UNIVERSITÉ DU QUÉBEC À MONTRÉAL

MODULATION OF MICROEVOLUTIONARY RESPONSES BY ANTHROPOGENIC IMPACTS AT THE BASE OF AQUATIC FOOD WEBS

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MODULATION DES RÉPONSES MICROÉVOLUTIVES PAR LES IMPACTS ANTHROPOGÉNIQUES À LA BASE DES RÉSEAUX TROPHIQUES AQUATIQUES

THÈSE

PRÉSENTÉE

COMME EXIGENCE PARTIELLE

DU DOCTORAT EN BIOLOGIE

PAR

MATHILDE SALAMON

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PREFACE

The present thesis is submitted in the manuscript format, and I am the first author of the articles presented below. All chapters were prepared to become distinct publications for submission. I have marginally edited the articles to fit the format of the thesis.

Chapter 1: Salamon, M., Astorg, L., Paccard, A., Chain, F., Hendry, A., Derry, A. M., Barrett, R.D.H (2023). Maladaptive migration from physiological refugia could constrain the rescue of native gastropods facing an invasive predator. Molecular Ecology, in revision

Chapter 2: Salamon, M., St-Martin, M., Barrett, R.D.H, Derry, A. M. (2023). Evolutionary rescue and adaptive reversal allowed the persistence of freshwater copepods during historical lake acidification and pH recovery. In preparation for submission to Proceedings of the Royal Society B: Biological Sciences

Chapter 2: Salamon, M., Barrett, R.D.H, Derry, A. M. (2023). Climate adaptation in freshwater copepods at latitudinal and microgeographic spatial scales. In preparation for submission to American Naturalist

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RÉSUMÉ

Les impacts anthropiques provoquent d'importantes perturbations environnementales dans les écosystèmes d'eaux douces nordiques. Les populations et les espèces pourraient réagir et persister face à ces impacts via l'adaptation et les flux de gènes, et subir des changements démographiques. Pour les organismes à la base des réseaux trophiques d'eau douce, ces processus et leur interaction sont relativement peu documentés. Ma thèse porte sur deux espèces non-modèles d'eau douce: le copépode *Leptodiaptomus minutus* et le gastéropode *Amnicola limosus*. J'ai étudié 1) l'adaptation locale des gastéropodes à un prédateur invasif (le gobie à tâches noires *Neogobius melanostomus*) et à la limitation en calcium, 2) le sauvetage évolutif et le renversement adaptatif des copépodes en réponse à l'acidification historique des lacs et 3) l'adaptation locale des copépodes au climat. J'ai utilisé du séquençage de génomes entiers groupés pour détecter les signatures de la sélection, quantifier les flux de gènes et inférer leur histoire démographique, en combinaison avec des expériences de transplant réciproque et d'écologie de la résurrection. Premièrement, douze populations de gastéropodes ont été séquencées, provenant d'habitats situés dans le fleuve Saint-Laurent et un de ses affluent, le long de gradients d'impact de prédateurs invasifs et de concentration de calcium qui limite la distribution des gobies. Deuxièmement, j'ai obtenu des séquences génomiques temporelles à partir d'œufs de résistance de copépodes extraits de carottes de sédiments, pour deux lacs touchés par l'acidification dans le parc de Killarney, ON, Canada, et j'ai mesuré les traits d'histoire de vie d'individus ressuscités. Enfin, des populations de copépodes ont été séquencées le long a) d'un gradient latitudinal du sud du Québec au Groenland, et b) d'un gradient micro-géographique de température estivale et de carbone organique dissous coloré lié au brunissement à Cape-Race, T.-N.-L., Canada.

Dans le premier chapitre, j'ai montré que les habitats de gastéropodes non envahis pourraient servir de refuges physiologiques et fournir des migrants aux populations envahies, par le biais d'un flux de gènes importants (i.e. un sauvetage démographique/génétique). Cependant, comme les populations sources et puits sont différentiellement adaptées (à la faible concentration de calcium et aux gobies), cela génère un conflit entre les deux types de sauvetage, car l'immigration d'individus adaptés aux refuges pourrait entraîner une dépression hybride dans les populations envahies et éroder leur adaptation locale à l'envahisseur.

Dans le deuxième chapitre, j'ai étudié la combinaison de facteurs (démographiques, génétiques et environnementaux) qui ont facilité le sauvetage évolutif des populations de copépodes soumises à un fort impact environnemental (acidification historique), et qui influencent les conséquences à long terme du sauvetage lors du rétablissement environnemental. Le rétablissement après l'acidification a entraîné un renversement adaptatif, mais des effets potentiellement néfastes sur le potentiel adaptatif des populations sauvées restent présents.

Enfin, j'ai documenté l'influence de la dérive génétique et des flux de gènes sur l'adaptation locale au climat, à différentes échelles spatiales. J'ai détecté des signaux putatifs d'adaptation au climat le long des gradients latitudinaux et micro-géographiques. À l'échelle latitudinale, le flux de gènes semble être limité entre les régions, avec la possibilité de flux de gènes longue-distance. La diversité génétique est réduite, combinée à de forts effets de dérive à la périphérie de l'aire de

répartition (Groenland). A l'échelle micro-géographique, les flux de gènes sont élevés avec une forte diversité génétique malgré un effet important de la dérive. Ces résultats indiquent une adaptation micro-géographique à Cape-Race, où les flux de gènes entre des populations divergentes pourrait atténuer les effets de la dérive.

En utilisant des espèces non-modèles, le travail présenté a permis d'obtenir des connaissances substantielles sur l'interaction entre les processus micro-évolutifs et écologiques qui déterminent les réponses des espèces et populations face aux impacts anthropogéniques. Ma thèse a également apporté des informations cruciales sur des concepts clés pour la conservation (sauvetage génétique et évolution), notamment en ce qui concerne leur application dans les populations naturelles. Ces contributions constituent un progrès significatif pour les espèces à la base des réseaux trophiques d'eau douce, qui sont sous-étudiées mais importantes écologiquement. Ma thèse s'inscrit dans le cadre plus général de combler les lacunes dans les connaissances sur les impacts globaux sur les écosystèmes, qui sont nécessaires pour implémenter des décisions en conservation, afin de mieux préserver la biodiversité d'eau douce qui subit un rapide déclin.

Mots clés : Adaptation rapide, démographie, flux de gènes, sauvetage génétique, sauvetage évolutif, génomique, expériences de transplant, écologie de la résurrection, écosystèmes d'eau douce, copépodes, gastéropodes, espèces envahissantes, acidification, changement climatique.

ABSTRACT

Anthropogenic impacts are causing significant environmental disturbances in Northern freshwater ecosystems. Populations and species could respond and persist through adaptation and gene flow and undergo demographic changes. For organisms at the base of freshwater food webs, these processes and their interaction are relatively unknown. My thesis focuses on two non-model freshwater species: the copepod *Leptodiaptomus minutus* and the gastropod *Amnicola limosus*. I investigated 1) the local adaptation of gastropods to an invasive predator (the round goby *Neogobius melanostomus*) and calcium limitation, 2) the evolutionary rescue and adaptive reversal of copepods in response to historical lake acidification, and 3) the local adaptation of copepods to climate. I used pooled whole-genome sequences to detect signatures of selection, quantify gene flow and infer their demographic history, combined with reciprocal transplant experiments and resurrection ecology. First, twelve gastropod populations were sequenced, from habitats located in the Saint Lawrence River system along gradients of invasive predator impact and calcium concentration that is limiting the distribution of the invader. Second, I obtained temporal genomic samples from copepod resting eggs extracted from sediment cores in two acid-impacted lakes from Killarney Park, ON, Canada, and measured life-history traits on resurrected individuals. Finally, copepod populations were sequenced along a) a latitudinal gradient from southern Quebec to Greenland, and b) a microgeographic gradient of summer temperature and colored dissolved organic carbon related to browning at Cape-Race, NL, Canada.

In the first chapter, I showed that uninvaded gastropod habitats could act as physiological refugia and provide migrants to invaded populations through strong gene flow (i.e., demographic/genetic rescue). However, as these source and sink populations are divergently adapted (to the low calcium concentration and the invader), this generates a conflict between the two types of rescues, as immigration of individuals adapted to the refuges could result in outbreeding depression in the invaded populations and erode their local adaptation to the invader.

In the second chapter, I investigated the combination of factors (demographic, genetic, and environmental) that facilitated evolutionary rescue in copepod populations from a strong environmental impact (historical acidification) and are influencing the long-term consequences of the rescue during environmental recovery. The recovery from acidification resulted in an adaptive reversal but with potentially detrimental effects on adaptive potential in the rescued populations.

Finally, I documented how drift and gene flow can influence local adaptation to climate across different spatial scales. I detected putative signals of climatic adaption along both the latitudinal and microgeographic gradients. At the latitudinal scale, gene flow appears to be limited among regions, with some possible long-distance gene flow, and genetic diversity is reduced combined with strong effects of drift at the range margin (Greenland). At the microgeographic scale, gene flow is strong with high genetic diversity despite important drift. This provides evidence for microgeographic adaptation at Cape-Race, where gene flow between divergent populations could alleviate the effects of drift.

This work employed non-model species to provide meaningful insights into the interaction between micro-evolutionary and ecological processes driving the responses to anthropogenic impacts. My thesis also provided critical knowledge on key concepts for conservation (genetic and evolutionary rescue), particularly regarding their application in natural populations. These contributions constitute important progress for understudied yet ecologically significant species at the base of freshwater food webs. They fit within the larger goal of filling knowledge gaps on global impacts in these ecosystems, which is needed to implement conservation decisions for the preservation of rapidly declining freshwater biodiversity.

Keywords: Rapid adaptation, demography, gene flow, genetic rescue, evolutionary rescue, genomics, transplant experiments, resurrection ecology, freshwater ecosystems, copepods, gastropods, invasive species, acidification, climate change

INTRODUCTION

1.1. Preamble

The present thesis focuses on the evolutionary and ecological responses to global changes (invasive species, historical acidification, and climate change) in non-model species at the base of freshwater food webs. Knowledge of micro-evolutionary and ecological processes is particularly lacking for these species despite their ecological significance and the vulnerability of freshwater ecosystems facing steep biodiversity declines. I also address knowledge gaps on important concepts in conservation (demographic, genetic, and evolutionary rescue) to better assess their applicability in nature. I used a combination of whole-genome pool sequencing and reciprocal transplant experiment as well as resurrection ecology to further our understanding of these processes and concepts. In the following introduction, I present anthropogenic impacts on freshwater ecosystems, evolutionary ecology as a tool to provide information on species and populations' responses to these impacts, state of knowledge on micro-evolutionary processes in freshwater species, genomic tools, and our study species, and the general approaches used in this work.

1.2. Anthropogenic impacts on northern freshwater ecosystems

Freshwater ecosystems are facing multiple direct threats from anthropogenic activities, such as climate change, pollution, and invasive species (Jaureguiberry et al., 2022). Despite their vulnerability and the ecosystem services they provide, these ecosystems have received less consideration than terrestrial and marine ecosystems (Maasri et al., 2022), even though freshwater ecosystem experience higher extinction rates than terrestrial ecosystems (Albert et al., 2021). Climate change is projected to strongly impact the physical, chemical, and ecological properties of northern freshwater ecosystems (Heino et al., 2009; Woolway et al., 2020). These impacts include disrupting the seasonal timing of break-up and freeze-up of lake ice (Derksen et al., 2012), shifting temperature and stratification patterns (Kraemer et al., 2015), and altering community composition, phenology, and seasonal succession of biological communities (Bradshaw & Holzapfel, 2008; Stoks et al., 2014; Vadadi-Fülöp & Hufnagel, 2014). Increased concentrations of colored dissolved organic matter through changing precipitation and temperature regimes (i.e., browning; Solomon et al., 2015) then disrupt light and temperature characteristics as well as food web dynamics (Lefébure et al., 2013; P. T. Kelly et al., 2016).

Another important threat to freshwater ecosystems is pollution (Jaureguiberry et al., 2022), with historical acidification in the Canadian regions surrounding Sudbury, ON, providing a good example (Keller et al., 2003). At Killarney Park, ON, acidified, recovered, and buffered lakes coexist at the local scale following anthropogenic acidification in the 1960s. This important environmental impact resulted from the activity of smelters and mines at Sudbury ON, located 60 km north of Killarney, which peaked in the 1950s. The acidification co-occurred with deposits of toxic metals (A. S. Dixit et al., 1992) from the atmosphere (Cu and Ni) and acid soil run-offs (Al and Mn). This provoked important mortalities and local extinctions of fishes (Beamish & Harvey, 1972), zooplankton (Sprules, 1975), and phytoplankton species (Kwiatkowski & Roff, 1976). Reduction of emissions in the 1970s led to the progressive chemical recovery of the lakes, with decreased metal concentrations and a return to pre-acidification pH values in some lakes, but others remain acidified (Keller et al., 2003). The biological recovery has however been slower than the chemical recovery and communities have not been able to return to their initial composition and abundance (Gray & Arnott, 2012). For zooplankton species, this is in part due to dispersal limitations and allee effects (Gray & Arnott, 2011, 2012), community resistance (Gray & Arnott, 2012), predation (Gray et al., 2012), and emergent threats, such as climate change (Meyer-Jacob et al., 2019), exotic invasion (Gray et al., 2012), and calcium limitation linked to the elemental depletion of watershed soils as a result of historical acidification and forest harvesting (Ross & Arnott, 2022).

Finally, invasive species also considerably threaten freshwater ecosystems (Carpenter et al., 2011) for instance as a key driver of freshwater biodiversity loss (Dextrase & Mandrak, 2006; Jaureguiberry et al., 2022). They have a strong negative effect on freshwater species abundance (Gallardo et al., 2016), and can trigger shifts in the physicochemical and biological properties of freshwater environments (Gallardo et al., 2016; Emery-Butcher et al., 2020). Overall, there is thus a pressing need to improve knowledge of global impacts on freshwater biodiversity (e.g., specie's ecological and evolutionary responses; Maasri et al., 2021), to inform conservation measures aimed at bending the downward curve of freshwater biodiversity loss (Tickner et al., 2020).

1.3. Evolutionary ecology as a tool to understand populations' and species' responses to rapid environmental change

Rapid and widespread ecological responses to anthropogenic impacts have been widely documented (Early et al., 2016; Jackson et al., 2016; Scheffers et al., 2016; Lehnherr et al., 2018; Jaureguiberry et al., 2022). Similarly, a large number of studies have reported changes at the phenotypic level in human-disturbed populations (Sanderson et al., 2021) as well as rapid adaptation (Geerts et al., 2015; Lee et al., 2017; Melotto et al., 2020; Arietta & Skelly, 2021). Improving our understanding and providing predictions on the rapid adaptation of species to global biodiversity threats are crucial targets in the field of evolutionary biology (Hoffmann & Sgrò, 2011; A. Waldvogel et al., 2020). This is because it could allow populations and species to withstand environmental disturbances (Carlson et al., 2014). Adaptive responses could reinforce the persistence of populations by enabling competitive advantage and colonization resistance against migrants via a "monopolization effect", whereby locally adapted populations have higher fitness than migrant genotypes or species and gain a numerical advantage, thus preventing their establishment (de Meester et al., 2016; van Doorslaer et al., 2009). On the other hand, if populations are unable to adapt or disperse and environmental conditions outstrip their tolerance range, they could undergo local extinctions, thereby favoring the invasion of genotypes and species that were previously unable to tolerate these conditions and shifting species assemblages (Hickling et al., 2006; Vanoverbeke et al., 2016; Meester et al., 2018).

The persistence of species and populations in the face of these threats depends on various ecological and evolutionary processes and their interaction: adaptive potential, dispersal and gene flow, demography, species interactions, and the speed and scale of environmental shifts (Urban et al., 2016b; Fig.1.1; Bay, Rose, Barrett, et al., 2017). Populations may be able to respond adaptively through evolution (Geerts et al., 2015; Barrett et al., 2019), plasticity (Merilä & Hendry, 2014; Levis et al., 2018; Fox et al., 2019), or the evolution of plasticity (Crispo et al., 2010; M. Kelly, 2019; Brennan, DeMayo, et al., 2022). Accurate predictions on future adaptive responses require integrated knowledge about the genetic architecture of adaptive traits, the spatial distribution of adaptive alleles, the importance of phenotypic plasticity, and the population dynamics of organisms; information which is often lacking for many non-model species (Urban et al., 2016b; Bay, Rose, Barrett, et al., 2017). The complexity of the interaction between mechanisms involved in adaptive

responses brings uncertainty about the fate of many populations and species (Kokko et al., 2017), and predictive models have only recently begun to incorporate evolutionary mechanisms (Bay, Rose, Logan, et al., 2017; Razgour et al., 2019; Aguirre-Liguori et al., 2021; Wuitchik et al., 2022). Accordingly, it is important to expand the evaluation and integration of evolutionary potential (the capacity of phenotypic traits to evolve leading to population growth under future conditions), to provide more accurate extinction risk assessment, and identify targets of conservation to preserve biodiversity (Forester et al., 2022).

The adaptive potential will depend to a large extent on selection acting on standing genetic variation (SGV) as it can facilitate rapid adaptation by increasing the speed of evolution (Barrett & Schluter, 2008; Hermisson & Pennings, 2017; Bitter et al., 2019; D. Ben Stern & Lee, 2020; Chaturvedi et al., 2021; Brennan, deMayo, et al., 2022). Identifying and locating the spatial distribution of adaptive alleles, as well as assessing the role of gene flow in bringing adaptive alleles into threatened populations, are thus important to evaluate the potential for rapid adaptation (Dixon et al., 2015; Bay, Rose, Barrett, et al., 2017; Bay, Rose, Logan, et al., 2017). In addition, the genetic architecture of adaptive loci (number of loci, fitness effects, linkage, epistasis, pleiotropy) will influence the speed and probability of adaptation (Bay, Rose, Barrett, et al., 2017). This factor can reinforce local adaptation in the face of gene flow by preventing swamping through, linkage, multiple small-effects loci, pleiotropy, and reduced recombination (Tigano & Friesen, 2016). Phenotypic plasticity will likewise influence positively or negatively adaptive responses. It can be maladaptive by masking the effect of selection and incurring energetic costs (Crispo, 2008), and decreasing population viability in unpredictable environments (Reed et al., 2010). Alternatively, plasticity can be adaptive, for example in fluctuating environments (Hallsson & Björklund, 2012), and can be under selection (Levis et al., 2018; M. Kelly, 2019). Finally, plasticity can also also be transmitted between generations and be either adaptive or maladaptive depending on the reliance of species on environmental predictability, which is often disrupted in the case of anthropogenic impacts (Donelan et al., 2020)

Reconstructing demographic changes can be helpful to assess potential population declines induced by anthropogenic impacts (Arietta & Skelly, 2021) and the vulnerability of populations due to small (effective) population size (Gompert et al., 2021). Demography is also important as it will influence local adaptation because adaptive alleles are lost more quickly in small populations from the effect

of drift (Blanquart et al., 2012), and effective population size has a positive relationship with the efficacy of selection (Lanfear et al., 2014). A special case where demography and selection interact is the evolutionary rescue, where an initial environmental disturbance induces a population decline, which is then offset by the increase in adaptive allele frequency (G. Bell & Gonzalez, 2009; Carlson et al., 2014; G. Bell, 2017). This process has previously been proposed as a conservation measure to avoid extinction (Mills et al., 2018), but important uncertainties remain regarding its applicability in natural populations (McDermott, 2019). Even though experimental and theoretical studies have explored the factors influencing the probability of evolutionary rescue (e.g., environmental impact, phenotypic plasticity, starting population size, new mutation or standing variation; Carlson et al., 2014; Orr & Unckless, 2014; Hufbauer et al., 2015; Ashander et al., 2016; Anciaux et al., 2018), and occurrences of rescue have been detected in the wild (Tinghitella, 2008; Carlson et al., 2014; Oziolor et al., 2019; Gignoux‐Wolfsohn et al., 2021), the role of these factors and their combination on promoting evolutionary rescue in natural populations is still not well understood. Additionally, the rescue could have negative consequences in the long term, particularly due to a loss of genetic diversity from demographic stochasticity and strong selection, jeopardizing the adaptive potential and persistence of populations (G. S. Stewart et al., 2017). These consequences are still relatively unknown in the wild, even though knowledge of the outcome of evolutionary rescue on a longer timescale is needed to fully evaluate its applicability for conservation (Carlson et al., 2014).

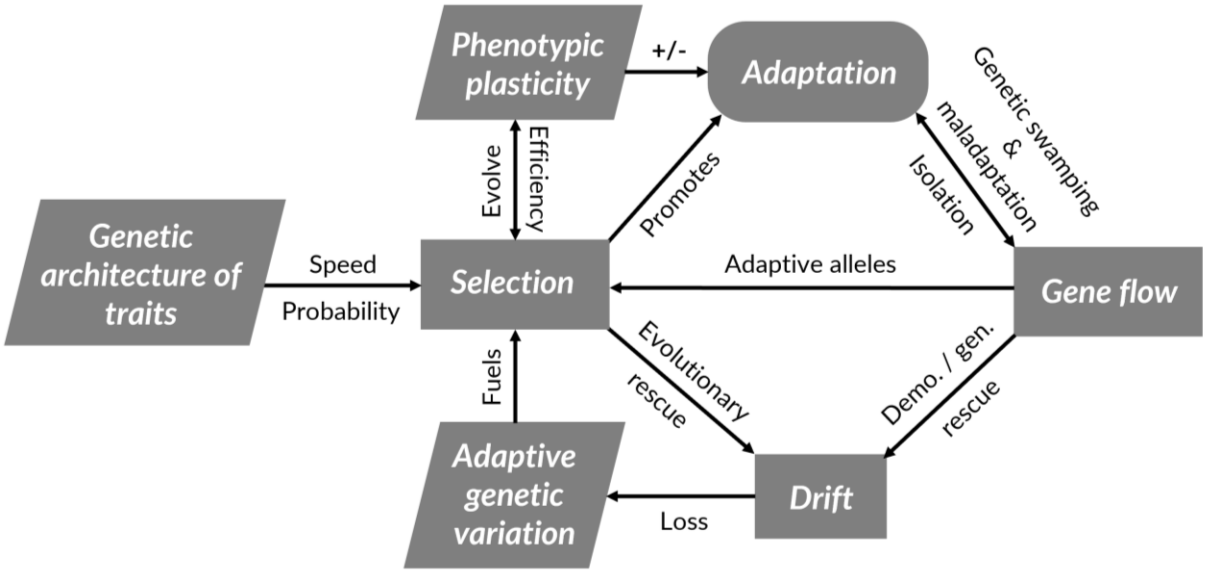
Quantifying the magnitude of gene flow is likewise critical in the context of environmental disturbance, to assess the possibility of habitat tracking (Matz et al., 2018) and range shifts (Razgour et al., 2019). Gene flow interacts positively and negatively with adaptation. Positive interactions include the preservation of genetic diversity in small populations by alleviating the effect of drift (Gompert et al., 2021) and as an input of adaptive alleles (Dixon et al., 2015; Matz et al., 2018). It can also reduce local adaptation (Crispo, 2008; Blanquart et al., 2012) through maladaptive migration (D. A. Bell et al., 2019) and genetic swamping (Tigano & Friesen, 2016). The special case when the input of new alleles through migration into small, inbred populations leads to population growth is termed genetic rescue (Hufbauer et al., 2015; Whiteley et al., 2015; Fitzpatrick et al., 2016; D. A. Bell et al., 2019), which has generated considerable interest for conservation (Weeks et al., 2017; Ralls et al., 2018; Hoffmann et al., 2021; Willi et al., 2022).

However, it can lead to outbreeding depression in the case of mixing between adaptively divergent populations (Frankham et al., 2011), a risk that is sometimes neglected (Hamilton & Miller, 2016; Ralls et al., 2020). Demographic rescue of small populations through immigration can also occur (Hufbauer et al., 2015), highlighting the tension between the positive outcome of immigration and the negative outcome, through the input of maladaptive gene flow, which can deter these effects.

Adaptation can, in turn, reduce gene flow (Crispo, 2008), as migrants have reduced fitness in divergent environments, through processes such as monopolization effects (de Meester et al., 2016) and selection against migrants (Bolnick & Nosil, 2007), which can result in patterns of isolation by environment (Wang & Bradburd, 2014). Even though local adaptation, drift, and gene flow interact through multiple processes to determine the population's responses to environmental shifts, they are not always studied conjointly (Ahrens et al., 2018), especially regarding the impact of climate change (Schmidt et al., 2021; but see Gamboa et al., 2022). Another aspect to consider is the spatial scale, as the effect of these processes can differ depending on the scale considered, which means that their inference may thus not be generalizable from one scale to another (Aguirre-Liguori et al., 2021). Signals of selection from climate are not necessarily consistent between spatial scales (Rellstab et al., 2017; Gugger et al., 2021), drift may be more related to demographic history at a broad scale (Thörn et al., 2021), but depend on population size at fine scale (Gompert et al., 2021), while gene flow may be disrupted at small spatial scale but not at a regional scale as a consequence of habitat degradation (Harrisson et al., 2012). It is thus important to better characterize the interaction between adaptation, demography, and gene flow and how they modulate populations' overall response to global change, a knowledge that is especially lacking in understudied and threatened freshwater ecosystems.

1.4. Insights into micro-evolutionary processes in freshwater ecosystems

Figure 1.1: Conceptual diagram illustrating the interactions between selection, drift, gene flow, and adaptation (emerging property), and some of the factors influencing these processes.



Significant insights into micro-evolutionary processes have been obtained for freshwater ecosystems (Grummer et al., 2019). Shifts in lake environments have been shown to induce rapid genetic adaptation, for example in *Daphnia magna* from temperature changes (Geerts et al., 2015) and predation pressure (Chaturvedi et al., 2021), the latter being facilitated by large amounts of standing variation. Other remarkable examples were observed in three-spined sticklebacks, for example through rapid adaptation to lake versus stream habitats (Marques et al., 2019; K. Reid et al., 2021). Inference of gene flow made it possible to assess population connectivity in an endangered freshwater snail and to identify barriers to gene flow from anthropogenic activities (Redak et al., 2021). Studies have likewise inferred demographic, adaptation, and gene flow in combination, as well as how they interact in some cases. Investigating the interaction between demographic processes and local adaptation demonstrated that small brook trout populations with low genetic diversity were able to maintain high fitness in novel pond environments (Yates et al., 2019). A study on freshwater midges uncovered climatic adaptation along a latitudinal gradient and estimated contemporary gene flow in addition to demographic changes in the distant past (A. M. Waldvogel et al., 2018). Demographic changes and patterns of gene flow have similarly been related to past climates, for instance by documenting colonization events post-glaciation in endangered freshwater mussels (Inoue et al., 2014). Inferring jointly demography and gene flow

has additionally made it possible to answer applied questions, for example by identifying fish populations in need of assisted migration (low effective population size and genetic diversity, river landscape fragmentation; Pavlova et al., 2017), or by applying and tracking genetic rescue in guppies (Fitzpatrick et al., 2016, 2020). Finally, some studies have examined the interaction between gene flow and selection in three-spined sticklebacks, finding that adaptive genetic polymorphisms can be maintained in populations even in the case of strong gene flow and weak selection (Raeymaekers et al., 2014) and that standing genetic variation can be maintained by being selectively neutral and at low frequency in ancestral populations (as opposed to positively selected in derived environments; Haenel et al., 2022). Despite these advances, knowledge of micro-evolutionary and ecological processes, as well as their combined effects, are still understudied for species at the basis of aquatic food webs, particularly relative to human impacts (Stoks et al., 2014; Loria et al., 2019).

1.5. Using genomic tools to study evolutionary processes

The reduction in sequencing costs of individual and population genomes (Schlötterer et al., 2014) has greatly increased the application of genomic techniques to characterize micro-evolutionary processes in model and non-model species (Davey et al., 2011; Ahrens et al., 2018). Genomic sequencing methods enable the identification of millions of Single Nucleotide Polymorphisms (SNPs) that can be used in downstream analyses of population genomics. More recently, genomics studies have transitioned from the use of reduced representation methods such as Restriction site-associated DNA sequencing (RADseq) to whole-genome-resequencing (WGS; Bourgeois & Warren, 2021). WGS, even though more costly than previous sequencing methods, has the advantage of a better representation of the genome and thus lowers the chance of missing putative loci under selection (Lowry et al., 2017). It can also be combined with pool-sequencing, thereby dramatically reducing sequencing costs (Schlötterer et al., 2014). A key aspect to consider when using a pool-sequencing approach and other types of genome sequencing is the availability of a reference genome (Schlötterer et al., 2014; Jung et al., 2020), which is necessary to align the raw sequence reads and to conduct analyses that require information about the position of SNPs on the genome (e.g., functional enrichment analyses; Kofler & Schlötterer, 2012). An additional caveat is that it does not allow to obtain individual haplotype data (Schlötterer et al., 2014) and offers limited information about linkage disequilibrium (LD, the association of alleles at two loci due to non-

random genetic processes; e.g., Feder et al., 2012), which is useful for a range of population genetic methods (e.g., Kwon et al., 2022). Analytical methods leveraging LD can for instance aid in characterizing the architecture of traits under selection (e.g., differentiating between direct causative variants vs indirect selection on neutral SNPs physically linked to causative variants; Rêgo et al., 2019) or demographic changes (Beichman et al., 2018). Despite this, the application of population genomic tools based on patterns of allele frequencies within and between populations can offer crucial insights into patterns of local adaptation (D. B. Stern et al., 2022), gene flow (Pfenninger et al., 2015) and demography (Christe et al., 2017).

Recent advances in bioinformatic tools offer enormous potential for providing important insights into evolutionary mechanisms shaping adaptive responses of non-model species to climate change and other forms of environmental stress (Hoban et al., 2016; A. Waldvogel et al., 2020). They also permit the assessment of population connectivity, the investigation of demographic and genetic diversity changes, and the detection of the potential for genetic adaptation (e.g., D. Ben Stern & Lee, 2020). To uncover putative loci under selection, genome scan analyses are employed and divided into two categories: Outlier (OA) and Environmental Association analyses (EAA). OA identify SNPs that are highly differentiated in their allele frequencies between compared populations, while EAA detects significant associations between allele frequencies and environmental variables of interest (Ahrens et al., 2018). Further investigations of outlier SNPs can give us insight into the physiological functions putatively under selection and the architecture of adaptive traits (e.g., Morales et al., 2019). Gene flow can be assessed indirectly through patterns of population structure (Grummer et al., 2019) and with population differentiation measures (e.g., pairwise F_{ST}), which can then be used to assess a pattern of isolation by distance (Rousset, 1997). Direct inference of gene flow rates can be obtained from different methods, for example by using coalescent and Bayesian approaches on phased (individual) spatialized data (House & Hahn, 2018) or using the allele frequency spectrum (SFS, Gutenkunst et al., 2009). To reconstruct demographic history, various methods exist as well: based on the SFS, on phased data from LD, from coalescent simulations, or by using Approximate Bayesian Computation on summary statistics (Beichman et al., 2018). A specific field where the study of local adaptation, gene flow, and demography intersect with environmental variation through the application of genomic tools is landscape genomics, which is still burgeoning for freshwater ecosystems (Grummer et al., 2019). There is also a species

bias in the use of genomic methods, to the detriment of freshwater invertebrates (Ahrens et al., 2018). Leveraging recent development in genomic analysis tools could thus bring important insights into micro-evolutionary and ecological processes in non-model species (A. Waldvogel et al., 2020) at the base of freshwater food webs.

1.6. Copepods and gastropods as non-model freshwater species to study rapid adaptation

Copepods are key taxa in freshwater and marine ecosystems due to their abundance and diversity (Bron et al., 2011). Further, in freshwater ecosystems, copepods provide essential nutritional compounds (DHA: Docosahexaenoic acid) to higher trophic levels (Grosbois et al., 2017), which cannot be synthesized *de novo* in many organisms, and is less abundant in other freshwater zooplankton taxa such as cladocerans (Burns et al., 2011; Strandberg et al., 2015; Twining et al., 2021). Calanoid copepods, which are the focus of the present thesis, have a seasonal life cycle in northern environments, with adults surviving under the ice or as diapausing eggs (bet-hedging strategy; Hairston et al., 1985), which allows early developmental stages (nauplii) to coincide with the phytoplankton bloom in spring and avoidance of predators (Grosbois et al., 2017). Phenological, demographic, and phenotypical changes have already been observed in freshwater and marine copepods from long-term time-series data, potentially as a result of climate change (Winder et al., 2009; Poloczanska et al., 2013; Vadadi-Fülöp & Hufnagel, 2014).

Copepods are an emerging model system to study responses to contemporary and rapid evolutionary change in zooplankton using genomics (Bron et al., 2011; Madoui et al., 2017; Brennan, deMayo, et al., 2022; D. B. Stern et al., 2022), although most of this research has focused on marine or estuarine copepods. Examples of copepod species used in studies of genetic adaptation include 1) the harpacticoid tidepool copepod *Tigriopus californicus* for the study of thermal adaptation (M. W. Kelly et al., 2013, 2017; Griffiths et al., 2021), 2) the calanoid *Eurytemora affinis* for understanding invasion and transition from saline to freshwater environments (Lee, 2016; D. Ben Stern & Lee, 2020; D. B. Stern et al., 2022) as well as adaptation to oil pollution (Lee et al., 2017), and 3) the cyclopoid *Oithona* spp. for studying responses to global oceanic changes (Madoui et al., 2017). Studies of marine copepods have also investigated adaptive responses to ocean warming and acidification (Bailey et al., 2017; Thor et al., 2018; Griffiths et al., 2021; Brennan, DeMayo, et al., 2022). Results were mixed for acidification, with

either small effects of selection or decreased fecundity and scope for growth, as well as divergent expression levels (Thor & Dupont, 2015; Bailey et al., 2017; Thor et al., 2018; Dam et al., 2021). On the contrary, the selection from warming was strong, resulting in positive changes in multiple life-history traits (Dam et al., 2021), increased heat tolerance as well as expression levels due to adaptive introgression from heat-tolerant populations (Griffiths et al., 2021) and synergistic action with acidification at the genomic level in addition to decreased plasticity (Brennan, DeMayo, et al., 2022; Brennan, deMayo, et al., 2022).

Even though significant insights into adaptive responses to multiple threats have been gained for marine copepods, similar evidence for freshwater copepods is lacking (Stoks et al., 2014). Documented examples of genetic adaptation are very scarce but include the adaptation of *Eurytemora affinis* to low salinity (D. ben Stern & Lee, 2020; D. B. Stern et al., 2022), of *Leptodiaptomus sicilis* to high lake salinity (Ortega‐Mayagoitia et al., 2022) and of *Onychodiaptomus sanguineus* to changes in predation levels affecting diapause timing (Hairston & Dillon, 1990; Ellner et al., 1999). Currently, there is additionally a paucity of data about gene flow between populations of copepods in freshwater landscapes at different spatial scales (Incagnone et al., 2015). Lacustrine zooplankton are passive dispersers (Incagnone et al., 2015) and can move over small spatial scales by rivers and floods, and over long distances through resting stages transported by wind and animals (Havel & Shurin, 2004). Gene flow in freshwater zooplankton is usually considered to be limited, potentially due to monopolization effects (de Meester et al., 2002; Havel & Shurin, 2004; but see Zeller et al., 2006; Ventura et al., 2014; Incagnone et al., 2015; Ortega-Mayagoitia et al., 2022). There are some indications that the freshwater calanoid copepod *Leptodiaptomus minutus* could have persisted during acute anthropogenic acidification through the rapid evolution of increased resistance to acidic pH (Frost et al., 2006; Derry & Arnott, 2007; Derry et al., 2010). *L. minutus* also appear to be sensitive to climate, as an extensive pond survey of Cape-Race, NF (Canada) showed that individuals had a larger body size and higher concentrations of essential fatty acids in warmer ponds (Charette & Derry, 2016). This species is broadly distributed along a latitudinal gradient from North-Eastern America (Stemberger, 1995; Pinel-Alloul et al., 2013), to Greenland (Samchyshyna et al., 2008; Oester et al., 2022) and Iceland (Antonsson & Antonsson, 1992), where it is dominant in zooplankton communities (Pinel-Alloul et al., 2013; St-Gelais et al., 2021). *L. minutus* is thus a

species of interest for the study of adaptation to climate and acidification, supported by its ecological importance, although no genomic resources were previously available.

Gastropods have also been used to study rapid adaptation, with a typical example being the response to predation (Seeley, 1986; Brookes & Rochette, 2007; Morales et al., 2019). *Littorina* snails were shown to respond adaptively to native predators through changes in allele frequencies resulting in phenotypic differences (Westram et al., 2018; thicker shells; Morales et al., 2019), and also by phenotypic plasticity with the development of thicker shells in response to invasive predatory crabs (Trussell & Smith, 2000; Brookes & Rochette, 2007). Adaptive responses to predators can be mediated by abiotic factors such as calcium concentration, which interact with the plastic response to affect morphological and behavioral traits (Rundle et al., 2004; Bukowski & Auld, 2014). In *Littorina* snails, the genetic differences in shell thickness between crab and wave ecotypes interacted with genetic differences in metabolism and physiology in ecotypes exposed to warmer, drier conditions of the high shore compared to the low shore ecotypes (Morales et al., 2019). Other examples of rapid adaptation in gastropods include the transition from high-current river habitats to low-current lake habitats through epigenetically determined plastic modifications of shell morphology (Thorson et al., 2017; Smithson et al., 2020), or adaptation to ocean acidification due to metabolism upregulation (Calosi et al., 2017). Gene flow between aquatic gastropod populations can be high or low depending on the mode of reproduction (broadcast spawner or direct development; J. J. Bell, 2008; Crandall et al., 2010; Keeney et al., 2009; Kyle & Boulding, 2000; Redak et al., 2021). In addition, gene flow can also be modulated by the behavior of individual gastropods, with dispersal by floating in the water column or drifting on floating vegetation for example (Little & Nix, 1976; Martel & Chia, 1991). *Amnicola limosus* is a small freshwater gastropod species with a large distribution (https://www.gbif.org/fr/species/5192461). It is a species of interest for the study of adaptation to invasive species, as it was shown to be impacted by the presence of an invasive predator (the round goby *Neogobius melanostomus*) in the Upper St. Lawrence River, resulting in strong demographic declines (up to 95-98% loss in abundance) and the decrease of its shell size of 0.5-1 mm to avoid predation (Kipp et al., 2012). However, *A. limosus* is also present at sites of this river system where the calcium concentration (Ca^{2+} concentrations below 22 mg/L, Sanderson, Derry, et al., 2021) is preventing round goby presence as they cannot tolerate low concentrations (Baldwin et al., 2012; Iacarella & Ricciardi,

2015), thereby creating refuges. Gene flow between the refuge and impacted populations might be limited as this species has a direct-development reproduction mode (Pinel-Alloul & Magnin, 1973). Developing genomic resources for the copepod *L. minutus* and the gastropod *A. limosus* will thus help fill a gap in the knowledge of micro-evolutionary and ecological processes of species at the basis of freshwater foodweb, and particularly their responses to global changes.

1.7. Thesis objectives

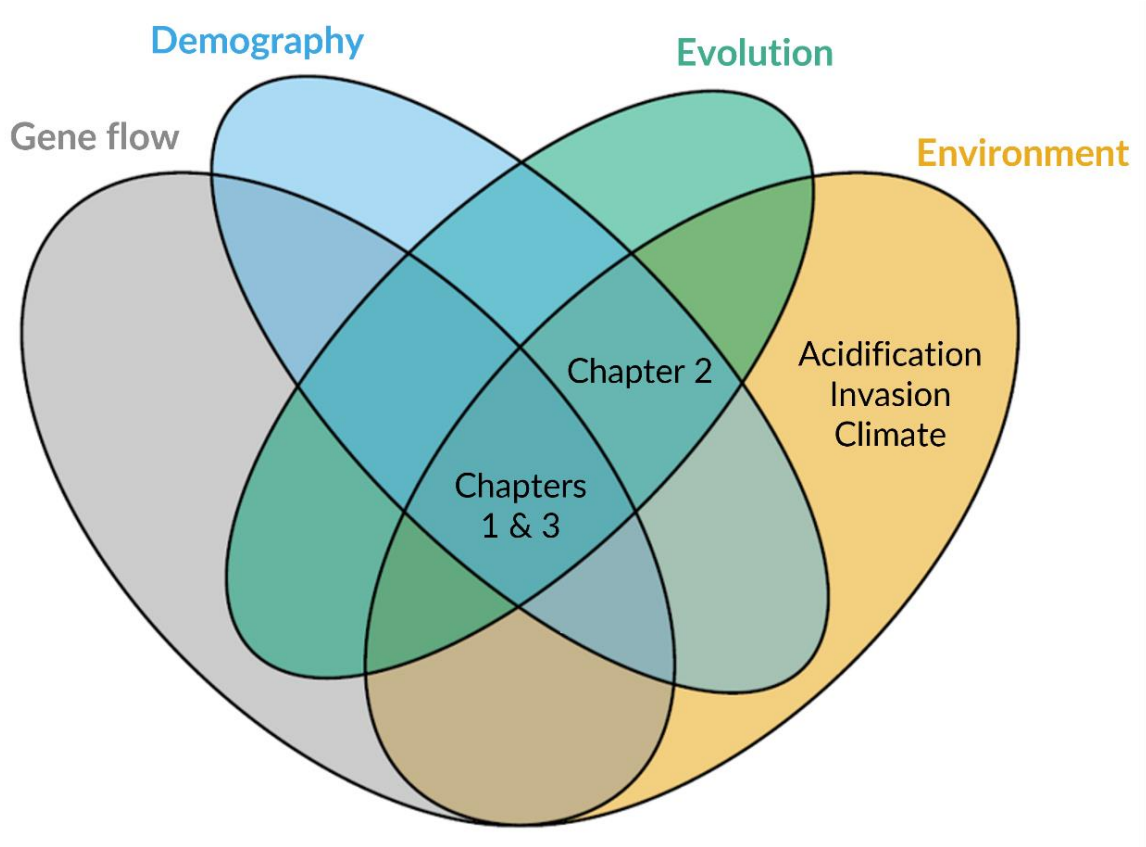
Despite important advances in the understanding of micro-evolutionary ecological processes in freshwater ecosystems, studies in landscape genomics on freshwater species are still rare (Grummer et al., 2019). Most genomics studies rely on model organisms (e.g., Daphnia genus and three-spined sticklebacks; Colbourne et al., 2011; K. Reid et al., 2021) whereas genomic information is still lacking for many non-model freshwater species (Ahrens et al., 2018). My thesis focuses on micro-evolutionary and ecological processes in understudied yet ecologically significant non-model invertebrate species at the base of freshwater food webs. It builds on the growing but limited literature on aquatic landscape genomics (Grummer et al., 2019). The objective of my thesis is thus to improve our understanding of rapid adaptation, gene flow patterns, and demographic processes, as well as their interactions, for two non-model freshwater species: the gastropod *Amnicola limosus* and the copepod *Leptodiaptomus minutus*. These processes are examined across different spatial and temporal scales, and as in response to three significant threats to freshwater biodiversity: invasive species, pollution, and climate change (Jaureguiberry et al., 2022). This overall objective was addressed through three independent chapters (Fig. 1.2):

- **Chapter 1:** To what extent does the local adaptation of gastropods (to an invasive predator and calcium limitation) interact with gene flow between the refuge and invaded populations? Does it generate a conflict between demographic and genetic rescue?

- **Chapter 2:** How do the combination of demographic, genetic, and environmental factors interact to facilitate the evolutionary rescue of the copepod *Leptodiaptomus minutus* from historical acidification? Do long-term effects of the rescue allow an adaptive reversal during recovery?

- **Chapter 3:** Are patterns of gene flow and genetic drift divergent at latitudinal versus microgeographic spatial scales? Can they differentially modulate adaptive responses of the copepod *L. minutus* to climate?

Figure 1.2 Venn diagram showing the four mechanisms addressed in the present thesis, illustrating the specific objectives of each chapter and the scale (temporal or spatial) studied.



1.8. General approaches

1.8.1. Development of a draft genome for the copepods

During my Ph.D. thesis, I worked on a draft assembly for the copepod *Leptodiaptomus minutus*, for which genomic resources did not exist, and no reference genome from a close species was available. Recently, a study used a genome assembly of the sister species *Leptodiaptomus sicilis*, but this assembly is still in its early stages (Ortega‐Mayagoitia et al., 2022). In partnership with the McGill Genome Center (MGC) and as part of the CanSeq150 project, the goal was to obtain an assembly that I could use for aligning the pool-seq Illumina pair-end reads. This was also motivated by the paucity of reference genomes available for copepods (Bron et al., 2011), with

only 13 reference genomes publicly available on NCBI as of 2023, all species from marine and brackish environments. An initial issue was to obtain large enough fragments of DNA in sufficient quantity after extraction, which are required for long-read sequencing. After numerous extraction tests (preserved or live individuals, individual or pooled, extraction methods), I was able to obtain satisfying results using the MagAttract HMW kit (QIAGEN, Toronto, ON, Canada) with pooled live individuals (between 6-76 copepods). Pooling individuals for the DNA extraction was motivated by the small size of the copepods (< 1 mm), and as it is the most popular approach for copepod genome assembly (Jørgensen et al., 2019b). I used this approach for all the libraries and sequencing platforms. Initially, two libraries were sequenced with 10X Genomics (one with 6 pooled individuals and one with 10 ind.). Then one library was sequenced on PromethION (Oxford Nanopore) from a separate sample (76 individuals), but the output of the ONT-seq was not very extensive (~16Gb) due to technical issues. I additionally used the Illumina pair-ended short-reads of some of the 22 populations sequenced (NovaSeq6000, PE150, between 120-300 million reads per population).

The initial rough estimate of the genome size was > 1 Gb based on the c-value of 1.77 from *L. sicilis*, using the genome size database: http://www.genomesize.com/. To estimate the genome size, I used a k-mer analysis with Jellyfish (Marçais & Kingsford, 2011) and GenomeScope (Vurture et al., 2017) to examine the k-mer (set of words of length *k*) size distribution. By combining two libraries to obtain sufficient coverage (19X), I was able to obtain the genome size and other parameters: the total estimated length was ≈ 750 Mb, there was a high level of heterozygosity (≈ 4%), and a very high content of repeats of ≈ 50%.

I initially tried to align one library of trimmed short Illumina reads to the reference genome of the copepod *Eurytemora affinis* downloaded from the NCBI repository, with the Burrows-Wheeler Aligner v0.7.17 (BWA; H. Li & Durbin, 2009), but only 4.75% of the ≈ 250 million trimmed reads were aligned, using a quality filter of 20. This low alignment rate was not very surprising as *E. affinis* diverged from *L. minutus* at least 150 m.y.a (Eyun, 2017). An initial assembly from the first 10X library was then attempted with Supernova (Weisenfeld et al., 2018) at the McGill Genome center, which gave an N50 size (median of the contig sizes) of 53.46 Kb and an assembly size of 548Kb (too small). A second assembly was conducted using the two 10X libraries, but it failed due to the low coverage (22X on 4.5Gb). The estimated genome size output by supernova was 1Gb,

close to my estimate with Jellyfish and GenomeScope. Another assembly was attempted by MGC using the two 10X libraries and using the Minia3 assembler (Chikhi & Rizk, 2013) but this assembly also failed.

I attempted to run a first assembly using an Illumina short reads library with the assembler MEGAHIT, which is optimized for metagenomic data but can be applied to single genomes (D. Li et al., 2015). This method produced a very fragmented assembly, with ≈ 6 million contigs, a total length of ≈ 3Gb, and N50 = 612 bp. I also used the wtdbg2 assembler with the ONTseq library only, which yielded promising statistics (11,141 contigs, assembly size ≈ 236Mb, N50 ≈ 32kb), but after aligning one Illumina library, only 0.7% of the reads aligned. I then attempted to combine the long-reads and short-reads sequencing in a hybrid assembler (one Illumina library, the ONTseq, and 10X libraries), first testing with the Platanus-allee assembler (Kajitani et al., 2019), which is designed for very heterogeneous genomes. This method performed very poorly, with a scaffolded assembly output of 25 MB of data. Finally, I then attempted another hybrid assembly with OPERA-MS (Bertrand et al., 2019) with one Illumina library and the ONTseq library. The results seemed better compared to using MEGAHIT but still very fragmented: ≈ 4 million contigs, an assembly size of 2 Gb, and N50 = 515 bp. Currently, this is the "best" assembly so far, onto which I was able to align 50% of the reads from one Illumina library successfully with BWA. The high heterozygosity and repeat content detected with the k-mer analysis are probably important factors that explain why the assembly attempts have been unsuccessful (Jung et al., 2020). Due to the difficulty in obtaining a satisfying assembly, I decided to use a reference-free option instead (see below).

1.8.2. Pool-sequencing and genomic analyses

I applied whole genome pool sequencing (pool-WGS) on the gastropod *Amnicola limosus* and the calanoid copepod *Leptodiaptomus minutus*. Samples were pooled by populations (≥ 40 individuals per pool) and whole-genome shotgun sequencing was applied on libraries with a goal of 20X coverage, using an Illumina HiSeqX (*Amnicola* project) at Genome Quebec and an Illumina NovaSeq (copepod projects) at MGC. As I was unable to obtain a draft genome of *L. minutus*, I relied on a reference-free algorithm for the discovery of SNPs implemented in Discosnp++ (Uricaru et al., 2015). This method is based on a de Bruijn graph analysis of k-mers and has

previously been successfully applied to copepod metagenomic datasets (Arif et al., 2019). This solution was preferred over the alignment of reads on the reference genome of another copepod species (e.g., *Eurytemora affinis*), as a significant divergence of sequences can occur even between closely related species (Jung et al., 2020). The reference-free method performed very well for the *L. minutus* study system, as I was able to obtain final datasets of 3-6 million SNPs for each project after the various filtering steps.

For both the *A. limosus* and *L. minutus* projects, I used population genomics methods to investigate population structure (e.g., PoPoolation2 and poolfstat: Kofler, Pandey, et al., 2011; Hivert et al., 2018) and history (Treemix; Pickrell & Pritchard, 2012), detect putative loci under selection to uncover local adaptation (FST scans, outlier and environmental association analyses with poolfstat and the hierarchical Bayesian models implemented in Baypass; Gautier, 2015) and to reconstruct the demographic history of populations (Diffusion Approximations for Demographic Inference ∂a∂I; Gutenkunst et al., 2009). Finally, I used poolfstat to obtain genetic diversity estimates (observed heterozygosity) and calculated additional diversity indices with a sliding window approach along the genome for the *Amnicola* project (Tajima's π, Watterson's theta, Tajima's D; Kofler, Orozco-terWengel, et al., 2011). Using these genomic analysis methods, I was able to make inferences on local adaptation, putative selection pressure, demography, and gene flow, as well as their interaction in these complex study systems.

1.8.3. Laboratory reciprocal transplant experiments and resurrection ecology

Reciprocal transplant experiments are typically used to uncover the local adaptation of populations, by comparing fitness-related traits in home and transplant environments and can be conducted in laboratory or natural settings (Brousseau et al., 2021; Wadgymar et al., 2022). They involve three steps: 1) testing local adaptation by showing that populations have higher fitness in their home environment, 2) testing the selection pressure by removing the selection agent, which should result in equal fitness between populations, and 3) testing the phenotypic traits under selection by showing that the fitness is associated to a given trait values in the presence or absence of the selection pressure (Wadgymar et al., 2022). They can be used to reveal a fitness trade-off in the transplant environment, for example, due to antagonistic pleiotropy, where the genetic basis of a trait conferring high fitness in the local environment results in reduced fitness in transplant

conditions (Bono et al., 2017). However, trade-offs do not always emerge in locally adapted populations (only 50% of the cases; Hereford, 2009), and are more likely to occur during local adaptation to homogenous environments than in heterogenous conditions (Bono et al., 2017). Reciprocal transplants can also be used to tease out phenotypic plasticity from the genetic basis of traits (Stoks et al., 2016), although common garden experiments are preferable in this case as they are dedicated to differentiating the role of these two processes (de Villemereuil et al., 2016; Wadgymar et al., 2022). However, transplant experiments require the reproduction of individuals for a few generations to eliminate maternal effects, which can be challenging depending on the species considered (de Villemereuil et al., 2016).

A special case of reciprocal transplant is resurrection ecology, where seeds or resting stages from previous generations are "resurrected" and exposed to historic versus current environmental conditions (Burge et al., 2018). Resting stages produced by many aquatic organisms accumulate in the sediments of lakes (Burge et al., 2018), and can be used in combination with resurrection ecology methods and genomics to retrace the evolutionary trajectory of populations (phenotypes and genomes), particularly concerning recent environmental disturbances (Weider et al., 2018; Ellegaard et al., 2020). Resurrection ecology has yielded significant insights into rapid adaptation in freshwater ecosystems with species of the genus *Daphnia*, notably to variable predation pressure and climate change (Geerts et al., 2015; Stoks et al., 2016; Chaturvedi et al., 2021). Transplant experiments and resurrection ecology, combined with genomics, are powerful approaches that allowed me to obtain significant insights into the interaction between micro-evolutionary and ecological processes for the two non-model freshwater species *Amnicola limosus* and *Leptodiaptomus minutus*.

1.9. Overall contributions and significance of the research

All the chapters of my thesis provide original contributions to knowledge. For each chapter, I am providing below the most significant features:

Chapter 1:

- I showed how strong gene flow between divergently adapted gastropod populations provides a potential demographic and genetic rescue (source: uninvaded physiological refugia, sink: invaded habitats; Hufbauer et al., 2015), but it also generates a conflict between these two types of rescues, as immigration of individuals adapted to the refuges could result in outbreeding depression in the invaded populations and erode their local adaptation to the invader. This has rarely been studied when assessing invasive species impact, and more generally when studying genetic rescue in natural populations.

- These results highlight the need for careful planning of genetic rescue as a conservation measure (D. A. Bell et al., 2019; Hoffmann et al., 2021), to avoid mixing between adaptively divergent populations (Frankham et al., 2011; Robinson et al., 2021), given that the risk of outbreeding depression is sometimes neglected (Hamilton & Miller, 2016; Ralls et al., 2020).

Chapter 2:

- I investigated the combination of factors (demographic, genetic, and environmental) that promoted evolutionary rescue in natural populations from a strong environmental impact and influenced the long-term consequences of the rescue during environmental recovery, leading to an adaptive reversal but with potentially detrimental effects on adaptive potential in the rescued populations.

- Demonstrations of evolutionary rescue in nature are still rare (G. Bell, 2017; McDermott, 2019; but see Gignoux‐Wolfsohn et al., 2021)

- The influence of evolutionary and ecological factors on the probability of rescue and its long-term consequences in natural populations are also largely unknown (Carlson et al., 2014; G. Bell, 2017).

Chapter 3:

- I documented how drift and gene flow can influence local adaptation to climate across different spatial scales, which are rarely studied in combination (Schmidt et al., 2021; but see Gamboa et al., 2022)

- I showed that these three processes diverge between scales, with implications for the generalization of inferences of micro-evolutionary processes and predictions of population adaptive responses to climate change (Aguirre-Liguori et al., 2021).

CHAPTER 1

MALADAPTIVE MIGRATION FROM PHYSIOLOGICAL REFUGIA COULD CONSTRAIN THE RESCUE OF NATIVE GASTROPODS FACING AN INVASIVE PREDATOR

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N.B. References cited in this chapter are presented at the end of the thesis.

ABSTRACT

Biological invasions have caused the loss of freshwater biodiversity worldwide. The interplay between adaptive responses and demographic characteristics is expected to be important for the resilience of populations to biological invasions, but the interaction between these factors is poorly understood. The native freshwater gastropod *Amnicola limosus* is distributed along spatial variation in impact from an invasive molluscivorous fish (*Neogobius melanostomus*), as well as in calcium concentrations, which is limiting the distribution of this invader (refuges). We investigated the potential for genetic adaptation of *A. limosus* to the invasive predator and the low calcium habitats. We conducted pooled whole-genome sequencing of twelve gastropod populations from the Upper St. Lawrence River, complemented with a laboratory reciprocal transplant of wild F0 *A. limosus* to measure survival and fecundity in treatments of water calcium concentration (low/high) and round goby cue (present/absent). We quantified gene flow between the habitat types to test how population structure might interact with adaptation. We found that uninvaded habitats with low calcium could act as refugia for the gastropods from the invasive fish and provide migrants to declining invaded gastropod populations through gene flow (i.e., demographic rescue), which also maintained genetic diversity (i.e., genetic rescue). However, we also detected signatures of divergent selection between habitat types and evidence of low fitness of individuals from refuge populations in both habitat types. This suggests that migrants from refuges could introduce maladapted alleles to recipient populations in high calcium, invaded habitats, thereby reducing fitness and producing a conflict between demographic, genetic, and evolutionary rescue.

2.1. Introduction

Invasive species represent a significant threat to global biodiversity (Early et al., 2016; Dueñas et al., 2021). They are an important driver of species extinction (Bellard et al., 2017), have a strong negative impact on native species abundance, and cause indirect adverse effects through physicochemical alterations of the native environment (Gallardo et al., 2016; Emery-Butcher et al., 2020). The strength of the impact of invasive species on native populations and communities depends on their abundance and trophic level, with invasive predators typically having the most substantial impact (Bradley et al., 2019). The extent of a biological invasion in a geographic location will be determined by the limits of habitat suitability in the area (Liu et al., 2020). Environmental gradients can be important barriers restricting invasive species' unchecked advance (Mothes et al., 2019). For example, the range of the invasive Asian clam in North America and Europe is currently limited by its inability to tolerate minimum temperatures lower than -10°C and by altitudes higher than 2000m (Crespo et al., 2015). Thus, impacted native species that can tolerate a broader range of environmental parameters than the invaders may have access to refuge habitats free from invaders (Chapman et al., 2002; A. J. Reid et al., 2013). However, across large spatial scales or strong environmental gradients, it is unclear whether this type of 'physiological refugia' results from universally broad physiological tolerance in native species or local adaptation of populations experiencing distinct environmental conditions. Moreover, invasive predators might impose additional divergent selection on native species because local populations that overlap with the invader could experience selection for anti-predator traits (Strauss et al., 2006; Brookes & Rochette, 2007). Indeed, among natural and anthropogenic stressors, invasive species are one of the strongest drivers of phenotypic change in native populations (Sanderson et al., 2022).

Local adaptation could interact with demographic processes to facilitate or hinder the co-existence of native species with an invasive predator by providing demographic subsidies, genetic rescue, or by introducing maladaptive alleles to recipient habitats. While some sites can have environmental conditions more conducive to invasion and therefore suffer stronger biotic selection from invasion, other sites can have environmental conditions that exclude invasive predators and act as uninvaded refuges (Derry et al., 2013; Astorg et al., 2021, 2022). In this scenario, refuge populations could potentially serve as a demographic subsidy of individuals for invaded populations experiencing population decline (Foppen et al., 2000; With et al., 2006), and prevent their extinction through

demographic rescue (Hufbauer et al., 2015). Genetic rescue can additionally occur when migrants prevent the extinction of declining populations through increased genetic diversity that reduces inbreeding depression (Carlson et al., 2014; Whiteley et al., 2015; Fitzpatrick et al., 2016); if this genetic variation includes adaptive alleles, then genetic rescue can also lead to an evolutionary rescue, i.e. the avoidance of extinction via adaptation (G. Bell & Gonzalez, 2011; Sexton et al., 2011; Hufbauer et al., 2015).

