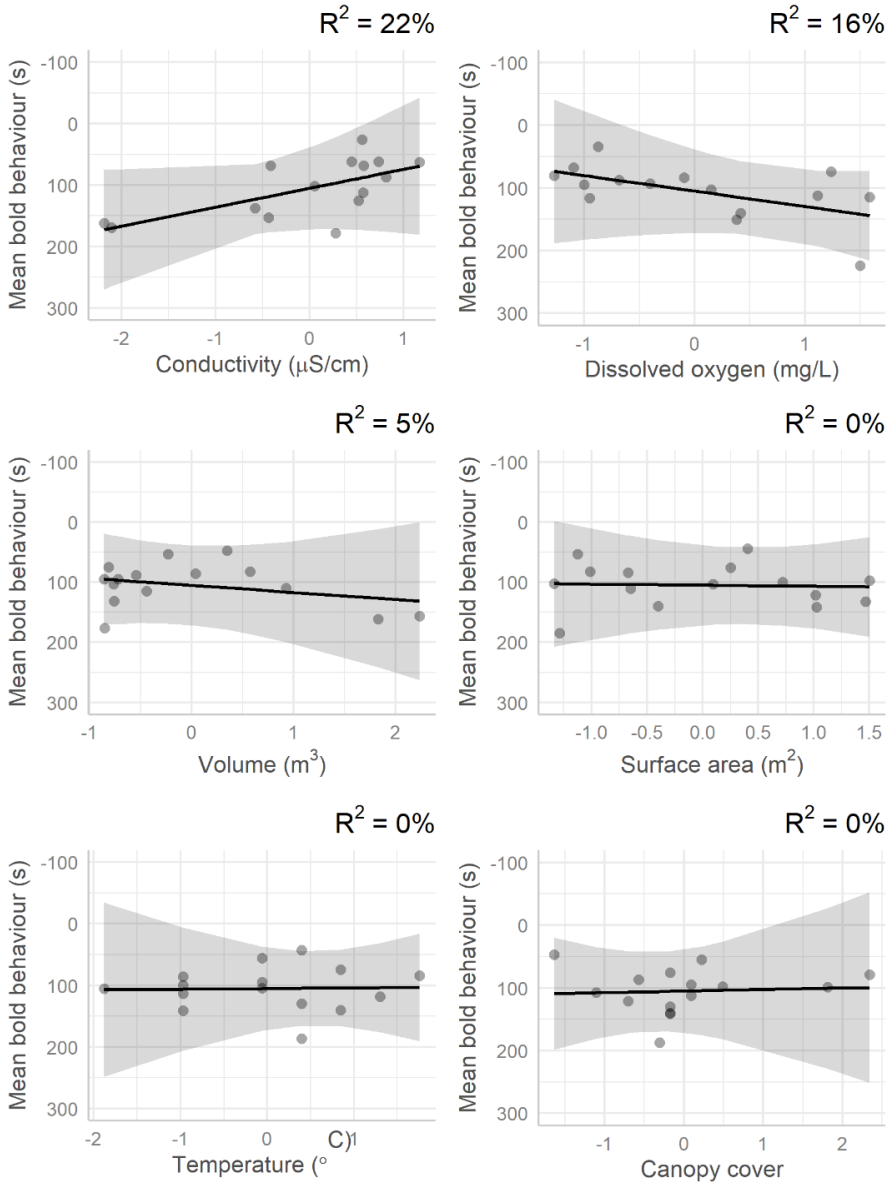

Figure 4. Effects of ecological variables on mean pool-level bold behaviour (the sum of time spent frozen in the open field and under the plant refuge). Data points are the partial model residuals and are alongside trend lines predicted by the linear model. The units for canopy cover are the number of grid sections crossed on a densiometer. The y-axis is inversed for more intuitive interpretation: bold behaviour increases as the y-axis approaches zero - towards the top of the plot. Conductivity was temperature standardized (specific conductance).

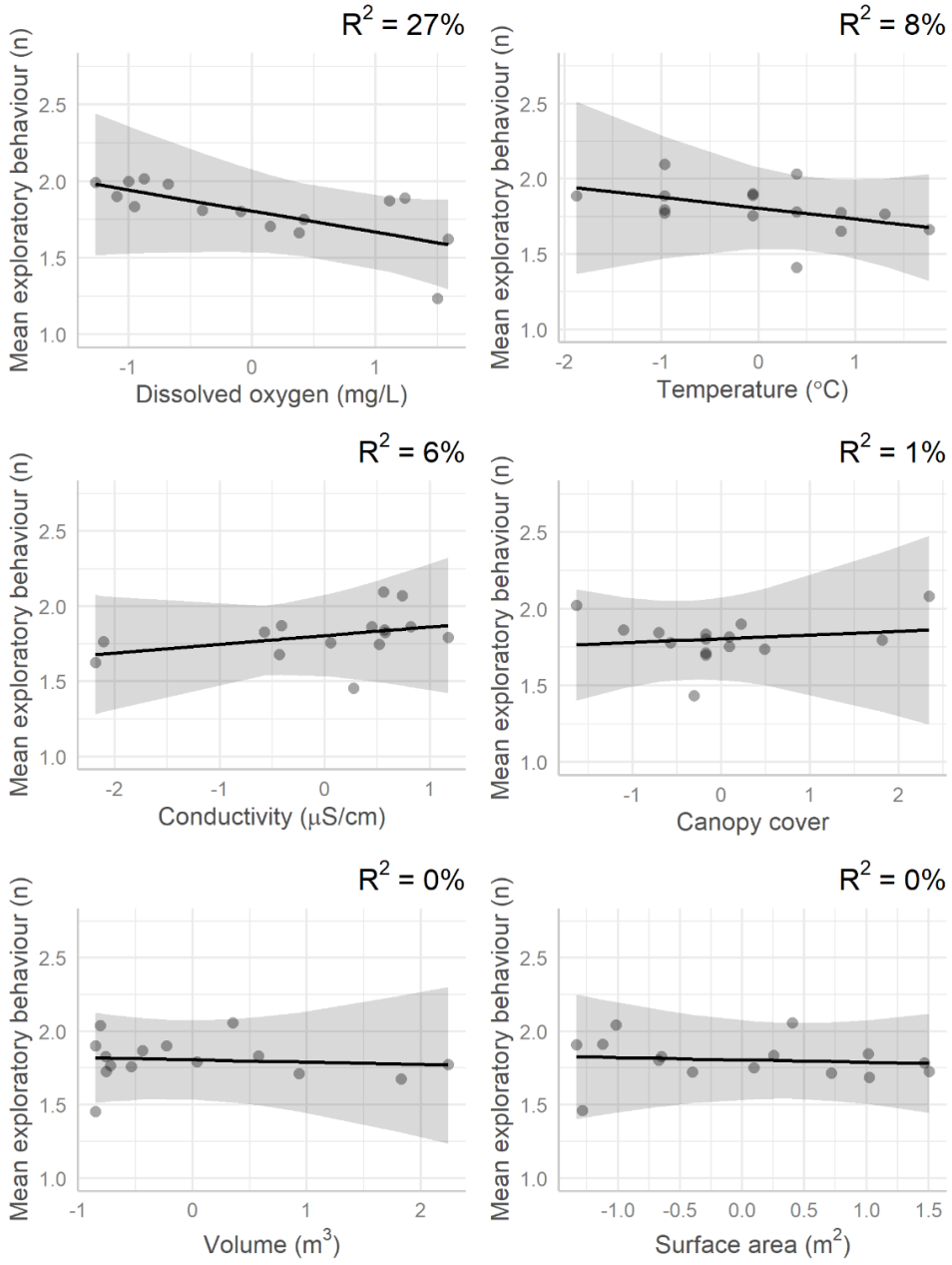

Figure 5. Effects of ecological variables on mean pool-level exploratory behaviour (number of squares crossed in the open field). Data points are the partial model residuals and are alongside trend lines predicted by the linear model. The units for canopy cover are the number of grid sections crossed on a densiometer. Exploratory behaviour values were log transformed to meet assumptions of linearity. Conductivity was temperature standardized (specific conductance).

Bridging statement 2

In Chapter 2, I found that the abiotic environment was associated with guppy behaviour even on very small spatial scales. Whereas individual-level attributes had no or weak effects on behaviour, some abiotic factors had much stronger effects, indicating that associations between abiotic factors and behaviour can be more predictable than associations between individual-level attributes and behaviour. Nevertheless, the most variance explained by a single abiotic factor was still only 27%, highlighting weak overall predictability.

This Chapter concludes Part One of my thesis, where I investigate the causes of intraspecific variation. Across Chapters 1 and 2, I found that the causes of phenotypic variation in guppies were weakly predictable because, in most cases, the majority of variance remained unexplained by the predictor variables we considered. For my next three chapters, I pivot to investigating the consequences of intraspecific variation. In doing so, I also switch from the Trinidadian guppy system to focus on another fish species: threespine stickleback. With stickleback, I explore the extent to which non-random movement is a predictable consequence of intraspecific variation, in ecological, evolutionary, and applied contexts.

In the next chapter, Chapter 3, I specifically investigate dispersal – an ecologically important process – as a possible consequence of intraspecific variation. To do so, I experimentally induce variation in peritoneal fibrosis, which is an inflammation and tissue repair response, to assess how fibrosis affects dispersal in a mark recapture experiment in nature.

Chapter 3: Does motility-restricting fibrosis influence dispersal? An experiment in nature with threespine stickleback

Heckley, A. M., Bolnick, D.I., Dinh, F., Hendry, A.P., Steinel, N.C. Does motility-restricting fibrosis influence dispersal? An experiment in nature with threespine stickleback. *Ecology and Evolution*. 10.1002/ece3.70697.

Abstract

Dispersal can affect individual-level fitness and population-level ecological and evolutionary processes. Factors that affect dispersal could therefore have important eco-evolutionary implications. Here, we investigated the extent to which an inflammation and tissue repair response – peritoneal fibrosis – which is known to restrict movement, could influence dispersal by conducting a mark-recapture experiment in a lake in Alaska with threespine stickleback (*Gasterosteus aculatus).* A subset of captured stickleback were injected with aluminum phosphate to experimentally induce fibrosis ('treatment group'), and another subset were injected with saline or received no injection– both of which do not induce fibrosis ('control group'). We released all fish at one introduction point and re-sampled stickleback throughout the lake for eight days. We recaptured 123 individuals (n = 47 fibrosis treatment; n = 76 control) and dissected them to determine fibrosis levels. Overall, fibrosis did not affect dispersal. Some compelling (but not statistically significant) trends suggest that early-stage inflammation may affect dispersal, providing opportunities for future work. By showing that effects to dispersal are not important side-effects of fibrosis, these findings improve our understanding of the ecological implications of immune responses.

Introduction

Dispersal, or the movement of organisms with potential for gene flow, can have implications for individual fitness (Ronce, 2007). Dispersal can have fitness benefits in cases where organisms disperse to avoid inbreeding, competition, or suboptimal environments (Bonte et al., 2012). Yet dispersal can be detrimental to individual fitness as it is energetically costly, does not guarantee access to superior habitats, and can increase exposure to predators or parasites (Bonte et al., 2012). Any factors that influence dispersal could therefore have important eco-evolutionary implications.

Immune responses represent one factor that can influence host dispersal (Brown and Shine, 2014; e.g., Møller et al., 2004; Suhonen et al., 2010). Following immune activation, dispersal could increase due to individuals seeking out resources or environments (e.g., behavioural fever; Rakus et al., 2017) or because some immune responses are associated with hormones that correspond to increased dispersal (e.g., testosterone can be associated with both dispersal and aspects of immune functioning in some mammals; Holekamp and Smale, 1998; Muehlenbein and Bribiescas, 2005). Alternatively, dispersal could decrease due to lethargy associated with sickness behaviour (Tizard, 2008), or physical changes to host structures or tissues in response to infection that affect motility. As an example of the latter, fibrosis is an early immunological (inflammatory) and tissue repair response characterized by the development of collagenous tissue in the host body cavity that can result in increased tissue stiffness (Thannickal et al., 2014; Wells, 2013).

Threespine stickleback (*Gasterosteus aculeatus;* hereafter 'stickleback') are often infected with *Schistocephalus solidus*, parasitic tapeworms that develop within the body cavity

of infected hosts (Barber and Scharsack, 2010). Stickleback are the intermediate hosts of *S. solidus* and, once the parasite reaches the infectious stage (~50 mg), infected stickleback display complex parasite-induced behavioural modifications that facilitate transmission to the final bird hosts (Barber, 2013). Stickleback anti-*S. solidus* responses partially occur via the development of fibrosis, which can limit the size of *S. solidus* (preventing parasites from reaching the infectious stage), and can even eliminate infections (Fuess et al., 2021; Weber et al., 2022). Although seemingly advantageous, stickleback that develop fibrosis must then bear the burden of this irreversible and costly immune response, which can include lower reproductive fitness and foraging rates (De Lisle and Bolnick, 2021). Fibrosis can also alter stickleback locomotion, including aspects of the C-start response (rapid, small-scale movement that typically occurs in response to an immediate threat, such as a predation attempt; Matthews et al., 2023). However, it is not yet known how fibrosis influences larger-scale movements - such as dispersal in a lake.

Our objective was to assess the extent to which fibrosis influences stickleback dispersal in nature. To investigate this, we conducted a mark-recapture experiment in a lake in Alaska, where fibrosis was experimentally induced in a subset of fish. We found that fibrosis does not affect dispersal (estimated as distance captured from the release point). However, interesting trends, although not statistically significant, suggest that the timing of fibrosis development may affect dispersal, but additional work will be needed to provide support for these suggestive trends.

Methods

Study location

The work for this project was conducted in June 2023 in Hope Lake (60.421467°, -151.187413°) on the Kenai Peninsula in Alaska. In 2019, stickleback were introduced into Hope Lake from four other populations as part of a series of stickleback introductions in Alaska (Hendry et al., 2024). We chose Hope Lake due to the low natural fibrosis rates in 2023 relative to other Kenai Peninsula lakes (Bolnick et al., 2024).

Capture, mark, and release

Minnow traps were set on June 18th at approximately midnight (D-2 on Figure 1) and were checked at 07 h on June 19th, 2023. Traps were set midway up the lake on both sides. Fish captured on either side of the lake from our access points were kept separate during handling and processing (D-1). We presumed all stickleback included in the experiment were adults based on body size, although it is possible that we included some sub-adults because stickleback show substantial among-population variation in adult body sizes (Reimchen et al., 2016).

Prior to injections, stickleback were anaesthetized using buffered MS-222 (40-75 mg/L pH 7.4). For injections, individual fish were placed on kitchen sponges covered in wet paper towel, with a second piece of wet paper towel covering the eyes and gills. A subset of fish (n = 300) were injected intraperitoneally with 10 µL of alumvax phosphate (OZ Biosciences, San Diego, CA USA; “alum”), a vaccine adjuvant that recruits immune cells to the injection site, causing strong innate immune responses (Kool et al., 2012), and inducing fibrosis in ray-finned

fishes (Vrtílek and Bolnick, 2020). The fibrotic response induced by these alum injections does not differ among any of the stickleback populations introduced into Hope Lake (Bolnick et al., 2024). Another subset of fish (n = 300) were injected with 10 µL of phosphate buffered saline (EMD Milipore, Billerica, MA, USA), which does not induce fibrosis. We used Ultra-Fine needle insulin syringes (capacity: 3/10 mL; length: 8 mm; gauge: 31 g; BD Bioscience, Franklin Lakes, NJ, USA) for the injections. A final subset of fish (n = 57) were anaesthetized but received no injections to ensure that the injections did not cause fibrosis. Fish had their dorsal spine, left pelvic spine, or right pelvic spine clipped to indicate if they received the alum, saline, or handling-only treatment, respectively.

Alum injections occurred from approximately 09 h to 19 h. Saline injections occurred from approximately 19 h to 22 h. Saline injections occurred over a much shorter time because the saline syringes were filled in advance, which is not possible for alum because the alum solution separates and requires frequent mixing – although individuals in both treatment groups were held in captivity, and handled during injections, for approximately the same amount of time. Dorsal spine clips for the handling-only control group took place from approximately 23 h to 01 h. Upon injection, the fish were immediately placed in a recovery bucket outfitted with a bubbler for at least 15 minutes where their swimming was monitored to ensure that they resumed normal behaviour. The fish were then transferred to larger coolers outfitted with bubblers, where they were held until they were released. Approximately 24 hours following the first alum injections (D0), we released 223 fish that received alum injections, 263 fish that received saline injections, and 50 fish that received no injection back into Hope Lake at one common introduction point.

Recapture and fibrosis scoring

Every day for the next eight days (D1 – D8), we set traps along both sides of the lake spaced approximately 20 m apart, starting from the introduction point. On the first day of recapturing (D1), we expected that fish would not move more than 200 m in 24 hours and so we set the farthest traps at 200 m (Bolnick et al., 2009). If marked fish were found in the farthest trap, we added additional traps at 20 m increments on the subsequent days (Figure 1). On D5 no marked fish were captured in the two farthest traps on the left side, and so we removed the single farthest trap on the left side for the subsequent day, so that we could add an additional trap on the right side. For the final three days (D6 – D8), we were trapping close to 500 m from the introduction point on both the left and right sides of the lake (475 m on the left side and 505 m on the right side).

We set the first traps each morning of the experiment at approximately 09 h along the shoreline of one side of the lake (i.e., the left or right side looking outward from the introduction point). Midway through the day, we would set traps along the opposite shoreline. On both sides, we started by setting the traps that were the farthest from the introduction point (it took approximately ten to twenty minutes to set all the traps). We started checking the traps approximately three hours after the first traps were set (~ 12 h). Each day we would check the farthest traps from the release point until we captured five marked fish on both sides of the lake - those presumed to have dispersed the farthest. If we captured more than five fish, the “extra” marked fish would be returned to the lake at the point of capture (n = 15); we returned these fish because fibrosis can take days to develop, and we wanted to maximize variation in fibrosis and

dispersal distance for our analyses. We would then return to the shore and score fibrosis (see below), after which we would return to the lake and check the closest traps until we captured five additional marked fish per side - those presumed to have dispersed the least. To ensure that time of day did not bias our results, we alternated daily which side of the lake was checked first (e.g., on day one we would set traps on the left side of the lake first, and then on day two we would set traps on the right side first). GPS coordinates were collected for each trap, and the distance between the common release point and the trap from which a given stickleback was sampled was used as a proxy of dispersal distance.

We euthanized the fish with a lethal dose of buffered MS-222. Fish were dissected and fibrosis was scored on a scale of 0-4 (Table 1) (Hund et al., 2022). Fish sex, mass (g), standard length (mm), and presence/absence of *S. solidus* was recorded. Carcasses were retained and stored in formalin and brought back to McGill University. These experiments were conducted in accordance with approved IACUC protocols at the University of Illinois Urbana-Champaign (Protocol #: 21031), with permission of the State of Alaska Department of Fish and Game (Permit #: SF2023-110).

Statistical analysis

Statistical analyses were performed in R v 4.3.1 (R Core Team, 2024). A Wilcoxon signed rank test confirmed that fibrosis did not differ between the injection control (saline injections) and the handling control (dorsal clips) groups ($W = 398.50$, $p = 0.23$), so the control and handling control fish were pooled to create a broader ‘control’ group variable for subsequent analyses. We also used a Wilcoxon signed rank test to confirm that alum injections successfully induced

higher extents of fibrosis, and we calculated the mean fibrosis score (+/- SD) for alum-injected and control group fish. We used a chi-squared test to confirm no differences in recapture rates between the treatment and control groups (see '*Results'*).

To investigate the effects of fibrosis on dispersal, we started by calculating the median dispersal distance for fish with or without fibrosis. We also compared the median dispersal distance between alum-injected and control-group fish, but only for fibrotic fish. This comparison accounts for two considerations. First, fish that received alum injections might not have developed fibrosis by the time they were captured; for some stickleback genotypes fibrosis can take ~ ten days to develop (Hund et al., 2022), and our experiment ended approximately nine days after we did the injections. Second, fish from the control group could have pre-existing fibrosis not caused by our experimental treatment. This comparison therefore allows us to roughly compare induced fibrosis (in the alum treatment fish) to pre-existing fibrosis (in the control group fish). We finally calculated the median dispersal distance between alum-injected non-fibrotic fish, and control group non-fibrotic fish. Differences between medians within these three groups were formally evaluated using Wilcoxon signed rank tests. Below, we present results from analyses conducted with the full dataset, but complementary analyses conducted using only fibrotic fish, and only non-fibrotic fish can be found in the supplementary materials (Tables S3-S7).

To investigate the effects of fibrosis on dispersal, we used a structural equation model (SEM) (Rosseel, 2012). This approach is appropriate because of the hierarchical nature of our analyses: a treatment (i.e., alum) induces fibrosis, which may or may not induce a change in

dispersal distance. Path analyses can be more effective than traditional linear mixed models for detecting the effects of alum-induced fibrosis on behaviour (Matthews et al., 2023).

Before modelling, continuous predictors were scaled and centered, and one data point (a saline-injected individual) was removed from the dataset owing to incomplete data collected during the dissections. We first built a base model, comprised of the direct effect of treatment on fibrosis, and the direct effects of treatment and fibrosis on dispersal distance. Treatment was therefore considered to have an indirect effect on dispersal via effects on fibrosis. In a second model, in addition to the base model, we also included the direct effects of sex on fibrosis and dispersal distance, as well as mass (g) on dispersal distance. Although we also collected standard length, we only included mass (g) in this model because mass (g) and standard length (mm) were highly correlated ($r = 0.88$). In this second model, we also included the direct effects of maximum trap distance on fibrosis, and maximum trap distance on dispersal distance. The former effect is important to consider because fibrosis can take a few days to develop, and so fibrosis could increase throughout the experiment. The latter effect is important because we set traps on both sides of the lake, and there could be a side bias, and, because we increased the number of traps during the experiment, the farthest distance the fish could be captured on D1 was not as far as on D8 (i.e., maximum trap distance covaries with the day of the experiment). Finally, although we also recorded the presence of *S. solidus*, prevalence was very low (3%; $n = 4$) and so we did not include *S. solidus* prevalence in the models. In summary, this second model included effects of treatment, fibrosis, sex, mass (g), and maximum trap distance (m). We compared the base model to the more complex model with AIC and the more complex model was considered superior ($\Delta AIC = 53.07$).

To corroborate the SEM findings, we constructed linear mixed models (LMM) (Bates et al., 2015). We constructed a base model with the independent effects of fibrosis, treatment, sex, and mass (g) on dispersal distance (no interactions). We also constructed a second model with a four-way interaction between fibrosis, treatment, sex, and mass (g). In both models, we included maximum trap distance (m) as a random effect to account for side and day effects. The complex model with interactions was the best model ($\Delta AIC = 71.83$), however no interactions were statistically significant (Table S2) and so the interactions were removed to optimize model fit (Engqvist, 2005). For effect sizes, we calculated part R^2 for each predictor in the final model using 1000 bootstrap iterations for 95% confidence interval estimation (Stoffel et al., 2020). No linear model assumptions were violated (Hartig, 2022).

Results

We recaptured 23% (n = 123) of the fish that we released into Hope Lake. Within each treatment group, we recaptured 21% of the alum (n = 47), 25% of the saline (n = 66), and 20% of the no injection (n = 10) fish. Alum injection induced higher extents of fibrosis ($W = 2343$, $p < 0.01$) (mean fibrosis +/- SD: alum-injected fish = 1.28 +/- 1.16; control-group fish = 0.66 +/- 0.97), and recapture rates did not differ between the treatment and control groups ($\chi^2 = 0.36$, $df = 1$, $p = 0.55$).

Only 24 hours post release (D1), half of the captured fish had dispersed more than 100 meters from the introduction point; and, by midway through the experiment (~ D4), over half the captured fish had dispersed more than 300 meters (Table 2). Median dispersal distances did not

significantly differ when comparing all fish (fibrotic vs non fibrotic: W = 1932, p = 0.45), only fibrotic fish (alum vs control: W = 491.5, p = 0.54), and only non-fibrotic fish (alum vs control: W = 392.5, p = 0.80). Nevertheless, irrespective of treatment, the median distance (and interquartile range; IQR) that fibrotic fish dispersed was 260 m (175 m) compared to 250 m (243.5 m) for fish without fibrosis (Figure 2A). Conversely, among only fibrotic fish, the median distance (IQR) that alum-injected fish dispersed was 293 m (175 m), compared to 250 m (189 m) for control group fish (Figure 2B). This comparison was even more exaggerated between the non-fibrotic alum-injected fish, which moved a median distance (IQR) of 300 m (259.5 m), compared to the non-fibrotic control-group fish, which moved 210 m (234.5 m) (Figure 2C).

With the SEM, we did not find an effect of fibrosis on dispersal (Table 3). Unsurprisingly, treatment had a direct effect increasing fibrosis, and maximum trap distance was significantly associated with dispersal distance, confirming the value in including these terms in the models (Figure 3; Table 3). Mass (g) and maximum trap distance (m) also significantly increased dispersal distance, whereas neither sex nor treatment were statistically significant (Figure 3; Table 3). Consistent with these SEM results, only mass (g) was significant in the LMMs and explained about 6% of the variance in dispersal (part R^2 [95% CI] = 0.06 [0.02 – 0.16]) (Table 4). The effect sizes for all other predictors were extremely low (part R^2 [95% CI]: treatment = 0.01 [0.00 – 0.11], fibrosis = 0.00 [0.00 – 0.10], sex = 0.00 [0.00 – 0.10]) (Figure 4).

Discussion

We conducted a mark-recapture experiment in a lake in Alaska to assess how an inflammatory and tissue repair response - peritoneal fibrosis - influences stickleback dispersal. We found that fibrosis does not affect dispersal. However, trends lacking statistical significance suggest that dispersal may be affected by the process of fibrosis development, highlighting opportunities for future work.

Given our large sample size (n = 123), our experiment is compelling in demonstrating that stickleback dispersal is not affected by fibrosis. This result is unexpected, owing to the physical side-effects of fibrosis (e.g., increased body stiffness), and because aspects of fish locomotion are altered by fibrosis in laboratory settings (Matthews et al., 2023). It's possible that dispersal is not affected by fibrosis in nature because stickleback are able to compensate for the physical impacts to tissues by altering aspects of their locomotion (as can sometimes be observed for animals with injuries; Hendry et al., 2022). However, fibrosis is also costly for stickleback in natural settings, negatively affecting foraging success and reproductive fitness (De Lisle and Bolnick, 2021), suggesting that not all costs of fibrosis can be rectified through compensatory behaviours, even when the severity of fibrosis is similar (mean fibrosis in De Lisle and Bolnick, 2021= 0.89 +/- 0.90 vs mean fibrosis here (ignoring treatment) = 0.89 +/- 1.09). One difference between this past study and ours, however, is that whereas they worked with established fibrosis from infection, we experimentally induced fibrosis and our study therefore likely comprises more early-stage fibrosis. Additionally, that study had much higher *S. solidus* prevalence (35% and 7% vs 3% here). In populations with higher *S. solidus* prevalence, behavioural changes – including to dispersal – could be owing to fibrosis, as well as effects of the parasite. These

effects of the parasite could emerge owing to morphological changes (e.g., distended abdomens and altered gait) or direct manipulation by the parasite on host behaviour (Barber, 2013). Nevertheless, our findings raise the question of what other ecological performance features are affected by fibrosis in nature, if any? These might include predator evasion, male nest construction, courtship dances, nest maintenance and egg care.

The effects of fibrosis on dispersal are possibly better reflected in the statistically non-significant trends suggesting that alum-injected fish may disperse farther than control-group fish. Although additional work is necessary to understand if the trend is indicative of something biologically meaningful, the trend suggests that although fibrosis itself does not affect dispersal, side-effects of innate immune (inflammation) activation might, particularly in the earliest stages (i.e., before fibrosis is even visible). Indeed, whereas the median dispersal distance only differed by 10 m for fibrotic fish vs non-fibrotic fish, this difference increased to 43 m for alum-injected vs control-group fibrotic fish, and 90 m for alum-injected vs control-group non-fibrotic fish.

There are several reasons why fish experiencing recent innate immune activation could disperse farther than fish with long-standing fibrosis or no fibrosis, and these mechanisms could be investigated in future research. First, the majority of energetic costs associated with fibrosis could occur in the earliest stages of the immune response when fibrosis is developing. This shift in energetic requirements would explain why fibrosis itself does not affect dispersal, but fibrosis development may. Second, fish might be motivated to disperse to find environmental conditions that alleviate symptoms of discomfort or facilitate the immune response (Rakus et al., 2017). Future work that specifically compares variation in dispersal throughout fibrosis development, along with traits that can be affected by early immune responses (e.g., physiological,

behavioural, metabolic) could provide insight into the possible role of early-stage immune activation on ecological processes.

One limitation to our study is that we could not continuously track stickleback, and capturing stickleback at various distances from the introduction point only serves as a "proxy" for dispersal. It is possible that a fish could have dispersed farther than we estimated, such as if a stickleback dispersed completely around the lake (~ 1500 m) before being trapped. This limitation could be circumvented if work is conducted in study systems where the cumulative dispersal distance can be acquired (e.g., using PIT-telemetry). Continuous tracking could also potentially account for fish that occupy deeper sections of the lake (we were unable to capture these individuals because we trapped along the shoreline). Similarly, half of the stickleback in our study dispersed more than 100 m after 24 hours in the lake, and the lake is < 800 m following the longest shoreline. Effects of fibrosis may have emerged had we conducted this study in a larger lake.

In conclusion, our study shows that stickleback dispersal in nature is not influenced by fibrosis. This finding can serve as a starting point for future work investigating other ecological processes that could be affected by fibrosis. The trends in our data that are not statistically significant also point to a possible utility of the stickleback-fibrosis system for improving our understanding of the ecological implications of innate immune activation. In showing that peritoneal fibrosis, a motility-restricting biological process, does not affect dispersal in a natural system, our study ultimately improves our understanding of how immune responses are associated with ecological processes in nature.

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References

Barber, I. (2013). Sticklebacks as model hosts in ecological and evolutionary parasitology. *Trends in Parasitology* **29**, 556–566.

Barber, I. and Scharsack, J. P. (2010). The three-spined stickleback- *Schistocephalus solidus* system: an experimental model for investigating host-parasite interactions in fish. *Parasitology* **137**, 411–424.

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Soft.* **67**,.

