UNIVERSITÉ DU QUÉBEC À MONTRÉAL

# VARIATION PHÉNOTYPIQUE DE COPÉPODES À TRAVERS DES GRADIENTS ABIOTIQUES ET BIOTIQUES AU NIVEAU DU PAYSAGE

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*"To be surprised and to marvel is to begin to understand"*

*- José Ortega y Gasset*

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*This work is dedicated to my*
*Canadian and European family, who*
*relentlessly and blindly supported me*
*throughout this entire process, and*
*most importantly deeply believed,*
*inspired and stimulated me immensely,*
*making this adventure so worthwhile.*

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**Fig. 4** Effects of YOY brook trout stocking and season on the phytoplankton community composition and seston fatty acid composition. In all panels, open symbols represent time of initial stocking in early summer and solid symbols represent late summer, which was after two months of brook trout exposure at the time of fish re-capture in fish-stocked ponds. **A.** NMDS of phytoplankton community composition (Hellinger-tranformed relative taxon biovolume; mg $m^{-3}$) and **B.** NMDS of seston phytoplankton biomarker fatty acid composition. In panels **A.** and **B.**, square symbols represent fish-stocked ponds and circle symbols represent fishless ponds. Arrows on the NMDS biplots in represent trajectories of phytoplankton communities between these two periods. The biovolume (mg $m^{-3}$) of three major phytoplankton groups (cryptophytes, dinoflagellates, and chlorophytes) are shown in **C.** fish-stocked ponds and in **D.** fishless ponds. In panels **C.** and **D.**, we used the following symbols to designate key phytoplankton groups: downward triangles, crytophytes; upward triangles, dinoflagellates; diamonds, chlorophytes. Asterisks (*) represent significant differences between initial stocking and post-stocking in the fish-stocked ponds ($P<0.05$).

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# RÉSUMÉ

Les variables abiotiques peuvent modifier considérablement les processus écosystémiques ainsi qu'agir comme agents sélectifs de la diversification évolutive à l'échelle du paysage. Cependant, l'influence de ces variables abiotiques sur la variation phénotypique pour des traits adaptatifs ou maladaptatifs à travers de paysages avec des niveaux différents d'hétérogénéité spatiale et temporelle est largement inexplorée. À l'aide d'une approche métacommunautaire, ma thèse examinera, les processus écologiques pertinents agissant sur l'étendue de la variation phénotypique à l'échelle locale et du paysage, dans un consommateur aquatique primaire clé et abondant. Le présent travail a été mené pour la plupart à de multiples niveaux trophiques dans un système peu étudié qui s'étend sur de larges gradients abiotiques naturels en acidité. L'acidité est une force écologique puissante qui peut agir de l'échelle individuelle à l'échelle écosystémique, en modifiant les tolérances physiologiques, les interactions de la communauté et les attributs de l'écosystème. L'objectif principal de mon travail sera le rôle de la variation phénotypique parmi les populations de copépodes calanoïdes de *Leptodiaptomus minutus* à travers de gradients en acidité à l'échelle, couvrant différents niveaux de complexité écologique. Ces copépodes sont souvent les principaux consommateurs primaires qui transfèrent l'énergie et des composés essentiels entre les ressources basales et les prédateurs d'invertébrés et de poissons dans les réseaux trophiques aquatiques nord-tempérés. Il existe trois principaux objectifs de thèse: **CHAPITRE I**) fera une comparaison au niveau du paysage en termes tolérance au pH de *L. minutus* dans trois régions qui diffèrent dans l'hétérogénéité spatiale et temporelle et quantifient les conséquences de ces différences écologiques pour la ma des traits intraspécifiques au niveau de la population; **CHAPITRE II**) produira un modèle métapopulationelle avec des caractéristiques source-puits comprenant la sélection asymétrique qui est basee sur de l'evidence empirique, afin de modéliser les conditions favorisant la persistance de la ma dans des environnements spatialement hétérogènes; **CHAPITRE III**) quantifiera les sources de variation phénotypique dans les copépodes de *L. minutus* (taille corporelle, fécondité et accumulation trophique d'acides gras essentiels) qui sont associés aux gradients d'acidité et de prédation à l'échelle du paysage. Dans ce dernier chapitre, je considérerai également les effets des cascades trophiques générées par la dynamique des nutriments entraînées par les consommateurs (Consumer-driven Nutrient Dynamic ou CND).