Local adaptation can also occur in the presence of gene flow through mechanisms that increase the strength of selection or reduce gene flow (Richardson et al., 2014) Therefore, this gene flow could instead bring maladapted alleles into invaded populations if populations have experienced strong divergent selection across the environmental gradient (Bolnick & Nosil, 2007). Migration from refuges consequently poses some risks when recipient and source populations are divergent due to local adaptation, which can cause genetic incompatibilities in hybrids and reduce local adaptation (Fenster & Galloway, 2000; Edmands, 2007; Frankham et al., 2011; i.e., outbreeding depression; D. A. Bell et al., 2019). This effect will be exacerbated if impacted/recipient populations are also adapted to the invasive species, thereby further contributing to maladaptation among immigrants coming from refuge populations. Thus, there could be a tension between demographic rescue (immigrants from refuge populations providing individuals to bolster the shrinking populations in invaded habitat) versus these immigrants potentially being maladapted and thereby reducing the mean fitness of the invaded populations. Standard thinking about genetic rescue is that immigrants will bolster the genetic variation in the populations they arrive in, without necessarily considering whether this additional genetic variation is locally adaptive or not (Pavlova et al., 2017; D. A. Bell et al., 2019). It is thus important to understand how these two sources of adaptation (to the refuge vs. to the predator) can interact with demographic processes to either facilitate or hinder the ability of a native species to avoid population decline from an invasive predator.

Genomic methods are increasingly used to understand species and populations' responses to sudden environmental changes induced by anthropogenic activities such as invasive species (D. Ben Stern & Lee, 2020) and are an important tool for informing conservation (Willi et al., 2022). They can enable the assessment of population connectivity, investigate demographic and genetic changes, and detect the potential for genetic adaptation (e.g., Marques et al., 2019). Reconstructing demographic changes can help assess potential population declines induced by invasive species.

Additionally, inference of gene flow can identify the source and recipient populations in a metapopulation impacted by the invaders. Finally, assessing genetic adaptation can determine if source and recipient populations are divergent because of local adaptation (Cure et al., 2017), thus altering the likelihood of genetic and/or evolutionary rescue from genetically differentiated populations. Hence, knowledge of evolutionary forces, which can be elucidated through genomic tools, is critical for understanding the overall response of native species to the impact of biological invasions.

Gastropods have been widely used to study adaptation in response to predation (Brookes & Rochette, 2007; Hooks & Padilla, 2021), with abiotic factors such as calcium concentration modulating this response through changes in shell morphology and behavior (Rundle et al., 2004; Bukowski & Auld, 2014). As such, they are a useful biological study model for addressing evolutionary responses to biological invasions. *Amnicola limosus* is a small dominant freshwater gastropod species with a wide geographical distribution in the USA and Canada (https://www.gbif.org/fr/species/5192461). This gastropod does not have a pelagic larval phase: egg masses are deposited on the substrate, and juveniles move from the substrate to the macro-algal substrate (Pinel-Alloul & Magnin, 1973). Part of the range of *A. limosus* has been invaded by the round goby (*Neogobius melanostomus*), a molluscivorous fish, from the lower Great Lakes and running downstream throughout the Upper St. Lawrence River (Hickey & Fowlie, 2005). *Amnicola limosus* is commonly found in the stomach contents of round gobies, and following the goby invasion of Lake Saint-Louis, *A. limosus* populations experienced a 0.5-1 mm reduction in shell size (Kipp et al., 2012). Because the mean gape size of the round goby is larger than the maximum size of *A. limosus*, round gobies do not have to crush the snail, which suggests that shell size reduction is likely to be due to reduced predation pressure on smaller and less visible individuals (round gobies are visual predators; Kipp et al., 2012). A considerable reduction in small gastropod abundance (down to 2-5% of the original population size, with *Amnicola* being the most abundant species) and species richness in the Upper St. Lawrence River were also reported since the invasion of round gobies in this ecosystem in 2005 (Kipp et al., 2012). However, round gobies cannot tolerate low calcium concentrations (Baldwin et al., 2012; Iacarella & Ricciardi, 2015), and have not invaded the Ottawa River (Ca^{2+} concentrations below 22 mg/L, Sanderson et al., 2021, present study) at its junction with the Upper St. Lawrence River. On the contrary, this low calcium

concentration is not a physiological limit for *A. limosus* (1.1 mg/L; Shaw & Mackie, 1990). These calcium-poor waters are thus acting as a refuge from goby predation in this system (Astorg et al., 2021). Calcium-poor waters could potentially provide demographic subsidies for the native populations at invaded sites (e.g., amphipods, Derry et al., 2013).

This study aims to investigate the potential adaptation of *A. limosus* to the water calcium gradient and the presence of round goby invasion in the Upper St. Lawrence River, as well as the demographic and genetic consequences of the goby invasion on the native gastropod. We tested for evidence of local adaptation via 1) genome scans for SNPs associated with calcium concentration and round goby presence and 2) a laboratory reciprocal transplant of wild *A. limosus* individuals to measure survival and fecundity in factorially-crossed treatments of water calcium concentration and round goby chemical cue. For the local adaptation to the low calcium and high goby predation environmental conditions, we expected to find outlier SNPs associated with one or both of these covariables. In the transplant experiment, we also expected populations to show a home versus transplant advantage in life-history traits relative to the calcium concentration and presence of goby cues. For the demography, because of the large decrease in the population size of gastropods observed in the Upper St. Lawrence River following round goby invasion (Kipp et al., 2012), we hypothesized that *A. limosus* populations in invaded habitats could have undergone a similar decrease in abundance, perhaps accompanied by a genetic bottleneck. If so, uninvaded populations could potentially provide demographic, genetic, and evolutionary rescue for invaded populations (Hufbauer et al., 2015; Whiteley et al., 2015). However, this possibility would depend on the level of gene flow between habitats and the extent of adaptive differentiation. Because the life history of *A. limosus* does not involve a pelagic larval phase (Pinel-Alloul & Magnin, 1973), we expected to observe low gene flow in the absence of strong water currents. Low gene flow impedes demographic and genetic rescue, and strong adaptive differentiation will likely hinder evolutionary rescue. As such, we hypothesized that local adaptation to the distinct habitat types might have led to reduced gene flow (isolation by environment; Wang & Bradburd, 2014). Our paper provides a rare empirical study to address how spatial heterogeneity in both abiotic conditions and invasive predator presence can interact with demographic processes to shape the response of a native species to biological invasion. Freshwater environments are deeply impacted by invasive species (Gallardo et al., 2016). It is important to not only consider ecological impacts

of invasion but also evolutionary and demographic responses in native species that can help foster invasive-native species coexistence in invaded freshwater ecosystems.

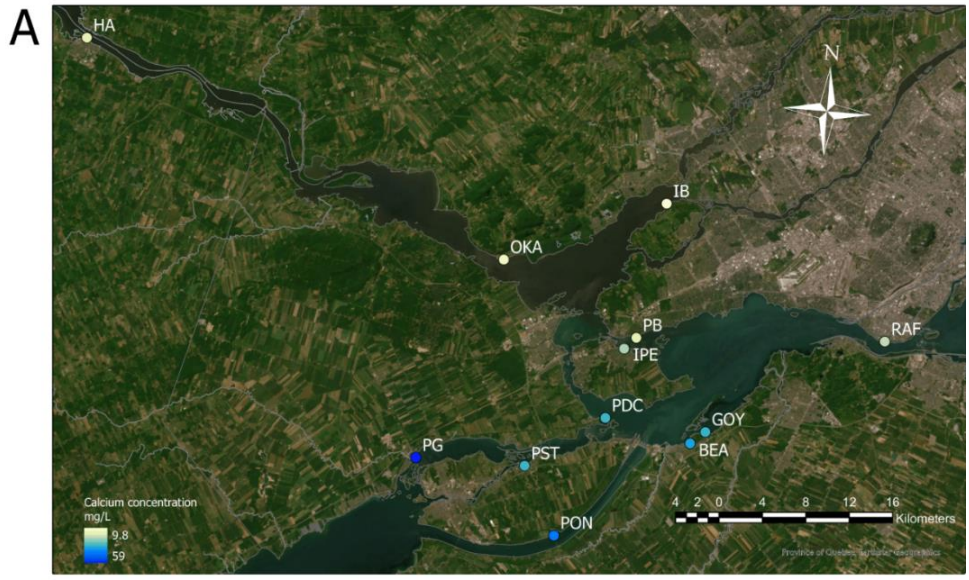
2.2. Material and Methods

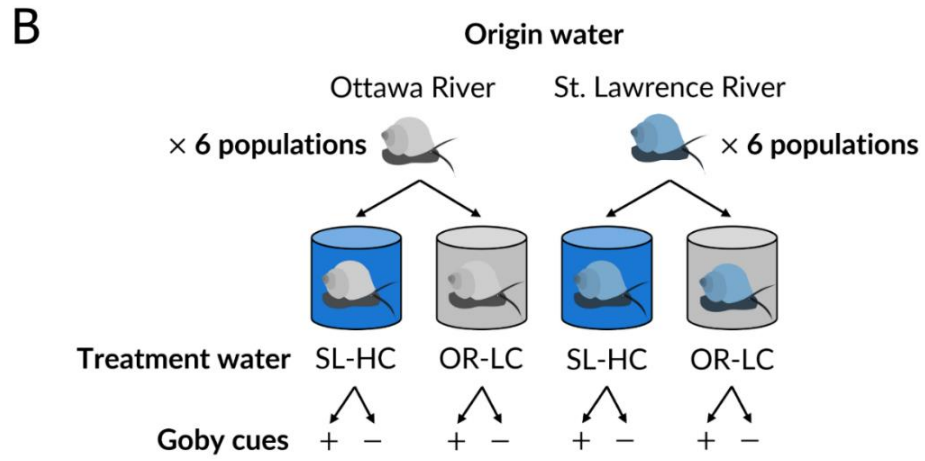
2.2.1. Study sites, sample, and physicochemical data collection

Our twelve study sites are located at the junction of the Ottawa river and the St. Lawrence River near Montreal, QC, Canada (Fig. 2.1). The Ottawa River water is calcium-poor (10-15 mg/L calcium), and the St. Lawrence River water is comparatively calcium-rich (30-40 mg/L) due to the different geological characteristics of their watersheds. These water masses mix at the junction of two major river systems at Lake Saint Louis, a widening of the St. Lawrence River, but the calcium gradient persists in the north and south shores, and water masses are distinct. In 2005, round gobies invaded the upper St. Lawrence River and the southern shore of Lake Saint-Louis, but not the calcium-poor Ottawa River nor calcium-poor sites on the north shore of Lake Saint-Louis (Kipp & Ricciardi, 2012). Twelve *Amnicola limosus* populations were sampled from the study sites in this fluvial ecosystem (Fig. 2.1), with three populations fully in the Ottawa River, three fully in the St. Lawrence River, and six populations in the Lake St-Louis, including three on the north shore and three on the south shore. We coded populations collected in the Ottawa River water as OR (calcium-poor water and gobies absent) and populations from the St. Lawrence River water as SL (calcium-rich water and gobies present). Two populations had inverted patterns: RAF is calcium-poor, but gobies are present, and PDC is calcium-rich, but gobies are absent. It is noteworthy that PDC is located in a refuge habitat (wetland, Astorg et al., 2021) close to invaded sites, and should thus receive strong gene flow from nearby invaded populations. Field-collected *Amnicola* snails were obtained near the shore via hand picking and brought back to the lab for further processing (DNA extractions and the common garden experiment) in June-October 2017. Goby abundance was measured in the field between July and September 2017 on a single occasion at each site. For this, each site was sampled using three seine net passes, with intermission periods between seining times. The seine net used for sampling nearshore habitats was 30 feet long by 6 feet deep and 1/8 mesh on a 10 m distance. Round gobies were placed into bins and released after the three hauls. The geographic location and environmental characteristics of our sampling sites are detailed in Table A.1. We measured dissolved oxygen (DO; mg/L), pH, water temperature ($^{\circ}$C), and conductivity ($\mu S.cm^{-2}$) using a Professional Plus Model YSI multi-parameter probe (model

10102030; Yellow Springs Inc.) at each study site in 2017 at the time of gastropod collection. On the same occasions, we collected water samples and analyzed them for calcium (Ca), total phosphorus (TP), total nitrogen (TN), as well as dissolved organic carbon (DOC) at the GRIL-UQAM analytical lab (Supplementary Methods). Site-specific invasion status by round goby (invaded / uninvaded) is defined by presence/absence (Table A.1).

Figure 2.1: Locations of study sites of collection of *Amnicola limosus* populations in the St. Lawrence River system and experimental design of reciprocal transplant experiment. A: The study sites are located near Montreal, QC, Canada. Sites are colored based on water calcium concentration (mg/L). Sites with gobies absent are: HA, OKA, IB, PB, IPE, and sites with gobies present are: PG, PST, PON, PDC, BEA, GOY. The two exceptions are RAF (low calcium/gobies present) and PDC (high calcium/gobies absent). B: Experimental design showing the two waters of origin (Ottawa River in grey or St. Lawrence River in blue) with six populations from each river (replicates), water treatment (OR-LC: Low calcium – Ottawa River; SL-HC: St. Lawrence River – high calcium) and goby cue treatment (+/−: with or without).





2.2.2. De novo genome assembly and pool-sequencing

For the *de novo* genome assembly, we extracted DNA from the tissue of one individual snail collected in 2017 using a standard Phenol Chloroform extraction method, after removing the shell and excising the mollusks guts to avoid contaminants. Briefly, tissue samples were placed in a digestion buffer containing proteinase K and digested at 55°C. DNA was then isolated using an isoamyl-phenol-chloroform solution, followed by ethanol precipitation. DNA quantity and quality were verified using a combination of different quality control methods: Qubit assay (Thermo Fisher Scientific Inc.), Tapestation (Agilent Inc.), and Femto Pulse (Agilent Inc,). Fragments longer than 1 kb were selected for further processing. Library preparation was performed using 10X Chromium Linked-Read library kit (10X Genomics Inc.) and sequenced on 3 lanes of Illumina HiSeqX PE150 at the McGill Genome Center. Reads were assembled with Supernova v.2.1.1. The assembled genome is 1,899,346,312 bp in length, with 815,134 scaffolds and a N50 of approximately 5kb. For the pooled sequencing, we extracted DNA from the tissues of 40 individuals per pool/population using the same standard Phenol Chloroform extraction method mentioned above. We quantified all samples using a Picogreen ds DNA assay (Thermo Fisher Scientific Inc.) on an Infinite 200 Nanoquant (Tecan Group Ltd). Samples were normalized to a dsDNA concentration of 15ng/µL, re-quantified, and pooled according to the sampling population. Thus, we created 12 pools of 40 individuals each at 15ng/µL. Libraries were prepared and sequenced on 5 lanes of HiSeq2500 125 bp pair-ended at the McGill University and Genome Quebec Innovation Center.

2.2.3. Read processing and SNPs calling

We prepared the assembled reference genome of *Amnicola limosus* by first indexing it with the Burrows-Wheeler Aligner (BWA; H. Li & Durbin, 2009) v0.7.17 and with Samtools faidx v1.12, and by creating a dictionary with Picard Tools v2.23.3. We then used a custom pipeline for pool-seq quality processing, read alignment, and SNP discovery. We first trimmed reads with the function trim-fastq.pl from popoolation v1.2.2 (Kofler, Orozco-terWengel, et al., 2011) for a base quality of 20 and a minimum length of 50 bp. We aligned trimmed reads to the reference genome with bwa-mem v0.7.17. We filtered out ambiguously aligned reads with samtools v1.13 using a score of 20 and sorted bam files with samtools. We called SNPs with samtools mpileup, then filtered SNPs with a minimum global coverage of 5. We converted the mpileup file to a sync file with Popoolation2 v1.10.03 (Kofler, Pandey, et al., 2011), with a quality score of 20. The sync file

was then converted to a "pooldata" object with the poolfstat package in R (Hivert et al., 2018), using a haploid pool size of 80 for all populations, a minimum read count per base of two, a minimum coverage of five and a maximum of 300, a minimal minor allele frequency of 0.0125 (to remove singletons) and discarding indels. This pipeline retained 21,312,700 biallelic SNPs.

2.2.4. Detecting genomic signatures of selection

To detect putative loci under selection, we used both outlier and environmental association analysis approaches. We conducted the outlier analysis using the core model from hierarchical Bayesian models implemented in Baypass, using default parameters (Gautier, 2015). Baypass is advantageous in the context of our study (potential bottlenecks in invaded populations) as it enables the detection of outlier SNPs after taking demographic history into account, thus avoiding the confounding effect of demography. The core model estimates the scaled covariance matrix Ω of population allele frequencies, which summarizes population history and is then explicitly accounted for through Ω. The full dataset was divided into 27 pseudo-independent datasets to overcome computing limitations. The "pooldata" object from poolfstat was converted to the 27 sub-dataset Baypass input files with the "thinning" subsampling method and sub-sample size of 750,000 SNPs. We used the core model to estimate the XtX statistic and associated p-value under a χ^2 distribution with 12 degrees of freedom (bilateral test, Baypass manual). We considered SNPs as outliers when their p-value derived from the XtX estimator was < 0.001. The shape of the histogram p-values derived from the XtX statistics confirmed that they were well-behaved (A peak close to 0 for loci putatively under selection and a uniform distribution between [0,1] for neutral loci; Fig. A.1B, François et al., 2016). The Ω matrices from the 27 sub-datasets were compared visually to assess the concordance of the results, then the statistics obtained for each SNP were combined.

For the environmental association analysis, we opted for the standard model STD under the Importance Sampling approach in Baypass, in which the association between covariables and SNP allele frequencies are assessed independently. This model computes for each SNP its regression coefficient β_{ik} of the association between the SNP allele frequencies and a covariable, from which a Bayes factor BF_{is} is derived. We selected two environmental covariables: invasion status (presence/absence of the gobies) and calcium concentration. We checked the Pearson correlation

coefficient between covariables with the function pairs.panel() in the package psych in R, which was r = 0.71 (slightly above the recommended threshold for the regression method of $|r| < 0.7$, Fig. A.2). We also estimated the C_2-statistic with the STD model, which is more appropriate for binary variables and was used for the association with goby presence/absence. Covariables were all standardized to $\hat{\mu} = 0$ and $\widehat{\alpha^2} = 1$. For the calcium association, SNPs were considered significantly associated with a covariable when $BF_{is} > 20$ dB (Jeffrey's rule for "decisive evidence"; Gautier, 2015). For the association with goby presence/absence, we used the R package qvalue to correct for multiple hypothesis testing on the p-values derived from the C_2-statistic and applied a False Discovery Rate of $\alpha = 0.01$ as a q-values cut-off for outlier detection. As a complementary analysis to investigate the potential for adaptation to the invasive predator and the low calcium concentration, we identified outlier SNPs showing consistent allele frequency differences between environment types using the approach implemented in poolFreqDiff (Wiberg et al., 2017). Note that in the present case, this pattern should be due to shared recent ancestry. This method relies on modeling allele frequencies with a generalized linear model (GLM) and a quasibinomial error distribution. It also accounts for bias in allele frequency estimation (e.g., Gautier et al., 2013) by rescaling allele counts to an effective sample size n_{eff} (Feder et al., 2012). We ran the analysis separately for the two covariables as binary comparisons: invasion status (presence/absence) and calcium concentration (low < 24.3 mg/L, high > 34.3 mg/L). For the minimum read count per base, and the minimum and maximum coverage, we used the same values as for the poolfstat filtering, and we also rescaled the allele counts with n_{eff} and added one to zero count cells. To account for demography and genetic structure, we applied the empirical-null hypothesis approach (François et al., 2016) to recalibrate p-values based on a genomic inflation factor of $\lambda = 0.85$. We confirmed that recalibrated p-values were well-behaved based on the shape of the histogram (Fig. A.3; François et al., 2016). We then transformed the recalibrated p-values into q-values with the R package qvalue, and defined outliers if their q-value was below the FDR $\alpha = 0.01$.

2.2.5. Reciprocal transplant experiment

We conducted a laboratory reciprocal transplant experiment at UQAM with field-collected F_0-generation *A. limosus* to investigate the response of gastropods with different source population habitat types (low calcium/uninvaded Ottawa River or high calcium/invaded St. Lawrence River) to home and transplant water (calcium-rich water from the St. Lawrence River or calcium-poor

water from the Ottawa River), in the presence or absence of goby cues. The goby cues treatment was used to test for their effect on traits (e.g., shell size, hiding behavior) correlated with life-history traits. *Amnicola limosus* snails used in the experiment were mostly at adult or sub-adult stages as we selected the largest individuals collected in the field and the dates of collection correspond to the presence of adult cohorts in the field (Pinel-Alloul & Magnin, 1973). Two additional water treatments were also tested: the artificial freshwater medium COMBO (Kilham et al., 1998), with and without the addition of calcium, to test for the specific effect of calcium (Ca) concentration on fitness components. The overall design was therefore a two (origin water: St. Lawrence River SL versus Ottawa River OR) × four (treatment water from St. Lawrence versus Ottawa River, growth media with/without Ca) × two (presence versus absence of round goby cue) factorial experiment, with 12 replicates (corresponding to our sampling populations) per treatment combination.

We raised wild F_0 individuals from the 12 populations in the laboratory for up to 73 days. Between 15 and 22 individuals (average: 19.6±1.3) were initially placed in 250 ml plastic cups with river water, and reared in growth chambers (Thermo Scientific Precision Model 818) at 18°C with a light:dark cycle of 12:12 hours. We fed *Amnicola* snails ad libitum with defrosted spinach every 2-3 days if needed or at each water change. Water in the water treatments was changed, and old spinach was removed every 3-4 days. For the goby cues treatment, gobies were kept in a 50-liter aquarium for two weeks prior to the experiment, set in a growth chamber at 18°C with a 12:12h light. Gobies were fed 3-4 times a week with flake fish food (TetraFin). The goby cue treatment was added as 5 mL of water from the goby aquarium per *Amnicola* culture at each water change (every 2-3 days), which represents 2% of the volume of the culture. The addition of water was done manually with a 30 mL syringe. We recorded survival and fecundity (total number of eggs produced per individual) as response variables every 19 ± 13 days throughout the experiment, using high-resolution stereomicroscopes (Olympus). However, due to the very low survival for all populations for the treatment testing the effects of calcium in growth media, we removed this comparison from further analyses (see Fig. A.4).

We analyzed fecundity (total number of eggs produced) and survival rates with a generalized linear model (GLM) and a generalized linear mixed effect model (GLMM) using the lme4 package in R

respectively. We modeled fecundity with a negative binomial distribution while survival was modeled with a binomial distribution and a logit link function. We checked the models for overdispersion using the overdisp_fun function from https://bbolker.github.io. We tested both models with and without the random effect of populations, using an AIC approach corrected for small sample size (AICc) and the ΔAIC criterion to evaluate the random effects (kept when ΔAIC > 2) with the R package bbmle. Likelihood ratio tests were used to evaluate the fixed effects for both the GLM and GLMM models. For the GLM model of fecundity, we checked for the influence of outliers on the model, by using both visual and quantitative diagnostics of the leverage and Cook's distance. We did not find a consistent effect of outliers on this model and thus did not remove outliers. Fixed effect coefficients and their confidence intervals were converted to incident rate ratios (fecundity) and odd ratios (survival) using an exponential function.

2.2.6. Population structure, genetic diversity, and demography

We first estimated population structure with the core model from Baypass, as the scaled covariance matrix Ω of population allele frequencies summarizes some aspects of population history. We also obtained a genome-wide pairwise F_{ST} matrix and the observed heterozygosity from the poolfstat package, using the same parameters as described above. We used the pairwise F_{ST} matrix to assess the potential for isolation by distance, using the relationship between the genetic distance (FST/(1-FST) Rousset, 1997) and the log of the geographical distance (2D distribution of populations) with a Mantel test (9999 permutations) using the vegan package in R. We also tested for isolation by environment, by first calculating the environmental distance between population pairs using the squared Mahalanobis distance, calculated from the calcium concentration and goby presence/absence with the R package ecodist. We verified that there was no correlation between the environmental distance and geographic distance (non-significant Mantel test with 9999 permutations: $r^2 = 0.01$, p-value = 0.11). Then we tested for a relationship between environmental distance and genetic distance as $F_{ST}/(1-F_{ST})$ with a Mantel test (9999 permutations).

We compared heterozygosity levels between habitat types with a t-test after checking for the assumptions of normality (qqplot) and homoscedasticity (Bartlett test). We calculated genome-wide diversity indices (Tajima's pi, Watterson theta, and Tajima's D) using popoolation (Kofler, Orozco-terWengel, et al., 2011). First, we generated mpileup files for each population separately

with samtools (H. Li et al., 2009) from the sorted.bam files output by the custom pipeline. Then we computed the genome-wide diversity indices using non-overlapping windows of 100kb, a minimum coverage of 20 (as recommended in Kofler, Orozco-terWengel, et al., 2011 except for Tajima's D with minimum coverage = 13, as the corrected estimator requires the pool size < 3 minimum coverage), a minimum quality of 20, a minimum fraction covered of 0.05 and a pool size of 40. It should be noted that popoolation calculates the diversity indices along chromosomes; thus, due to the fragmentation of our draft genome, the diversity indices were calculated mostly among separate contigs and in windows < 100kb. We used Hedge's G to detect a potential difference in the three diversity indices between St. Lawrence and Ottawa rivers.

We also investigated the demographic history of two populations using the diffusion approximation method implemented in δaδi (Gutenkunst et al., 2009). We aimed to detect a potential bottleneck in the invaded population and to quantify the magnitude and direction of gene flow between the two habitats. We selected the populations PG and PB for our dataset, PG being the most impacted population (Kipp et al., 2012) and PB as an uninvaded population with a relatively low pairwise F_{ST} with PG (F_{ST} = 0.07). Our most complex model has defined population sizes after the split (nu1 and nu2), then a bottleneck in both PG and PB (modeling a scenario in which the goby invasion impacted population abundance at the whole ecosystem scale) followed by exponential recovery in both populations, with T_S, the scaled time between the split and the bottleneck, (Fig. 2.5A) and T_B the scaled time between the bottleneck and present. As we knew the time of the potential bottleneck (12 years before sampling with one generation per year), we set T_B as a fixed parameter. Migration rates are asymmetric but constant through time after the split, with m_{ir} the migration from PB (refuge) to PG (invaded) and m_{ri} in the opposite direction. As we set T_B as a fixed parameter, the parameter $\theta = 4\mu L$ was an explicit parameter in the models that included a bottleneck. We defined θ with μ the mutation rate of 7.6×10^{-9} substitutions per site per year from the Caenograstropoda species *Nucella lamellose* (McGovern et al., 2010), and L the effective sequenced length of 28,982,622 bp, calculated as $L \approx total\ length\ of\ sequence\ analyzed \times SNPs\ retained\ for\ use\ in\ dadi/total\ SNPs\ in\ analyzed\ sequence$.

We investigated two non-nested models: a) without the bottleneck in PB, or b) a simple population split at T_S with asymmetric migration. The default local optimizer was used on the log of

parameters with random perturbation of the parameters to obtain a set of parameter values with the highest composite likelihood. To accommodate for large computation time, optimization was conducted over at least 10 independent runs or until convergence was reached. Finally, we compared our three models based on the large differences in the likelihoods and residuals of the models. As we obtained unlikely results during the conversion of parameters in our best model (split with migration; e.g., 5,647,161 years since the split defined as $t_S = T_S \times 2 \times Nref \times 1\ generation\ per\ year$), possibly due to imprecise mutation rates, we did not conduct parameter conversion. To obtain uncertainties on our parameters while accounting for the effect of linkage, we used bootstrapping and the Godambe Information Matrix approach (Coffman et al., 2016). For this, we generated 100 bootstrapped datasets with a chunk size of 1×10^5 bp.

To accommodate the potential effect of using a pool-seq approach on the variance in allele frequency estimates stemming from differences in coverage between pools (Gautier et al., 2013), we used a filter to obtain relatively homogenous coverage between our two selected populations/pools. From the initial SNPs dataset output by poolfstat (21,312,700 SNPs), we retained SNPs that fell within the 1st and 3rd quartiles of coverage in both populations (11-19X for PB and 10-18X for PG). We also filtered out SNPs that were detected as outliers (putatively under selection) in the Baypass (core and aux models) and poolFreqDiff analyses. Finally, we removed uninformative SNPs (fixed or lost in both populations). These filters retained a dataset of 4,236,876 SNPs. We used a custom script and the dadi_input_pools function from the genomalicious R package (Thia & Riginos, 2019) with the "probs" parameter in the methodSFS option to transform allele frequency data into the SNP data format from δaδi. We used δaδi to infer the folded SFS as we did not have information on the ancestral allele state. Due to low confidence in the low-frequency estimates, we masked entries from 0 to 5 reads.

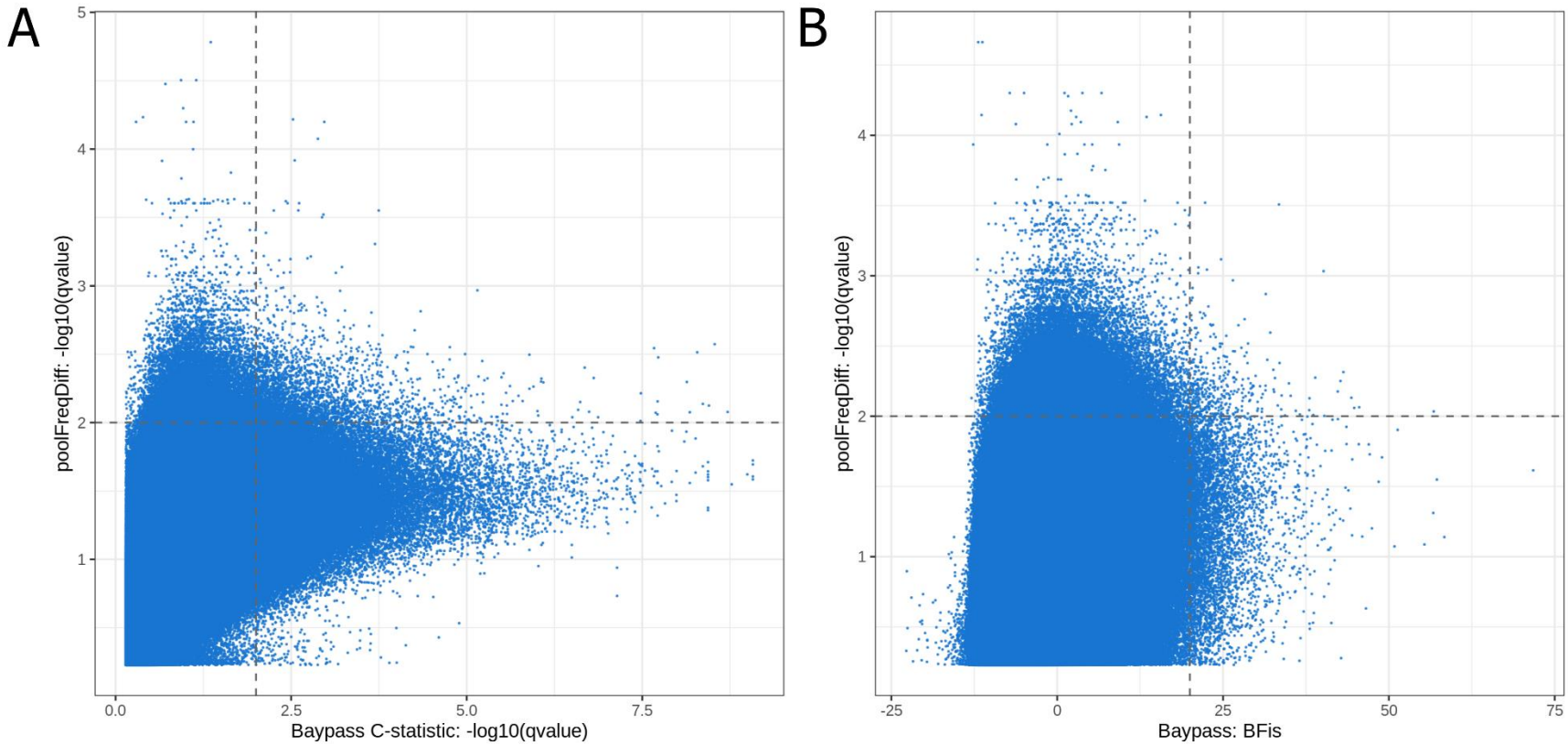
2.3. Results

2.3.1. Genomic signatures of local adaptation to round goby invasion and water calcium

Using the core model in Baypass as our outlier analysis, we found 226,794 outliers SNPs with p-value < 0.001 and either high or low XtX values, which represented ≈1.1% of the dataset (Fig. A.1). Outlier SNPs with high XtX values can be interpreted as putatively under positive selection, while low XtX values indicate balancing selection (Gautier, 2015). We also investigated the association

of SNP allele frequencies with the selected environmental variables (invasion status and calcium concentration) using the STD model in Baypass. We found 88,277 SNPs associated with the goby presence/absence (q-value < 0.01, ≈ 0.4% of the dataset, Fig. 2.2A) and 5,367 outlier SNPs significantly associated with the calcium concentration (BFis > 20, ≈ 0.03% of the dataset, Fig. 2.2B). Most of the outliers were uniquely associated with a single covariable, with 1,305 SNPs (≈1.4% of outliers) associated with both calcium and invasion status (Fig. A.5). We also identified 23,651 outlier SNPs displaying consistent differences in allele frequency in the same direction between the populations from the invaded and uninvaded habitats, with a FDR of 1% (Fig. 2.2A) and 54,285 outliers between the low and high calcium habitats (Fig. 2.2B). Of those outliers, 405 were in common between the Baypass STD model and the poolFreqDiff analysis of the calcium, and 3,324 between C_2-statistic from Baypass and poolFreqDiff for the goby presence/absence (Fig. 2.2, Fig. A.5). Overall, we found 1,050 SNPs in common between the Baypass core and STD models, as well as 7,009 SNPs in common between the Baypass STD model and the poolFreqDiff analyses including both the invasion status and calcium concentration (Fig. A.6).

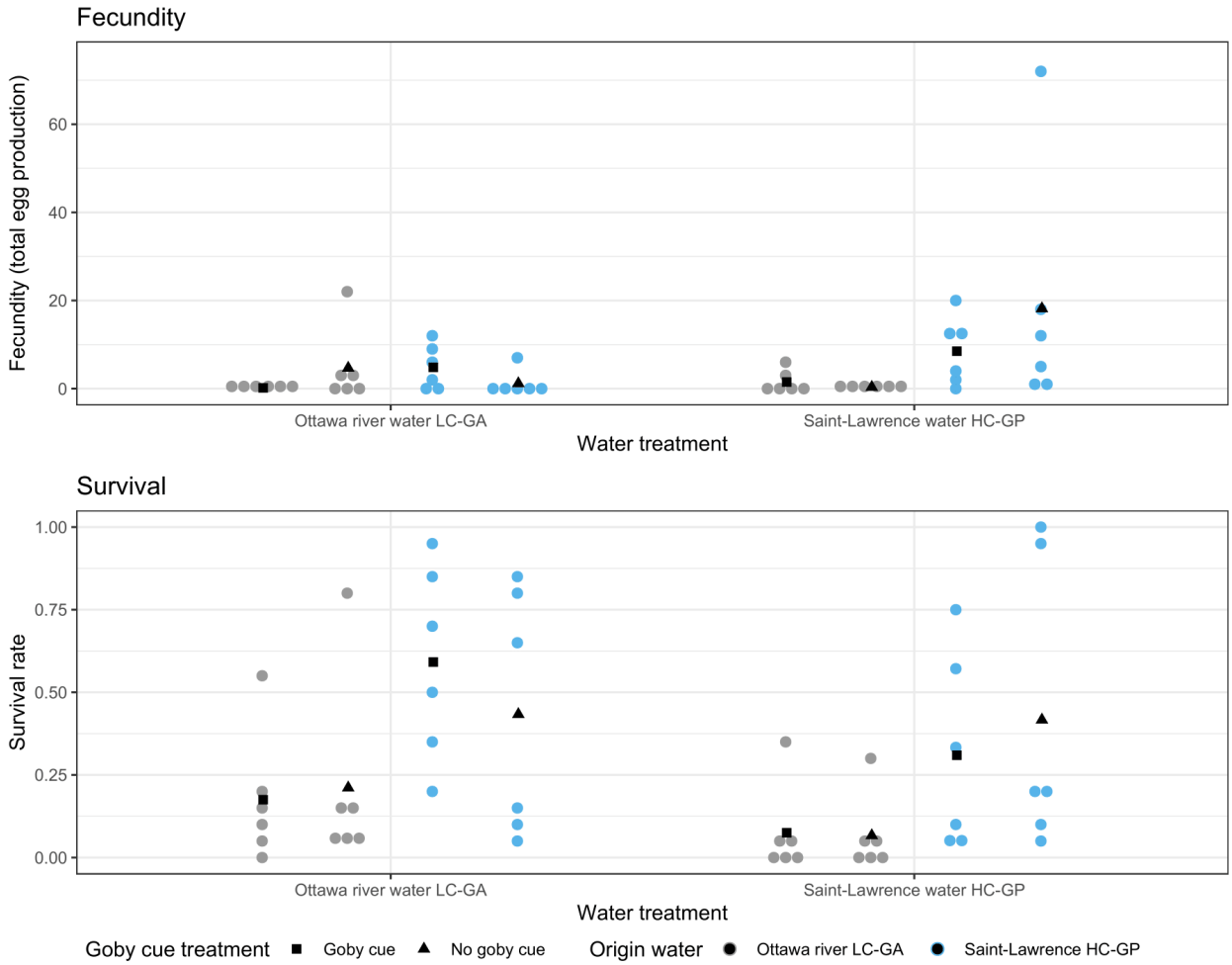
Figure 2.2: Results of the environmental association analyses. A: Biplot of the q-values obtained from the poolFreqDiff analysis testing for consistent differences in allele frequencies between populations from invaded and uninvaded as a function of the q-values derived from the C_2-statistic from the STD model in Baypass assessing the association of SNP allele frequencies with goby presence/absence. The dashed vertical and horizontal grey lines indicate the FDR of α 0.01. B: Biplot of the q-values for the poolFreqDiff analysis comparing populations of low and high calcium habitats as a function of the Bayes Factor (BFis) from the Baypass STD model for the association with calcium concentration. Colors and symbols are the same as in A. The dashed vertical line (BFis > 20 dB) indicates outlier SNPs significantly associated with the calcium covariable and the horizontal line gives the q-value FDR of α 0.01.



2.3.2. (Mal)adaptive responses to round goby invasion and water calcium levels

We found life history differences between the gastropod populations from the two environments, using fecundity and survival as fitness components (Fig. 2.3). For fecundity, the model with the random effect of population origin was not better than the model without (ΔAICc = 1.2), and only the origin water (OR vs SL) effect was significant (p = 0.015). Even though the interaction between origin water and treatment water was marginally significant (p = 0.076), fecundity was higher for SL and OR populations in home water (13.30 SD 19.70 and 2.42 SD 6.27 respectively) than in transplant water (3.00 SD 4.33 and 0.92 SD 1.83 respectively). SL populations produced ≈ 5 times more eggs than OR populations (4.90, 95% CI [1.54-15.58]). For survival, the model with a random effect of the population was better than without (ΔAIC = 250.7). The fixed effects of origin and treatment water were significant (p = 0.020 and p = 1.496×10^{-9}, respectively), but their interaction and the goby cue effect were not (p = 0.203 and p = 0.794, respectively). SL populations were 10 times more likely to survive compared to OR populations (9.55, 95% CI [1.70-53.73]). However, exposure to treatment water from the St. Lawrence River significantly lowered the odds of survival, with survival rates less than one-third that of populations exposed to Ottawa River water (0.31, 95% CI [0.21-0.46]). There was considerable variation in survival rates between populations, as demonstrated by the significant effect of population on survival. Variation in survival among populations (random effect of the population of origin) did not depend on geographical location or habitat of origin (Fig. A.7): OKA, PG, and PON had significantly higher survival rates, while BEA, PDC, and PST had significantly lower survival rates.

Figure 2.3: Fecundity (total number of eggs produced) and survival as a function of water treatment, origin water, and goby cue treatment in the reciprocal transplant experiment. Each dot represents a measurement for one population (blue: St. Lawrence populations, grey: Ottawa river populations), the black dots the average for treatment, with squares and triangles differentiating treatment with or without goby cues. LC-GA: low calcium/gobies absent, HC-GP: high calcium goby present. The significance of effects and converted fixed effect coefficients are given in the text.



2.3.3. Demographic and genetic effects of the invasion

Genome-wide nucleotide diversity π was relatively high overall with 1.11% (SD 0.64) on average. Diversity was similar between the populations from the invaded St. Lawrence River habitats (1.14 %, SD 0.72) and the populations of the uninvaded Ottawa River (1.09%, SD 0.60), with a negligible effect size of habitat type (Fig. 2.4A; Hedges' g = 0.09, 95% CI [0.16, 0.02]). Estimates of $\theta_{\text{Watterson}}$ were identical between the two habitat types (Fig. 2.4B; SL populations: 0.015 SD 0.008; OR populations: 0.015 SD 0.008), and the effect size of habitat was therefore negligible (Hedges' g = 0.06, 95% CI [0.13, 0.01]). This resulted in a slightly negative overall Tajima's D (-0.38 SD

0.43), which was lower for SL populations (-0.41 SD 0.44) than for OR populations (-0.36 SD 0.43), but the effect size of the difference was negligible (Hedges' g = 0.11, 95% CI [0.10, 0.13], Fig. 4C). Observed heterozygosity was not significantly different (p-value = 0.289, t = -1.153, df = 6.5; Fig. 2.4D) between the populations from uninvaded (0.168 SD 0.003) and invaded sites (0.171 SD 0.003). Based on the small branch lengths in the admixture tree in drift units ($drift\ parameter = \frac{t}{2Ne}$, with t the number of generations since the populations split and N_e the effective population size), we infer that the populations have been relatively little affected by genetic drift (Pickrell & Pritchard, 2012), except for PG (length of the branch connecting PG and the rest of the populations). Despite the negative Tajima's D values and the known population declines in invaded habitats, we found that our two focal populations (PB and PG) were not affected by a genetic bottleneck, as the model with a simple population split with migration had the highest likelihood compared to the two models with bottlenecks followed by growth (Fig. 2.5, Fig. A.8, Table A.2). However, we found that the scaled population size was ≈ 11 times higher in the refuge population PB than in the invaded population PG.

Figure 2.4: Genome-wide diversity indices. A: violin plots of nucleotide diversity pi, B: Watterson's Theta, C: and Tajima's D according to habitats (OR: Ottawa river with low calcium and goby absent; SL: St. Lawrence River with high calcium and goby present), with median and interquartile ranges shown in the box plot insert. D: Observed heterozygosity per population, comparing habitat types (black dots: average per habitat, error bars show one standard error interval).

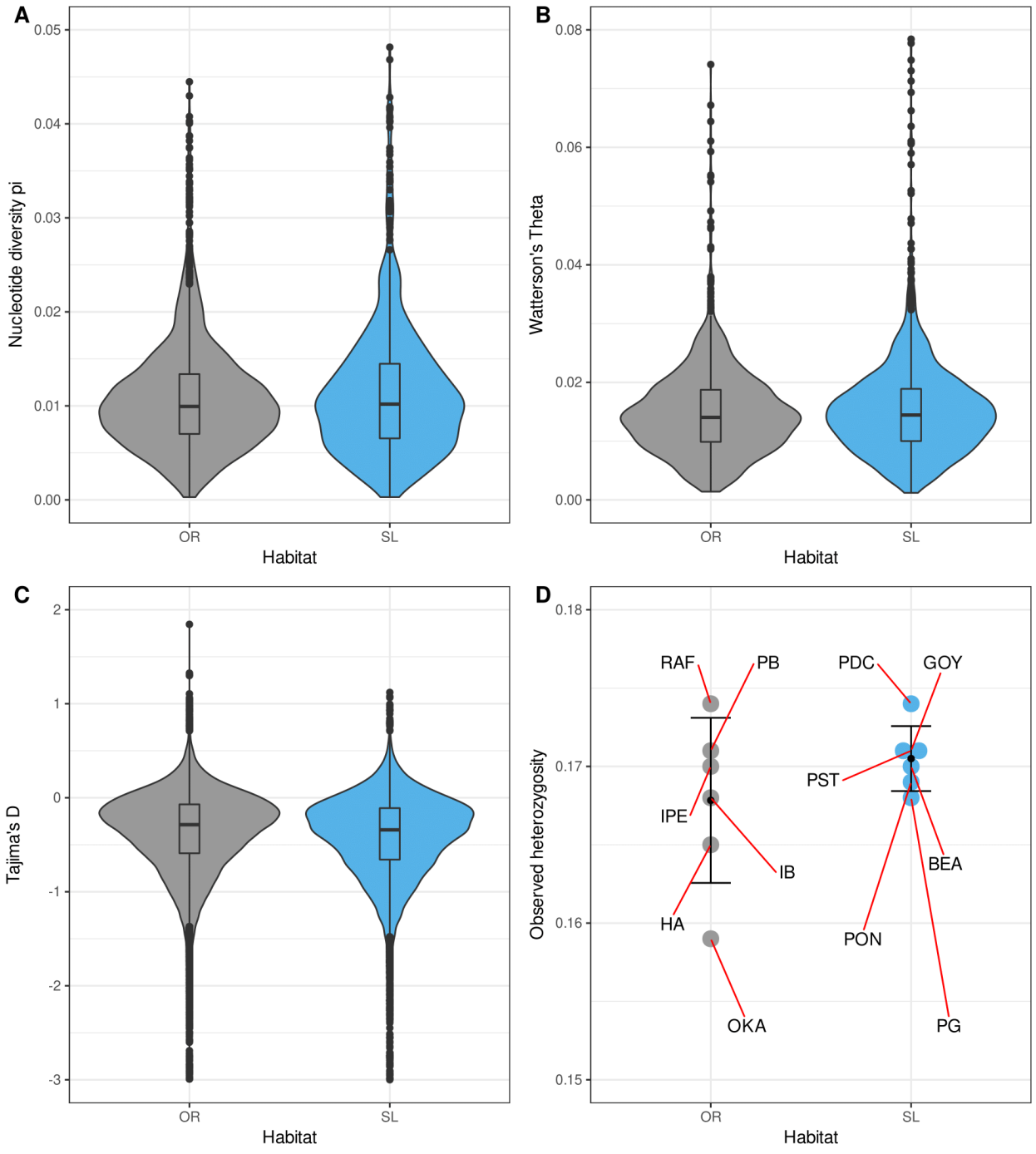
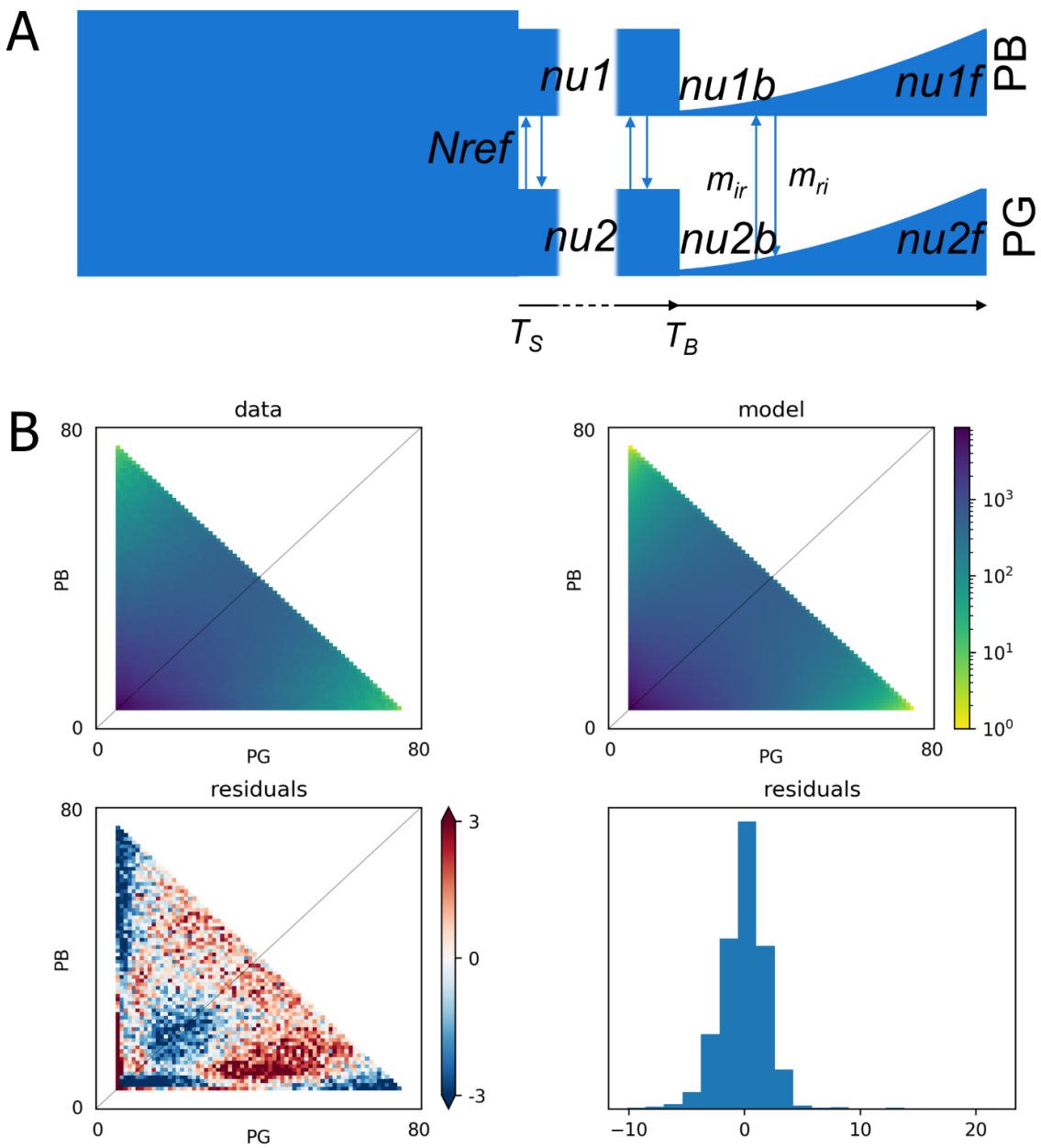


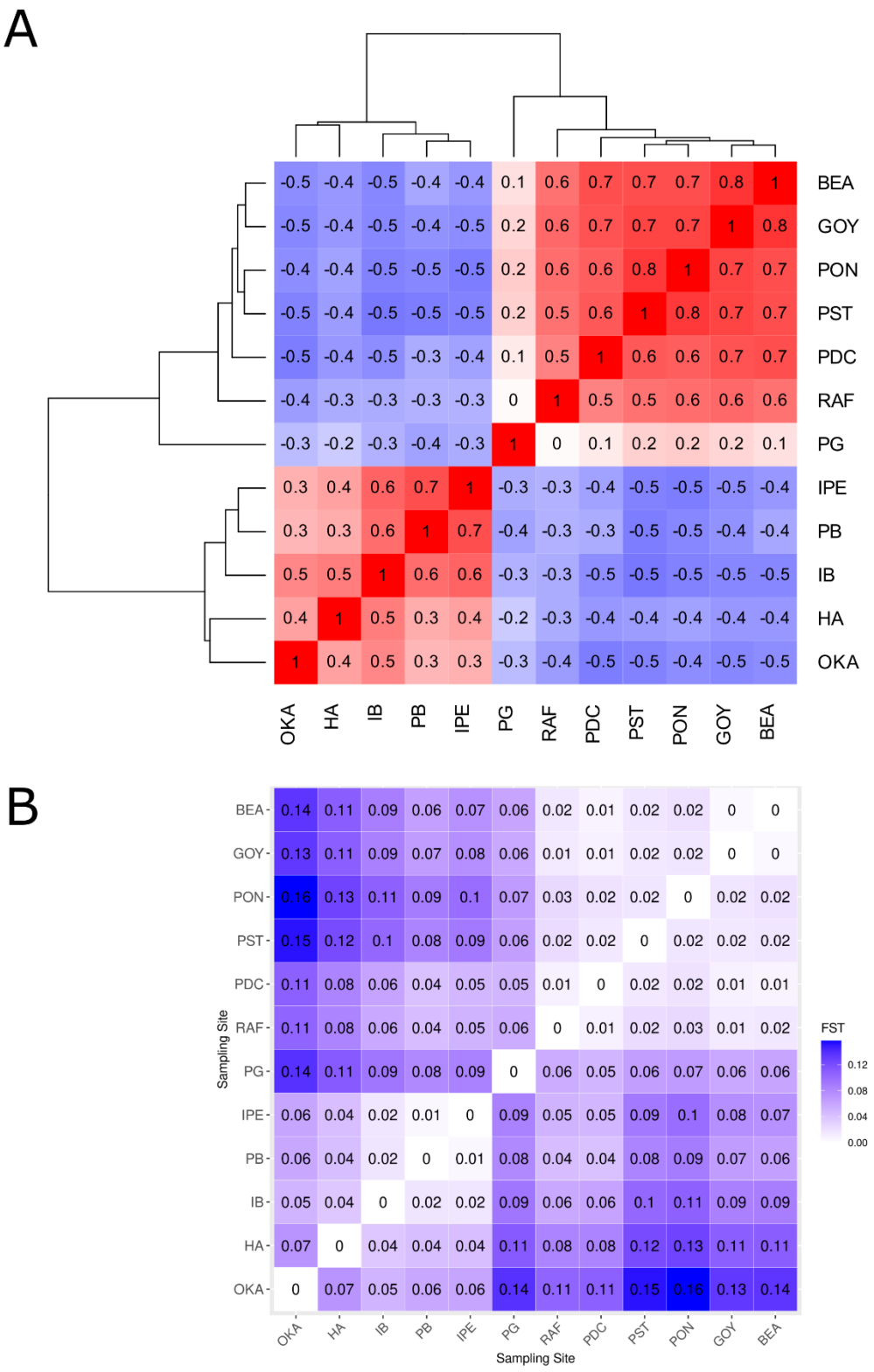
Figure 2.5: Demographic model tested with δaδi. A: Visual model with population split and constant migration, then bottlenecks in both populations, followed by exponential recovery. nu1 and nu2 are the scaled population sizes after the split, nu1b/nu1f and nu2b/nu2f represent the scaled population sizes of PB and PG after the bottlenecks and during the recovery respectively. T_S is the scaled time between the split and the bottleneck, and T_B is between the bottleneck and the present. m_{ri} is the migration rate from PG toward PB, and m_{ir} from PB toward PG. B: Top: Folded joint site frequency spectrum (SFS) of PB and PG for a sample size of 80 (two times the number of individuals) for the observed data (left) and the model (right). The colored scale indicates the logarithm of the number of sites for a given read count. Note that the data was masked from 0 to 5. Bottom: residuals of the normalized differences between the observed data and the model (left), shown as a histogram (right).



2.3.4. Isolation by environment and strong gene flow between habitat types

We found that populations clustered by environment type (Fig. 2.6), particularly by the presence/absence of the round goby, with RAF (invaded site with low calcium concentration) and PDC (uninvaded site with high calcium concentration but located very close to invaded sites) both clustering with invaded populations and showing lower pairwise F_{ST} values within those clusters. Our results from the scaled covariance Ω matrix of population allele frequencies inferred with Baypass indicate that there is also positive covariance in allele frequencies within clusters and negative covariances between clusters. These population structure results were concordant between the Ω matrix (Fig. 2.6A) and the pairwise F_{ST} matrix (Fig. 2.6B). Population structure was also explained by isolation by distance and by the environment (Fig. A.9), with the positive correlation between the genetic distance $F_{ST}/(1-F_{ST})$ and the log of the geographic distance or the Mahalanobis being significant (Mantel test, 9999 permutations: $p = 0.007$, $r^2 = 0.209$ and $p = 0.044$, $r^2 = 0.078$ respectively). We detected low to moderate gene flow based on pairwise F_{ST} across populations, with a relatively low average pairwise F_{ST} of 0.06 (SD 0.04, Fig. 2.6B). We found that the scaled migration rates estimated from δaδi were asymmetric, low but significant, with the scaled migration rate $2N_em$ from the refuge (PB) toward the invaded population (PG) being two times higher than the inverse ($m_{ir} = 1.51$, 95% CI [1.47,1.53] and $m_{ri} = 0.88$, 95% CI [0.86,0.94] respectively Table A.2).

Figure 2.6: Population genetic structure. A: Heatmap of the scaled covariance matrix Ω (with ρ_{ij} the correlation coefficient between pairs of populations) with hierarchical clustering tree (using the average agglomeration method), obtained from the core model of Baypass. B: Pairwise F_{ST} matrix between the twelve study populations.



2.4. Discussion

Knowledge of evolutionary and demographic processes is crucial for our understanding of how native species will respond to biological invasion and the mechanisms that may facilitate or inhibit their co-existence with invasive species. We investigated the interaction between adaptation and demography to gain insight into the persistence of a native gastropod (*Amnicola limosus*) following approximately 12 years of exposure to an invasive predator, the round goby, in the Upper St. Lawrence River. Our genomic results indicate that *A. limosus* has locally adapted to the invasion in the span of $\leq$12 generations. We also find evidence for adaptation to differences in water calcium over the longer geological history of the ecosystem. Moderate to strong asymmetric gene flow detected among study sites indicated that uninvaded habitats could serve as refuges from the invasive predator and provide migrants to invaded habitats, thereby helping to reduce negative demographic and genetic consequences of round goby predation on invaded populations (i.e., demographic/genetic rescue). However, individuals in uninvaded refuges were doubly maladapted for life history traits: they had low fitness overall and were not adapted to the invasive predator. Therefore, despite the current persistence of native *A. limosus* gastropods in the Upper St. Lawrence River system following the invasion by round gobies, this native gastropod could become vulnerable due to migration of maladapted individuals from uninvaded habitats, triggering a possible "genetic sabotage", as opposed to the genetic rescue.

2.4.1. Genomic signatures of local adaptation to round goby invasion and water calcium

Our genomic data (population structure and environmental association analyses) provides general evidence for local adaptation to the two distinct environment types in *A. limosus*, i.e., low calcium/goby absent and high calcium/goby present. The exceptions were for two populations that experienced inverse conditions for selection than the other sampled populations and clustered with the invaded populations: RAF, an invaded site with low calcium concentrations, and PDC, an uninvaded site with high calcium concentration that is located nearby invaded sites; both populations clustered with invaded populations. For RAF, the results support strong selection from goby predation even under lower calcium conditions, which are less optimal environmental conditions for round goby feeding and performance (Iacarella & Ricciardi, 2015). For PDC, the results suggest strong migration from adjacent invaded sites; PDC itself remained uninvaded despite higher water calcium concentrations, likely because the site was located within a wetland,

which provides less optimal conditions for round-goby establishment because of substrate properties (Astorg et al., 2021).

Our results confirmed that this pattern of isolation by environment was significant (Wang & Bradburd, 2014). This pattern could be generated by selection against migrants (Nosil et al., 2008; Orsini et al., 2013; Tigano & Friesen, 2016) related to calcium limitation and round goby predation. For example, individuals from the OR populations had lower fitness overall compared to SL populations and thus might have low reproductive output and survival in invaded habitats. In addition, given that goby predation has been shown to cause selection for smaller shell sizes at maturity (Kipp et al., 2012), OR individuals might be more vulnerable to predation than SL individuals if they are more conspicuous due to larger shell sizes. Similarly, hybrids might be selected against if intermediate phenotypes have lower fitness in their local environment (Thompson et al., 2022). Our EA analyses with Baypass and poolFreqDiff potentially allowed us to disentangle the signals of the two putative selective pressures (i.e., the effect of selection from goby predation at invaded sites and low calcium levels at uninvaded sites), even though invasion status and calcium concentration were strongly correlated. We found SNPs uniquely associated with invasion status and calcium concentration, which can be interpreted as signatures of local adaptation to predation by the round goby fish, and to the more limiting calcium concentrations at uninvaded sites.