Bolnick, D. I., Snowberg, L. K., Patenia, C., Stutz, W. E., Ingram, T. and Lau, O. L. (2009). Phenotype-dependent native habitat preference facilitates divergence between parapatric and stream stickleback. *Evolution* **63**, 2004–2016.

Bolnick, D. I., Barrett, R. D. H., Choi, E., Eckert, L., Hendry, A. P., Kerns, E. V., Lind, Å. J., Milligan-McClellan, K., Peichel, C. L., Sasser, K., et al. (2024). Destabilized host-parasite dynamics in newly founded populations.

Bonte, D., Van Dyck, H., Bullock, J. M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Saastamoinen, M., et al. (2012). Costs of dispersal. *Biological Reviews* **87**, 290–312.

Brown, G. P. and Shine, R. (2014). Immune Response Varies with Rate of Dispersal in Invasive Cane Toads (*Rhinella marina*). *PLoS ONE* **9**, e99734.

De Lisle, S. P. and Bolnick, D. I. (2021). Male and female reproductive fitness costs of an immune response in natural populations *. *Evolution* **75**, 2509–2523.

Engqvist, L. (2005). The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour* **70**, 967–971.

Fuess, L. E., Weber, J. N., Den Haan, S., Steinel, N. C., Shim, K. C. and Bolnick, D. I. (2021). Between-population differences in constitutive and infection-induced gene expression in threespine stickleback. *Molecular Ecology* **30**, 6791–6805.

Hartig, F. (2022). DHARMa: residual diagnostics for hierarchical (multi-Level / mixed) regression models.

Hendry, A. P., Hendry, C. A., Hendry, A. S., Roffey, H. L. and Hendry, M. A. (2022). Performance of wild animals with “broken” traits: Movement patterns in nature of moose with leg injuries. *Ecology and Evolution* **12**, e9127.

Hendry, A. P., Barrett, R. D. H., Bell, A. M., Bell, M. A., Bolnick, D. I., Gotanda, K. M., Haines, G. E., Lind, Å. J., Paccard, M., Peichel, C. L., et al. (2024). Designing eco-evolutionary experiments for restoration projects: Opportunities and constraints revealed during stickleback introductions. *Ecology and Evolution* **14**, e11503.

Holekamp, K. E. and Smale, L. (1998). Dispersal Status Influences Hormones and Behavior in the Male Spotted Hyena. *Hormones and Behavior* **33**, 205–216.

Hund, A. K., Fuess, L. E., Kenney, M. L., Maciejewski, M. F., Marini, J. M., Shim, K. C. and Bolnick, D. I. (2022). Population-level variation in parasite resistance due to differences in immune initiation and rate of response. *Evolution Letters* **6**, 162–177.

Kool, M., Fierens, K. and Lambrecht, B. N. (2012). Alum adjuvant: some of the tricks of the oldest adjuvant. *Journal of Medical Microbiology* **61**, 927–934.

Matthews, D. G., Maciejewski, M. F., Wong, G. A., Lauder, G. V. and Bolnick, D. I. (2023). Locomotor effects of a fibrosis-based immune response in stickleback fish. *Journal of Experimental Biology* **226**, jeb246684.

Møller, A. P., Martín-Vivaldi, M. and Soler, J. J. (2004). Parasitism, host immune defence and dispersal. *Journal of Evolutionary Biology* **17**, 603–612.

Muehlenbein, M. P. and Bribiescas, R. G. (2005). Testosterone-mediated immune functions and male life histories. *Am. J. Hum. Biol.* **17**, 527–558.

R Core Team (2024). *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rakus, K., Ronsmans, M. and Vanderplasschen, A. (2017). Behavioral fever in ectothermic vertebrates. *Developmental & Comparative Immunology* **66**, 84–91.

Reimchen, T., E., Steeves, D. and Bergstrom, C. A. (2016). Sex matters for defence and trophic traits of threespine stickleback. *Evolutionary Ecology Research* **17**, 459–485.

Ronce, O. (2007). How Does It Feel to Be Like a Rolling Stone? Ten Questions About Dispersal Evolution. *Annu. Rev. Ecol. Evol. Syst.* **38**, 231–253.

Rosseel, Y. (2012). **lavaan** : An *R* Package for Structural Equation Modeling. *J. Stat. Soft.* **48**,.

Stoffel, M. A., Nakagawa, S. and Schielzeth, H. (2020). *partR2: Partitioning R^2 in generalized linear mixed models*. Bioinformatics.

Suhonen, J., Honkavaara, J. and Rantala, M. J. (2010). Activation of the immune system promotes insect dispersal in the wild. *Oecologia* **162**, 541–547.

Thannickal, V. J., Zhou, Y., Gaggar, A. and Duncan, S. R. (2014). Fibrosis: ultimate and proximate causes. *J. Clin. Invest.* **124**, 4673–4677.

Tizard, I. (2008). Sickness behavior, its mechanisms and significance. *Anim. Health. Res. Rev.* **9**, 87–99.

Vrtílek, M. and Bolnick, D. I. (2020). Phylogenetically conserved peritoneal fibrosis response to an immunologic adjuvant in ray-finned fishes.

Weber, J. N., Steinel, N. C., Peng, F., Shim, K. C., Lohman, B. K., Fuess, L. E., Subramanian, S., Lisle, S. P. D. and Bolnick, D. I. (2022). Evolutionary gain and loss of a pathological immune response to parasitism. *Science* **377**, 1206–1211.

Wells, R. G. (2013). Tissue mechanics and fibrosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1832**, 884–890.

Tables

Table 1. Definitions used to score fibrosis (Hund et al., 2022)

Fibrosis score	Definition
0	No fibrosis - visceral organs move around freely.
1	Mild fibrosis - visceral organs are slightly fused together but still move around relatively easily.
2	Moderate fibrosis - visceral organs are fused together, but are not fused to the body wall.
3	Moderate-severe fibrosis - visceral organs are fused together and to the body wall. Skin does not tear when the fish is dissected.
4	Severe fibrosis - visceral organs are completely fused together and to the body wall. Skin tears when the fish is dissected.

Table 2. The median distance (and interquartile range; IQR) at which stickleback were captured for each day of recapturing, along with the maximum possible distance that they could be captured. See Table S1 for trapping information specific to each side of the lake.

Day	n	Median capture distance (m)	IQR	Maximum trap distance (m)
D1	23	100	109.50	250
D2	12	181	77.50	293
D3	15	293	145.50	356
D4	19	320	119.50	398
D5	14	347	278.75	498
D6	21	398	150.00	505
D7	15	340	138.50	505
D8	4	356	56.00	505

Table 3. SEM regression results. Fibrosis does not affect dispersal, but maximum trap distance (m) and mass (g) do. Fibrosis is affected by treatment and maximum trap distance (m). Significant terms ($p < 0.05$) are bolded.

Response variable	Explanatory variable	Estimate	SE	z	p
Fibrosis	**Treatment**	**0.50**	**0.17**	**2.85**	**<0.01**
	Maximum trap distance (m)	**0.19**	**0.09**	**2.17**	**0.03**
	Sex	-0.30	0.17	-1.76	0.08
Dispersal distance	Fibrosis	-3.75	10.08	-0.97	0.71
	Treatment	-4.58	20.06	-0.23	0.82
	Maximum trap distance (m)	**73.92**	**9.69**	**7.63**	**<0.001**
	Sex	-0.40	19.15	-0.02	0.98
	Mass (g)	**32.41**	**9.38**	**3.46**	**<0.01**

Table 4. Results from the linear mixed models. Mass (g) is the only statistically significant factor affecting dispersal. Significant terms ($p < 0.05$) are bolded.

	χ^2	df	p
Fibrosis	0.01	1	0.92
Treatment	1.08	1	0.30
Sex	0.06	1	0.80
Mass (g)	**11.82**	**1**	**<0.001**

Figures

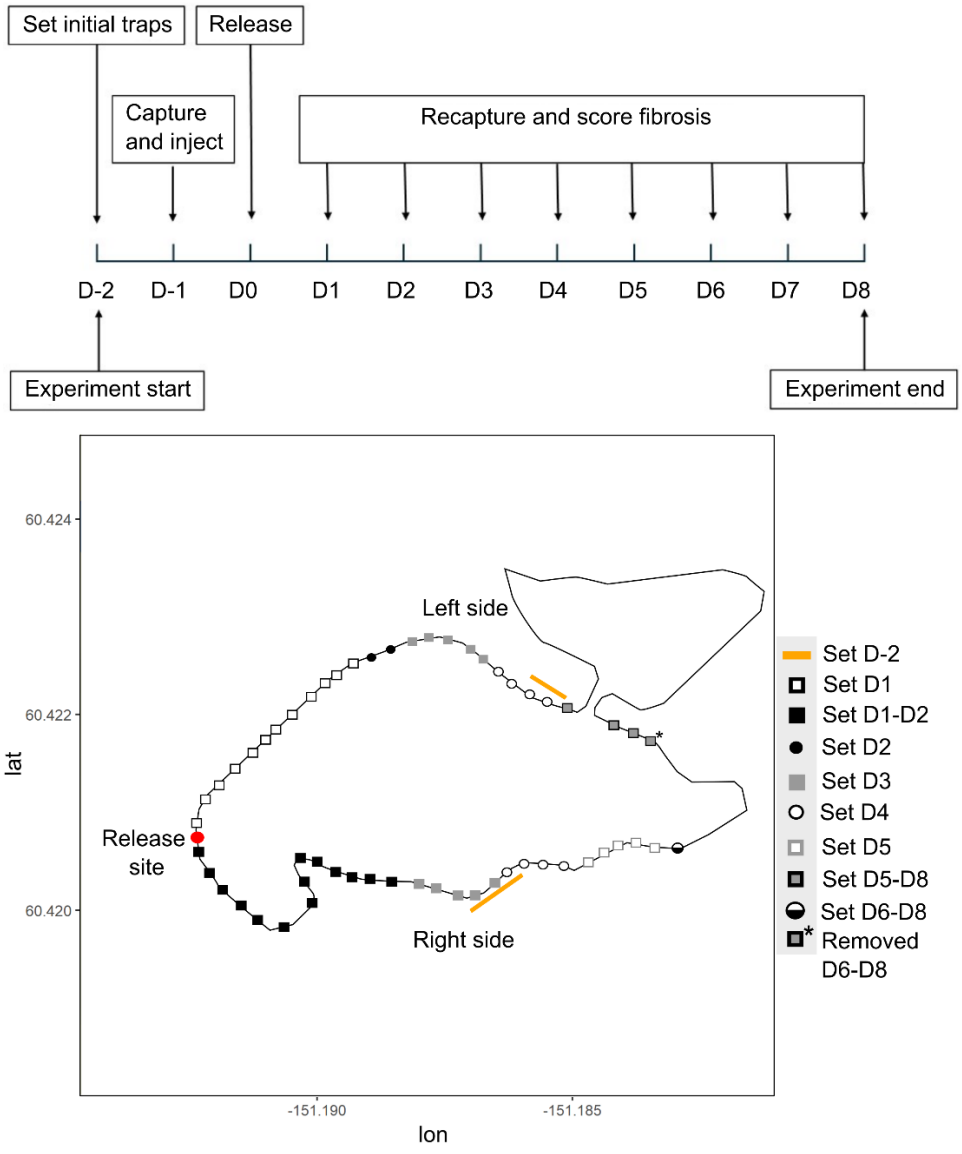

Figure 1. Experimental timeline and map of Hope Lake. On the map, the red circular point represents the release point, and the orange lines represent the stretch of shoreline where twenty traps were set (ten on either side) to capture stickleback for use in the experiment (this line was shifted latitudinally to avoid covering the other points). The other points represent trap locations

set throughout the experiment. The colours and shapes of the data points indicate the day at which the traps were set; the traps that were added on a given day (abbreviated “D”) were set in the same location for all subsequent days of the experiment, except in one case where a trap was removed after only being set for a single day (indicated by the asterisk). The sides of the lake are labeled relative to the release site (left/right).

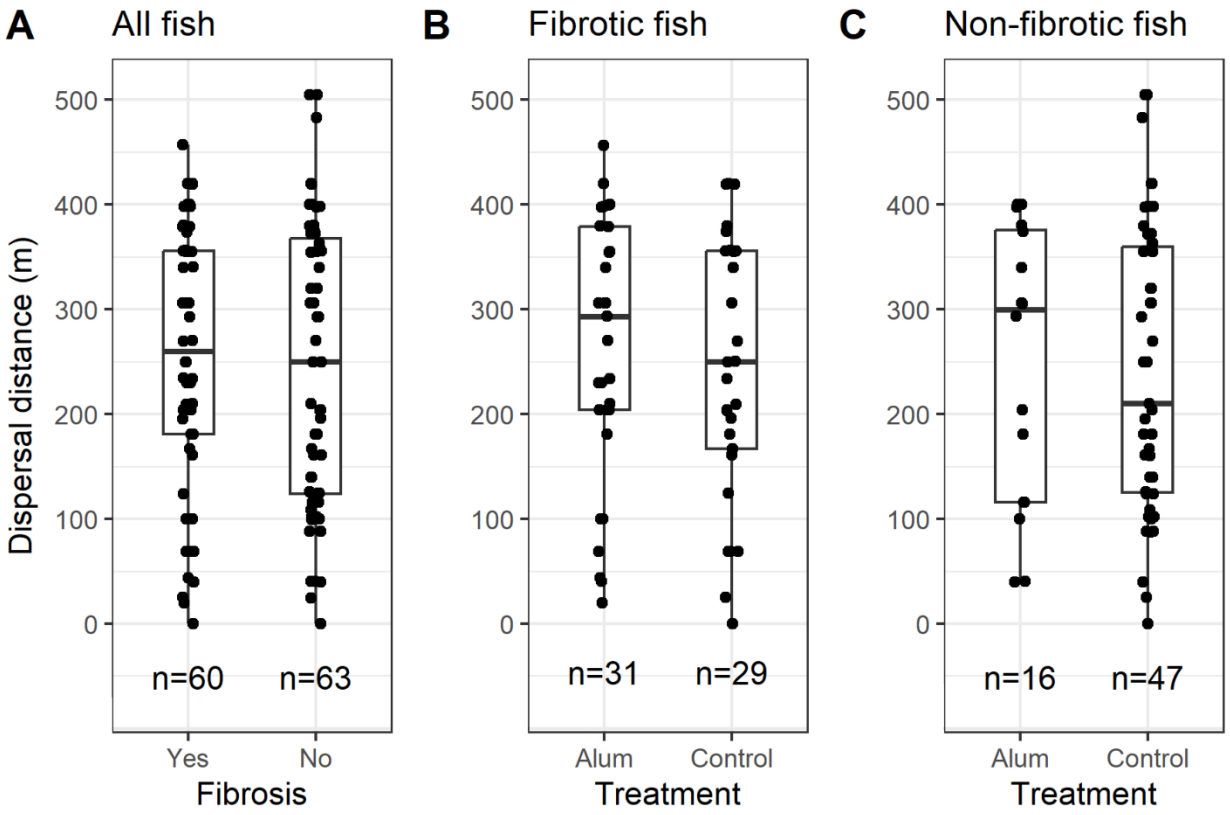

Figure 2. Although (A) fibrosis does not affect dispersal overall, non-statistically significant trends suggest alum-injected fish may disperse farther than control-group fish when only considering (B) fibrotic fish (comparing induced fibrosis to long-standing fibrosis) and (C) only non-fibrotic fish (comparing induced, early-stage fibrosis that is not yet visible to no fibrosis). The midline of the boxplot denotes the median, and the jittered data points represent individual fish. Sample sizes are displayed for each group. The differences presented within each panel are not statistically significant.

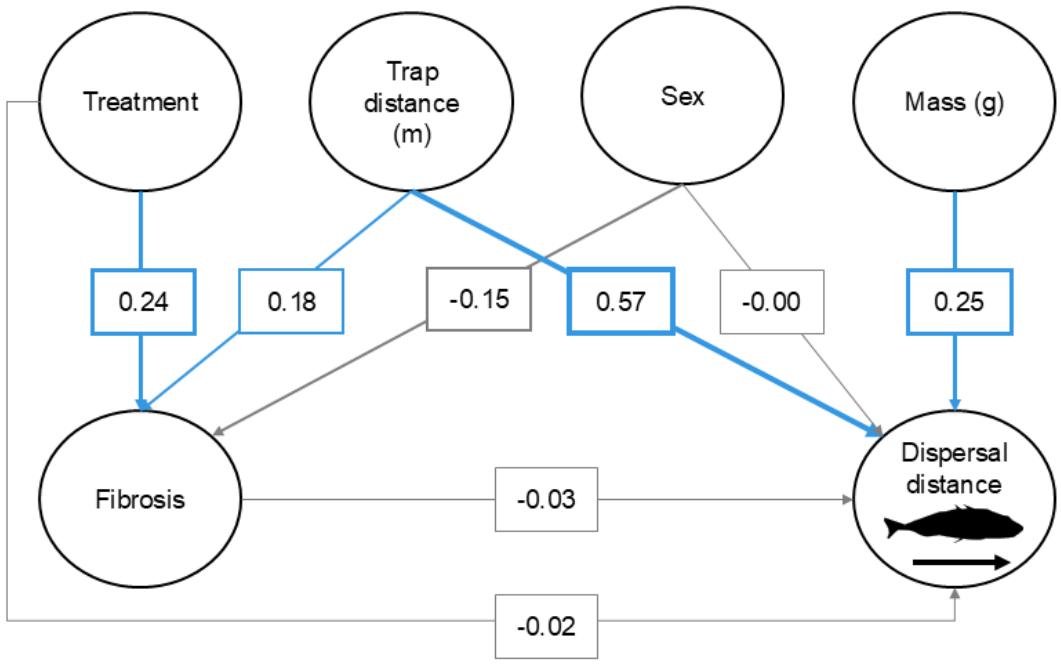

Figure 3. SEM results showing that fibrosis does not affect dispersal, although some other factors do. The blue lines indicate positive associations, the grey lines indicate negative associations, and the numeric values represent standardized path coefficients, which are also reflected in the width of the lines.

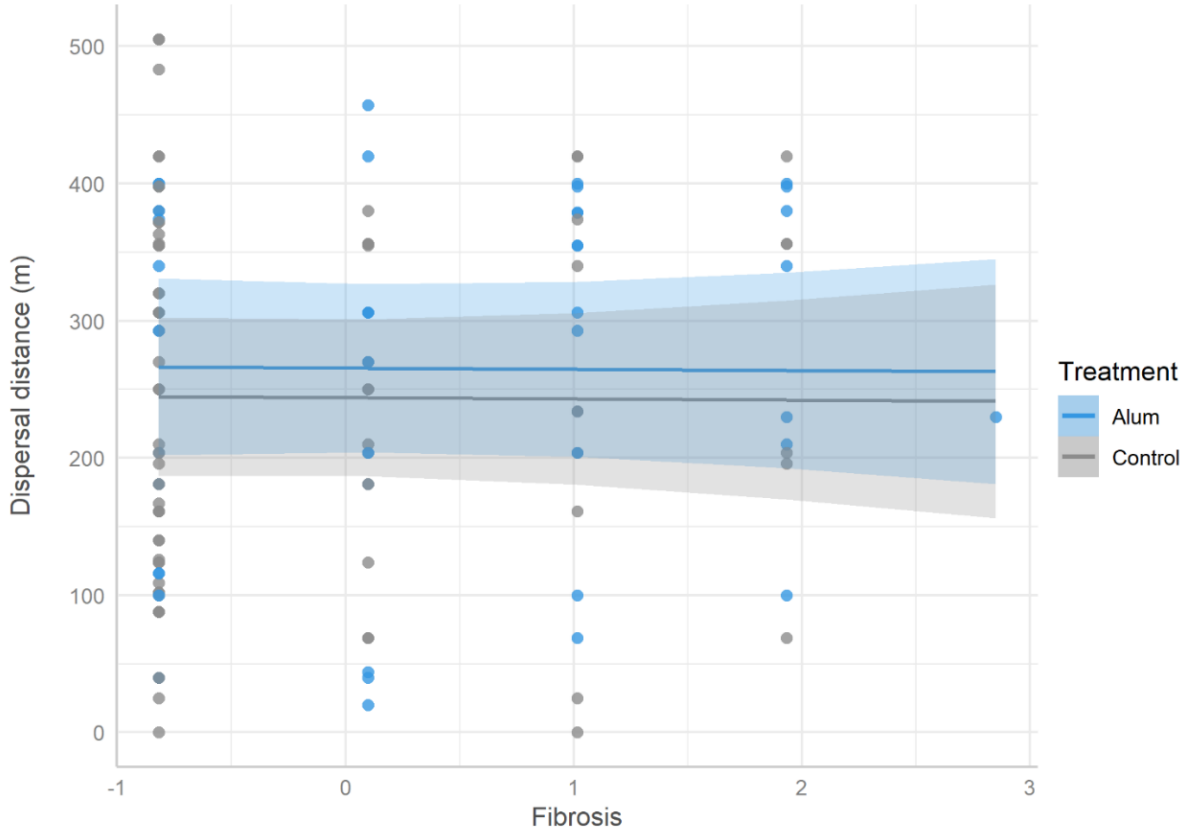

Figure 4. LMM results showing that fibrosis does not affect dispersal. The lines and ribbons are predicted by the model, and the data points are the raw data. Fibrosis is z-score standardized, so values on the x-axis do not correspond to the literal measured fibrosis scores.

Bridging statement 3

In Chapter 3, I investigate the extent to which variation in dispersal, which is an ecologically important process, might arise as a consequence of variation in peritoneal fibrosis. My results show that the effects of fibrosis on dispersal are not predictable, because fibrosis explained 0% of the variance in dispersal distance. I did observe some possible effects of early-stage inflammation on dispersal, although these results were not statistically significant, and more work will be required to scrutinize these suggestive trends.

For my next two chapters, to investigate the evolutionary and applied consequences of intraspecific variation, I leverage conservation translocations where stickleback populations were experimentally founded (as opposed to in Chapter 3 where I experimentally induced variation). In these translocations, stickleback from up to eight 'source' populations were introduced into lakes with no other stickleback. Because the identity of the source populations are known, this experimental design enables me to ask how variation within and among the source populations can translate to movement in novel environments.

In Chapter 4, I specifically investigate how intraspecific variation (among-population differences) can affect spatial assortment following introduction into a novel environment. By shaping the extent of admixture, spatial assortment can provide insight into how evolution might proceed in those environments.

Chapter 4: A test for spatial assortment following introduction into a novel environment: An experiment with threespine stickleback

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Abstract

When individuals from multiple populations are introduced into a novel environment, the extent to which they assort non-randomly will shape how evolution unfolds and the outcomes of introductions. However, the ancestry of introduced organisms is typically unknown in the early stages of introductions, and investigating assortment with respect to ancestry can be challenging. We introduced 2098 threespine stickleback (*Gasterosteus aculeatus*) from eight 'source' populations into a 'recipient' lake with no stickleback. Three years after introduction, we set traps around the entire recipient lake perimeter, genotyped two fish from every trap to determine the proportion of their ancestry represented by each 'source' population, and recorded microhabitat information for each trap (e.g., substrate type, depth). We assessed the extent to which individuals assorted by ancestry within a trap and by geographic distance. Furthermore, we explored how microhabitat features associated with ancestry. Assortment was random with respect to ancestry (by trap and distance). There was a weak trend suggesting that individuals in deeper traps might be more genetically similar than individuals in shallow traps. Overall, our results indicate that genetic variation (and evolutionary potential) appear to be high in the recipient lake.

Introduction

During biological introductions, such as invasions or conservation translocations, individuals are sometimes introduced from multiple populations (Dlugosch & Parker, 2008; Nistelberger et al., 2023). Introductions with multiple founding populations can be more successful than single-source introductions, owing in part to increased genetic diversity (e.g., Doorduin et al., 2010; Nistelberger et al., 2023). However, the extent of genetic diversity – and thus the evolutionary trajectories of introduced populations – might be more complex than just the number of founding populations. These dynamics could also be shaped by how individuals from different populations spatially assort in novel environments. Spatial assortment can be random or non-random, and non-random assortment can be further divided into two types based on the direction of the association (Jiang et al., 2013). With negative assortment, individuals are more likely to associate with more dissimilar individuals, and with positive assortment individuals are more likely to associate with more similar individuals.

There are at least three mechanisms that could generate non-random spatial assortment following introductions into novel environments. First, individuals could preferentially assort with other individuals that have particular phenotypes or genotypes (e.g., Brask et al., 2019; Elmer et al., 2009) – this is sometimes called 'social' assortment (Brodie et al., 2022). Second, individuals could non-randomly assort by geographic distance, such that individuals that are physically closer in proximity could be more similar (e.g., Hudson et al., 2016). These patterns could emerge owing to social assortment reflected at broader-scales or could also be generated following introductions if individuals differ in dispersal propensity or in key traits that are correlated with dispersal distance (i.e., spatial sorting; Shine et al., 2011). Third, individuals

could non-randomly assort owing to variation in (micro)habitat selection, which would be expected to occur if individuals colonize sites with (micro)habitat features to which they are better adapted (i.e., matching habitat choice; Edelaar et al., 2008). In all cases, non-random spatial assortment could lead to assortative mating (Jiang et al., 2013), resulting in offspring that are also more similar to nearby conspecifics than would be expected by chance, reinforcing the effects of social, geographic, and (micro)habitat assortment (e.g., Munar-Delgado et al., 2024).

How individuals from different populations assort in novel environments can shape the evolution of introduced populations owing to consequences for admixture. Both random and negative assortment could generate high extents of admixture, which can be beneficial for introduced organisms owing to increased standing genetic variation, the introduction of novel genetic variation via recombination, and reduced inbreeding load (Verhoeven et al., 2011). However, admixture could also lead to outbreeding depression, hindering local adaptation. By contrast, positive assortment could limit admixture, possibly increasing inbreeding load but facilitating local adaptation. Examples exist of both positive and negative social assortment (e.g., Brask et al., 2019; Chock et al., 2017; Elmer et al., 2009; Hedrick et al., 2016; Takahashi & Hori, 2008), suggesting that this mechanism could increase or decrease admixture in novel environments if preference is associated with ancestry. Both dispersal-mediated geographic assortment and (micro)habitat assortment could reduce admixture if populations differ in their dispersal patterns or in the habitats to which they are better adapted. (Micro)habitat assortment via habitat matching differs from social and geographic assortment in that individuals are selecting habitats to which they are already well-adapted. Microhabitat assortment thus could provide the fastest route to divergence and speciation following introductions.

In the present study, we conducted a manipulative experiment in a natural setting by translocating 2098 threespine stickleback (*Gasterosteus aculeatus*; hereafter simply 'stickleback') from eight "source" populations into a single "recipient" lake that had no other stickleback. In doing so, our study offers a nearly unprecedented opportunity for observation of how individuals from multiple populations spatially assort in the early stages following introduction into a novel environment. We explored three questions to investigate possible mechanisms of spatial assortment. First, to what extent are individuals socially assorting by shared ancestry? Here, our proxy for a social interaction was two individuals being captured together. Second, to what extent are individuals that are geographically closer more ancestrally similar? With this question we investigated both spatial autocorrelation and capture distance from the introduction point. These metrics could reflect broader-scale social assortment and/or dispersal-associated spatial assortment. Finally, to what extent are environmental variables related to microhabitat associated with shared ancestry and ecotype? The goal of this final question was to provide insight into the role of matching habitat choice in generating spatial assortment in novel environments.

Methods

Study system and field methods

Stickleback are a small teleost fish found in marine and coastal freshwater environments throughout the northern hemisphere (Reynolds et al., 1995). We focused on stickleback populations inhabiting lakes. Lacustrine stickleback consume a combination of benthic prey on the substrate (e.g., chironomids) and limnetic prey in the water column (e.g., zooplankton).

Lacustrine stickleback populations show morphological adaptations that reflect these prey choice preferences, and populations can be categorized into two ecotypes: benthics and limnetics (Willacker et al., 2010). Stickleback have been found to assort based on various factors (and to varying extents), such as ecotype (Vines & Schluter, 2006), kinship (Frommen & Bakker, 2006), diet (e.g., Ingram et al., 2015; Snowberg & Bolnick, 2008, 2012) and body size (e.g., Peuhkuri et al., 1997).

This experiment took place on the Kenai Peninsula in Alaska in an 8.9-hectare lake called Loon Lake (60.519245°, -151.049865°). Loon Lake is one of many lakes involved in a series of whole-lake restoration experiments (Hendry et al., 2024). These lakes were treated with rotenone in 2018 to eradicate the invasive northern pike (*Esox lucius*), which rendered the lake fishless. The following year, we collected stickleback from eight other naturally occurring "source" populations in Alaska to repopulate these fishless lakes. The source populations were deliberately selected as they fall along the extreme ends of the benthic-limnetic axis relative to other nearby populations (i.e., they are the most benthic or the most limnetic) (Hendry et al., 2024). In Loon Lake, we introduced a total of 2098 stickleback from four populations with phenotypes corresponding to the benthic ecotype: Finger Lake (n = 299), Tern Lake (n = 170), Watson Lake (n = 294), Walby Lake (n = 301); and four populations with phenotypes corresponding to the limnetic ecotype: Long Lake (n = 287), South Rolly Lake (n = 275), Spirit Lake (n = 300), Wik Lake (n = 172) (Hendry et al., 2024) (Figure 1). Individuals from all eight populations were introduced together at one common point.

In 2023, three years after the introduction, we set 100 traps around the perimeter of Loon Lake. For each trap, we took GPS coordinates, trap depth (m) measurements, and reported the

total number of stickleback in each trap and the total time the trap was set. We also recorded several microhabitat variables that have been previously considered for stickleback (Stuart et al., 2017). We recorded distance to aquatic vegetation (m), vegetation 'type' (none, emergent macrophytes only, emergent macrophytes and logs, and submerged macrophytes and logs – there were no instances with only submerged macrophytes). We also recorded substrate 'type' (detritus, sand, silt), fringing terrestrial habitat (forest, grassy marsh, brushy marsh, muskeg, forest/muskeg), and water habitat (along the shore, in open water, in an inlet). Lastly, we recorded any bycatch in the traps (dragonfly larvae, caddisfly larvae, trout).

We collected fin clips from up to five individuals per trap. We did not sample any stickleback when traps had <2 individuals, we sampled all stickleback when traps contained between two and five individuals, and we haphazardly selected five fish to sample when traps contained >5 stickleback. Prior to collecting fin clips, individuals were anaesthetized with MS-222. We removed the upper or lower half of the caudal fin, and we stored these fin clips in ethanol-filled vials labelled with trap number. All fish were released at the point of capture.