Mots clés: Aquatique, Maladaptation, Metapopulations, Reseaux trophiques, Traits

# ABSTRACT

Abiotic variables can significantly alter ecosystem processes as well as act as selective agents of evolutionary diversification across landscape gradients. However how abiotic variables can affect phenotypic variation for adaptive or maladaptive traits across landscapes with differing levels of spatial and temporal heterogeneity is largely unexplored. Using a metacommunity approach, my thesis will examine ecologically relevant processes acting to influence the extent of phenotypic variation operating at the local and landscape levels, in a key and abundant primary aquatic consumer. The present work was conducted for the most part at multiple food web trophic levels in an understudied system that spans broad natural abiotic gradients in acidity. Acidity is a potent ecological force that can operate on different levels, from individuals to the ecosystem scale, acting on physiological tolerances, community interactions and ecosystem attributes. The main focus of my work will be on the role of phenotypic variation among *Leptodiaptomus minutus* calanoid copepod populations across landscape gradients in acidity, spanning different levels of ecological complexity. These copepods are often the dominant primary consumers that transfer energy and essential compounds between primary producers and both invertebrate and fish predators in north-temperate aquatic food webs. There are three main thesis objectives: **CHAPTER I)** will make a landscape level comparison of *L. minutus* pH tolerance across three regions that differ in spatial and temporal heterogeneity and quantify the consequences of these ecological differences for copepod population-level maladaptatation in terms of phenotype-environment mismatches; **CHAPTER II)** will produce a two-patch metapopulation model based on empirical evidence, with source-sink characteristics and asymmetric selection to model the conditions that favour the persistence of phenotype-environment mismatches in spatially heterogeneous environments; **CHAPTER III**) will quantify sources of phenotypic variation in *L. minutus* copepods (body size, fecundity, and the trophic accumulation of essential fatty acids) that are associated with landscape gradients in acidity and predation. In my final chapter, I will also consider the effects of trophic cascades generated through consumer-driven nutrient dynamics (CND).

Keywords: Aquatic, Food webs, Maladaptation, Metapopulations, Traits,

# INTRODUCTION

Human disturbance is becoming increasingly problematic for the persistence of populations and species worldwide. As a result of anthorpogenic disturbance and climate change, biodiversity is declining globally with major consequences for key ecosystems processes (Hooper et al. 2012; Cardinale et al. 2012; Dirzo et al. 2014). This is of concern for the survival of native species and resident natural populations when disturbed in their wild natural habitat. In combination with environmental changes, human activity on short contemporary scales is already producing unexpected ecological and evolutionary outcomes. Ecology and evolution must be inextricably linked if they are occurring on similar contemporary timescales, with current day ecology being in large part what shapes adaptive evolutionary change (Darwin 1859). Such anthropogenic alterations can be a potent source of selection for evolutionary responses that occur over ecological time frames (<100 years) (Stockwell et al. 2003; Hendry et al. 2011). This has led scientists to ask on the effect of evolution on ecological interactions (Yoshida et al. 2003; Hairston 2005; Palkovacs et al. 2009; Post and Palkovacs 2009; Fussmann et al. 2007; Walsh et al. 2012; Ellner 2013) and ecosystem processes (Harmon et al. 2009; Palkovacs et al. 2009; Bassar et al. 2010; Matthews et al. 2011; Zandona et al. 2011; Bassar et al. 2012). Changes in the performance of ecologically important traits, could potentially have cascading effects at the gene, individual, population, community, and ecosystem levels (Whitham et al. 2006; Fussmann et al. 2007; Post and Palkovacs 2009; Matthews et al. 2011; Schoener 2011; Hendry et al. 2011; Ellner 2013; Reznick 2013). Analysis of rapid evolution as an ecological process has the potential to make evolutionary ecology one of the most central of applied biological sciences (Thompson 1998; Stockwell et al. 2003; Hendry et al. 2011).

How natural populations are able to cope and respond to these disturbances has important implications for predicting population responses to multiple sources of disturbance. There is ample evidence in the scientific literature to suggest that populations can evolve rapid responses to these ecological disturbances in contemporary timescales (Thompson 1998; Hairston et al. 1999; Hendry and Kinnison 1999; Palumbi 2001; Bradshaw and Holzapfel 2001; Stockwell et al. 2003; Reale et al. 2003; Yeh 2004; Olsen et al. 2004; Hairston et al. 2005; Hendry et al. 2006; Derry and Arnott 2007; Fisk et al. 2007; Hendry et al. 2011; De Leon et al. 2011), and, as a result, have cascading effects on communities and ecosystems. Deeper insights into how disturbance in native habitats affects evolution, and also physiological responses of populations to these stressors would give us greater capacity to predict how interactions between ecology and evolution mediate responses in altered aquatic food webs. This work is important as a global unit because it allows us to better comprehend interactions between ecology and evolution in natural, unperturbed ecosystems, and because it can inform us of possible consequences of human disturbance in anthropogenic-challenged ecosystems in other regions.