Our study thus joins a larger body of the literature documenting rapid adaptation to anthropogenic environmental disturbance through shifts in allele frequencies (e.g., Brennan, deMayo, et al., 2022). Due to the unavailability of an annotated reference genome for *A. limosus* or a closely related species, we were unable to investigate putative physiological functions underlying the adaptations to the environmental covariables described here. Adaptation to calcium likely involves different functions from adaptation to predation. Differences in calcium water concentrations between the water masses from the two rivers are related to the geological characteristics of the river watersheds and therefore represent environmental differences over the long evolutionary history of this species in the St. Lawrence River. On the other hand, predation from the invasive round goby on mollusks is a recent and novel stressor in the St. Lawrence River. Putative physiological functions that would be worth investigating in future studies include transmembrane calcium transport and biomineralization pathways that might be involved in adaptation to low calcium concentration

(Clark et al., 2020), as well as shell development regulatory genes that could play a role in the evolution of smaller-sized shells at maturity (Kipp et al., 2012; as has been observed in populations subject to goby predation; Johnson et al., 2019).

2.4.2. (Mal)adaptive responses to round goby invasion and water calcium levels

Our results from the reciprocal transplant experiment give insight into potential adaptive and maladaptive responses in life history traits between SL and OR populations to divergent calcium concentrations and round goby predation regimes. Indeed, we found important differences in life-history traits (fecundity and survival as fitness components) between the populations from the two habitat types. The potential for local adaptation to the calcium gradient is suggested by a home advantage in fecundity for populations from both habitats in their origin water versus transplant water (water treatment SL vs OR), although the interaction between origin and treatment water was only marginally significant. While our laboratory reciprocal transplant experiment could indicate that *A. limosus* responded to round goby invasion through shifts in life-history traits, populations from the uninvaded habitats are also possibly maladapted, as shown by low fitness across treatment water and goby cue treatments. Both fecundity and survival were higher in the invaded SL population than in the uninvaded OR populations, regardless of water treatment. This suggests the OR populations might be generally maladapted (Brady et al., 2019), which could potentially occur through a trade-off of adaptation to low calcium water, as intracellular transport of calcium is energetically costly (Clark et al., 2020). In addition, individuals from the Ottawa River might allocate more resources toward calcium transport and be less able to invest in life-history traits such as reproduction.

Survival rates between populations varied widely, especially for the SL populations. This could reflect potential local (mal)adaptation to other biotic or abiotic parameters that we did not consider in the present study (e.g., temperature, substrate, nutrient availability, and food quality). As it was conducted using a single generation, we acknowledge that our reciprocal transplant experiment did not allow us to differentiate plastic vs genetic vs maternal effects on the measured traits. Our genomic results support the idea that the life-history differences observed between the two population types could be at least partially explained by adaptive genetic differences between the environments.

2.4.3. Demographic and genetic effects of the invasion

Given prior findings showing a decline in gastropod population abundance following invasion by round gobies, we hypothesized that invaded populations might suffer from population bottlenecks and reduced genetic diversity. However, we did not find any negative effect of the invasion on genetic diversity, with high levels of nucleotide diversity found in all populations. The nucleotide diversity reported here is relatively high compared to other species observed across phyla (Leffler et al., 2012), although lower than what has been observed in other gastropod species (Redak et al., 2021; Oswald et al., 2022). The slightly negative Tajima's D found in all our populations indicates that there was an excess of rare alleles compared to the neutral model, which could reflect a recent bottleneck followed by population expansion. However, as our demographic analysis did not indicate the occurrence of genetic bottlenecks in invaded or uninvaded populations, the magnitude of the population declines appears to have been insufficient to trigger major declines in genetic diversity levels. This implies that invaded populations are not currently in need of genetic rescue from refuge populations to recover genetic diversity, or perhaps that genetic rescue has already occurred or is ongoing and is the reason for high genetic diversity in invaded sites. Indeed, our δaδi models indicated that effective population size was an order of magnitude less in the invaded vs. refuge site, and scaled migration rates were asymmetric, with two times higher migration rates from the refuge toward the invaded population. This suggests that continuous migration of individuals from the larger uninvaded/refuge populations might be providing not only a demographic subsidy (demographic rescue; Hufbauer et al., 2015) but could also be replenishing the genetic diversity potentially lost by population declines in invaded populations (genetic rescue; Whiteley et al., 2015). Indeed, even though the scaled migration rate that we report here is low ($2Nem < 2$; Blanquart et al., 2012), they imply that the populations are connected ($0.1 << 4Nem << 11$; Hämälä et al., 2018). These rates are also significant, as comparable gene flow levels are sufficient to maintain a high level of genetic diversity despite strong genetic drift (Gompert et al., 2021).

2.4.4. Potential “genetic sabotage” through migration of maladapted individuals from refuge populations

A core goal of this study was to determine if local adaptation could interact with demographic rescue. We found relatively low levels of gene flow between the populations from the two habitat

types, with pairwise-FST values within the range of what has been observed in other egg-laying marine gastropod species (J. J. Bell, 2008; Keeney et al., 2009). Based on the gene flow levels found in the present study, dispersal is likely limited by the absence of a pelagic larval phase (Pinel-Alloul & Magnin, 1973), but drift by floatation in the water column or rafting on floating vegetation (Little & Nix, 1976; Martel & Chia, 1991) could also be important modes of dispersal, enhanced by strong water currents of the dynamic river system. We found that refuge populations provide migrants to invaded populations, thereby potentially providing both demographic and genetic rescue. However, because these migrants have low fitness overall (as demonstrated by low survival and fecundity across water treatments in our reciprocal transplant experiment) and are not adapted to the invasive predator, the input of genetic diversity from these populations toward invaded populations might provide a net detrimental effect to the recipient populations. For instance, genetic swamping and outbreeding derived from genetic admixture with maladapted populations could diminish the efficiency of local adaptation to the invasive predator in recipient populations (D. A. Bell et al., 2019). In addition, because individuals from uninvaded populations have lower reproductive output and survival, they will provide a more limited demographic subsidy to invaded populations. Thus, not all genetic rescue events might provide the same benefit to their recipients - the influx of maladapted alleles from refuge populations could potentially turn genetic rescue into genetic sabotage that imperils the success of populations under threat from novel selection pressures.

This is particularly important because strong selection such as the one observed in the invaded populations can also lead to reduced population sizes and drift, with negative effects on population fitness (Falk et al., 2012). The net outcome of this conflict will depend on the severity of population decline in recipient populations (i.e., how in need of demographic and genetic rescue they are; Hufbauer et al., 2015) and the magnitude of relative fitness difference between source and recipient populations (i.e., how detrimental migrant alleles will be for the recipient populations; Bolnick & Nosil, 2007). Over the short term, any form of demographic rescue may be beneficial for a population experiencing a strong decline. However, continuous migration of maladapted alleles is unlikely to permit long-term viability, regardless of the demographic need (Tufto, 2001).

2.4.5. The need to consider the potential costs of genetic rescue during population management

This study documents the impact of an aquatic invasive predator on evolutionary and demographic processes in a native prey species. Evaluating the potential evolutionary impacts of invasive species on native species is important because they can lead to surprising, unforeseen negative effects, such as the disruption of existing local adaptation (Melotto et al., 2020) and outbreeding depression through the influx of maladapted migrants from refuge habitats. Although genetic rescue has been proposed as a valuable tool for the conservation of small, isolated populations (Whiteley et al., 2015; Ralls et al., 2018), it has also been recognized that genetic rescue carries the risk of outbreeding depression if there is an adaptive genetic divergence between source and recipient populations (Frankham et al., 2011). The present study highlights a case from natural, unmanaged populations where the input of maladaptive alleles into invaded populations could lead to negative consequences. It thus reiterates the importance of considering the local adaptation of donor and recipient populations during managed introductions that aim to produce genetic rescue (Hoffmann et al., 2021).

Author contributions: MS, LA, APH, AMD, and RDHB designed the study. LA collected the field samples. LA and MS conducted the laboratory experiment. LA and AP performed the DNA extractions. LA prepared the initial pool-seq processing pipeline with input from FC, finalized by MS. MS ran the bioinformatic and statistical analyses with input from AMD and RDHB. The article was written by MS with input from AMD and RDHB. All authors contributed to the review and editing. AMD and RDHB funded the study.

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CHAPTER 2

EVOLUTIONARY RESCUE AND ADAPTIVE REVERSAL ALLOWED THE PERSISTENCE OF FRESHWATER COPEPODS DURING HISTORICAL LAKE ACIDIFICATION AND PH RECOVERY

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N.B. References cited in this chapter are presented at the end of the thesis.

ABSTRACT

The persistence of populations facing severe environmental disturbance can be enabled by natural selection on heritable phenotypic variation – a process known as an evolutionary rescue. Examples of evolutionary rescue have been documented in wild populations, but few studies have investigated the combination of factors (demographic, genetic, and environmental) that facilitate the rescue of populations in complex natural settings. This is important because the interplay of these factors in wild populations can cause uncertainty about the long-term outcome of evolutionary rescue: the persistence of rescued populations could potentially be threatened during environmental recovery due to a loss of genetic diversity from strong selection and demographic effects, and reduced fitness in the recovered environment. Here, we used copepod resting eggs of *Leptodiaptomus minutus* from three time periods of lake ecological history spanning ≈200 generations in two populations impacted by historical acidification (pre-acidification, acidification, and recovered). Whole genome sequencing of the resting eggs allowed us to uncover allele frequency shifts associated with the acidification followed by pH recovery. Our resurrection ecology approach was used to retrace adaptive shifts concomitant to the environmental transitions. Resurrected copepods from the pre-acidification period showed sensitivity to acidity while individuals from the acidification period were adapted to acidic pH and showed high fitness overall. This tolerance was subsequently lost during pH recovery. We found evidence of rapid directional selection in genome scans, consistent with the results of the phenotypic assays. Significant changes in allele frequencies of outlier loci during acidification were followed by a reversal in the recovered populations. Demographic models and a decrease in genetic diversity in the acidified populations indicated a demographic decline during the acidification process followed by population recovery, suggesting evolutionary rescue. The genomic results indicated that the loss of acid tolerance could be due to a combination of adaptive reversal and drift. By employing temporal genomic data and resurrection ecology experiments in lake ecosystems that have been impacted by historical human disturbance, this study fills a critical knowledge gap about the long-term implications of evolutionary rescue in the wild.

3.1. Introduction

Understanding and predicting adaptive responses to rapid anthropogenic impacts on the environment are important goals in evolutionary biology (Hoffmann & Sgrò, 2011; Urban et al., 2016b; Sanderson et al., 2022). The process of evolutionary rescue (i.e., an increase in the frequency of adaptive alleles following an initial population decline generating demographic recovery) has been the focus of several recent studies because of its potential to allow populations to avoid extinction from sudden environmental degradation (Gomulkiewicz & Holt, 1995; G. Bell & Gonzalez, 2011; Carlson et al., 2014; Hufbauer et al., 2015; G. Bell, 2017; Rêgo et al., 2019). The general predictions of evolutionary rescue have been first demonstrated empirically with yeast (G. Bell & Gonzalez, 2009). Factors that influence the probability of evolutionary rescue have been explored theoretically and experimentally (Carlson et al., 2014), such as the use of new mutations versus standing variation (Orr & Unckless, 2014), starting population size (Hufbauer et al., 2015), the strength of environmental disturbance, and the capacity for phenotypic plasticity (Ashander et al., 2016). Examples of evolutionary rescue have also been hinted at in wild populations (Carlson et al., 2014; Oziolor et al., 2019; Gignoux‐Wolfsohn et al., 2021). For instance, field crickets are hypothesized to have escaped high mortality rates from invasive parasitoid flies via a single gene mutation that suppresses the sexual song of males which attracted the flies (Tinghitella, 2008). Despite the potential short-term benefits of evolutionary rescue, most studies that have addressed the factors affecting evolutionary rescue have been laboratory experiments and theoretical modeling, and validation of the role of these factors and their interaction under natural conditions is still lacking (G. Bell, 2017). This knowledge gap may be due to the difficulty of documenting the interplay between demographic, genetic, and environmental factors during an evolutionary rescue event, particularly in complex, natural settings where many ecological and evolutionary processes act concurrently (Carlson et al., 2014).

An additional knowledge gap is that the long-term stability of evolutionary rescue in the wild is largely unknown, likely due to the intensive requirements of long-term monitoring of natural populations following an environmental disturbance (Carlson et al., 2014). Although evolutionary rescue has been proposed as a conservation measure for populations facing extinction (Mills et al., 2018), laboratory experiments suggest that it could potentially have detrimental effects over the long term (G. S. Stewart et al., 2017). Indeed, the long-term benefits of evolutionary rescue could

potentially be mitigated by two effects: 1) a loss of genetic diversity due to strong selection on adaptive alleles and demographic stochasticity, thereby reducing the ability of populations to adapt to future disturbances (G. S. Stewart et al., 2017) and 2) when ecosystems eventually recover or move toward another equilibrium, the phenotypic trait value that was selected for during the disturbance may be distant from the adaptive peak in the restored environmental conditions (unpredictable evolutionary directions: Grant & Grant, 2002; lags in adaptive reversals: Derry & Arnott, 2007; e.g., evolutionary traps: Carlson et al., 2014). However, environmental recovery may also lead to positive outcomes such as an adaptive reversal (i.e., a reversal in the direction of selection resulting in alleles and phenotypes reverting to their ancestral state; Chaturvedi et al., 2021) due to the relaxation of selection (Lahti et al., 2009). The maintenance of standing genetic variation is likely a key factor allowing adaptive reversal in fluctuating environments (D. Ben Stern & Lee, 2020; Chaturvedi et al., 2021; Garcia-Elfring et al., 2021). It is thus critical to study population demography, genetics, and environmental characteristics jointly during and after evolutionary rescue in the wild, as this combination of factors may be especially important in determining the outcome of evolutionary rescue (G. Bell, 2017)

Resurrection ecology provides a powerful toolkit to explore how environmental conditions impact demography and genetics factors during evolutionary rescue over long time periods (Franks et al., 2018; Weider et al., 2018). Many aquatic organisms produce resting stages as part of their life cycle, which can accumulate in sediments over time and form 'seed banks' (Hairston et al., 1995). These resting eggs can be hatched in the lab after being exposed to the proper hatching cues and reared in different environmental conditions to be used in a common garden or reciprocal transplant experiment setup (Hairston & Kearns, 2002; Franks et al., 2018). With a combination of resurrection ecology and genomics, these resting eggs can be used to retrace the evolutionary trajectories of populations relative to environmental disturbances (Burge et al., 2018; Weider et al., 2018; Ellegaard et al., 2020). The synthesis of these two methods offers a powerful approach to addressing evolutionary questions because it has the potential to illuminate past phenotypic and genetic changes that occurred in a population (Franks et al., 2018; Ellegaard et al., 2020). Resurrection ecology has been used extensively in the model species of the genus *Daphnia* (*Daphnia magna* and *Daphnia pulicularia*), for instance, to study adaptive responses to changes in predation regimes (Stoks et al., 2016; Chaturvedi et al., 2021), temperature (Geerts et al., 2015)

and eutrophication (Frisch et al., 2014). However, this approach has been comparatively less developed for other aquatic organisms (Burge et al., 2018), although some species such as diatoms (Burge et al., 2018), brine shrimp (Lenormand et al., 2018) and copepods (Derry et al., 2010) are emerging as new study systems for resurrection ecology studies. Adding whole-genome sequencing approaches to this research will yield important new insights into the genetic mechanisms underlying adaptive responses to historical environmental change (A. Waldvogel et al., 2020; Ellegaard et al., 2020). Genomics tools allow us to document (adaptive) allele frequency changes over time (e.g., Brennan, deMayo, et al., 2022) and to reconstruct the demographic history through modeling (e.g., Matz et al., 2018).

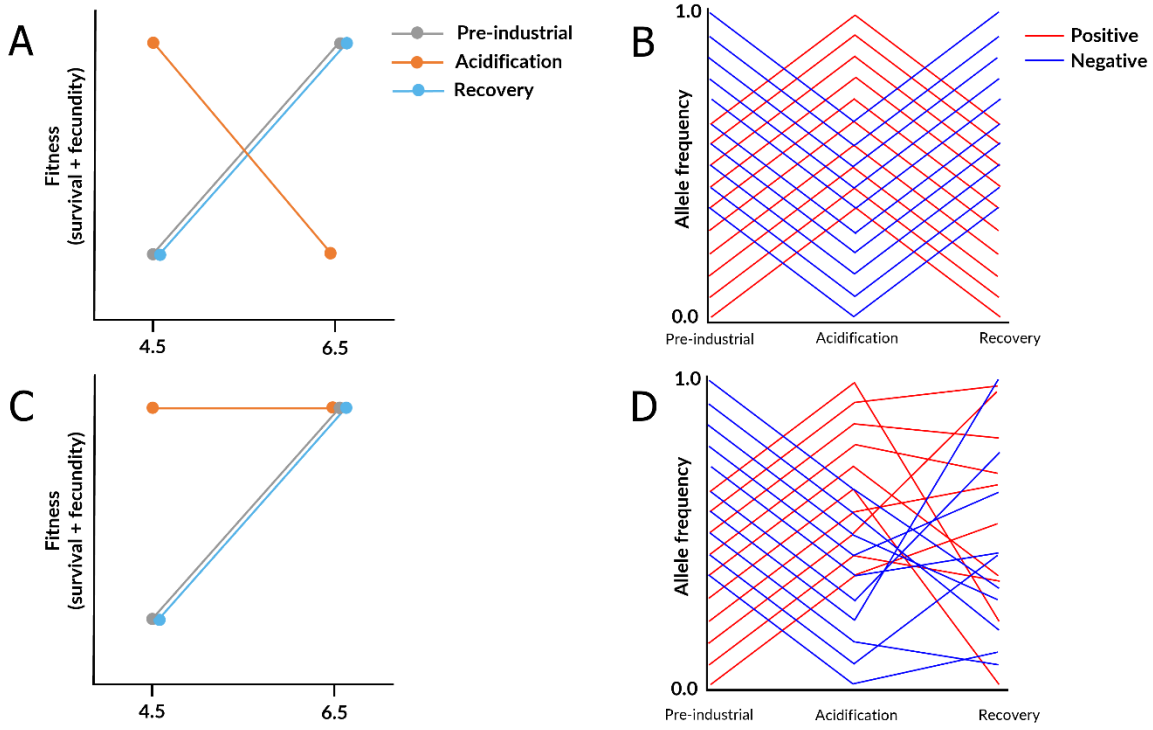
The ecological history of disturbance and recovery in the lakes of Killarney Park, ON (Canada) provides a powerful study system for testing for evolutionary rescue and its potential effects on population resilience in aquatic organisms. Many lakes in this region were acidified in the 1960s as a result of metal mining emissions but have undergone chemical recovery in water pH over the last several decades following government controls on emissions in the 1970s (Keller et al., 2007). Lake acidification resulted in high mortality rates and local extinctions of fish (Beamish & Harvey, 1972), zooplankton (Sprules, 1975), and phytoplankton (Kwiatkowski & Roff, 1976). However, a species of a calanoid copepod, *Leptodiaptomus minutus*, developed resistance to acidic pH and subsequently became dominant in zooplankton communities across this acidified landscape (Sprules, 1975; Derry et al., 2010; Gray et al., 2012). Resurrected nauplii from resting eggs of *L. minutus* dated from the pre-industrial period (i.e., unexposed to acidity) showed variable survival to acidity in contrast with the consistently high survival of resurrected individuals from the acidification period. However, this was then followed by a return to lower but variable survival to acidic pH during the recovery period (Derry & Arnott, 2007; Derry et al., 2010). The sensitivity of unexposed individuals suggests that the acute acidification could have resulted in high levels of mortality, which was the case in a whole lake acidification experiment in which the population of *L. minutus* crashed until extinction (Frost et al., 2006). While many Killarney lakes have undergone progressive pH recovery over the last several decades, biological recovery has been slower and zooplankton communities have not returned to their initial composition and abundance (Gray & Arnott, 2012).

This study aimed to provide evidence for and evaluate the factors underlying evolutionary rescue and adaptive reversal of copepod populations in two pH-recovered lakes with a history of acute acidification. We tested for evidence of the selection of acid-tolerant phenotypes and alleles during the lake acidification, followed by the selection of acid-sensitive phenotypes and alleles during the lake pH recovery, by using resting eggs sampled from sediment cores at three time points of lake ecological history (pre-acidification, acidification, and recovered). Our approach is two-fold: First, we conducted a laboratory reciprocal transplant that employed resurrected *L. minutus* individuals to measure survival, fecundity, and development time at two pH treatments (acidic and neutral pH). Second, we analyzed temporal changes in allele frequencies based on whole genome sequencing (WGS) across the time series. At the phenotypic level, resurrected individuals from the pre-industrial period should show a variable (but on average low) survival at acidic pH. Conversely, resurrected individuals from the acidification period should have high survival at acidic pH. The return to variable (low) survival of *L. minutus* to acidic pH during the recovery period (Derry & Arnott, 2007; Derry et al., 2010) implies that some acid-sensitive phenotypes could have either persisted during the acidification or been reintroduced to the population through immigration from other lakes or the resting egg bank. Acid resistance (survival) could incur a fitness cost (trade-off) in neutral pH conditions, for example, due to antagonistic pleiotropy (Lahti et al., 2009; Bono et al., 2017).

If the rise and fall of acid tolerance (indicated by high survival rates and its correlated trait fecundity) represents evolutionary rescue followed by an adaptive reversal, we expect that resurrected individuals from the acidification period should have lower fitness at neutral pH than at acidic pH (i.e., a fitness trade-off) and that copepods from the recovery period should have lower fitness at acidic pH (Fig. 3.1A). At the genetic level, in the case of an adaptive reversal, we expect to observe signatures of directional selection between the pre-acidification and the acute phase of lake acidification, followed by a reversal in the trajectory of allele frequency change between the period of acidification and pH recovery (Fig. 3.1B). Alternatively, acid sensitivity could have re-evolved in the population due to loss of tolerance mutations through genetic drift when selection for tolerance was removed in the absence of acidic pH (Fig. 3.1C) In the case of acid tolerance loss due to genetic drift, we would expect to observe a signal of directional selection during the acidification phase followed by random changes in allele frequency during the recovery phase (Fig.

3.1D). The potential demographic effects of the initial low acid tolerance, combined with the development of acid tolerance over time and the high density of *L. minutus* during the acidification period, hints at the potential U-shaped demographic signature of evolutionary rescue (Gomulkiewicz & Holt, 1995). At the genomic level, we should thus observe a pattern of reduction in effective population size during the acidification phase followed by a rebound. Our study leverages historical data and samples to document a valuable example of evolutionary rescue and its long-term consequences in nature. By combining resurrection ecology with WGS, we were able to characterize the interaction between demography, the genetics underlying rapid adaptation, and environmental selection that promoted the evolutionary rescue of populations from acute acidification and adaptive reversal during recovery.

Figure 3.1: Conceptual illustration of the expected shifts in life-history traits and allele frequencies in response to historical lake acidification and pH recovery. A: Adaptive divergence in life-history traits between individuals from the three time periods (pre-acidification, acidification, recovery) exposed to acidic or neutral pH. A trade-off for acid tolerance in individuals from the acidification period at neutral pH would indicate adaptive reversal during lake pH recovery. B: Shifts in adaptive allele frequencies due to acidification followed by a reversal in frequencies during lake recovery. C: Adaptive shifts in life-history traits due to the acidification followed by loss of acid tolerance due to genetic drift, indicated by an absence of a trade-off for acid tolerance in individuals from the acidification period at neutral pH. D: Shifts in adaptive allele frequency due to directional selection from acidification, followed by random changes in allele frequencies due to drift.



3.2. Methods

3.2.1. Study sites, sampling, and physicochemical data collection

Killarney Park, Ontario, Canada (46°01′ N, 81°24′ W) contains over 600 boreal lakes from the Canadian Shield geological region with pH ranging from 4.3 to 7. This wide range in pH among lakes is the result of natural pH variation, as well as anthropogenically induced acidification associated with the long-range transport of acid deposition from SO_2 emissions from nearby metal mining smelters during the mid-1900s (Keller et al., 2007). Subsequent emission reductions in the mid-1970s led to differences in pH recovery trajectories between lakes due to differences in buffering capacities originating from the geological properties of their bedrock (S. S. Dixit et al., 2002).

We collected sediment cores from two lakes that acidified and are now at different stages of pH recovery: George (N46°01.7960 W81°23.8310) for which the pH was recovered to circumneutral pH in 1994, and Lumsden (N46°01.5240 W81°25.9730) which is still recovering (pH < 6; Suenaga, 2018). These two lakes were also impacted differently by the acidification (Fig. B.1B), with a lower and more progressive drop in pH for George, and a faster, stronger acidification in Lumsden (A. S. Dixit et al., 1992). We sampled 53 sediment cores from each lake during July 2018 and June-July 2019 from the deepest point of each lake. We divided sediment cores into 1 cm-thick sections on-site using a custom-made sediment core extruder and then stored each sediment section in airtight Whirl-pack® bags that were refrigerated in the dark until analysis. We isolated resting eggs of *Leptodiaptomus minutus* from the sediment cores of each lake, representing different periods of ecological history (pre-acidification, acidification, and recovery). The exception was that we were unable to obtain viable *L. minutus* resting eggs from the pre-acidification period of George Lake due to low resting egg density.

We conducted ^{210}Pb dating on one sediment core per lake at the Geotop Center (Université du Québec à Montréal). For each section, we weighed the sediments before (wet weight) and after drying (dry weight) to obtain the dry bulk density ρ, which is needed to calculate sediment age with the constant rate of supply (CRS) model. For each section, 100 mg of dry weight was isolated, and alpha-particle radiation activity was counted for 48 h in an alpha counter. We then applied the CRS (constant rate of supply) model (Binford, 1990) to calculate ^{210}Pb dates for core intervals. ^{210}Pb

dates for the sediment cores collected from each lake and the temporal sections selected for the resurrection and genomics experiments are presented in Fig. B.1B. Because we conducted the resurrection ecology experiment before we completed the ^{210}Pb dating of our sediment cores, we used published ^{210}Pb dates for sediment cores from these two lakes (Labaj et al., 2015) to determine the depth intervals for the three periods of ecological history for each lake (pre-acidification, acidification and recovered). This led to the inclusion of a larger period of time than intended for the acidification period for George Lake, which based on our ^{210}Pb dating encompassed the years 1887 to 1963, whereas similar depths encompassed the years 1940 to 1980 in Labaj et al. (2015). However, because the acidification period for George Lake started earlier and lasted longer than the dates we used from Labaj et al. (2015), and given the results of our resurrection ecology experiment (showing responses consistent with acid-adapted individuals, see below), we are confident that the sediment section included in our study is representative of the acidification period for this lake. We used a YSI Pro Plus multiparameter probe in situ (model 10102030; Yellow Springs Inc.) in 2018 and collected water samples in 2019 to obtain physicochemical properties for George and Lumsden lakes (surface temperature, pH, conductivity, dissolved organic carbon, total phosphorus, total nitrogen). Details of the analytical methods for water chemistry and physicochemical parameters of the lakes can be found in the supplementary methods and Table B.1.

3.2.2. Resurrection ecology experiment

From the resting eggs recovered from the lake cores, we applied a resurrection ecology experimental approach in which F_1-generation copepods were hatched from resting eggs originating from three time periods (pre-acidification, acidification, and recovery) and exposed to neutral (pH 6.5) and acidic pH (4.5) to measure survival, fecundity, and development time throughout their lifetime (≈ one month, Fig. 3.2). The copepod mothers who produced the eggs in the lake were considered F_0-generation copepods. We collected their resting eggs; these eggs remained dormant in the lake, arrested in time, and hatched for the experiment, making them F1. No maternal effects were standardized in this experiment as we were unable to obtain enough hatchlings from the reproduction of the F1 individuals to carry on the experiment on the F2 generation. We used two different methods to hatch the resting eggs as the older eggs (pre-acidification and acidification periods) could not be hatched directly (Derry et al., 2010). The first

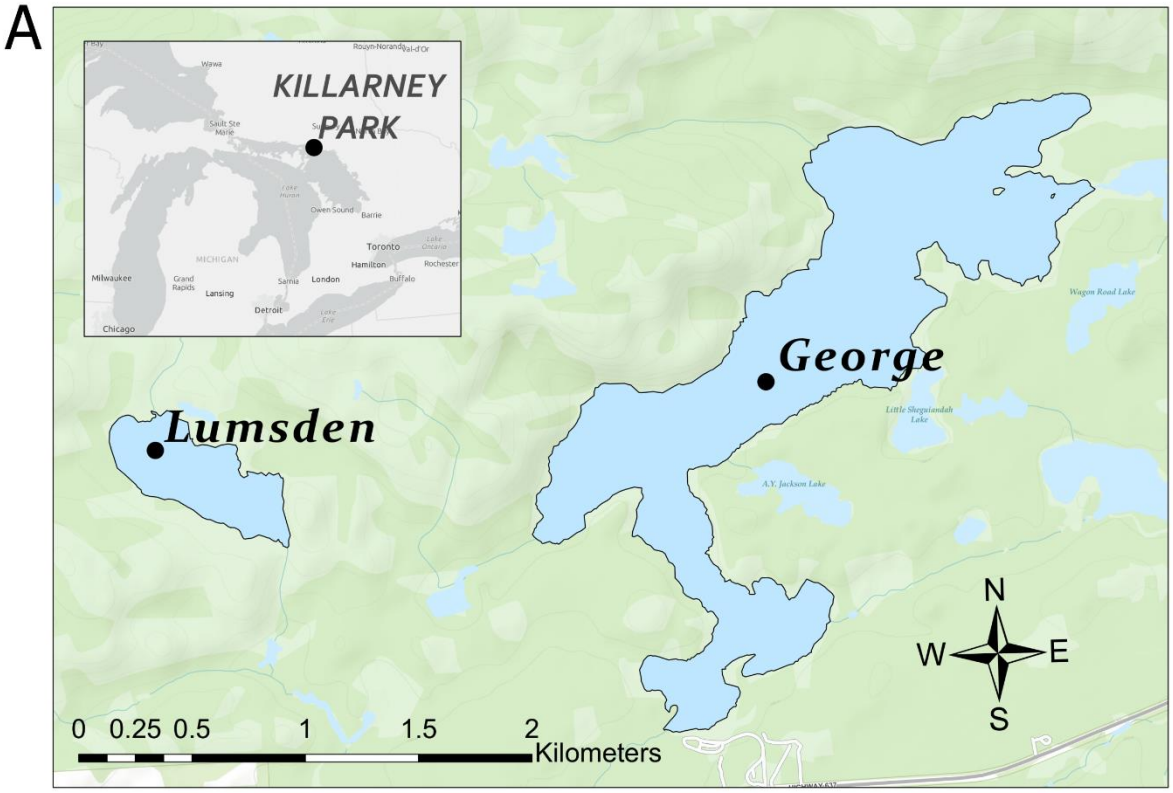
method was implemented for the eggs corresponding to the recovery period (from the past 10-20 years). Following the method in Hairston & Kearns (2002), we added the equivalent of 1 cm of the core sediment to the bottom of a plastic tray and then covered the eggs with 1-2 cm of COMBO culture medium (Kilham et al., 1998) prepared in the laboratory and pH adjusted to 4.5 or 6.5 with 0.02M sulfuric acid. In the second method, used for eggs corresponding to the pre-acidification and acidification periods, we separated the eggs from the sediments for each time period (acidification and recovery) with a sugar flotation method modified by Marcus (1990). We then stored the isolated resting eggs from the acidification and pre-acidification periods in distilled water, in a dark refrigerator at 4°C. Prior to exposure to hatching signals (luminosity, temperature, and oxygenation), we stored the eggs from the acidification period for 2 weeks to 1 month under the above conditions, and the eggs from the pre-acidification period for 2 months, following a developed method (Derry et al., 2010). This is necessary for resting eggs that have been buried for longer periods in lake history, as more time is required to stimulate them to hatch (Hairston & Kearns, 2002).

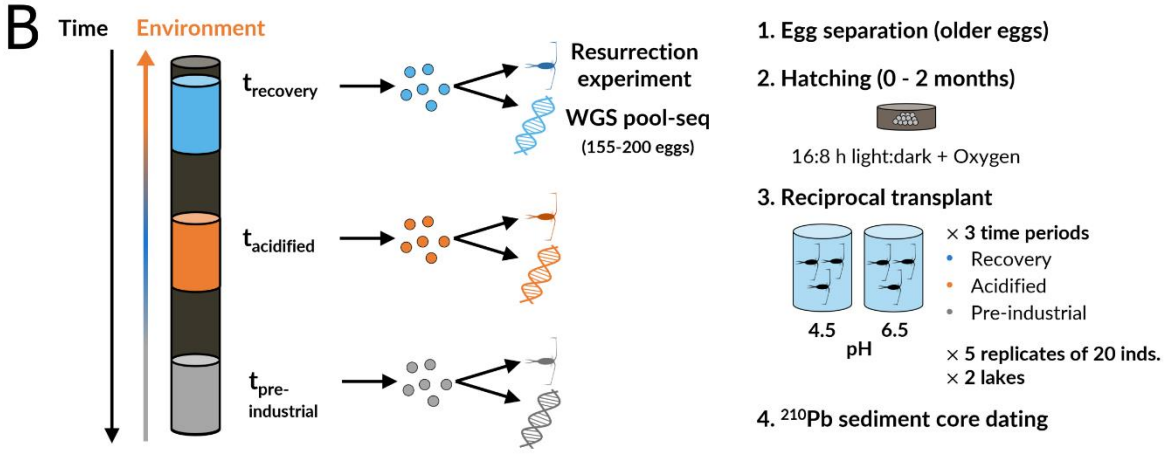
After these storage periods, we transferred all the resting eggs to a hatching beaker containing 200 mL of COMBO medium. For both methods, resting eggs from several sediment cores sections had to be combined in the beakers and hatching trays presented to obtain enough hatchlings for the resurrection experiment, but we did sediments from different time periods or lakes. We exposed resting eggs to the following hatching signals: 16h:8h light:dark cycle, 20°C, and oxygenation (Derry et al., 2010; Broman et al., 2015). We kept the hatching trays and beakers in a Thermo Scientific Precision Model 818 Incubator growth chamber and examined them regularly for the presence of nauplii. We verified the identity of nauplii using the list of species present in our study lakes (Suenaga, 2018) as well as a key from the Department of Biological Sciences at the University of New Hampshire (Haney et al., 2013). We removed a few nauplii from the beakers and datasets which were misidentified (N = 5 cyclopoid copepods and N = 1 calanoid copepod: *Epischura lacustris*).

We transferred hatchling nauplii (≈ 20 individuals per replicate) to beakers with 200 ml of COMBO medium adjusted to either an acidic pH of 4.5 or a circumneutral pH of 6.5. We changed the culture medium regularly (once to three times a week). We fed the copepods with a mixture of 1 mL of

brown algae (*Cryptomonas sp.* CPCC 336) and 1 mL of green algae (*Chlamydomonas sp.* CPCC 243) cultured at a stationary phase. We cultured the green algae in a modified Bold's Basal medium (Stein, 1973), and the brown algae in COMBO adjusted to pH 6.5, in the growth chambers containing the copepod cultures at 16h:8h light:dark cycle and 20°C. We noticed algal growth in the copepod cultures after 2 days, indicating sufficient food abundance. We observed copepods in each replicate during water changes to record: 1) their survival, 2) the development stage, and 3) the number of eggs or directly hatched nauplii produced throughout their lifetime (from hatching to metamorphosis, and from metamorphosis to sexual maturity). We measured the survival of copepods by counting the number of living and dead individuals. We obtained the developmental stage of copepods by counting the number of individuals observed at each life stage: nauplii, copepodites, and adult copepods. Their fecundity was measured by counting the number of eggs or nauplii produced relative to the number of adult females present in the culture.

Figure 3.2: Geographical location of the sampled lakes and experimental design of the resurrection ecology experiment. A: George and Lumsden are located in Killarney Provincial Park ON, Canada. B (left): Schematic representation of the resurrection experiment and the temporal genomics. B (right): Steps and details of the resurrection experiment.





3.2.3. Analysis of life-history datasets

We analyzed survival, fecundity, and development times with generalized linear mixed effect models (GLMM), generalized linear models (GLM), and t-tests. We modeled survival for Lumsden and George lakes with GLMMs using a binomial distribution and logit link function. We modeled fecundity with GLMs using a quasi-Poisson distribution to account for overdispersion. For development time, as the number of replicates was low for George Lake, we used different statistical tests for the two lakes. For Lumsden, we used a GLM with a Gamma distribution and a log link function, and for George a t-test pooling data over the pH treatment (testing for the effect of time period). For the t-test, the assumption of normality was verified with Shapiro-Wilk tests and the equality of variance with Bartlett's tests. We checked all GLMM and GLM models for overdispersion using the overdisp_fun function from https://bbolker.github.io. To evaluate the fixed effect on survival in our GLMMs, we used an AIC approach corrected for small sample size (AICc) with the ΔAIC criterion from the R package 'bbmle'. We kept the effects when $\Delta AIC > 2$. To evaluate the fixed effect on fecundity in our GLMs, we used an analysis of deviance with an F approximation due to the overdispersion. For the development time from Lumsden, we used the likelihood ratio test to examine the fixed effects. The fixed effect coefficients of the GLMs and GLMMs and their confidence intervals were converted to the odds ratio (survival), incident rate ratios (fecundity), and fold change (development time) using an exponential function.

3.2.4. Whole genome sequencing and SNP calling

We applied a pool-seq approach to the sequencing of temporal samples from the two lakes (Lumsden: pre-acidification, acidification, recovery, George: acidification, recovery). First, we separated the eggs from the sediments with the sugar flotation method described above. Each pool consisted of 200 eggs of *L. minutus* (except for Lumsden pre-acidification: 155 eggs) that were isolated in 70% ethanol after identification under high-resolution stereomicroscopes (Olympus). The resting eggs were first crushed with a plastic pestle after being flash-frozen with liquid nitrogen. We then extracted DNA from the crushed eggs with the QIAGEN MagAttract HMW kit following the manufacturer's instructions. DNA quality control (QC) was done with the Agilent gDNA 165 kb kit on the Femto pulse system. Libraries were prepared for Illumina sequencing with the Lucigen NxSeq AmpFREE kit and then had QC performed, then PCR enriched for 6 cycles followed by a final QC. Libraries were then sequenced on an Illumina NovaSeq6000 Sprime v1.5 to generate 150

bp pair-end reads. Quality control, library preparation, and sequencing were conducted at the McGill Genome Center. We trimmed reads with fastp (Chen et al., 2018) with a minimum base quality score of 20 in a sliding window starting at the 3' end and a minimum length of 50 bp. As a draft genome for *L. minutus* or a closely related species was unavailable, we applied the reference-free SNP discovery method from discosnp++ (Uricaru et al., 2015). This method uses a de Bruijn graph analysis of k-mers, produces SNPs datasets equivalent to reference-based approaches (Uricaru et al., 2015), and has been previously used for a copepod genomic dataset (Arif et al., 2019). We ran discosnp++ with the branching strategy (option -b 1), which allows a good compromise between precision and recall (Uricaru et al., 2015), extending polymorphism with left and right unitigs (only one SNP per contig) and a minimum coverage of 5 per pool. We converted the vcf file output by discosnp++ into a "pooldata" object with the poolfstat package in R (Hivert et al., 2018), with a haploid pool size of 400 for all populations (except for the size of 310 for Lumsden pre-acidification) and a minimum coverage of five per pool. We also set maximum coverage of 300, a minimum read count per base of two, a minimal minor allele frequency of 0.0032 (to remove singletons) and removing indels. Finally, we used the pooldata object from poolfstat to estimate bi-allelic frequencies (read counts for the reference allele / overall read coverage). This generated a dataset of 3,053,984 biallelic SNPs after filtering.

3.2.5. Detecting signals of selection

To assess the presence of putative loci under selection during the acidification and pH recovery time periods, we used two approaches: 1) an F_{ST} scan for loci showing exceptional differentiation between time periods, and 2) an environmental association analysis. We conducted the F_{ST} scan approach with poolfstat to detect outlier SNPs showing large differences in allele frequency between time points. SNP-specific pairwise F_{ST} values were obtained with the compute.pairwiseFST function using the ANOVA method (default) and the same parameters as described in the filtering step above. We identified outliers SNPs as those included within the 95% percentile (top 5%) of the distribution of pairwise F_{ST} values for each temporal comparison. Because genomic scans using pairwise F_{ST} do not consider population structure and demographic processes, we also used the hierarchical models implemented in Baypass (Gautier, 2015), with the core model as an outlier analysis and the auxiliary (Aux) model as an environmental association analysis. To avoid computing limitations, we divided the poolfstat dataset into four pseudo-

independent datasets by converting the pooldata object to four Baypass input files, using the "thinning" subsampling method with a sub-sample size of 763,496 SNPs. We ran both the core and auxiliary models with default parameters. We first used the core model to estimate the scaled covariance matrix of population allele frequencies Ω (Fig. B.2), which reflects population demography and is then explicitly accounted for during the detection of outlier SNPs. This model also allows the estimation of the XtX statistics, which is equivalent to SNP-specific F_{ST} and is based on the variance in allele frequencies between all populations (Günther & Coop, 2013), accounting for Ω and derived p-values under a χ^2 distribution with 5 degrees of freedom (N = 5 pools, bilateral test). We confirmed that the p-values behaved well based on the shape of their histogram (Fig. B.3B; François et al., 2016). We selected the aux model to detect SNPs significantly associated with the acidification (coded as a binary covariable; -1: pre-acidification and recovery, 1: acidification) in both the Lumsden and George datasets, as it is suitable for analyses of time samples such as experimental evolution datasets (see Baypass manual). This model computes the regression coefficient β_{ik} of the association between the SNP allele frequencies and the covariable (here positive and negative association with the acidification), as well as an associated Bayesian auxiliary variable δ_{ik} from which a Bayes factor BF_{mc} is derived. We considered SNPs significantly associated with the acidification covariable when their $BF_{mc} > 20$ dB (Jeffrey's rule for "decisive evidence"; Gautier, 2015). The acidification covariable was standardized to $\hat{\mu} = 0$ and $\widehat{\alpha^2} = 1$ as recommended. We compared the four Ω matrices visually to ensure that the results were concordant between sub-datasets, then we combined the statistics estimated for each SNP.

3.2.6. Population structure and demography.

Population structure was first estimated with the core model from Baypass. Results of the core model were compared to a genome-wide pairwise F_{ST} matrix estimated with the poolfstat package, using the same parameters as described above. To evaluate the potential effect of demography on genetic diversity levels, we also estimated observed heterozygosity for each temporal sample with poolfstat. To assess the possibility of evolutionary rescue (a demographic decline from the acidification followed by population recovery due to adaptation), we used the diffusion approximation approach from δaδi (Gutenkunst et al., 2009). We selected the recovery temporal samples of Lumsden and George for our dataset and analyzed each population separately (1D

model). The most complex model ("bottlegrowth") has a U-shaped demography as described above (Fig. 3.4A), with a genetic bottleneck at time T_B (scaled time between the bottleneck and present) corresponding to a decrease in effective population size N_e followed by exponential recovery in N_e. As we knew the approximate time of the potential bottleneck (≈1970, set as 48 years before sampling, with two generations per year; Boers & Carter, 1978), T_B was defined as a fixed parameter, and the parameter $\theta = 4\mu L$ as an explicit parameter, for all models except the neutral model. We defined θ with μ the mutation rate of 2.64×10^{-9} substitutions per site per year from the snapping shrimp *Alpheus spp.* (Silliman et al., 2021) and L the effective sequenced length of 617,993,816 bp, calculated as L ≈ total length of sequence analyzed × SNPs retained for use in δaδi/total SNPs in analyzed sequence.

We also analyzed three simpler models, including two nested models: a) genetic bottleneck without recovery ("two epochs" model), b) growth starting at T_B, and c) neutral. We filtered out SNPs that were detected as outliers (putatively under selection) in the Baypass (core and aux models) and F_{ST} scan analyses from the initial dataset output by poolfstat, which retained 4,315,136 SNPs. To transform the allele frequency data into the input format of δaδi, we used the dadi_input_pools function from the genomalicious R package (Thia & Riginos, 2019) applying the "probs" parameter in the methodSFS option. As we lacked information on the ancestral allele state, we inferred the folded SFS with δaδi, masking entries from 0 to 20 reads because of our limited confidence in the low-frequency estimates. We used the default local optimizer on the log of parameters and optimized the parameters for each model until convergence was reached (three runs falling within 1% of the best likelihood). We compared our nested models (bottlegrowth and two epochs) with an adjusted likelihood ration test, and the two others (growth and neutral) based on the large differences in the likelihoods and residuals of these models with our models showing the highest likelihood (bottlegrowth and two epochs). As we obtained unlikely results during the conversion of parameters in our best model (e.g., Effective population size after the bottleneck in George: 2 individuals for the bottlegrowth model defined as $NuB = nuB \times Nref$; and 20 individuals for the two epochs model defined as $Nu = nu \times Nref$), possibly due to imprecise mutation rates, we do not report the results of parameter conversion. To obtain uncertainties on our parameters while accounting for the effect of linkage, we used bootstrapping and the Godambe Information Matrix

approach (Coffman et al., 2016). For this, we generated 100 bootstrapped datasets with a chunk size of 1×10^5 bp.

3.3. Results

3.3.1. Resurrection ecology experiment: Adaptive shifts in life-history traits following acidification and pH recovery

The resurrection ecology experiment revealed population life history traits shifts (lifetime survival, development time, and fecundity) that were consistent with the adaptation of *L. minutus* to lake acidification followed by a reversal during recovery (Fig. 3.3, Table 3.1 and 3.2). For the survival of copepods from nauplii to adulthood (lifetime survival) in both Lumsden and George lakes (Fig. 3.3A), we found significantly greater survival rates of the resurrected individuals from the acidification period than from the recovery period. Specifically, for Lumsden, there were no significant differences in survival between individuals from the pre-acidification and recovery periods. These results hold regardless of pH (non-significant interaction). Although this interaction was not significant, survival was also significantly higher at neutral pH compared to acidic pH. We found similar results, albeit less pronounced, for early survival (from hatching to metamorphosis into copepodites), except for the effect of pH, which was not significant (Fig. B.4A, Table 3.1 and 3.2). Copepods from the acidification period developed significantly faster to adulthood compared to the pre-acidification period for Lumsden, and for both Lumsden and George, there was no difference in the development time between acidification and recovery individuals (Fig. 3.3C, Table 3.1 and 3.2). The pH treatment and the interaction between time period and pH did not affect development time to adulthood for both lakes (Table 3.1). The results were similar for early development (nauplii to copepodites) except for a significant difference between acidification and recovery for Lumsden (Fig. B.4B, Table 3.1 and 3.2). Female copepods from Lumsden were significantly more fecund during the acidification period than during the pre-acidification and recovery periods, but the pH and the interaction between time period and pH did not affect their fecundity (Fig. 3.3B, Table 3.1 and 3.2). However, George Lake female fecundity was only marginally affected by time period and the time period × pH interaction. To summarize, our experiment suggests there was an acquisition of acid tolerance during the acidification period followed by a loss of tolerance, shown through differences in survival and fecundity. The increase in acid tolerance was accompanied by faster development, but this change in development rate did

not reverse to pre-acidification levels following pH recovery. Our results were consistent between both lakes despite having experienced different strengths of historical acidification.

Figure 3.3: Observed shifts in life-history traits in freshwater copepods from different ecological periods (pre-acidification, acidification, recovery) in two historically acidified lakes as a response to two levels of pH treatment as revealed by the resurrection ecology experiment. A: Lifetime survival, B: development time from birth to adulthood, C: Fecundity (number of eggs or nauplii per female). Each dot represents a measurement for one replicate, and the black bars indicate the mean response for each treatment.

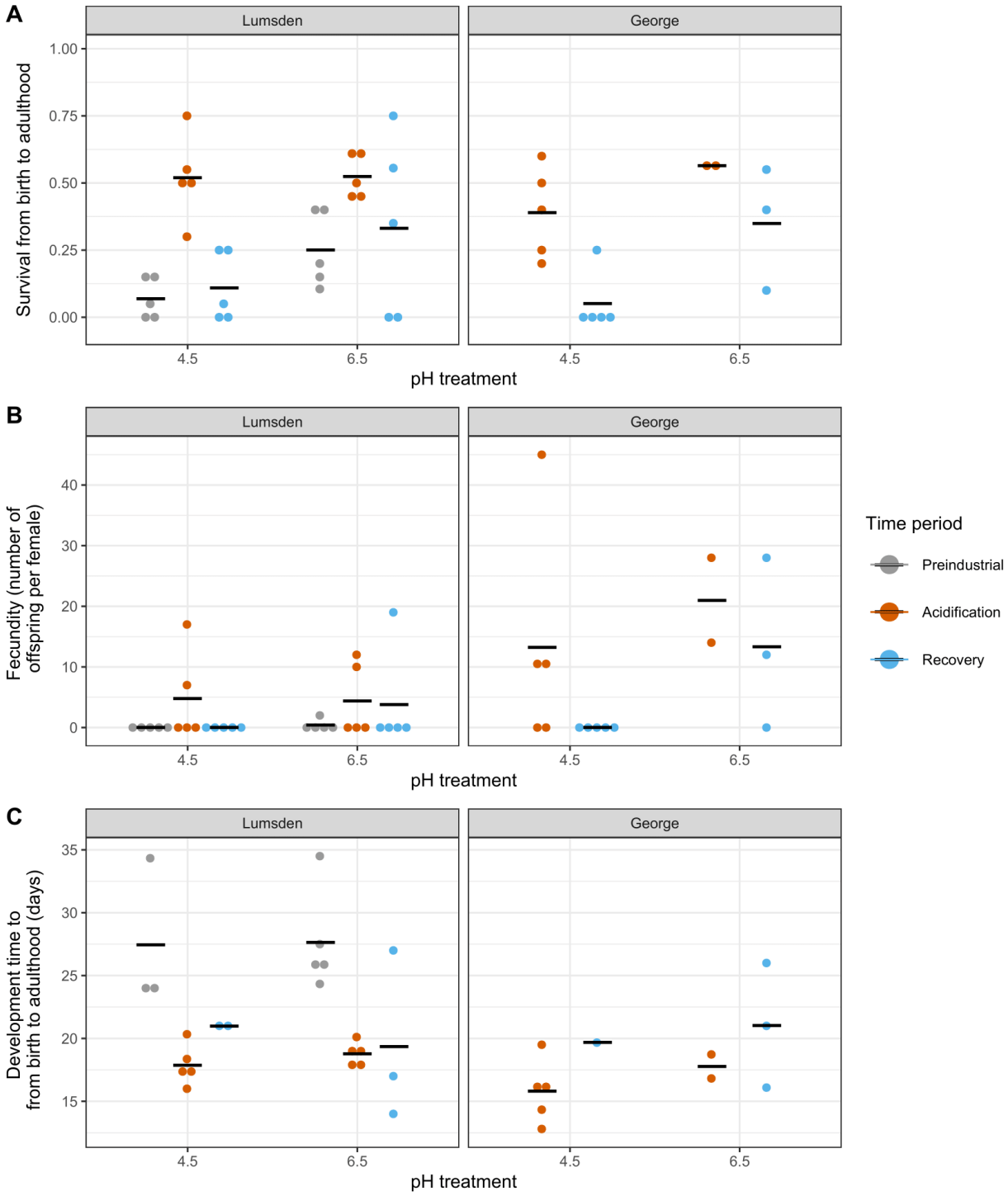


Table 3.1: Results of the analysis of survival, fecundity, and development times with generalized linear mixed effect models (GLMM), generalized linear models (GLM), and t-tests. Lifetime survival indicates survival from hatching to adulthood and early survival from hatching to the copepodid stage. For the GLMMs, a ΔAIC > 2 indicates that the effect is significant. Significant fixed effects are indicated in bold.

Response variable	Lake	Type model	Test	Fixed effects	df	p-value ΔAIC
Lifetime survival	Lumsden	GLMM binomial logit	AICc	Time period × pH	5	-2.95
				Time period	3	**10.83**
				pH	4	**2.01**
Early survival	Lumsden	GLMM binomial logit	AICc	Time period × pH	5	-5.9
				Time period	3	**8.41**
				pH	4	-0.90
Lifetime survival	George	GLMM binomial logit	AICc	Time period × pH	4	-1.87
				Time period	3	**7.55**
				pH	3	**3.48**
Early survival	George	GLMM binomial logit	AICc	Time period × pH	4	- 4.44
				Time period	3	**5.94**
				pH	3	-3.23
Fecundity	Lumsden	GLM quasi-poisson	Quasi-F	Time period × pH	24	0.20
				Time period	26	0.04
				pH	28	0.38
Fecundity	George	GLM quasi-poisson	Quasi-F	Time period × pH	11	0.08
				Time period	12	0.07
				pH	13	0.15
Lifetime development	Lumsden	GLM gamma log	LRT	Time period × pH	17	0.78
				Time period	20	$\mathbf{5.59\times 10^{-7}}$
				pH	19	0.93
Early development	Lumsden	GLM gamma log	LRT	Time period × pH	22	0.16
				Time period	25	**0.002**
				pH	24	0.39
Lifetime development	George	-	t-test	Time period	4	0.121
Early development	George	-	t-test	Time period	12	0.07

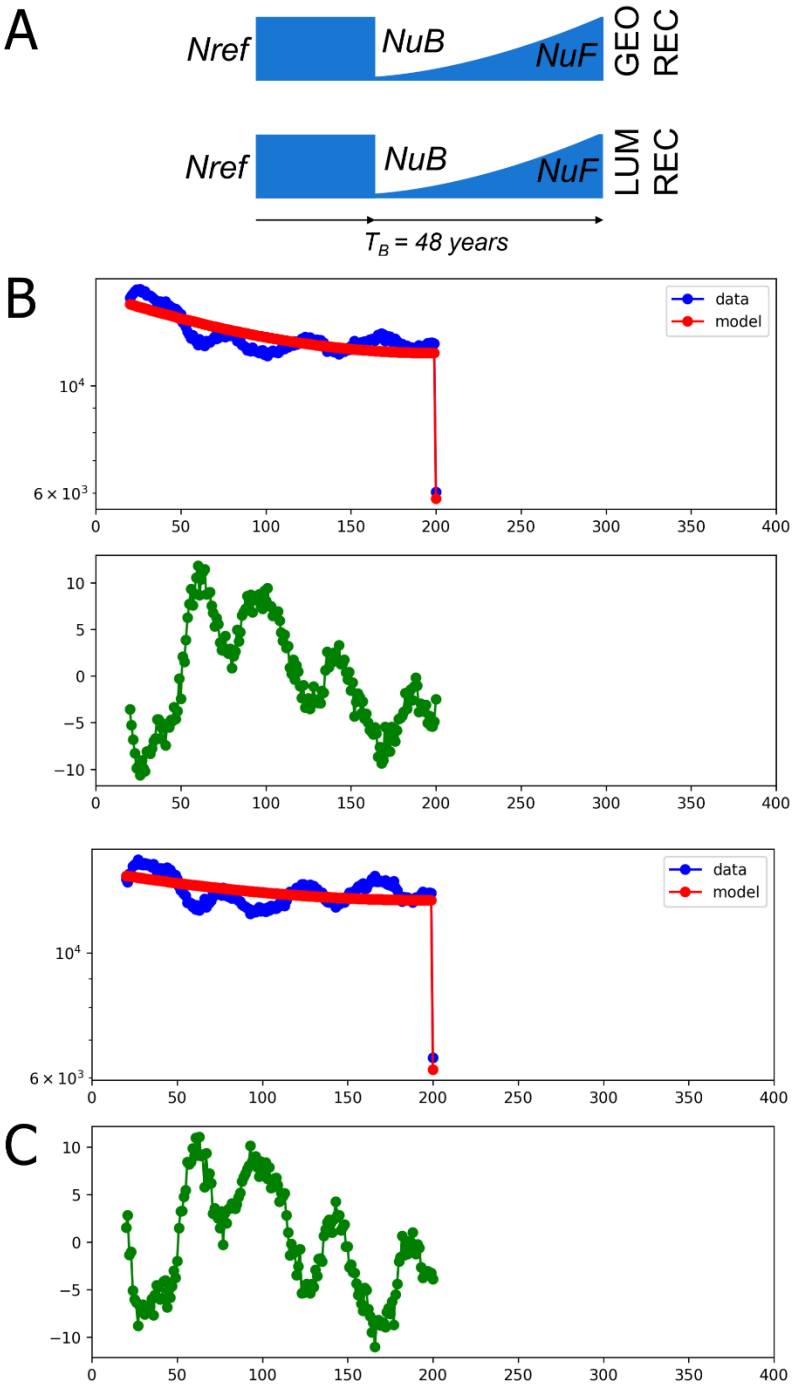
Table 3.2: Fixed effect coefficients of the GLMs and GLMMs and their confidence intervals after conversion with an exponential function, for each response variable and lake. The converted coefficients are given as the first level compared to the second (reference) level. The relationship is significant if the converted coefficients do not include 1 (in bold), is positive if > 1, and negative if < 1. When the converted coefficient is < 1, the inverse is given in parenthesis and indicates how much less likely an event is to be observed. For the results of the t-test (development time for George), the estimate represents the difference (in days) between time periods.