Genotyping

To infer the ancestry of fish, we genotyped individuals for autosomal single nucleotide polymorphisms (SNPs) that are specific to each source population. In 2018, our larger team sampled between 58-100 fish from each source lake and sequenced them as pools (see Weber et al. (2022). We selected 24 SNPs specific to each population, maximizing the frequency of the SNPs in each source population, and ensuring that every chromosome had at least one SNP from each source to minimize potential effects of linkage, while also filtering out SNPs with low read

numbers (more than 1.5 SDs fewer than the mean read number). To validate the efficacy of these SNPs in correctly assigning ancestry, we genotyped 16 individuals from each source population from samples collected in 2018 (from Haines et al. (2023); separate from those used to generate the pool-seq data). We found that all these individuals were assigned the correct ancestry based on the genotyping results. However, this trial run did illuminate some SNPs that were completely unsuccessful or that underperformed (i.e., were present in the trial fish in much lower frequency than expected from the pool-seq data). We omitted these SNPs from the subsequent analyses. The final list of SNPs included 154 in total with an average frequency of 82% in the respective source populations. To infer the ancestry of fish from the genotyping data, we computed an ancestry "score" for each source population for each individual. This score was based on the number of unique SNPs identified from each source in that individual, weighted to account for differences in the number of SNPs included for each source and the average frequency of those alleles in the source populations.

We assessed the accuracy of this method in correctly inferring ancestry across generations by simulating the scenario in R (v 4.4.1; R Core Team, 2024). Given that these fish were sampled three years post-introduction, and that stickleback can live for several years and have a minimum generation time of 1 year, we assumed that our sample was likely a mix of the F2 and F3 generations. In these simulations, the real and inferred ancestries are rounded to the closest proportion that makes sense for that generation (0.25 in F2, and 0.125 in F3) before being compared. In the F2 generation, we have an average accuracy of 93.5%; for the individuals whose proportion of ancestry we mis-infer, we still identify the correct set of ancestral populations about half the time, leaving just 3.5% of F2 individuals where we fail to identify one of ancestral populations. For the F3 generations, the level of admixture often causes our method

to round to an incorrect proportion, as we only infer the exact ancestry for 33% of the time. However, we still identify the correct set of ancestral populations 86.5% of the time. Even though this method is prone to error in inferring the exact ancestry, it is still highly accurate in identifying the correct ancestral populations and is therefore appropriate for our downstream analyses.

We genotyped 192 individuals from fin clips collected from individuals from 96 traps. Three fin-clips failed genotyping (ancestry was unable to be determined), and the traps from which they were sampled were excluded from subsequent analyses. Our final dataset thus comprised 186 individual fish distributed across 93 traps.

Statistical analysis

Statistical analyses were conducted in R v 4.4.1. We first assessed the extent to which the spatial assortment of individuals within the lake was non-random with respect to social interactions (where our proxy for a social interaction was that two individuals were captured together). For this objective, we compared our real dataset (n = 93 traps) to a simulated dataset that was generated by combining every possible combination of two individuals from our dataset (n = 17,205 ‘traps’). We then calculated the proportion of ancestry that was shared for both individuals within a given trap (real and simulated; Table 1). To compare the differences between the proportion of shared ancestry in the real and simulated traps, we built linear models using the lme4 package (Bates et al., 2015) with the proportion of shared ancestry as the response variable, and the data ‘type’ (real or simulated) as the predictor variable. For an effect

size, we extracted eta-squared from this model using the effectsize package (Ben-Shachar et al., 2020).

We next assessed the extent to which the spatial assortment of individuals within the lake was non-random with respect to geographic distance. First, we calculated Mantel correlations and Moran's *I* test statistics using the vegan (Oksanen et al., 2022) and ape (Paradis & Schliep, 2019) packages, respectively. Mantel tests and Moran's *I* tests both assess spatial autocorrelation, but Mantel test assesses correlations between two distance matrices and Moran's *I* is used for univariate analyses. For the Mantel test, we used (1) the geographic distance matrix, calculated using the latitudinal and longitudinal coordinates taken for each trap from which stickleback were sampled and (2) the genetic distance matrix, calculated using the source population ancestry estimates (i.e., all of the source lake columns in Table 1). We calculated Moran's *I* using the same geographic distance matrix as the Mantel test (but taking the inverse), along with the extent of shared ancestry within a given trap (i.e., the 'shared ancestry' column in Table 1). Next, we built a linear model with proportion of shared ancestry in a trap as the response variable, and the Euclidian distance between each trap and the introduction release point (i.e., the location where all stickleback were introduced into the environment) as the predictor variable.

We lastly explored the extent to which the spatial assortment of individuals within the lake was non-random with respect to microhabitat. For this, we built two "global" models. The first global model was a linear model in which the proportion of shared ancestry was the response variable, and microhabitat variables were included as predictor variables. Only the independent effects of each predictor were considered (i.e., the predictor variables were not

considered in interactions). The second global model was a linear model in which the mean limnetic ancestry within a trap was the response variable, and microhabitat variables were included as predictor variables (again with no interactions). This second model was originally a mixed effects model with individual-level limnetic ancestry as the response variable, microhabitat variables as predictor variables, and trap number as a random effect to account for the fact that two individuals were sampled from the same trap with the same environmental characteristics. However, the variance explained by trap for this mixed model was extremely low (variance ± SD = 0.00 ± 0.00), which could be owing to the small number of observations within each random effect level (i.e., only two individuals per trap) preventing the within-trap variance from being appropriately estimated. We therefore took the mean limnetic ancestry within the fish sampled for each trap and used this as our response variable to minimize the potential for Type I errors. The microhabitat variables included as predictor variables in both models were: depth (m; z-score standardized), distance to vegetation (m; z-score standardized), substrate composition (detritus, silt, sand), bank slope (marsh, shallow step), and protected water habitat (inlet, open shore, open water). Fringing habitat type (forest, marsh, muskeg, muskeg/forest) was removed owing to high collinearity with bank slope (marsh, shallow step). The presence of underwater logs (yes, no), density of lilies (dense, intermediate, sparse, none), and grass density (dense, intermediate sparse none) were not included owing to high collinearity with distance to vegetation (m).

Because these microhabitat analyses were not intended to assess specific hypotheses, for each global model we built candidate models with every possible combination of predictor variables. The full list of models were compared using Akaike's Information Criterion with small sample size correction (i.e., AICc; hereafter simply 'AIC'), where a lower AIC value is

indicative of a "better" model fit. Although it is most typically suggested that models with ΔAIC ≤ 2 are essentially equivalent at explaining the data, simulations have demonstrated that models with ΔAIC ≤ 6 should be considered to ensure that the best model is included in the final competitive model set (Burnham et al., 2011; Harrison et al., 2018; Richards et al., 2011; Symonds & Moussalli, 2011). We thus ran parallel analyses using both cut-off values. (Another recommended approach is to select all models where the cumulative Akaike Weight is ≥ 95%; Symonds & Moussalli, 2011 – the conclusions gained from this approach are consistent with our ΔAIC ≤ 6 cut-off.) We simplified the competitive model list by selecting the simplest version of competitive models (Harrison et al., 2018; Richards et al., 2011). For instance, if two models were competitive (i.e., ΔAIC ≤ 2 or ΔAIC ≤ 6, depending on the analyses), and one model had only depth as a predictor variable, whereas another model had both depth and substrate type as predictors, the model with only depth would be retained. To obtain parameter estimates, we ran the most parsimonious model if that model had a predictor variable, and we used full model averaging if the model had no predictor (i.e., the intercept-only model was the most parsimonious).

To ensure that the number of stickleback in a trap or the total time that a trap was set did not affect the proportion of shared ancestry in a trap, we constructed two linear models. In these models, the proportion of shared ancestry was the response variable, and either the number of stickleback in a trap or the total time a trap was set as the predictor variables.

Results

Every fish in our dataset had ancestry represented by more than a single source population; as in, there were no 'pure' types (Figure 2). Whereas 14% of individuals had no benthic ancestry (n = 26), all individuals had limnetic ancestry, and the lowest percentage of limnetic ancestry for any individual was 32%. The mean benthic ancestry across all individuals was 23% ± 16% (SD), and the mean limnetic ancestry was 77% ± 16% (SD). Accordingly, the most successful populations were limnetic (Table 2), and the vast majority of individuals had ancestry from two limnetic source populations: South Rolly lake (94% of individuals had this ancestry) and Long Lake (91%). By contrast, the two least successful populations were benthic: Walby Lake (10%) and Tern Lake (9%).

Individuals that were captured in the same trap were not more closely related than would be expected by random chance, as data 'type' (real or simulated) had no effect on the proportion of shared ancestry ($eta^2 = 0.00$; Table 3; Figure 3). Furthermore, we did not find any evidence that individuals were more genetically similar if they were geographically closer (Mantel r = 0.01, p = 0.21) or that the extent of shared ancestry within a given trap was associated with geographic space (Moran's $I = 0.02$, p = 0.23). The distance between the introduction point and capture location also did not affect the proportion of shared ancestry ($F_{(1, 91)} = 0.39$, p = 0.53).

For the microhabitat analysis, starting with proportion of shared ancestry, four models were competitive with $\Delta AIC \leq 2$. Because all four models included depth, the model with only depth ($\Delta AIC = 0.00$) was the most parsimonious and thus considered the best (Table 4). The results from this model indicate that individuals share more ancestry at deeper depths ($F_{(1, 91)} = 5.00$, p = 0.03; $eta^2 = 0.05$). By contrast, 22 models were competitive with $\Delta AIC \leq 6$ (Table S1)

and the intercept-only model (i.e., no predictor variable) was within this cut-off (ΔAIC = 2.84) and thus was considered the most parsimonious (Table 4). This finding differs from the prior result in that it suggests no microhabitat variable explained shared ancestry. However, the model with depth had higher weight than the intercept-only model (w_i = 0.18 vs 0.04, respectively), and depth was notably a parameter in 68% of the models that fell within this more conservative cut-off (n = 14) and had the highest cumulative weight among all terms (w_i = 0.74; Table S1). Nevertheless, the effect of depth was no longer statistically significant when estimated using the ΔAIC ≤ 6 cut-off and full-model averaging (β = 0.03, p = 0.17; Table S2).

Next considering mean limnetic ancestry, 7 models were competitive with ΔAIC ≤ 2 and 21 models were competitive with ΔAIC ≤ 6 (Table S3). The intercept-only model was the most parsimonious in both cases (ΔAIC = 0.00), indicating that microhabitat environmental variables do not affect the mean proportion of limnetic ancestry in a trap (see model-averaged parameter estimates in Table S4).

General linear models confirmed that the proportion of shared ancestry in a trap was not affected by the total number of stickleback captured in a trap (p = 0.57), and nor was it affected by the total time over which a trap was set (s) (p = 0.57) (Table S5).

Discussion

How individuals spatially assort following introduction into novel environments will shape the evolutionary trajectories of newly founded populations. To investigate various mechanisms that could generate non-random spatial assortment, we experimentally translocated stickleback from

eight populations into a single lake with no other stickleback. We analysed spatial assortment in three ways: via social interactions (two fish trapped together was our proxy for an interaction), geographic distance, and microhabitat environmental features. We found that neither social interactions nor geographic assortment differed from random. Some results do point to the role of depth for explaining the extent of shared ancestry in a trap, although the importance of depth varied depending on the model selection approach. Taken together, these findings provide exciting insight into how evolution can unfold during the early stages of stickleback introductions, by showing that stickleback randomly assort, maximizing admixture in newly founded populations.

Population-level spatial patterns

After only three generations in the novel environment, there was not a single individual in our sample of 186 fish that had a 'pure' ancestry fully represented by a single source population. The high degree of admixture suggests limited opportunities for inbreeding effects and indicates that evolutionary potential could be high in newly founded stickleback populations. If similar effects play-out in the earliest stages of other stickleback introductions, then this might have contributed to the wide-ranging success of stickleback as invaders throughout the northern hemisphere (Makhrov et al., 2024).

Despite the high overall admixture, some populations still dominated. At the extreme cases, 94% of all fish had some extent of ancestry represented by South Rolly Lake, whereas only 9% of fish had ancestry from Tern Lake. One implication of this dramatic difference in colonization success is that subsequent evolution will be shaped by the starting phenotypic and

genetic variation of the successful source populations. In the current case, most of the variation in the newly established Loon Lake population can be attributed to South Rolly (the most common ancestry) and other limnetic populations (the least limnetic individual still had 32% limnetic ancestry, whereas many individuals had no benthic ancestry). In the present study, we only investigated how these effects play out in a single lake, which prevents us from commenting on the extent to which this pattern would be repeatable in other introduction events. By focusing our efforts on a single lake, however, we were able to focus our efforts on genotyping individuals from around the entire perimeter of the lake, providing much finer-scale resolution than most past studies, in stickleback and other species, especially those that do single-point sampling.

One logical next step would be to identify the extent to which these colonization patterns are generalizable to other introduction events. For instance, we might expect that the outcomes of other introductions with these populations would be the same if certain populations dominate in all introductions, such that success mostly depends on the identity of populations introduced. However, the outcomes could differ if success mostly depends on features of the recipient environment, or if success is largely owing to chance events. The success of limnetic populations in our study suggests that the second scenario (the local environment) might not be the most important, because Loon Lake is relatively shallow and thus one might have expected that benthic populations would be more successful (Hendry et al., 2024).

Social assortment

Social assortment in the lake, assessed as two individuals being captured together, was random with respect to ancestry. The dominance of particular populations could contribute to this

randomness for social assortment. In other words, perhaps individuals *are* assorting with similar or dissimilar individuals or environments– it just so happens that most individuals are quite similar. It is also possible that patterns of non-random assortment did not emerge because we only sampled two fish per trap, or that trap is an insufficient 'proxy' for a social interaction. These concerns may seem especially plausible because we often captured many more than two fish in a single trap (mean number of individuals per trap = 45, max = 92), and our traps we set for about 7.5 hours, over which time we could capture multiple shoals. However, we found that neither of these factors affected the proportion of shared ancestry in a trap.

A larger explanation at hand – which is not mutually exclusive with the methodological considerations – is population density. Or, more specifically, the change in density over time. Indeed, stickleback were introduced into low-density conditions; we introduced only 2098 stickleback into an 8.9-hectare lake – which, assuming zero mortality, equates to 1 individual per 42 m^2. After three years in the lake, however, the population of stickleback ballooned and became far denser. In < 8 hours of trapping with 100 traps we captured almost 4500 stickleback along the perimeter, representing only a tiny fraction of total fish in the 8.9-hectare lake. Initially, in low density conditions, proximity could be the most important determinant of a social interaction rather than active preference (i.e., because options are limited). Later, in denser conditions, preference could be more highly associated with the fish with which individuals initially shoaled (F0), or the individuals near to where they were born (F1/F2) – in both cases, these preferences would be owing to dynamics occurring in the recipient lake, rather than source population characteristics of the founding individuals. This idea could be tested by doing dichotomous choice tests for familiar vs unfamiliar individuals with individuals collected from

the source populations, and then with individuals reared in low-density/low-sociality environments.

Geographic assortment

Non-random geographic assortment might be expected to arise in introductions owing to variation in dispersal. For instance, individuals from particular populations could differ in dispersal propensity, or in the locations to which they disperse (Edelaar et al., 2008). In testing for variation in geographic assortment, we are investigating the former (dispersal propensity; the latter concerns microhabitat selection – see below). We found that stickleback do not assort by geographic distance. Random geographic assortment is also consistent with some other work that found no difference in dispersal distance for most of these same source populations following introduction into another recipient lake (Heckley et al. In Prep). That other study, however, was much coarser in resolution, only comparing binary dispersal distances (near to where they were introduced vs far from where they were introduced) and was conducted on shorter timescales (one month and one year after introduction vs three years here). Taken together, that study and the present study show that stickleback rapidly and widely disperse throughout novel environments, seemingly independently of any variation in dispersal.

Microhabitat assortment

Pairs of individuals captured together in deeper traps seemed to share more ancestry than individuals captured together in shallow traps. This effect was strongly supported using the most common cut-off value for model selection ($\Delta AIC \leq 2$) but dissipated using more recent

recommendations ($\Delta AIC \leq 6$). Nevertheless, because depth still appeared in 70% of competitive models using the more conservative cut-off, we interpret these findings together to suggest that depth might be associated with the proportion of shared ancestry in a trap, although this effect is likely quite weak, as reflected by the small effect size ($eta^2 = 0.05$) and the near-zero slope for depth ($\beta = 0.04$) with the $\Delta AIC \leq 2$ cut-off.

We did not test for a mechanism explaining why depth would be associated with shared ancestry, and we therefore cannot provide a specific explanation for this result, and nor can we confidently state that it reflects biological relevance. Nevertheless, we report it here because this trend could be worth exploring in future work. One biological process that could explain this depth effect is risk avoidance at depths. Loon Lake is stocked with salmonids – stickleback predators that tend to occupy deeper water depths as adults (Kennedy & Strange, 1982). When predation risks are high, other fish species non-randomly assort with individuals of similar body sizes and shapes (Krause & Godin, 1994). Such a mechanism could result in stickleback in deeper areas (higher risk areas) assorting with more similar individuals. One explanation for why this depth effect was so weak could be the limited depth variation in our dataset. Although Loon Lake is generally quite shallow throughout its entirety, and we would expect stickleback to be near the shoreline during the breeding season because both benthic and limnetic stickleback nest on the benthos, our traps were (as is customary for trapping lacustrine stickleback) only set along the shoreline. Loon Lake is approximately 6.5 m at the deepest point (Hendry et al., 2024), and the mean depth at which our traps were set was 0.7 m (± 0.36 m SD; min – max = 0.3 m – 1.9 m). It is possible stronger effects might have emerged if we were trapping at deeper depths and capturing a wider range of phenotypes. Future studies that replicate our methods could deliberately trap along a wider depth gradient to try and capture a greater extent of variation.

Conclusions

We were motivated to understand how stickleback spatially assort following introduction to novel environments, because non-random spatial assortment will shape the evolutionary trajectories of newly founded populations. Three years after we introduced stickleback from eight populations into a lake with no other stickleback, we set traps around the lake perimeter and genotyped two individuals from each trap, allowing us to investigate how stickleback assort in the earliest stages of introductions. We found that stickleback from all populations were fully dispersed throughout this environment in only a few generations and interacted seemingly indiscriminately with individuals from other populations – although some populations outperformed others in terms of colonization success. In doing so, stickleback seemingly maximize genetic variation and evolutionary potential, which likely contributes to their overall success at colonizing novel environments. Our study thus raises a magnifying glass to how stickleback spatially assort in novel environments, providing some insight into how evolutionary processes might unfold following introductions.

References

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Ben-Shachar, M., Lüdecke, D., & Makowski, D. (2020). effectsize: Estimation of effect size indices and standardized parameters. *Journal of Open Source Software*, *5*(56), 2815. https://doi.org/10.21105/joss.02815

Brask, J. B., Croft, D. P., Edenbrow, M., James, R., Bleakley, B. H., Ramnarine, I. W., Heathcote, R. J. P., Tyler, C. R., Hamilton, P. B., Dabelsteen, T., & Darden, S. K. (2019). Evolution of non-kin cooperation: Social assortment by cooperative phenotype in guppies. *Royal Society Open Science*, *6*(1), 181493. https://doi.org/10.1098/rsos.181493

Brodie, E. D., Cook, P. A., Costello, R. A., & Formica, V. A. (2022). Phenotypic assortment changes the landscape of selection. *Journal of Heredity*, *113*(1), 91–101. https://doi.org/10.1093/jhered/esab062

Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: Some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, *65*(1), 23–35. https://doi.org/10.1007/s00265-010-1029-6

Chock, R. Y., Wey, T. W., Ebensperger, L. A., & Hayes, L. D. (2017). Evidence for a behavioural syndrome and negative social assortment by exploratory personality in the communally nesting rodent, Octodon degus. *Behaviour*, *154*(5), 541–562. https://doi.org/10.1163/1568539X-00003433

Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, *17*(1), 431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Doorduin, L. J., Van Den Hof, K., Vrieling, K., & Joshi, J. (2010). The lack of genetic bottleneck in invasive Tansy ragwort populations suggests multiple source populations. *Basic and Applied Ecology*, *11*(3), 244–250. https://doi.org/10.1016/j.baae.2009.12.007

Edelaar, P., Siepielski, A. M., & Clobert, J. (2008). Matching habitat choice causes directed gene flow: A neglected dimension in evolution and ecology. *Evolution*, *62*(10), 2462–2472. https://doi.org/10.1111/j.1558-5646.2008.00459.x

Elmer, K. R., Lehtonen, T. K., & Meyer, A. (2009). Color assortative mating contributes to sympatric divergence of Neotropical cichlid fish. *Evolution*, *63*(10), 2750–2757. https://doi.org/10.1111/j.1558-5646.2009.00736.x

Frommen, J. G., & Bakker, T. C. M. (2006). Inbreeding avoidance through non-random mating in sticklebacks. *Biology Letters*, *2*(2), 232–235. https://doi.org/10.1098/rsbl.2005.0432

Haines, G. E., Moisan, L., Derry, A. M., & Hendry, A. P. (2023). Dimensionality and modularity of adaptive variation: Divergence in threespine stickleback from diverse environments. *The American Naturalist*, *201*(2), 175–199. https://doi.org/10.1086/722483

Harrison, X. A., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D. N., Goodwin, C. E. D., Robinson, B. S., Hodgson, D. J., & Inger, R. (2018). A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ*, *6*, e4794. https://doi.org/10.7717/peerj.4794

Hedrick, P. W., Smith, D. W., & Stahler, D. R. (2016). Negative-assortative mating for color in wolves. *Evolution*, *70*(4), 757–766. https://doi.org/10.1111/evo.12906

Hendry, A. P., Barrett, R. D. H., Bell, A. M., Bell, M. A., Bolnick, D. I., Gotanda, K. M., Haines, G. E., Lind, Å. J., Paccard, M., Peichel, C. L., Peterson, C. R., Poore, H. A., Massengill, R. L., Milligan-McClellan, K., Steinel, N. C., Sanderson, S., Walsh, M. R., Weber, J. N., & Derry, A. M. (2024). Designing eco-evolutionary experiments for restoration projects: Opportunities and constraints revealed during stickleback introductions. *Ecology and Evolution*, *14*(6), e11503. https://doi.org/10.1002/ece3.11503

Hudson, C. M., Brown, G. P., & Shine, R. (2016). It is lonely at the front: contrasting evolutionary trajectories in male and female invaders. *Royal Society Open Science*, *3*(12), 160687. https://doi.org/10.1098/rsos.160687

Ingram, T., Jiang, Y., Rangel, R., & Bolnick, D. I. (2015). Widespread positive but weak assortative mating by diet within stickleback populations. *Ecology and Evolution*, *5*(16), 3352–3363. https://doi.org/10.1002/ece3.1609

Jiang, Y., Bolnick, D. I., & Kirkpatrick, M. (2013). Assortative mating in animals. *The American Naturalist*, *181*(6), E125–E138. https://doi.org/10.1086/670160

Kennedy, G. J. A., & Strange, C. D. (1982). The distribution of salmonids in upland streams in relation to depth and gradient. *Journal of Fish Biology*, *20*(5), 579–591. https://doi.org/10.1111/j.1095-8649.1982.tb03956.x

Krause, J., & Godin, J. J. (1994). Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae): effects of predation risk, fish size, species composition and size of shoals. *Ethology*, *98*(2), 128–136. https://doi.org/10.1111/j.1439-0310.1994.tb01063.x

Makhrov, A. A., Houle, E. Y. K., Hendry, A. P., Derry, A. M., & Lajus, D. L. (2024). Widespread evidence for rapid recent changes in global range and abundance of threespine stickleback (*Gasterosteus aculeatus*). *Fish and Fisheries*, 1–18. https://doi.org/10.1111/faf.12866

Munar-Delgado, G., Pulido, F., & Edelaar, P. (2024). Performance-based habitat choice can drive rapid adaptive divergence and reproductive isolation. *Current Biology*, S0960982224013605. https://doi.org/10.1016/j.cub.2024.10.006

Nistelberger, H. M., Roycroft, E., Macdonald, A. J., McArthur, S., White, L. C., Grady, P. G. S., Pierson, J., Sims, C., Cowen, S., Moseby, K., Tuft, K., Moritz, C., Eldridge, M. D. B., Byrne, M., & Ottewell, K. (2023). Genetic mixing in conservation translocations increases diversity of a keystone threatened species, *Bettongia lesueur*. *Molecular Ecology*, mec.17119. https://doi.org/10.1111/mec.17119

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., … Weedon, J. (2022). *vegan: community ecology package*. https://github.com/vegandevs/vegan

Paradis, E., & Schliep, K. (2019). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*(3), 526–528. https://doi.org/10.1093/bioinformatics/bty633

Peuhkuri, N., Ranta, E., & Seppä, P. (1997). Size-assortative schooling in free-ranging sticklebacks. *Ethology*, *103*(4), 318–324. https://doi.org/10.1111/j.1439-0310.1997.tb00021.x

R Core Team. (2024). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing.

Reynolds, J. D., Bell, M. A., & Foster, S. A. (1995). The evolutionary biology of the threespine stickleback. *The Journal of Animal Ecology*, *64*(3), 418. https://doi.org/10.2307/5902

Richards, S. A., Whittingham, M. J., & Stephens, P. A. (2011). Model selection and model averaging in behavioural ecology: the utility of the IT-AIC framework. *Behavioral Ecology and Sociobiology*, *65*(1), 77–89. https://doi.org/10.1007/s00265-010-1035-8

Shine, R., Brown, G. P., & Phillips, B. L. (2011). An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences*, *108*(14), 5708–5711. https://doi.org/10.1073/pnas.1018989108

Snowberg, L. K., & Bolnick, D. I. (2008). Assortative mating by diet in a phenotypically unimodal but ecologically variable population of stickleback. *The American Naturalist*, *172*(5), 733–739. https://doi.org/10.1086/591692

Snowberg, L. K., & Bolnick, D. I. (2012). Partitioning the effects of spatial isolation, nest habitat, and individual diet in causing assortative mating within a population of threespine stickleback. *Evolution*, *66*(11), 3582–3594. https://doi.org/10.1111/j.1558-5646.2012.01701.x

Stuart, Y. E., Veen, T., Weber, J. N., Hanson, D., Ravinet, M., Lohman, B. K., Thompson, C. J., Tasneem, T., Doggett, A., Izen, R., Ahmed, N., Barrett, R. D. H., Hendry, A. P., Peichel, C. L., & Bolnick, D. I. (2017). Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nature Ecology & Evolution*, *1*(6), 0158. https://doi.org/10.1038/s41559-017-0158

Symonds, M. R. E., & Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology*, *65*(1), 13–21. https://doi.org/10.1007/s00265-010-1037-6

Takahashi, T., & Hori, M. (2008). Evidence of disassortative mating in a Tanganyikan cichlid fish and its role in the maintenance of intrapopulation dimorphism. *Biology Letters*, *4*(5), 497–499. https://doi.org/10.1098/rsbl.2008.0244

Verhoeven, K. J. F., Macel, M., Wolfe, L. M., & Biere, A. (2011). Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1702), 2–8. https://doi.org/10.1098/rspb.2010.1272

Vines, T. H., & Schluter, D. (2006). Strong assortative mating between allopatric sticklebacks as a by-product of adaptation to different environments. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1589), 911–916. https://doi.org/10.1098/rspb.2005.3387

Weber, J. N., Steinel, N. C., Peng, F., Shim, K. C., Lohman, B. K., Fuess, L. E., Subramanian, S., Lisle, S. P. D., & Bolnick, D. I. (2022). Evolutionary gain and loss of a pathological

immune response to parasitism. *Science*, *377*(6611), 1206–1211. https://doi.org/10.1126/science.abo3411

Willacker, J. J., Von Hippel, F. A., Wilton, P. R., & Walton, K. M. (2010). Classification of threespine stickleback along the benthic-limnetic axis. *Biological Journal of the Linnean Society*, *101*(3), 595–608. https://doi.org/10.1111/j.1095-8312.2010.01531.x

Tables

Table 1. Example from our dataset showing how the proportion of shared ancestry was calculated. The values in the columns ‘Finger’, ‘Tern’, ‘Walby’, ‘Watson, ‘Long’, ‘Spirit’, ‘South Rolly’, and ‘Wik’ show the estimated proportion of ancestry from that source population for a given individual (these are the raw values obtained during genotyping). The highlighted grey cells show instances where ancestry is estimated to be shared between individuals within a trap. The ‘Shared ancestry’ column sums the lowest value of shared ancestry (bolded) for a given trap.

Trap	**ID**	**Finger**	**Tern**	**Walby**	**Watson**	**Long**	**Spirit**	**South Rolly**	**Wik**	**Shared ancestry**
1	A	0.00	0.00	0.00	**0.06**	**0.08**	0.20	0.66	0.00	0.52
	B	0.00	0.02	0.00	0.25	0.34	**0.16**	**0.22**	0.00	
2	A	0.00	0.00	0.00	0.00	0.37	0.00	**0.46**	0.16	0.65
	B	0.00	0.16	0.00	0.08	**0.19**	0.00	0.57	0.00	

Table 2. The percent of individuals in our dataset with ancestry from each of the source populations that were introduced into Loon Lake.

Source population	**Ecotype**	**Individuals with ancestry**
South Rolly	Limnetic	94%
Long Lake	Limnetic	91%
Watson Lake	Benthic	76%
Spirit Lake	Limnetic	51%
Finger Lake	Benthic	27%
Wik Lake	Limnetic	13%
Walby Lake	Benthic	10%
Tern Lake	Benthic	9%

Table 3. Linear model showing that the proportion of shared ancestry in a trap does not differ from random.

	β	**SE**	**z**	**p**
Intercept	0.56	0.00	639.34	<0.001
Data type (real)	0.01	0.02	0.74	0.46

Table 4. Model comparison table used to select the "top" model for analyses investigating the microhabitat environmental features that best explain proportion of shared ancestry in a trap. Blank cells indicate that a given term was not present in a model. This table only presents models with ΔAICc ≤ 3 because these models were most relevant for model selection (see explanation in text and supplemental Table S1 for the full table).

Intercept	Bank slope	Depth (m)	Distance to vegetation (m)	Water Habitat	Substrate 'type'	df	ΔAICc	weight
0.58		0.04				3	0.00	0.18
0.58		0.03	0.02			4	1.37	0.09
0.59	+	0.04				4	1.53	0.08
0.58		0.04			+	5	1.93	0.07
0.59		0.05		+		5	2.07	0.06
0.59	+	0.03	0.02			5	2.78	0.04
0.58						2	2.84	0.04
0.60		0.05		+	+	7	2.84	0.04
0.58		0.04	0.02		+	6	2.86	0.04
0.58			0.02			3	2.95	0.04

Figures

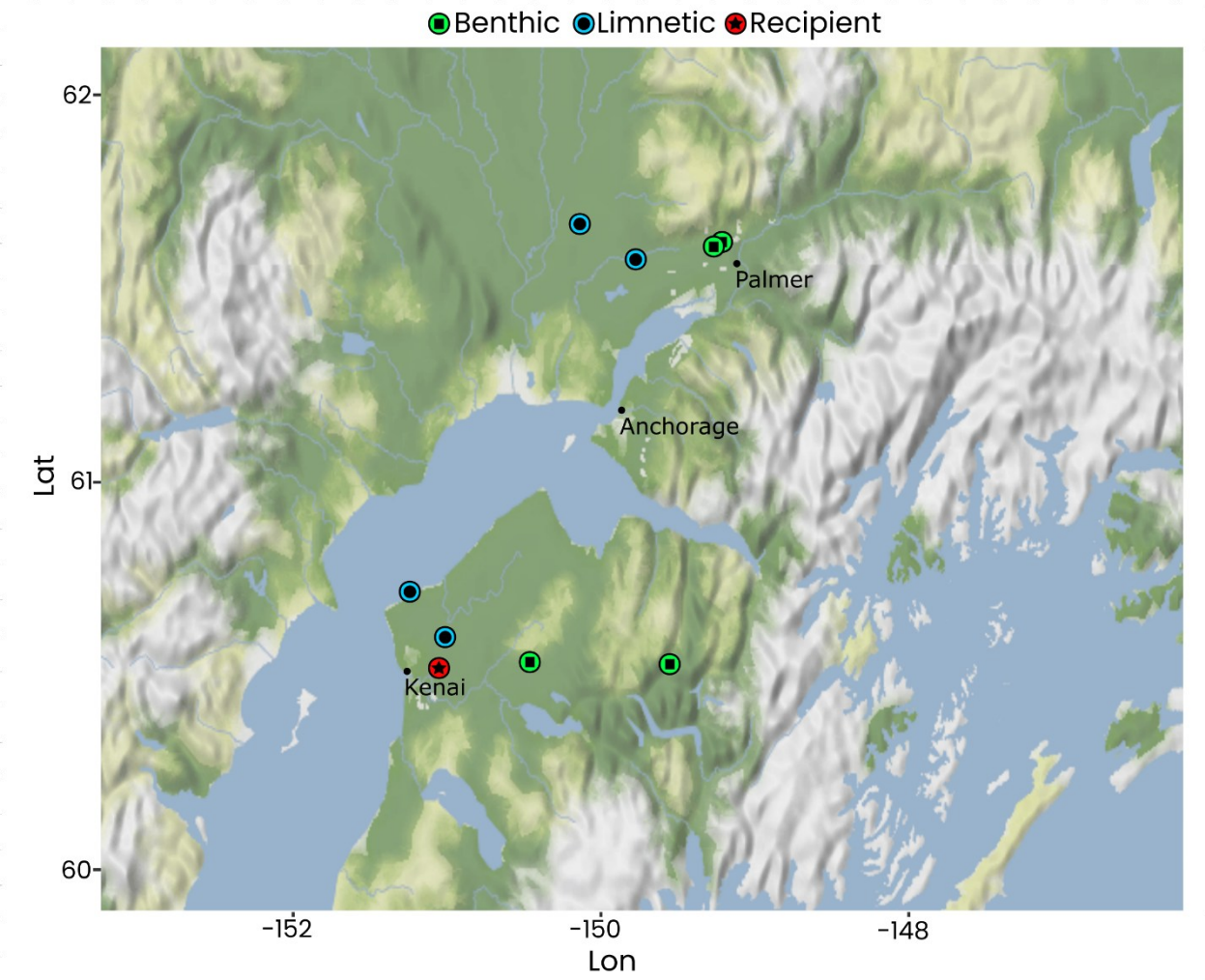

Figure 1. Map showing locations of the source lakes (coloured by ecotype) and the recipient lake. Major cities are labelled in black.

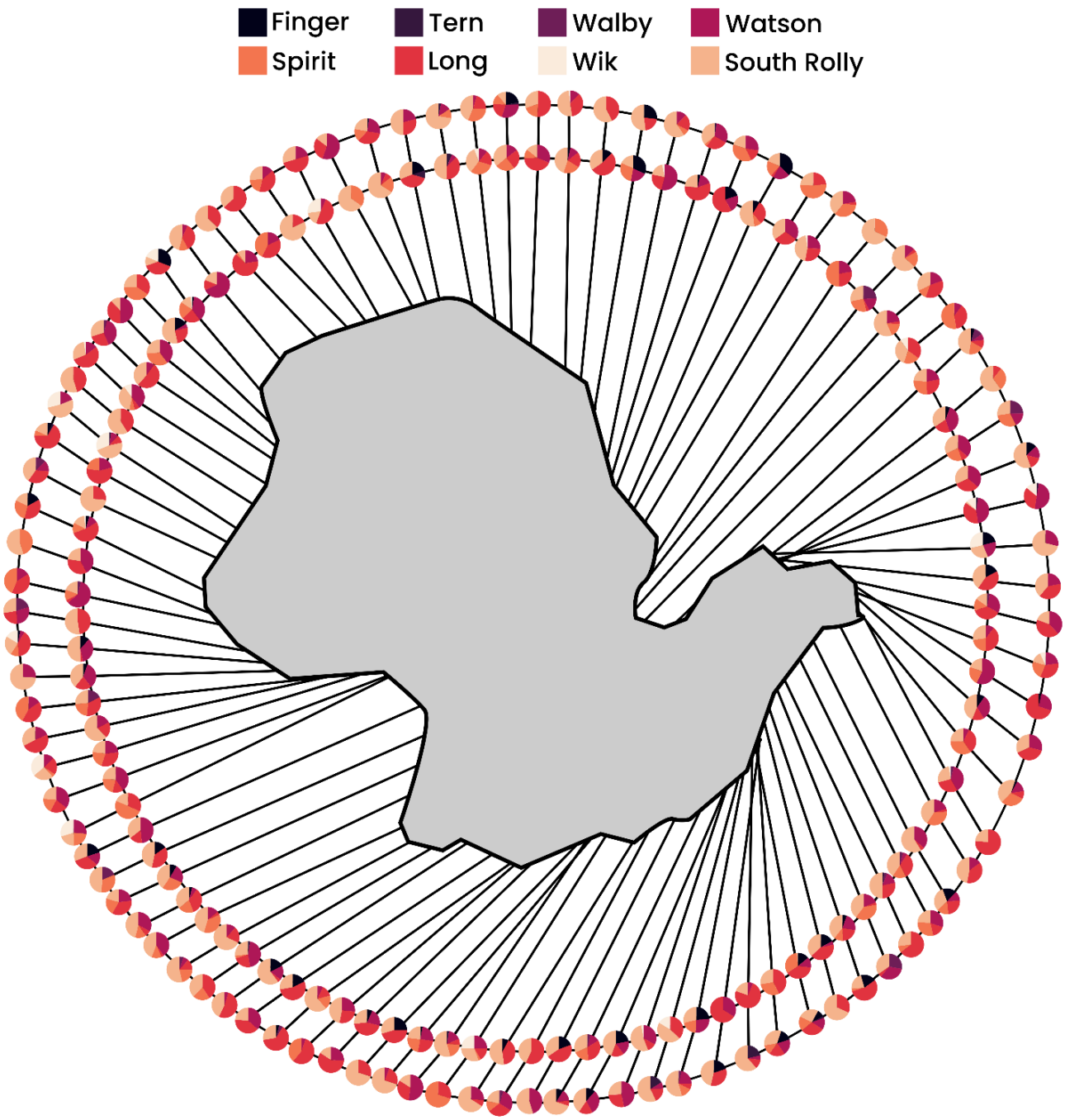

Figure 2. The distribution of genotypes around Loon Lake three years after the introduction. Each pie shows the genotype for a given individual, and the lines connecting the pies to the outline of the lake show the approximate location around the lake perimeter where an individual was trapped. Because we genotyped two individuals from every trap, there are two pies

connecting to the same location on the lake. The colours on the pies indicate the source populations from which they have ancestry; the four darkest colours (the top row of the legend) denote benthic source populations, and the lightest colours (the bottom row of the legend) are limnetic source populations.

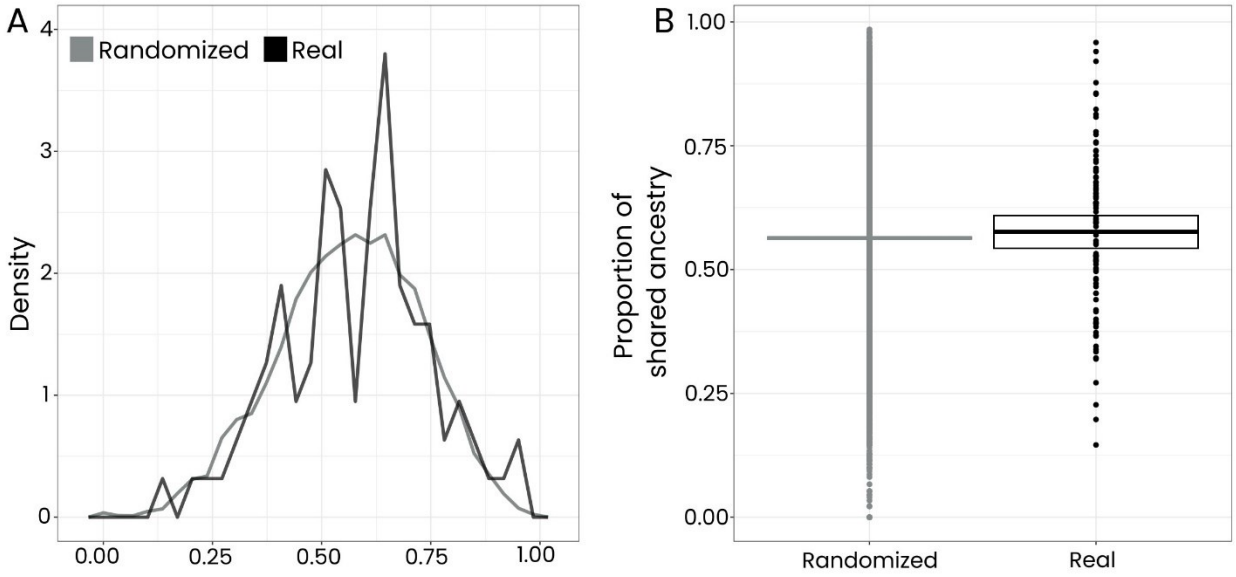

Figure 3. Individuals within a trap do not share more ancestry than would be expected by random chance. (a) Distribution of the proportion of shared ancestry within a trap for the real data and the data that were simulated by pairing every possible combination of fish in our dataset. (b) Effect of data type (real or simulated) on the proportion of shared ancestry within a 'trap'. The proportion of shared ancestry is predicted by the linear model. The midline of the crossbar denotes the mean and the top and bottom horizonal lines are the 95% confidence intervals (these lines are too close to distinguish for the simulated data). The data points are the raw data.

Bridging statement 4

In Chapter 4, I investigated how individuals from multiple populations spatially assort following introduction into a novel environment. I found that stickleback do not assort socially or geographically. Additionally, although I did detect a possible role of depth, this effect was weak. Social and geographic assortment were therefore not predictable, but microhabitat assortment was possibly weakly predictable. The high degree of admixture suggests that evolutionary potential could be high in this recipient population. Additionally, I found that some populations dominated in the introductions, and so subsequent evolution could be shaped by the phenotypes or genotypes of the populations that dominated.

In Chapter 5, my final chapter, I again focus on dispersal (as in Chapter 3), but with a focus on how dispersal could be predicted for use in applied contexts. Because dispersal can affect ecological and evolutionary processes, predicting dispersal could be useful to facilitate desirable (or mitigate undesirable) effects that introduced organisms can have on their environments following translocations or biological invasions. Here, I specifically investigate the extent to which dispersal can be predicted by source population and behavioural variation following a conservation translocation.

Chapter 5: Testing the determinants of dispersal in introductions: insights from a whole-lake conservation translocation of threespine stickleback

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Abstract

Predicting dispersal could be particularly useful during introduction events, such as invasions or translocations, because dispersal can affect how ecological and evolutionary processes play out in novel environments. Bold and exploratory behaviours can influence dispersal and these behaviours vary within and among populations, which could create challenges for predicting how dispersal will unfold in a new environment. We leveraged a conservation translocation where threespine stickleback (*Gasterosteus aculeatus*) from seven "source" populations were introduced into a single lake to explore how source population and behaviour can affect dispersal distance following introduction (our proxy for dispersal was capture location 'near' or 'far' from the introduction point). We found that fish from the different source populations had different bold and exploratory behaviours – but that these differences did not significantly influence dispersal after release into the new lake. At the individual level, however, we found that "weaker" dispersers explored more. This association between dispersal and behaviour documented one month after release disappeared a year later. Our results underscore the value of

conducting experimental manipulations in nature to understand how processes like dispersal unfold in natural systems.

Introduction

Animal dispersal connects organisms within and among environments, influencing individual fitness as well as population-level ecological and evolutionary dynamics (Bonte et al. 2012). Dispersal can be highly variable within and among populations because individuals with different traits (e.g., Stevens et al. 2013), experiences (e.g., Zepeda et al. 2021), genotypes (Saastamoinen et al. 2018), or conditions (e.g., Pan et al. 2019) can differ in their dispersal propensity, or in the locations to which they disperse. The resulting variation in dispersal can influence evolutionary processes. For instance, dispersal can increase gene flow, making local adaptation more difficult (Garant et al. 2007) – but, if genotypes or phenotypes differ in dispersal probabilities, distances, or locations, then dispersal might sometimes facilitate local adaptation (e.g., if organisms settle in habitats to which they are already well-adapted; Edelaar et al. 2008, Edelaar and Bolnick 2012). Similarly, because trait variation can impact processes such as resource consumption and trophic cascades, dispersal variation could also result in the ecological effects that organisms have on their environments varying in space (Hendry 2017, Des Roches et al. 2018). Predicting how dispersal unfolds in natural systems is therefore important to understand the evolution of populations and species and the ecological effects of organisms.

The value of predicting dispersal could be especially useful in situations where systems are perturbed. For instance, environmental disturbances, such as hurricanes or floods, can

influence rates and patterns of dispersal; and also whether or not the dispersers encounter established populations or particular habitats (Blondel et al. 2021, Comerford et al. 2023). Similar effects can attend human-mediated environmental disturbances or introductions of organisms, such as in biological invasions, agricultural contexts, habitat restorations, or conservation translocations (Lindström et al. 2013, Richardson et al. 2015, Hanson et al. 2017, Bilby and Moseby 2024). Our focus in the present paper will be on the translocation context – specifically considering the extent to which source population identity and various measures of behaviour might predict dispersal patterns of individuals introduced to new locations.

The source population of individuals can be relevant in introduction contexts for several reasons. First, biological invasions often involve individuals from many source populations, and it is not always clear which of those populations were the strongest contributors to invasion, and why (e.g., Zardus and Hadfield 2005, Austin et al. 2006). Among-population variation can thus exacerbate challenges associated with predicting and controlling invasions following an introduction event. Second, source population effects on dispersal could be useful when planning and implementing conservation translocations. That is, predictable variation in dispersal could be used to select organisms for translocation that disperse within a target range of distances; that is, enough to spread throughout the desirable area but not so much as to spread into undesirable areas. These are common challenges in translocation efforts (Le Gouar et al. 2012).

How might we predict *a priori* the dispersal of source populations in an invasion or conservation translocation? Perhaps the most direct approach would be to release individuals from multiple populations into a new location and directly measure their dispersal – an experimental approach that is hard to implement in most contexts, whether for logistical, legal,

or ethical reasons. Another approach would be to conduct assays to measure various aspects of behaviour expected to influence dispersal after release into a new location. Two especially likely candidate behaviours are boldness (risk taking) and exploration (movement in an unfamiliar environment) (e.g., Fraser et al. 2001, Myles-Gonzalez et al. 2015, Botero-Delgadillo et al. 2020). These behaviours could influence dispersal in several ways. As one example, bolder individuals might disperse farther because they are less fearful in novel environments (Sih et al. 2012). Of course, such behavioural assays typically need to be conducted in controlled settings, and so uncertainty would remain as to whether they would be effective predictors of dispersal following introduction into a natural environment.

Threespine stickleback (*Gasterosteus aculeatus* – henceforth just "stickleback") are an excellent system to explore these topics because they are used for conservation translocations in their native range (e.g., Bell et al. 2016, Swan et al. 2018, Hendry et al. 2024) and they are also invasive in many freshwater systems (Makhrov et al. 2024). In the present study, we leveraged a conservation translocation where stickleback from seven natural populations ("source" lakes) were introduced at one common release point in a "recipient" lake (Hendry et al. 2024). We first used controlled behavioural assays to measure bold and exploratory behaviours on individuals collected from the seven source populations used for the introduction. (Although boldness and exploration are often studied as "personalities", we do not investigate them as personalities here but rather point-in-time measurements of behaviour that include among-individual differences and individual-level plasticity; Réale and Montiglio 2021.) We then collected individuals one month following introduction into the recipient lake, both near the point of release and at farthest point from the release point in the lake and genotyped them to determine their source lake.

Capture location thus served as our proxy for dispersal, where it was presumed that "strong" dispersed were more likely to be captured far from the introduction point, whereas "weak" dispersers were more likely to be captured near that point. We repeated the collections and behavioural assays (but did not genotype the fish) one year later to determine whether any associations between behaviour and capture location persisted a year after the introduction. Our analyses were designed to answer three specific questions. First, at the population-level: to what extent can source population identity, and the average behaviour of fish from those populations, predict capture location when individuals are introduced into a new environment? Second, at the individual-level: how are source population identity (individual ancestry) and individual behaviour associated with capture location following release into the recipient lake? Third, to what extent do behavioural patterns change between initial release into the recipient lake and a year later? Whereas the first two questions provide insight into the predictive utility of factors that might influence dispersal, the third question provides insight into how the ecological or evolutionary impacts of dispersal variation might persist or change over time.

Methods

Lake descriptions and stickleback introductions

The translocation was conducted in G Lake (hereafter the 'recipient lake' or simply 'the lake') on the Kenai Peninsula in Alaska (60°25'47.6", 151°10'37.7"). The recipient lake is small, at 7 ha and a maximum depth of 9.4 m (Hendry et al. 2024). In 2018, the Alaska Department of Fish and Game treated the lake with rotenone to eradicate the invasive Northern Pike (*Esox lucius*), rendering the lake fishless (Couture et al. 2022). As part of an effort to re-establish the native

fish fauna, members of our team were given the opportunity to repopulate the lake with stickleback. A first attempt to introduce stickleback was made in June of 2019 – but that introduction was unsuccessful, as confirmed by subsequent sampling over the next three years (Hendry et al. 2024).

Our team therefore re-introduced stickleback into the recipient lake in May 2022 (Figure 1). The introduced fish originated from seven "source lakes" in Alaska (Table 1; for a map see Figure 3 in Hendry et al. 2024). Three of the source populations (South Rolly, Spirit, Wik) have stickleback that correspond to the limnetic ecotype and four of the source populations (Finger, Tern, Walby, Watson) have stickleback that correspond to the benthic ecotype (Haines et al. 2023, Hendry et al. 2024). In total, 3495 stickleback (495-500 from each of the seven source populations were introduced into the recipient lake between May 19 and May 25, 2022; Hendry et al. 2024). The releases took place over six days in a series of "waves," with each wave consisting of approximately 50 fish from each of the 7 populations. Additional details on the introductions and source populations can be found in Hendry et al (2024).

Behavioural trials

Minnow traps were used to collect stickleback from each of the source populations and the recipient lake. For the source populations, traps were set in easy-to-access locations near the road or at public access points. Thirty fish were collected from four of the source lakes, except for Watson Lake (n = 28), South Rolly Lake (n = 25), and Wik Lake (n = 0). Because we did not capture any stickleback over several hours in Wik Lake, it was excluded from the analyses corresponding to Question 1 (To what extent can mean source population behaviour predict

dispersal in the recipient lake?). In the recipient lake, to capture potentially "weaker" versus "stronger" dispersers, minnow traps were set along the shoreline near to the introduction point ("near" traps; ~ 0 – 50 m from the introduction point, both 'as the crow flies' and along the shoreline) and on the opposite shoreline farthest from the introduction point ("far" traps; ~ 250m – 300 m from the introduction point 'as the crow flies' or ~ 435 – 500 m along the shoreline) (Figure 2).

Source lake collections for behavioural trials took place in 2023, whereas recipient lake collections took place in both 2022 and 2023 (Figure 1). The goal in sampling the recipient lake twice was to assess behavioural differences shortly after introduction (June 22 – 26, 2022; 31 – 37 days after the first fish were introduced) versus a full year after introduction (June 14 – 17, 2023). In 2022, we captured only 60 individuals, which was expected given that we introduced only 3495 fish into a 7 ha lake. In 2023, reproduction by the introduced fish had increased stickleback density, and we were able to easily capture 100 individuals. Captured fish were held in trap-specific holding buckets equipped with air bubblers before being used in the trials, which were conducted at the side of the lake from which fish were collected.

The experimental "arena" was a 1.8 metre–diameter kiddie pool filled with about 200 L of lake water. This design is similar to other approaches that have been used to measure stickleback behaviour (e.g., Laskowski and Bell 2014, Bensky et al. 2017). Two divisions were used to divide the bottom of the experimental arena into sixteen sections (Figure 3). The first division was into eight equal wedges (like a pie), and the second division was a circle midway from the edge to the centre. A "holding container" made from a red plastic cup with a magnetic door was placed in the centre of the arena. To avoid direct sunlight and other external influences

that might scare the fish or influence their movement, the arena was always inside a gazebo with the sides covered with blackout curtains (Figure 3). After every trial, we removed one bucket of "old" water from the kiddie pool and added one bucket of "new" lake water. Note that fish from each population were tested in their home lake water to reduce any stress associated with non-home water chemistry.

From the holding buckets, we haphazardly selected one fish at a time for use in the trials. The fish was placed in the central holding container and left there for a three-minute acclimation period, after which a magnetic door was removed by hand, granting the fish access to the rest of the arena. Once the magnetic door was removed, the trial began. We first recorded the amount of time, in seconds, that it took for the entire length of the fish to emerge from the holding container (latency to emerge, our measure of "bold" behaviour). If the fish did not emerge after 10 minutes, the container was gently tilted from behind by the observer until the fish emerged. After emergence, we recorded – over a five-minute period – the number of unique sections of the grid drawn along the arena bottom that were entered by the fish as well as the total number of sections crossed. These last two behavioural measures are sometimes used to represent separate behaviours (i.e., exploration vs. activity). However, they were highly correlated in our dataset; the weakest correlation was $r = 0.74$ (South Rolly Lake) and the strongest was $r = 0.88$ (Watson Lake). For subsequent analyses we thus used only the number of unique sections crossed. We consider this behaviour closer to "exploration" than to "activity" because the setting of our assay is more consistent with the common definition that exploration represents movement in an unfamiliar environment, whereas activity is often thought to represent movement in a familiar environment (Réale et al. 2007). After each fish's five-minute trial, it was euthanized with an

overdose of buffered MS-222. Fin-clips were taken for each individual and preserved in 95% ethanol, after which the fish was preserved in formalin.

DNA sequencing

The fin clip samples (n = 60 for the recipient lake fish collected one month after introduction) were transported to McGill University. At McGill, we extracted DNA and genotyped the fish to assign population ancestry for the samples collected one month after introduction. To isolate DNA, we first placed fin clips into a solution of tissue digestion buffer and proteinase K, which was kept overnight at 55°C, and we then conducted a series of phenol-chloroform and ethanol washes. To assign individuals to source populations, we genotyped each fish for fixed or high-frequency Single Nucleotide Polymorphisms (SNP) specific to each source population, which were identified using poolseq data derived from Weber et al. (2022) – see also Hendry et al. (2024). Specifically, we used between 13 and 24 SNPs per population, with an average allele frequency of 81% in their respective source populations. The details of the development of the genotyping arrays and downstream analysis can be found in Bolnick et al. (2024). Using this method, we can accurately assign individual stickleback to the source population from which they were collected 100% of the time (Bolnick et al. 2024). Genotyping failed for two fin clips, and so our final dataset includes n = 58 samples for the recipient lake collected one-month after introduction.

Statistical analysis

Analyses were conducted in the R environment v 4.3.1 (R Core Team 2024). Because we did not catch any stickleback in Wik, Wik could not be included in the analyses investigating behaviour with fish captured from the source lakes. We also did not catch any stickleback with Tern Lake ancestry in the recipient lake, and so Tern Lake is excluded from analyses investigating capture distance.

To first examine how source population identity and behaviour might explain capture location at the population level (Question 1), we explored how behaviour (measured in the assays) and capture location (in the recipient lake) differed among the source populations. For behaviour, we used linear models (LM) in lme4 (Bates et al. 2015) with bold (latency to emerge) or exploratory (number of unique sections crossed) behaviour as the response variable in separate models, with source population as a fixed effect in both models. Source population was fixed rather than random because we were interested in differences among specific source populations, which were not a random selection from all potential source populations. Data in the exploratory behaviour model fit the assumptions of normality and homogeneity of variances, and bold behaviour was cube-root transformed to meet those assumptions (model diagnostics were assessed with the *DHARMa* R package; Hartig 2022). For all models (including those described below), effects of the explanatory variables were assessed with ANOVAs using the *car* package (Fox et al. 2023), with a type 2 sum of squares for models without interactions between predictor variables and type 3 sum of squares for models with interactions.

We then assessed (still Question 1) whether those source-lake properties (identity and average behaviour) might predict immediate dispersal (i.e., the 2022 data) in the recipient lake.

We started with a generalized linear model (GLM) with capture location (near vs. far) as the response variable and source population (inferred by genotyping) as the fixed effect. Because capture location was binary in our study, this GLM model used a binomial error family (i.e., logistic regression). The goal here was to assess the integrated effects of "all" properties that might vary among the source lakes. Next, we built LMs with the population-level mean bold or exploratory behaviour score as the fixed effect, and the proportion of fish from each source population that were captured in far traps in the recipient lake as the response variable (i.e., the proportion of fish from each population that were captured far from the release point). The goal here was to assess the potential effects of specific behaviours that might vary among the source lakes. Note that it was not possible to fit an individual-level GLMM with population as a random variable to these data, and so the analysis is only a rough assessment based on the mean values for the six source populations.

For Question 2, we examined how behaviour and source population were associated with immediate (i.e., 2022) dispersal at the individual level within the recipient lake. For this inference, we used a binomial GLM with capture location (near vs. far) as the response variable. The predictor variables in this model included individual-level data on bold behaviour, exploratory behaviour, and source population (bold and exploratory behaviours were standardized by converting each value into a z-score).

For Question 3, we assessed the extent to which behaviours associated with dispersal distance changed over time by analyzing data for fish collected from the recipient lake in 2022 and 2023. We constructed binomial GLMs with capture location as the response variable, and bold and exploratory behaviours (standardized) as predictor variables, and interactions between

those behavioural variables and year (as a fixed effect as were specifically interested in the order of the two years). Source population was not included in this model because, in 2023, fish had been interbreeding and so could not be genetically assigned to a specific sole-origin source population.

For effect sizes, we extracted eta^2 for each term in the linear models (Ben-Shachar et al. 2020). Following recommendations by Cohen (1988, 1992), as reported by the *effectsize* package (Ben-Shachar et al. 2020), we interpret eta^2 as: very small < 0.02, small <= 0.02 – 0.13, medium <= 0.13 – 0.25, and large >= 0.26.

Results

Population-level variation (Question 1)

The source populations differed in bold and exploratory behaviours (bold: $F_{5, 167} = 7.15$; $p = <0.001$; exploratory: $F_{5, 167} = 3.88$, $p < 0.01$), and source population explained almost a fifth of the total variance in bold behaviour and a tenth of the total variance in exploratory behaviours among all individuals assayed (bold: $eta^2 = 0.18$; exploratory: $eta^2 = 0.10$). Source populations with the highest scores for bold and exploratory behaviours were Finger, Spirit, and Walby; whereas South Rolly, Tern, and Watson individuals were at the other end of the spectrum (Figure 4). Although populations where fish exhibited the boldest behaviour also tended to explore more, the strength of correlations between these behaviours varied within the source lakes (Finger: r = -0.05, n = 30; South Rolly: r = -0.13, n = 25; Spirit: r = -0.68, n = 30; Tern: r = -0.45, n = 30; Walby: r = -0.71, n = 30; Watson: r = -0.18, n = 28).

Despite this variation in behaviour among fish from the source lakes, capture location in the recipient lake was not significantly associated with source population ($X^2_5 = 4.00$, $p = 0.55$; Table 2). Unsurprisingly, then, the mean bold and exploratory behaviours of stickleback collected from each source population was not significantly associated with the proportion of individuals that were captured near versus far in the recipient lake (Bold: $F_{(1,3)} = 0.84$, $p = 0.43$; Exploratory: $F_{(1,3)} = 2.74$, $p = 0.20$). However, despite the lack of statistical significance owing to the need for population-level analysis (i.e., n = 6), it is perhaps worth noting that variation in capture location seemed to be qualitatively associated with mean bold behaviour ($eta^2 = 0.22$) and mean exploratory behaviour ($eta^2 = 0.48$). Indeed, in the greatest apparent contrast, 78% (n = 7) of the Wik fish were captured at the far sites (we were unable to include Wik in the formal analyses for this question because we did not catch any fish in Wik), whereas only 38% (n = 6) of the Walby fish were captured at the far sites (Table 2). We provide this qualitative interpretation only to suggest that additional work might reveal effects of some population-level behavioural differences on movement following release into new locations.