Response variable	Lake	Fixed effect coeff.	Comparison	Estimate	Lower limit	Upper limit
Lifetime survival	Lumsden	Odds ratio	Acidification vs pre-acidification	**7.98**	2.96	21.51
			Recovery vs pre-acidification	1.31	0.46	3.72
			Neutral pH vs acidic pH	**2.59**	1.14	5.87
Early survival	Lumsden	Odds ratio	Acidification vs pre-acidification	**3.36**	1.42	7.97
			Recovery vs pre-acidification	0.59 (1.69)	0.25	1.41
			Neutral pH vs acidic pH	1.70	0.84	3.45
Lifetime survival	George	Odds ratio	Recovery vs acidification	**0.12 (8.33)**	0.04	0.40
			Neutral pH vs acidic pH	**5.35**	1.66	17.19
Early survival	George	Odds ratio	Recovery vs acidification	**0.27 (3.70)**	0.11	0.67
			Neutral pH vs acidic pH	1.45	0.57	3.75
Fecundity	Lumsden	Incident rate ratios	Acidification vs pre-acidification	**23.0**	1.32	9.30×10^5
			Recovery vs pre-acidification	9.5	0.36	3.96×10^5
Fecundity	George	Incident rate ratios	Recovery vs acidification	0.32 (3.13)	0.05	1.41
Lifetime development	Lumsden	Fold change	Acidification vs pre-acidification	**0.67 (1.49)**	0.58	0.77
			Recovery vs pre-acidification	**0.73 (1.36)**	0.61	0.86
Early development	Lumsden	Fold change	Acidification vs pre-acidification	**0.82 (1.22)**	0.72	0.93
			Recovery vs pre-acidification	0.98 (1.02)	0.87	1.13
Lifetime development	George	-	Recovery vs acidification	4.34	-	-
Early development	George	-	Recovery vs acidification	1.68	-	-

3.3.2. Population genomics: demography, population structure, and rapid genetic adaptation to acidification and lake pH recovery

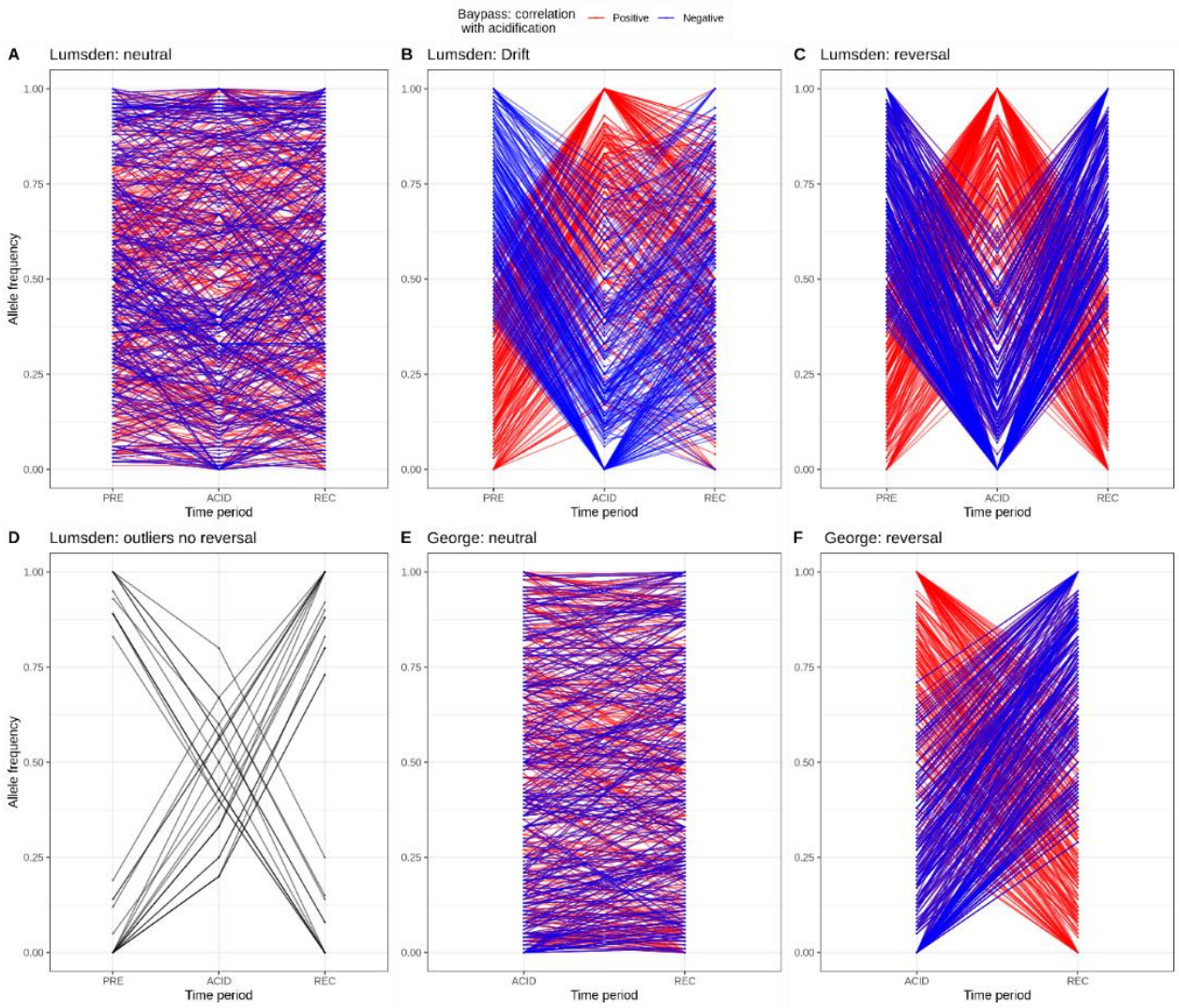
Using the recovery time period samples from George and Lumsden lakes, we found indications of significant declines in their effective population sizes during the acidification period. Our best models (highest likelihood) were the models including a genetic bottleneck at a known fixed time 48 years in the past (bottlegrowth, Fig. 3.4, and two epochs, Table B2 and Fig. B.5). However, we were not able to reject the simpler model of bottleneck with no recovery (two epochs) based on the likelihood ratio tests (adjusted D-statistic = -2.88, p-value = 1.00; adj. D-statistic = -2.71, p-value = 1.00; for George and Lumsden respectively), and thus we cannot conclusively infer that recovery of effective population size occurred after the bottleneck event. Our analyses suggest that the ancestral effective population sizes were possibly quite large, with estimated θ values of 1.3×10^6 for George and 1.6×10^6 for Lumsden (see Table B.2; $\theta = 4N_{ref}\mu L$, with N_{ref} the ancestral effective population sizes, μ the mutation rate, and L the effective sequenced length). In contrast, the decrease in effective population size was likely very pronounced, with very low inferred values for the ratio of effective population size after the genetic bottleneck to the ancient population size (*nuB*: 5.2×10^{-6} - 1.0×10^{-4}, Table B.2). This demographic decline was reflected by a decrease in heterozygosity in Lumsden from 0.36 to 0.33. This was followed by a smaller increase to 0.34 during the recovery phase. Conversely, for George, we did not find a change in heterozygosity during the recovery (observed heterozygosity = 0.31 for both the acidification and recovery time periods). We found that pairwise F_{ST} values were higher between lakes across time periods (moderate, 0.09 on average) than between time periods within lakes (low, 0.03 on average). For Lumsden, genetic differentiation was lower between the pre-acidification and the recovery periods than between the acidification and the pre-acidification or recovery (Fig. B.2 and B.6).

Figure 3.4: Demographic model tested with δaδi. A: Visual 1D models with genetic bottlenecks in both George and Lumsden populations at T_B, followed by exponential recovery. NuB, NuF: effective population sizes after the bottlenecks and during the recovery, respectively. T_B: known scaled time between the bottleneck and present. B) Results for George (copepods from the recovery period) with the bottlegrowth model. Top: Folded site frequency spectrum (SFS) for a sample size of 400 for the observed data (blue line) and the model (red line), showing the logarithm of the number of sites (y-axis) as a function of a given read count (x-axis). The data was masked from 0 to 20. Bottom: Histogram of the residuals (normalized differences between the observed data and the model). C) Results for Lumsden Lake copepods from the recovery period.



The genome scan of pairwise F_{ST} values detected outlier loci in temporal comparisons of both study lakes (Lumsden: pre-acidification vs acidification and acidification vs recovery, George: acidification vs recovery). For Lumsden, we found 149,269 SNPs in the top 5% of F_{ST} values in the pre-acidification vs acidification comparison ($F_{ST} > 0.26$, ≈ 5% of the dataset), and 146,436 outliers in the acidification vs recovery comparison ($F_{ST} > 0.32$, ≈ 5% of the dataset). For George, we found 144,398 outlier SNPs in the acidification vs recovery comparison ($F_{ST} > 0.26$, ≈ 5% of the dataset). We also found 43,558 outlier SNPs in common between the two-time comparisons for Lumsden (≈1% of the dataset and ≈30% of the outliers). Remarkably, 99.93% of these outliers showed a reversal in allele frequencies during the recovery period (Fig. 3.5C). These SNPs are considered candidates for directional selection during the acidification followed by adaptive reversal during recovery. The 29 outliers that did not reverse either showed a consistent increase or decrease in allele frequency over both time comparisons (Fig. 3.5D). We identified 92,692 SNPs that were outliers in the Lumsden F_{ST} scan for the pre-acidification to acidification comparison, but not for the acidification to recovery comparison, and these SNPs were not significantly associated with the acidification in Baypass (BFmc < 20, ≈3% of the dataset, Fig. 3.5B). We consider these outlier SNPs in the pre-acidification to acidification comparison as candidates for directional selection from acidification. However, during the recovery time period, these SNPs that were outliers in the first temporal comparison were possibly subject to drift instead of selection for the second temporal comparison (non-outliers). The remaining Lumsden outliers were not significantly differentiated for the pre-acidification to the acidification comparison, but they were significant for the acidification to recovery comparison.

Figure 3.5: Allele frequency changes of outliers detected with the FST scan and Baypass. The red and blue colors indicate positive and negative associations with acidification in Baypass (regression coefficient βik > 0 and βik < 0 respectively). A: A random subset of 500 non-outlier SNPs for Lumsden (non-significant in the FST scan for both time comparisons and not significantly associated with lake acidification with Baypass, BFmc < 20). B: A random subset of 500 SNP candidates for drift following the lake acidification in Lumsden (significant in the FST scan for the pre-acidification to acidification comparison but not for the acidification to recovery comparison and not significantly associated with lake acidification with Baypass, BFmc < 20). C: A random subset of 500 outliers SNP candidates for directional selection during acidification and an adaptive reversal during recovery for Lumsden lake copepods (significant in the FST scan for both temporal comparisons and with Baypass, BFmc > 20). D: Lumsden outliers significant in the FST scan for both temporal comparisons but not showing a reversal (N = 29). E: A random subset of 500 non-outlier SNPs for George Lake copepods (non-significant in the FST scan and Baypass BFmc < 20). F: A random subset of 500 outlier SNPs for George Lake copepods (significant in the FST scan and Baypass BFmc > 20).



We found 10,934 outlier SNPs in common between the acidification to recovery comparisons for both Lumsden and George lakes (≈ 0.4% of the dataset and ≈7-8% of the acidification vs recovery outliers). These SNPs are considered candidates for parallel selection during the recovery phase. Using the core model in Baypass as our outlier analysis, we found 33,239 outlier SNPs with p-value < 0.001 and either high or low XtX values, which represented ≈1% of the dataset (Fig. B.3). The XtX estimate is based on the variance in allele frequencies across populations, thus in the present case, outlier SNPs are significantly differentiated considering both the populations (Lumsden and George) and temporal samples (pre-acidification, acidification, and recovery). For the Aux model testing the association between SNP allele frequencies and acidification, we found 92,095 outlier SNPs significantly associated with acidification (BFmc > 20; ≈ 3% of the dataset). These outlier SNPs have allele frequencies both negatively (46,664 outliers) and positively associated (45,431 outliers) with acidification (Fig. 3.5, Fig. B.7), and some showed a strong association, with their correlation coefficient $|\beta_i| > 0.2$ (20,474 outliers, Fig. B.7). Finally, we found outliers in common between the F_{ST} scan and the auxiliary model in Baypass (Fig. B.8): 13,339 outliers in Lumsden (using the outliers of the F_{ST} scan in common between both time periods), 30,941 in George, and 1,277 outliers common to both Lumsden and George Lake (candidates for parallel selection).

3.4. Discussion

Understanding the factors that determine the long-term success of the evolutionary rescue is crucial for predicting populations' responses to historical and ongoing environmental change (Carlson et al., 2014), but knowledge of how demographic, genetic, and environmental mechanisms interact as this process unfolds in complex natural ecosystems is lacking. Here, we investigated the possibility of evolutionary rescue in freshwater calanoid copepod (*Leptodiaptomus minutus*) populations that were historically exposed to acute lake acidification, followed by an adaptive reversal during lake pH recovery. We combined a resurrection ecology experiment with whole-genome data spanning ≈ 200 generations in two lakes with histories of acidification. We found adaptive shifts during acidification, with increased acid tolerance and fecundity as well as faster growth rate, which were probably mediated by directional selection at the genomic level. We also found evidence for a genetic bottleneck from lake acidification followed by high population density and a potential recovery of the effective population size, suggestive of evolutionary rescue.

Resurrected copepods from the recovery period showed reduced acid tolerance and fecundity, accompanied by a reversal in allele frequency at outlier loci. Finally, we found some indications of parallel phenotypic and genomic shifts between the copepod populations from two historically acidified lakes. Overall, our results support the possibility of evolutionary rescue in these wild populations and help shed light on the factors (demographic, genetic, and environmental) that likely promoted the rescue.

3.4.1. Contemporary adaptation to acute historical acidification

Using a resurrection ecology experimental approach, we detected shifts in life-history traits between the pre-acidification and acidification time periods in both study lakes. We documented an increase in lifetime survival and fecundity as well as faster development rates overall (across pH treatments) for copepods from the acidification period. As our resurrection experiment was conducted within a single generation without standardizing maternal effects, we acknowledge that our experimental setup did not allow us to disentangle the influence of genetic from plastic and maternal effects on the life-history traits. Apart from survival, for which the effect of pH was significant, fecundity and development time showed flat reaction norms across pH treatments, with most differences occurring between individuals from distinct time periods. Considering that the individuals from different time periods correspond to distinct genotypes within the same continuous population, this implies that we did not find indications of genotype × environment interactions for these traits. This suggests that plasticity may play a limited role in changes in fecundity and development as a response to acidity. There were some signs of initial intraspecific variation in acid tolerance (Des Roches et al., 2018), as a few individuals from the pre-acidification period from Lumsden Lake were able to survive at acidic pH until sexual maturity. These acid-tolerant individuals could have been positively selected during the process of acidification, leading to adaptation to acidic pH. Our experiment also revealed an important concomitant decrease in development time following the acidification, for both early and late development stages. Our results are consistent with adaptation to historical lake acidification leading toward a faster pace of life, with higher survival, fecundity, and increased growth rates, which have also been reported in response to stressful environments, such as urbanization (Brans & De Meester, 2018). However, the increased survival and fecundity we found in our experiments for the individuals from the acidification period contrast with studies of marine copepods, which have reported little selective

effects of acidity in response to ocean acidification (Thor & Dupont, 2015; Dam et al., 2021). Given these contradictions, and apart from a few relevant examples (Dam et al., 2021; Brennan, DeMayo, et al., 2022; Brennan, deMayo, et al., 2022), studies examining the full scope of responses to acidification (phenotypic, genetic, and demographic) are still lacking and will require more efforts (Loria et al., 2019).

The adaptive shifts in life-history traits that we detected were accompanied by significant allele frequency changes between the pre-acidification and the acidification periods. This implies that the life-history shifts observed between these two time periods could be due in part to shifts in adaptive allele frequency, resulting from the directional selection from the acidification. As these allele frequency changes were both positively and negatively associated with lake acidification and we do not know the ancestral allele states, adaptation could have occurred through both positive selection as well as purifying selection (Johri et al., 2020; Griffiths et al., 2021). Given the number of outlier SNPs detected with our various analyses, it is also likely that linked selection is playing an important role in structuring genomic responses in these populations (Buffalo & Coop, 2020). Based on the number of generations between the pre-acidification and acidification periods and the presence of a few acid-resistant copepods initially, we hypothesize that selection acted on standing variation rather than on *de novo* mutation (hard sweep; Hermisson & Pennings, 2017).

Leptodiaptomus minutus does not currently have an annotated genome, and thus we were unable to conduct functional analyses of the SNPs putatively under selection from the acidification. Possible functions under selection to explore in future studies could be related to osmoregulation and homeostasis as these physiological functions have consistently been identified as a mechanism of response to acidification in copepods (Havens, 1993; De Wit et al., 2016; Thor et al., 2018; Brennan, deMayo, et al., 2022; Brennan, DeMayo, et al., 2022). Regulation of diapause (Gyllström & Hansson, 2004; Roncalli et al., 2021) and lipid metabolism (Roncalli et al., 2019) are also of interest as they tend to be favored in stressful environments. Taken together, the changes in life-history traits (survival, fecundity, and development) as well as significant shifts in the frequency of putatively adaptive alleles provide support for the occurrence of rapid adaptive evolution due to historical changes in pH.

3.4.2. Demographic consequences of acidification and evolutionary rescue

The universal increase in survival, fecundity, and growth rates that we observed in copepods that experienced lake acidification suggests that individuals from this time period have higher fitness than individuals from the pre-acidification period. During the initial process of acidification, the lower survival and fecundity at acidic pH, combined with a slow development rate, likely had important demographic effects, potentially triggering a population decline. This was indeed confirmed with our demographic analysis, in which the best models included a genetic bottleneck, with a significant decrease in effective population size for both populations. This decline resulted in a moderate reduction in genetic diversity during the acidification period for Lumsden. The population decline due to acidification was likely followed by a population rebound, as *L. minutus* populations were documented to be at high density in acidified lakes by 1972-1973 (8 ind./L in George, 17 ind./L in Lumsden; Suenaga, 2018). The genetic bottlenecks detected in our study populations during the acidification, in conjunction with a probable population recovery and an increase in the frequency of adaptive phenotypes and putative alleles, indicate that our study populations likely avoided local extinction (e.g., Frost et al., 2006) through evolutionary rescue.

This rescue could have been facilitated by several factors: large initial population size, standing genetic variation, and progressive rate of environmental change. Population size was probably large initially, as we inferred a high effective population size prior to the genetic bottleneck. Larger population sizes have been shown to increase the likelihood of evolutionary rescue (Hufbauer et al., 2015). It is also likely that the evolutionary rescue was fuelled by adaptation from standing genetic variation rather than from new mutations, as acid-tolerant phenotypes appeared to be present in the populations during the pre-acidification period. Theory suggests that adaptation from standing variation can require less time and a smaller initial population size for evolutionary rescue to occur (Orr & Unckless, 2014). A recent study highlighted the role of standing genetic variation in the evolutionary rescue of bats from white-nose syndrome (Gignoux‐Wolfsohn et al., 2021). It has also been suggested that evolutionary rescue from high levels of environmental stress should be driven by large-effect mutations that are present as standing genetic variation (Anciaux et al., 2018) which might be the case in our study system and will require further investigations. The populations from both our study lakes showed similar responses despite the different trajectories during acidification (much faster and steeper for Lumsden Lake; A. S. Dixit et al., 1992). These

results confirm that the rate of acidification that lakes in the Killarney Park region experienced (progressive or abrupt) represented a very stressful environmental event for aquatic organisms, with a genetic bottleneck in *L. minutus*, which was one of the most tolerant zooplankton species in this region (Sprules, 1975; Derry et al., 2010). This is consistent with the high extirpation rate recorded at the community level for other zooplankton species and aquatic taxa (Beamish & Harvey, 1972; Sprules, 1975).

Despite evidence for evolutionary rescue in *L. minutus* populations, our demographic analyses highlight the potential negative consequences of this process. Indeed, the strong selection and drift that occurred during evolutionary rescue appear to be associated with a genetic bottleneck with a decline in effective population size. Despite the following environmental recovery and increased population abundance, it is unclear if the effective population size rebounded as well because our models with and without recovery were both equally likely. This is consistent with a previous study that found that evolutionary rescue led to genetic load due to the reduction of genetic diversity from the strong selection and drift at reduced population sizes (G. S. Stewart et al., 2017). Prior experimental studies of evolutionary rescue have also shown demographic and genetic bottlenecks with large reductions in effective population size, followed by limited N_e recovery (Rêgo et al., 2019). This suggests that the short-term benefits of evolutionary rescue might be accompanied by detrimental effects in the long term.

3.4.3. Evidence for an adaptive reversal during lake pH recovery and the role of drift

The results of the resurrection experiment indicated a loss of acid tolerance (survival and fecundity) in individuals from the recovery period for both lakes. In the case of an adaptive reversal, we might expect to find a trade-off between survival to acidity and survival or fecundity at neutral pH for the copepods from the acidification period, for example, due to antagonistic pleiotropy (Lahti et al., 2009; Bono et al., 2017). This would lead to the selection of acid-sensitive phenotypes during pH recovery in the lakes. This pattern has been previously shown in populations of *L. minutus* from acidic lakes and ponds (Derry & Arnott, 2007; Negrín Dastis & Derry, 2016; Negrín Dastis et al., 2019). Surprisingly, we did not find indications of such a trade-off in individuals from the acidification period, which showed high fitness across neutral and acidic pH treatments. This suggests that loss of acid tolerance could have occurred via drift rather than a positive selection of

acid-sensitivity. However, it is also possible that this pattern could be due to unmeasured maternal effects that had positive effects on copepod fitness from the acidification period, possibly masking the expected trade-off. Although we did not quantify lipid content or fatty acid profiles, we observed that eggs and nauplii from the acidification time period contained more pronounced lipid droplets with red carotenoid pigments compared to the two other time periods, a trend that continued to adulthood. This coloration can be indicative of the accumulation of fatty acids from their algal diet (Grosbois & Rautio, 2018), which are known to affect egg production (Schneider et al., 2017) and survival (Grosbois et al., 2017). Changes in the species composition and biomass of phytoplankton were documented during the acidification and recovery period (Kwiatkowski & Roff, 1976; Vinebrooke et al., 2002; Findlay, 2003), and the important reduction in zooplankton diversity during the acidification (Sprules, 1975) could have led to reduced competition and facilitate the accumulation of fatty acids. Additionally, our results are based on a laboratory experiment in a simplified environment, whereas *L. minutus* fitness might have been affected by ecological interactions such as increased competition and predation during the progressive ecological and chemical recovery (Gray et al., 2012). Finally, fitness trade-offs from local adaptation are not always detected (Hereford, 2009). This might be the case in the results presented here, due to the complexity of the study system and its inherent stochasticity, which could have masked these effects.

Despite the apparent absence of a fitness trade-off for acid tolerance, we did find signs of an evolutionary reversal at the genomic level. Indeed, a large proportion of the Lumsden outliers were significant for both temporal comparisons (pre-acidification to acidification and acidification to recovery) and almost all of these outliers showed a reversal in the direction of allele frequency change (either positive or negative) during recovery. This result was confirmed at the whole genome level because the pre-acidification population sample was more closely related to the recovery sample than to the acidification sample (both for the covariance matrix of population allele frequencies and pairwise F_{ST}). Our results thus indicate that the reversal in acid tolerance at the phenotypic level is at least in part due to a reversal in adaptive allele frequency at the genomic level. A similar adaptive reversal at both the phenotypic and genomic levels has previously been shown in *Daphnia magna* in response to shifts in predation levels (Chaturvedi et al., 2021). However, a large proportion of outliers were also significant between the pre-acidification to

acidification but not in the acidification to recovery comparison, indicating that not all loci that were putatively selected during acidification also experienced selection during the recovery. These loci are thus candidates for directional selection from acidification and changes in allele frequency due to drift during the recovery. This explanation is supported by the large reduction in effective population size during acidification, which could have led to increased drift after evolutionary rescue. These results imply that the loss of acid tolerance during pH recovery in the lakes could therefore be due to a combination of directional selection (i.e., adaptive reversal) and drift.

In contrast to survival and fecundity, development time showed no sign of reverting to pre-acidification trait values or being responsive to pH. This could be because the developmental rate has responded to a selection pressure that we did not measure in the present study. This would mean that the acceleration of developmental rate during the acidification period and persistence during recovery does not result from adaptation to acidity but rather from a distinct selection pressure occurring on a similar timescale. A potential cause for the acceleration of development times is temperature increase linked to climate change, which has been documented in the region of Killarney (Meyer-Jacob et al., 2019). A previous study on marine copepods found that selection from increased temperature resulted in faster growth rates, whereas acidification slowed down development (Dam et al., 2021). Ecological monitoring has also revealed that a population of *Leptodiaptomus ashlandi* shifted from one to two generations per year concurrently with increasing temperatures, implying faster growth rates due to climate warming (Winder et al., 2009). Our results thus highlight that the reversal of life-history traits (fecundity, survival) is due in part to directional selection and drift, whereas development time may be responding to other sources of selection and therefore did not experience a reversal.

3.4.4. Parallelism of evolutionary rescue and future trends

George and Lumsden lakes are not at the same stage of ecological recovery, but we nonetheless found comparable phenotypic responses over time between the two populations, suggestive of parallelism in life-history trait shifts (Bolnick et al., 2018). We also found evidence for parallel responses at the genomic level (7% of the outliers), with comparable proportions of genetic parallelism (2-50%) as has been found in other study systems (Stuart et al., 2017; Bolnick et al., 2018; Morales et al., 2019; Garcia‐Elfring et al., 2021). Despite this, we also found differences

in the genomic responses between lakes (significant outliers during the transition from acidification to recovery time period in one lake but not the other), likely due to the difference in pH recovery trajectories and the extent of historical acidification (A. S. Dixit et al., 1992; Suenaga, 2018). Finally, our results indicate that both populations went through a similar pattern of evolutionary rescue followed by an adaptive reversal, implying that these processes may have been repeated in other *L. minutus* populations with similar ecological histories of lake acidification and pH recovery. Adaptive reversals were indeed documented in other populations of the region from historically acidified lakes (Derry & Arnott, 2007), implying that the evolutionary rescue followed by adaptive reversal could be repeatable and predictable in this study system. These processes might not be highly repeatable at the SNP level, but they could be at the gene or functional level due to redundancy (Bolnick et al., 2018; Barghi et al., 2020).

Even though *Leptodiaptomus minutus* populations were sufficiently resilient to undergo an evolutionary rescue and an adaptive reversal from historical acidification and lake pH recovery, this might not be repeatable under future threats. Our genomic results show that a set of alleles are potentially under selection from a distinct environmental pressure. These SNPs were not significant in the pre-acidification to acidification comparison but were significant in the acidification to recovery comparison, or significant for both time period comparisons but not showing a reversal in allele frequency trajectories. Indeed, acidified lakes in the Killarney, Ontario (Canada) region have not reverted to their original ecological state (Gray et al., 2012), and additional stressors relevant to copepod biology appeared during the same time period. A potential selection pressure that arose during the acidification period and continued during the pH recovery period is climate change(Meyer-Jacob et al., 2019), which is known to be a potent selection pressure in marine copepods (Dam et al., 2021; Griffiths et al., 2021; Brennan, DeMayo, et al., 2022; Brennan, deMayo, et al., 2022). However, the reduced effective population size found in the present study indicates that *L. minutus* populations from historically acidified lakes likely experienced significant genetic drift, potentially reducing their present and future adaptive potential (Blanquart et al., 2012). Decreases in *L. minutus* populations were recently reported across several ecosystems along with ongoing physicochemical and biotic changes (shifts in productivity, browning, predation, and competition levels; Barbiero et al., 2019; Leach et al., 2019; Ross & Arnott, 2022), highlighting

the need for an assessment of the adaptive potential to climate change and other stressors in ecologically significant zooplankton, such as freshwater calanoid copepods.

Author contributions: MS, AMD, and RDHB designed the study. MS and MSM collected the field samples and conducted the laboratory experiment. MS performed the DNA extractions, and the pool-seq processing pipeline, and ran the bioinformatic and statistical analyses with input from AMD and RDHB. The article was written by MS with input from AMD and RDHB. All authors contributed to the review and editing. AMD and RDHB funded the study.

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CHAPTER 3

CLIMATE ADAPTATION IN FRESHWATER COPEPODS AT LATITUDINAL AND MICROGEOGRAPHIC SPATIAL SCALES

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N.B. References cited in this chapter are presented at the end of the thesis.

ABSTRACT

The relative influences of evolutionary forces underpinning freshwater invertebrate population responses to climate at different spatial scales are largely unknown. Our study focused on a non-model freshwater calanoid copepod, *Leptodiaptomus minutus*, which has a broad northern distribution. We applied pooled whole-genome sequencing to detect signatures of selection to two climatic variables, summer temperature and colored dissolved organic matter (CDOM), and documented patterns of gene flow and genetic drift in *L. minutus* populations at two spatial scales: across a latitudinal gradient from Southern Québec to Greenland and a microgeographic climatic gradient at Cape Race, NL, Canada. Our results provided evidence of local adaptation to temperature and CDOM at both spatial scales, while the relative influence of genetic drift and gene flow differed between spatial scales. For the latitudinal gradient, gene flow was limited by distance, with strong genetic drift and reduced heterozygosity at the range edge in Greenland. We also detected strong genetic drift at the microgeographic scale, but without a loss in heterozygosity, possibly due to high gene flow. This study has implications for understanding how distinct patterns of gene flow and drift at latitudinal versus microgeographic spatial scales can potentially impact the evolutionary responses of freshwater invertebrates to climate change.

4.1. Introduction

Climate change is predicted to have strong physical, chemical, and ecological impacts on freshwater ecosystems (Adrian et al., 2009; Heino et al., 2009; Sharma et al., 2019). Documented environmental changes associated with climate change include altered temperature and stratification regimes (Kraemer et al., 2015), disruption of the seasonal timing of break-up and freeze-up of ice cover (Derksen et al., 2012), and higher precipitation and temperatures resulting in increased concentrations of colored dissolved organic matter (CDOM; "freshwater browning") in northern temperate lakes (Solomon et al., 2015; i.e., browning; Meyer-Jacob et al., 2019). In turn, freshwater browning induces changes in light and thermal environments, which can have cascading effects on food web dynamics (Lefébure et al., 2013; P. T. Kelly et al., 2016). Evolutionary responses of populations and species to climatic disturbances are anticipated to be mediated by demography, gene flow, adaptive potential, and the rate and magnitude of environmental change (Bay, Rose, Barrett, et al., 2017). To be able to predict the evolutionary responses and resilience of impacted populations to climate change, there is a need for studies that integrate the knowledge of these multiple processes (Urban et al., 2016b), particularly at different spatial scales.

Climate change is a broad-scale disturbance that impacts freshwater ecosystems at both regional (through broad-scale differences among regions across latitude and longitude; Kraemer et al., 2017) and local scales (through ecosystem-specific and region-specific effects; Lehnherr et al., 2018). However, microevolutionary responses may not be comparable between different spatial scales. The few studies that have investigated evolutionary patterns across these different spatial scales have found that signals of local adaptation to climate can indeed be divergent depending on the spatial scale considered (Rellstab et al., 2017; Gugger et al., 2021). At a large spatial scale (e.g., latitudinal), the magnitude of genetic drift can often be related to historical demographic events such as postglacial colonization (Thörn et al., 2021), whereas at a local spatial scale, it is expected that genetic drift will be primarily influenced by contemporary population size (Gompert et al., 2021). Gene flow can also be disrupted at a local scale due to habitat fragmentation, whereas at broader spatial scales these disturbances have been less impactful (Harrisson et al., 2012). Nevertheless, very few studies have explicitly compared the relative influence of microevolutionary processes in response to climate factors that vary across both latitudinal and

microgeographic spatial scales (Schmidt et al., 2021; but see Gamboa et al., 2022). Moreover, there is a paucity of research to understand the evolutionary responses of non-model freshwater organisms (Stoks et al., 2014), which will be critical for understanding their adaptive responses to climate change, especially considering the global freshwater biodiversity crisis (Tickner et al., 2020; Albert et al., 2021). Therefore, the objective of this study was to investigate how microevolutionary processes at different spatial scales might impact the responses of non-model freshwater calanoid copepods to climate gradients.

At a broad, latitudinal scale, demographic events such as range expansion can increase genetic drift and lead to reduced genetic diversity at the species range edge (de Kort et al., 2021; Thörn et al., 2021), for instance through allele surfing (Paulose & Hallatschek, 2020) and founder effects (Excoffier et al., 2009). In turn, limited genetic diversity at distribution margins can constrain adaptation to novel habitats (Takahashi et al., 2016; Sánchez-Castro et al., 2022). In the context of climate change, information about gene flow between populations of a species is key to assessing their ability to extend their distribution northward or to more suitable habitats (Matz et al., 2018; Razgour et al., 2019). Gene flow from populations at the core of the distribution can also affect the fitness of populations at the edges: positively by maintaining genetic diversity in small or inbred populations and as a source of pre-adapted alleles, or negatively through maladaptation and genetic swamping (Whiteley et al., 2015; D. A. Bell et al., 2019; Angert et al., 2020). While broad-scale latitude gradients can inform us about standing genetic variation and evolutionary responses to the combined influences of historical biogeography and climate, care must be taken when inferring adaptive responses of populations to future climate change (Damgaard, 2019; Aguirre-Liguori et al., 2021).

At smaller, microgeographic spatial scales, where populations share similar historical biogeography and evolutionary history, space-for-time substitution studies can provide important insight into fine-scale microevolutionary processes (Wogan & Wang, 2018). At local spatial scales within regions, gene flow can potentially have a strong influence on population genetic structure, but there is the potential for microgeographic adaptation along local-scale clines in the presence of mechanisms that reduce gene flow or increase the strength of selection (Richardson et al., 2014). Based on the migration-selection balance theory (Haldane, 1930; Wright, 1931; Bulmer, 1972; Lenormand, 2002), strong gene flow should reduce the possibility of microgeographic adaptation

through genetic swamping (Tigano & Friesen, 2016), whereas strong selection can sometimes overwhelm the effect of migration (Margres et al., 2019) and permit local adaptation. Selection against migrants that have low fitness in the new habitat can reinforce microgeographic adaptation by preventing migrants from contributing maladapted alleles to the gene pool (Richardson et al., 2014; Tigano & Friesen, 2016; Hämälä et al., 2018; Thompson et al., 2022). This can result in a pattern of isolation by environment (Nosil et al., 2008; Wang & Bradburd, 2014), where neutral diversity correlates with the degree of environmental difference between sites. Monopolization effects occur when early colonizers initially adapt to a new environment and gain a numerical and fitness advantage over later colonizers (de Meester et al., 2016), and this process can also promote fine-scale adaptation (Richardson et al., 2014). Demography should be considered in the context of microgeographic adaptation because smaller populations will lose genetic diversity (and adaptive alleles) from drift at higher rates and thus have a lower potential for adaptation (Blanquart et al., 2012). Recent studies have provided good examples of microgeographic adaptation in Amazonian trees (Brousseau et al., 2021), urban grasshoppers (Edelaar et al., 2019), and stickleback fish (Maciejewski et al., 2020), but studies evaluating the smallest spatial scales of adaptation and the processes mediating microgeographic adaptation are still lacking for many (non-model) species (Richardson et al., 2014). Space-for-time approaches combined with genomic tools are useful in this context, to help us improve our understanding of the mechanisms underlying climate adaptation in non-model organisms at different spatial scales (Micheletti & Narum, 2018; Kurland et al., 2019)

Copepods are increasingly used in studies of contemporary and rapid evolution (Bron et al., 2011; Madoui et al., 2017; Jørgensen et al., 2019a; D. Ben Stern & Lee, 2020; Brennan, DeMayo, et al., 2022). They are key organisms in freshwater and marine habitats because of their abundance and diversity (Bron et al., 2011; Pinel-Alloul et al., 2013). There is evidence for the rapid adaptation of marine/brackish copepods to ocean warming and acidification (Brennan, deMayo, et al., 2022), changes in salinity (D. B. Stern et al., 2022), and oil spill pollution (Lee et al., 2017). However, equivalent studies on freshwater copepods are lacking and there are still very few studies testing if they can genetically adapt to divergent climatic environments, (Hairston et al., 1990; Ellner et al., 1999). In addition, estimates of gene flow are generally lacking for freshwater copepods; it is often assumed that gene flow between fragmented lake/pond populations of passively dispersing

zooplankton is low, even at small scales (de Meester et al., 2002; but see Zeller et al., 2006; Ventura et al., 2014; Incagnone et al., 2015; Ortega‐Mayagoitia et al., 2022).

This study aimed to evaluate the influence of evolutionary mechanisms (genetic drift, gene flow) modulating adaptive responses to selection from climatic clines in a freshwater calanoid copepod at latitudinal and microgeographic spatial scales. To do this, we investigated patterns of genetic variation in copepod lake populations across a latitudinal gradient spanning southern Quebec (Canada) to Greenland (from 47°N to 61°N of latitude) and a microgeographic gradient in temperature and CDOM in a fragmented pondscape with steep spatial variation in environmental selection (Cape Race, NL Canada). Using pooled whole-genome sequencing (Schlötterer et al., 2014; Bourgeois & Warren, 2021), we investigated patterns of local adaptation with genome scans to detect SNPs associated with two climate variables (temperature and CDOM). We also documented patterns of gene flow within and between regions and evaluated the potential effect of genetic drift on genetic diversity.

We predicted that we would detect putative signals of local genetic adaptation with our genome scans among copepod populations both along the latitudinal gradient and along the microgeographic gradient due to differences in summer temperature (the average temperature range of the warmest quarter is between 8-16°C for the latitudinal gradient and 16-26°C summer pond temperature at Cape Race) and potentially due to among-habitat differences in CDOM (Tannic acid equivalent varies between 0.43-1.41 mg/L for the latitudinal gradient and 0.79-9.07 mg/L at Cape Race). As *L. minutus* probably underwent a range expansion at the end of the last glacial maximum (Stemberger, 1995), combined with potential dispersal limitation in this species and smaller population sizes, we also expected to find that populations at the range edge (Greenland) would be more affected by genetic drift and show lower genetic diversity than mainland populations (Pironon et al., 2017). Finally, from prior knowledge of freshwater zooplankton dispersal abilities (based on FST; de Meester et al., 2002; Zeller et al., 2006; Ventura et al., 2014; Ortega‐Mayagoitia et al., 2022), we hypothesized that gene flow should be higher within than between regions, with low migration rates overall, which would produce a pattern of isolation by distance at both the latitudinal and microgeographic scale. Our study documents how the interactions between genetic drift, gene flow, and climatic selection depend on a geographic scale,

from latitudinal to microgeographic-specific responses. Inference of micro-evolutionary responses may thus not be transferable from one spatial scale to another, and this has important implications for understanding and predicting adaptive responses to climate change.

4.2. Material and methods

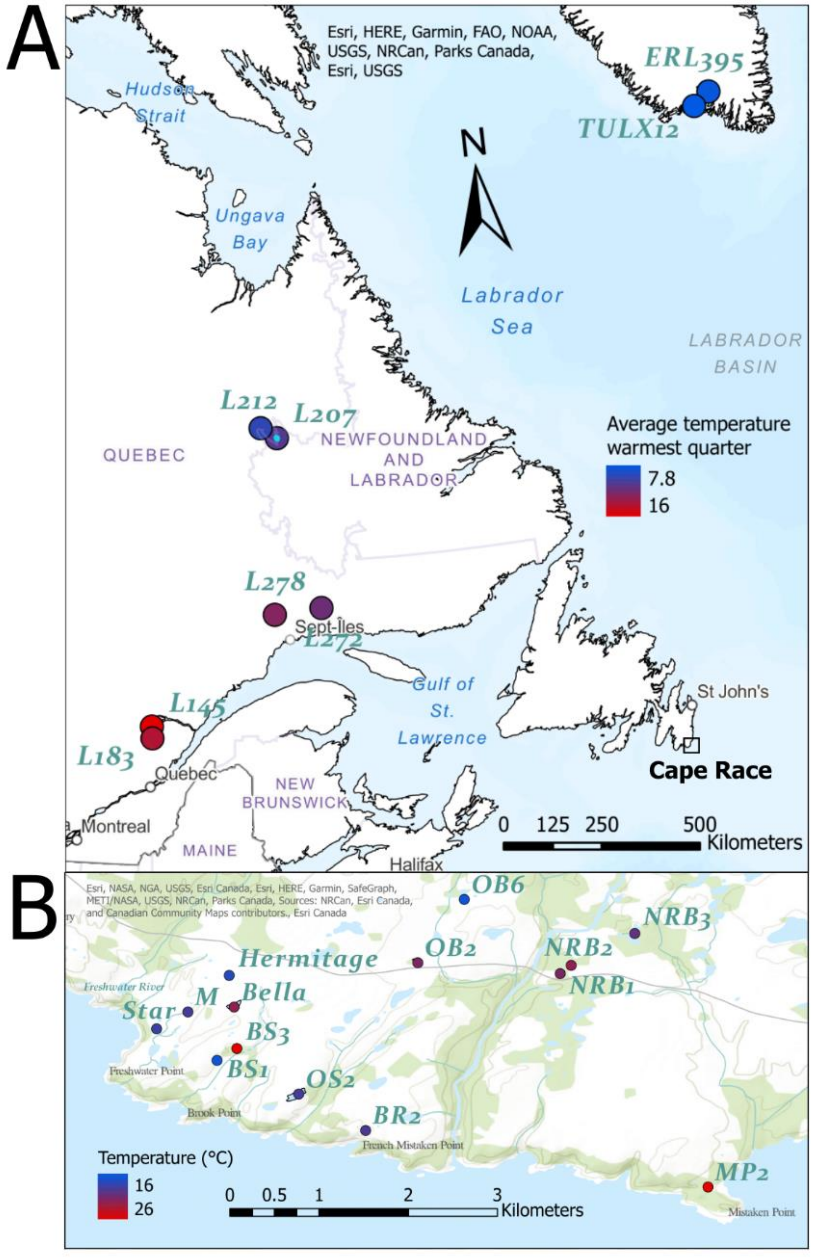
4.2.1. Study system

The freshwater calanoid copepod *Leptodiaptomus minutus* is broadly distributed across a latitudinal climatic gradient from Eastern North America (Stemberger, 1995; Pinel-Alloul et al., 2013) to Greenland (Samchyshyna et al., 2008) and Iceland (Antonsson & Antonsson, 1992). The species is often dominant in zooplankton communities in northeastern North America (Pinel-Alloul et al., 2013). For the latitudinal scale, our study included a total of eight copepod populations that spanned a latitudinal gradient (Fig. 4.A, Table 4.1) from Québec, Canada (latitude: 47.933, longitude: -71.203) to Greenland (latitude: 61.079, longitude: -45.640). Within Québec, samples of *Leptodiaptomus minutus* were collected and preserved at -80°C in ethanol from three regions in a paired design (N = 6, two lake populations per region) in the summer of 2013: 1) boreal shield lakes in the Côte-Nord, 2) boreal shield lakes near Chicoutimi and 3) taiga shield lakes close to the northern treeline near Schefferville. Environmental and biological data were collected for each of the six lakes (Martin et al., 2021; St-Gelais et al., 2021). Extending north of Canada, two *L. minutus* pond populations were collected in arctic steppe lakes of south-western Greenland in the summer of 2019 and preserved in ethanol at -20°C. To obtain a measure of colored dissolved organic matter, we converted absorbance values at 440 nm to absorption coefficients and then into a tannic acid equivalent (quantitative estimate of CDOM) using standard equations (Cuthbert & del Giorgio, 1992). We downloaded climate data for the latitudinal gradient (average annual temperature, the average temperature of the warmest and coldest quarters, total annual precipitation; Table C.2) from the worldclim database (Fick & Hijmans, 2017) at a resolution of 30 seconds (≈1 km^2) with the raster package in R v.4.0.4.

Table 4.1: Physico-chemical characteristics of latitudinal gradient populations. Reg.: region, SA: surface area, max. d.: maximum depth, Chla: chlorophyll A concentration, TAE: tannic acid equivalent. The climatic dataset was downloaded from the worldclim database at a resolution of 30 seconds (≈1 km^2). Tmean: Average temperature, WQTmean: Average temperature of the warmest quarter, CQTmean: Average temperature of the coldest quarter, Prec.mm: annual precipitations (mm).

Lake	Reg.	Latitude	Longitude	SA (m^2)	Max. D. (m)	Chla (µg/L)	pH	TAE (mg/L)	T mean	WQT mean	CQT mean	Prec. mm
ERL395	GL	61.079077	-45.639876	8,110	2	2.41	6.71	0.55	1.8	15.7	-13.8	1045
TULX12	GL	60.866271	-46.490463	7,321	2.75	1.34	7.96	0.49	0.2	13.9	-14.9	1261
L145	CH	48.20125	-71.23432	1,913,972	6.70	3.16	7.77	0.72	-1.8	11.6	-16.4	1014
L183	CH	47.93300	-71.20300	366,531	2.60	3.54	6.86	1.41	-1.6	12.4	-16.8	1099
L207	SC	54.86770	-66.67668	1,695,155	1.80	1.41	6.96	0.68	-4.7	10.7	-21.1	751
L212	SC	55.10626	-67.32345	309,854	2.60	0.41	7.71	0.43	-5.9	9.5	-22.4	792
L272	CN	50.91879	-65.21399	161,237	40.0	0.81	6.40	0.58	0.5	8.1	-6.4	815
L278	CN	50.79037	-66.91415	4,448,919	40.0	0.69	6.77	0.68	1.3	7.8	-4.6	863

Figure 4.1: Study sites along the climatic gradients. A: The study sites along the latitudinal gradient (N = 8) are located in four regions: Chicoutimi (L145, L183), Côte-Nord (L272, L278), Schefferville (L207, L212), Greenland (ERL395, TULX12). The location of the microgeographic gradient at Cape Race, NF, Canada is indicated by the square. The temperature corresponds to the average temperature of the warmest quarter. B: The study sites along the microgeographic gradient at Cape Race (N = 14). The temperature indicates the summer pond temperature.



At Cape Race, NL (Canada), *L. minutus* occurs in abundance in a complex of unconnected ponds embedded in a coastal barren landscape, where the distance between ponds is on average 2033m (SD 1133m). The distance connecting some pairs of ponds corresponds approximately to the smallest distance between populations for this species (minimum distance: 124 m). This landscape is environmentally heterogeneous for several climate-related variables that are important determinants of *L. minutus* population attributes (body size, fecundity, and acid tolerance: temperature, CDOM, and pH; Charette & Derry, 2016a). Cape Race thus provides a useful study system for examining the potential for and the processes modulating microgeographic adaptation. At Cape Race, *L. minutus* individuals were isolated from samples collected from 14 ponds in the summer of 2014 (Fig. 4.1B, Table 4.2). We preserved the copepod samples in 99% molecular-grade ethanol at -80C until genomic analyses were performed.

Table 4.2: Physico-chemical characteristics of the microgeographic gradient populations at Cape Race. SA: surface area, max. d.: maximum depth, Chla: chlorophyll A concentration, TAE: tannic acid equivalent, Temp: summer pond temperature.

Pond	Latitude	Longitude	SA (m^2)	Max. D. (cm)	Chla (µg/L)	pH	TAE (mg/L)	Temp. (°C)
BS1	46.641055	-53.2128564	454	41	1.70	5.54	6.12	16.4
Hermitage	46.649522	-53.211645	631	20	0.58	6.31	1.91	18.1
OS2	46.637701	-53.204711	9538	60	0.31	6.90	0.85	19.60
BR2	46.634070	-53.198041	447	30	0.61	4.33	2.94	19.9
Star	46.644200	-53.218880	101	20	1.23	6.19	4.53	19
M	46.645882	-53.215759	181	38	0.31	6.61	0.79	19.50
NRB3	46.653639	-53.171203	1185	70	1.38	4.40	2.85	20.70
NRB1	46.649701	-53.178641	2981	100	1.07	4.48	5.16	21.80
NRB2	46.650520	-53.177543	1577	50	1.07	4.41	3.15	22.70
OB2	46.650778	-53.192833	3238	15	0.77	6.90	1.10	21.80
Bella	46.646370	-53.211152	4738	40	0.79	7.30	1.25	22.70
BS3	46.642268	-53.210923	531	10	0.61	4.40	3.51	26.3
MP2	46.628551	-53.164188	374	30	3.38	4.35	9.07	26.30
OB6	46.657389	-53.188028	1281	28	0.38	5.10	0.93	16.60

4.2.2. Sequencing and SNP calling

We applied a pool-seq approach to the sequencing of copepod population samples (N = 8 pools for the latitudinal gradient and N = 14 pools for the Cape Race gradient). Each pooled population sample contained a total of 200 adults of *L. minutus* (except for the following ponds at Cape Race: BS1 = 122 and BS3 = 97 individuals). We identified the adult specimens of *L. minutus* under a high-resolution stereomicroscope (Olympus SZX2-ILLT, Tokyo, Japan) with the aid of a taxonomic key (Haney et al., 2013) and zooplankton community species lists provided from these samples by other studies (latitude gradient: St-Gelais et al., 2021; Cape Race: Charette & Derry, 2016). Copepods were subsequently isolated for DNA extraction, and pooled individuals were extracted for DNA using the MagAttract HMW kit (QIAGEN, Toronto, ON, Canada) following manufacturer steps. DNA was run for QC with the Agilent gDNA 165 kb kit on the Femto pulse system. Libraries were prepared for Illumina sequencing with the Illumina Truseq DNA PCR-free kit and QCed. Libraries were then sequenced on one lane of an Illumina NovaSeq6000 Sprime v1.5 at 150 bp pair-ended. Quality control, library preparation, and sequencing were conducted at the McGill Genome Center.

We used fastp to trim reads (Chen et al., 2018) with a minimum base quality of 20 in a sliding window moving from tail (3') to front and a minimum length of 50 bp. There is currently no reference genome for *L. minutus* or a closely related species, so we used a reference-free method for SNP discovery implemented in discosnp++ (Uricaru et al., 2015), which is based on a de Bruijn graph analysis of k-mers. This method yields SNP datasets comparable to traditional reference-based methods (Uricaru et al., 2015) and has previously been successfully applied to a copepod genomic dataset (Arif et al., 2019). We used discosnp++ on the combined dataset from the two gradients (N = 22 pools) and ran the analysis with the branching strategy -b 1 (which gives a good compromise between precision and recall; Uricaru et al., 2015), and a minimum global coverage of five per pool. We then converted the vcf file output by discosnp++ into input files for downstream analyses with the poolfstat package in R (Hivert et al., 2018), with a haploid pool size of 400 for all populations except for BS1 and BS3 (244 and 194 respectively), minimum coverage of five per pool, maximum coverage of 400, a minimum read count per base of two, a minimal minor allele frequency of 0.0052 (to remove singletons) and removing indels. This yielded a dataset of 6,056,212 biallelic SNPs after filtering.

4.2.3. Detecting signals of selection

To assess the presence of putative loci under selection across the climate gradients, we used the hierarchical Bayesian models implemented in Baypass (Gautier, 2015), with the core model as an outlier analysis and the auxiliary (aux) model as an environmental association analysis. As *L. minutus* possibly underwent a range expansion at the end of the last glacial maximum (Stemberger, 1995), it was important to account for demographic processes when searching for putative loci under selection (Hoban et al., 2016). Baypass is advantageous as it estimates the scaled covariance matrix Ω of population allele frequencies in the core model, which summarizes population history and is then explicitly accounted for during outlier detection. We analyzed the datasets from the latitudinal and Cape Race gradients separately as the environmental parameters collected were different for each dataset (climate data: worldclim versus pond summer temperature measurements *in situ*). This scheme was also used to identify putative SNPs under selection in common between the two gradients as evidence of independent parallel adaptation (Bolnick et al., 2018), and that would correspond to strong candidates for climatic adaptation.

We first divided the full poolfstat dataset into seven pseudo-independent input datasets each containing <1 million SNPs to avoid computing limitations in Baypass, using the "thinning" subsampling method with a sub-sample size of 865,173 SNPs, which were then split between latitudinal and Cape Race samples. We used the core model to estimate the statistic XtX and the associated p-value under a χ^2 distribution with 8 or 14 degrees of freedom corresponding to the number of populations (bilateral test; Gautier, 2015). The XtX statistic uses the variance in allele frequencies across populations to identify outliers (i.e., overly differentiated SNPs; Günther & Coop, 2013). The shape of the histogram p-values derived from the XtX statistics showed that they were not well-behaved in the Cape Race dataset (too liberal, Fig. C.1; François et al., 2016). Consequently, a threshold value for XtX was computed to distinguish between selection and neutrality (i.e., overly differentiated SNPs) and detect outlier SNPs. First, we simulated a Pseudo-Observed Dataset (POD) of 865,174 SNPs by randomly sampling vectors of read count data with the simulate.baypass() function as detailed in the manual, using the first of the seven sub-datasets with its associated a_π, b_π parameters, and Ω. This POD was analyzed with the core model with Baypass and we then calculated the XtX threshold by using the 99% quantile of XtX distribution.

We used this threshold of XtX > 21.7 to define SNPs as outliers for Cape Race, and a p-value threshold of $p < 0.001$ for the latitudinal dataset.

The aux model was then used to detect SNPs significantly associated with the climatic variables. We selected two covariables of interest for each gradient (latitudinal populations: temperature of the warmest quarter and tannic acid equivalent TAE; Cape Race populations: summer pond temperature and TAE) after checking the correlation between our available environmental variables, using the function pairs.panel() in the package psych in R (Fig. C.2, C.3). The Pearson's correlation coefficients r between the selected covariables were < 0.6, lower than the recommended threshold for the regression method ($|r| < 0.7$, Fig. C.2). The aux model allows us to obtain the coefficient β_{ik} of the regression between the SNP allele frequencies and the covariable. This coefficient is associated with a Bayesian auxiliary variable δ_{ik} from which a Bayes factor BF_{mc} is derived. The covariables were standardized to $\hat{\mu} = 0$ and $\widehat{\alpha^2} = 1$ as recommended in the Baypass manual. We compared visually the seven Ω matrices from each dataset to ensure that the results were similar between sub-datasets, then we combined the statistics estimated for each SNP. We considered SNPs significantly associated with the climatic covariable when their $BF_{mc} > 20$ dB (Jeffrey's rule for "decisive evidence"; Gautier, 2015). Both the core and auxiliary models were used with default parameters.

4.2.4. Population structure, demography, and genetic diversity

We assessed an aspect population structure with the covariance matrix Ω of population allele frequencies obtained from the core model of Baypass. We also calculated the genome-wide pairwise F_{ST} matrix and observed population heterozygosity with the poolfstat package, with the same options described for the filtering. We used the pairwise F_{ST} matrix to evaluate population structure as well as the possibility of isolation by distance and by the environment for the two gradients. This was based on the relationship between the genetic distance (FST/(1- FST); Rousset, 1997) and the log of the geographical distance (2D distribution of populations) with a Mantel test (9999 permutations) implemented in the vegan package in R v4.2.1. For the isolation by environment, we first calculated the environmental distance between pairs of populations as the squared Mahalanobis distance from the temperature and TAE data with the package ecodist in R. We checked the correlation between environmental distance and geographic distance to avoid

confounding effects (non-significant: $r^2 = 0.10$, p-value = 0.09). Then, we tested for a relationship between environmental distance and genetic distance as $F_{ST}/(1-F_{ST})$ with a Mantel test (9999 permutations).

We also used the admixture graph analysis Treemix to explore population history (including migration or admixture events) and the effect of genetic drift (Pickrell & Pritchard, 2012). Note that this analysis does not allow to differentiate between historical and contemporary events(Gompert et al., 2014). We selected the population L145 for the latitudinal gradient and NRB1 for Cape Race as roots, based on the results of the Ω matrices inferred with Baypass. We increased the number of edges -m (migration/admixture events) between zero and 8-14 (number of populations) and used blocks of 1000 SNPs (-k option) to account for linkage disequilibrium. To estimate the number of migration edges, we selected a given number of edges when the model reached the threshold of 99.8% of the variance in population relatedness explained (Pickrell & Pritchard, 2012). This threshold was reached for m = 1 migration edge for the latitudinal dataset and m = 8 edges for the microgeographic dataset. We confirmed the robustness of the tree topologies with 100 bootstrap replicates using the R package BITE (Milanesi et al., 2017). The length of the branches in the admixture tree is expressed in drift units ($drift\ parameter = \frac{t}{2Ne}$, with t the number of generations after the populations diverged and N_e the effective population size), with the drift parameter corresponding to the diffusion approximation of a Wright-Fisher model of genetic drift and being closely related to F_{ST} (Pickrell & Pritchard, 2012). For the genetic diversity, we assessed the relationship between heterozygosity and latitude/longitude for the latitudinal dataset with a Spearman rank correlation test with the function cor.test() in R.

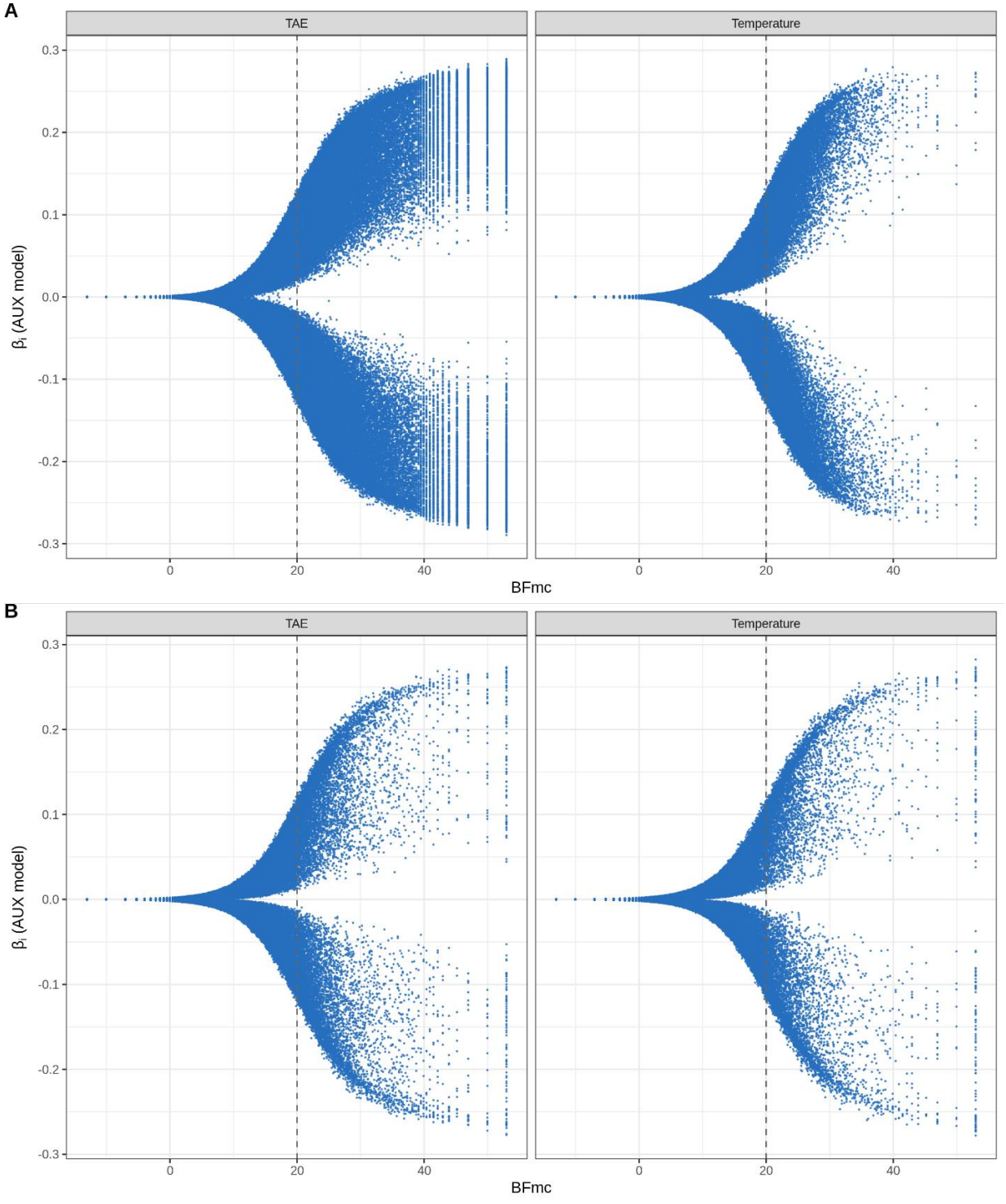
4.3. Results

4.3.1. Detecting signals of selection: Putative signals of selection along the climate clines.

We found putative signals of selection with the Baypass core model (outlier analysis) in both gradient datasets by identifying 53,753 outliers for the latitudinal dataset ($p < 0.001$, 0.8% of the dataset, Fig. C.4) and 665,762 outlier SNPs for the Cape Race dataset (XtX > 21.7, 10.2% of the total SNPs; Fig. C.5), with 10,594 outliers in common between the datasets (0.2% of the total dataset). Outliers with high XtX values, (Fig. C.4, C.5) are indicative of positive selection while

low XtX values suggest balancing selection (Gautier, 2015). Using the auxiliary model, we also found a putative pattern of local adaptation to climate along the latitudinal and Cape Race gradients through significant associations between SNP allele frequencies and the selected climatic variables (temperature of warmest quarter, summer pond temperature, and tannic acid equivalent; Fig. 4.2). For the latitudinal gradient, we found 151,583 SNPs that were significantly associated (BFmc > 20) with one or more covariables (≈ 2.5% of the dataset), with 43,611 SNPs uniquely associated with the temperature covariable and 104,707 SNPs uniquely associated with the TAE gradient (Fig. C.6). The Cape Race dataset yielded 24,089 SNPs significantly associated with the covariables (0.4% of the total SNPs), with 9,439 SNP uniquely associated with summer temperature and 13,848 SNPs uniquely associated with TAE. Fewer outlier SNPs were shared between datasets (N = 62 outlier SNPs for the temperature of the warmest quarter/summer pond temperature and N = 276 for TAE concentrations, Fig. C.6). We found both negative and positive associations between the allele frequencies of the outlier SNPs and the climate variables, with some SNPs displaying strong associations (correlation coefficient $|\beta_i| > 0.2$: 10,157 SNPs for the latitudinal dataset and 1077 SNPs for Cape Race; Fig. 4.2). Finally, we found 6,162 outlier SNPs in common between the core and auxiliary models for the latitudinal dataset and 9,366 for Cape Race.