Individual-level variation (Question 2)

At the individual level, the GLM considering bold behaviour, exploratory behaviour, and source population explained about a third of the variance in capture location ($eta^2 = 0.29$). The vast majority of this explanatory power could be attributed to exploratory behaviour, which accounted for over a fifth of the variance in capture location ($eta^2 = 0.22$). Specifically, individuals that were captured near (rather than far from) the introduction point were more likely to cross more unique sections in the arena ($X^2_1 = 13.31$, $p = <0.001$; Figure 5). Stated another

way, individuals that seemed to disperse farther in the lake explored less in the behavioural assays. By contrast, neither bold behaviour nor source population were significantly associated with capture location (bold: $X^2_1 = 2.04$, $p = 0.15$, $eta^2 = 0.00$; source population: $X^2_5 = 4.20$, $p = 0.52$, $eta^2 = 0.07$; Figure 5).

Temporal variation (Question 3)

The interaction between exploratory behaviour and year was statistically significant ($X^2_1 = 8.11$, $p < 0.01$). In 2022, fish at the capture site far from the release location explored less than those at the capture site near to the release location. In 2023, however, no association between capture location and exploratory behaviour was evident (Figure 6a). Neither bold behaviour nor year (independently or in an interaction) affected capture location, and effect sizes for bold behaviour and year were very weak (bold: $eta^2 = 0.00$; year: 0.01; eta^2 = bold-year interaction: $eta^2 = 0.00$; Table 3; Figure 6b).

Discussion

Predicting how dispersal takes place can help to understand ecological and evolutionary processes in nature, and such predictions could be especially useful during introduction events, such as biological invasions or conservation translocations. We approached this topic by asking how source population and behaviour (bold and exploratory behaviours) might explain capture location (near the introduction point vs far from the introduction point; our proxy for dispersal)

in a novel environment – and how any 'dispersal'-associated spatial patterning might change over time. We were able to implement this work by leveraging a large-scale conservation translocation in Alaska where stickleback from seven source populations were introduced into a single lake that required restoration. We found that the different source populations showed differences in behaviour – but that source population, and their average behaviours, were not strongly associated with capture location after introduction. Instead, we found that capture location was associated with exploratory behaviour at the individual level, such that stickleback captured farther from the introduction point (seemingly longer-distance dispersers) explored less than stickleback captured near the introduction point (seemingly shorter-distance dispersers) when tested in a common arena. However, this last effect disappeared by the following year. We now discuss these findings in more detail, highlighting opportunities for future research and some possible applied interpretations of our results.

Population-level variation (Question 1)

We found considerable variation among source populations in bold and exploratory behaviours, a finding that matches previous work showing how these behaviours can differ dramatically among populations of stickleback (Lacasse and Aubin-Horth 2012, De Winter et al. 2016) and other fish species (e.g., Brown and Braithwaite 2004, Brown et al. 2005, Archard and Braithwaite 2011). These results confirm (1) that the methods used in our assays were suitable for discerning behavioural differences among populations, and (2) that the potential was present for population-level differences in the experiment to shape dispersal in the recipient lake (i.e., the populations differed in the behaviours hypothesized to influence dispersal). However, we then

found that source population and average behaviour were not significantly associated with capture location (and, therefore, we assume dispersal) following release into the recipient lake.

Several potential explanations can be advanced for why source population, and average source population behaviour, were not associated with capture location after release. First, it's possible that capture location is not an accurate reflection of dispersal distance; individuals might not have settled in the location at which they were captured, or they may have travelled greater distances before settling in that location. Second, behavioural plasticity might be present such that bold and exploratory behaviours in the assays did not reflect bold and exploratory behaviours after release into the lake. Indeed, the experimental arenas were very different from the real lake and, further, source population behaviour was assessed in source lake water. Third, the recipient lake might be too small (7 ha) for us to have observed population-level differences in dispersal. Indeed, our personal observations confirmed that some fish dispersed to the other side of the lake within a few days following introduction. After a month, it's possible that fish from all populations - even those that could be less inclined to rapidly disperse - were fully dispersed throughout the lake. Population-level differences in dispersal might therefore be relevant only on larger spatial scales or during immediate sampling of the "dispersal front" days, hours, or even minutes after an introduction. Fourth, perhaps other behaviours in experimental assays would have been better predictors of dispersal; although the "source population" effect would be expected to integrate all such behaviours – and it was not significant. Fifth, some source population effects might be present, and we simply would have needed more power (i.e., more individuals) to statistically confirm them (see suggestive trends noted in the Results). Although larger sample sizes would have been beneficial, we would like to reiterate the scale of

the experiment: that is, 3495 fish released into a 7 ha lake would be < 1 fish per m^2 of surface area and our "near" versus "far" sampling could only represent a small fraction of the overall lake.

Regardless, the upshot of our experiment from a stickleback perspective is that, within a month following the introduction, fish from all source populations were found along both shorelines near and far to where they were introduced. Thus, the population from which stickleback are introduced during translocations or invasions is unlikely to strongly impact ecological and evolutionary outcomes that could occur via associations with dispersal – at least when the introductions occur in small and isolated lakes. Given this result for dispersal, other ecologically-important processes or traits (e.g., body size, reproductive rate, local adaptation) could be more useful for mitigating or facilitating the impacts of introduced stickleback, and possibly other freshwater fishes.

Individual-level variation (Question 2)

Despite an absence of clear population-level influences, behaviour was strongly associated with capture location at the individual level immediately (~ 5 weeks) after the introduction. In particular, individuals captured nearest the introduction point (i.e., "weaker" dispersers) explored less than individuals captured farthest from the introduction point (i.e., "stronger" dispersers). This difference is the opposite of what would typically be expected (including by us before the study) given that it would seem intuitive for individuals that explore more (i.e., those that move more on a small scale) to also be more dispersive (i.e., they would move more on a large scale).

We suggest two potential reasons for why seemingly "weaker" dispersers might have been more exploratory in our study.

First, although exploratory behaviour and dispersal are both associated with movement, the two behaviours also have other functions. For instance, exploration is primarily about information acquisition (Rojas-Ferrer et al. 2020), whereas the motivation to disperse can be driven by many factors, such as avoiding predators, parasites, or inbreeding, as well seeking out breeding grounds or other resources (Bowler and Benton 2005). Further, acquiring information about the environment (exploration) could trade off with dispersal, such that more exploratory individuals spend more time investigating their environments, but then move more slowly through those environments overall (dispersal). At least one other study has found a similar pattern to what we observed here: juvenile flying squirrels (*Pteromys volans*) dispersing longer distances explore less than those dispersing shorter distance (Selonen and Hanski 2006). However, that study was done in the context of natal dispersal, where motivations to disperse could differ from conservation translocations where individuals are placed in new environments (Stamps and Swaisgood 2007). With stickleback, one mark-recapture study did not find that exploratory behaviour was associated with movement in a river (Laskowski et al. 2015). One difference between that study and ours is that the stickleback in our study were introduced into a novel environment, whereas in that study the stickleback were not displaced; factors contributing to movement in a completely novel environment might differ from factors that contribute to movement at the fringes of novelty.

Second, the direction of causality might be murky because we assayed behaviour after dispersal, rather than before. Specifically, we did not assay the behaviour of individuals before

release because the assays take time (we averaged approximately three fish per hour), and so could only be done for a small subset of the 3495 fish that were released. The chances of then catching back the specific individuals we assayed before release would be very low – leading to extremely low sample sizes. (Indeed, the 60 total individuals that we re-captured over five days of effort represented only 1.7% of the number released). Owing to this order of assessment (dispersal first, then the behavioural assays), lower exploratory behaviour (or something associated with it) could have caused increased dispersal or increased dispersal (or something associated with it) could have caused lower exploratory behaviour. Regardless of causality, the strong association between exploratory behaviour in an experimental arena and dispersal distance in a lake suggests the value of further work to exporting this association for the extent to which it might be predictable in other contexts as well.

Spatial variation over time (Question 3)

We last assessed how spatial patterns of behavioural variation might shift over time in the recipient lake by also sampling a year after the introduction. In that sampling, we found no notable spatial patterns of behavioural variation; in contrast to the above individual-level association between exploration and dispersal one month after the introduction. It is possible that environmental factors that differ between the shorelines changed between the two years and are underlying this association. Given that we did not measure habitat features on either side of the lake, subsequent work that investigates similar associations between behaviours and dispersal-proxies should attempt to characterize the habitats to rule out any environmental drivers.

On the other hand, this inter-annual change could also reasonably suggest that the earlier behavioural difference is indeed associated with dispersal. If this result is associated with dispersal, it suggests that behavioural associations do not persist or accumulate through time, which could possibly be explained by admixture eliminating this association between behaviour and capture location. This temporal change has several practical implications. First, it suggests that associations between phenotypes and dispersal can be ephemeral – and therefore should be tested early on before they disappear. This idea supports previous assertions of interesting behavioural (and other trait) differences specifically on the "wave front" of invasions or range expansions (e.g., Liebl and Martin 2012, Myles-Gonzalez et al. 2015, Gruber et al. 2017). Such wave-front effects are expected to be transitory and can disappear after the trailing edge of phenotypes "catches up" to the wave front. In the case of stickleback in our 7 ha lake, this shift spears to have taken only a single generation – and perhaps much less as we only assayed one month after introduction (a near vs. far behavioural difference was evident) and twelve months after introduction (no behavioural difference was evident).

Conclusions

Because dispersal is strongly associated with ecological and evolutionary processes, predicting dispersal can provide insight into how these eco-evolutionary processes will unfold in nature (Edelaar et al. 2008, Bonte et al. 2012). In our study, we investigated the extent to which source population and behaviour could predict individual dispersal (with 'capture location' as our proxy for dispersal) in threespine stickleback from seven populations translocated into a single lake in Alaska. Although the populations did differ considerably in behaviour (bold and exploratory),

neither source population nor their average behaviour predicted whether they were found near or far from the release point a month after introduction. From a practical standpoint, this result simply means that pretty much any source population will yield similar dispersive outcomes for stickleback released into lakes. Our study thus complements previous findings that individual stickleback can move long distances (Ward et al. 2013) and that invasive stickleback spread widely to new locations (Makhrov et al. 2024). Specifically, our findings suggest that these outcomes are general to the species rather than specific to the individual populations that are introduced. As a result, future work considering source populations in introduction scenarios would better focus on other stickleback traits, such as foraging traits, defensive armour, parasite resistance, or life history – all of which vary dramatically in our study area (Baker et al. 1995, 1998, Willacker et al. 2010, Haines et al. 2023, Hendry et al. 2024, Bolnick et al. 2024).

Yet we did find a strong – and surprising – association between exploratory behaviour and initial dispersal distance: individuals captured farther from the release site explored less in experimental arenas. The fact this behavioural difference was gone a year later suggests that it may be specifically associated with dispersal – but additional work will be needed to disentangle cause and effect, and whether other environmental features are contributing to this result. Regardless, this result illustrates that exploration could serve as a possible tool to predict dispersal – although its utility will require deeper scrutiny. It also underscores the value of conducting experimental manipulations in nature to investigate how complex ecological processes, such as dispersal, play out in natural systems.

References

Archard, G. A., and V. A. Braithwaite. 2011. Variation in aggressive behaviour in the poeciliid fish Brachyrhaphis episcopi: Population and sex differences. Behavioural Processes 86:52–57.

Austin, J. W., A. L. Szalanski, R. H. Scheffrahn, M. T. Messenger, J. A. McKern, and R. E. Gold. 2006. Genetic evidence for two introductions of the Formosan subterranean termite, Coptotermes formosanus (Isoptera: Rhinotermitidae), to the United States. Florida Entomologist 89:183–193.

Baker, J. A., S. A. Foster, and M. A. Bell. 1995. Armor morphology and reproductive output in threespine stickleback, Gasterosteus aculeatus. Pages 225–233 in J. J. Luczkovich, P. J. Motta, S. F. Norton, and K. F. Liem, editors. Ecomorphology of fishes. Springer Netherlands, Dordrecht.

Baker, J. A., S. A. Foster, D. C. Heins, M. A. Bell, and R. W. King. 1998. Variation in female life-history traits among Alaskan populations of the threespine stickleback, Gasterosteus aculeatus L. (Pisces: Gasterosteidae). Biological Journal of the Linnean Society 63:141–159.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67.

Bell, M. A., D. C. Heins, M. A. Wund, F. A. Von Hippel, R. Massengill, K. Dunker, G. A. Bristow, and E. A. Windsor. 2016. Reintroduction of threespine stickleback into Cheney and Scout Lakes, Alaska. Evolutionary Ecology Research 17:157–178.

Ben-Shachar, M., D. Lüdecke, and D. Makowski. 2020. effectsize: estimation of effect size indices and standardized parameters. Journal of Open Source Software 5:2815.

Bensky, M. K., R. Paitz, L. Pereira, and A. M. Bell. 2017. Testing the predictions of coping styles theory in threespined sticklebacks. Behavioural Processes 136:1–10.

Bilby, J., and K. Moseby. 2024. Review of hyperdispersal in wildlife translocations. Conservation Biology 38:e14083.

Blondel, L., I. G. Paterson, P. Bentzen, and A. P. Hendry. 2021. Resistance and resilience of genetic and phenotypic diversity to "black swan" flood events: A retrospective analysis with historical samples of guppies. Molecular Ecology 30:1017–1028.

Bolnick, D. I., R. D. H. Barrett, E. Choi, L. Eckert, A. P. Hendry, E. V. Kerns, Å. J. Lind, K. Milligan-McClellan, C. L. Peichel, K. Sasser, A. R. Thornton, C. Wolf, N. C. Steinel, and J. N. Weber. 2024, June 27. Destabilized host-parasite dynamics in newly founded populations. bioRxiv.

Bonte, D., H. Van Dyck, J. M. Bullock, A. Coulon, M. Delgado, M. Gibbs, V. Lehouck, E. Matthysen, K. Mustin, M. Saastamoinen, N. Schtickzelle, V. M. Stevens, S. Vandewoestijne, M. Baguette, K. Barton, T. G. Benton, A. Chaput-Bardy, J. Clobert, C. Dytham, T. Hovestadt, C. M. Meier, S. C. F. Palmer, C. Turlure, and J. M. J. Travis. 2012. Costs of dispersal. Biological Reviews 87:290–312.

Botero-Delgadillo, E., V. Quirici, Y. Poblete, E. Poulin, B. Kempenaers, and R. A. Vásquez. 2020. Exploratory behavior, but not aggressiveness, is correlated with breeding dispersal

propensity in the highly philopatric thorn-tailed rayadito. Journal of Avian Biology 51:jav.02262.

Bowler, D. E., and T. G. Benton. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80:205–225.

Brown, C., and V. A. Braithwaite. 2004. Size matters: a test of boldness in eight populations of the poeciliid Brachyraphis episcopi. Animal Behaviour 68:1325–1329.

Brown, C., F. Jones, and V. Braithwaite. 2005. In situ examination of boldness–shyness traits in the tropical poeciliid, Brachyraphis episcopi. Animal Behaviour 70:1003–1009.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences. 2nd edition. Routledge, New York.

Cohen, J. 1992. A power primer. Psychological Bulletin 112:155–159.

Comerford, M. S., T. M. La, S. Carroll, and S. P. Egan. 2023. Spatial sorting promotes rapid (mal)adaptation in the red-shouldered soapberry bug after hurricane-driven local extinctions. Nature Ecology & Evolution 7:1856–1868.

Couture, J. M., Z. C. Redman, J. Bozzini, R. Massengill, K. Dunker, B. R. Briggs, and P. L. Tomco. 2022. Field and laboratory characterization of rotenone attenuation in eight lakes of the Kenai Peninsula, Alaska. Chemosphere 288:132478.

De Winter, G., R. M. Henrique, A. T. Rafael, and C. Ben. 2016. Knights in shining armour are not necessarily bold: Defensive morphology correlates negatively with boldness, but

positively with activity, in wild threespine stickleback, Gasterosteus aculeatus. Evolutionary Ecology Research 17:279–290.

Des Roches, S., D. M. Post, N. E. Turley, J. K. Bailey, A. P. Hendry, M. T. Kinnison, J. A. Schweitzer, and E. P. Palkovacs. 2018. The ecological importance of intraspecific variation. Nature Ecology & Evolution 2:57–64.

Edelaar, P., and D. I. Bolnick. 2012. Non-random gene flow: an underappreciated force in evolution and ecology. Trends in Ecology & Evolution 27:659–665.

Edelaar, P., A. M. Siepielski, and J. Clobert. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. Evolution 62:2462–2472.

Fox, J., S. Weisberg, and B. Price. 2023. car: Companion to Applied Regression.

Fraser, D. F., J. F. Gilliam, M. J. Daley, A. N. Le, and G. T. Skalski. 2001. Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. The American Naturalist 158:124–135.

Garant, D., S. E. Forde, and A. P. Hendry. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. Functional Ecology 21:434–443.

Gruber, J., G. Brown, M. J. Whiting, and R. Shine. 2017. Is the behavioural divergence between range-core and range-edge populations of cane toads (Rhinella marina) due to evolutionary change or developmental plasticity? Royal Society Open Science 4:170789.

Haines, G. E., L. Moisan, A. M. Derry, and A. P. Hendry. 2023. Dimensionality and modularity of adaptive variation: divergence in threespine stickleback from diverse environments. The American Naturalist 201:175–199.

Hanson, H. I., K. Birkhofer, H. G. Smith, E. Palmu, and K. Hedlund. 2017. Agricultural land use affects abundance and dispersal tendency of predatory arthropods. Basic and Applied Ecology 18:40–49.

Hartig, F. 2022. DHARMa: residual diagnostics for hierarchical (multi-Level / mixed) regression models.

Hendry, A. P. 2017. Eco-evolutionary dynamics. Princeton University Press, Princeton.

Hendry, A. P., R. D. H. Barrett, A. M. Bell, M. A. Bell, D. I. Bolnick, K. M. Gotanda, G. E. Haines, Å. J. Lind, M. Paccard, C. L. Peichel, C. R. Peterson, H. A. Poore, R. L. Massengill, K. Milligan-McClellan, N. C. Steinel, S. Sanderson, M. R. Walsh, J. N. Weber, and A. M. Derry. 2024. Designing eco-evolutionary experiments for restoration projects: opportunities and constraints revealed during stickleback introductions. Ecology and Evolution 14:e11503.

Lacasse, J., and N. Aubin-Horth. 2012. A test of the coupling of predator defense morphology and behavior variation in two threespine stickleback populations. Current Zoology 58:53–65.

Laskowski, K. L., and A. M. Bell. 2014. Strong personalities, not social niches, drive individual differences in social behaviours in sticklebacks. Animal Behaviour 90:287–295.

Laskowski, K. L., S. Pearish, M. Bensky, and A. M. Bell. 2015. Predictors of individual variation in movement in a natural population of threespine stickleback (Gasterosteus aculeatus). Pages 65–90 Advances in Ecological Research. Elsevier.

Le Gouar, P., J. Mihoub, and F. Sarrazin. 2012. Dispersal and habitat selection: behavioural and spatial constraints for animal translocations. Pages 138–164 in J. G. Ewen, D. P. Armstrong, K. A. Parker, and P. J. Seddon, editors. Reintroduction Biology. First edition. Wiley.

Liebl, A. L., and L. B. Martin. 2012. Exploratory behaviour and stressor hyper-responsiveness facilitate range expansion of an introduced songbird. Proceedings of the Royal Society B: Biological Sciences 279:4375–4381.

Lindström, T., G. P. Brown, S. A. Sisson, B. L. Phillips, and R. Shine. 2013. Rapid shifts in dispersal behavior on an expanding range edge. Proceedings of the National Academy of Sciences 110:13452–13456.

Makhrov, A. A., E. Y. K. Houle, A. P. Hendry, A. M. Derry, and D. L. Lajus. 2024. Widespread evidence for rapid recent changes in global range and abundance of threespine stickleback (Gasterosteus aculeatus). Fish and Fisheries:1–18.

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

Pan, M., H. Zhang, L. Zhang, and H. Chen. 2019. Effects of starvation and prey availability on predation and dispersal of an omnivorous predator Arma chinensis Fallou. Journal of Insect Behavior 32:134–144.

R Core Team. 2024. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Réale, D., and P. Montiglio. 2021. Animal personality, the study of individual behavioral differences. Pages 342–366 in J. J. Bolhuis, L. Giraldeau, and J. A. Hogan, editors. The Behavior of Animals, 2nd Edition. First edition. Wiley.

Réale, D., S. M. Reader, D. Sol, P. T. McDougall, and N. J. Dingemanse. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews 82:291–318.

Richardson, K. M., V. Doerr, M. Ebrahimi, T. G. Lovegrove, and K. A. Parker. 2015. Considering dispersal in reintroduction and restoration planning. Pages 59–72 Advances in reintroduction biology of Australian and New Zealand fauna.

Rojas-Ferrer, I., M. J. Thompson, and J. Morand-Ferron. 2020. Is exploration a metric for information gathering? Attraction to novelty and plasticity in black-capped chickadees. Ethology 126:383–392.

Saastamoinen, M., G. Bocedi, J. Cote, D. Legrand, F. Guillaume, C. W. Wheat, E. A. Fronhofer, C. Garcia, R. Henry, A. Husby, M. Baguette, D. Bonte, A. Coulon, H. Kokko, E. Matthysen, K. Niitepõld, E. Nonaka, V. M. Stevens, J. M. J. Travis, K. Donohue, J. M. Bullock, and M. del Mar Delgado. 2018. Genetics of dispersal. Biological Reviews 93:574–599.

Selonen, V., and I. K. Hanski. 2006. Habitat exploration and use in dispersing juvenile flying squirrels. Journal of Animal Ecology 75:1440–1449.

Sih, A., J. Cote, M. Evans, S. Fogarty, and J. Pruitt. 2012. Ecological implications of behavioural syndromes. Ecology Letters 15:278–289.

Stamps, J. A., and R. R. Swaisgood. 2007. Someplace like home: Experience, habitat selection and conservation biology. Applied Animal Behaviour Science 102:392–409.

Stevens, V. M., A. Trochet, S. Blanchet, S. Moulherat, J. Clobert, and M. Baguette. 2013. Dispersal syndromes and the use of life-histories to predict dispersal. Evolutionary Applications 6:630–642.

Swan, K. D., N. A. Lloyd, and A. Moehrenschlager. 2018. Projecting further increases in conservation translocations: A Canadian case study. Biological Conservation 228:175–182.

Ward, A. J. W., R. James, A. D. M. Wilson, and M. M. Webster. 2013. Site fidelity and localised homing behaviour in three-spined sticklebacks (Gasterosteus aculeatus). Behaviour 150:1689–1708.

Weber, J. N., N. C. Steinel, F. Peng, K. C. Shim, B. K. Lohman, L. E. Fuess, S. Subramanian, S. P. D. Lisle, and D. I. Bolnick. 2022. Evolutionary gain and loss of a pathological immune response to parasitism. Science 377:1206–1211.

Willacker, J. J., F. A. Von Hippel, P. R. Wilton, and K. M. Walton. 2010. Classification of threespine stickleback along the benthic-limnetic axis. Biological Journal of the Linnean Society 101:595–608.

Zardus, J. D., and M. G. Hadfield. 2005. Multiple origins and incursions of the Atlantic barnacle Chthamalus proteus in the Pacific. Molecular Ecology 14:3719–3733.

Zepeda, E., E. Payne, A. Wurth, A. Sih, and S. Gehrt. 2021. Early life experience influences dispersal in coyotes (Canis latrans). Behavioral Ecology 32:728–737.

Tables

Table 1. Information on the location and ecotype of the source populations, as well as the number of fish from each source population that were released into the lake.

Source population	**Coordinates**	**Ecotype**	**Number released**
Finger	61°36'34.3"N, 149°15'52.2"W	Benthic	500
South Rolly	61°40'00.3"N, 150°08'12.7"W	Limnetic	500
Spirit	60°36'01.1"N, 151°00'45.2"W	Limnetic	500
Tern	60°31'49.7"N, 149°33'12.6"W	Benthic	500
Wik	60°43'02.8"N, 151°14'30.3"W	Limnetic	495
Walby	61°04'23.3"N, 149°46'18.1"W	Benthic	500
Watson	60°32'09.1"N, 150°27'42.7"W	Benthic	500

Table 2. Number of individuals captured in ‘near’ and ‘far’ traps in 2022.

Source population	**Near**	**Far**
Finger	3	3
South Rolly	4	5
Spirit	3	3
Tern	0	0
Walby	10	6
Watson	6	6
Wik	2	7

Table 3. Anova output from generalized linear model assessing the effects of behaviour and year on capture location (near vs. far).

Coefficient	**X^2**	**df**	**p**
Boldness	1.55	1	0.21
Year	2.32	1	0.13
Exploration	13.03	1	<0.001
Boldness: Year	2.20	1	0.14
Exploration: Year	8.11	1	<0.01

Figures

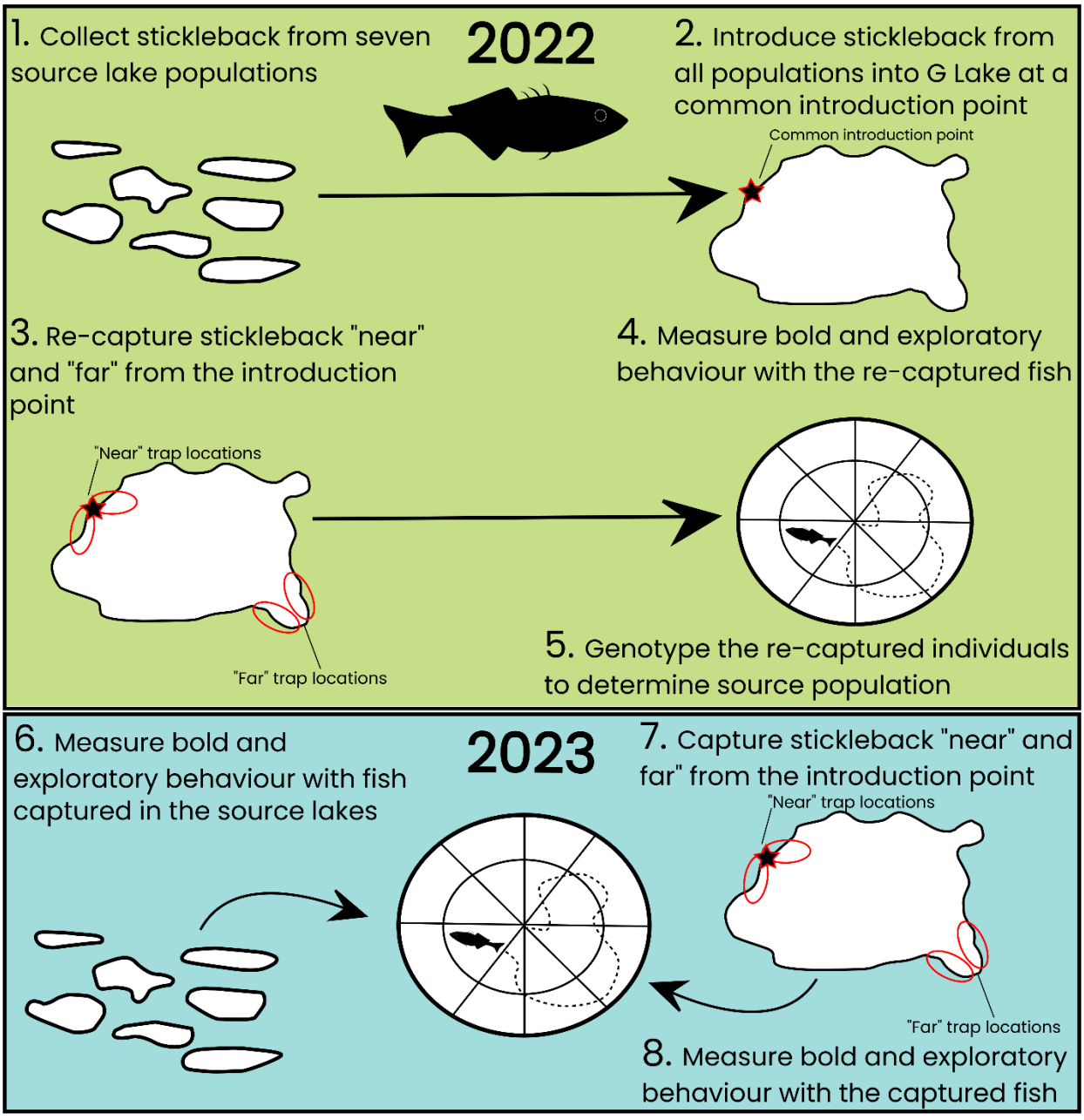

Figure 1. Experimental timeline.

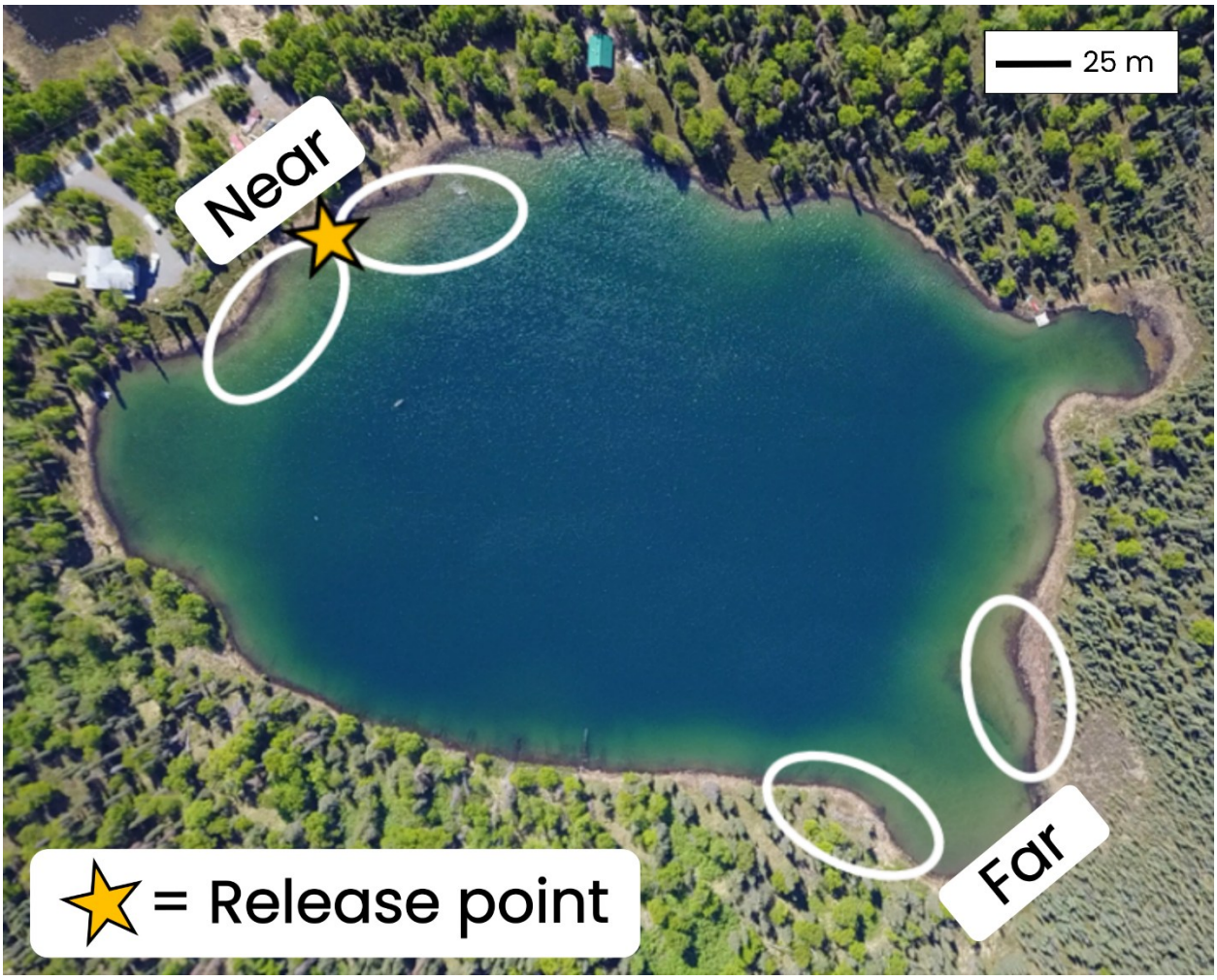

Figure 2. Aerial photograph of the recipient lake, showing the release point, and the general areas (ellipses) where traps were set “near” to or “far” from to the release point to capture potentially “weaker” and “stronger” dispersers.

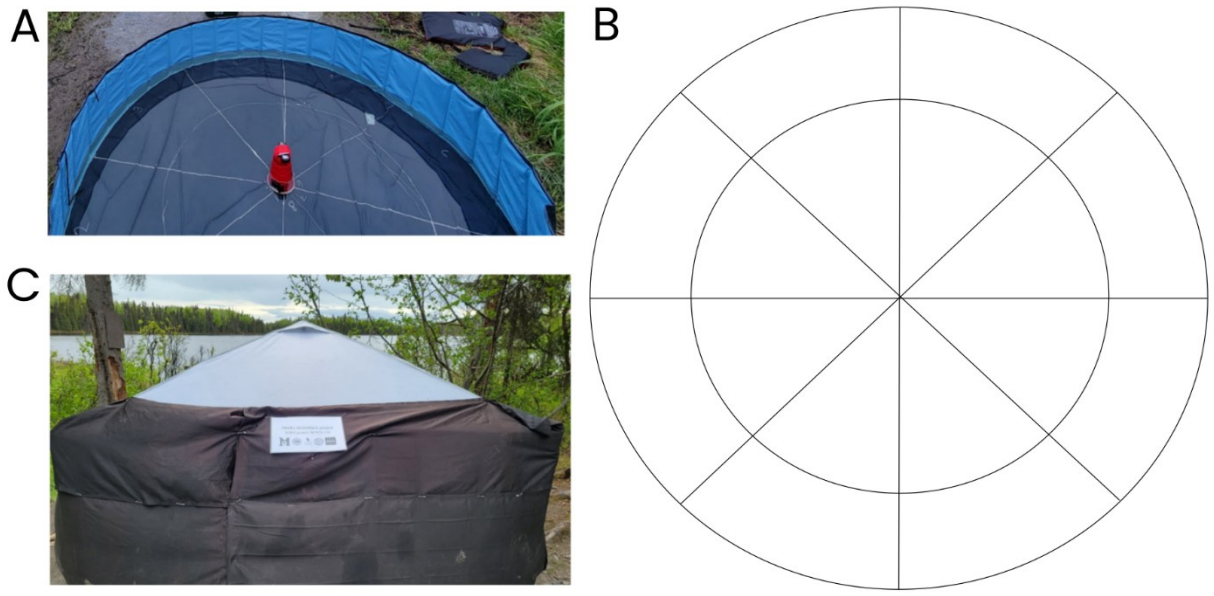

Figure 3. The experimental arena used for behavioural trials. The water-filled kiddie pool (Panel A) had a grid on the bottom (Panel B) and was covered by a gazebo and surrounded by black-out curtains (Panel C).

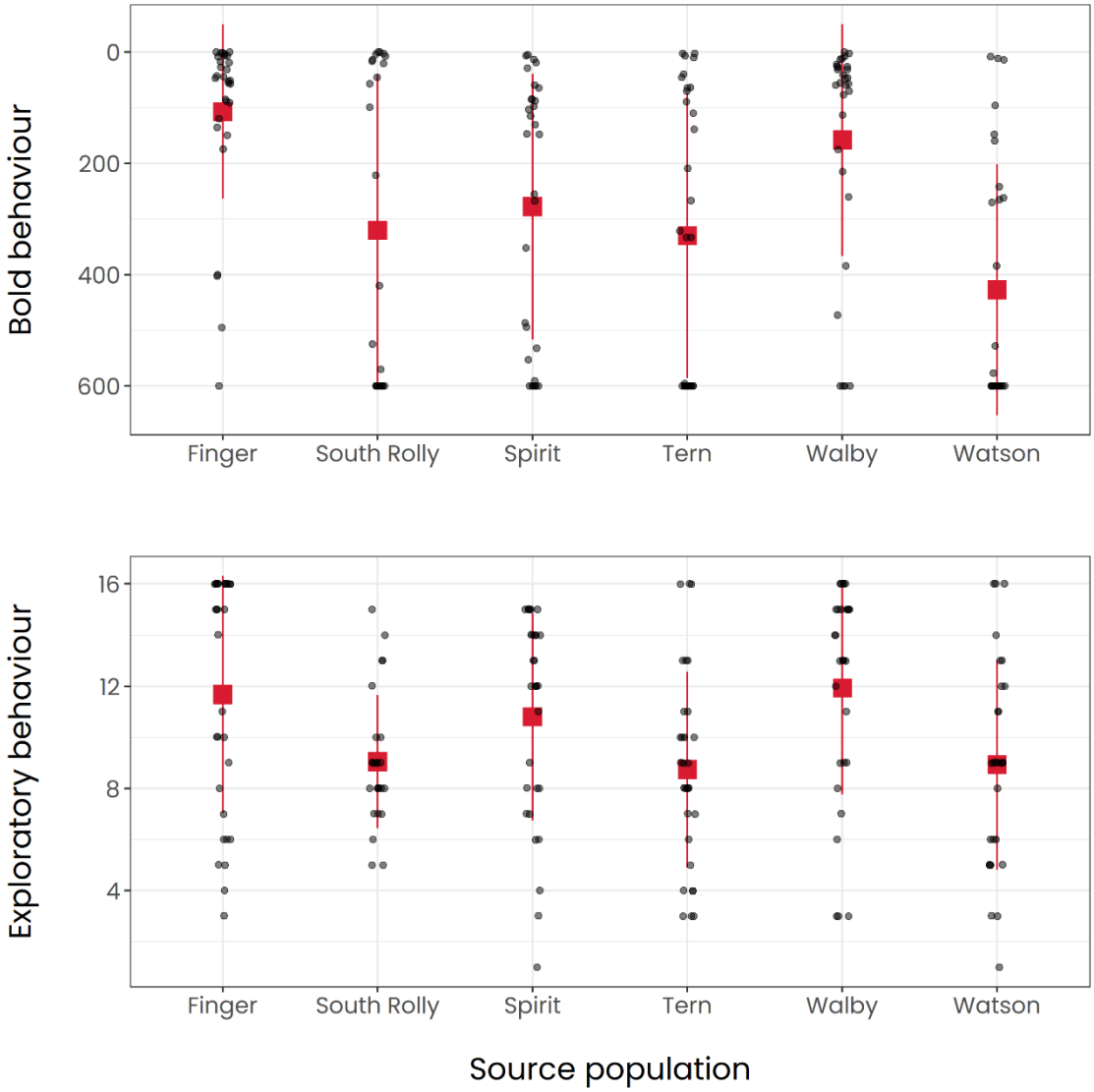

Figure 4. Beeswarm plot showing behavioural differences among the source populations. Raw boldness (Panel A) and exploration scores (Panel B) are displayed for each fish captured from each source population. The red square and error bars denote the mean and standard deviation for each population. The y-axis for boldness is reversed for more intuitive interpretation (i.e., boldness increases approaching the top of the figure). Wik Lake fish were introduced into the recipient lake but are excluded from this figure because we were unable to perform behavioural assays on fish captured directly from that population (see text).

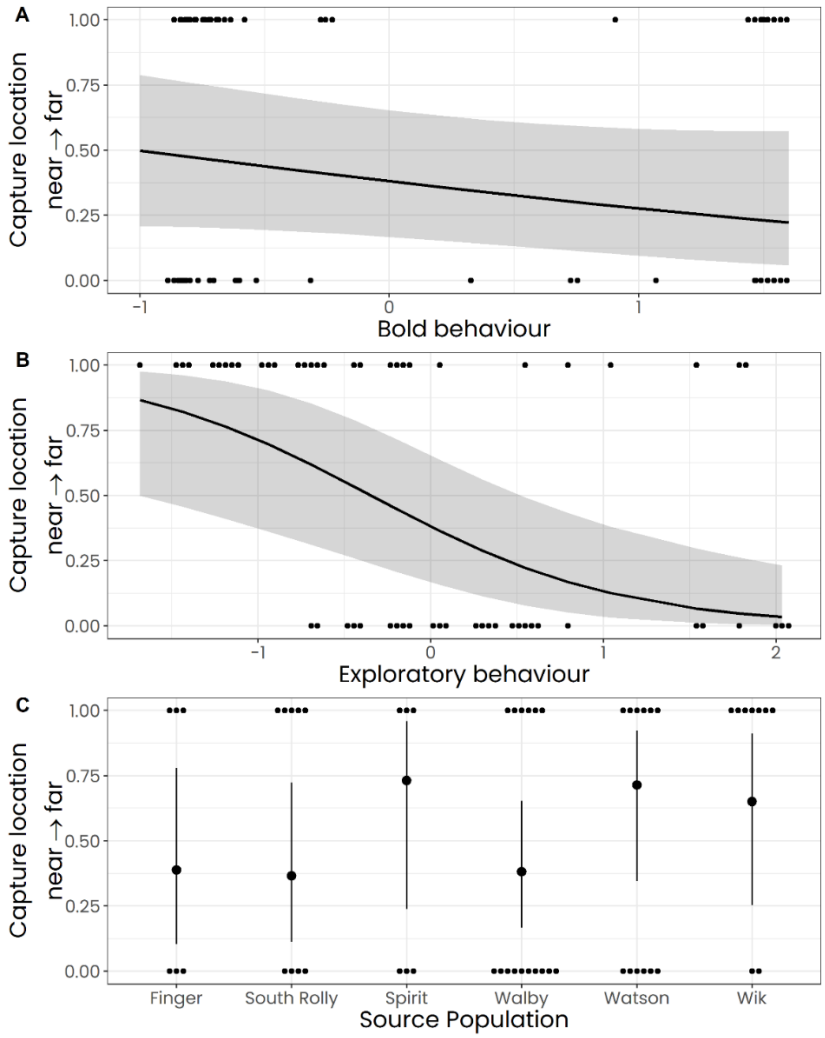

Figure 5. Individuals captured near the introduction point ("weaker" dispersers) crossed more sections in an artificial arena (more exploratory) one month after introduction (Panel B), but neither boldness (Panel A) nor source population (Panel C) significantly influenced dispersal. The trend lines with confidence intervals (for boldness and exploration) or error bars (for population) are predicted by the model, and the data points are the raw data. Tern Lake fish were introduced into the recipient lake but are excluded from this figure because we did not capture any fish with Tern ancestry (see text). Because the behaviours and source population were in the same model, the trends predicted by the model will not correspond exactly to the raw data.

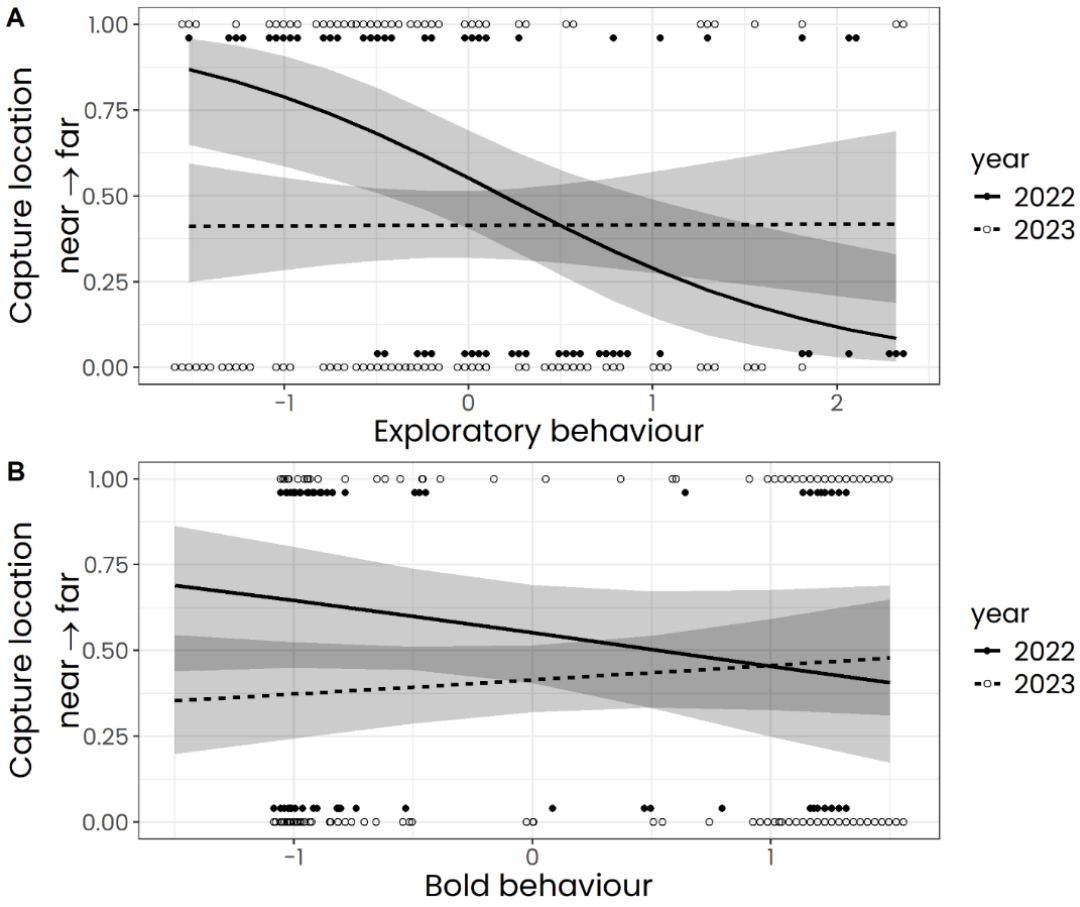

Figure 6. The association between exploration and dispersal distance that was evident in 2022 was no longer evident by 2023. The trend lines with confidence intervals (for boldness and exploration) are predicted by the model, and the data points are the raw data.

General Discussion and Conclusion

Investigating the extent to which the causes and consequences of intraspecific variation are predictable is essential for understanding ecological and evolution processes and manipulating their outcomes for desired benefits. In this thesis, I specifically define predictability as the variance that can be explained by statistical models. Variance explained (R^2) is also used as a measure of parallel evolution, and so, throughout this discussion, the terms parallelism and predictability are used interchangeably when discussing my chapter about parallel evolution. In Part One (*The Causes of Intraspecific Variation)*, I first investigated the extent to which the causes of phenotypic variation are predictable in Trinidadian guppies. Investigating the causes of variation in guppies is important, because guppy research has contributed to our foundational knowledge of evolutionary biology, and it might be assumed that the causes of phenotypic variation in guppies are highly predictable. In Part Two (*The Consequences of Intraspecific Variation)*, I next investigated the predictability of movement as a consequence of variation in threespine stickleback, a species that is often introduced into novel environments. The extent to which stickleback movement is predictable provides insight into how ecological and evolutionary processes might unfold in novel environments, and whether movement could be leveraged for practical applications. In the sections below, I discuss the extent to which the causes and consequences of intraspecific variation are predictable and then how we can work to improve predictability going forward.

How predictable are the causes of intraspecific variation?

In Chapter 1, I found that about a quarter of phenotypic variance in guppy population-level trait means can be explained by predation regime. I also found that colour appears to be especially weakly parallel and that differences in evolutionary history can decrease estimates of parallelism. Other studies that also use R^2 as a measure of parallelism consider 'moderate' parallelism as $R^2 \geq 0.33$, and 'strong' parallelism as $R^2 \geq 0.50$ (Langerhans 2018). For the overall estimate of parallelism and the mean estimates of the within-population and among-population factors, our estimates of R^2 only surpass $R^2 \geq 0.50$ in one instance ('other' traits, where n = 3), and only three estimates of R^2 (13% of all R^2 estimates) surpass 'moderate' parallelism (R^2: Life history = 0.39; Other morphology = 0.34; Common Garden (F2) rearing environment = 0.35). These findings in Chapter 1 emphasize that most phenotypic variation in guppy population-level trait means is not explained by the simplified predation regime dichotomy, but, rather, reflects variation within the regimes. In Chapter 2, when focusing on only low predation sites, I found that the local abiotic environment can have associations with guppy behaviour. This suggests that non-biotic aspects of the environment could reduce predictability of the predation regime dichotomy by contributing to phenotypic variation at local levels. However, the most important abiotic factors still only explained about a quarter of the variance in guppy behaviour.

Across both chapters in Part One of my thesis, the majority of phenotypic variance was therefore not explained by statistical models, and we can conclude that the causes of phenotypic variation in guppies are typically only weakly predictable. These findings show that even famous examples of parallel evolution – where we would likely expect predictability to be highest – show substantial non-parallel (or, unpredictable) components. Weak predictability of the causes

of phenotypic variation emphasizes that currently the most typical research approach lacks nuance surrounding the complexities of natural systems and suggests that we lack a robust understanding of what underlies phenotypic variation in nature. My findings are particularly troubling given that I conducted this work with a species that provides many 'textbook' examples of parallel evolution. Predictability could therefore be even lower for non-model systems or systems where dichotomous selection regimes are not as easily leveraged.

Improving predictability on the causes of phenotypic variation will require deliberate sampling of additional sources of environmental variation, beyond 'focal' sources of selection. In guppies, for instance, this could include the many factors that can vary within a single predation regime and are known to shape phenotypes (e.g., mate choice, predators, or parasites; Endler and Houde 1995, Jacquin et al. 2016). Some researchers are already investigating the role of multiple sources of selection for shaping the phenotypes of guppies and other organisms inhabiting dichotomous environments. For example, Gotanda et al. (2013) investigated the role that parasites play in shaping guppy phenotypes within the specific context of the predation regimes, and Langerhans (2018) investigated how interspecific competition affected phenotypic variation within dichotomous predation regimes with Bahamas mosquitofish. Because the factors that shape phenotypes can vary among populations, among individuals within populations, and within individuals (Kingsolver et al. 2001, Hendry 2017), future studies will be especially valuable if also conducted at multiple scales. Habitat contrasts will continue to serve as important venues for this future work investigating the causes of phenotypic variation in nature. For over 50 years we've leveraged Trinidad as a natural laboratory to establish that predation regimes can shape guppy phenotypes. The next 50 years will be better spent untangling the role that other factors can play within these regimes to shape phenotypes.

How predictable are the consequences of intraspecific variation?

In the last three chapters of this thesis, I investigated the extent to which the consequences of intraspecific variation are predictable in stickleback. I specifically focused on how intraspecific variation can predict movement (dispersal and assortment). Non-random movement can lead to spatially structured ecological and evolutionary effects that might be particularly important following introductions into novel environments (Edelaar and Bolnick 2012). In Chapter 3 I found that variation in fibrosis does not affect dispersal, in Chapter 4 I found that stickleback do not assort non-randomly by ancestry, and in Chapter 5 I demonstrate that ancestry does not predict dispersal. Although I did find that some factors might affect stickleback movement in lakes, specifically early-stage inflammation (Chapter 3), microhabitat (Chapter 4), and exploratory behaviour (Chapter 5), more work is needed to assess the extent to which statistically not significant or weak trends (in the case of early stage inflammation and microhabitat, respectively) are biologically meaningful, or if unexpected results are generalizable to other populations or species (in the case of weaker dispersers being more exploratory).

Taken together, Part Two of my thesis demonstrates that variation in stickleback movement is at best weakly predictable, and is often not predictable (i.e., $R^2 = 0$). Stickleback movement is therefore not likely to result in spatially structured effects to population dynamics, community structure, or ecosystem functioning – although, these population, community and ecosystem-level effects could still occur following stickleback introductions, but we would not expect to see any associated spatial structuring. Stickleback movement in lakes is also unlikely to

have spatially structured evolutionary effects owing to implications for gene flow, local adaptation, or assortative mating.

The weak predictability of stickleback movement might be surprising given the evidence that suggests stickleback sometimes move non-randomly (e.g., Bolnick et al. 2009, Maciejewski et al. 2020). One important consideration is that all work presented in this thesis investigates movement following experimental displacement (Chapter 3) or introductions into novel environments (Chapters 4, 5). Non-random movement might be more important in 'undisturbed' environments where stickleback are exhibiting more 'normal' movement patterns. As such, future work in these same lakes that uses less disruptive approaches, such as passive trapping and genotyping to infer non-random gene flow in established populations (e.g., Maciejewski et al. 2020), might uncover that finer-scale movement patterns or spatial dynamics emerge over time as these stickleback populations reach equilibrium. Nevertheless, the consistently high movement capacity exhibited by stickleback is perhaps not surprising because stickleback are rapidly expanding their range, illustrating that they are highly successful at colonizing new environments (Makhrov et al. 2024). Random movement following introductions might therefore facilitate colonization and establishment success by enabling settlement throughout entire lakes (as evidenced by random dispersal) and high genetic admixture and evolutionary potential (as evidenced by random spatial assortment).

One advantage of consistently weak predictability is that we might 'rule out' associations that are extremely weakly predictable as not being worth trying to predict. For instance, future work would likely not benefit from scrutinizing among-population variation in stickleback movement following introductions. Other consequences might be more predictable and thus

better for focusing our efforts to anticipate or manipulate ecological and evolutionary outcomes following stickleback introductions. Traits that are less sensitive to the external environment might be more predictable, and thus could be considered for this future work. For instance, variation in stickleback foraging traits can have important ecological effects (Harmon et al. 2009, Des Roches et al. 2013), and some of these traits are highly heritable (e.g., gill raker number; Aguirre et al. 2004). Focusing on highly heritable traits might result in higher predictability.

Next steps for improving predictability

Across the board, the causes and consequences of intraspecific variation that I investigated were weakly predictable. Given that predictability is so desirable for understanding the natural world, protecting vulnerable ecosystems, and benefiting human societies, is there a way forward given the weak predictability that I generally found here or is predictability a pie-in-the-sky ambition?

There are at least three general ways to improve predictability. The first approach, which I pointed to when discussing the causes of variation, is that we can improve our understanding of natural systems by increasing the number of variables on which we collect data (i.e., looking beyond 'key' sources of variation or dichotomous habitat contrasts) (Møller and Jennions 2002). The second approach, which I pointed to when discussing the consequences of variation, is to focus our efforts on 'important' traits or processes that we might expect to be the most predictable (or, stated another way, 'discounting' the traits or processes that are found to be unpredictable). The third approach is to try and reduce the complexity of the associations we are investigating. My work suggests that two effective ways for reducing complexity could be focusing on smaller spatial scales and 'simpler' traits or processes (note that I do not mean

simpler to measure, which might actually result in decreased predictability as we discuss in Chapter 1, but rather traits that are shaped by fewer processes or in simpler ways).

The importance of spatial scale was made evident in Part One of this thesis. Parallelism in Chapter 1 was lower when pooling populations with different evolutionary histories (from different drainages), and guppy behaviour in Chapter 2 was more strongly associated with the specific pool, rather than the stream, from which guppies were sampled. These results emphasize that predictability could decrease as spatial scales – and associated sources of variation – increase. Possibly higher predictability at smaller spatial scales does not mean that we should only be conducting research at small scales (I emphasize above that research should be conducted at multiple spatial scales), but rather that the effect of scale must be thoroughly considered in experimental design, analysis, and interpretation.

The impact of trait complexity on predictability became apparent in Chapter 1 where I found that colour was less predictable than the other trait type categories. This result is especially surprising for colour, because colour is one of the most famous examples of parallel evolution in guppies (Endler 1978, 1980). As we discuss in that chapter, however, this finding does not discount the substantial body of research that has found low-predation guppies are more colourful than their high-predation counterparts (Haskins and Haskins 1951, Endler 1978, 1980). Rather, the low predictability of colour is likely owing to the fact that colour traits are highly multidimensional and are an example of many-to-one mapping – which, in a phenotypic context, refers to when multiple phenotypes are able to serve the same function (Thompson et al. 2017, Bolnick et al. 2018). The negative association between complexity and predictability could also explain why stickleback movement was so weakly predictable. Movement is a highly complex

process, and large-scale movements such as dispersal can be broken down into multiple phases (e.g., pre-emigration, initiation, transfer, settlement), each of which will be associated with its own risks and costs (Bonte et al. 2012). “Breakdowns” in predictability could occur at each phase owing to individual-level (e.g., genetics, body condition, learned experience) or environmental sources of variation (e.g., temperature, predators, parasites), decreasing the extent to which movement is a predictable process (Bonte et al. 2012, Edelaar and Bolnick 2012). Investigating ‘simple’ traits (such as traits with 1:1, rather than many-to-one, mapping; Bolnick et al. 2018) could therefore increase predictability, and might be more effectively leveraged or manipulated for applied benefits.

Research in natural settings

Natural populations and environments are awash with historical contingencies and sources of heterogeneity that can reduce predictability. The quickest and most effective way to reduce complexity, and thus maximize predictability, might therefore be to take our investigations out of natural settings and into controlled environments (e.g., laboratories or mesocosms). However, the value of predictability lies in understanding and predicting ecological and evolutionary processes as they occur despite, or in interaction with, these complexities.

In Chapter 3, we investigated effects of fibrosis on dispersal in a lake in Alaska. One study that motivated this work showed that fibrosis can affect aspects of locomotion, specifically the c-start response, which is a startle response that occurs in response to immediate threats (Matthews et al. 2023). When we submitted this manuscript, one anonymous reviewer suggested that, because the Matthews et al. (2023) study found an effect, our study required additional

experiments as we did not find a significant effect in the field. The reviewer stated, “field data can be negative for many reasons” and implied that the negative result we observed could be a flaw in our study. In my perspective, the fact that field data can be negative for many reasons is a strength of our study. It is not straightforward to compare what we investigated in Chapter 3 to the Matthews et al. (2023) study because we investigated different traits (an immediately startle response vs a more sustained movement). I also generally agree with the reviewer that more research could help elucidate what specific factors are reducing predictability. However, the reviewer comment serves to highlight an important discussion that is sometimes raised in ecological and evolutionary research (e.g., Hendry 2019). That is, that most empirical research in ecology and evolution is conducted in controlled settings. Although these settings are critical for providing insight into the mechanisms underlying ecological and evolutionary processes, and can be important starting points for deriving hypotheses, they cannot inform what actually occurs in natural environments (Hendry 2019). Therefore, studies with natural populations are required to validate the ecological and evolutionary relevance of findings from controlled settings.

All the research in my thesis was conducted with natural populations because my objective was to understand the extent to which the causes and consequences of intraspecific variation are predictable in nature. The lack of effects of fibrosis on dispersal in nature suggests that this effect is not strong relative to other factors at play. Had I conducted this work in a controlled setting I might have found higher predictability (Peek et al. 2003). However, high predictability in controlled settings that does not translate to high, or even moderate, predictability in natural settings does very little to improve our understanding of the natural world and likely cannot be leveraged for practical applications.

Conclusions

Across the five chapters presented in this thesis, I found that the causes and consequences of intraspecific variation were only weakly predictable. These results suggest that our current understanding of the causes of intraspecific variation might be limited and that we lack the breadth of knowledge required to predict or manipulate the ecological and evolutionary outcomes of intraspecific variation. Nevertheless, I do not think this conclusion paints a grim picture of our ability to understand the natural world. Although I found that some traits or processes were not predictable, I found that others were more predictable. In identifying what is more and less predictable, we can focus our efforts on attempting to understand or manipulate the processes that might be more highly predictable. Further, although we will likely never be able to explain 100% of the causes and consequences of intraspecific variation in natural systems (Møller and Jennions 2002, Peek et al. 2003), predictability increases with more information on a system. This reality is highly motivating because it serves as a reminder that the work we are doing as researchers, even when delving into the nitty-gritty details of a system, is all helping to improve our predictive ability and thus our ability to understand, benefit from, and protect the natural world.

References

Aguirre W, Doherty P, Bell M. 2004. Genetics of lateral plate and gillraker phenotypes in a rapidly evolving population of threespine stickleback. Behaviour 141: 1465–1483.

Bolnick DI, Barrett RDH, Oke KB, Rennison DJ, Stuart YE. 2018. (Non)parallel evolution. Annual Review of Ecology, Evolution, and Systematics 49: 303–330.

Bolnick DI, Snowberg LK, Patenia C, Stutz WE, Ingram T, Lau OL. 2009. Phenotype-dependent native habitat preference facilitates divergence between parapatric and stream stickleback. Evolution 63: 2004–2016.

Bonte D, Van Dyck H, Bullock JM, Coulon A, Delgado M, Gibbs M, Lehouck V, Matthysen E, Mustin K, Saastamoinen M, Schtickzelle N, Stevens VM, Vandewoestijne S, Baguette M, Barton K, Benton TG, Chaput-Bardy A, Clobert J, Dytham C, Hovestadt T, Meier CM, Palmer SCF, Turlure C, Travis JMJ. 2012. Costs of dispersal. Biological Reviews 87: 290–312.