Figure 4.2: Putative SNPs for climate adaptation. Regression coefficients βi between SNPs allele frequencies and each covariable as a function of the Bayes-Factor (BF_{MC} in dB) from the Baypass Aux model. SNPs significantly associated have $BF_{MC} > 20$ (dashed vertical line). A: Results for the latitudinal gradient populations (covariables: Tannic acid equivalent and temperature of the warmest quarter). B: Results for the Cape Race populations along the microgeographic gradient (covariables: TAE and summer temperature).



4.3.2. Population structure: differences between the latitudinal and microgeographic scale

For the latitudinal gradient, populations clustered by region (Fig. 4.3A), with the Greenland populations being the most distantly related group. Within Québec, the Chicoutimi populations appeared as the basal group, while the Cote-Nord and Schefferville populations are more closely related. The results were strongly concordant between the covariance matrix of population allele frequencies Ω and the admixture graph analysis, using L145 as a root (Fig. 4.3A and 4.5A), both in terms of the clusters defined and the position of the populations within the trees. For these populations, we identified one migration or admixture event from the Chicoutimi population L183 toward the ancestor population of the Greenland populations, accounting for a high fraction of the ancestry (w = 19%, Fig. 5A). Cape Race populations grouped into three clusters (Fig. 4.3B). The Cape Race clusters did not appear to be related to environmental characteristics or spatial location (see results for IBD and IBE below), with one cluster including Star and MP2 and another cluster with OB6 and BR2, which are populations separated by a comparatively large distance (4,528 and 3,691 m respectively). The results were relatively similar between the Ω matrix and the admixture graph with NRB1 as root (Fig. 4.3B and 4.5B), although some populations did not have the same position within the two trees. We identified eight migration or admixture events with the two most important ones from MP2 toward BR2 and from OB2 toward the ancestral population of Bella, Star, and M, explaining a large proportion of the ancestry (w = 40% and 52% respectively). The bootstrap replicates supported some of the population splits well (e.g., BR2 and NRB3), while others were less well supported (e.g., OB2 and OS2, Fig. 4.5B).

We found a pattern of isolation by distance for the latitudinal populations (Fig. 4.4A), with a significant positive correlation between the linearized genetic distance $F_{ST}/(1-F_{ST})$ and the log of the geographic distance (Mantel test, 9999 permutations, $p = 0.001$, $r^2 = 0.41$). We also tested for isolation by environment in the latitudinal populations but found no significant pattern (Mantel test, 9999 permutations, $p = 0.18$, $r^2 = 0.09$, Fig. 4.4C). For the microgeographic scale at Cape Race, we similarly found a significant pattern of isolation by distance (Fig. 4.4B, Mantel test, 9999 permutations, $p = 0.02$). However, this correlation accounted for a relatively small proportion of the variance explained ($r^2 = 0.08$), and it was driven by the population pair NRB1 and NRB2, which are very close genetically (pairwise $F_{ST} = 0.02$) and spatially (124 m). The significance of the IBD

relationship was not maintained when this pair was removed ($p = 0.139$, $r^2 = 0.03$). There was no significant pattern of isolation by environment in the Cape Race populations (Mantel test, 9999 permutations, $p = 0.10$, $r^2 = 0.07$, Fig. 4.4D). Finally, we detected a wide range of genetic differentiation between pairwise comparisons across the latitudinal gradient (Fig. C.6), with the lowest pairwise F_{ST} between the pair of Schefferville populations ($F_{ST} = 0.06$) and the highest between the Greenland population TULX12 and the Chicoutimi population L145 ($F_{ST} = 0.20$). Notably, pairwise F_{ST} was relatively low between some populations in different regions separated by large distances, for instance between L207 (Schefferville) and L278 (Cote-Nord; $F_{ST} = 0.07$) which are separated by ≈ 450 km. For Cape Race, genetic differentiation was low to moderate (Fig. C.7), with the lowest pairwise F_{ST} between the closest pair of populations NRB1 and NRB2 ($F_{ST} = 0.02$, pairwise distance = 124 m), and the highest F_{ST} between Hermitage Pond and most of the other pond populations ($F_{ST} = 0.16$ on average).

4.3.3. Demography and genetic diversity: Strong effects of genetic drift and reduced genetic diversity at the range margins.

Based on the results of the admixture tree analysis, Québec populations appeared less affected by genetic drift than in Greenland as they showed short branches and nodes (except for L183, Fig. 4.5A). The length of the branches is expressed in drift units ($drift\ parameter = \frac{t}{2Ne}$, see methods above) and is proportional to the amount of genetic drift occurring in each population (Pickrell & Pritchard, 2012). For Cape Race, the relatively long length of the branches and nodes indicated that most of the copepod populations in this landscape were strongly affected by genetic drift (Fig. 4.5B). The drift observed for Cape Race populations was comparable to the populations from Greenland. We also found that genetic heterozygosity was lower at the range margin for the latitudinal gradient in Greenland populations. We detected a moderate to strong negative correlation between observed heterozygosity and latitude/longitude (rho = -0.40 and -0.52 respectively), but this relationship was not significant ($p = 0.197$ and $p = 0.327$ respectively; Fig. 4.5C and 4.5D). On the contrary, Cape Race populations showed higher observed heterozygosity than Québec continental populations on average, with a few exceptions (mean 0.17 SD 0.01 and mean 0.16 SD 0.01 respectively; Fig. 4.5C and 4.5D).

Figure 4.3: Population genetic structure along the two climatic gradients. Heatmaps of the scaled covariance matrix Ω (with ρij the correlation coefficient between pairs of populations) with hierarchical clustering tree (using the average agglomeration method), obtained from the core model of Baypass. A: Results for the latitudinal gradient, B: Results for the microgeographic gradient at Cape Race

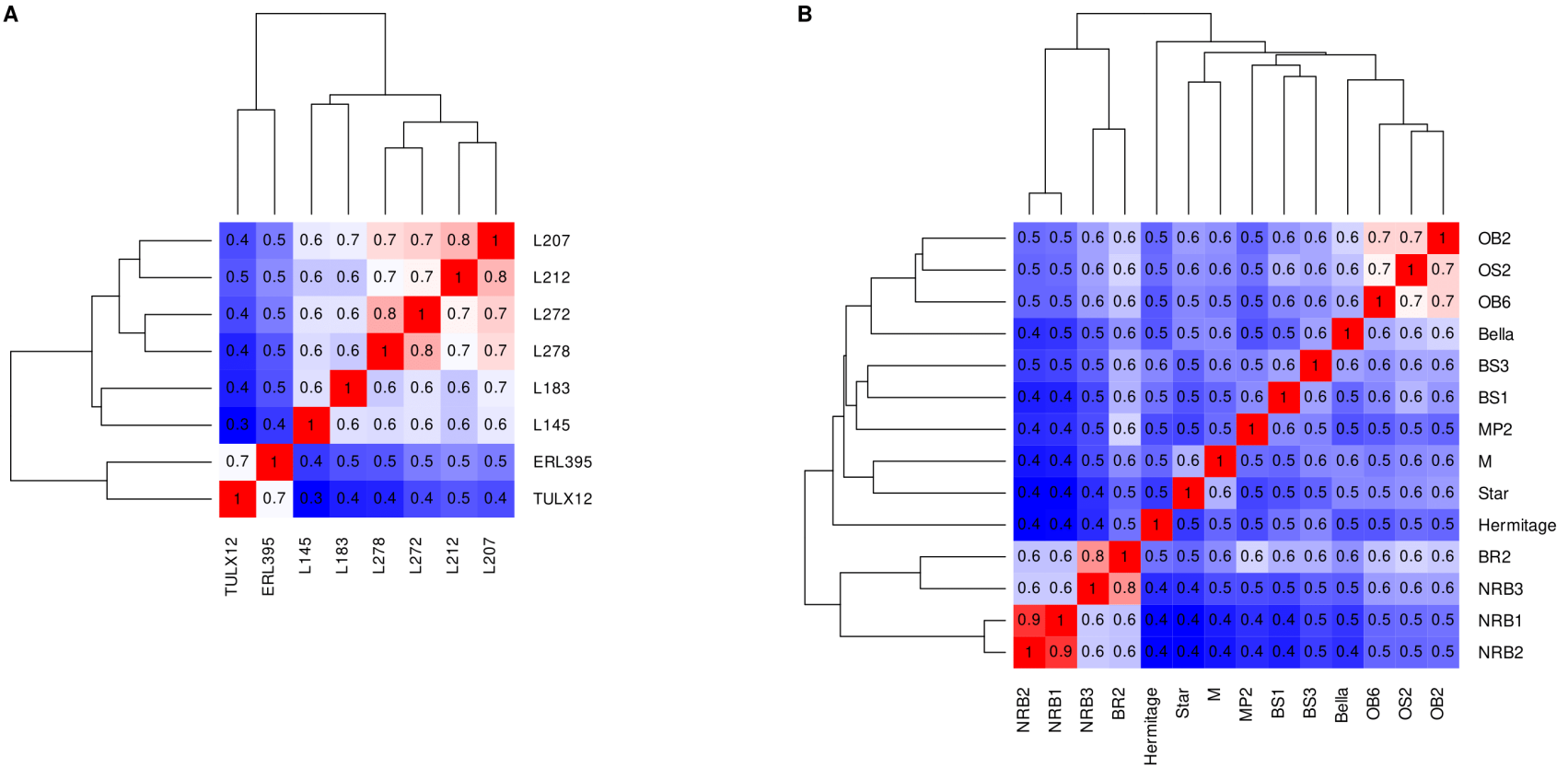


Figure 4.4: Tests of isolation by distance (IBD) and isolation by environment (IBE). IBD: correlation between the log of the geographical distance (in m, 2D distribution of populations) and the linearized genetic distance FST/(1-FST) between pairs of populations. IBE: correlation between the environmental distance (squared Mahalanobis distance) and the linearized genetic distance FST/(1-FST) between pairs of populations. A: Significant IBD detected for the latitudinal gradient (Mantel test: p = 0.001, r2 = 0.41), B: Significant IBD for the microgeographic gradient at Cape Race (Mantel test: p = 0.02, r2 = 0.08). Note that the relationship is not significant after removing the population's pair NRB1-NRB2 (red, see results). C: Non-significant IBE for the latitudinal gradient (Mantel test: p = 0.18, r2 = 0.09). D: Non-significant IBE for the microgeographic gradient at Cape Race (Mantel test: p = 0.10, r2 = 0.07).

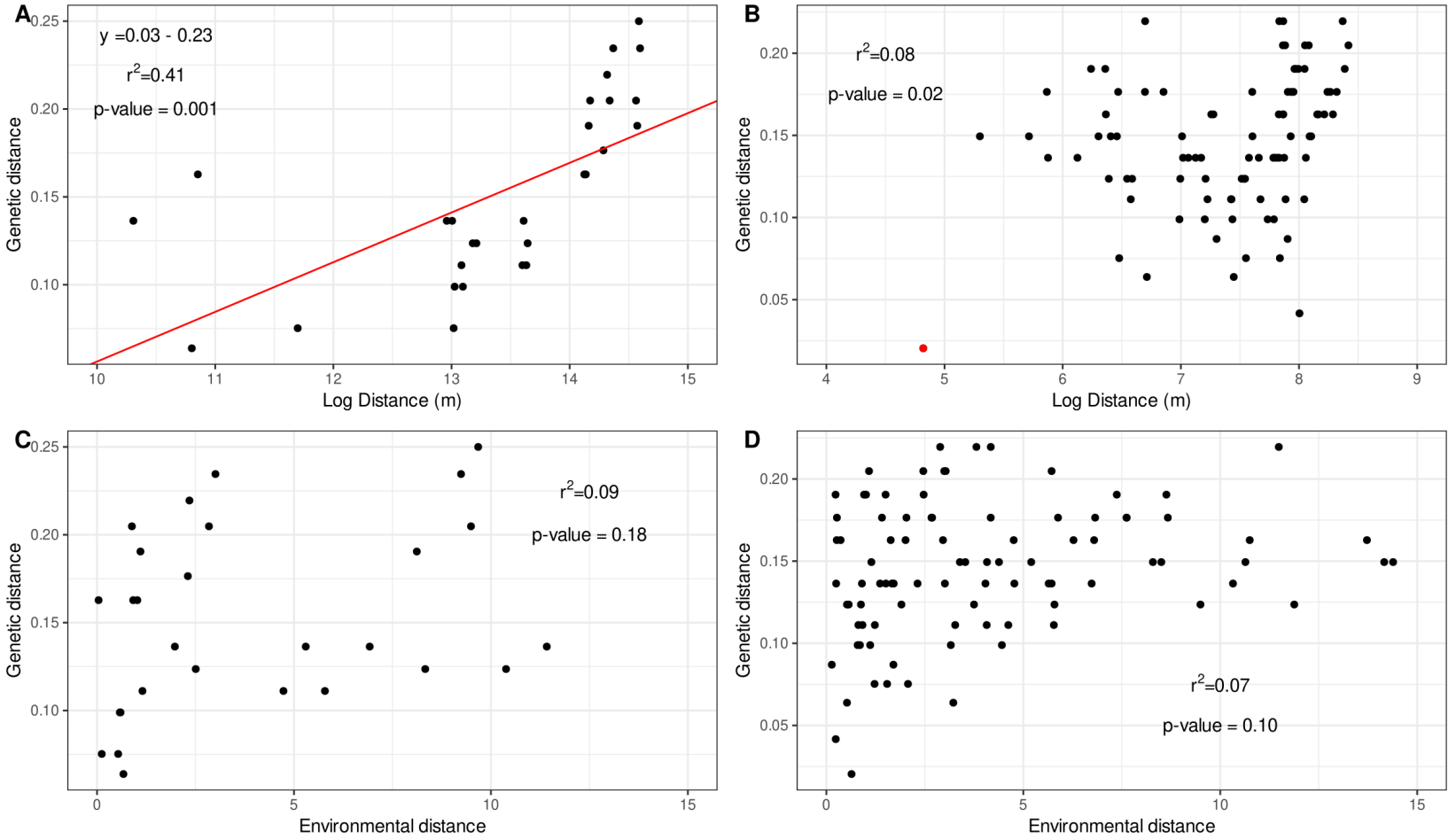
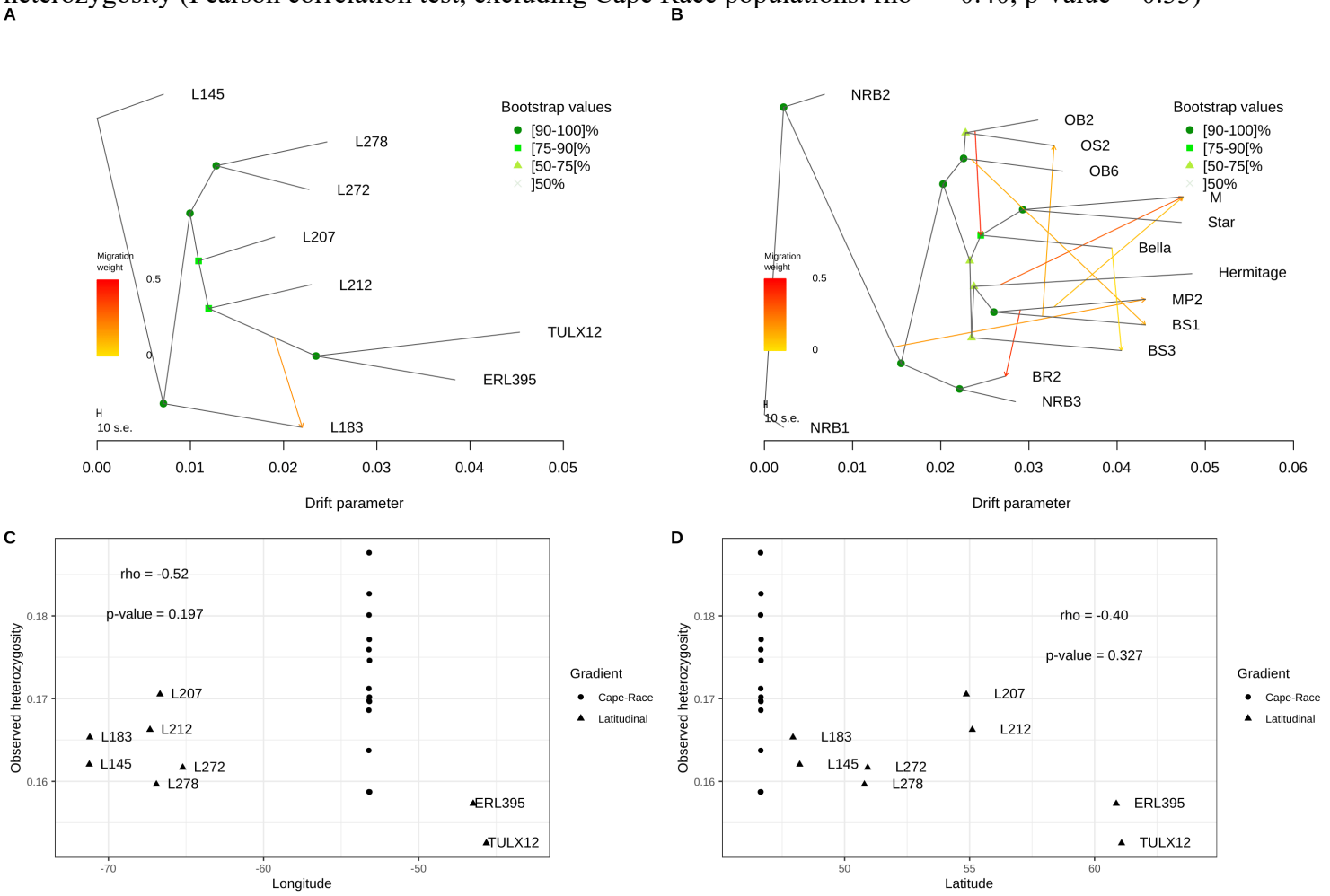


Figure 4.5: Genetic drift and heterozygosity along the climatic gradients. Top: Admixture graphs output by Treemix. Confidence in the tree topology is shown as % of bootstrap replicates out of 100 bootstraps (dots on the nodes). Note that the lack of dots on some nodes indicates that Treemix modified the structure of the consensus tree. A: Tree of the latitudinal gradient populations using L145 as the root, and one admixture/gene flow event, B: Tree of the microgeographic gradient populations at Cape Race with NRB1 as the root, and eight admixture/gene flow event, C: Negative relationship between longitude and observed heterozygosity (Pearson correlation test, excluding Cape Race populations shown as dots: rho = - 0.52, p-value = 0.20), D: Negative relationship between longitude and observed heterozygosity (Pearson correlation test, excluding Cape Race populations: rho = - 0.40, p-value = 0.33)



4.4. Discussion

We investigated patterns of population genetic structure, genetic diversity, and putative signals of selection from two climatic variables in the northern calanoid copepod *Leptodiaptomus minutus* at broad, latitudinal, and microgeographic spatial scales (latitudinal from southern Quebec to Greenland and microgeographic at Cape Race, NL, Canada). While the selection was important for potential adaptive genetic divergence among copepod populations at both spatial scales, the relative influence of genetic drift and gene flow differed between spatial scales. Our genome scans enabled inference of potential local adaptation of *L. minutus* to differences in summer temperature and CDOM at both latitudinal and microgeographic scales (i.e., microgeographic adaptation). At the latitudinal scale, populations were structured by regions, although we did find evidence for some long-distance gene flow (> 400 km). Notably, populations at the range edge (Greenland) were more affected by genetic drift and showed reduced heterozygosity in comparison to populations at the center of the species distribution range in Québec. In contrast, at the microgeographic scale, copepod populations were neither structured by distance nor by environment, suggesting strong gene flow throughout the landscape (a few hundred meters to a few kilometers). Our results suggest that Cape Race populations have been strongly affected by genetic drift. However, the strong gene flow detected between closely located populations at Cape Race could potentially reduce the negative impact of genetic drift on (adaptive) genetic diversity. This is because genetic drift reduces local adaptation due to the loss of adaptive alleles (Blanquart et al., 2012), whereas gene flow can provide an input of (adaptive) genetic diversity from nearby populations (Gompert et al., 2021).

We contribute a rare study that compares influences of microevolutionary processes along climatic gradients at different spatial landscape scales in non-model freshwater calanoid copepods. Freshwater organisms are especially vulnerable to the direct and indirect effects of climate change (Meyer-Jacob et al., 2019; Jaureguiberry et al., 2022). Our findings suggest that climate adaptation in freshwater copepods is possible, but that the relative importance of genetic drift and gene flow can diverge at microgeographic and latitudinal scales, implying that inferences about these processes are not transferable between different scales. Knowledge of the processes that underlie adaptive evolutionary responses in natural populations at different spatial scales is key to understanding biological responses to climate change and being able to predict population resilience (Aguirre-Liguori et al., 2021).

4.4.1. Detecting signals of selection: Potential adaptation to climatic variables at latitudinal and microgeographic scales

The results of the environmental association analyses suggested a pattern of local adaptation of *L. minutus* copepods to spatial variation in climate (temperature and CDOM) at both the latitudinal and microgeographic scales. The detection of SNPs that were strongly associated with the temperature and TAE gradients constitute important candidate loci for climatic adaptation, and these should be examined more closely in future studies. In addition, the significant outliers that were detected with the core model but were not associated with our selected environmental covariables could correspond to candidates for local adaptation to other (a)biotic variables that we did not consider in this study (Gautier, 2015). For instance, evidence for adaptive responses in *L. minutus* to acidic pH at Cape-Race is supported by laboratory and field reciprocal transplant experiments between neutral and acidic ponds (Negrín Dastis & Derry, 2016; Negrín Dastis et al., 2019), as well as resurrection experiments and genetic analyses from historically acidified boreal shield Canadian lakes (Derry et al., 2009, 2010). Notably, we only found a few outliers SNPs in common between the latitudinal and Cape Race gradients, which has also been the case for other studies investigating climate adaptation at different spatial scales (Rellstab et al., 2017; Gugger et al., 2021). These few shared outlier SNPs could represent limited parallelism (Bolnick et al., 2018), combined with possible allele loss from drift associated with the colonization history of Cape Race following the last glacial maximum, as well as the effects of island biogeography. Indeed, previous studies have reported reduced genetic diversity in Newfoundland populations due to either founder effects or drift with isolation between post-glacial relic populations (D. T. Stewart & Baker, 1992; Paetkau & Strobeck, 1996). Even though significant SNP association with climatic variables does not mean that these SNPs are causative variants underlying adaptation (Barrett & Hoekstra, 2011; Gautier, 2015), this is a useful first step toward characterizing the genetic basis of climate adaptation in *Leptodiaptomus minutus*.

Prior research has suggested that for organisms that response to seasonal cues, such as copepods in north-temperate regions, selection by climate change should be stronger on the timing of phenological events than on thermal tolerance (Bradshaw & Holzapfel (2008). However, it is unclear how spatial scale might influence this relationship. Given the different spatial scales between the latitudinal and microgeographic gradients in our study, climatic adaptations in *L.*

minutus could be related to different biological processes. At the microgeographic scale, where the length of the winter and ice-free seasons should be relatively similar among ponds, thermal tolerance could play a more important role (Geerts et al., 2015; Griffiths et al., 2021). Climate warming has also been shown to induce positive changes in multiple life-history traits in an experimental evolution study on the marine copepod *Acartia tonsa* (Dam et al., 2021). Warming was found to be a strong selection pressure in this experiment, and subsequent sequencing revealed SNPs enriched in functions associated with development (Brennan, deMayo, et al., 2022). In contrast, at the latitudinal scale, where habitats are divergent in their seasonality (e.g., onset and termination of ice cover), climate adaptation could be more related to the onset of diapausing eggs production and voltinism (A. M. Waldvogel et al., 2018; Häfker et al., 2018; Roncalli et al., 2019, 2021). Indeed, *L. minutus* populations are divergent in the number of generations per year in the south (Quebec: two generations; Boers & Carter, 1978) and the north (Iceland: one generation; Antonsson & Antonsson, 1992) of the species distribution. In the absence of a reference genome for *L. minutus* (or a close relative), we lack functional information about the candidate loci identified here that could be used to test these hypotheses. This is an important area for future work, as it will also permit tests of less understood mechanisms of climate adaptation.

Little is presently known about potential zooplankton adaptive responses to freshwater habitat browning associated with climate change (Stoks et al., 2014). Browning of freshwater habitats through increased concentrations of CDOM has the potential to impose selection on zooplankton through altered light and thermal environments (Solomon et al., 2015), and ensuing changes in predator-prey interactions (Estlander et al., 2010). A handful of studies have addressed the effects of browning on zooplankton interspecific (Robidoux et al., 2015) and intraspecific traits (Robidoux et al., 2015; Charette & Derry, 2016; Minguez et al., 2020). A possible adaptation could be based on the relationship between CDOM and exposure to UV radiation, as zooplankton display a large array of behavioral and physiological protective strategies from the latter (Rautio & Tartarotti, 2010). We argue that there is a need for -omics and integrative studies to examine the adaptive response of zooplankton to projected changes in CDOM and their interaction with temperature shifts (Lefébure et al., 2013). Despite the present study being an exploration of putative adaptation at the genomic level and not explanatory, the various phenotypic and physiological functions that could be the target of climatic selection in *Leptodiaptomus minutus* and that have been documented

in other copepod species warrants further investigation. To improve our functional understanding of the mechanisms that could contribute to the success or failure of climate adaptation, future studies could involve developing an annotated reference genome (e.g., D. B. Stern et al., 2022) for *L. minutus*, transcriptomics (e.g., Brennan, DeMayo, et al., 2022) and transplant experiments (e.g., Lee et al., 2017).

4.4.2. Population genetic structure and patterns of gene flow within/between regions

In terms of range expansion, our genetic connectivity results indicate the possibility of habitat tracking by *L. minutus* at the microgeographic scale through short-distance gene flow and northward range expansion at the latitudinal scale through long-distance gene flow, although its range limit is already located at high latitudes (e.g., 74°N in East Greenland, Samchyshyna et al., 2008) and further movement northward could be limited by oceanic barriers. However, no range shift has been documented in this species yet.

Both Greenland and Cape Race copepod populations were characterized by a strong influence of genetic drift compared to the continental populations in Quebec. Yet, we also found that observed heterozygosity in copepod populations at Cape Race was surprisingly high, often higher than in mainland populations. This suggests that different processes could explain the observed patterns of genetic drift in Greenland versus Cape Race populations. In Greenland, populations occupy deeper ponds of > 16,000 m^3 (compared with < 6000 m^3 in Cape Race ponds) and should be able to support relatively large populations. Consequently, patterns of genetic drift in Greenland could be due to a legacy of the demographic history of range expansion and colonization (Thörn et al., 2021) rather than small population size. In contrast, at Cape Race, the pattern of genetic drift might instead be explained by small population sizes in small ponds, and the relatively high heterozygosity could result from gene flow from nearby ponds (Gompert et al., 2021).

4.4.3. Strong effects of genetic drift and reduced genetic diversity at the range margin

Both Greenland and Cape Race copepod populations were characterized by a strong influence of genetic drift compared to the continental populations in Quebec. In both Greenland and Cape Race, island biogeography could play an important role in the pattern of genetic diversity of copepod populations as both habitats are isolated from continental populations. Also, both Greenland and

Cape Race could be considered as range limit populations, the former north-eastern biogeographical limit to Europe and the latter a physical eastern range limit on the Atlantic Ocean. However, we found that observed heterozygosity in copepod populations at Cape Race was surprisingly high, often higher than in mainland populations. This suggests that different processes could explain the observed patterns of genetic drift in Greenland versus Cape Race populations. In Greenland, populations occupy deeper ponds of > 16,000 m3 (compared with < 6000 m3 in Cape Race ponds) and should be able to support relatively large populations. Consequently, patterns of genetic drift in Greenland could be due to a legacy of the demographic history of range expansion and colonization (Thörn et al., 2021) rather than small population size. In contrast, at Cape Race, the pattern of genetic drift might instead be explained by small population sizes in small ponds, and the relatively high heterozygosity could result from gene flow from nearby ponds. Indeed, even low levels of gene flow can maintain high levels of genetic diversity in populations strongly affected by drift (Gompert et al., 2021).

4.4.4. Diverging effects of gene flow and drift on climatic adaptation at microgeographic and latitudinal scale

At the latitudinal spatial scale, our finding of reduced gene flow between continental and Greenland populations suggests that range margin populations are not at risk of migration load (i.e., the input of migrant alleles that have lower fitness than local alleles; Bolnick & Nosil, 2007; Angert et al., 2020). However, the surprisingly strong connectivity among some continental regions implies that there is a potential for southern warm-adapted genotypes to migrate northward (Matz et al., 2018; Razgour et al., 2019). The absence of isolation by environment in latitudinal populations, despite the signals of climate adaptation, suggests that other mechanisms could have a stronger effect on population structure, e.g., demographic history along the gradient. At Cape Race, our findings suggest that gene flow was probably not restricted in this landscape, and these results, combined with our genome scan, support a pattern of local adaptation at a very fine spatial scale in *L. minutus* (i.e., microgeographic adaptation; Richardson et al., 2014). Our genomic findings are consistent with previous studies that detected a pattern of local adaptation to differences in pond pH among Cape Race populations (Negrín Dastis & Derry, 2016; Negrín Dastis et al., 2019) as well as resurrection experiments and genetic analyses from historically acidified boreal shield Canadian lakes (Derry et al., 2009, 2010).

As we did not detect a pattern of isolation by environment in either spatial scale, the putative local adaptation that we detected could potentially be maintained by monopolization effects (Richardson et al., 2014; de Meester et al., 2016) instead of selection against migrants (Wang & Bradburd, 2014) in the presence of strong gene flow. Indeed, monopolization effects can be important for genetically structuring zooplankton populations, and reinforcing local adaptation (de Meester et al., 2002; van Doorslaer et al., 2009). *L. minutus* individuals overwinter under the ice as adults and subsequently reproduce in spring to coincide with the algal spring bloom, with nauplii emerging from resting eggs deposited in the sediments in fall (Grosbois et al., 2017). Therefore, locally adapted copepods originating from the spring reproduction peak and the resting eggs in ponds or lakes may have a numerical and fitness advantage over immigrants arriving later in the season, particularly from divergently adapted populations. At Cape-Race, another possible explanation for the maintenance of adaptation in the face of strong gene flow is the genetic architecture of adaptive traits, for example, if they are based on recessive alleles or multiple alleles with small effects (Tigano & Friesen, 2016).

Importantly, we found that the relative influence of genetic drift was dependent on spatial scale. In Greenland, where genetic diversity was reduced, and genetic drift was strong, adaptive resilience to future climate change could potentially be restricted. This is because genetic drift is expected to impede local adaptation (Blanquart et al., 2012), and because lower genetic diversity at range edges has been shown to reduce local adaptation to climate (Takahashi et al., 2016; Sánchez-Castro et al., 2022). However, the role of genetic load in reducing local adaptation at range edges is still not well understood (accumulated deleterious alleles; Angert et al., 2020). In the continental populations in this study, the effect of genetic drift appeared weaker, and should thus not constrain climatic adaptation. At the microgeographic spatial scale, we found indications of local adaptation despite evidence for strong genetic drift. It is thus possible that the high gene flow inferred from the minimal population genetic structure at Cape Race could counteract the loss of alleles due to genetic drift through the input of (adaptive) genetic diversity from nearby, locally adapted populations (Gompert et al., 2021). Microgeographic adaptation could therefore reduce the impact of genetic drift in these small pond populations through the beneficial effect of gene flow among divergent populations at the metapopulation level.

4.5. Conclusion

Overall, our results highlight the relevance of studying micro-evolutionary processes (gene flow, drift, and selection) conjointly at different spatial scales to gain a comprehensive knowledge of species responses to climate change (Forester et al., 2022). Future studies could focus on uncovering traits under selection from climate change and their relationship with fitness, which could be translated into evolutionary potential and incorporated into predictive models (Aguirre-Liguori et al., 2021; Forester et al., 2022). This information is particularly needed in freshwater ecosystems (Maasri et al., 2021) to inform conservation measures aimed at reducing freshwater biodiversity loss (Tickner et al., 2020; Albert et al., 2021).

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CONCLUSION

5.1. Key contributions

Population responses to rapid anthropogenic environmental changes will depend on the complex interaction between evolutionary and ecological processes such as population dynamics (demography), gene flow, adaptive potential, and the speed/magnitude of the shifts in environmental conditions (Bay, Rose, Barrett, et al., 2017; Urban et al., 2016b). By focusing on these interactions, my thesis addresses knowledge gaps in evolutionary biology. Overall, I provided insights into interactions between microevolutionary processes that could be harnessed for future studies aiming at predicting species responses to anthropogenic impacts (Bay, Rose, Barrett, et al., 2017; Aguirre-Liguori et al., 2021; Forester et al., 2022), particularly in face of global freshwater biodiversity threats (Tickner et al., 2020; Jaureguiberry et al., 2022). My research findings also elaborated on key concepts proposed as conservation "solutions" (Alexander et al., 2014; Willi et al., 2022): evolutionary and genetic rescue. My results suggest that genetic and evolutionary rescue could indeed alleviate demographic declines and genetic load combined with increasing adaptive potential in species imperiled by abrupt environmental changes (Carlson et al., 2014; Hufbauer et al., 2015; Robinson et al., 2021), although care must be taken in understanding the contribution of potentially maladapted migrants and the risk of outbreeding depression in imperiled recipient populations.

Information on the microevolutionary processes underlying population responses to environmental change is still lacking for many species (Ahrens et al., 2018; Loria et al., 2019; Urban et al., 2016b). This is particularly true for non-model species at the base of freshwater food webs, despite the important ecological functions that they provide to aquatic ecosystems. My thesis focuses on two non-model freshwater invertebrates that provide essential ecological links in aquatic food webs: the gastropod *Amnicola limosus* and the calanoid copepod *Leptodiaptomus minutus*. *A. limosus* is relevant for the study of rapid adaptive response to an invasive species, in a highly connected river ecosystem, with the presence of refuge and invaded habitats due to a natural gradient in calcium concentrations, generating a second axis of local adaptation. *L. minutus* is interesting for understanding the adaptation to historical acidification and climate change. I studied these two species through a combination of whole-genome pool sequencing, reciprocal transplant

experiments, and resurrection ecology, which allowed me to obtain significant insights into the processes of genetic and evolutionary rescue, as well as the interaction between gene flow, drift, and climatic adaptation across different spatial scales.

In the first chapter, I detected interactions between (rapid) adaptation, population demography, and gene flow in a continuous river ecosystem with strong selection gradients and high connectivity. In this system, uninvaded populations of native gastropods of the species *Amnicola limosus* from low-calcium habitats provided demographic and genetic subsidies to populations from high-calcium habitats invaded by the molluscivorous round goby (*Neogobius melanostomus*). However, these migrants were maladapted (adapted to the low-calcium habitats but not to the invasive predator, with low fitness overall). As a result, they do not provide a good genetic subsidy, which could result in outbreeding depression and compromise the resilience of invaded populations in the long term. This study adds to growing evidence that genetic rescue using adaptively divergent populations could yield negative outcomes (Edmands, 2007; Frankham et al., 2011; D. A. Bell et al., 2019; Robinson et al., 2021). This is particularly important because genetic rescue is promoted as a key conservation approach for the management of small populations (Ralls et al., 2018; Gaitán-Espitia & Hobday, 2021), to maintain the genetic diversity and adaptive potential of increasingly threatened populations (Weeks et al., 2011; Hoffmann et al., 2021; Willi et al., 2022), while sometimes dismissing the risks of outbreeding depression (Hamilton & Miller, 2016; Ralls et al., 2020).

In the second chapter, I examined the interaction between population demography and rapid adaptation through time and found that freshwater copepod populations underwent an evolutionary rescue as a response to historical lake acidification, which enabled their persistence in anthropogenically acidified lakes on the Canadian boreal shield. The evolutionary rescue was followed by an adaptive reversal during the pH recovery when atmospheric metal mining emissions were reduced and lakes underwent chemical recovery. Demonstrations of evolutionary rescue in nature are very rare (G. Bell, 2017; but see Gignoux‐Wolfsohn et al., 2021), and I additionally investigated some of the factors that allowed the rescue of the freshwater copepods (starting population size, standing genetic variation, length, and strength of the environmental degradation), which combined role is poorly understood in natural populations (Carlson et al., 2014). Further, I

addressed the long-term consequences of evolutionary rescue on the resilience of copepod populations to future environmental changes, a knowledge that is also lacking in nature (Carlson et al., 2014; G. S. Stewart et al., 2017). Our study showed that evolutionary rescue did not prevent an adaptive reversal during pH recovery, implying that the loss of genetic diversity that occurs during evolutionary rescue does not necessarily prevent future adaptation. Questions however remain regarding the adaptive potential of these populations, as a legacy of the bottleneck which occurred during the acidification remains in present populations, which could imply a strong effect of drift and genetic load.

In the third chapter, I examined local adaptation, gene flow, and drift, and found that the interaction between these micro-evolutionary processes is dependent on the spatial scale considered, with evidence for climatic adaptation at both latitudinal and microgeographic scales. There was strong gene flow between freshwater copepod populations at the fine spatial scale, but it was limited at the latitudinal scale despite evidence for some long-distance dispersal. Genetic drift was stronger at the range edge and in small populations, but its effect on local adaptation at the fine spatial scale was probably offset by high gene flow. These processes are rarely studied conjointly at different spatial scales, and the findings in this chapter thus raise questions about the generality of inferences of micro-evolutionary processes across spatial scales. This has implications for climatic response predictions because results obtained at a given spatial scale will not necessarily be representative of processes occurring at a different spatial scale (Aguirre-Liguori et al., 2021; Urban et al., 2016b). In addition, my findings highlight the need for the conservation of landscape-level parameters such as environmental heterogeneity, habitat size, and connectivity that promote evolutionary potential to mitigate against future potential extinction risk in populations (Walsworth et al., 2019).

5.2. Future directions

Building on the advances in understanding interactions between micro-evolutionary and ecological processes presented here, future research could further improve our understanding of freshwater invertebrate evolutionary responses to anthropogenic impacts through the development of new genomic and experimental resources that are currently still very limited for non-model organisms such as freshwater copepods and gastropods (Stoks et al., 2014; Loria et al., 2019). The transplant experiments presented in chapters 1 and 2 revealed that the adaptation of *A. limosus* to the invasive

predator and to lower calcium concentration, as well as *L. minutus* to the acidification, likely involved life-history traits (survival, fecundity, and development time). Future studies on *A. limosus* could focus on the shell morphology hypothesis (reduced size of the shell at maturity in invaded habitats; Kipp et al., 2012) as well as the metabolism and calcium transport involved in biomineralization (Johnson et al., 2019; adaptation to the low calcium environment; Clark et al., 2020). For the acidification response in *L. minutus*, investigating the physiological mechanisms of adaptation to acidity would be of interest as osmoregulation and homeostasis have consistently been identified as a mechanism of response to acidification in copepods (Havens, 1993; De Wit et al., 2016; Thor et al., 2018; Brennan, deMayo, et al., 2022). Finally, for the adaptation of freshwater calanoid copepods to climate, relevant traits include phenology (e.g., the onset of production of diapausing eggs; Czypionka et al., 2019; Häfker et al., 2018; Hairston et al., 1990; Roncalli et al., 2021), voltinism (Boers & Carter, 1978; Antonsson & Antonsson, 1992; A. M. Waldvogel et al., 2018), lipid metabolism (Grosbois et al., 2017; Roncalli et al., 2019) and life-history (Dam et al., 2021).

Investigation of relevant traits potentially involved in rapid adaptation could be feasible with common garden experiments and quantitative genetics methods. However, rigorous tests would require standardizing maternal effects for at least two generations and measuring relatedness between individuals, thus requiring sufficient reproductive rates. This might be amenable for *L. minutus* but is less likely for *Amnicola limosus*. For *L. minutus*, some populations and temporal samples exhibited high fecundity rates, but commonly reproduction yielded resting eggs that would not hatch under the lab conditions applied in Chapter 2, and directly hatching subitaneous eggs were only produced in a few cases. This implies that cultivating *L. minutus* over several generations would entail pinpointing population samples that produce subitaneous eggs or developing a method to hatch newly produced resting eggs. For *A. limosus*, fecundity was consistently low, with rates < 1 egg/individual in most replicates. This species might thus not be ideal for lab rearing or would require extensive testing of culture protocols or multi-year field experiments. For *L. minutus*, common garden experiments could be combined with resurrection ecology to study climate adaptation (e.g., studies with Daphnia; Burge et al., 2018; Weider et al., 2018), as was presented in Chapter 2. This would allow measuring additive genetic variance within and between populations as well as quantitative traits genetic differentiation Q_{ST} (Spitze, 1993).

An interesting avenue to explore would be to combine common garden experiments with genomic sequencing and dedicated statistical analyses, which can yield powerful insights into adaptive traits (de Villemereuil et al., 2016). Based on the complexity of the traits involved in adaptive responses reported for *L. minutus* and *A. limosus*, the underlying genetic basis is likely to be polygenic (Morales et al., 2019; Barghi et al., 2020; Brennan, deMayo, et al., 2022). This means that genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping are appropriate approaches to characterize these traits (Barghi et al., 2020; Czypionka et al., 2019; Wuitchik et al., 2022). However, linking putative underlying loci with effects on fitness leading to adaptation requires knowledge of the genetic architecture of those traits (Barrett & Hoekstra, 2011): linkage disequilibrium (e.g., causal or linked to a causal SNP), epistasis (gene interactions), pleiotropy (loci with multiple effects on phenotypes) and dominance. Using dedicated methods for the study of genetic architecture then allows for obtaining effect sizes (Boyle et al., 2017; proportion of a trait variance explained by a locus; Barghi et al., 2020). Despite the promising insights uncovered by these methods, a necessary tool to apply them is the availability of high-quality reference genome (i.e., fully assembled and annotated; Jung et al., 2020). This would involve several additional steps for *A. limosus* and new approaches for the development of a reference genome for *L. minutus*. For *A. limosus*, as the draft genome is currently highly fragmented into contigs, scaffolding using additional long reads sequencing (e.g., PacBio HiFi) and an assembled de novo transcriptome (RNA-sequencing are also important for genome annotation, i.e., identifying protein-coding genes) could be combined with chromatin conformation capture methods such as Hi-C to obtain a chromosome-level assembly (Jung et al., 2020). For *L. minutus*, the genome assembly that I attempted likely failed due to the high repeat content and heterozygosity of the genome (Jung et al., 2020). I believe that a similar pipeline could be started successfully by using single individual data with recent development for ultra-low-input methods involving PCR for long-read technologies and chromatin conformation, and deeper sequencing.

An important intraspecific process that can modulate responses to environmental disturbances and that was not addressed by this thesis is the contribution of phenotypic plasticity (i.e., the expression of different phenotypic traits under diverging environmental conditions, with the same underlying genetic background; Bay, Rose, Barrett, et al., 2017). Indeed, the role of phenotypic plasticity in rapid adaptive responses to novel environments has been outlined by numerous studies and reviews

(Merilä & Hendry, 2014; Levis et al., 2018; Fox et al., 2019; M. Kelly, 2019). Information on the proportion of phenotypic shifts explained by phenotypic plasticity is key for predicting population and species responses to environmental change (Bay, Rose, Barrett, et al., 2017). Understanding the relative role of plasticity is also critical as there are limitations to rapid adaptation through plasticity (Gunderson & Stillman, 2015; M. W. Kelly et al., 2017; Brennan, DeMayo, et al., 2022). Further, phenotypic plasticity can sometimes be maladaptive (Brady et al., 2019; Duputié et al., 2015; Ghalambor et al., 2007), and questions remain as to whether phenotypic plasticity will have positive or negative effects on rapid adaptation (Fox et al., 2019). The role of phenotypic plasticity is particularly relevant in the context of this thesis for the study systems involving freshwater gastropods in the St. Lawrence River and freshwater calanoid copepods at Cape Race, where plasticity should be favored due to the combination of high gene flow and high spatial environmental heterogeneity (Crispo, 2008). Some of the possible traits involved in the adaptation of gastropods to invasive predators (e.g., shell morphology; Brookes & Rochette, 2007; Clark et al., 2020) and copepods to climate (e.g., thermal tolerance; M. W. Kelly et al., 2017) are likely to be at least in part explained by phenotypic plasticity. Teasing out the contribution of genetic adaptation and plasticity in these study systems would thus be necessary to obtain a complete understanding of the adaptive potential in the face of global threats and to be able to formulate predictions (Bay, Rose, Barrett, et al., 2017).

Complementary sequencing tools could be developed to investigate the contribution of plasticity to adaptive responses by investigating changes in gene expression levels: transcriptomics (M. W. Kelly et al., 2017; Brennan, DeMayo, et al., 2022) and epigenomics (Hu & Barrett, 2017). Transcriptomics has the advantage of not requiring a reference genome as transcriptomes can be assembled *de novo* (Jørgensen et al., 2019b; Roncalli et al., 2018), although a reference genome would be helpful to interpret the transcriptomic results. This would allow comparing expression levels between groups of individuals or populations exposed to different environmental conditions (e.g., heat stress or ambient temperature in copepods; M. W. Kelly et al., 2017) in lab cultures (DeBiasse et al., 2018) or wild populations (Roncalli et al., 2019). This technique, combined with experimental evolution, can make it possible to tease out differences in expression due solely to plasticity or genetic changes (M. W. Kelly et al., 2017; Brennan, DeMayo, et al., 2022), whereas differences uncovered by reciprocal transplant experiments within one generation typically reflect

plasticity (Bailey et al., 2017). Finally, transcriptomics used jointly with trait measurements could also help to tease out the relative roles of plasticity and genetic adaptation to modulate relevant trait responses (morphological or physiological) in the context of rapid environmental changes (M. Kelly, 2019; Brennan, DeMayo, et al., 2022). Epigenomics is a tool that has been relatively recently applied to wild populations (Hu & Barrett, 2017) and is still underused in invertebrates (Suarez-Ulloa et al., 2015). Epigenetic modifications involve, for instance, DNA methylation or histone modifications, resulting in changes in gene expression level and thus play an important role in phenotypic plasticity (Hu & Barrett, 2017). These modifications can be transmitted between generations (Smithson et al., 2020; Hu et al., 2021) and evolve between different ecotypes (Hu & Barrett, 2022). Epigenomics can be used without a reference genome, but this will limit the potential for inferences (Hu & Barrett, 2017). It thus appears as a valuable tool to understand rapid adaptation, particularly regarding the role of plasticity (Hu & Barrett, 2017; Caizergues et al., 2022).

Knowledge of traits under selection from global change and their underlying genetic basis could be used to evaluate species' evolutionary potential, which can then be incorporated into predictive models to evaluate extinction risks (Forester et al., 2022). However, fully evaluating evolutionary potential is very challenging for many species, but proxies such as Genotype Environmental Associations (applied for all the chapters presented in my thesis) linked with fitness and preserving of genetic diversity (standing variation) across various environmental conditions can be useful for conservation purposes (Bay, Rose, Logan, et al., 2017; Forester et al., 2022). An important conservation tool is species distribution models, which forecast changes in species distribution under future environmental conditions, and has recently begun to incorporate evolutionary potential (Wuitchik et al., 2022), gene flow (Razgour et al., 2019), and genetic load, which can be used to estimate the genetic offset (difference between allele frequencies in the present and the future due to adaptation) and thus the vulnerability of populations across a species range (Bay et al., 2018; Aguirre-Liguori et al., 2021). Models aimed at evaluating extinction risk are additionally expanded by incorporating adaptive potential (evolution and phenotypic plasticity), demography, environmental shifts, gene flow, and drift as well as species interactions (Dynamic ecological-evolutionary models DEEM; Forester et al., 2022; Urban et al., 2016b), but these models are still underdeveloped despite their effectiveness for conservation (Bay, Rose, Logan, et al., 2017; Forester et al., 2022). Based on the knowledge developed in my thesis (proxies for evolutionary

potential, gene flow, and drift), species distribution modeling could be applied and extended with additional information on phenotypic plasticity and species interaction to integrate into a DEEM. A similar approach could be used across trophic levels to better assess the impacts of global changes on freshwater ecosystems (Maasri et al., 2022), and implement conservation measures to limit further loss of freshwater biodiversity (Tickner et al., 2020).

An important goal of evolutionary biology is to improve our understanding of rapid adaptation due to global changes to be able to predict future trajectories at the population and species level (Hoffmann & Sgrò, 2011; A. Waldvogel et al., 2020). Employing non-model species can provide meaningful insights into the interaction between micro-evolutionary and ecological processes driving the responses to anthropogenic impacts. The work presented in this thesis also provided critical knowledge on key concepts (genetic and evolutionary rescue) for conservation, particularly for their application in natural populations. These contributions constitute important progress for understudied yet ecologically significant species at the base of freshwater food webs, within the larger goal of filling knowledge gaps on global impacts in these ecosystems, which is needed to implement conservation decisions for the preservation of rapidly declining freshwater biodiversity.

APPENDIX A

MALADAPTIVE MIGRATION FROM PHYSIOLOGICAL REFUGIA COULD CONSTRAIN THE RESCUE OF NATIVE GASTROPODS FACING AN INVASIVE PREDATOR

Supplementary Methods: Analytical methods for water chemistry

We collected water samples to quantify dissolved calcium (Ca; mg/L), total phosphorous (TP; µg/L), total nitrogen (TN; mg/L), and dissolved organic carbon (DOC; mg/L). TP, TN, and DOC samples were analyzed at the GRIL- Université du Québec à Montréal (UQAM) analytical laboratory. Water calcium samples were analyzed with a Thermo ICAP-6300 Inductively Coupled Argon Plasma - Optical Emission Spectrometer (ICP-OES) following protocols described by US EPA (1994) at the University of Alberta Biogeochemical Analytical Service Laboratory (U of A – BASL; Edmonton, Alberta, Canada). Total Phosphorus (TP) was measured spectrophotometrically on the same machine by the molybdenum blue method after persulfate digestion (Griesbach and Peters, 1991). Total Nitrogen (TN) was analyzed with a continuous flow analyzer (OI Analytical Flow Solution 3100 ©) using an alkaline persulfate digestion method, coupled with a cadmium reactor, following a standard protocol (Patton and Kryskalla, 2003). DOC concentrations of 0.45 µm filtered samples (surfactant-free membrane filters) were analyzed with an OI Analytical Aurora 1030W TOC Analyzer (https://www.oico.com/1030W) using a persulfate oxidation method at the GRIL- Université du Québec à Montréal (UQAM) analytical laboratory.

Citations:

EPA 1994. Method 200.7, Revision 4.4: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry. Environmental Monitoring Systems Laboratory Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

Griesbach S.J. & Peters R.H. (1991) The effects of analytical variations on estimates of phosphorus concentration in surface waters. Lake Reservoir Management, 7, 97–106.

Patton CJ, Kryskalla JR. Methods of analysis by the US Geological Survey National Water Quality Laboratory: Evaluation of alkaline persulfate digestion as an alternative to kjeldahl digestion for determination of total and dissolved nitrogen and phosphorus in water: US Department of the Interior, US Geological Survey; 2003.

Table A.1: Environmental characteristics of the study sites. Ca: calcium concentration, TP: total phosphorus, TN: total nitrogen, DOC: dissolved organic carbon, Temp: water temperature, DO: dissolved oxygen, Cond: conductivity, Alk: alkalinity.

Site name	Latitude	Longitude	Goby abund.	Time since invasion (years)	Ca (mg/L)	TP (ug/L)	TN (ppm)	DOC (mg/L)	Temp (C)	DO (%)	Cond.	Alk.	pH
RAF	45.415387	-73.633328	6	<12	22.45	35.28	0.462	7.37	18.4	96.0	182.8	118.95	7.76
PST	45.290666	-74.044296	32	13-16	34.33	14.54	0.338	8.95	22.9	120.7	313.8	204.10	8.60
PON	45.234976	-74.003697	41	13-16	44.04	18.59	0.411	3.64	21.3	98.5	311.2	202.15	8.28
PG	45.290416	-74.172167	54	13-16	58.84	97.32	1.594	17.10	21.9	137.2	367.0	238.55	8.50
PDC	45.335278	-73.954167	0	Uninvaded	34.57	53.91	0.496	28.79	18.9	98.0	300.3	195.00	8.40
PB	45.403333	-73.925000	0	Uninvaded	17.95	44.12	0.660	21.95	24.0	98.4	130.3	84.50	7.81
OKA	45.459444	-74.087500	0	Uninvaded	12.79	76.67	0.765	13.52	22.9	106.4	115.2	74.75	7.86
IPE	45.393611	-73.938333	0	Uninvaded	24.26	46.26	0.431	29.76	22.8	92.0	191.9	124.80	7.90
IB	45.515989	-73.901314	0	Uninvaded	9.80	42.94	0.497	6.79	20.0	89.3	80.7	52.70	7.59
HA	45.614444	-74.598611	0	Uninvaded	15.66	64.32	0.590	19.70	21.3	98.6	127.2	80.25	7.51
GOY	45.329859	-73.835825	4	13-16	34.74	42.39	0.393	12.82	20.3	76.8	317.3	206.05	7.73
BEA	45.319491	-73.852733	37	13-16	37.56	66.41	0.442	19.42	19.9	94.9	307.9	199.55	8.05

Table A.2: Demographic analysis of PB and PG for three models (bottleneck followed by growth in both populations, bottlegrowth in PG, split with asymmetric migration). Models are presented according to their log likelihood (log L), with the number of parameters in each model (k). For the best model (split with migration), we show the estimated scaled parameters nu1 (scaled PB population size), nu2 (scaled PG population size), m_{ri} (scaled migration from invaded to refuge population), m_{ir} (scaled migration from refuge to invaded population) and T_S (scaled time since the population split), with the upper and lower bounds of the 95% confidence interval shown in brackets.

Model	**Log-likelihood**	**k**	**nu1**	**nu2**	$\boldsymbol{m_{ri}}$	$\boldsymbol{m_{ir}}$	$\mathbf{T_S}$
Split-mig	-15,563	5	4.28 [4.15-4.45]	0.39 [0.39-0.41]	0.88 [0.86-0.94]	1.51 [1.47-1.53]	3.40 [3.26-3.54]
Bottlegrowth PG	-62,231	8	-	-	-	-	-
Bottlegrowth PB-PG	$\mathbf{-3.8018 \times 10^6}$	10	-	-	-	-	-

Figure A.1: Outliers SNPs identified with the core model in Baypass. A: P-values associated to the XtX statistic (on -log10 scale) as a function of the XtX estimate. Horizontal line indicates the threshold of p-values < 0.001. Low XtX values indicate

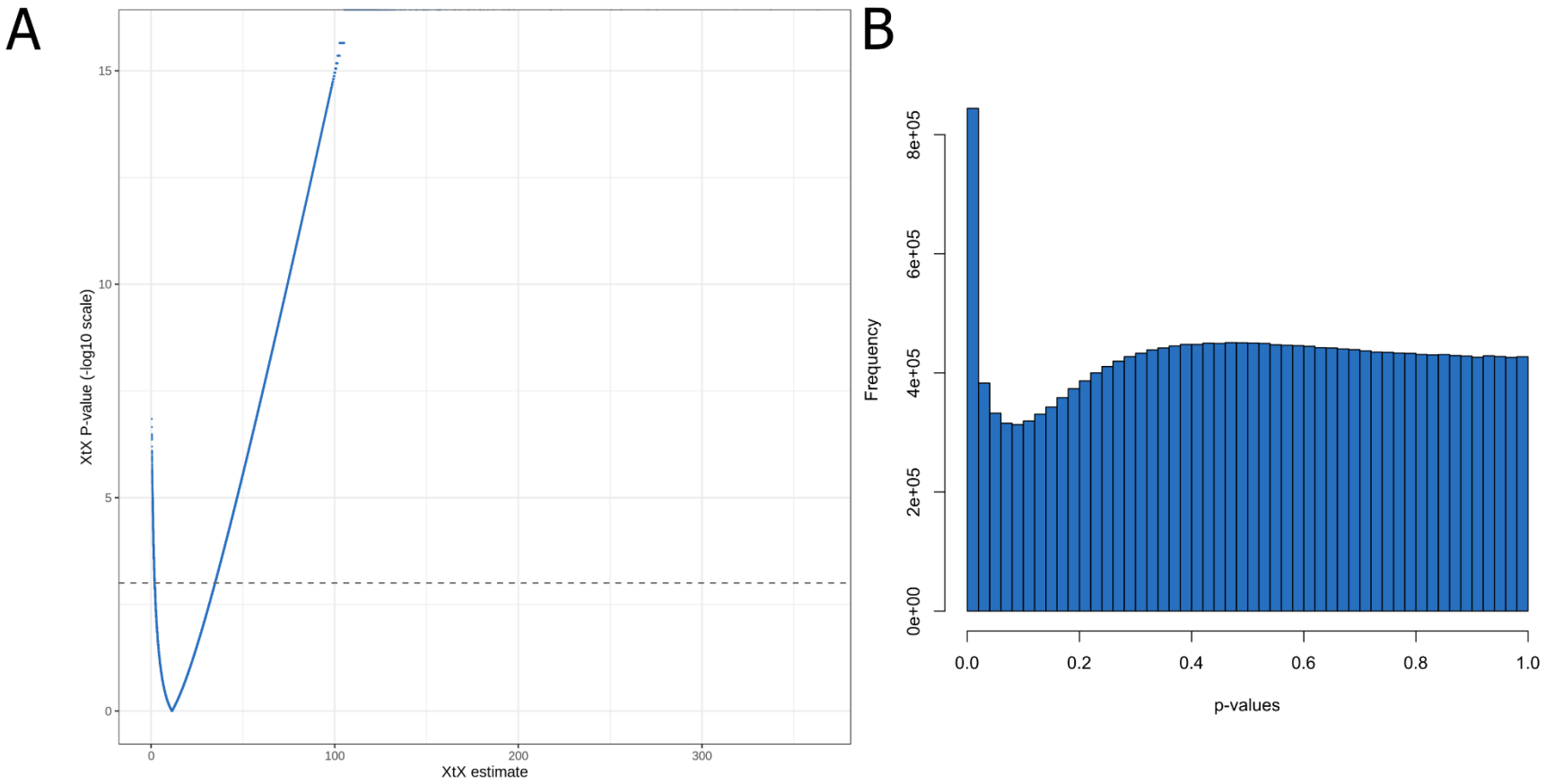


Figure A.2: Correlation between selected environmental covariables for the EA analyses with Baypass and RDA. Inv_status: absence/presence of round gobies, calcium_mg.L: calcium concentration in mg/L

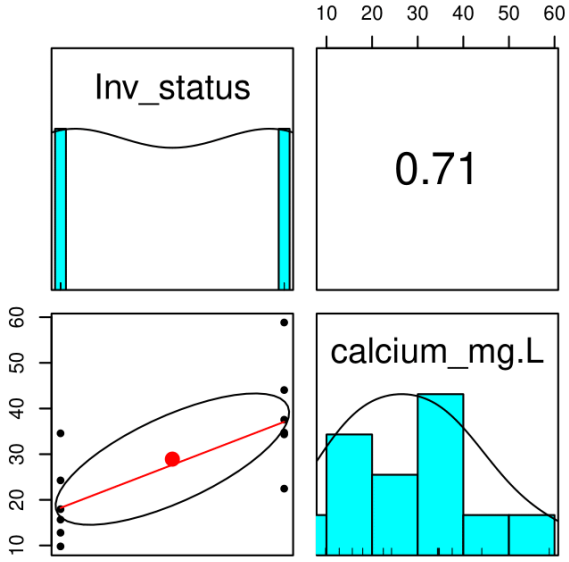


Figure A.3: Histograms of the recalibrated p-values output by poolFreqDiff. P-values were recalibrated based on the empirical-null hypothesis approach, with a genomic inflation factor of λ = 0.85. A: Recalibrated p-values for the goby presence/absence association. B: Recalibrated p-values for the calcium concentration association. = 0.85

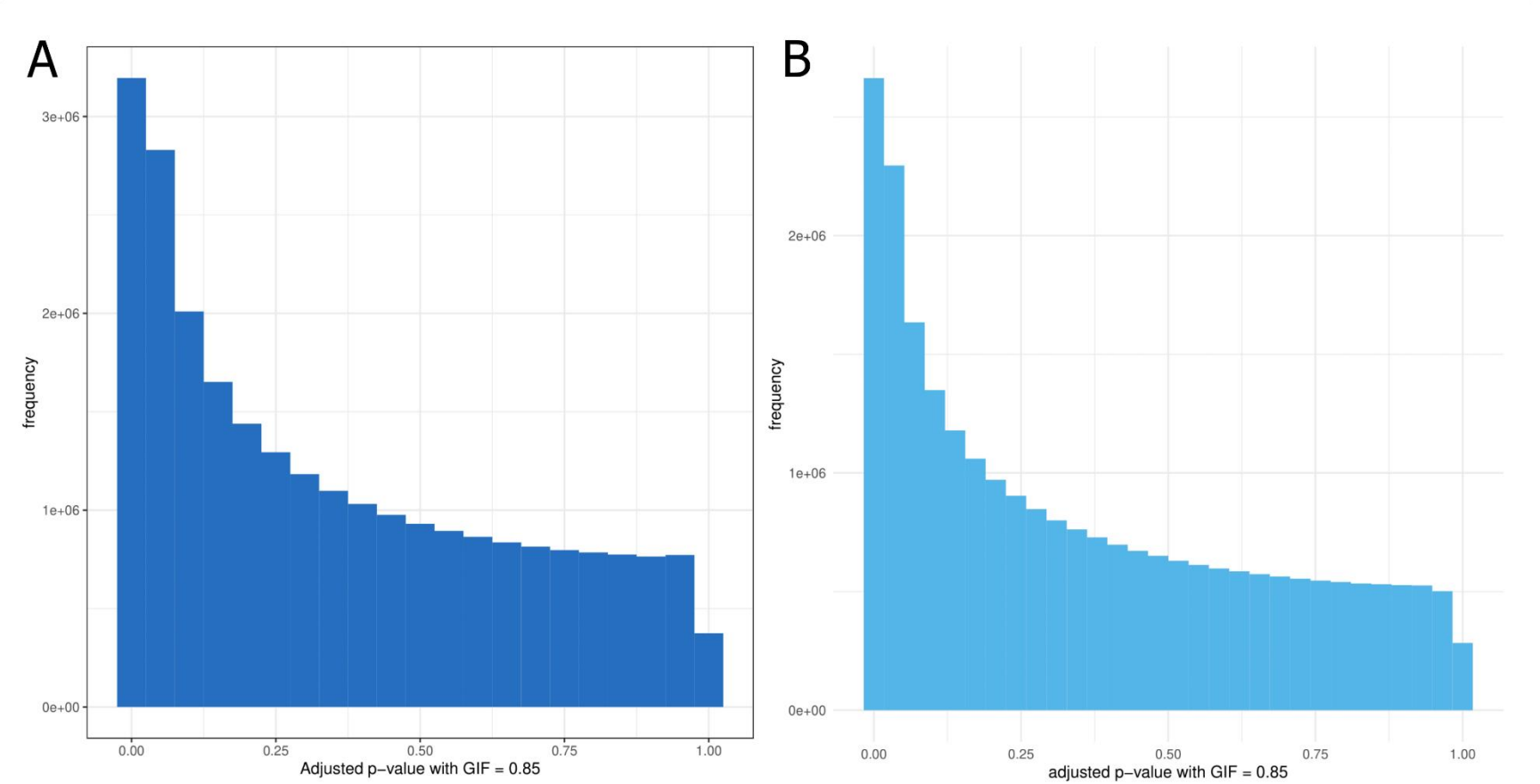


Figure A.4: Fecundity (total number of eggs produced) and survival rates measured in a reciprocal transplant as a function of water treatment, goby cue, and origin water. Treatments with combo water (High Calcium HC and Low Calcium LC) had very low survival and fecundity overall and were thus not included in the statistical analyses.