Des Roches S, Shurin JB, Schluter D, Harmon LJ. 2013. Ecological and evolutionary effects of stickleback on community structure. PLoS ONE 8: e59644.

Edelaar P, Bolnick DI. 2012. Non-random gene flow: an underappreciated force in evolution and ecology. Trends in Ecology & Evolution 27: 659–665.

Endler JA. 1978. A predator's view of animal color patterns. Pages 319–364 in Hecht MK, Steere WC, and Wallace B, eds. Evolutionary Biology. Springer US.

Endler JA. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34: 76.

Endler JA, Houde AE. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. Evolution 49: 456–468.

Gotanda KM, Delaire LC, Raeymaekers JAM, Pérez-Jvostov F, Dargent F, Bentzen P, Scott ME, Fussmann GF, Hendry AP. 2013. Adding parasites to the guppy-predation story: insights from field surveys. Oecologia 172: 155–166.

Harmon LJ, Matthews B, Des Roches S, Chase JM, Shurin JB, Schluter D. 2009. Evolutionary diversification in stickleback affects ecosystem functioning. Nature 458: 1167–1170.

Haskins CP, Haskins EF. 1951. The inheritance of certain color patterns in wild populations of *Lebistes reticulatus* in Trinidad. Evolution 5: 216–225.

Hendry AP. 2017. Eco-evolutionary dynamics. Princeton University Press.

Hendry AP. 2019. A critique for eco-evolutionary dynamics. Functional Ecology 33: 84–94.

Jacquin L, Reader SM, Boniface A, Mateluna J, Patalas I, Pérez-Jvostov F, Hendry AP. 2016. Parallel and nonparallel behavioural evolution in response to parasitism and predation in Trinidadian guppies. Journal of Evolutionary Biology 29: 1406–1422.

Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001. The strength of phenotypic selection in natural populations. The American Naturalist 157: 245–261.

Langerhans RB. 2018. Predictability and parallelism of multitrait adaptation. Journal of Heredity 109: 59–70.

Maciejewski MF, Jiang C, Stuart YE, Bolnick DI. 2020. Microhabitat contributes to microgeographic divergence in threespine stickleback. Evolution 74: 749–763.

Makhrov AA, Houle EYK, Hendry AP, Derry AM, Lajus DL. 2024. Widespread evidence for rapid recent changes in global range and abundance of threespine stickleback (*Gasterosteus aculeatus*). Fish and Fisheries 1–18.

Matthews DG, Maciejewski MF, Wong GA, Lauder GV, Bolnick DI. 2023. Locomotor effects of a fibrosis-based immune response in stickleback fish. Journal of Experimental Biology 226: jeb246684.

Møller A, Jennions MD. 2002. How much variance can be explained by ecologists and evolutionary biologists? Oecologia 132: 492–500.

Oke KB, Rolshausen G, LeBlond C, Hendry AP. 2017. How parallel is parallel evolution? A comparative analysis in fishes. The American Naturalist 190: 1–16.

Peek MS, Leffler AJ, Flint SD, Ryel RJ. 2003. How much variance is explained by ecologists? Additional perspectives. Oecologia 137: 161–170.

Stuart YE, Veen T, Weber JN, Hanson D, Ravinet M, Lohman BK, Thompson CJ, Tasneem T, Doggett A, Izen R, Ahmed N, Barrett RDH, Hendry AP, Peichel CL, Bolnick DI. 2017. Contrasting effects of environment and genetics generate a continuum of parallel evolution. Nature Ecology & Evolution 1: 0158.

Thompson CJ, Ahmed NI, Veen T, Peichel CL, Hendry AP, Bolnick DI, Stuart YE. 2017. Many-to-one form-to-function mapping weakens parallel morphological evolution. Evolution 71: 2738–2749.

Appendix

Supplementary material for Chapter 1

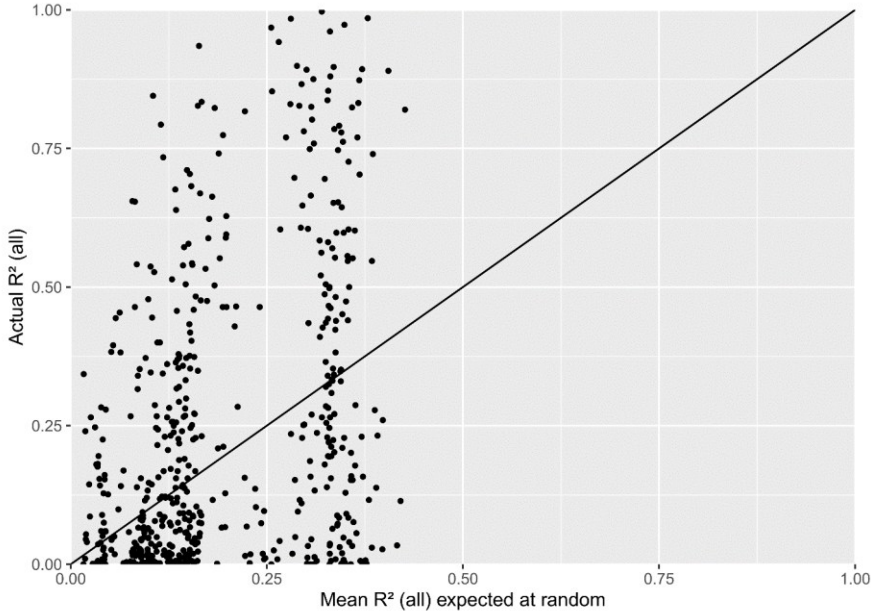

Figure S1. The mean 'permutated' R^2 values generated from permuting each trait within each predation regime 100 times against the actual 'observed' R^2 values. Points along the 1:1 line indicate that the 'permuted' and 'observed' estimates are the same. Points above the 1:1 line indicate that the 'observed' estimates are higher than expected by chance, and below the 1:1 line indicate that the 'observed' values are lower than expected by chance.

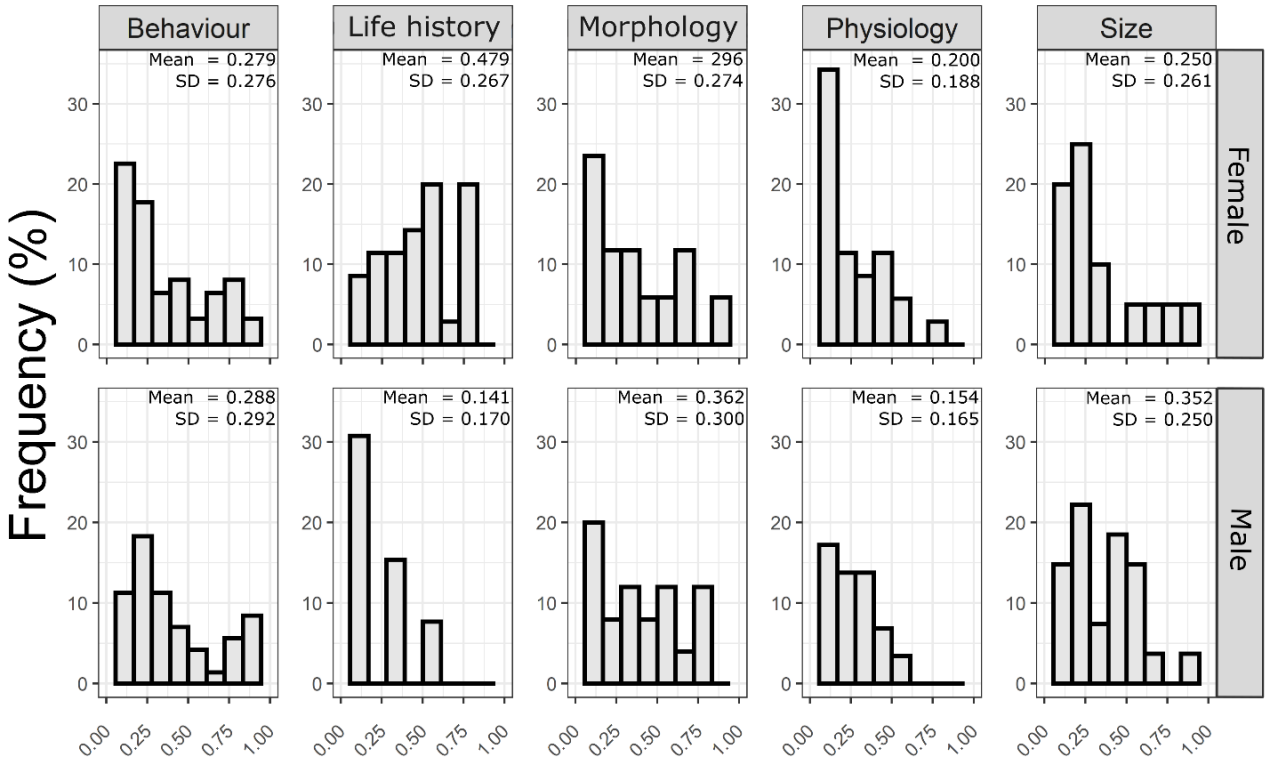

Figure S2. Frequency distribution of parallelism, separated by trait type and sex. Trait type categories only represented by one sex (i.e., colour and other) are not shown.

Table S1. Summary of the studies used in the analyses

Study	# of traits	Slope	Drainage	Population type	Introduction sites
Burns and Rodd 2008	10	South	Caroni, Oropouche	Natural	NA
Burns et al. 2009	6	South	Caroni, Oropouche	Natural	NA
de Lira et al. 2021	4	North, South	Caroni, Northern, Oropouche	Introduction, Natural	El Cedro
Devigili et al. 2019	8	North, South	Caroni, Northern, Oropouche	Introduction, Natural	Turure
Easty et al. 2011	7	North	Northern	Introduction, Natural	Damier
Edenbrow et al. 2017	6	Other, South	Caroni, Oropouche, Other	Introduction, Natural	Turure
Egset et al. 2011	2	North, Northeastern, South	Caroni, Northern, Oropouche, Other	Introduction, Natural	El Cedro
Elgee et al. 2010	7	South	Caroni, Oropouche	Introduction, Natural	Turure
Evans and Magurran 1999	4	South	Caroni	Natural	NA
Evans et al. 2003	1	South	Caroni, Oropouche	Introduction, Natural	Turure
Evans et al. 2011	6	South	Caroni, Oropouche	Introduction, Natural	Turure

Fischer et al. 2013	24	North, South	Northern, Oropouche	Natural	NA
Fischer et al. 2016	26	South	Caroni, Oropouche	Natural	NA
Gordon et al. 2012	2	North, South	Caroni, Northern, Oropouche	Introduction, Natural	Tururе
Gordon et al. 2017b	22	North	Northern	Introduction, Natural	Damier
Gotanda et al. 2013	36	North, South	Caroni, Northern, Oropouche	Introduction, Natural	Arima, Damier, El Cedro, Tururе
Harris et al. 2010	6	South	Caroni, Oropouche	Natural	NA
Herbert-Read et al. 2017	68	South	Caroni, Oropouche	Natural	NA
Herbert-Read et al. 2019	22	North, South	Caroni, Northern, Oropouche	Introduction, Natural	Arima, Tururе
Huizinga et al. 2009	2	South	Caroni, Oropouche	Introduction, Natural	Tururе
Ioannou et al. 2017	20	South	Caroni, Oropouche	Introduction, Natural	Tururе
Sandkam et al. 2015	28	North, South	Caroni, Northern	Natural	NA
Magurran and Seghers 1994a	2	South	Caroni	Introduction, Natural	Aripo
Magurran and Seghers 1994b	2	North, South	Caroni, Northern	Introduction, Natural	Aripo
Millar and Hendry 2011	66	North, South	Caroni, Northern, Oropouche	Introduction, Natural	El Cedro, Tururе

Neff et al. 2008	1	South	Caroni, Oropouche	Introduction, Natural	Turure
Reddon et al. 2018	4	North, South	Caroni, Northern	Natural	NA
Reznick and Bryant 2007	1	South	Caroni	Introduction, Natural	Aripo, El Cedro
Reznick and Endler 1982	3	Other, South	Caroni, Oropouche, Other	Introduction, Natural	El Cedro
Reznick et al. 2004	8	North, South	Northern, Oropouche	Natural	NA
Reznick et al. 2005	2	North, South	Northern, Oropouche	Natural	NA
Schwartz and Hendry 2007	7	North, South	Caroni, Northern, Oropouche	Natural	NA
Stephenson et al. 2015	4	North, Northeastern, Other, South	Caroni, Northern, Oropouche, Other	Introduction, Natural	Arima, Turure
Valvo et al. 2019	15	North, South	Caroni, Northern, Oropouche	Introduction, Natural	El Cedro, Turure
Weese et al. 2010	6	North, South	Caroni, Northern	Introduction, Natural	Damier
Zandona et al. 2015	8	South	Caroni	Natural	NA

Table S2. The sites in our database that we labelled as introductions, with specific information about each introduction event from the published literature. GPS coordinates of introduction sites are provided where available.

Site	Source population	Year	Reference(s) documenting introductions	Comments
Damier	Yarra	1996	Karim et al., 2007 Gordon et al., 2009	No guppies prior to introduction
Aripo I Lat: 10.668712 Lon: -61.234599	Aripo 6	1976	Endler, 1980 Reznick and Bryant, 2007	No guppies prior to introduction
Middle Aripo Lat: 10.666879 Lon: -61.23004	Unknown	1980	Seghers and Magurran, 1994	Predators (not guppies) introduced
El Cedro	Guanapo	1981	Reznick and Bryga, 1987 Reznick and Bryant, 2007	
Upper Lalaja, Lower Lalaja	Lower Guanapo	2008	Reznick et al., 2019	All introduction sites are in the Upper Guanapo.
Taylor,	Lower Guanapo	2009	Reznick et al., 2019	All introduction sites are in the Upper Guanapo.

Caigual				
Turure	Guanapo river	1957	Haskins, 1961 Blondel et al., 2019	Exact source population is still unknown
Lower Arima	Mixed source populations	2001	Fraser et al., 2015	Accidental introduction

Table S3. Summary of parallelism between the sexes (R^2 – the proportion of variance among site means explained by predation regime for a given trait in a given sex in a given study), (A-C) for each of the (B-D) within-population and (D-F) among-population factors.

	Factor			Mean R^2	SD
A	Sex		Females	0.310	0.276
			Males	0.206 (with colour)	0.243 (with colour)
				0.275 (without colour)	0.269 (without colour)
B	Trait type	Colour	Females	NA	NA
			Males	0.102	0.146
		Behaviour	Females	0.279	0.276
			Males	0.288	0.292
		Life history	Females	0.479	0.267
			Males	0.141	0.170
		Size	Females	0.250	0.261
			Males	0.352	0.250
		Other morphology	Females	0.296	0.274
			Males	0.362	0.300
		Physiology	Females	0.200	0.188
			Males	0.154	0.165
		Other	Females	0.760	0.097
			Males	NA	NA
C	Rearing environment	Common garden (F2)	Females	0.489	0.259
			Males	0.204	0.224
		Wild-caught	Females	0.263	0.260
			Males	0.207	0.248
D	Ecological complexity	Within one slope	Females	0.281	0.238
			Males	0.164	0.188
		Between both slopes	Females	0.243	0.248
			Males	0.117	0.158

E	Evolutionary history	Within one drainage	Females	0.287	0.235
			Males	0.233	0.253
		Between both drainages	Females	0.218	0.211
			Males	0.164	0.182
F	Time since colonization	Only natural	Females	0.309	0.284
			Males	0.140	0.157
		Natural and introduced	Females	0.245	0.250
			Males	0.115	0.148

Table S4. The results of generalized linear mixed models with mean sample size per trait included as a fixed effects term.

Factor		$\chi 2$	df	p
Trait type	Trait type	12.598	5	0.0275
	Mean number	0.829	1	0.363
Sex (with colour)	Sex	0.442	1	0.506
	Mean number	0.171	1	0.680
Sex (without colour)	Sex	1.378	1	0.240
	Mean number	0.156	1	0.693
Rearing environment	Rearing environment	0.982	1	0.322
	Mean number	0.185	1	0.668
Sex and traits	Trait type	18.402	6	0.00530
	Sex	2.118	1	0.146
	Mean number	0.551	1	0.458
Sex and rearing environment	Rearing environment	0.882	1	0.348
	Sex	0.360	1	0.548

	Mean number	0.123	1	0.725
Ecological complexity	Slope (within or between the slopes)	7.153	1	0.00749
	Sex	1.055	1	0.307
	Mean number	0.170	1	0.680
Time since colonization	With only natural or with both natural and introduced populations	4.721	1	0.0298
	Mean number	0.0257	1	0.873
Evolutionary history	Drainage (With only the Caroni or the Caroni and Oropouche)	12.977	1	0.000315
	Sex	0.305	1	0.581
	Mean number	0.0969	1	0.756

Table S5. The results of generalized linear mixed models with the mean 'permuted' R^2 values as the response variable. The 'permuted' R^2 values were obtained by permuting each trait within each predation regime 100 times without replacement.

Factor		$\chi 2$	df	p
Trait type	Trait type	10.234	5	0.0687
Sex (with colour)	Sex	0.472	1	0.492
Sex (without colour)	Sex	0.0778	1	0.780
Rearing model	Rearing environment	1.14	1	0.285
Sex and trait	Trait type	55.82	6	3.167x10-10
	Sex	0.0218	1	0.883
Sex and rearing environment	Rearing environment	1.06	1	0.302
	Sex	0.212	1	0.646
Ecology	Sex (within or between the slopes)	0.587	1	0.444

	Sex	2.31	1	0.129
Time since colonization	With only natural or with both natural and introduced populations	1.39	1	0.239
Evolutionary history	Drainage (With only the Caroni or the Caroni and Oropouche)	7.93	1	0.00487
	Sex	2.47	1	0.116

References cited only in the online appendixes (data sources)

Burns, J.G., Di Nardo, P. & Rodd, F.H. 2009. The role of predation in variation in body shape in guppies *Poecilia reticulata*: a comparison of field and common garden phenotypes. *Journal of Fish Biology* **75**: 1144–1157.

Burns, J.G. & Rodd, F.H. 2008. Hastiness, brain size and predation regime affect the performance of wild guppies in a spatial memory task. *Animal Behaviour* **76**: 911–922.

de Lira, J.J.P.R., Yan, Y., Levasseur, S., Kelly, C.D. & Hendry, A.P. 2021. The complex ecology of genitalia: Gonopodium length and allometry in the Trinidadian guppy. *Ecol. Evol.* **11**: 4564–4576.

Devigili, A., Evans, J.P. & Fitzpatrick, J.L. 2019. Predation shapes sperm performance surfaces in guppies. *Proc. R. Soc. B.* **286**: 20190869.

Easty, L.K., Schwartz, A.K., Gordon, S.P. & Hendry, A.P. 2011. Does sexual selection evolve following introduction to new environments? *Animal Behaviour* **82**: 1085–1095.

Edenbrow, M., Bleakley, B.H., Darden, S.K., Tyler, C.R., Ramnarine, I.W. & Croft, D.P. 2017. The evolution of cooperation: interacting phenotypes among social partners. *The American Naturalist* **189**: 630–643.

Egset, C.K., Bolstad, G.H., Rosenqvist, G., Endler, J.A. & Pélabon, C. 2011. Geographical variation in allometry in the guppy (*Poecilia reticulata*): Variation in static allometry in guppies. *Journal of Evolutionary Biology* **24**: 2631–2638.

Elgee, K.E., Evans, J.P., Ramnarine, I.W., Rush, S.A. & Pitcher, T.E. 2010. Geographic variation in sperm traits reflects predation risk and natural rates of multiple paternity in the guppy. *Journal of Evolutionary Biology* **23**: 1331–1338.

Evans, J.P., Gasparini, C., Holwell, G.I., Ramnarine, I.W., Pitcher, T.E. & Pilastro, A. 2011. Intraspecific evidence from guppies for correlated patterns of male and female genital trait diversification. *Proc. R. Soc. B.* **278**: 2611–2620.

Evans, J.P. & Magurran, A.E. 1999. Geographic variation in sperm production by Trinidadian guppies. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **266**: 2083–2087.

Evans, J.P., Pilastro, A. & Ramnarine, I.W. 2003. Sperm transfer through forced matings and its evolutionary implications in natural guppy (*Poecilia reticulata*) populations. *Biological Journal of the Linnean Society* **78**: 605–612.

Fischer, E.K., Ghalambor, C.K. & Hoke, K.L. 2016. Plasticity and evolution in correlated suites of traits. *J. Evol. Biol.* **29**: 991–1002.

Fischer, E.K., Soares, D., Archer, K.R., Ghalambor, C.K. & Hoke, K.L. 2013. Genetically and environmentally mediated divergence in lateral line morphology in the Trinidadian guppy (*Poecilia reticulata*). *Journal of Experimental Biology* jeb.081349.

Gordon, S.P., Hendry, A.P. & Reznick, D.N. 2017. Predator-induced Contemporary Evolution, Phenotypic Plasticity, and the Evolution of Reaction Norms in Guppies. *Copeia* **105**: 514–522.

Gordon, S.P., López-Sepulcre, A. & Reznick, D.N. 2012. Predation-associated differences in sex linkage of wild guppy coloration. *Evolution* **66**: 912–918.

Gotanda, K.M., Delaire, L.C., Raeymaekers, J.A.M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., *et al.* 2013. Adding parasites to the guppy-predation story: insights from field surveys. *Oecologia* **172**: 155–166.

Harris, S., Ramnarine, I.W., Smith, H.G. & Pettersson, L.B. 2010. Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos* **119**: 1711–1718.

Herbert-Read, J.E., Rosén, E., Szorkovszky, A., Ioannou, C.C., Rogell, B., Perna, A., *et al.* 2017. How predation shapes the social interaction rules of shoaling fish. *Proc. R. Soc. B.* **284**: 20171126.

Herbert-Read, J.E., Wade, A.S.I., Ramnarine, I.W. & Ioannou, C.C. 2019. Collective decision-making appears more egalitarian in populations where group fission costs are higher. *Biol. Lett.* **15**: 20190556.

Huizinga, M., Ghalambor, C.K. & Reznick, D.N. 2009. The genetic and environmental basis of adaptive differences in shoaling behaviour among populations of Trinidadian guppies, *Poecilia reticulata*. *Journal of Evolutionary Biology* **22**: 1860–1866.

Ioannou, C.C., Ramnarine, I.W. & Torney, C.J. 2017. High-predation habitats affect the social dynamics of collective exploration in a shoaling fish. *Sci. Adv.* **3**: e1602682.

Leinonen, T., McCairns, R.J.S., Herczeg, G. & Merilä, J. 2012. Multiple evolutionary pathways to decreased lateral plate coverage in freshwater threespine sticklebacks: functional convergence in sticklebacks. *Evolution* **66**: 3866–3875.

Magurran, A.E. & Seghers, B.H. 1994a. Predator inspection behaviour covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. *Behav* **128**: 121–134.

Magurran, A.E. & Seghers, B.H. 1994b. Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata* , populations in Trinidad. *Proc. R. Soc. Lond. B* **255**: 31–36.

Millar, N.P. & Hendry, A.P. 2012. Population divergence of private and non-private signals in wild guppies. *Environ Biol Fish* **94**: 513–525.

Neff, B.D., Pitcher, T.E. & Ramnarine, I.W. 2008. Inter-population variation in multiple paternity and reproductive skew in the guppy. *Mol Ecol* **17**: 2975–2984.

Reddon, A.R., Chouinard-Thuly, L., Leris, I. & Reader, S.M. 2018. Wild and laboratory exposure to cues of predation risk increases relative brain mass in male guppies. *Funct Ecol* **32**: 1847–1856.

Reznick, D., Bryant, M. & Holmes, D. 2005. The evolution of senescence and post-reproductive lifespan in guppies (*Poecilia reticulata*). *PLoS Biol* **4**: e7.

Reznick, D.N. & Bryant, M.J. 2007. Comparative long-term mark-recapture studies of guppies (*Poecilia reticulata*): differences among high and low predation localities in growth and survival. *Annales Zoologici Fennici* **44**: 152–160.

Reznick, D.N., Bryant, M.J., Roff, D., Ghalambor, C.K. & Ghalambor, D.E. 2004. Effect of extrinsic mortality on the evolution of senescence in guppies. *Nature* **431**: 1095–1099.

Reznick & Endler, J.A. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**: 160.

Sandkam, B., Young, C.M. & Breden, F. 2015. Beauty in the eyes of the beholders: colour vision is tuned to mate preference in the Trinidadian guppy (*Poecilia reticulata*). *Mol Ecol* **24**: 596–609.

Schwartz, A. & Hendry, A.P. 2007. A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies. *Evolutionary Ecology Research* **9**: 71–90.

Schwartz, A.K., Weese, D.J., Bentzen, P., Kinnison, M.T. & Hendry, A.P. 2010. Both Geography and Ecology Contribute to Mating Isolation in Guppies. *PLoS ONE* **5**: e15659.

Stephenson, J.F., van Oosterhout, C. & Cable, J. 2015. Pace of life, predators and parasites: predator-induced life-history evolution in Trinidadian guppies predicts decrease in parasite tolerance. *Biol. Lett.* **11**: 20150806.

Valvo, J.J., Rodd, F.H. & Hughes, K.A. 2019. Consistent female preference for rare and unfamiliar male color patterns in wild guppy populations. *Behavioral Ecology* **30**: 1672–1681.

Weese, D.J., Gordon, S.P., Hendry, A.P. & Kinnison, M.T. 2010. Spatiotemporal variation in linear natural selection on body color in wild guppies (*Poecilia reticulata*). *Evolution* **64**: 1802–1815.

Zandonà, E., Dalton, C.M., El-Sabaawi, R.W., Howard, J.L., Marshall, M.C., Kilham, S.S., *et al.* 2017. Population variation in the trophic niche of the Trinidadian guppy from different predation regimes. *Sci Rep* **7**: 5770.

Supplementary material for Chapter 2

Table S1. Published repeatability estimates for studies that have measured boldness and exploration with wild-caught guppies or descendants of guppies from Trinidad.

ID*	Behaviour	Measure	R	p-value	Method to estimate R	Sex	Location in-text	Notes
	Boldness	Latency to emerge	0.64	NA	Lessells and Boag (1987)	M	Table 2	Two measures of repeatability for the same measurements.
	Exploration	Swimming rate	0.6	NA	Lessells and Boag (1987)	M	Table 2	Two measures of repeatability for the same measurements.
1	Boldness	Time frozen	0.65	NA	Lessells and Boag (1987)	M	Table 2	Two measures of repeatability for the same measurements.
	Boldness	Latency to emerge	0.63	<0.001	Spearman	M	Table 2	Two measures of repeatability for the same measurements.
	Exploration	Swimming rate	0.43	0.001	Spearman	M	Table 2	Two measures of repeatability for the same measurements.
	Boldness	Time frozen	0.22	0.123	Spearman	M	Table 2	Two measures of repeatability for the same measurements.
	Exploration	Number of grid squares traversed	0.126	0.199	Nakagawa and Schielzeth (2010)	F	Table 2	NA
2	Exploration	Proportion of the environment visited	0.111	0.035	Nakagawa and Schielzeth (2010)	F	Table 2	NA
	Boldness	Latency to emerge	0.409	0.008	Nakagawa and Schielzeth (2010)	F	Table 2	NA

3	Boldness	Number of predator inspections	0	0.731	Nakagawa and Schielzeth (2010)	F	Table 2	NA
	Exploration	Distance travelled	0.591	<0.001	Stoffel et al., 2017	M	Table 1	NA
	Exploration	Area explored	0.45	<0.001	Stoffel et al., 2017	M	Table 1	NA
	Boldness	Latency to emerge	0.194	0.079	Stoffel et al., 2017	M	Table 1	NA
	Boldness	Number of inspections	0.227	0.044	Stoffel et al., 2017	M	Table 1	NA
	Boldness	Time inspecting	0.301	0.013	Stoffel et al., 2017	M	Table 1	NA
	Boldness	Number of times re-entering the refuge	0.339	0.019	Stoffel et al., 2017	M	Table 1	NA
	Boldness	Time spent in refuge	0.467	<0.001	Stoffel et al., 2017	M	Table 1	NA
4	Boldness	Total area used (model predator)	0.354	0.032	Spearman	M	Table 4	Did predator "training" after first trial to associate simulated predator with aversive experience; don't call it "repeatability"
	Boldness	Latency to approach (s) model predator	0.264	0.107	Spearman	M	Table 4	Did predator "training" after first trial to associate simulated predator with aversive experience; don't call it "repeatability"
	General activity	Experimental area used (open field test)	0.418	0.01	Spearman	M	Table 4	Did predator "training" after first trial to associate simulated predator with aversive experience; don't call it "repeatability"; Activity seems to be equivalent to our measure of exploration

	General activity	Total area used (open field test)	0.488	0.002	Spearman	M	Table 4	Did predator "training" after first trial to associate simulated predator with aversive experience; don't call it "repeatability"; Activity seems to be equivalent to our measure of exploration
	General activity	Total time moving (s) (open field test)	0.409	0.012	Spearman	M	Table 4	Did predator "training" after first trial to associate simulated predator with aversive experience; don't call it "repeatability"; Activity seems to be equivalent to our measure of exploration
	Exploration	Total area used (novel object)	0.328	0.048	Spearman	M	Table 4	Did predator "training" after first trial to associate simulated predator with aversive experience; don't call it "repeatability"
	Exploration	Number of approaches (novel object)	0.367	0.026	Spearman	M	Table 4	Did predator "training" after first trial to associate simulated predator with aversive experience; don't call it "repeatability"
	Exploration	Time moving toward object (s)	0.466	0.004	Spearman	M	Table 4	Did predator "training" after first trial to associate simulated predator with aversive experience; don't call it "repeatability"
5	Boldness	Predator inspection	0.24	0.05	Spearman	NR	In-text	NA
	Boldness	Emergence time	0.33	<0.001	Lessells and Boag (1987)	MF	Table 1	NA
6	Boldness	Emergence time	0.29	0.001	Lessells and Boag (1987)	F	Table 1	NA
	Boldness	Emergence time	0.34	0.004	Lessells and Boag (1987)	M	Table 1	NA

	Boldness	Time in hesitancy zone	0.21	0.003	Lessells and Boag (1987)	MF	Table 1	NA
	Boldness	Time in hesitancy zone	0.31	0.001	Lessells and Boag (1987)	F	Table 1	NA
	Boldness	Time in hesitancy zone	0.011	0.47	Lessells and Boag (1987)	M	Table 1	NA
	Boldness	Emergence time	0.33	0.001	Nakagawa & Schielzeth, 2010	F	Table 1	NA
	Boldness	Emergence time	0.31	0.005	Nakagawa & Schielzeth, 2010	F	Table 1	NA
	Boldness	Activity	0.37	0.001	Nakagawa & Schielzeth, 2010	F	Table 1	NA
7	Boldness	Order caught	0.27	<0.001	Nakagawa & Schielzeth, 2010	F	Table 1	NA
	Boldness	Area covered	0.46	<0.001	Nakagawa & Schielzeth, 2010	F	Table 1	NA
	Boldness	Time in middle	0.42	<0.001	Nakagawa & Schielzeth, 2010	F	Table 1	NA
	Exploration	Distance moved	0.523	<0.001	Stoffel, Nakagawa, & Schielzeth, 2017	F	Table 1	NA
	Exploration	Area explored	0.265	0.034	Stoffel, Nakagawa, & Schielzeth, 2017	F	Table 1	NA
	Boldness	Latency to exit refuge	0.256	<0.001	Stoffel, Nakagawa, & Schielzeth, 2017	F	Table 1	NA
8	Boldness	Time in refuge	0	0.105	Stoffel, Nakagawa, & Schielzeth, 2017	F	Table 1	NA
	Boldness	Number of times in refuge	0.092	0.278	Stoffel, Nakagawa, & Schielzeth, 2017	F	Table 1	NA
	Boldness	Number of inspections	0	1	Stoffel, Nakagawa, & Schielzeth, 2017	F	Table 1	NA
9	Boldness/exploration	Area (pre-predator)	0.2	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA

	Boldness/exploration	Exposed (pre-predator)	0.17	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Freezings (pre-predator)	0.27	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Shelter (pre-predator)	0.27	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Tracklength (pre-predator)	0.27	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Area (post-bird strike)	0.14	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Exposed (post-bird strike)	0.26	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Freezings (post-bird strike)	0.21	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Shelter (post-bird strike)	0.17	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Tracklength (post-bird strike)	0.22	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Area (post-cichlid reveal)	0.3	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Exposed (post-cichlid reveal)	0.13	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Freezings (post-cichlid reveal)	0.27	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Shelter (post-cichlid reveal)	0.18	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
	Boldness/exploration	Tracklength (post-cichlid reveal)	0.23	NR	Nakagawa & Schielzeth, 2010	MF	Table 3	NA
10	Boldness	Latency to leave refuge	0.48	<0.001	Nakagawa & Schielzeth, 2010	MF	In-text	NA

Activity	Number of zone changes and total time spent swimming	0.44	<0.001	Nakagawa & Schielzeth, 2010	MF	In-text	Activity seems to be equivalent to our measure of exploration.