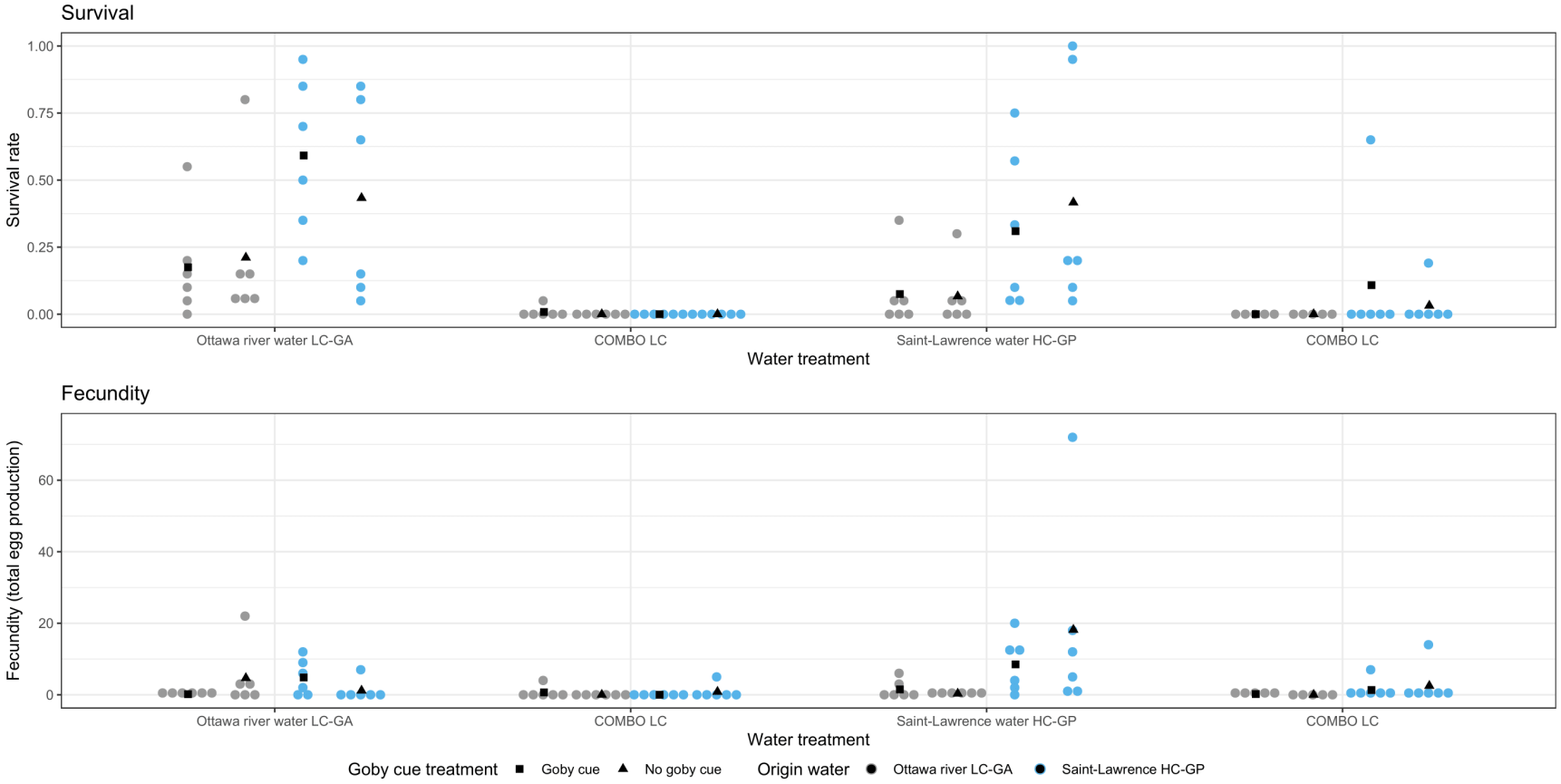


Figure A.5 Venn diagram of the outliers from the Environmental association analyses. Compared methods are the Baypass STD model (Calcium concentration), the Baypass C-statistic (Goby presence) and the poolfreqdiff analyses of parallel changes in allele frequencies, for both the calcium concentration and invasion status (presence/absence). Outlier SNPs in common between the analyses are shown in the shaded areas.

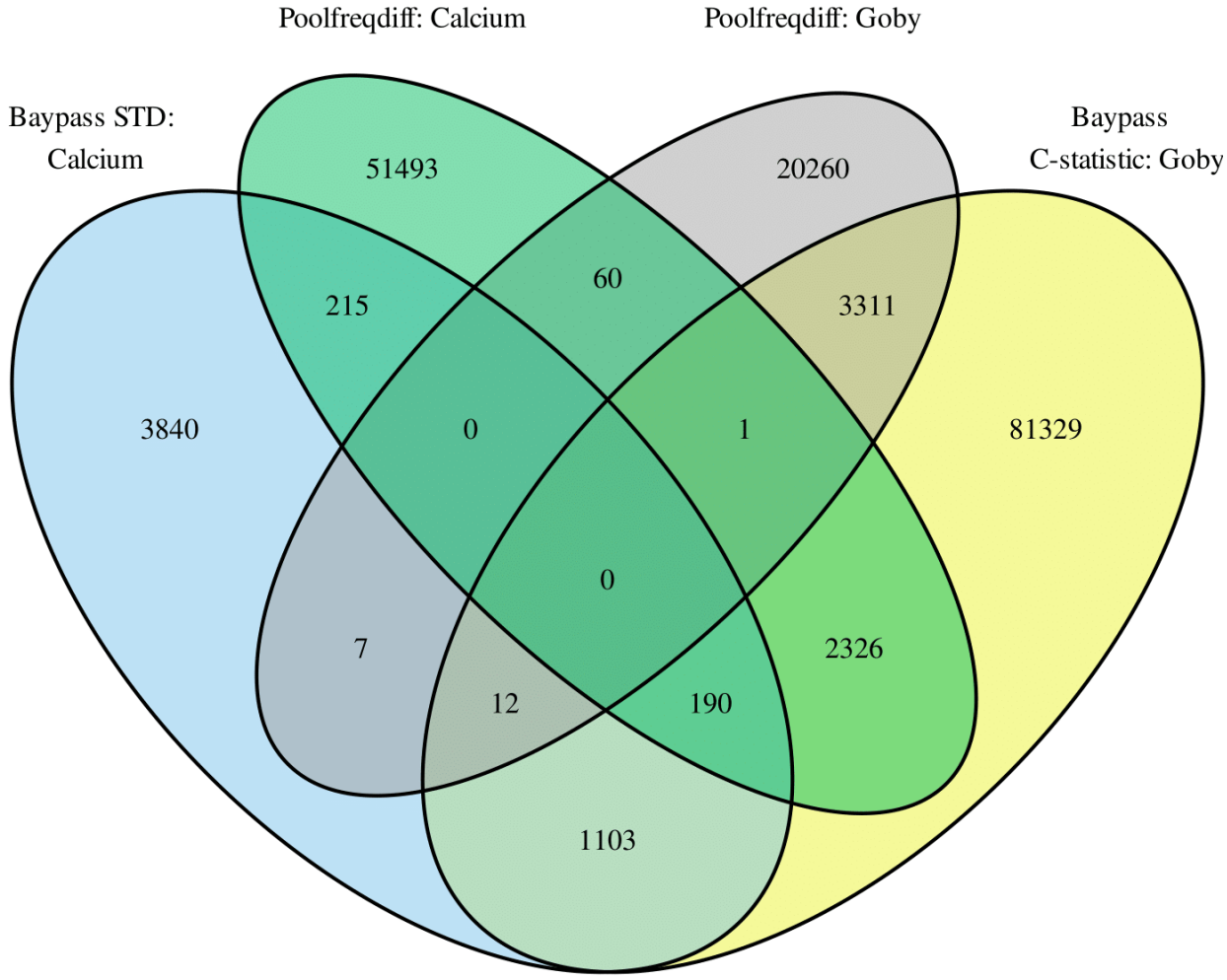


Figure A.6: Venn diagram of the outlier and Environmental association analyses. Outliers SNPs from Baypass (Core and STD models, C-statistic) and the poolFreqDiff analysis of parallel changes in allele frequencies comparing the goby absent/present and low/high calcium habitats. Outlier SNPs in common between the three methods are shown in the shaded areas.

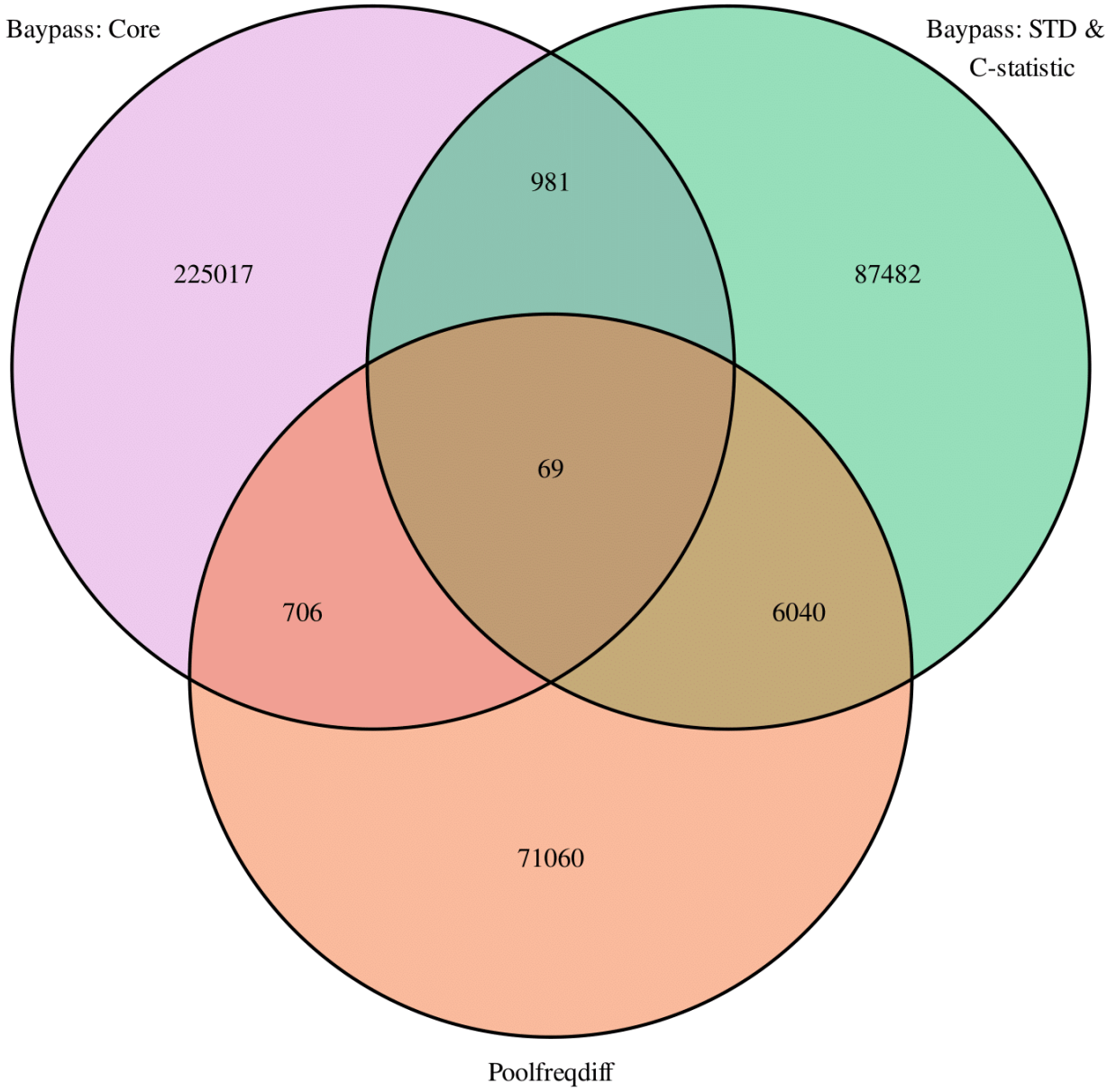


Figure A.7: Random effect of the populations of origin from the GLMM model for survival (binomial distribution and logit link function). Random effect deviations showing shifts of populations relative to the fixed intercept (0).

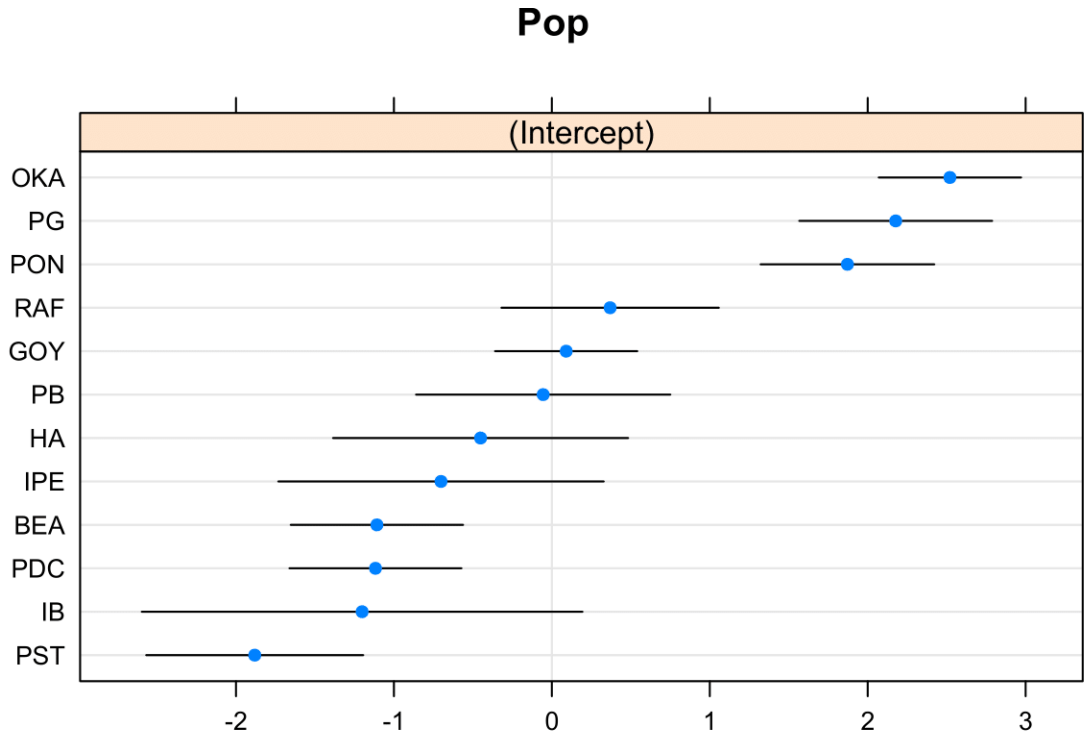


Figure A.8: Alternative demographic model tested with δaδi. A: Results for the model with bottleneck followed by growth in PG. Top: Folded joint site frequency spectrum (SFS) of PB and PG for a sample size of 80 (two times the number of individuals) for the observed data (left) and the model (right). The colored scale indicates the logarithm of the number of sites for a given read count. Note that the data was masked from 0 to 5. Bottom: residuals of the normalized differences between the observed data and the model (left), shown as histogram (right) B. Results for the model of a bottleneck followed by growth in both populations.

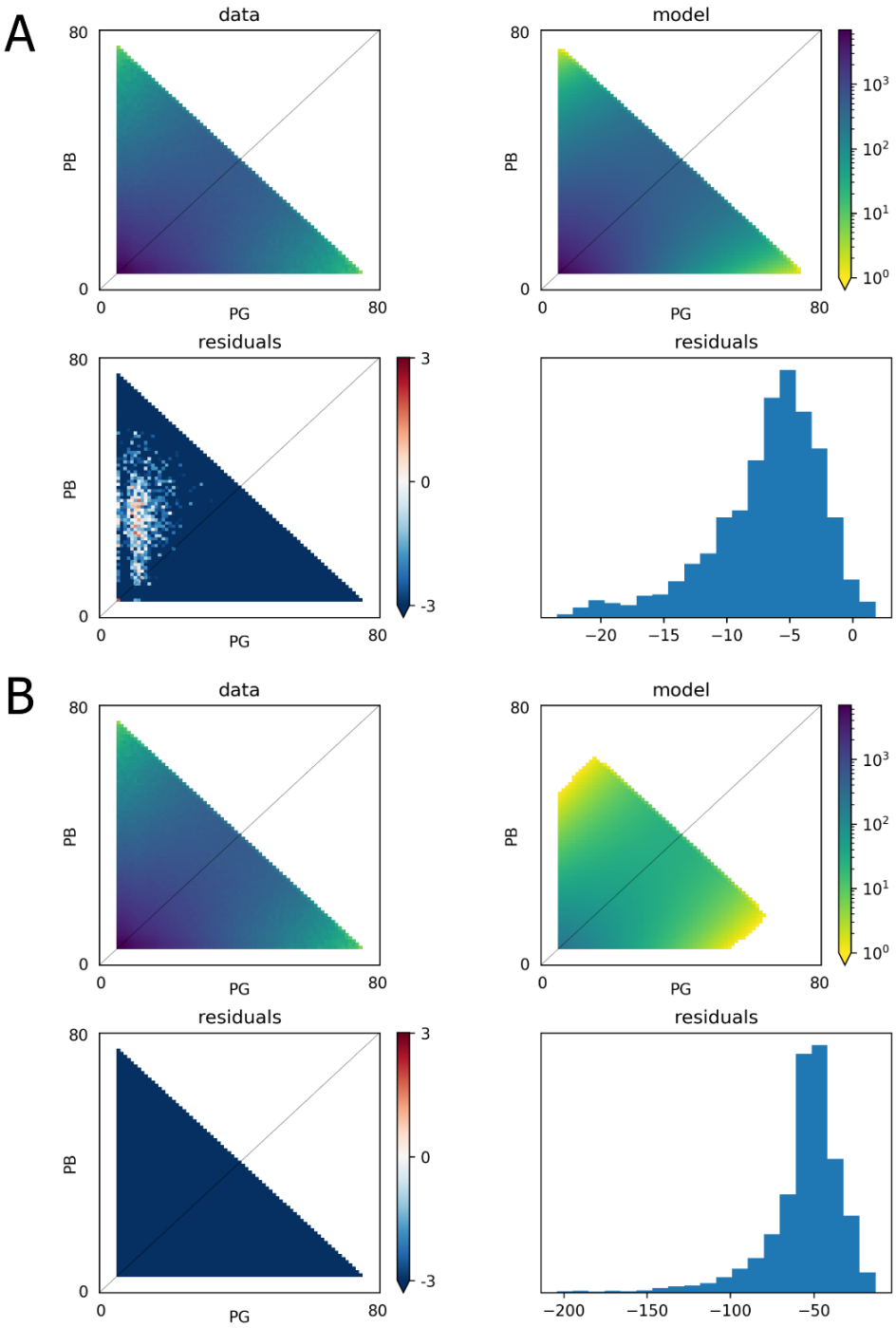
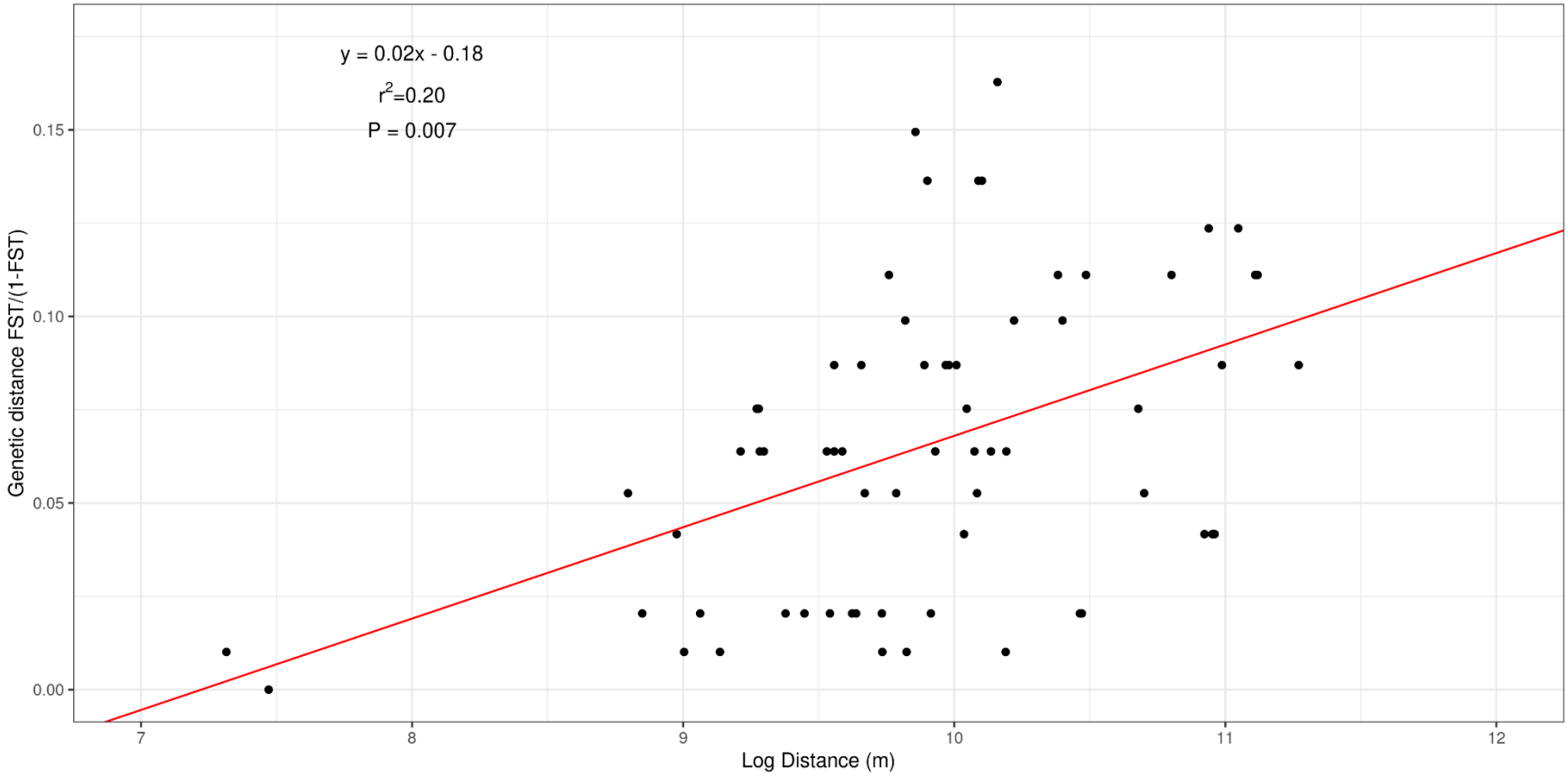


Figure A.9: Pattern of isolation by distance between the study populations. Significant positive correlation (Mantel test: $p = 0.007$, $r^2 = 0.20$) between the log of the geographical distance (in m, 2D distribution of populations) and the linearized genetic distance $F_{ST}/(1-F_{ST})$



APPENDIX B

EVOLUTIONARY RESCUE AND ADAPTIVE REVERSAL ALLOWED THE PERSISTENCE OF FRESHWATER COPEPODS DURING HISTORICAL LAKE ACIDIFICATION AND PH RECOVERY

Supplementary Methods – analytical methods for water chemistry

The pH, specific conductivity (SPC; µS/cm and temperature (°C) were measured at the surface in August 2018 with a YSI pro plus multiparameter probe (model 10102030; Yellow Springs Inc.). The other physicochemical parameters were analyzed in the GRIL-UQAM water chemistry laboratory on water samples collected in late June 2019. Dissolved organic carbon concentration was measured from water filtrates passed through 0.45 µm filters (surfactant-free membrane filters) and then acidified (5% phosphoric acid), followed by sodium persulfate oxidation and concentration reading with a 1010 total organic carbon analyzer (O.I. Analytical, College Station, TX, U.S.A.). Total phosphorus was measured spectrophotometrically on the same instrument by the molybdenum blue method after persulfate digestion (Griesbach and Peters, 1991). Total nitrogen samples were also analyzed on this instrument with the continuous flow analyzer by the alkali persulfate digestion method and then coupled to a cadmium reactor, following a standard protocol (Patton and Kryskalla, 2003).

Table B.1: Physico-chemical characteristics of the study sites (June 2018-2019)

Lake	Maximum depth (m)	Surface pH temperature		Conductivity (µS/cm)	Dissolved organic carbon (mg/L)	Total Phosphorus (µg/L)	Total Nitrogen (mg/L)
George	36.5	25.4	7.05	18.9	2.7	3.2	0.20
Lumsden	25	25.0	6.13	11.1	2.5	3.4	0.18

Table B.2: Demographic analysis with δaδi of Lumsden and George recovery samples for four models (genetic bottleneck followed by growth "bottlegrowth", genetic bottleneck only "two epochs", growth and neutral). Models are presented according to their log likelihood (log L), with the number of parameters in each model (k). For the best models (bottlegrowth and two epochs), we show the estimated scaled parameters nuB (scaled effective population size after the bottleneck or growth event), nuF (scaled effective population size after the recovery), and Theta ($\theta = 4\mu L$, with μ the mutation rate and L the effective sequenced length; see main text), with the upper and lower bounds of the 95% confidence interval shown in brackets.

Population	Model	Log-likelihood	k	nuB	nuF	Theta
George	Bottlegrowth	-3,979	3	8.4×10^{-6} [-1.2×10^{-2}- 1.2×10^{-2}]	29.9 [11.6 - 48.3]	1.3×10^{6} [$1.2 \times 10^{6} - 1.4 \times 10^{6}$]
	Two epochs	-3,972	2	1.0×10^{-4}[9.3×10^{-5}- 1.1×10^{-4}]	-	1.3×10^{6} [$1.1 \times 10^{6} - 1.5 \times 10^{6}$]
	Growth	-176,115	2	4.61	-	772,830
	Neutral	-29,024,901	1	-	-	773,216
Lumsden	Bottlegrowth	-3793	3	5.2×10^{-6} [-1.2×10^{-2}- 1.2×10^{-2}]	11.2 [3.3 - 6.34]	1.6×10^{6} [1.4×10^{6}- 1.8×10^{6}]
	Two epochs	-3774	2	7.4×10^{-5} [-1.0×10^{-2}- 1.0×10^{-2}]	-	1.6×10^{6} [1.0×10^{6}- 2.3×10^{6}]
	Growth	-219,734	2	8.5	-	781,042
	Neutral	-29,399,621	1			781,433

Figure B.1: ^{210}Pb-dating of sediment cores and historical pH trajectory for George and Lumsden lakes. A: ^{210}Pb sediment core dating reconstructed using the Constant Rate of Supply (CRS) model. Grey boxes indicate the selected depth sections and approximate start and end date for each time period (chronologically: pre-industrial, acidification, recovery). B: Historical lake pH based on reconstruction from diatom and chrysophyte paleo-fossils (A. S. Dixit et al., 1992), and contemporary lake pH as measured by the Ontario Ministry of the Environment and from the literature (Swanage, 2018) for George and Lumsden lakes. Grey boxes approximate

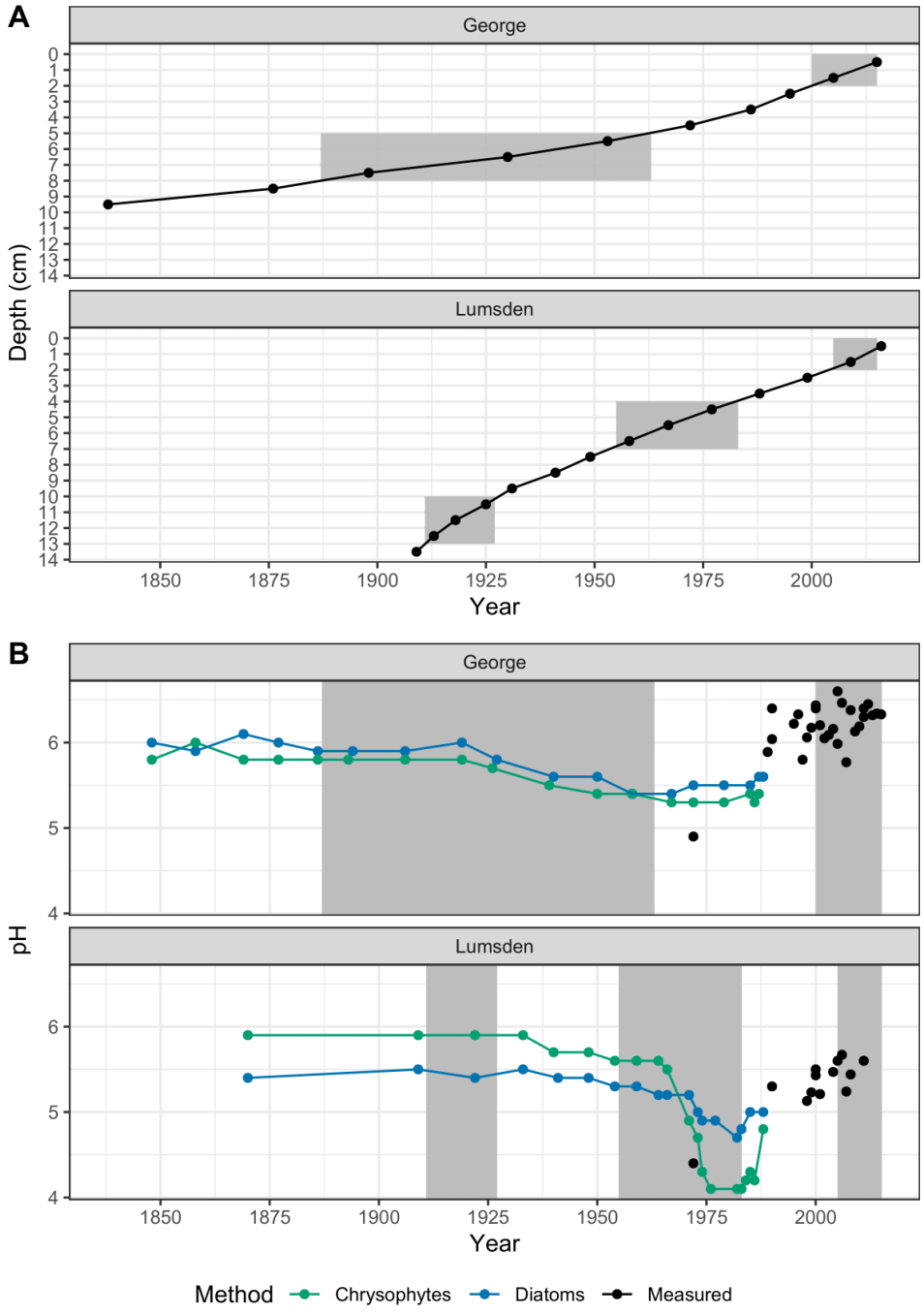


Figure B.2: Population genetic structure and demographic history. Heatmap of the scaled covariance matrix Ω (with ρ_{ij} the correlation coefficient between pairs of populations) with hierarchical clustering tree (using the average agglomeration method), obtained from the core model of Baypass

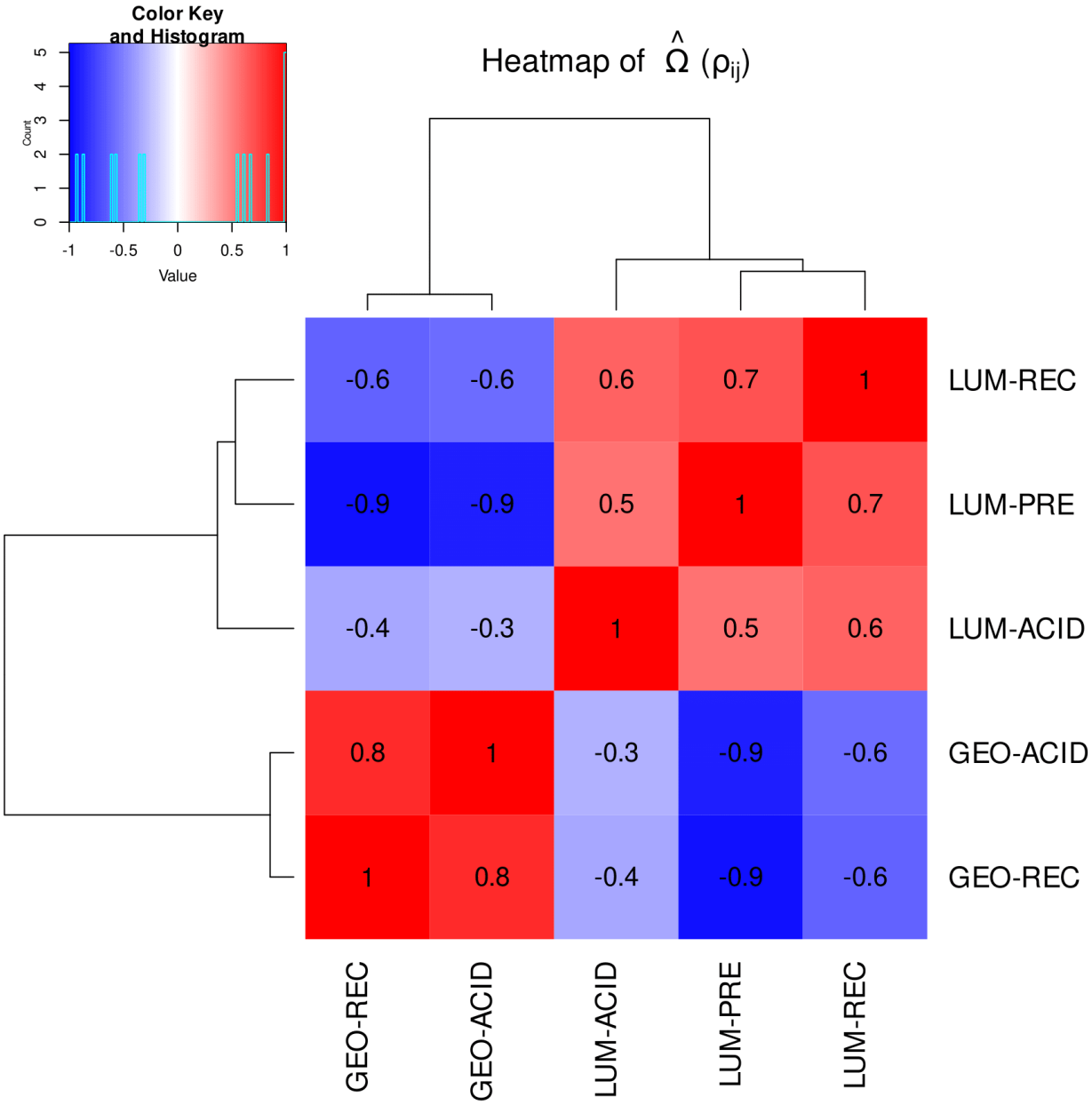


Figure B.3: Outliers SNPs identified with the core model in Baypass. Left: Histogram of the p-values derived from the XtX estimate. Right: P-values associated with the XtX statistic (on -log10 scale) as a function of the XtX estimate. The horizontal line indicates the threshold of p-values < 0.001.

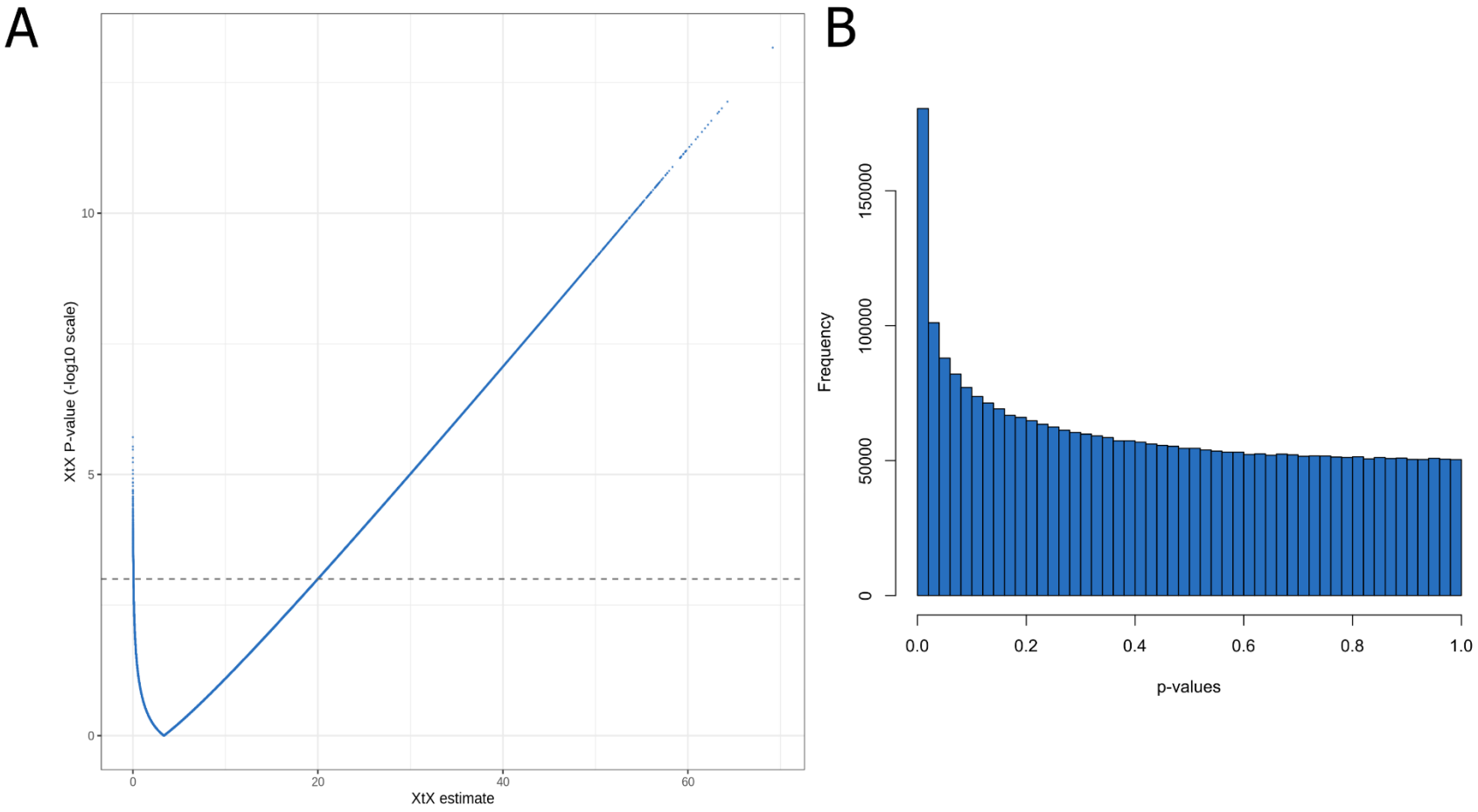


Figure B.4: Results of the resurrection ecology experiment for George and Lumsden lakes as a function of time periods of origin for lake acidification history and pH treatment. A: Lifetime survival, B: development time from birth to adulthood, C: Fecundity (number of eggs or nauplii per female). Each dot represents a measurement for one replicate, the black horizontal bars are the mean responses for each treatment.

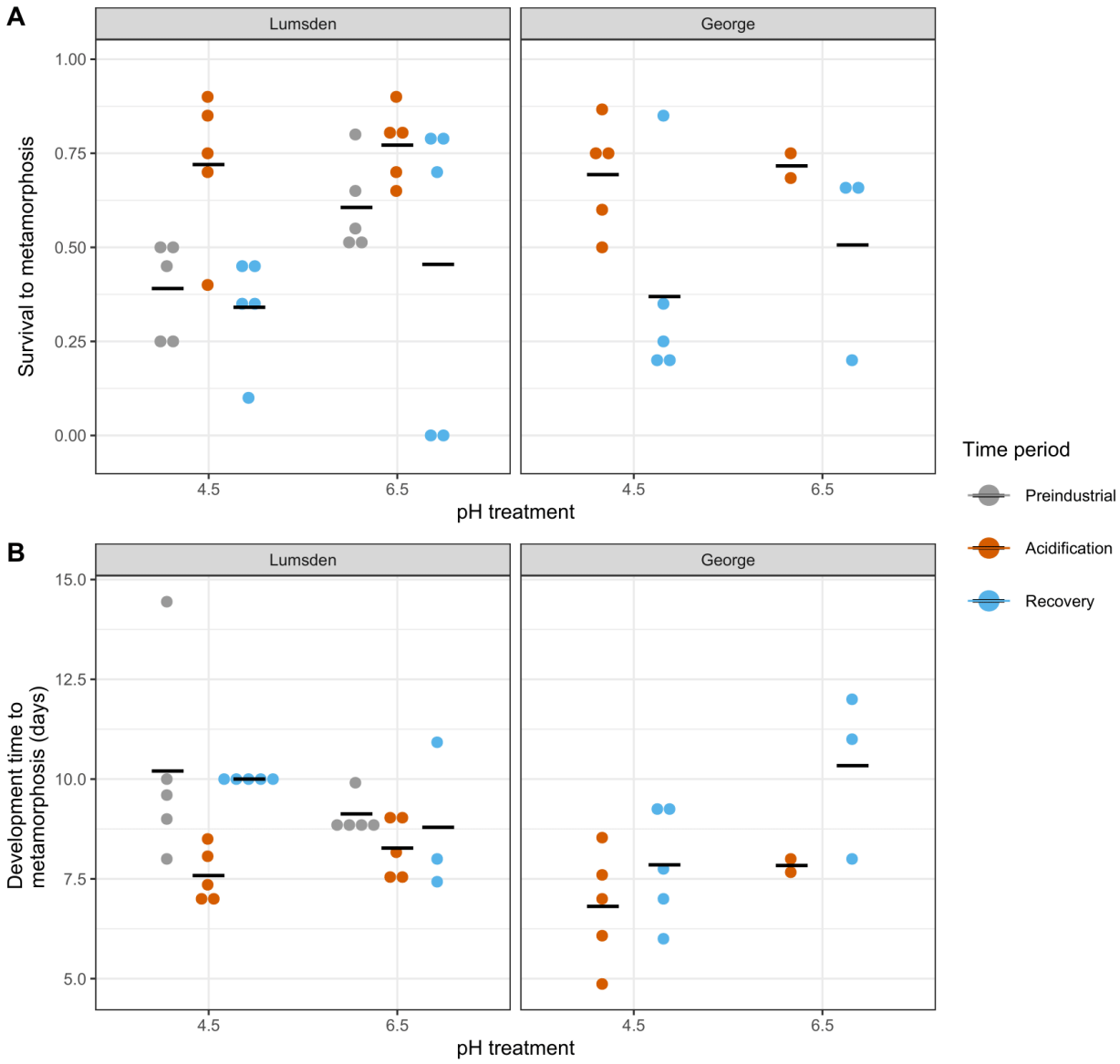


Figure B.5: Alternative bottleneck model (two epochs) tested with δaδi. A: Results for George's copepods from the recovery period. Top: Folded site frequency spectrum (SFS) for a sample size of 400 (two times the number of individuals) for the observed data (blue line) and the model (red line), showing the logarithm of the number of sites (y-axis) as a function of a given read count (x-axis). Note that the data was masked from 0 to 20. Bottom: Histogram of the residuals of the normalized differences between the observed data and the model. B: Results for Lumsden's copepods from the recovery period.

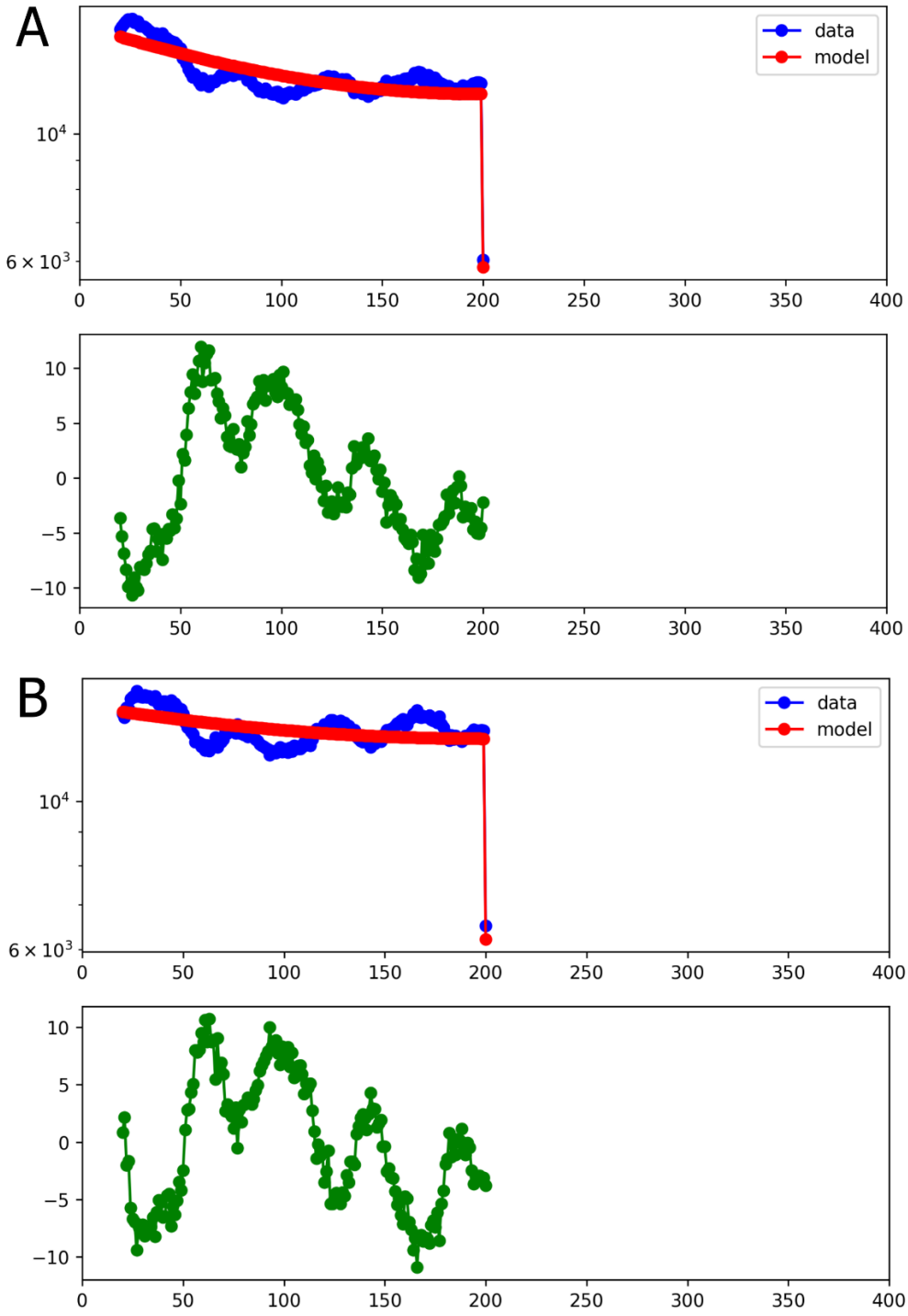


Figure B.6: Pairwise F_{ST} matrix between the 5 temporal samples

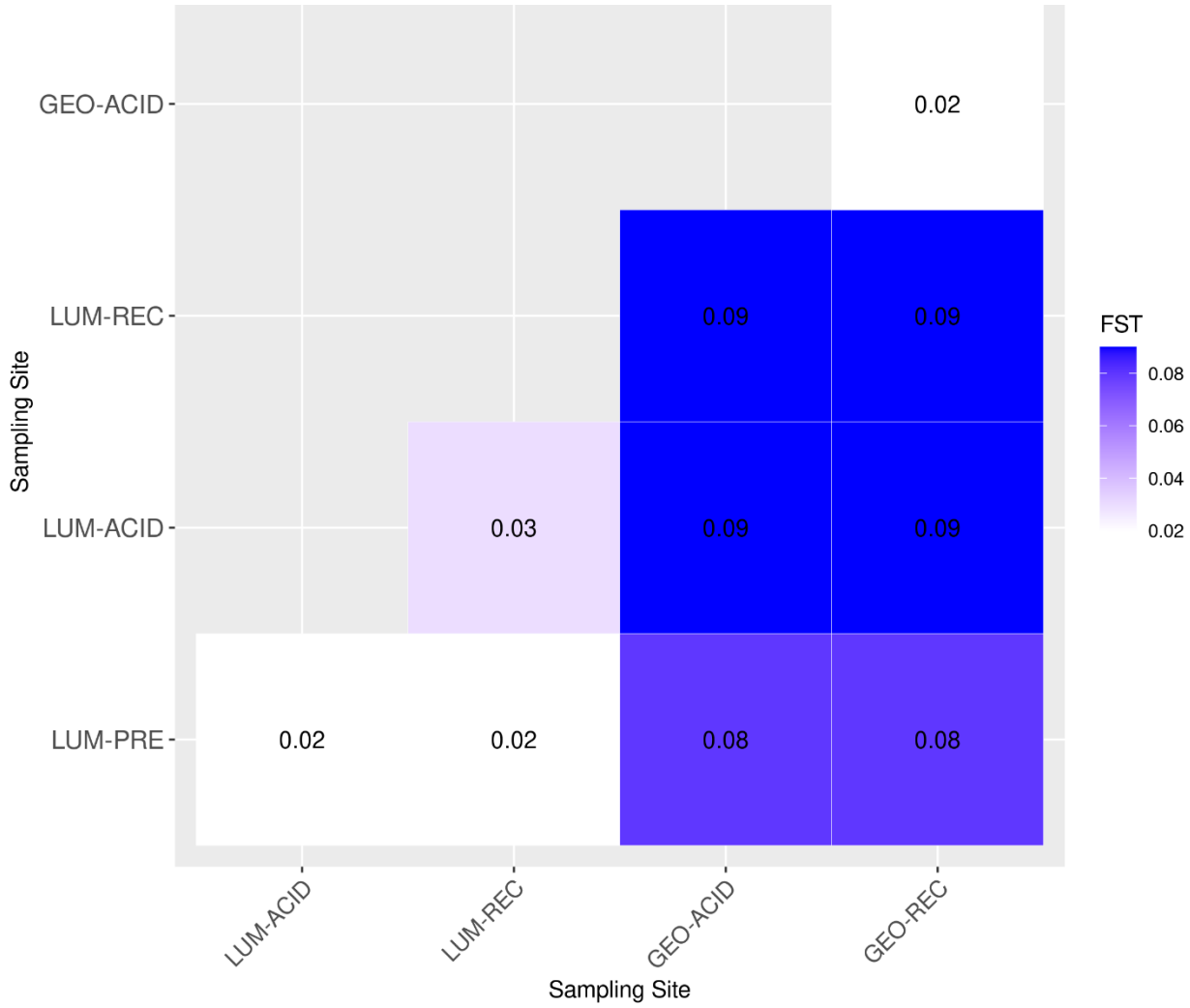


Figure B.7: Correlation coefficient β_i between allele frequencies and the acidification as a function of Bayes Factor in Baypass. Outliers were detected when BFmc > 20

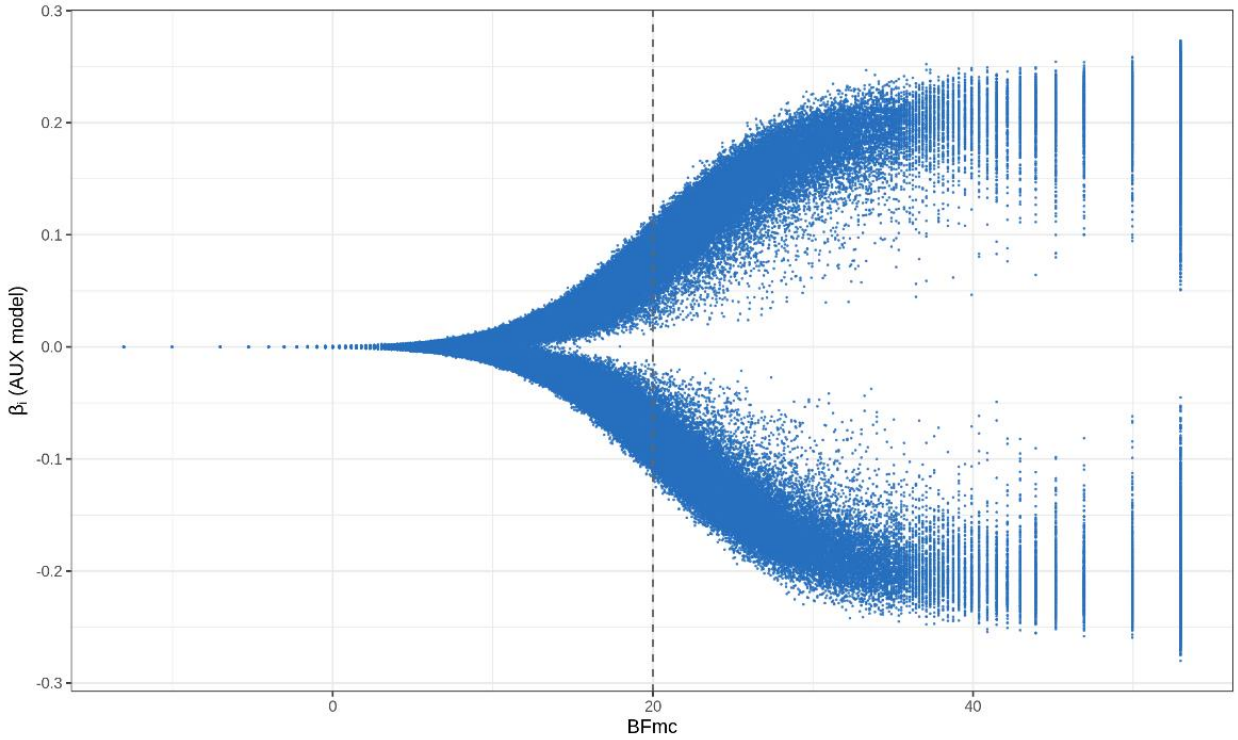
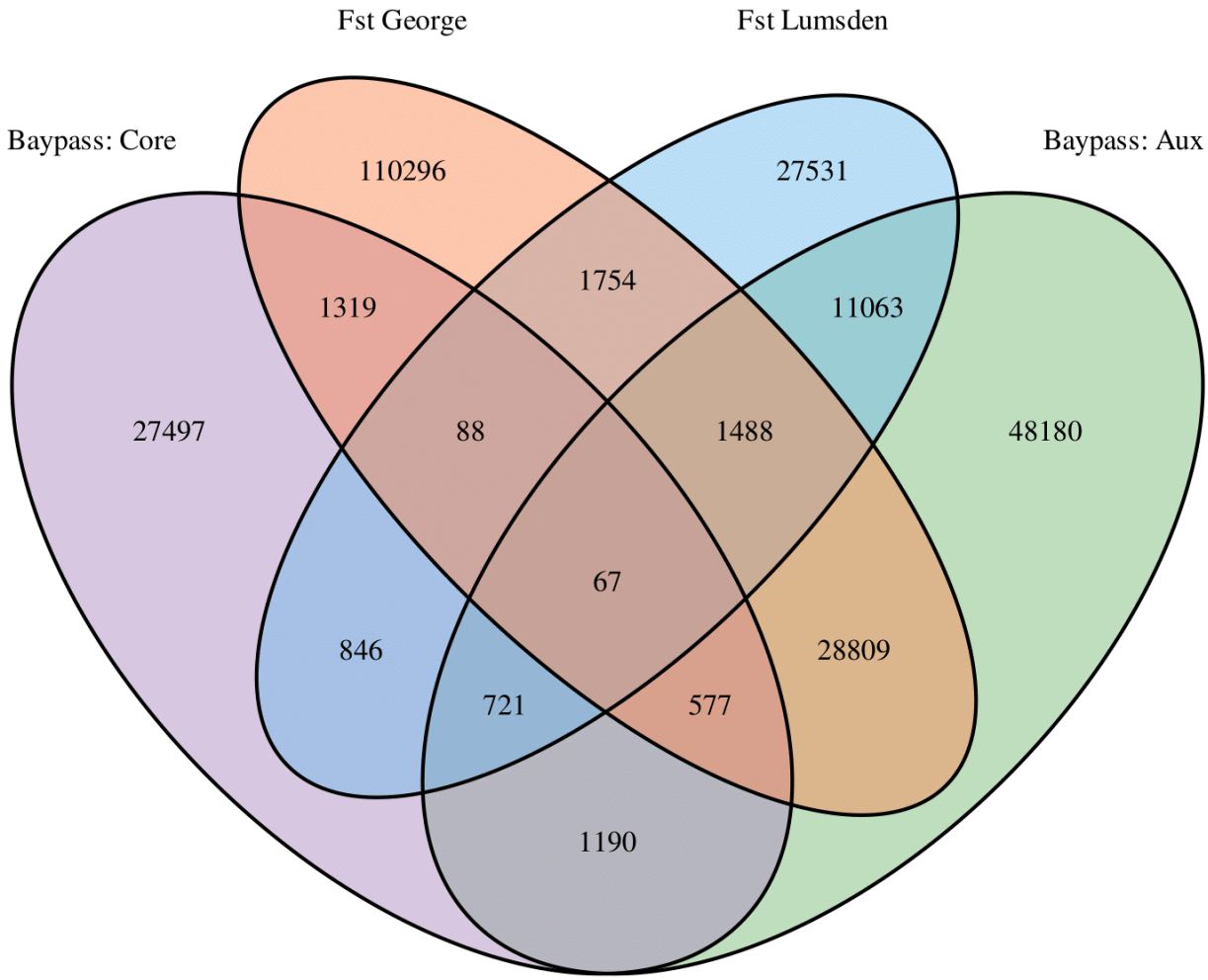


Figure B.8: Venn diagram of the outliers detected with the core and auxiliary models in Baypass in common with the outliers of the FST scan for George (acidification vs recovery comparison) and Lumsden (outliers in pre-acidification vs acidification and acidification vs recovery comparisons). Outlier SNPs shared by the different analyses are shown in the shaded areas.



APPENDIX C

CLIMATE ADAPTATION IN FRESHWATER COPEPODS AT LATITUDINAL AND MICROGEOGRAPHIC SPATIAL SCALES

Figure C.1: Histogram of p-values derived from the XtX statistics output by the core model in Baypass for Cape Race. The p-values were generated from χ^2 distribution with 14 degrees of freedom.

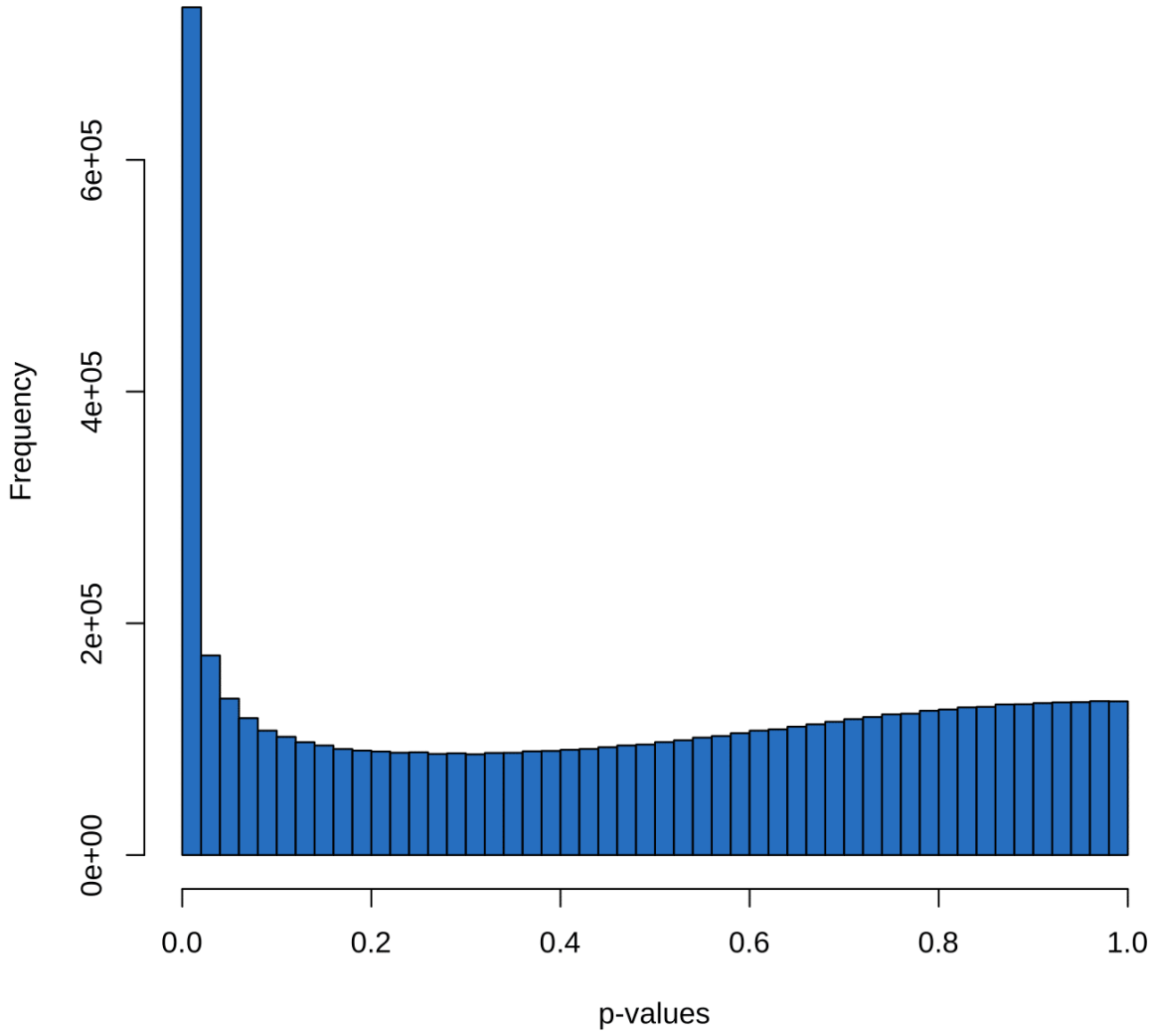


Figure C.2: Correlation between environmental covariables for the latitudinal gradient. Pearson correlation coefficient r is shown in the upper diagonal. Tmean: Average annual temperature, WQTmean: Average temperature of the warmest quarter, CQTmean: Average temperature of the coldest quarter, Prec.mm: annual precipitations (mm), Chla: chlorophyll A concentration µg/L, TAE: tannic acid equivalent mg/L. Note that the correlation between the selected covariables for the Baypass aux model (WQTmean and TAE) is 0.6.

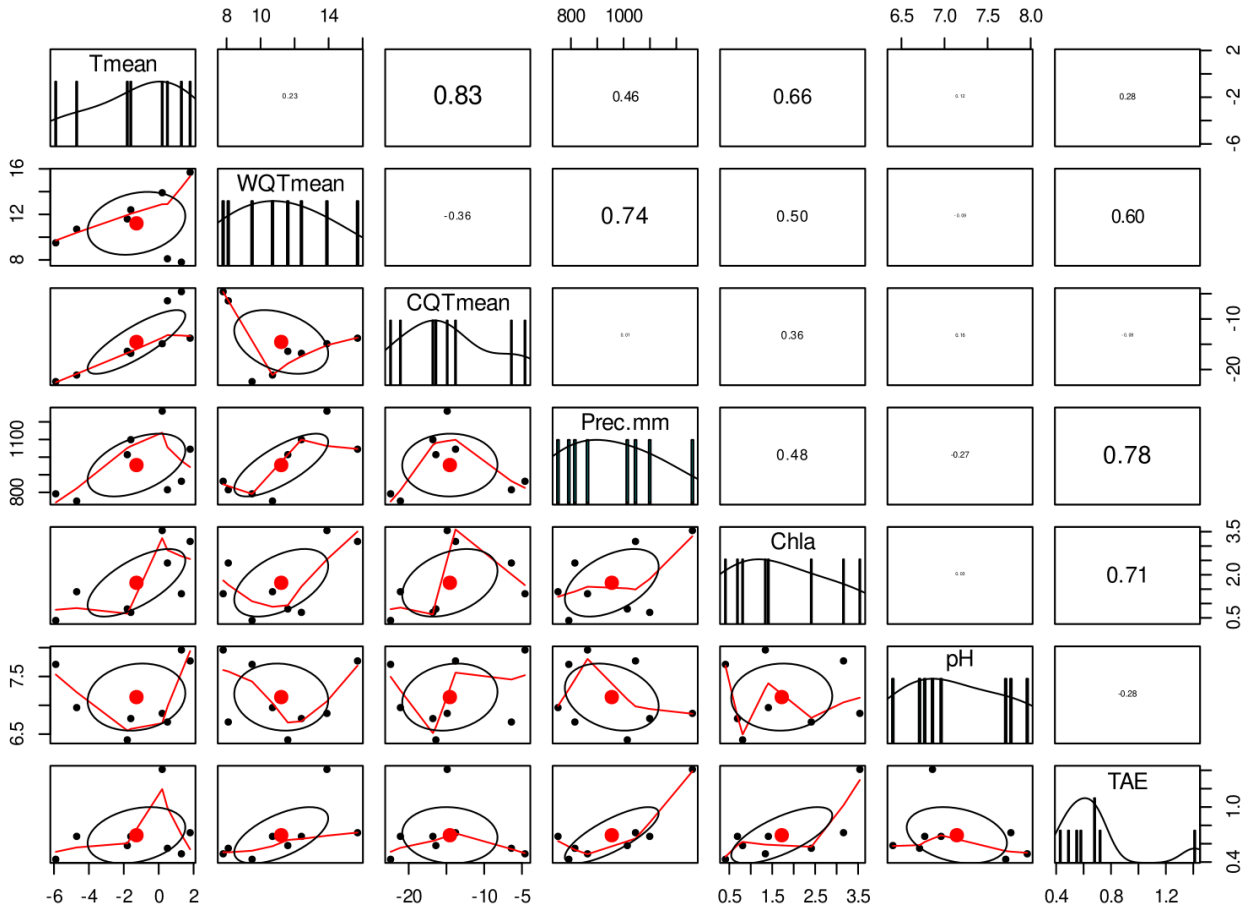


Figure C.3: Correlation between environmental covariables for the microgeographic gradient at Cape Race. Chla: chlorophyll A concentration in µg/L, Temperature_C: summer pond temperature, TAE: tannic acid equivalent in mg/L. Selected covariables for the aux model in Baypass are summer temperature and TAE.

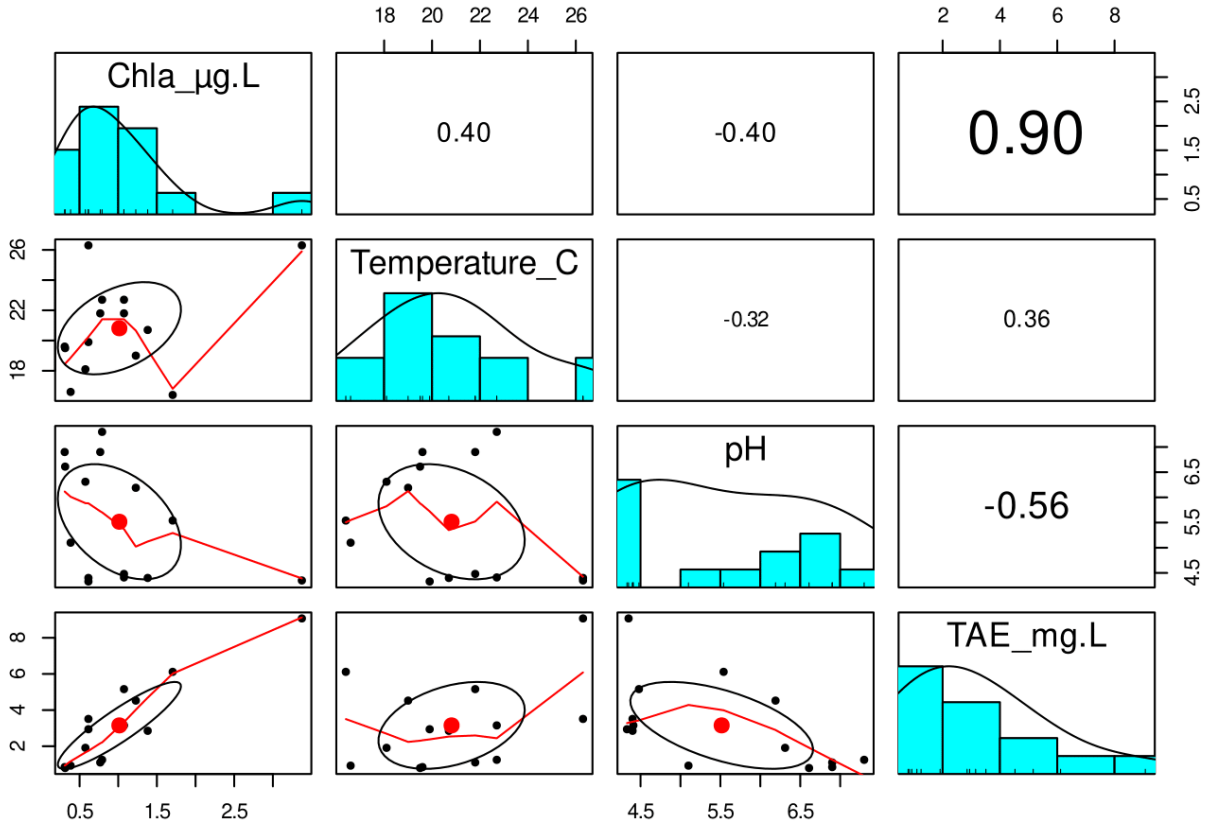


Figure C.4: Outliers SNPs identified in the latitudinal dataset with the core model in Baypass. P-values derived from the XtX statistic (on -log10 scale) are shown as a function of the XtX estimate. The horizontal dotted line indicates the threshold of p-values < 0.001 (threshold for outlier detection). Low XtX values indicate SNPs putatively under balancing selection, high XtX values indicate SNPs putatively under positive selection (overly differentiated SNPs)

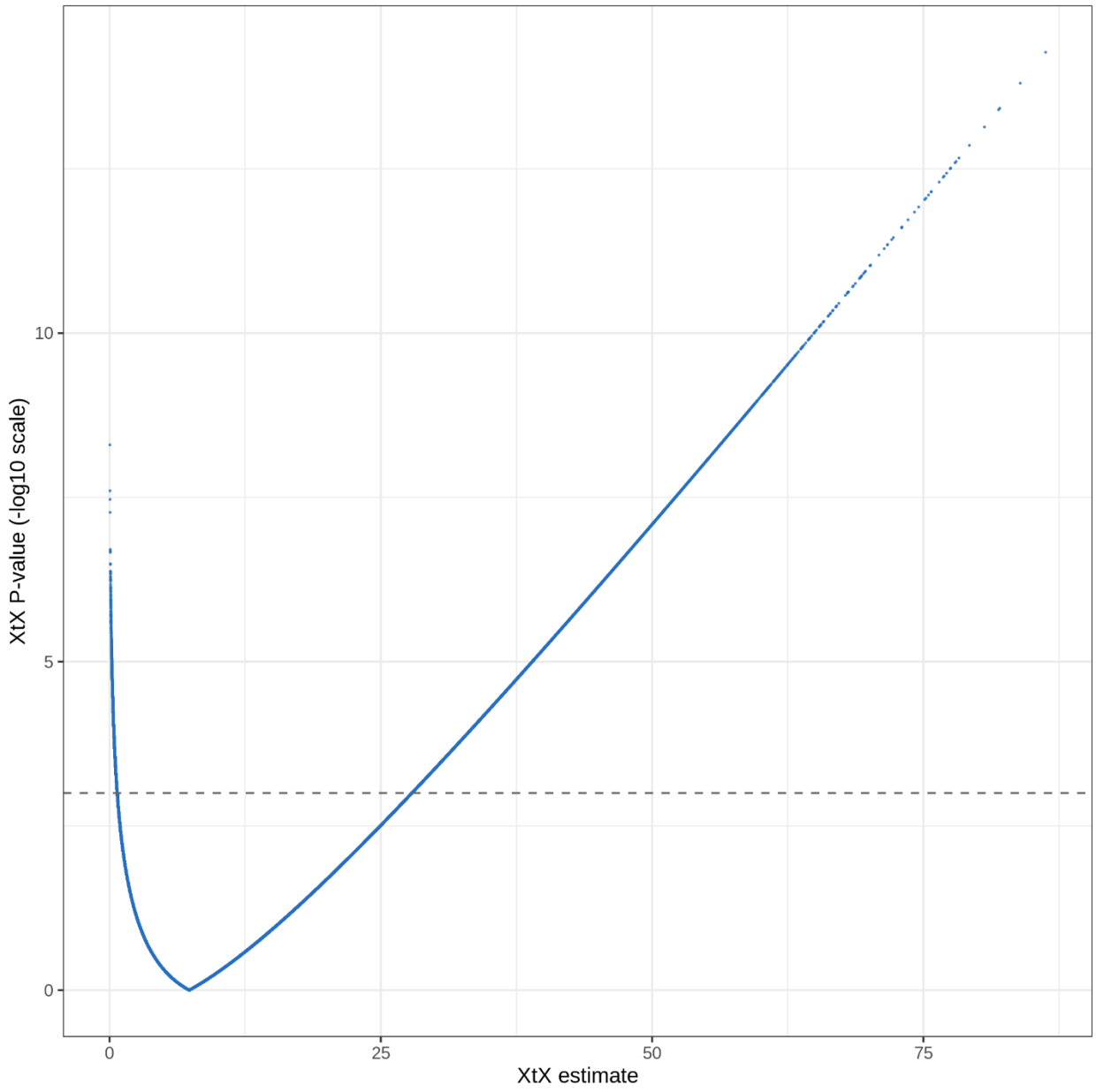


Figure C.5: Outliers SNPs identified in the Cape Race dataset with the core model in Baypass. As the shape of the histogram p-values derived from the XtX statistics showed that they were not well-behaved, we used a threshold value for XtX computed from a simulated pseudo-observed dataset (POD) to distinguish between selection and neutrality, which yielded a threshold value of XtX > 21.7 (shown as the vertical dotted line)

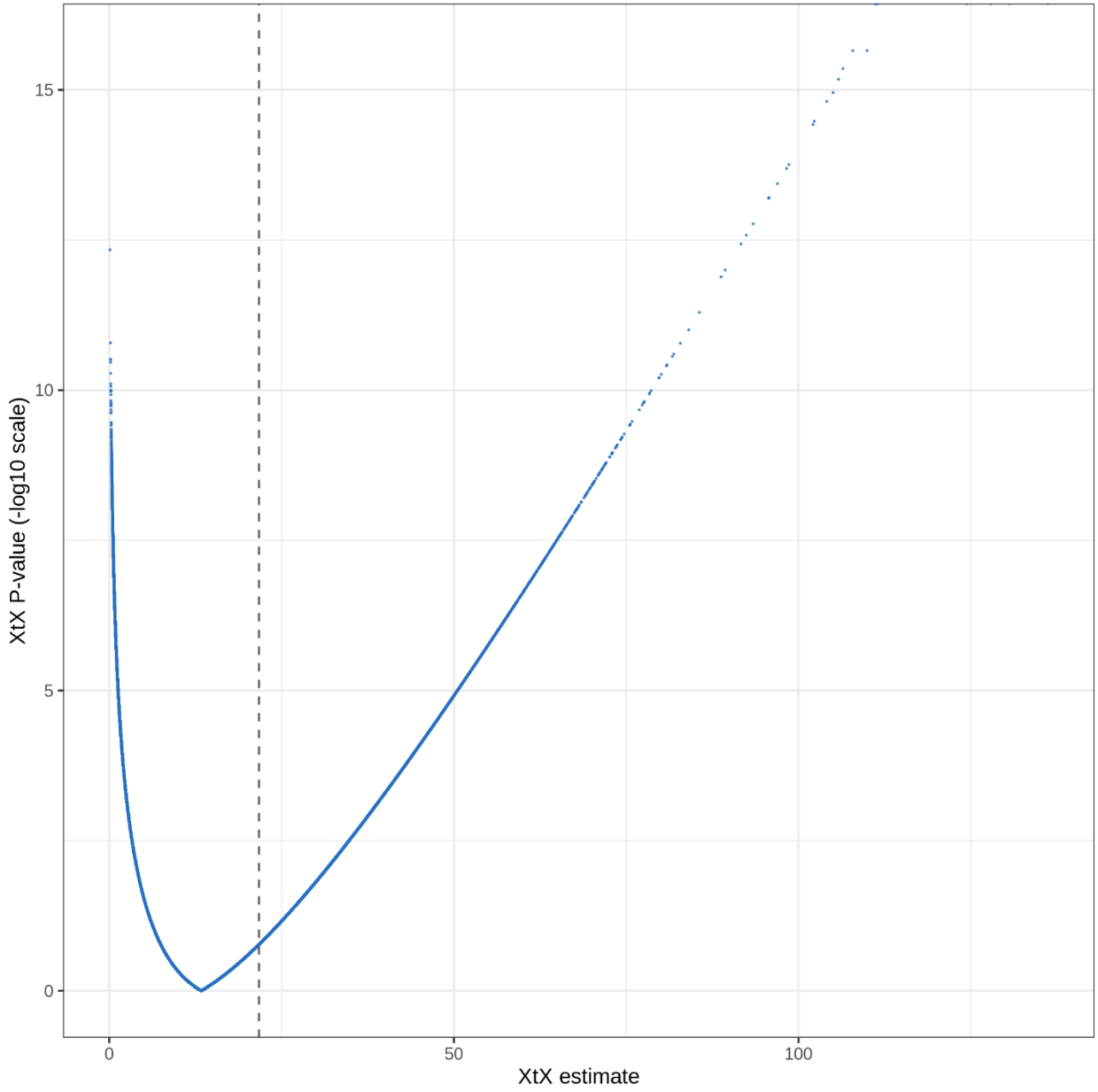


Figure C.6: Venn diagram of the outliers detected with the auxiliary model in Baypass in common between the covariables (temperature and TAE) and the spatial grdients. Outlier SNPs shared by the covariables/gradients are shown in the shaded areas.

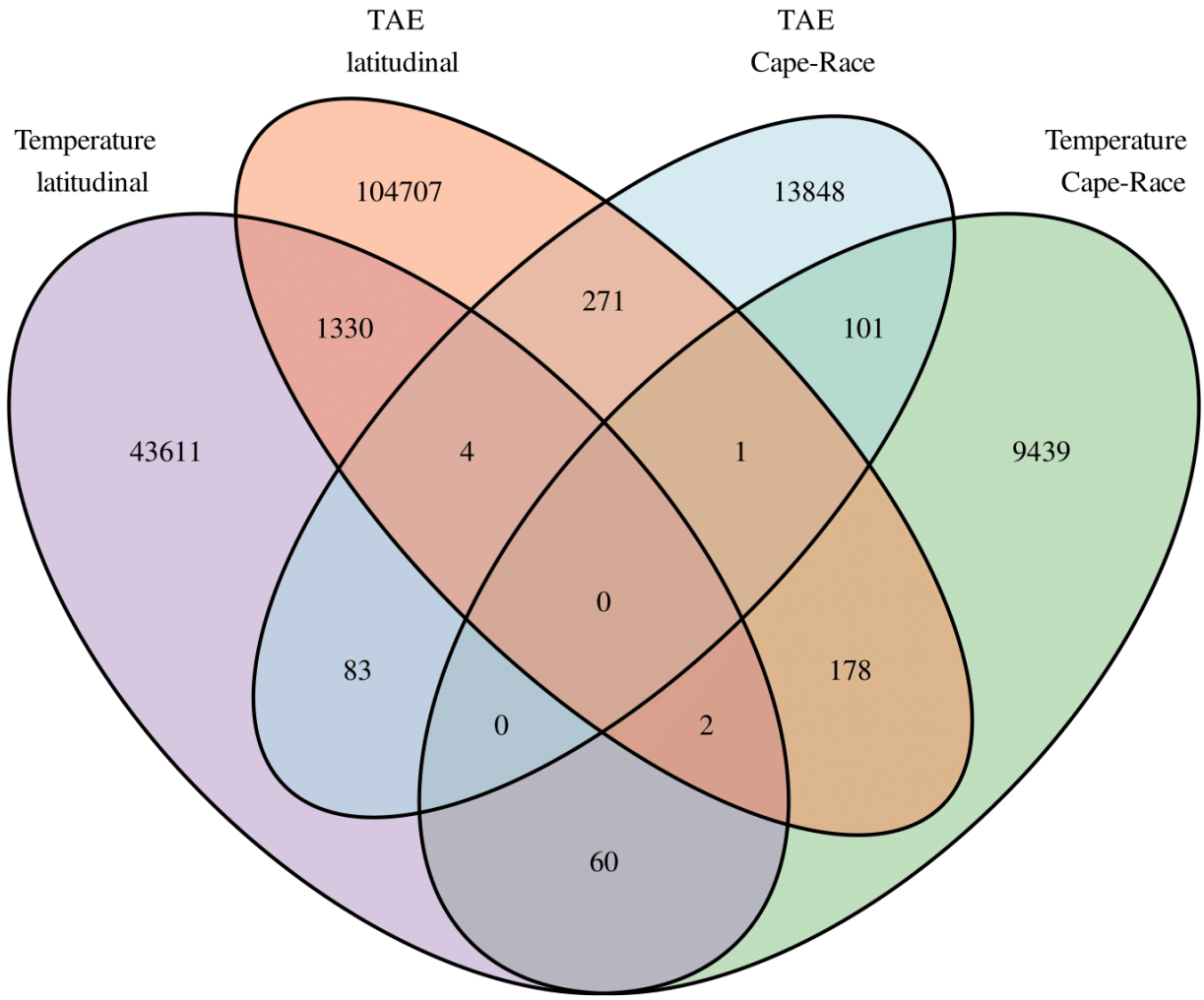


Figure C.7: Pairwise F_{ST} matrix between the eight latitudinal gradient populations

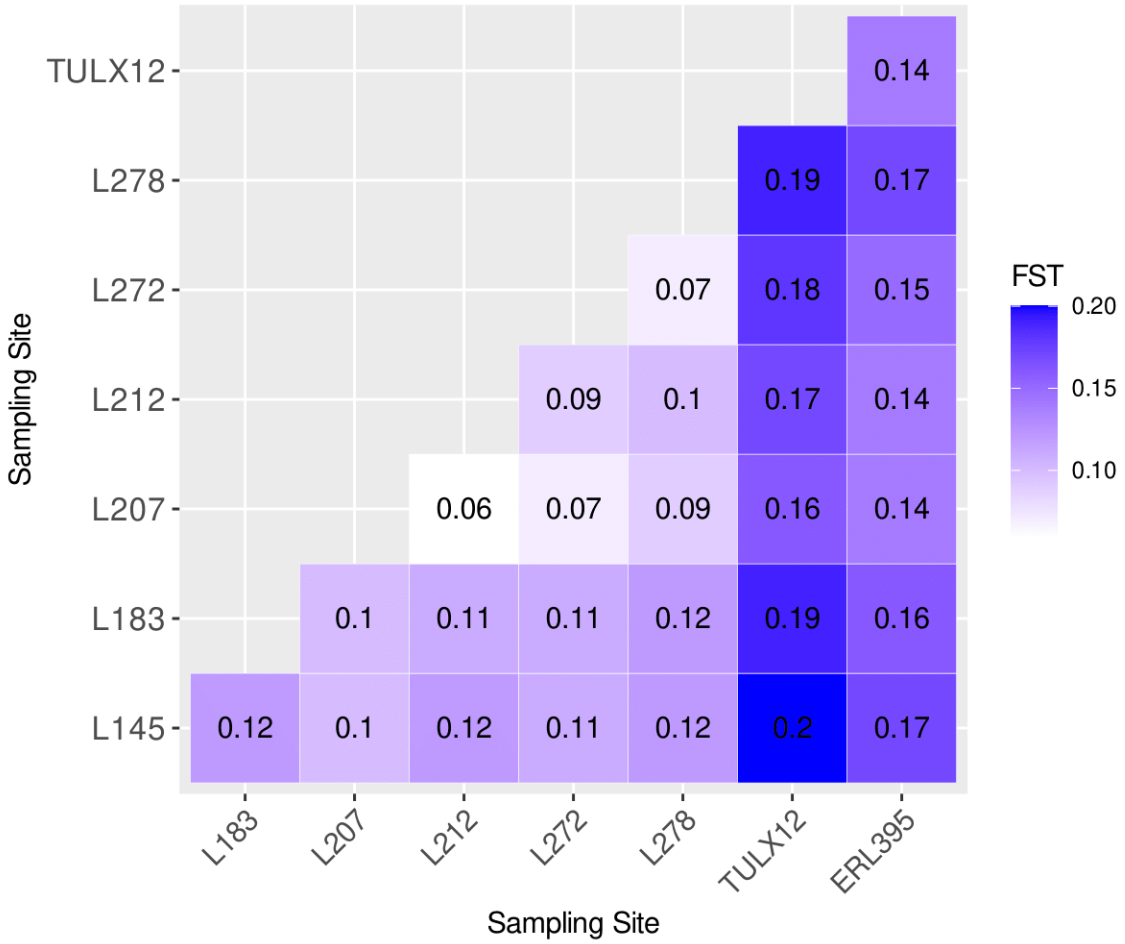
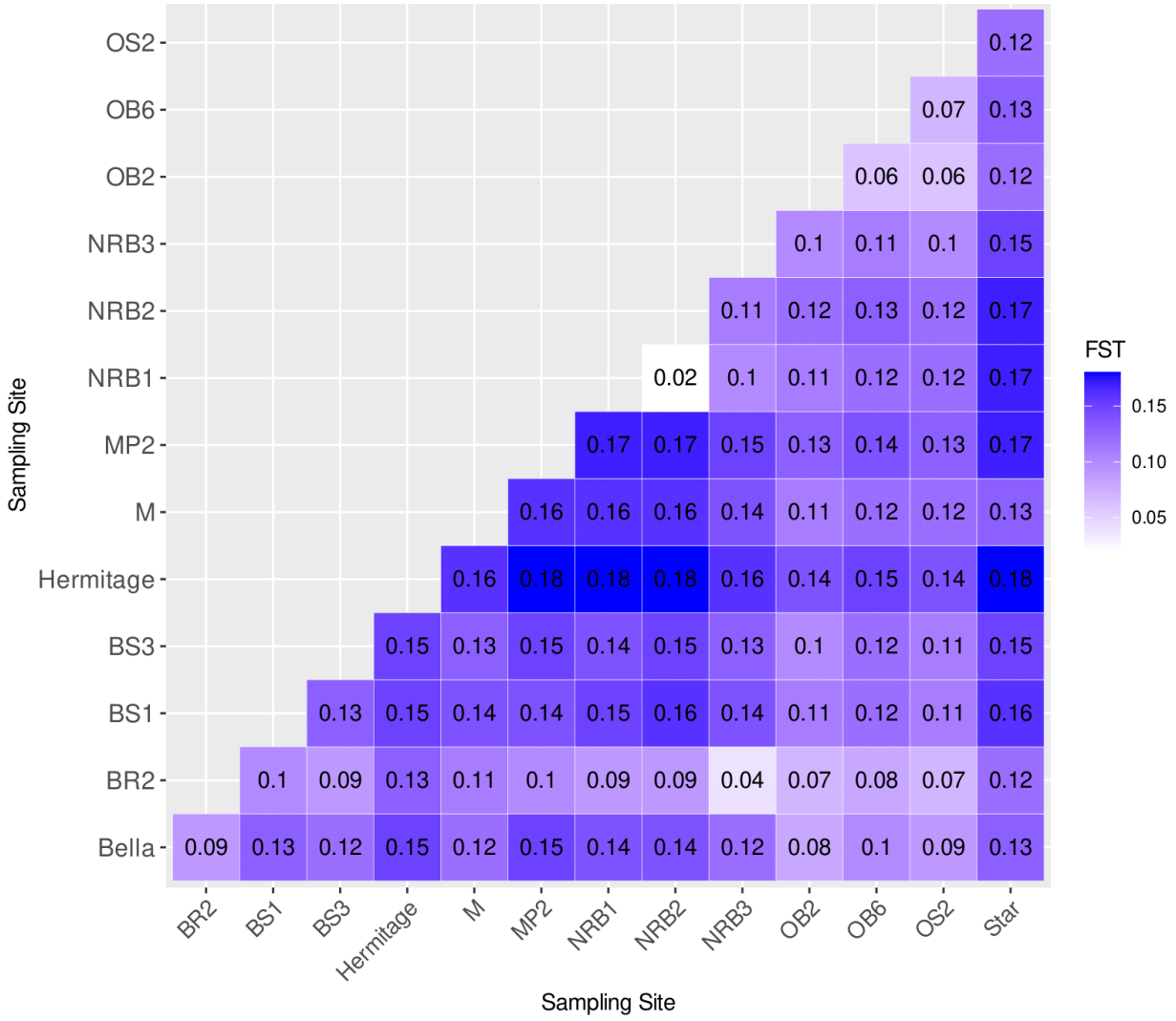


Figure C.8: Pairwise FST matrix between the 14 Cape Race populations



BIBLIOGRAPHY

Adrian, R., O'Reilly, C. M., Zagarese, H., Baines, S. B., Hessen, D. O., Keller, W., Livingstone, D. M., Sommaruga, R., Straile, D., van Donk, E., Weyhenmeyer, G. A., & Winder, M. (2009). Lakes as sentinels of climate change. Limnology and Oceanography, 54(6part2), 2283–2297.

Aguirre-Liguori, J. A., Ramírez-Barahona, S., & Gaut, B. S. (2021). The evolutionary genomics of species' responses to climate change. Nature Ecology and Evolution, 5(10), 1350–1360.

Ahrens, C. W., Rymer, P. D., Stow, A., Bragg, J., Dillon, S., Umbers, K. D. L., & Dudaniec, R. Y. (2018). The search for loci under selection: trends, biases and progress. Molecular Ecology, 27(6), 1342–1356.

Albert, J. S., Destouni, G., Duke-Sylvester, S. M., Magurran, A. E., Oberdorff, T., Reis, R. E., Winemiller, K. O., & Ripple, W. J. (2021). Scientists' warning to humanity on the freshwater biodiversity crisis. Ambio, 50(1), 85–94.

Alexander, H. K., Martin, G., Martin, O. Y., & Bonhoeffer, S. (2014). Evolutionary rescue: Linking theory for conservation and medicine. Evolutionary Applications, 7(10), 1161–1179.

Anciaux, Y., Chevin, L.-M., Ronce, O., & Martin, G. (2018). Evolutionary Rescue over a Fitness Landscape. Genetics, 209(1), 265–279.

Angert, A. L., Bontrager, M. G., & Ågren, J. (2020). What Do We Really Know About Adaptation at Range Edges? Annual Review of Ecology, Evolution, and Systematics, 51(1), 341–361.

Antonsson, Ú., & Antonsson, U. (1992). The Structure and Function of Zooplankton in Thingvallavatn, Iceland. Oikos, 64(1/2), 188.

Arietta, A. Z. A., & Skelly, D. K. (2021). Rapid microgeographic evolution in response to climate change. Evolution, 75(11), 2930–2943.

Arif, M., Gauthier, J., Sugier, K., Iudicone, D., Jaillon, O., Wincker, P., Peterlongo, P., & Madoui, M. A. (2019). Discovering millions of plankton genomic markers from the Atlantic Ocean and the Mediterranean Sea. Molecular Ecology Resources, 19(2), 526–535.

Ashander, J., Chevin, L.-M., & Baskett, M. L. (2016). Predicting evolutionary rescue via evolving plasticity in stochastic environments. Proceedings of the Royal Society B: Biological Sciences, 283(1839), 20161690.

Astorg, L., Charette, C., Windle, M. J. S., & Derry, A. M. (2022). Two decades since first invasion: Revisiting round goby impacts on nearshore aquatic communities in the Upper St. Lawrence River. Journal of Great Lakes Research, 48(2), 581–592.

Astorg, L., Sanderson, S., Côté-Gravel, V., Sorbara, F., Windle, M. J. S., Hendry, A. P., & Derry, A. M. (2021). Different refuge types dampen exotic invasion and enhance diversity at the

whole ecosystem scale in a heterogeneous river system. Biological Invasions, 23(2), 443–460.

Bailey, A., de Wit, P., Thor, P., Browman, H. I., Bjelland, R., Shema, S., Fields, D. M., Runge, J. A., Thompson, C., & Hop, H. (2017). Regulation of gene expression is associated with tolerance of the Arctic copepod Calanus glacialis to CO2-acidified sea water. Ecology and Evolution, 7(18), 7145–7160.

Baldwin, B. S., Carpenter, M., Rury, K., & Woodward, E. (2012). Low dissolved ions may limit secondary invasion of inland waters by exotic round gobies and dreissenid mussels in North America. Biological Invasions, 14(6), 1157–1175.

Barbiero, R. P., Rudstam, L. G., Watkins, J. M., & Lesht, B. M. (2019). A cross-lake comparison of crustacean zooplankton communities in the Laurentian Great Lakes, 1997–2016. Journal of Great Lakes Research, 45(3), 672–690.

Barghi, N., Hermisson, J., & Schlötterer, C. (2020). Polygenic adaptation: a unifying framework to understand positive selection. Nature Reviews Genetics, 21(12), 769–781.

Barrett, R. D. H., & Hoekstra, H. E. (2011). Molecular spandrels: Tests of adaptation at the genetic level. Nature Reviews Genetics, 12(11), 767–780.

Barrett, R. D. H., Laurent, S., Mallarino, R., Pfeifer, S. P., Xu, C. C. Y., Foll, M., Wakamatsu, K., Duke-Cohan, J. S., Jensen, J. D., & Hoekstra, H. E. (2019). Linking a mutation to survival in wild mice. Science, 363(6426), 499–504.

Barrett, R. D. H., & Schluter, D. (2008). Adaptation from standing genetic variation. Trends in Ecology and Evolution, 23(1), 38–44.

Bay, R. A., Harrigan, R. J., Underwood, V. le, Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. Science, 359(6371), 83–86.

Bay, R. A., Rose, N., Barrett, R., Bernatchez, L., Ghalambor, C. K., Lasky, J. R., Brem, R. B., Palumbi, S. R., & Ralph, P. (2017). Predicting Responses to Contemporary Environmental Change Using Evolutionary Response Architectures. The American Naturalist, 189(5), 463–473.

Bay, R. A., Rose, N. H., Logan, C. A., & Palumbi, S. R. (2017). Genomic models predict successful coral adaptation if future ocean warming rates are reduced. Science Advances, 3(11), 1–10.

Beamish, R. J., & Harvey, H. H. (1972). Acidification of the La Cloche Mountain Lakes, Ontario, and Resulting Fish Mortalities. Journal of the Fisheries Research Board of Canada, 29(8), 1131–1143.

Beichman, A. C., Huerta-Sanchez, E., & Lohmueller, K. E. (2018). Using Genomic Data to Infer Historic Population Dynamics of Nonmodel Organisms. Annual Review of Ecology, Evolution, and Systematics, 49(1), 433–456.

Bell, D. A., Robinson, Z. L., Funk, W. C., Fitzpatrick, S. W., Allendorf, F. W., Tallmon, D. A., & Whiteley, A. R. (2019). The Exciting Potential and Remaining Uncertainties of Genetic Rescue. Trends in Ecology and Evolution, 34(12), 1070–1079.

Bell, G. (2017). Evolutionary Rescue. Annual Review of Ecology, Evolution, and Systematics, 48(1), 605–627.

Bell, G., & Gonzalez, A. (2009). Evolutionary rescue can prevent extinction following environmental change. Ecology Letters, 12(9), 942–948.

Bell, G., & Gonzalez, A. (2011). Adaptation and Evolutionary Rescue in Metapopulations Experiencing Environmental Deterioration. Science, 332(6035), 1327–1330.

Bell, J. J. (2008). Similarity in connectivity patterns for two gastropod species lacking pelagic larvae. Marine Ecology Progress Series, 357, 185–194.

Bellard, C., Rysman, J., Leroy, B., Claud, C., & Mace, G. M. (2017). A global picture of biological invasion threat on islands. Nature Ecology & Evolution, 1(12), 1862–1869.

Bertrand, D., Shaw, J., Kalathiyappan, M., Ng, A. H. Q., Kumar, M. S., Li, C., Dvornicic, M., Soldo, J. P., Koh, J. Y., Tong, C., Ng, O. T., Barkham, T., Young, B., Marimuthu, K., Chng, K. R., Sikic, M., & Nagarajan, N. (2019). Hybrid metagenomic assembly enables high-resolution analysis of resistance determinants and mobile elements in human microbiomes. Nature Biotechnology, 37(8), 937–944.

Binford, M. W. (1990). Calculation and uncertainty analysis of 210Pb dates for PIRLA project lake sediment cores. Journal of Paleolimnology, 3(3), 253–267.

Bitter, M. C., Kapsenberg, L., Gattuso, J. P., & Pfister, C. A. (2019). Standing genetic variation fuels rapid adaptation to ocean acidification. Nature Communications, 10(1), 1–10.

Blanquart, F., Gandon, S., & Nuismer, S. L. (2012). The effects of migration and drift on local adaptation to a heterogeneous environment. Journal of Evolutionary Biology, 25(7), 1351–1363.

Boers, J. J., & Carter, J. C. H. (1978). Instar development rates of Diaptomus minutus (Copepoda: Calanoida) in a small lake in Quebec. Canadian Journal of Zoology, 56(8), 1710–1714.

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(1), 303–330.

Bolnick, D. I., & Nosil, P. (2007). Natural selection in populations subject to a migration load. Evolution, 61(9), 2229–2243.

Bono, L. M., Smith, L. B., Pfennig, D. W., & Burch, C. L. (2017). The emergence of performance trade-offs during local adaptation: insights from experimental evolution. Molecular Ecology, 26(7), 1720–1733.

Bourgeois, Y. X. C., & Warren, B. H. (2021). An overview of current population genomics methods for the analysis of whole-genome resequencing data in eukaryotes. Molecular Ecology, 30(23), 6036–6071.

Boyle, E. A., Li, Y. I., & Pritchard, J. K. (2017). An Expanded View of Complex Traits: From Polygenic to Omnigenic. Cell, 169(7), 1177–1186.

Bradley, B. A., Laginhas, B. B., Whitlock, R., Allen, J. M., Bates, A. E., Bernatchez, G., Diez, J. M., Early, R., Lenoir, J., Vilà, M., & Sorte, C. J. B. (2019). Disentangling the abundance–impact relationship for invasive species. Proceedings of the National Academy of Sciences of the United States of America, 116(20), 9919–9924.

Bradshaw, W. E., & Holzapfel, C. M. (2008). Genetic response to rapid climate change: It's seasonal timing that matters. Molecular Ecology, 17(1), 157–166.

Brady, S. P., Bolnick, D. I., Angert, A. L., Gonzalez, A., Barrett, R. D. H., Crispo, E., Derry, A. M., Eckert, C. G., Fraser, D. J., Fussmann, G. F., Guichard, F., Lamy, T., McAdam, A. G., Newman, A. E. M., Paccard, A., Rolshausen, G., Simons, A. M., & Hendry, A. P. (2019). Causes of maladaptation. Evolutionary Applications, 12(7), 1229–1242.

Brans, K. I., & De Meester, L. (2018). City life on fast lanes: Urbanization induces an evolutionary shift towards a faster lifestyle in the water flea Daphnia. Functional Ecology, 32(9), 2225–2240.

Brennan, R. S., DeMayo, J. A., Dam, H. G., Finiguerra, M. B., Baumann, H., & Pespeni, M. H. (2022). Loss of transcriptional plasticity but sustained adaptive capacity after adaptation to global change conditions in a marine copepod. Nature Communications, 13(1), 1–13.

Brennan, R. S., deMayo, J. A., Dam, H. G., Finiguerra, M., Baumann, H., Buffalo, V., & Pespeni, M. H. (2022). Experimental evolution reveals the synergistic genomic mechanisms of adaptation to ocean warming and acidification in a marine copepod. Proceedings of the National Academy of Sciences of the United States of America, 119(38), 1–10.

Broman, E., Brüsin, M., Dopson, M., & Hylander, S. (2015). Oxygenation of anoxic sediments triggers hatching of zooplankton eggs. Proceedings of the Royal Society B: Biological Sciences, 282(1817), 20152025.

Bron, J. E., Frisch, D., Goetze, E., Johnson, S. C., Lee, C., & Wyngaard, G. A. (2011). Observing copepods through a genomic lens. Frontiers in Zoology, 8(1), 22.

Brookes, J. I., & Rochette, R. (2007). Mechanism of a plastic phenotypic response: Predator-induced shell thickening in the intertidal gastropod Littorina obtusata. Journal of Evolutionary Biology, 20(3), 1015–1027.

Brousseau, L., Fine, P. V. A., Dreyer, E., Vendramin, G. G., & Scotti, I. (2021). Genomic and phenotypic divergence unveil microgeographic adaptation in the Amazonian hyperdominant tree Eperua falcata Aubl. (Fabaceae). Molecular Ecology, 30(5), 1136–1154.

Buffalo, V., & Coop, G. (2020). Estimating the genome-wide contribution of selection to temporal allele frequency change. Proceedings of the National Academy of Sciences of the United States of America, 117(34), 20672–20680.

Bukowski, S. J., & Auld, J. R. (2014). The effects of calcium in mediating the inducible morphological defenses of a freshwater snail, Physa acuta. Aquatic Ecology, 48(1), 85–90.

Bulmer, M. G. (1972). Multiple Niche Polymorphism. The American Naturalist, 106(948), 254–257.

Burge, D. R. L., Edlund, M. B., & Frisch, D. (2018). Paleolimnology and resurrection ecology: The future of reconstructing the past. Evolutionary Applications, 11(1), 42–59.

Burns, C. W., Brett, M. T., & Schallenberg, M. (2011). A comparison of the trophic transfer of fatty acids in freshwater plankton by cladocerans and calanoid copepods. Freshwater Biology, 56(5), 889–903.

Caizergues, A. E., le Luyer, J., Grégoire, A., Szulkin, M., Senar, J., Charmantier, A., & Perrier, C. (2022). Epigenetics and the city: Non ‐ parallel DNA methylation modifications across pairs of urban ‐ forest Great tit populations. Evolutionary Applications, 15(1), 149–165.

Calosi, P., Melatunan, S., Turner, L. M., Artioli, Y., Davidson, R. L., Byrne, J. J., Viant, M. R., Widdicombe, S., & Rundle, S. D. (2017). Regional adaptation defines sensitivity to future ocean acidification. Nature Communications, 8(May 2016).

Carlson, S. M., Cunningham, C. J., & Westley, P. A. H. (2014). Evolutionary rescue in a changing world. Trends in Ecology and Evolution, 29(9), 521–530.

Carpenter, S. R., Stanley, E. H., & vander Zanden, M. J. (2011). State of the World's Freshwater Ecosystems: Physical, Chemical, and Biological Changes. Annual Review of Environment and Resources, 36(1), 75–99.

Chapman, L. J., Chapman, C. A., Nordlie, F. G., & Rosenberger, A. E. (2002). Physiological refugia: Swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 133(3), 421–437.

Charette, C., & Derry, A. M. (2016). Climate alters intraspecific variation in copepod effect traits through pond food webs. Ecology, 97(5), 1239–1250.

Chaturvedi, A., Zhou, J., Raeymaekers, J. A. M., Czypionka, T., Orsini, L., Jackson, C. E., Spanier, K. I., Shaw, J. R., Colbourne, J. K., & De Meester, L. (2021). Extensive standing genetic variation from a small number of founders enables rapid adaptation in Daphnia. Nature Communications, 12(1), 1–10.

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34(17), i884–i890.

Chikhi, R., & Rizk, G. (2013). Space-efficient and exact de Bruijn graph representation based on a Bloom filter. Algorithms for Molecular Biology, 8(1), 1–9.

Christe, C., Stölting, K. N., Paris, M., Fraïsse, C., Bierne, N., & Lexer, C. (2017). Adaptive evolution and segregating load contribute to the genomic landscape of divergence in two tree species connected by episodic gene flow. Molecular Ecology, 26(1), 59–76.

Clark, M. S., Peck, L. S., Arivalagan, J., Backeljau, T., Berland, S., Cardoso, J. C. R., Caurcel, C., Chapelle, G., de Noia, M., Dupont, S., Gharbi, K., Hoffman, J. I., Last, K. S., Marie, A., Melzner, F., Michalek, K., Morris, J., Power, D. M., Ramesh, K., … Harper, E. M. (2020). Deciphering mollusc shell production: the roles of genetic mechanisms through to ecology, aquaculture and biomimetics. Biological Reviews, 95(6), 1812–1837.

Coffman, A. J., Hsieh, P. H., Gravel, S., & Gutenkunst, R. N. (2016). Computationally efficient composite likelihood statistics for demographic inference. Molecular Biology and Evolution, 33(2), 591–593.

Colbourne, J. K., Pfrender, M. E., Gilbert, D., Thomas, W. K., Tucker, A., Oakley, T. H., Tokishita, S., Aerts, A., Arnold, G. J., Basu, M. K., Bauer, D. J., Cáceres, C. E., Carmel, L., Casola, C., Choi, J.-H., Detter, J. C., Dong, Q., Dusheyko, S., Eads, B. D., … Boore, J. L. (2011). The ecoresponsive genome of Daphnia pulex. Science (New York, N.Y.), 331(6017), 555–561.

Crandall, E. D., Taffel, J. R., & Barber, P. H. (2010). High gene flow due to pelagic larval dispersal among South Pacific archipelagos in two amphidromous gastropods (Neritomorpha: Neritidae). Heredity, 104(6), 563–572.

Crespo, D., Dolbeth, M., Leston, S., Sousa, R., & Pardal, M. Â. (2015). Distribution of Corbicula fluminea (Müller, 1774) in the invaded range: a geographic approach with notes on species traits variability. Biological Invasions, 17(7), 2087–2101.

Crispo, E. (2008). Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. Journal of Evolutionary Biology, 21(6), 1460–1469.

Crispo, E., DiBattista, J. D., Correa, C., Thibert-Plante, X., McKellar, A. E., Schwartz, A. K., Berner, D., de León, L. F., & Hendry, A. P. (2010). The evolution of phenotypic plasticity in response to anthropogenic disturbance. Evolutionary Ecology Research, 12(1), 47–66.

Cure, K., Thomas, L., Hobbs, J. P. A., Fairclough, D. v., & Kennington, W. J. (2017). Genomic signatures of local adaptation reveal source-sink dynamics in a high gene flow fish species. Scientific Reports, 7(1), 1–10.

Cuthbert, I. D., & del Giorgio, P. (1992). Toward a standard method of measuring color in freshwater. Limnology and Oceanography, 37(6), 1319–1326.

Czypionka, T., Fields, P. D., Routtu, J., van den Berg, E., Ebert, D., & de Meester, L. (2019). The genetic architecture underlying diapause termination in a planktonic crustacean. Molecular Ecology, 28(5), 998–1008.

Dam, H. G., deMayo, J. A., Park, G., Norton, L., He, X., Finiguerra, M. B., Baumann, H., Brennan, R. S., & Pespeni, M. H. (2021). Rapid, but limited, zooplankton adaptation to simultaneous warming and acidification. Nature Climate Change, 11(9), 780–786.

Damgaard, C. (2019). A Critique of the Space-for-Time Substitution Practice in Community Ecology. Trends in Ecology & Evolution, 34(5), 416–421.

Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., & Blaxter, M. L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics, 12(7), 499–510.

de Kort, H., Prunier, J. G., Ducatez, S., Honnay, O., Baguette, M., Stevens, V. M., & Blanchet, S. (2021). Life history, climate and biogeography interactively affect worldwide genetic diversity of plant and animal populations. Nature Communications, 12(1), 516.

de Meester, L., Gómez, A., Okamura, B., & Schwenk, K. (2002). The Monopolization Hypothesis and the dispersal-gene flow paradox in aquatic organisms. Acta Oecologica, 23(3), 121–135.

de Meester, L., Vanoverbeke, J., Kilsdonk, L. J., & Urban, M. C. (2016). Evolving Perspectives on Monopolization and Priority Effects. Trends in Ecology and Evolution, 31(2), 136–146.

de Villemereuil, P., Gaggiotti, O. E., Mouterde, M., & Till-Bottraud, I. (2016). Common garden experiments in the genomic era: new perspectives and opportunities. Heredity, 116(3), 249–254.

De Wit, P., Dupont, S., & Thor, P. (2016). Selection on oxidative phosphorylation and ribosomal structure as a multigenerational response to ocean acidification in the common copepod Pseudocalanus acuspes. Evolutionary Applications, 9(9), 1112–1123.

DeBiasse, M. B., Kawji, Y., & Kelly, M. W. (2018). Phenotypic and transcriptomic responses to salinity stress across genetically and geographically divergent Tigriopus californicus populations. Molecular Ecology, 27(7), 1621–1632.

Derksen, C., Smith, S. L., Sharp, M., Brown, L., Howell, S., Copland, L., Mueller, D. R., Gauthier, Y., Fletcher, C. G., Tivy, A., Bernier, M., Bourgeois, J., Brown, R., Burn, C. R., Duguay, C., Kushner, P., Langlois, A., Lewkowicz, A. G., Royer, A., & Walker, A. (2012). Variability and change in the Canadian cryosphere. Climatic Change, 115(1), 59–88.

Derry, A. M., & Arnott, S. E. (2007). Adaptive reversals in acid tolerance in copepods from lakes recovering from historical stress. Ecological Applications, 17(4), 1116–1126.

Derry, A. M., Arnott, S. E., & Boag, P. T. (2010). Evolutionary shifts in copepod acid tolerance in an acid-recovering lake indicated by resurrected resting eggs. Evolutionary Ecology, 24(1), 133–145.

Derry, A. M., Arnott, S. E., Shead, J. A., Hebert, P. D. N., & Boag, P. T. (2009). Ecological linkages between community and genetic diversity in zooplankton among boreal shield lakes. Ecology, 90(8), 2275–2286.

Derry, A. M., Kestrup, Å. M., & Hendry, A. P. (2013). Possible influences of plasticity and genetic/maternal effects on species coexistence: Native Gammarus fasciatus facing exotic amphipods. Functional Ecology, 27(5), 1212–1223.

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. Nature Ecology & Evolution, 2(1), 57–64.

Dextrase, A. J., & Mandrak, N. E. (2006). Impacts of alien invasive species on freshwater fauna at risk in Canada. Biological Invasions, 8(1), 13–24.

Dixit, A. S., Dixit, S. S., & Smol, J. P. (1992). Long-Term Trends in Lake Water pH and Metal Concentrations Inferred from Diatoms and Chrysophytes in Three Lakes near Sudbury, Ontario. Canadian Journal of Fisheries and Aquatic Sciences, 49(S1), 17–24.

Dixit, S. S., Dixit, A. S., & Smol, J. P. (2002). Diatom and chrysophyte transfer functions and inferences of post-industrial acidification and recent recovery trends in Killarney lakes (Ontario, Canada). Journal of Paleolimnology, 27(1), 79–96.

Dixon, G. B., Davies, S. W., Aglyamova, G. v., Meyer, E., Bay, L. K., & Matz, M. v. (2015). Genomic determinants of coral heat tolerance across latitudes. Science, 348(6242), 1460–1462.

Donelan, S. C., Hellmann, J. K., Bell, A. M., Luttbeg, B., Orrock, J. L., Sheriff, M. J., & Sih, A. (2020). Transgenerational Plasticity in Human-Altered Environments. Trends in Ecology and Evolution, 35(2), 115–124.

Dueñas, M.-A., Hemming, D. J., Roberts, A., & Diaz-Soltero, H. (2021). The threat of invasive species to IUCN-listed critically endangered species: A systematic review. Global Ecology and Conservation, 26, e01476.

Duputié, A., Rutschmann, A., Ronce, O., & Chuine, I. (2015). Phenological plasticity will not help all species adapt to climate change. Global Change Biology, 21(8), 3062–3073.

Early, R., Bradley, B. A., Dukes, J. S., Lawler, J. J., Olden, J. D., Blumenthal, D. M., Gonzalez, P., Grosholz, E. D., Ibañez, I., Miller, L. P., Sorte, C. J. B., & Tatem, A. J. (2016). Global threats from invasive alien species in the twenty-first century and national response capacities. Nature Communications, 7(1), 12485.

Edelaar, P., Baños-Villalba, A., Quevedo, D. P., Escudero, G., Bolnick, D. I., & Jordán-Andrade, A. (2019). Biased movement drives local cryptic coloration on distinct urban pavements. Proceedings of the Royal Society B: Biological Sciences, 286(1912), 20191343.

Edmands, S. (2007). Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology, 16(3), 463–475.

Ellegaard, M., Clokie, M. R. J., Czypionka, T., Frisch, D., Godhe, A., Kremp, A., Letarov, A., McGenity, T. J., Ribeiro, S., & John Anderson, N. (2020). Dead or alive: sediment DNA archives as tools for tracking aquatic evolution and adaptation. Communications Biology, 3(1), 1–11.

Ellner, S. P., Hairston Jr., N. G., Kearns, C. M., & Babai, D. (1999). The roles of fluctuating selection and long-term diapause in microevolution of diapause timing in a freshwater copepod. Evolution, 53(1), 111–122.

Emery-Butcher, H. E., Beatty, S. J., & Robson, B. J. (2020). The impacts of invasive ecosystem engineers in freshwaters: A review. Freshwater Biology, 65(5), 999–1015.

Estlander, S., Nurminen, L., Olin, M., Vinni, M., Immonen, S., Rask, M., Ruuhijärvi, J., Horppila, J., & Lehtonen, H. (2010). Diet shifts and food selection of perch Perca fluviatilis and roach Rutilus rutilus in humic lakes of varying water colour. Journal of Fish Biology, 77(1), 241–256.

Excoffier, L., Foll, M., & Petit, R. J. (2009). Genetic Consequences of Range Expansions. Annual Review of Ecology, Evolution, and Systematics, 40(1), 481–501.

Eyun, S. il. (2017). Phylogenomic analysis of Copepoda (Arthropoda, Crustacea) reveals unexpected similarities with earlier proposed morphological phylogenies. BMC Evolutionary Biology, 17(1), 1–12.

Falk, J. J., Parent, C. E., Agashe, D., & Bolnick, D. I. (2012). Drift and selection entwined asymmetric reproductive isolation in an experimental niche shift. Evolutionary Ecology Research, 14(4), 403–423.