The following references correspond to the IDs in Table S1 (see the first column):

1. Herdegen-Radwan, M. (2019). Does inbreeding affect personality traits?. Ecology and Evolution, 9(19), 10929-10937.
2. Kniel, N., & Godin, J. G. J. (2019). Characterizing the (co) variance of personality traits in female Trinidadian guppies (*Poecilia reticulata*). *Environmental biology of fishes*, *102*(11), 1351-1363.
3. Kniel, N., & Godin, J. G. J. (2020). Does individual personality predict male mating preference for female body size in the Trinidadian guppy?. *Ethology*, *126*(11), 1019-1030.
4. Smith, B. R., & Blumstein, D. T. (2012). Structural consistency of behavioural syndromes: does predator training lead to multi-contextual behavioural change?. *Behaviour*, *149*(2), 187-213.
5. Croft, D. P., Krause, J., Darden, S. K., Ramnarine, I. W., Faria, J. J., & James, R. (2009). Behavioural trait assortment in a social network: patterns and implications. *Behavioral Ecology and Sociobiology*, *63*, 1495-1503.
6. Harris, S., Ramnarine, I. W., Smith, H. G., & Pettersson, L. B. (2010). Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos*, *119*(11), 1711-1718.
7. White, S. J., Kells, T. J., & Wilson, A. J. (2016). Metabolism, personality and pace of life in the Trinidadian guppy, *Poecilia reticulata*. Behaviour, 153(13-14), 1517-1543.
8. Kniel, N., Guenther, A., & Godin, J. G. J. (2020). Individual personality does not predict learning performance in a foraging context in female guppies, *Poecilia reticulata*. Animal Behaviour, 167, 3-12.
9. Houslay, T. M., Vierbuchen, M., Grimmer, A. J., Young, A. J., & Wilson, A. J. (2018). Testing the stability of behavioural coping style across stress contexts in the Trinidadian guppy. Functional Ecology, 32(2), 424-438.
10. Jacquin, L., Reader, S. M., Boniface, A., Mateluna, J., Patalas, I., Pérez-Jvostov, F., & Hendry, A. P. (2016). Parallel and nonparallel behavioural evolution in response to parasitism and predation in Trinidadian guppies. Journal of Evolutionary Biology, 29(7), 1406-1422.

Studies for Table S1 were compiled on Web of Science using the exact search string on September 2nd, 2024:

TS = ((guppy OR guppies OR 'poecilia reticulata') AND (boldness OR bold OR exploration OR exploratory OR 'risk taking' OR 'open field test' OR 'open field trial') AND (repeatable OR repeatability OR 'interindividual consistency' OR 'intraclass' OR 'behavioural consistency' OR 'behavioral consistency'))

Records from Web of Science were filtered to only include studies where boldness and exploration were measured in guppies collected from Trinidad, or descendants of guppies collected from Trinidad. We also only included studies where repeatability estimates were provided.

Table S2. Raw ecological data and number of guppies sampled for each pool.

Stream	Pool	n	Temp (° C)	DO (mg/L)	Conductivity (µS/cm)	pH	Canopy cover (squares covered on densiometer)	SA (m^2)	Volume (m^3)	Mean depth (m)	Max depth (m)
1	1	13	22.50	5.74	110.00	9.11	7.50	0.35	0.05	0.13	0.19
1	2	19	22.50	4.74	118.20	8.86	4.00	0.85	0.62	0.73	0.15
1	3	40	22.50	3.27	111.50	8.53	3.25	0.46	0.06	0.13	0.23
1	4	14	22.90	4.98	105.90	8.26	2.75	0.53	0.06	0.12	0.18
1	5	24	22.30	4.17	97.10	7.90	6.50	0.67	0.07	0.11	0.20
1	6	6	22.50	3.60	87.90	7.15	2.75	0.94	0.91	0.97	0.15
1	7	10	22.70	3.68	88.30	7.18	3.50	0.32	0.24	0.74	0.12
2	1	7	22.70	3.88	56.40	6.73	1.00	0.26	0.03	0.12	0.16
2	2	33	22.70	3.45	54.90	6.32	3.25	1.08	0.13	0.12	0.27
2	3	84	23.00	3.55	85.20	6.61	1.75	0.94	0.17	0.18	0.27
2	4	9	23.10	4.49	106.90	7.07	2.00	0.45	0.32	0.72	0.13
2	5	4	22.90	6.23	104.50	7.37	2.75	0.72	0.50	0.70	0.13
2	6	7	22.80	6.14	101.30	7.53	2.50	0.27	0.03	0.12	0.16
2	7	6	22.80	5.87	106.60	7.48	0.00	0.76	0.43	0.56	0.83
2	8	27	22.80	5.02	106.80	7.40	2.75	1.06	1.05	0.98	0.14

Table S3. Correlations between the ecological variables. The grey shading indicates high correlations (r > 0.8) that were used to justify excluding two terms from the models (mean depth (m) and pH were excluded).

	Dissolved oxygen (mg/L)	Surface area (m^2)	Temperature (C)	Conductivity (S/cm)	Volume (m3)	Maximum depth (m)	pH	Canopy cover (squares covered on densiometer)	Mean depth (m)
Dissolved oxygen (mg/L)	1.00	-0.21	-0.06	0.47	0.38	-0.10	0.40	0.25	0.41
Surface area (m2)		1.00	0.36	-0.43	0.46	0.22	-0.60	-0.31	0.22
Temperature (C)			1.00	-0.29	0.06	0.19	-0.67	-0.78	0.05
Conductivity (S/cm)				1.00	0.29	-0.21	0.83	0.27	0.36
Volume (m3)					1.00	-0.23	0.02	-0.14	0.93
Maximum depth (m)						1.00	-0.26	-0.37	-0.29
pH							1.00	0.59	0.09

canopy	1.00	-0.11
Mean depth (m)		1.00

Table S4. Partial-eta squared for linear models with only sex and mass as fixed effects (sensitivity analysis).

Behaviour	**Fixed effect**	**η_p^2**	**95% CI**
Boldness	Sex	0.00	0.00 – 1.00
	Mass (g)	0.02	0.00 – 1.00
	Stream	0.00	0.00 – 1.00
Exploration	Sex	0.00	0.00 – 1.00
	Mass (g)	0.00	0.00 – 1.00
	Stream	0.00	0.00 – 1.00

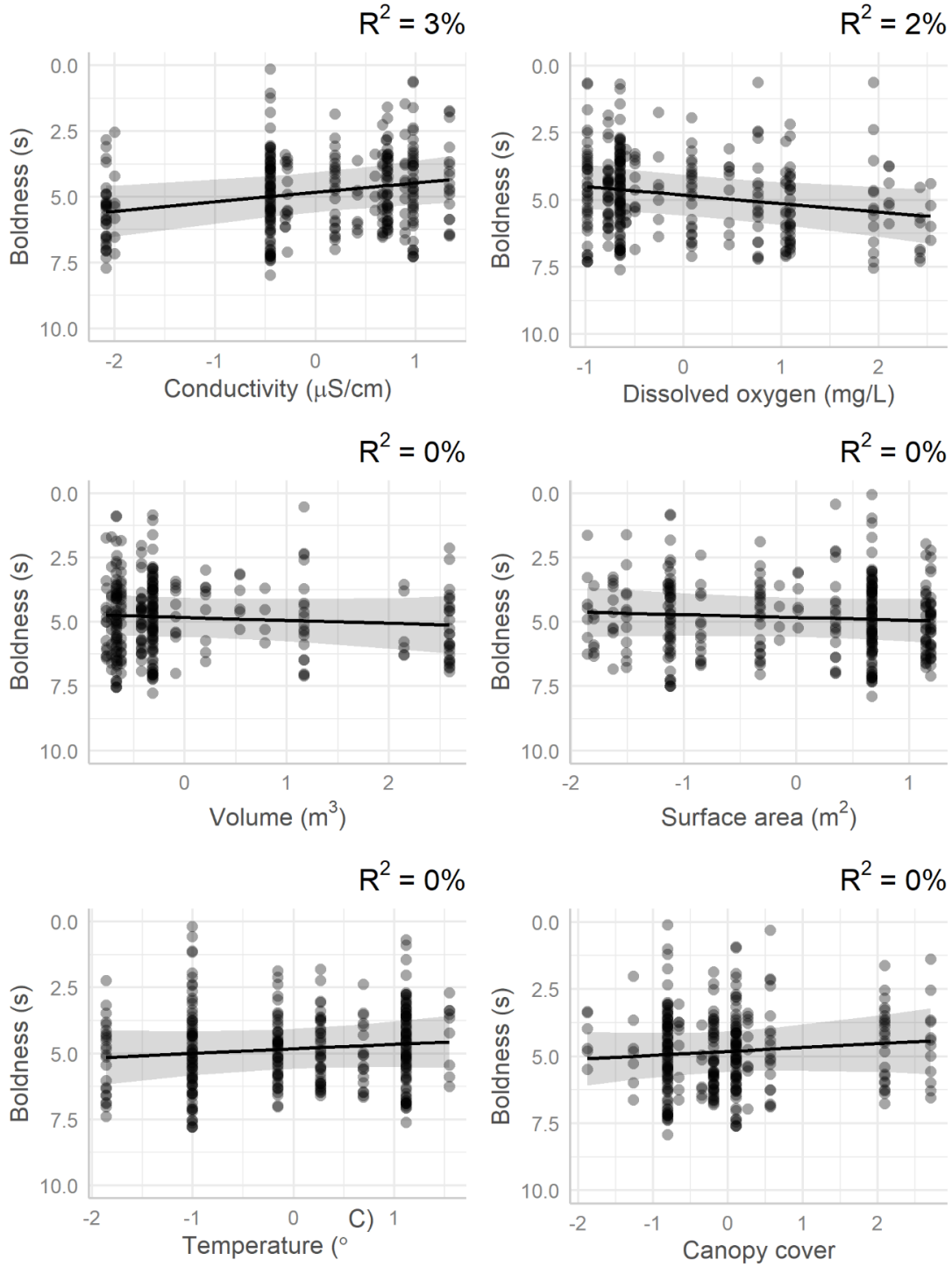

Figure S1. Effects of ecological variables on boldness (the sum of time spent frozen in the open field and under the plant refuge). Data points are the model residuals and are alongside trend lines predicted by the linear mixed model. Boldness was cube-root transformed to meet assumptions of linearity. The y-axis is inversed for more intuitive interpretation: boldness increases as the y-axis approaches zero - towards the top of the plot.

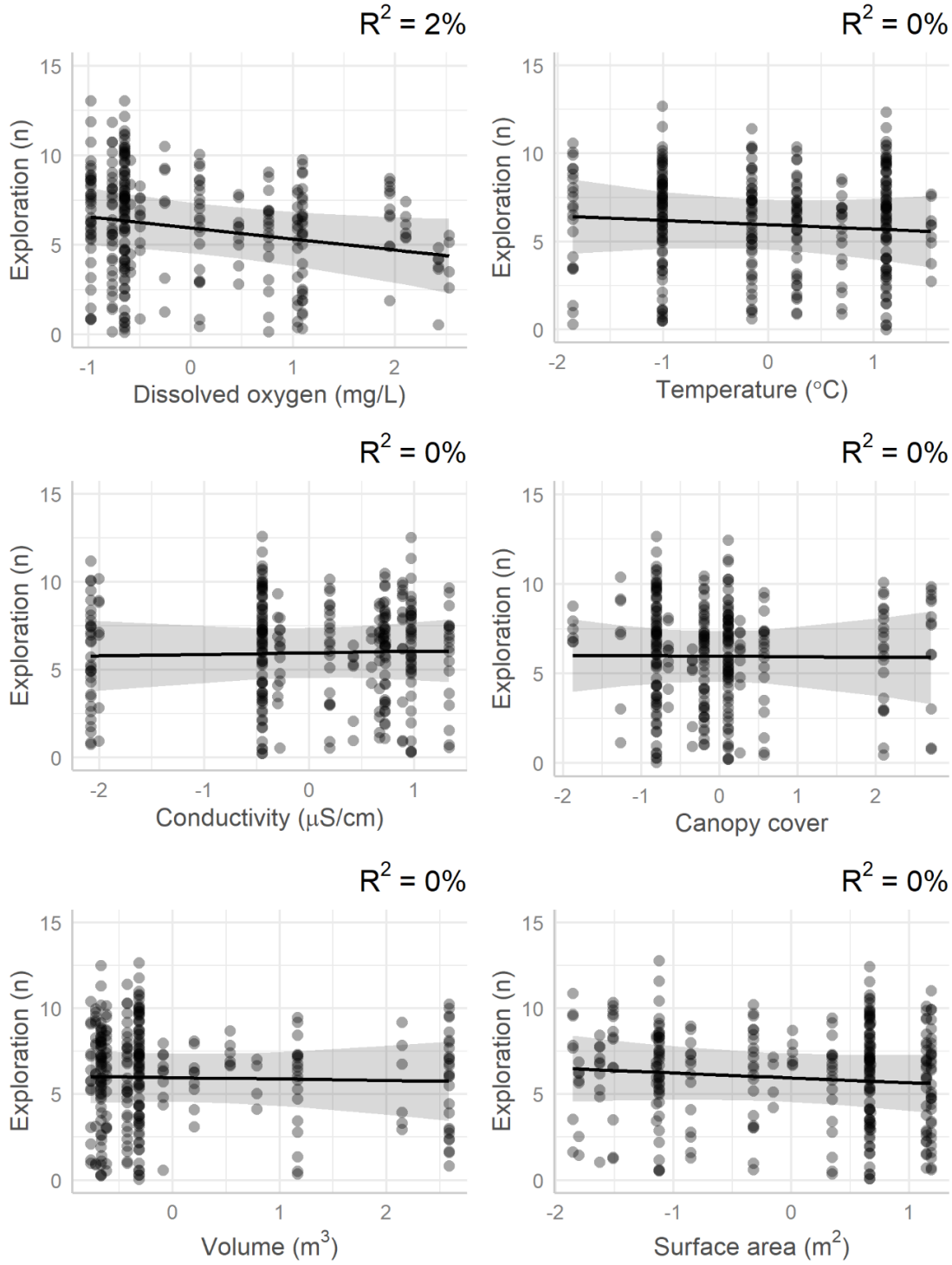

Figure S2. Effects of ecological variables on exploration (number of squares crossed in the open field). Data points are the model residuals and are alongside trend lines predicted by the linear mixed model. Exploration values were square root transformed to meet assumptions of linearity.

Supplementary material for Chapter 3

Table S1. The median distance (and interquartile range; IQR) at which stickleback were captured on either side of the lake (left/right) for each day of recapturing, along with the maximum possible distance that they could be captured.

Day	Capture side	n	Maximum trap distance (m)	Median capture distance (m)	IQR
D1	Left	11	250	167	182
	Right	12	204	100	42
D2	Left	8	293	189	186
	Right	4	204	181	6
D3	Left	5	356	293	232
	Right	10	306	255	125
D4	Left	9	398	315	256
	Right	9	379	320	49
	Release point	1	398	0	NA
D5	Left	4	498	374	34
	Right	9	483	139	291
	Release point	1	498	0	NA
D6	Left	11	498	398	50
	Right	10	505	347	195
D7	Left	4	475	398	16
	Right	11	505	230	136
D8	Left	4	475	356	56
	Right	0	505	NA	NA

Table S2. Results from the linear mixed model with a four-way interaction between fibrosis, treatment, sex, and mass, with a type 3 sum of squares. No explanatory variables are statistically associated with dispersal when considered independently or in an interaction.

	χ^2	df	p
Intercept	**51.86**	**1**	**<0.001**
Fibrosis	0.17	1	0.68
Treatment	0.06	1	0.81
Sex	0.15	1	0.70
Mass (g)	0.00	1	0.99
Fibrosis : Treatment	0.27	1	0.60
Fibrosis : Sex	0.00	1	0.95
Treatment : Sex	0.64	1	0.42
Fibrosis : Mass (g)	0.20	1	0.65
Treatment : Mass (g)	2.25	1	0.13
Sex : Mass (g)	0.44	1	0.51
Fibrosis : Treatment : Sex	0.02	1	0.88
Fibrosis : Treatment : Mass (g)	0.02	1	0.87
Fibrosis : Sex : Mass (g)	0.26	1	0.61
Treatment : Sex : Mass (g)	1.52	1	0.22
Fibrosis : Treatment : Sex : Mass (g)	1.02	1	0.31

Analyses with the subset datasets

To complement the results in the main-text, we ran additional analyses with (1) a subset dataset comprised of only fibrotic fish (Tables S3-S5), and (2) a subset dataset comprised of only non-fibrotic fish (Tables S6-S7). For the SEMs, we only used the fibrotic fish dataset, because the objective of using SEMs was to assess possible effects of treatment on dispersal via fibrosis, and this analysis was therefore not possible without variation in fibrosis. The LMMs were conducted with both subset datasets – the only difference to the main-text is that the fixed effect of fibrosis was removed for the non-fibrotic fish LMM.

Table S3. SEM regression results conducted with the fibrotic fish dataset. Significant terms ($p < 0.05$) are bolded. Maximum trap distance (m) and sex significantly affected fibrosis, and only maximum trap distance significantly affected dispersal.

Response variable	Explanatory variable	Estimate	SE	z	p
Fibrosis	Treatment	0.31	0.23	1.33	0.18
	Maximum trap distance (m)	**0.33**	**0.11**	**2.91**	**<0.01**
	Sex	**-0.66**	**0.23**	**-2.86**	**<0.01**
Dispersal distance	Fibrosis	-7.53	16.17	-0.47	0.64
	Treatment	12.61	29.17	0.43	0.67
	Maximum trap distance (m)	**62.99**	**15.25**	**4.13**	**<0.001**
	Sex	-0.88	30.73	-0.03	0.98
	Mass (g)	21.46	14.34	1.50	0.14

Table S4. Results from the linear mixed model conducted with the fibrotic fish dataset. Maximum trap distance (m) was included as a random effect in the model. None of the measured factors had a statistically significant effects on dispersal distance.

	χ^2	df	p
Fibrosis	0.06	1	0.81
Treatment	2.34	1	0.13
Sex	0.00	1	0.97
Mass (g)	0.91	1	0.34

Table S5. R^2 values with confidence intervals from the linear mixed model conducted with the fibrotic fish dataset. Effect sizes were consistently weak. Confidence intervals were calculated using 1000 bootstrap iterations.

	R^2	95% CI
Model	0.04	0.01-0.21
Treatment (control)	0.03	0.00-0.19
Mass (g)	0.01	0.00-0.17
Fibrosis	0.00	0.00-0.17
Sex (male)	0.00	0.00-0.17

Table S6. Results from the linear mixed model conducted with the non-fibrotic fish dataset. Maximum trap distance (m) was included as a random effect in the model. Significant terms ($p < 0.05$) are bolded.

	χ^2	df	p
Treatment	0.20	1	0.65
Sex	0.01	1	0.93
Mass (g)	**8.73**	**1**	**<0.01**

Table S7. R^2 values with confidence intervals from the linear mixed model conducted with the non-fibrotic fish dataset. Effect sizes were consistently weak. Confidence intervals were calculated using 1000 bootstrap iterations.

	R^2	95% CI
Model	0.10	0.02-0.28
Mass (g)	0.09	0.02-0.28
Treatment (control)	0.00	0.00-0.19
Sex (male)	0.00	0.00-0.18

Supplementary material for Chapter 4

Table S1. Full AICc table used for model selection for the proportion of shared ancestry in a trap. Blank cells indicate that a term was not present in a given model.

Intercept	Bank slope	Depth (m)	Distance to vegetation (m)	Water Habitat	Substrate 'type'	df	ΔAICc	weight
0.58		0.04				3	0.00	0.18
0.58		0.03	0.02			4	1.37	0.09
0.59	+	0.04				4	1.53	0.08
0.58		0.04			+	5	1.93	0.07
0.59		0.05		+		5	2.07	0.06
0.59	+	0.03	0.02			5	2.78	0.04
0.58						2	2.84	0.04
0.60		0.05		+	+	7	2.84	0.04
0.58		0.04	0.02		+	6	2.86	0.04
0.58			0.02			3	2.95	0.04
0.60	+		0.03			4	3.38	0.03
0.60	+					3	3.39	0.03
0.59	+	0.04			+	6	3.58	0.03
0.57		0.04	0.01	+		6	3.81	0.03

0.59	+	0.04		+		6	4.05	0.02
0.57		0.05	0.02	+	+	8	4.22	0.02
0.60	+	0.03	0.02		+	7	4.43	0.02
0.60	+	0.05		+	+	8	5.04	0.01
0.58			0.03		+	5	5.16	0.01
0.60	+		0.03		+	6	5.55	0.01
0.56	+	0.04	0.01	+		7	5.70	0.01
0.58					+	4	5.71	0.01
0.60	+				+	5	6.11	0.01
0.57	+	0.05	0.02	+	+	9	6.36	0.01
0.53				+		4	6.56	0.01
0.50			0.03	+		5	6.67	0.01
0.50	+		0.03	+		6	7.19	0.00
0.53	+			+		5	7.26	0.00
0.49			0.03	+	+	7	8.63	0.00
0.49	+		0.03	+	+	8	9.16	0.00
0.53				+	+	6	9.29	0.00
0.53	+			+	+	7	9.89	0.00

Table S2. Full-model averaged coefficients for the proportion of shared ancestry using delta ΔAIC ≤ 2 and ΔAIC ≤ 6 cut-offs.

ΔAIC cut-off		β	Standard error	z	p
≤ 2	Intercept	0.58	0.02	28.75	<0.001
	Depth	0.04	0.02	2.10	0.04
	Distance to vegetation	0.00	0.01	0.32	0.75
	Bank slope: Shallow step	-0.01	0.02	0.29	0.77
	Substrate: Sand	-0.01	0.03	0.33	0.74
	Substrate: Silt	0.00	0.02	0.21	0.84
≤ 6	Intercept	0.58	0.08	7.24	<0.001
	Depth	0.03	0.02	1.37	0.17
	Distance to vegetation	0.01	0.01	0.50	0.62
	Bank slope: Shallow step	-0.01	0.03	0.40	0.69
	Substrate: Sand	-0.02	0.04	0.48	0.63
	Substrate: Silt	0.01	0.03	0.31	0.76
	Protected water habitat: Open shore	0.00	0.08	0.00	1.00
	Protected water habitat: Open water	-0.03	0.10	0.25	0.80

Table S3. Full AICc table used for model selection for the proportion of limnetic ancestry in a trap. Blank cells indicate that a term was not present in a given model.

Intercept	Bank slope	Depth (m)	Distance to vegetation (m)	Water Habitat	Substrate 'type'	df	ΔAICc	weight
0.77						2	0.00	0.15
0.75	+					3	0.63	0.11
0.75					+	4	0.68	0.11
0.79		-0.03				3	1.40	0.07
0.77			-0.01			3	1.48	0.07
0.76			-0.02		+	5	1.81	0.06
0.78		-0.03			+	5	1.98	0.06
0.76	+		-0.01			4	2.07	0.05
0.75	+				+	5	2.31	0.05
0.77	+	-0.02				4	2.45	0.04
0.79		-0.02	-0.01			4	3.21	0.03
0.75	+		-0.02		+	6	3.49	0.03
0.78		-0.02	-0.01		+	6	3.54	0.03
0.77	+	-0.03			+	6	3.96	0.02
0.77	+	-0.01	-0.01			5	4.16	0.02

0.79				+		4	4.25	0.02
0.79	+			+		5	4.92	0.01
0.79				+	+	6	5.07	0.01
0.77	+	-0.02	-0.01		+	7	5.51	0.01
0.80		-0.03		+		5	5.62	0.01
0.81			-0.01	+		5	5.77	0.01
0.82			-0.02	+	+	7	6.18	0.01
0.81	+		-0.01	+		6	6.37	0.01
0.80		-0.03		+	+	7	6.43	0.01
0.79	+			+	+	7	6.76	0.01
0.80	+	-0.02		+		6	6.85	0.00
0.81		-0.03	-0.01	+		6	7.56	0.00
0.82	+		-0.02	+	+	8	7.88	0.00
0.82		-0.03	-0.01	+	+	8	8.06	0.00
0.80	+	-0.03		+	+	8	8.55	0.00
0.81	+	-0.01	-0.01	+		7	8.61	0.00
0.82	+	-0.02	-0.01	+	+	9	10.12	0.00

Table S4. Full-model averaged coefficients for the mean limnetic ancestry in a given trap using delta $\Delta AIC \leq 2$ and $\Delta AIC \leq 6$ cut-offs.

ΔAIC cut-off		β	Standard error	z	p
≤ 2	Intercept	0.77	0.02	36.13	<0.001
	Bank slope: Shallow step	0.00	0.01	0.34	0.74
	Substrate: Sand	0.01	0.02	0.44	0.66
	Substrate: Silt	0.02	0.03	0.31	0.54
	Depth (m)	-0.01	0.02	0.32	0.75
	Distance to vegetation (m)	-0.00	0.01	0.32	0.75
≤ 6	Intercept	0.77	0.04	20.85	<0.001
	Bank slope: Shallow step	0.01	0.02	0.46	0.64
	Substrate: Sand	0.01	0.02	0.44	0.66
	Substrate: Silt	0.02	0.03	0.63	0.53
	Depth (m)	-0.01	0.02	0.34	0.73
	Distance to vegetation (m)	-0.00	0.01	0.39	0.70
	Protected water habitat: Open shore	-0.00	0.03	0.06	0.95
	Protected water habitat: Open water	-0.00	0.03	0.05	0.96

Table S5. General linear model showing that the total number of stickleback captured in a trap and the total time over which a trap was set do not affect proportion of shared ancestry in a trap.

Model	Coefficient	β	SE	z	p
Total number of stickleback in a trap	Intercept	0.41	0.19	2.11	0.04
	Number of stickleback	-0.00	0.00	-0.57	0.57
Total time over which a trap was set	Intercept	3.36	5.35	0.63	0.53
	Time	-0.41	0.71	-0.57	0.57

List of abbreviations

°C	Degrees Celsius
µL	Microlitre
µS	Microsecond
AIC	Akaike's information criterion
AICc	Akaike's information criterion with small sample size correction
ΔAIC	The difference between the model with the lowest AIC(c) and a given model
Alum	Aluminum phosphate
ANOVA	Analysis of variance
AUP	Animal use protocol
CI	Confidence interval
cm	Centimetres
D	Day
df	Degrees of freedom
F	F statistic or female
F0	Parental generation
F1	First filial generation
F2	Second filial generation
F3	Third filial generation
g	Gram
GLM	Generalized linear model
GLMM	Generalized linear mixed model
GPS	Global Positioning System
h	Hour
IACUC	Institutional Animal Care and Use Committee
IQR	Interquartile range
L	Litre
LM	Linear model
LMM	Linear mixed model
m/M	Metre or male
mg	Milligram
mL	Millilitre
mm	Millimetre
MS-222	Tricaine methanesulfonate
n/N	Number or sample size

NA	Not applicable
NR	Not reported
p	P-value
PC	Principal component
PC1	The first principal component
PC2	The second principal component
PCA	Principal component analysis
pH	Potential of hydrogen
PIT	Passive Integrated Transponder
PVC	Polyvinyl chloride
r	Correlation coefficient
R^2	Coefficient of determination
sd/SD	Standard deviation
SE	Standard error
SEM	Structural equation model
SNP	Single nucleotide polymorphism
t	T-test statistic
v	Version
W	Wilcoxon test statistic
X^2	Chi-square test statistic
YSI	Yellow strings instrument
z	Z value
β	Regression coefficient
η_p^2	Partial eta-squared