Feder, A. F., Petrov, D. A., & Bergland, A. O. (2012). LDx: Estimation of Linkage Disequilibrium from High-Throughput Pooled Resequencing Data. PLoS ONE, 7(11), 1–7.

Fenster, C. B., & Galloway, L. F. (2000). Inbreeding and outbreeding depression in natural populations of Chamaecrista fasciculata (Fabaceae). Conservation Biology, 14(5), 1406–1412.

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology, 37(12), 4302–4315.

Findlay, D. L. (2003). Response of Phytoplankton Communities to Acidification and Recovery in Killarney Park and the Experimental Lakes Area, Ontario. AMBIO: A Journal of the Human Environment, 32(3), 190–195.

Fitzpatrick, S. W., Bradburd, G. S., Kremer, C. T., Salerno, P. E., Angeloni, L. M., & Funk, W. C. (2020). Genomic and Fitness Consequences of Genetic Rescue in Wild Populations. Current Biology, 30(3), 517-522.e5.

Fitzpatrick, S. W., Gerberich, J. C., Angeloni, L. M., Bailey, L. L., Broder, E. D., Torres-Dowdall, J., Handelsman, C. A., López-Sepulcre, A., Reznick, D. N., Ghalambor, C. K., & Chris Funk, W. (2016). Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. Evolutionary Applications, 9(7), 879–891.

Foppen, R. P. B., Chardon, J. P., & Liefveld, W. (2000). Understanding the role of sink patches in source-sink metapopulations: Reed Warbler in an agricultural landscape. Conservation Biology, 14(6), 1881–1892.

Forester, B. R., Beever, E. A., Darst, C., Szymanski, J., & Funk, W. C. (2022). Linking evolutionary potential to extinction risk: applications and future directions. Frontiers in Ecology and the Environment, 20(9), 507–515.

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. Philosophical Transactions of the Royal Society B: Biological Sciences, 374(1768), 20180174.

François, O., Martins, H., Caye, K., & Schoville, S. D. (2016). Controlling false discoveries in genome scans for selection. Molecular Ecology, 25(2), 454–469.

Frankham, R., Ballou, J. D., Eldridge, M. D. B., Lacy, R. C., Ralls, K., Dudash, M. R., & Fenster, C. B. (2011). Predicting the Probability of Outbreeding Depression. Conservation Biology, 25(3), 465–475.

Franks, S. J., Hamann, E., & Weis, A. E. (2018). Using the resurrection approach to understand contemporary evolution in changing environments. Evolutionary Applications, 11(1), 17–28.

Frisch, D., Morton, P. K., Chowdhury, P. R., Culver, B. W., Colbourne, J. K., Weider, L. J., & Jeyasingh, P. D. (2014). A millennial-scale chronicle of evolutionary responses to cultural eutrophication in Daphnia. Ecology Letters, 17(3), 360–368.

Frost, T. M., Fischer, J. M., Klug, J. L., Arnott, S. E., & Montz, P. K. (2006). Trajectories of zooplankton recovery in the Little Rock Lake whole-lake acidification experiment. Ecological Applications, 16(1), 353–367.

Gaitán‐Espitia, J. D., & Hobday, A. J. (2021). Evolutionary principles and genetic considerations for guiding conservation interventions under climate change. Global Change Biology, 27(3), 475–488.

Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology, 22(1), 151–163.

Gamboa, M. P., Ghalambor, C. K., Scott Sillett, T., Morrison, S. A., & Chris Funk, W. (2022). Adaptive divergence in bill morphology and other thermoregulatory traits is facilitated by

restricted gene flow in song sparrows on the California Channel Islands. Molecular Ecology, 31(2), 603–619.

Garcia‐Elfring, A., Paccard, A., Thurman, T. J., Wasserman, B. A., Palkovacs, E. P., Hendry, A. P., & Barrett, R. D. H. (2021). Using seasonal genomic changes to understand historical adaptation to new environments: Parallel selection on stickleback in highly‐variable estuaries. Molecular Ecology, 30(9), 2054‐2064.

Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. Genetics, 201(4), 1555–1579.

Gautier, M., Foucaud, J., Gharbi, K., Cézard, T., Galan, M., Loiseau, A., Thomson, M., Pudlo, P., Kerdelhué, C., & Estoup, A. (2013). Estimation of population allele frequencies from next-generation sequencing data: Pool-versus individual-based genotyping. Molecular Ecology, 22(14), 3766–3779.

Geerts, A. N., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feuchtmayr, H., Atkinson, D., Moss, B., Davidson, T. A., Sayer, C. D., & De Meester, L. (2015). Rapid evolution of thermal tolerance in the water flea Daphnia. Nature Climate Change, 5(7), 665–668.

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(3), 394–407.

Gignoux‐Wolfsohn, S. A., Pinsky, M. L., Kerwin, K., Herzog, C., Hall, M., Bennett, A. B., Fefferman, N. H., & Maslo, B. (2021). Genomic signatures of selection in bats surviving white‐nose syndrome. Molecular Ecology, 30(22), 5643‐5657.

Gompert, Z., Lucas, L. K., Buerkle, C. A., Forister, M. L., Fordyce, J. A., & Nice, C. C. (2014). Admixture and the organization of genetic diversity in a butterfly species complex revealed through common and rare genetic variants. Molecular Ecology, 23(18), 4555–4573.

Gompert, Z., Springer, A., Brady, M., Chaturvedi, S., & Lucas, L. K. (2021). Genomic time‐series data show that gene flow maintains high genetic diversity despite substantial genetic drift in a butterfly species. Molecular Ecology, 30(20), 4991‐5008.

Gomulkiewicz, R., & Holt, R. D. (1995). When does Evolution by Natural Selection Prevent Extinction? Evolution, 49(1), 201.

Grant, P. R., & Grant, B. R. (2002). Unpredictable Evolution in a 30-Year Study of Darwin's Finches. Science, 296(5568), 707–711.

Gray, D. K., & Arnott, S. E. (2011). Does dispersal limitation impact the recovery of zooplankton communities damaged by a regional stressor? Ecological Applications, 21(4), 1241–1256.

Gray, D. K., & Arnott, S. E. (2012). The role of dispersal levels, Allee effects and community resistance as zooplankton communities respond to environmental change. Journal of Applied Ecology, 49(6), 1216–1224.

Gray, D. K., Arnott, S. E., Shead, J. A., & Derry, A. M. (2012). The recovery of acid-damaged zooplankton communities in Canadian Lakes: The relative importance of abiotic, biotic and spatial variables. Freshwater Biology, 57(4), 741–758.

Griffiths, J. S., Kawji, Y., & Kelly, M. W. (2021). An Experimental Test of Adaptive Introgression in Locally Adapted Populations of Splash Pool Copepods. Molecular Biology and Evolution, 38(4), 1306–1316.

Grosbois, G., Mariash, H., Schneider, T., & Rautio, M. (2017). Under-ice availability of phytoplankton lipids is key to freshwater zooplankton winter survival. Scientific Reports, 7(1), 11543.

Grosbois, G., & Rautio, M. (2018). Active and colorful life under lake ice. Ecology, 99(3), 752–754.

Grummer, J. A., Beheregaray, L. B., Bernatchez, L., Hand, B. K., Luikart, G., Narum, S. R., & Taylor, E. B. (2019). Aquatic Landscape Genomics and Environmental Effects on Genetic Variation. Trends in Ecology and Evolution, 34(7), 641–654.

Gugger, P. F., Fitz-Gibbon, S. T., Albarrán-Lara, A., Wright, J. W., & Sork, V. L. (2021). Landscape genomics of Quercus lobata reveals genes involved in local climate adaptation at multiple spatial scales. Molecular Ecology, 30(2), 406–423.

Gunderson, A. R., & Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proceedings of the Royal Society B: Biological Sciences, 282(1808).

Günther, T., & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. Genetics, 195(1), 205–220.

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the Joint Demographic History of Multiple Populations from Multidimensional SNP Frequency Data. PLoS Genetics, 5(10), e1000695.

Gyllström, M., & Hansson, L.-A. (2004). Dormancy in freshwater zooplankton: Induction, termination and the importance of benthic-pelagic coupling. Aquatic Sciences, 66(3), 274–295.

Haenel, Q., Guerard, L., MacColl, A. D. C., & Berner, D. (2022). The maintenance of standing genetic variation: Gene flow vs. selective neutrality in Atlantic stickleback fish. Molecular Ecology, 31(3), 811–821.

Häfker, N. S., Teschke, M., Last, K. S., Pond, D. W., Hüppe, L., & Meyer, B. (2018). Calanus finmarchicus seasonal cycle and diapause in relation to gene expression, physiology, and endogenous clocks. Limnology and Oceanography, 63(6), 2815–2838.

Hairston, N. G., & Dillon, T. A. (1990). Fluctuating selection and response in a population of freshwater copepods. Evolution, 44(7), 1796–1805.

Hairston, N. G., Dillon, T. A., & de Stasio, B. T. (1990). A Field Test for the Cues of Diapause in a Freshwater Copepod. Ecology, 71(6), 2218–2223.

Hairston, N. G., & Kearns, C. M. (2002). Temporal dispersal: ecological and evolutionary aspects of zooplankton egg banks and the role of sediment mixing. Integrative and Comparative Biology, 42(3), 481–491.

Hairston, N. G., Olds, E. J., & Munns, W. R. (1985). Bet-hedging and environmentally cued diapause strategies of diaptomid copepods. SIL Proceedings, 1922-2010, 22(5), 3170–3177.

Hairston, N. G., Van Brunt, R. A., Kearns, C. M., & Engstrom, D. R. (1995). Age and Survivorship of Diapausing Eggs in a Sediment Egg Bank. Ecology, 76(6), 1706–1711.

Haldane, J. B. S. (1930). A mathematical theory of natural and artificial selection (Part IV, Isolation). Mathematical Proceedings of the Cambridge Philosophical Society, 26(2), 220–230.

Hallsson, L. R., & Björklund, M. (2012). Selection in a fluctuating environment leads to decreased genetic variation and facilitates the evolution of phenotypic plasticity. Journal of Evolutionary Biology, 25(7), 1275–1290.

Hämälä, T., Mattila, T. M., & Savolainen, O. (2018). Local adaptation and ecological differentiation under selection, migration, and drift in Arabidopsis lyrata. Evolution, 72(7), 1373–1386.

Hamilton, J. A., & Miller, J. M. (2016). Adaptive introgression as a resource for management and genetic conservation in a changing climate. Conservation Biology, 30(1), 33–41.

Haney, J. F., Aliberti, M. A., Allan, E., Allard, Stephanie Bauer, D. J., Beagen, W., Bradt, S. R., Carlson, B., Carlson, S. C., Doan, U. M., Dufresne, J., Godkin, W. T., Greene, S., Kaplan, A., Maroni, E., Melillo, S., Murby, L. A., Smith (Nowak), J. L., Ortman, B., Quist, J. E., … Travers, B. (2013). An Image-Based Key To The Zooplankton Of North America version 5.0 released 2013. University of New Hampshire Center for Freshwater Biology. http://cfb.unh.edu/cfbkey/html/

Harrisson, K. A., Pavlova, A., Amos, J. N., Takeuchi, N., Lill, A., Radford, J. Q., & Sunnucks, P. (2012). Fine-scale effects of habitat loss and fragmentation despite large-scale gene flow for some regionally declining woodland bird species. Landscape Ecology, 27(6), 813–827.

Havel, J. E., & Shurin, J. B. (2004). Mechanisms, effects, and scales of dispersal in freshwater zooplankton. Limnology and Oceanography, 49(4part2), 1229–1238.

Havens, K. E. (1993). Acid and aluminum effects on osmoregulation and survival of the freshwater copepod Skistodiaptomus oregonensis. Journal of Plankton Research, 15(6), 683–691.

Heino, J., Virkkala, R., & Toivonen, H. (2009). Climate change and freshwater biodiversity: Detected patterns, future trends and adaptations in northern regions. Biological Reviews, 84(1), 39–54.

Hereford, J. (2009). A Quantitative Survey of Local Adaptation and Fitness Trade‐Offs. The American Naturalist, 173(5), 579–588.

Hermisson, J., & Pennings, P. S. (2017). Soft sweeps and beyond: understanding the patterns and probabilities of selection footprints under rapid adaptation. Methods in Ecology and Evolution, 8(6), 700–716.

Hickey, M. B. C., & Fowlie, A. R. (2005). First occurrence of the Round Goby, Neogobius melanostomus, in the St. Lawrence River at Cornwall, Ontario. Canadian Field-Naturalist, 119(4), 582–583.

Hickling, R., Roy, D. B., Hill, J. K., Fox, R., & Thomas, C. D. (2006). The distributions of a wide range of taxonomic groups are expanding polewards. Global Change Biology, 12(3), 450–455.

Hivert, V., Leblois, R., Petit, E. J., Gautier, M., & Vitalis, R. (2018). Measuring genetic differentiation from pool-seq data. Genetics, 210(1), 315–330.

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., Poss, M. L., Reed, L. K., Storfer, A., & Whitlock, M. C. (2016). Finding the Genomic Basis of Local Adaptation: Pitfalls, Practical Solutions, and Future Directions. The American Naturalist, 188(4), 379–397.

Hoffmann, A. A., Miller, A. D., & Weeks, A. R. (2021). Genetic mixing for population management: From genetic rescue to provenancing. Evolutionary Applications, 14(3), 634–652.

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. Nature, 470(7335), 479–485.

Hooks, A. P., & Padilla, D. K. (2021). Introduced predator elicits population-specific responses from prey. Biological Invasions, 23(2), 477–490.

House, G. L., & Hahn, M. W. (2018). Evaluating methods to visualize patterns of genetic differentiation on a landscape. Molecular Ecology Resources, 18(3), 448–460.

Hu, J., & Barrett, R. D. H. (2017). Epigenetics in natural animal populations. Journal of Evolutionary Biology, 30(9), 1612–1632.

Hu, J., & Barrett, R. D. H. (2022). The role of plastic and evolved DNA methylation in parallel adaptation of threespine stickleback (Gasterosteus aculeatus). Molecular Ecology, December, 1–11.

Hu, J., Wuitchik, S. J. S., Barry, T. N., Jamniczky, H. A., Rogers, S. M., & Barrett, R. D. H. (2021). Heritability of DNA methylation in threespine stickleback (Gasterosteus aculeatus). Genetics, 217(1).

Hufbauer, R. A., Szűcs, M., Kasyon, E., Youngberg, C., Koontz, M. J., Richards, C., Tuff, T., & Melbourne, B. A. (2015). Three types of rescue can avert extinction in a changing environment. Proceedings of the National Academy of Sciences, 112(33), 10557–10562.

Iacarella, J. C., & Ricciardi, A. (2015). Dissolved ions mediate body mass gain and predatory response of an invasive fish. Biological Invasions, 17(11), 3237–3246.

Incagnone, G., Marrone, F., Barone, R., Robba, L., & Naselli-Flores, L. (2015). How do freshwater organisms cross the “dry ocean”? A review on passive dispersal and colonization processes with a special focus on temporary ponds. Hydrobiologia, 750(1), 103–123.

Inoue, K., Monroe, E. M., Elderkin, C. L., & Berg, D. J. (2014). Phylogeographic and population genetic analyses reveal Pleistocene isolation followed by high gene flow in a wide ranging, but endangered, freshwater mussel. Heredity, 112(3), 282–290.

Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: A meta-analysis. Global Change Biology, 22(1), 180–189.

Jaureguiberry, P., Titeux, N., Wiemers, M., Bowler, D. E., Coscieme, L., Golden, A. S., Guerra, C. A., Jacob, U., Takahashi, Y., Settele, J., Díaz, S., Molnár, Z., & Purvis, A. (2022). The direct drivers of recent global anthropogenic biodiversity loss. Science Advances, 8(45), 1–12.

Johnson, A. B., Fogel, N. S., & Lambert, J. D. (2019). Growth and morphogenesis of the gastropod shell. Proceedings of the National Academy of Sciences, 116(14), 6878–6883.

Johri, P., Charlesworth, B., & Jensen, J. D. (2020). Toward an Evolutionarily Appropriate Null Model: Jointly Inferring Demography and Purifying Selection. Genetics, 215(1), 173–192.

Jørgensen, T. S., Petersen, B., Petersen, H. C. B., Browne, P. D., Prost, S., Stillman, J. H., Hansen, L. H., & Hansen, B. W. (2019a). The genome and mRNA transcriptome of the cosmopolitan calanoid copepod acartia tonsa dana improve the understanding of copepod genome size evolution. Genome Biology and Evolution, 11(5), 1440–1450.

Jørgensen, T. S., Petersen, B., Petersen, H. C. B., Browne, P. D., Prost, S., Stillman, J. H., Hansen, L. H., & Hansen, B. W. (2019b). The Genome and mRNA Transcriptome of the Cosmopolitan Calanoid Copepod Acartia tonsa Dana Improve the Understanding of Copepod Genome Size Evolution. Genome Biology and Evolution, 11(5), 1440–1450.

Jung, H., Ventura, T., Sook Chung, J., Kim, W. J., Nam, B. H., Kong, H. J., Kim, Y. O., Jeon, M. S., & Eyun, S. il. (2020). Twelve quick steps for genome assembly and annotation in the classroom. PLoS Computational Biology, 16(11), 1–25.

Kajitani, R., Yoshimura, D., Okuno, M., Minakuchi, Y., Kagoshima, H., Fujiyama, A., Kubokawa, K., Kohara, Y., Toyoda, A., & Itoh, T. (2019). Platanus-allee is a de novo haplotype assembler enabling a comprehensive access to divergent heterozygous regions. Nature Communications, 10(1), 1702.

Keeney, D. B., King, T. M., Rowe, D. L., & Poulin, R. (2009). Contrasting mtDNA diversity and population structure in a direct-developing marine gastropod and its trematode parasites. Molecular Ecology, 18(22), 4591–4603.

Keller, W., Heneberry, J. H., & Dixit, S. S. (2003). Decreased acid deposition and the chemical recovery of Killarney, Ontario, lakes. Ambio, 32(3), 183–189.

Keller, W., Yan, N. D., Gunn, J. M., & Heneberry, J. (2007). Recovery of Acidified Lakes: Lessons From Sudbury, Ontario, Canada. Water, Air, & Soil Pollution: Focus, 7(1–3), 317–322.

Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. Philosophical Transactions of the Royal Society B: Biological Sciences, 374(1768), 20180176.

Kelly, M. W., Grosberg, R. K., & Sanford, E. (2013). Trade-Offs, Geography, and Limits to Thermal Adaptation in a Tide Pool Copepod. The American Naturalist, 181(6), 846–854.

Kelly, M. W., Pankey, M. S., DeBiasse, M. B., & Plachetzki, D. C. (2017). Adaptation to heat stress reduces phenotypic and transcriptional plasticity in a marine copepod. Functional Ecology, 31(2), 398–406.

Kelly, P. T., Craig, N., Solomon, C. T., Weidel, B. C., Zwart, J. A., & Jones, S. E. (2016). Experimental whole-lake increase of dissolved organic carbon concentration produces unexpected increase in crustacean zooplankton density. Global Change Biology, 22(8), 2766–2775.

Kilham, S. S., Kreeger, D. A., Lynn, S. G., Goulden, C. E., & Herrera, L. (1998). COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia, 377(1–3), 147–159.

Kipp, R., Hébert, I., Lacharité, M., & Ricciardi, A. (2012). Impacts of predation by the Eurasian round goby (Neogobius melanostomus) on molluscs in the upper St. Lawrence River. Journal of Great Lakes Research, 38(1), 78–89.

Kipp, R., & Ricciardi, A. (2012). Impacts of the eurasian round goby (neogobius melanostomus) on benthic communities in the upper st. lawrence river. Canadian Journal of Fisheries and Aquatic Sciences, 69(3), 469–486.

Kofler, R., Orozco-terWengel, P., de Maio, N., Pandey, R. V., Nolte, V., Futschik, A., Kosiol, C., & Schlötterer, C. (2011). PoPoolation: A Toolbox for Population Genetic Analysis of Next Generation Sequencing Data from Pooled Individuals. PLoS ONE, 6(1), e15925.

Kofler, R., Pandey, R. V., & Schlötterer, C. (2011). PoPoolation2: Identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). Bioinformatics, 27(24), 3435–3436.

Kofler, R., & Schlötterer, C. (2012). Gowinda: Unbiased analysis of gene set enrichment for genome-wide association studies. Bioinformatics, 28(15), 2084–2085.

Kokko, H., Chaturvedi, A., Croll, D., Fischer, M. C., Guillaume, F., Karrenberg, S., Kerr, B., Rolshausen, G., & Stapley, J. (2017). Can Evolution Supply What Ecology Demands? Trends in Ecology and Evolution, 32(3), 187–197.

Kraemer, B. M., Anneville, O., Chandra, S., Dix, M., Kuusisto, E., Livingstone, D. M., Rimmer, A., Schladow, S. G., Silow, E., Sitoki, L. M., Tamatamah, R., Vadeboncoeur, Y., & McIntyre, P. B. (2015). Morphometry and average temperature affect lake stratification responses to climate change. Geophysical Research Letters, 42(12), 4981–4988.

Kraemer, B. M., Chandra, S., Dell, A. I., Dix, M., Kuusisto, E., Livingstone, D. M., Schladow, S. G., Silow, E., Sitoki, L. M., Tamatamah, R., & McIntyre, P. B. (2017). Global patterns in lake ecosystem responses to warming based on the temperature dependence of metabolism. Global Change Biology, 23(5), 1881–1890.

Kurland, S., Wheat, C. W., de la Paz Celorio Mancera, M., Kutschera, V. E., Hill, J., Andersson, A., Rubin, C. J., Andersson, L., Ryman, N., & Laikre, L. (2019). Exploring a Pool-seq-only approach for gaining population genomic insights in nonmodel species. Ecology and Evolution, 9(19), 11448–11463.

Kwiatkowski, R. E., & Roff, J. C. (1976). Effects of acidity on the phytoplankton and primary productivity of selected northern Ontario lakes. Canadian Journal of Botany, 54(22), 2546–2561.

Kyle, C. J., & Boulding, E. G. (2000). Comparative population genetic structure of marine gastropods (Littorina spp.) with and without pelagic larval dispersal. Marine Biology, 137(5–6), 835–845.

Labaj, A. L., Kurek, J., Jeziorski, A., & Smol, J. P. (2015). Elevated metal concentrations inhibit biological recovery of Cladocera in previously acidified boreal lakes. Freshwater Biology, 60(2), 347–359.

Lahti, D. C., Johnson, N. A., Ajie, B. C., Otto, S. P., Hendry, A. P., Blumstein, D. T., Coss, R. G., Donohue, K., & Foster, S. A. (2009). Relaxed selection in the wild. Trends in Ecology and Evolution, 24(9), 487–496.

Lanfear, R., Kokko, H., & Eyre-Walker, A. (2014). Population size and the rate of evolution. Trends in Ecology and Evolution, 29(1), 33–41.

Leach, T. H., Winslow, L. A., Hayes, N. M., & Rose, K. C. (2019). Decoupled trophic responses to long-term recovery from acidification and associated browning in lakes. Global Change Biology, 25(5), 1779–1792.

Lee, C. E. (2016). Evolutionary mechanisms of habitat invasions, using the copepod Eurytemora affinis as a model system. Evolutionary Applications, 9(1), 248–270.

Lee, C. E., Remfert, J. L., Opgenorth, T., Lee, K. M., Stanford, E., Connolly, J. W., Kim, J., & Tomke, S. (2017). Evolutionary responses to crude oil from the Deepwater Horizon oil spill by the copepod Eurytemora affinis. Evolutionary Applications, 10(8), 813–828.

Lefébure, R., Degerman, R., Andersson, A., Larsson, S., Eriksson, L. O., Båmstedt, U., & Byström, P. (2013). Impacts of elevated terrestrial nutrient loads and temperature on pelagic food-web efficiency and fish production. Global Change Biology, 19(5), 1358–1372.

Leffler, E. M., Bullaughey, K., Matute, D. R., Meyer, W. K., Ségurel, L., Venkat, A., Andolfatto, P., & Przeworski, M. (2012). Revisiting an Old Riddle: What Determines Genetic Diversity Levels within Species? PLoS Biology, 10(9), e1001388.

Lehnherr, I., St Louis, V. L., Sharp, M., Gardner, A. S., Smol, J. P., Schiff, S. L., Muir, D. C. G., Mortimer, C. A., Michelutti, N., Tarnocai, C., St Pierre, K. A., Emmerton, C. A., Wiklund, J. A., Köck, G., Lamoureux, S. F., & Talbot, C. H. (2018). The world's largest High Arctic lake responds rapidly to climate warming. Nature Communications, 9(1), 1–9.

Lenormand, T. (2002). Gene flow and the limits to natural selection. Trends in Ecology & Evolution, 17(4), 183–189.

Lenormand, T., Nougué, O., Jabbour-Zahab, R., Arnaud, F., Dezileau, L., Chevin, L. M., & Sánchez, M. I. (2018). Resurrection ecology in Artemia. Evolutionary Applications, 11(1), 76–87.

Levis, N. A., Isdaner, A. J., & Pfennig, D. W. (2018). Morphological novelty emerges from pre-existing phenotypic plasticity. Nature Ecology & Evolution, 2(8), 1289–1297.

Li, D., Liu, C.-M., Luo, R., Sadakane, K., & Lam, T.-W. (2015). MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics, 31(10), 1674–1676.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 25(14), 1754–1760.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics, 25(16), 2078–2079.

Little, C., & Nix, W. (1976). The burrowing and floating behaviour of the gastropod Hydrobia ulvae. Estuarine and Coastal Marine Science, 4(5), 537–544.

Liu, C., Wolter, C., Xian, W., & Jeschke, J. M. (2020). Most invasive species largely conserve their climatic niche. Proceedings of the National Academy of Sciences of the United States of America, 117(38), 23643–23651.

Loria, A., Cristescu, M. E., & Gonzalez, A. (2019). Mixed evidence for adaptation to environmental pollution. Evolutionary Applications, 12(7), 1259–1273.

Lowry, D. B., Hoban, S., Kelley, J. L., Lotterhos, K. E., Reed, L. K., Antolin, M. F., & Storfer, A. (2017). Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation. Molecular Ecology Resources, 17(2), 142–152.

Maasri, A., Jähnig, S. C., Adamescu, M. C., Adrian, R., Baigun, C., Baird, D. J., Batista‐Morales, A., Bonada, N., Brown, L. E., Cai, Q., Campos‐Silva, J. v., Clausnitzer, V., Contreras‐MacBeath, T., Cooke, S. J., Datry, T., Delacámara, G., de Meester, L., Dijkstra, K. B., Do, V. T., … Worischka, S. (2021). A global agenda for advancing freshwater biodiversity research. Ecology Letters, 25(March), 1–9.

Maciejewski, M. F., Jiang, C., Stuart, Y. E., & Bolnick, D. I. (2020). Microhabitat contributes to microgeographic divergence in threespine stickleback. Evolution, 74(4), 749–763.

Madoui, M. A., Poulain, J., Sugier, K., Wessner, M., Noel, B., Berline, L., Labadie, K., Cornils, A., Blanco-Bercial, L., Stemmann, L., Jamet, J. L., & Wincker, P. (2017). New insights into global biogeography, population structure and natural selection from the genome of the epipelagic copepod Oithona. Molecular Ecology, 26(17), 4467–4482.

Marçais, G., & Kingsford, C. (2011). A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics, 27(6), 764–770.

Marcus, N. H. (1990). Calanoid copepod, cladoceran, and rotifer eggs in sea-bottom sediments of northern Californian coastal waters: Identification, occurrence and hatching. Marine Biology, 105(3), 413–418.

Margres, M. J., Patton, A., Wray, K. P., Hassinger, A. T. B., Ward, M. J., Lemmon, E. M., Lemmon, A. R., & Rokyta, D. R. (2019). Tipping the scales: The migration-selection balance leans toward selection in snake venoms. Molecular Biology and Evolution, 36(2), 271–282.

Marques, D. A., Lucek, K., Sousa, V. C., Excoffier, L., & Seehausen, O. (2019). Admixture between old lineages facilitated contemporary ecological speciation in Lake Constance stickleback. Nature Communications, 10(1), 4240.

Martel, A., & Chia, F. S. (1991). Drifting and dispersal of small bivalves and gastropods with direct development. Journal of Experimental Marine Biology and Ecology, 150(1), 131–147.

Martin, G. K., Beisner, B. E., Chain, F. J. J., Cristescu, M. E., del Giorgio, P. A., & Derry, A. M. (2021). Freshwater zooplankton metapopulations and metacommunities respond differently to environmental and spatial variation. Ecology, 102(1), 1–11.

Matz, M. V., Treml, E. A., Aglyamova, G. V., & Bay, L. K. (2018). Potential and limits for rapid genetic adaptation to warming in a Great Barrier Reef coral. PLoS Genetics, 14(4), 1–19.

McDermott, A. (2019). Probing the limits of "evolutionary rescue." Proceedings of the National Academy of Sciences, 116(25), 12116–12120.

McGovern, T. M., Keever, C. C., Saski, C. A., Hart, M. W., & Marko, P. B. (2010). Divergence genetics analysis reveals historical population genetic processes leading to contrasting phylogeographic patterns in co-distributed species. Molecular Ecology, 19(22), 5043–5060.

Meester, L. De, Stoks, R., & Brans, K. I. (2018). Genetic adaptation as a biological buffer against climate change: Potential and limitations. Integrative Zoology, 13(4), 372–391.

Melotto, A., Manenti, R., & Ficetola, G. F. (2020). Rapid adaptation to invasive predators overwhelms natural gradients of intraspecific variation. Nature Communications, 11(1), 3608.

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. Evolutionary Applications, 7(1), 1–14.

Meyer-Jacob, C., Michelutti, N., Paterson, A. M., Cumming, B. F., Keller, W., & Smol, J. P. (2019). The browning and re-browning of lakes: Divergent lake-water organic carbon trends linked to acid deposition and climate change. Scientific Reports, 9(1), 1–10.

Mi Kwon, Y., Vranken, N., Hoge, C., Lichak, M. R., Camacho-Garcia, J., Bista, I., Wood, J., McCarthy, S., Hui Tan, H., Howe, K., Bandara, S., von Lintig, J., Durbin, R., Svardal, H., & Bendesky, A. (2021). Genomic consequences of domestication of the Siamese fighting fish. BioRxiv, 2021.04.29.442030. https://doi.org/10.1101/2021.04.29.442030

Micheletti, S. J., & Narum, S. R. (2018). Utility of pooled sequencing for association mapping in nonmodel organisms. Molecular Ecology Resources, 18(4), 825–837.

Milanesi, M., Capomaccio, S., Vajana, E., Bomba, L., Garcia, J. F., Ajmone-Marsan, P., & Colli, L. (2017). BITE: An R package for biodiversity analyses. BioRxiv, 181610.

Mills, L. S., Bragina, E. V., Kumar, A. V., Zimova, M., Lafferty, D. J. R., Feltner, J., Davis, B. M., Hackländer, K., Alves, P. C., Good, J. M., Melo-Ferreira, J., Dietz, A., Abramov, A. V., Lopatina, N., & Fay, K. (2018). Winter color polymorphisms identify global hot spots for evolutionary rescue from climate change. Science, 359(6379), 1033–1036.

Minguez, L., Sperfeld, E., Berger, S. A., Nejstgaard, J. C., & Gessner, M. O. (2020). Changes in food characteristics reveal indirect effects of lake browning on zooplankton performance. Limnology and Oceanography, 65(5), 1028–1040.

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(12).

Mothes, C. C., Stroud, J. T., Clements, S. L., & Searcy, C. A. (2019). Evaluating ecological niche model accuracy in predicting biotic invasions using South Florida's exotic lizard community. Journal of Biogeography, 46(2), 432–441.

Negrín Dastis, J. O., & Derry, A. M. (2016). The impact of regional landscape context on local maladaptive trait divergence: a field test using freshwater copepod acid tolerance. Evolutionary Ecology, 30(5), 841–859.

Negrín Dastis, J. O., Milne, R., Guichard, F., & Derry, A. M. (2019). Phenotype–environment mismatch in metapopulations—Implications for the maintenance of maladaptation at the regional scale. Evolutionary Applications, 12(7), 1475–1486.

Nosil, P., Egan, S. P., & Funk, D. J. (2008). Heterogeneous genomic differentiation between walking-stick ecotypes: "Isolation by adaptation" and multiple roles for divergent selection. Evolution, 62(2), 316–336.

Oester, R., Greenway, R., Moosmann, M., Sommaruga, R., Tartarotti, B., Brodersen, J., & Matthews, B. (2022). The influence of predator community composition on photoprotective traits of copepods. Ecology and Evolution, 12(4), 1–10.

Orr, H. A., & Unckless, R. L. (2014). The Population Genetics of Evolutionary Rescue. PLoS Genetics, 10(8), 1–9.

Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J., & de Meester, L. (2013). Drivers of population genetic differentiation in the wild: Isolation by dispersal limitation, isolation by adaptation and isolation by colonization. Molecular Ecology, 22(24), 5983–5999.

Ortega‐Mayagoitia, E., Alcántara‐Rodríguez, J. A., Urbán‐Olivares, J., Campos, J. E., & Ciros‐Pérez, J. (2022). Genomic signatures of adaptive divergence in lacustrine copepods. Freshwater Biology, 67(6), 1045–1062.

Oswald, J. A., Roth, B., Faske, T. M., Allen, J. M., Mestre, C., Rivers-Pankratz, D., van Norman, K., & Guralnick, R. P. (2022). Population genomics of Monadenia (Gastropoda: Stylommatophora: Xanthonychidae) land snails reveals structuring but gene-flow across distinct species and morphotypes. Conservation Genetics, 23(2), 299–311.

Oziolor, E. M., Reid, N. M., Yair, S., Lee, K. M., Guberman VerPloeg, S., Bruns, P. C., Shaw, J. R., Whitehead, A., & Matson, C. W. (2019). Adaptive introgression enables evolutionary rescue from extreme environmental pollution. Science, 364(6439), 455–457.

Paetkau, D., & Strobeck, C. (1996). Mitochondrial DNA and the phylogeography of Newfoundland black bears. Canadian Journal of Zoology, 74(1), 192–196.

Paulose, J., & Hallatschek, O. (2020). The impact of long-range dispersal on gene surfing. Proceedings of the National Academy of Sciences of the United States of America, 117(14), 7584–7593.

Pavlova, A., Beheregaray, L. B., Coleman, R., Gilligan, D., Harrisson, K. A., Ingram, B. A., Kearns, J., Lamb, A. M., Lintermans, M., Lyon, J., Nguyen, T. T. T., Sasaki, M., Tonkin, Z., Yen, J. D. L., & Sunnucks, P. (2017). Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: A call for assisted gene flow. Evolutionary Applications, 10(6), 531–550.

Pfenninger, M., Patel, S., Arias-Rodriguez, L., Feldmeyer, B., Riesch, R., & Plath, M. (2015). Unique evolutionary trajectories in repeated adaptation to hydrogen sulphide-toxic habitats of a neotropical fish (Poecilia mexicana). Molecular Ecology, 24(21), 5446–5459.

Pickrell, J. K., & Pritchard, J. K. (2012). Inference of Population Splits and Mixtures from Genome-Wide Allele Frequency Data. PLoS Genetics, 8(11), e1002967.

Pinel-Alloul, B., André, A., Legendre, P., Cardille, J. A., Patalas, K., & Salki, A. (2013). Large-scale geographic patterns of diversity and community structure of pelagic crustacean zooplankton in Canadian lakes. Global Ecology and Biogeography, 22(7), 784–795.

Pinel-Alloul, B., & Magnin, E. (1973). Observations sur le cycle vital et la croissance d' Amnicola limosa (Say) (Mollusca, Gastropoda, Prosobranchia) du lac Saint-Louis près de Montréal. Canadian Journal of Zoology, 51(2), 311–313.

Pironon, S., Papuga, G., Villellas, J., Angert, A. L., García, M. B., & Thompson, J. D. (2017). Geographic variation in genetic and demographic performance: new insights from an old biogeographical paradigm. Biological Reviews, 92(4), 1877–1909.

Poloczanska, E. S., Brown, C. J., Sydeman, W. J., Kiessling, W., Schoeman, D. S., Moore, P. J., Brander, K., Bruno, J. F., Buckley, L. B., Burrows, M. T., Duarte, C. M., Halpern, B. S., Holding, J., Kappel, C. V., O'Connor, M. I., Pandolfi, J. M., Parmesan, C., Schwing, F., Thompson, S. A., & Richardson, A. J. (2013). Global imprint of climate change on marine life. Nature Climate Change, 3(10), 919–925.

Raeymaekers, J. A. M., Konijnendijk, N., Larmuseau, M. H. D., Hellemans, B., de Meester, L., & Volckaert, F. A. M. (2014). A gene with major phenotypic effects as a target for selection vs. homogenizing gene flow. Molecular Ecology, 23(1), 162–181.

Ralls, K., Ballou, J. D., Dudash, M. R., Eldridge, M. D. B., Fenster, C. B., Lacy, R. C., Sunnucks, P., & Frankham, R. (2018). Call for a Paradigm Shift in the Genetic Management of Fragmented Populations. Conservation Letters, 11(2), 1–6.

Ralls, K., Sunnucks, P., Lacy, R. C., & Frankham, R. (2020). Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation. Biological Conservation, 251, 108784.

Rautio, M., & Tartarotti, B. (2010). UV Radiation and Freshwater Zooplankton: Damage, Protection and Recovery. Freshwater Reviews, 3(2), 105–131.

Razgour, O., Forester, B., Taggart, J. B., Bekaert, M., Juste, J., Ibáñez, C., Puechmaille, S. J., Novella-Fernandez, R., Alberdi, A., & Manel, S. (2019). Considering adaptive genetic

variation in climate change vulnerability assessment reduces species range loss projections. Proceedings of the National Academy of Sciences of the United States of America, 116(21), 10418–10423.

Redak, C. A., Williams, A. S., Garner, J. T., Halanych, K. M., & Whelan, N. v. (2021). Assessing Genomic Diversity, Connectivity, and Riverscape Genetics Hypotheses in the Endangered Rough Hornsnail, Pleurocera Foremani, Following Habitat Disruption. The Journal of Heredity, 112(7), 635–645.

Reed, T. E., Robin, S. W., Schindler, D. E., Hard, J. J., & Kinnison, M. T. (2010). Phenotypic plasticity and population viability: The importance of environmental predictability. Proceedings of the Royal Society B: Biological Sciences, 277(1699), 3391–3400.

Rêgo, A., Messina, F. J., & Gompert, Z. (2019). Dynamics of genomic change during evolutionary rescue in the seed beetle Callosobruchus maculatus. Molecular Ecology, 28(9), 2136–2154.

Reid, A. J., Chapman, L. J., & Ricciardi, A. (2013). Wetland edges as peak refugia from an introduced piscivore. Aquatic Conservation: Marine and Freshwater Ecosystems, 23(5), 646–655.

Reid, K., Bell, M. A., & Veeramah, K. R. (2021). Threespine Stickleback: A Model System for Evolutionary Genomics. Annual Review of Genomics and Human Genetics, 22(1), 357–383.

Rellstab, C., Fischer, M. C., Zoller, S., Graf, R., Tedder, A., Shimizu, K. K., Widmer, A., Holderegger, R., & Gugerli, F. (2017). Local adaptation (mostly) remains local: Reassessing environmental associations of climate-related candidate SNPs in Arabidopsis halleri. Heredity, 118(2), 193–201.

Richardson, J. L., Urban, M. C., Bolnick, D. I., & Skelly, D. K. (2014). Microgeographic adaptation and the spatial scale of evolution. Trends in Ecology and Evolution, 29(3), 165–176.

Robidoux, M., del Giorgio, P., & Derry, A. (2015). Effects of humic stress on the zooplankton from clear and DOC-rich lakes. Freshwater Biology, 60(7), 1263–1278.

Robinson, Z. L., Bell, D. A., Dhendup, T., Luikart, G., Whiteley, A. R., & Kardos, M. (2021). Evaluating the outcomes of genetic rescue attempts. Conservation Biology, 35(2), 666–677.

Roncalli, V., Cieslak, M. C., Castelfranco, A. M., Hopcroft, R. R., Hartline, D. K., & Lenz, P. H. (2021). Post-diapause transcriptomic restarts: insight from a high-latitude copepod. BMC Genomics, 22(1), 409.

Roncalli, V., Cieslak, M. C., Germano, M., Hopcroft, R. R., & Lenz, P. H. (2019). Regional heterogeneity impacts gene expression in the subarctic zooplankter Neocalanus flemingeri in the northern Gulf of Alaska. Communications Biology, 2(1), 1–13.

Roncalli, V., Cieslak, M. C., Sommer, S. A., Hopcroft, R. R., & Lenz, P. H. (2018). De novo transcriptome assembly of the calanoid copepod Neocalanus flemingeri: A new resource for emergence from diapause. Marine Genomics, 37, 114–119.

Ross, A. J., & Arnott, S. E. (2022). Similar zooplankton responses to low pH and calcium may impair long-term recovery from acidification. Ecological Applications, 32(3), 1–16.

Rousset, F. (1997). Genetic Differentiation and Estimation of Gene Flow from F -Statistics Under Isolation by Distance. Genetics, 145(4), 1219–1228.

Rundle, S. D., Spicer, J. I., Coleman, R. A., Vosper, J., & Soane, J. (2004). Environmental calcium modifies induced defences in snails. Proceedings of the Royal Society of London. Series B: Biological Sciences, 271(suppl_3), 2001–2004.

Samchyshyna, L., Hansson, L. A., & Christoffersen, K. (2008). Patterns in the distribution of Arctic freshwater zooplankton related to glaciation history. Polar Biology, 31(12), 1427–1435.

Sánchez-Castro, D., Perrier, A., & Willi, Y. (2022). Reduced climate adaptation at range edges in North American Arabidopsis lyrata. Global Ecology and Biogeography, 31(6), 1066–1077.

Sanderson, S., Beausoleil, M., O’ Dea, R. E., Wood, Z. T., Correa, C., Frankel, V., Gorné, L. D., Haines, G. E., Kinnison, M. T., Oke, K. B., Pelletier, F., Pérez‐Jvostov, F., Reyes‐Corral, W. D., Ritchot, Y., Sorbara, F., Gotanda, K. M., & Hendry, A. P. (2022). The pace of modern life, revisited. Molecular Ecology, 31(4), 1028–1043.

Sanderson, S., Derry, A. M., & Hendry, A. P. (2021). Phenotypic stability in scalar calcium of freshwater fish across a wide range of aqueous calcium availability in nature. Ecology and Evolution, 11(11), 6053–6065.

Scheffers, B. R., de Meester, L., Bridge, T. C. L., Hoffmann, A. A., Pandolfi, J. M., Corlett, R. T., Butchart, S. H. M., Pearce-Kelly, P., Kovacs, K. M., Dudgeon, D., Pacifici, M., Rondinini, C., Foden, W. B., Martin, T. G., Mora, C., Bickford, D., & Watson, J. E. M. (2016). The broad footprint of climate change from genes to biomes to people. Science, 354(6313), aaf7671.

Schlötterer, C., Tobler, R., Kofler, R., & Nolte, V. (2014). Sequencing pools of individuals — mining genome-wide polymorphism data without big funding. Nature Reviews Genetics, 15(11), 749–763.

Schmidt, D. A., Waterhouse, M. D., Sjodin, B. M. F., & Russello, M. A. (2021). Genome-wide analysis reveals associations between climate and regional patterns of adaptive divergence and dispersal in American pikas. Heredity, 127(5), 443–454.

Schneider, T., Grosbois, G., Vincent, W. F., & Rautio, M. (2017). Saving for the future: Pre-winter uptake of algal lipids supports copepod egg production in spring. Freshwater Biology, 62(6), 1063–1072.

Seeley, R. H. (1986). Intense natural selection caused a rapid morphological transition in a living marine snail. Proceedings of the National Academy of Sciences, 83(18), 6897–6901.

Sexton, J. P., Strauss, S. Y., & Rice, K. J. (2011). Gene flow increases fitness at the warm edge of a species' range. Proceedings of the National Academy of Sciences of the United States of America, 108(28), 11704–11709.

Sharma, S., Blagrave, K., Magnuson, J. J., O'Reilly, C. M., Oliver, S., Batt, R. D., Magee, M. R., Straile, D., Weyhenmeyer, G. A., Winslow, L., & Woolway, R. I. (2019). Widespread loss of lake ice around the Northern Hemisphere in a warming world. Nature Climate Change, 9(3), 227–231.

Shaw, M. A., & Mackie, G. L. (1990). Effects of Calcium and pH on the Reproductive Success of Amnicola limosa (Gastropoda). Canadian Journal of Fisheries and Aquatic Sciences, 47(9), 1694–1699.

Silliman, K., Indorf, J. L., Knowlton, N., Browne, W. E., & Hurt, C. (2021). Base-substitution mutation rate across the nuclear genome of Alpheus snapping shrimp and the timing of isolation by the Isthmus of Panama. BMC Ecology and Evolution, 21(1), 1–14.

Smithson, M., Thorson, J. L. M., Sadler-Riggleman, I., Beck, D., Skinner, M. K., & Dybdahl, M. (2020). Between-generation phenotypic and epigenetic stability in a clonal snail. Genome Biology and Evolution, 12(9), 1604–1615.

Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam, I., Fork, M. L., Karlsson, J., Larsen, S., Lennon, J. T., Read, J. S., Sadro, S., & Saros, J. E. (2015). Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. Ecosystems, 18(3), 376–389.

Spitze, K. (1993). Population structure in Daphnia obtusa: Quantitative genetic and allozymic variation. Genetics, 135(2), 367–374.

Sprules, W. G. (1975). Midsummer Crustacean Zooplankton Communities in Acid-Stressed Lakes. Journal of the Fisheries Research Board of Canada, 32(3), 389–395.

Stemberger, R. S. (1995). Pleistocene refuge areas and postglacial dispersal of copepods of the northeastern United States. Canadian Journal of Fisheries and Aquatic Sciences, 52(10), 2197–2210.

Stern, D. Ben, & Lee, C. E. (2020). Evolutionary origins of genomic adaptations in an invasive copepod. Nature Ecology & Evolution, 4(8), 1084–1094.

Stern, D. B., Anderson, N. W., Diaz, J. A., & Lee, C. E. (2022). Genome-wide signatures of synergistic epistasis during parallel adaptation in a Baltic Sea copepod. Nature Communications, 13(1), 4024.

Stewart, D. T., & Baker, A. J. (1992). Genetic differentiation and biogeography of the masked shrew in Atlantic Canada. Canadian Journal of Zoology, 70(1), 106–114.

Stewart, G. S., Morris, M. R., Genis, A. B., Szűcs, M., Melbourne, B. A., Tavener, S. J., & Hufbauer, R. A. (2017). The power of evolutionary rescue is constrained by genetic load. Evolutionary Applications, 10(7), 731–741.

St-Gelais, N. F., Vogt, R. J., del Giorgio, P. A., & Beisner, B. E. (2021). The taxonomic and functional biogeographies of phytoplankton and zooplankton communities across boreal lakes. Peer Community Journal, 1, e69.

Stoks, R., Geerts, A. N., & de Meester, L. (2014). Evolutionary and plastic responses of freshwater invertebrates to climate change: Realized patterns and future potential. Evolutionary Applications, 7(1), 42–55.

Stoks, R., Govaert, L., Pauwels, K., Jansen, B., & de Meester, L. (2016). Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea Daphnia magna. Ecology Letters, 19(2), 180–190.

Strandberg, U., Jelkänen, E., Taipale, S. J., Kankaala, P., Brett, M. T., Kainz, M. J., & Hiltunen, M. (2015). Selective transfer of polyunsaturated fatty acids from phytoplankton to planktivorous fish in large boreal lakes. Science of The Total Environment, 536, 858–865.

Strauss, S. Y., Lau, J. A., & Carroll, S. P. (2006). Evolutionary responses of natives to introduced species: What do introductions tell us about natural communities? Ecology Letters, 9(3), 357–374.

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(6), 5–12.

Suarez-Ulloa, V., Gonzalez-Romero, R., & Eirin-Lopez, J. M. (2015). Environmental epigenetics: A promising venue for developing next-generation pollution biomonitoring tools in marine invertebrates. Marine Pollution Bulletin, 98(1–2), 5–13.

Suenaga, E. K. (2018). Assessing the Chemical and Biological Recovery of Killarney [MSc thesis]. Queen's University.

Takahashi, Y., Suyama, Y., Matsuki, Y., Funayama, R., Nakayama, K., & Kawata, M. (2016). Lack of genetic variation prevents adaptation at the geographic range margin in a damselfly. Molecular Ecology, 25(18), 4450–4460.

Thia, J. A., & Riginos, C. (2019). genomalicious: serving up a smorgasbord of R functions for population genomic analyses. BioRxiv, 667337.

Thompson, K. A., Peichel, C. L., Rennison, D. J., McGee, M. D., Albert, A. Y. K., Vines, T. H., Greenwood, A. K., Wark, A. R., Brandvain, Y., Schumer, M., & Schluter, D. (2022). Analysis of ancestry heterozygosity suggests that hybrid incompatibilities in threespine stickleback are environment dependent. PLOS Biology, 20(1), e3001469.

Thor, P., Bailey, A., Dupont, S., Calosi, P., Søreide, J. E., de Wit, P., Guscelli, E., Loubet-Sartrou, L., Deichmann, I. M., Candee, M. M., Svensen, C., King, A. L., & Bellerby, R. G. J. (2018). Contrasting physiological responses to future ocean acidification among Arctic copepod populations. Global Change Biology, 24(1), e365–e377.

Thor, P., & Dupont, S. (2015). Transgenerational effects alleviate severe fecundity loss during ocean acidification in a ubiquitous planktonic copepod. Global Change Biology, 21(6), 2261–2271.

Thörn, F., Rödin-Mörch, P., Cortazar-Chinarro, M., Richter-Boix, A., Laurila, A., & Höglund, J. (2021). The effects of drift and selection on latitudinal genetic variation in Scandinavian common toads (Bufo bufo) following postglacial recolonisation. Heredity, 126(4), 656–667.

Thorson, J. L. M., Smithson, M., Beck, D., Sadler-Riggleman, I., Nilsson, E., Dybdahl, M., & Skinner, M. K. (2017). Epigenetics and adaptive phenotypic variation between habitats in an asexual snail. Scientific Reports, 7(1), 1–11.

Tickner, D., Opperman, J. J., Abell, R., Acreman, M., Arthington, A. H., Bunn, S. E., Cooke, S. J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A. J., Leonard, P., McClain, M. E., Muruven, D., Olden, J. D., … Young, L. (2020). Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan. BioScience, 70(4), 330–342.

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. Molecular Ecology, 25(10), 2144–2164.

Tinghitella, R. M. (2008). Rapid evolutionary change in a sexual signal: Genetic control of the mutation "flatwing" that renders male field crickets (Teleogryllus oceanicus) mute. Heredity, 100(3), 261–267.

Trussell, G. C., & Smith, L. D. (2000). Induced defenses in response to an invading crab predator: An explanation of historical and geographic phenotypic change. Proceedings of the National Academy of Sciences of the United States of America, 97(5), 2123–2127.

Tufto, J. (2001). Effects of releasing maladapted individuals: A demographic-evolutionary model. American Naturalist, 158(4), 331–340.

Twining, C. W., Bernhardt, J. R., Derry, A. M., Hudson, C. M., Ishikawa, A., Kabeya, N., Kainz, M. J., Kitano, J., Kowarik, C., Ladd, S. N., Leal, M. C., Scharnweber, K., Shipley, J. R., & Matthews, B. (2021). The evolutionary ecology of fatty ‐ acid variation: Implications for consumer adaptation and diversification. Ecology Letters, 24(8), 1709–1731.

Urban, M. C., Bocedi, G., Hendry, A. P., Mihoub, J. B., Pe'er, G., Singer, A., Bridle, J. R., Crozier, L. G., de Meester, L., Godsoe, W., Gonzalez, A., Hellmann, J. J., Holt, R. D., Huth, A., Johst, K., Krug, C. B., Leadley, P. W., Palmer, S. C. F., Pantel, J. H., … Travis, J. M. J. (2016a). Improving the forecast for biodiversity under climate change. Science, 353(6304).

Urban, M. C., Bocedi, G., Hendry, A. P., Mihoub, J.-B., Pe'er, G., Singer, A., Bridle, J. R., Crozier, L. G., de Meester, L., Godsoe, W., Gonzalez, A., Hellmann, J. J., Holt, R. D., Huth, A., Johst, K., Krug, C. B., Leadley, P. W., Palmer, S. C. F., Pantel, J. H., … Travis, J. M. J. (2016b). Improving the forecast for biodiversity under climate change. Science, 353(6304), aad8466.

Uricaru, R., Rizk, G., Lacroix, V., Quillery, E., Plantard, O., Chikhi, R., Lemaitre, C., & Peterlongo, P. (2015). Reference-free detection of isolated SNPs. Nucleic Acids Research, 43(2), e11–e11.

Vadadi-Fülöp, C., & Hufnagel, L. (2014). Climate change and plankton phenology in freshwater: Current trends and future commitments. Journal of Limnology, 73(1).

van Doorslaer, W., Vanoverbeke, J., Duvivier, C., Rousseaux, S., Jansen, M., Jansen, B., Feuchtmayr, H., Atkinson, D., Moss, B., Stoks, R., & de Meester, L. (2009). Local adaptation to higher temperatures reduces immigration success of genotypes from a warmer region in the water flea Daphnia. Global Change Biology, 15(12), 3046–3055.

Vanoverbeke, J., Urban, M. C., & de Meester, L. (2016). Community assembly is a race between immigration and adaptation: eco-evolutionary interactions across spatial scales. Ecography, 39(9), 858–870.

Ventura, M., Petrusek, A., Miró, A., Hamrová, E., Buñay, D., De Meester, L., & Mergeay, J. (2014). Local and regional founder effects in lake zooplankton persist after thousands of years despite high dispersal potential. Molecular Ecology, 23(5), 1014–1027.

Vinebrooke, R. D., Dixit, S. S., Graham, M. D., Gunn, J. M., Chen, Y.-W., & Belzile, N. (2002). Whole-lake algal responses to a century of acidic industrial deposition on the Canadian Shield. Canadian Journal of Fisheries and Aquatic Sciences, 59(3), 483–493.

Vurture, G. W., Sedlazeck, F. J., Nattestad, M., Underwood, C. J., Fang, H., Gurtowski, J., & Schatz, M. C. (2017). GenomeScope: fast reference-free genome profiling from short reads. Bioinformatics, 33(14), 2202–2204.

Wadgymar, S. M., DeMarche, M. L., Josephs, E. B., Sheth, S. N., & Anderson, J. T. (2022). Local Adaptation: Causal Agents of Selection and Adaptive Trait Divergence. Annual Review of Ecology, Evolution, and Systematics, 53(1), 87–111.

Waldvogel, A., Feldmeyer, B., Rolshausen, G., Exposito‐Alonso, M., Rellstab, C., Kofler, R., Mock, T., Schmid, K., Schmitt, I., Bataillon, T., Savolainen, O., Bergland, A., Flatt, T., Guillaume, F., & Pfenninger, M. (2020). Evolutionary genomics can improve prediction of species' responses to climate change. Evolution Letters, 4(1), 4–18.

Waldvogel, A. M., Wieser, A., Schell, T., Patel, S., Schmidt, H., Hankeln, T., Feldmeyer, B., & Pfenninger, M. (2018). The genomic footprint of climate adaptation in Chironomus riparius. Molecular Ecology, 27(6), 1439–1456.

Walsworth, T. E., Schindler, D. E., Colton, M. A., Webster, M. S., Palumbi, S. R., Mumby, P. J., Essington, T. E., & Pinsky, M. L. (2019). Management for network diversity speeds evolutionary adaptation to climate change. Nature Climate Change, 9(8), 632–636.

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. Molecular Ecology, 23(23), 5649–5662.

Weeks, A. R., Heinze, D., Perrin, L., Stoklosa, J., Hoffmann, A. A., van Rooyen, A., Kelly, T., & Mansergh, I. (2017). Genetic rescue increases fitness and aids rapid recovery of an endangered marsupial population. Nature Communications, 8(1), 1071.

Weeks, A. R., Sgro, C. M., Young, A. G., Frankham, R., Mitchell, N. J., Miller, K. A., Byrne, M., Coates, D. J., Eldridge, M. D. B., Sunnucks, P., Breed, M. F., James, E. A., & Hoffmann, A. A. (2011). Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evolutionary Applications, 4(6), 709–725.

Weider, L. J., Jeyasingh, P. D., & Frisch, D. (2018). Evolutionary aspects of resurrection ecology: Progress, scope, and applications—An overview. Evolutionary Applications, 11(1), 3–10.

Weisenfeld, N. I., Kumar, V., Shah, P., Church, D. M., & Jaffe, D. B. (2018). Corrigendum: Direct determination of diploid genome sequences. Genome Research, 27(5), 757–767.

Westram, A. M., Rafajlović, M., Chaube, P., Faria, R., Larsson, T., Panova, M., Ravinet, M., Blomberg, A., Mehlig, B., Johannesson, K., & Butlin, R. (2018). Clines on the seashore: The genomic architecture underlying rapid divergence in the face of gene flow. Evolution Letters, 2(4), 297–309.

Whiteley, A. R., Fitzpatrick, S. W., Funk, W. C., & Tallmon, D. A. (2015). Genetic rescue to the rescue. Trends in Ecology and Evolution, 30(1), 42–49.

Wiberg, R. A. W., Gaggiotti, O. E., Morrissey, M. B., & Ritchie, M. G. (2017). Identifying consistent allele frequency differences in studies of stratified populations. Methods in Ecology and Evolution, 8(12), 1899–1909.

Willi, Y., Kristensen, T. N., Sgro, C. M., Weeks, A. R., Ørsted, M., & Hoffmann, A. A. (2022). Conservation genetics as a management tool: The five best-supported paradigms to assist the management of threatened species. Proceedings of the National Academy of Sciences of the United States of America, 119(1), 1–10.

Winder, M., Schindler, D. E., Essington, T. E., & Litt, A. H. (2009). Disrupted seasonal clockwork in the population dynamics of a freshwater copepod by climate warming. Limnology and Oceanography, 54(6 PART 2), 2493–2505.

With, K. A., Schrott, G. R., & King, A. W. (2006). The implications of metalandscape connectivity for population viabilityin migratory songbirds. Landscape Ecology, 21(2), 157–167.

Wogan, G. O. U., & Wang, I. J. (2018). The value of space-for-time substitution for studying fine-scale microevolutionary processes. Ecography, 41(9), 1456–1468.

Woolway, R. I., Kraemer, B. M., Lenters, J. D., Merchant, C. J., O'Reilly, C. M., & Sharma, S. (2020). Global lake responses to climate change. Nature Reviews Earth & Environment, 1(8), 388–403.

Wright, S. (1931). Evolution in Mendelian populations. Genetics, 16(2), 97–159.

Wuitchik, S. J. S., Mogensen, S., Barry, T. N., Paccard, A., Jamniczky, H. A., Barrett, R. D. H., & Rogers, S. M. (2022). Evolution of thermal physiology alters the projected range of threespine stickleback under climate change. Molecular Ecology, 31(8), 2312–2326.

Yates, M. C., Bowles, E., & Fraser, D. J. (2019). Small population size and low genomic diversity have no effect on fitness in experimental translocations of a wild fish. Proceedings of the Royal Society B: Biological Sciences, 286(1916), 20191989.

Zeller, M., Reusch, T. B. H., & Lampert, W. (2006). A comparative population genetic study on calanoid freshwater copepods: Investigation of isolation-by-distance in two Eudiaptomus species with a different potential for dispersal. Limnology and Oceanography, 51(1), 117–124.