I here provide a brief overview, structured in subsections, of the theoretical and empirical work that was carried out throughout this research. The thesis made use of an excellent model species, the calanoid copepod (*Leptodiaptomus minutus*), with widespread distribution across lakes and ponds, but also rapid generation times, sexual species, often dispersal limited, but counter-intuitively the most dominant zooplankton species found across freshwater environments in the northeastern North American biogeographic range. First I will empirically quantify the influence of the regional landscape context on local trait divergence in natural populations. Then at the local level, I incorporate the dynamic forces of asymmetry in selection and migration into a theoretical heterogeneous two-patch model with source-sink characteristics, to explain the underlying mechanisms that maintain adaptation or maladaptation in natural populations. I then expand my research at the community level, to quantify the importance of the abiotic and biotic environment in determining these responses. Finally and again at the local level, I address the important role of food webs and the nutritional state of aquatic ecosystems situated along these environmental gradients.

There are three specific thesis objectives: **CHAPTER I** will explore ecological differences in *L. minutus* pH tolerance at the landscape level at broad scales across three regions that are subject to a combination of environmental, spatial, and historical influences; **CHAPTER II** will produce a mathematical two-patch model based on the results obtained from a two-year experiment conducted in nature, to better understand the effect of asymmetric selection for maladaptive trait-environmental mismatch in natural populations of *L. minutus*; **CHAPTER III** will quantify sources of phenotypic variation in *L. minutus* copepods that are associated with landscape gradients in acidity and fish predation, such as body size, fecundity, and the trophic accumulation of lipids and essential fatty acids (HUFAs) across a simplified food web. These chapter objectives are addressed experimentally by means of common environment transplant experiments in both the laboratory and the field (**CHAPTERS I and II**), a discrete two-patch mathematical model (**CHAPTER II)** and by means of a whole-pond stocking experiment (**CHAPTER III).** The first two chapters make unique contributions to the scientific literature in the fields of metapopulation ecology and aquatic ecology, and the third chapter makes a novel contribution to our understanding of food web ecology in shallow aquatic ecosystems. Overall the thesis is a unique blend of metapopulation ecology, landscape ecology, and food web ecology, giving new insights into factors that determine intraspecific trait variation (adaptive and maladaptive) of crustacean zooplankton in aquatic ecosystems.

### *0.1 Maladaptation*

Widespread maladaptation in nature is undisputed by theoretical (Stearns and Sage 1980; Gillespie 1994; Lande and Shannon 1996; García-Ramos and Kirkpatrick 1997; Holt and Gomulkiewicz 1997; Kirkpatrick and Barton 1997; Crespi 2000; Ronce and Kirkpatrick 2001; Thompson et al. 2002; Nesse 2005; Roy et al. 2005; Frank 2007; Garant et al. 2007; Hanski 2010) and empirical (Stearns and Sage 1980; Järvinen and Välsänen 1984; Møller 1995; Thomas et al. 1996; Dias and Blondel 1996b; Post and Forchhammer 2008; Rolshausen et al. 2015) studies. The reasons for widespread maladaptation in nature are numerous (Haldane 1956; Barton and Partridge 2000), with a general agreement that phenotypes tend to be usually lagging behind changes in the environment and that maladaptation is more of a persistent state rather than an outcome of many natural populations. If maladaptation is widespread in nature, then it is important to study it, explain how it persists over extended spatial and temporal scales (Hendry and Gonzalez 2008).

Maladaptation can be defined as mismatches between the mean population phenotype and the optimal phenotype in relation to the environment in a point in time, and can cause a decrease in the local fitness of a population (Lande and Shannon 1996), with a lack of plasticity also leading to maladaptation. Maladapted populations are therefore dominated by phenotypes with lower fitness than other available phenotypes (Dias and Blondel 1996b). In fragmented landscapes in particular, dispersal (Hanski and Gaggiotti 2004; Leibold et al. 2004) and gene flow (Ronce 2007) between habitat patches constrain local adaptation and ensure persistent maladaptation because of migration load (Lenormand 2002; Hendry and Taylor 2004; Bolnick and Nosil 2007; Moore et al. 2007). The extent of local maladaptation and population differentiation will therefore highly depend upon dispersal rates and spatial patterns in fragmented landscapes (Legrand et al. 2017). The conventional view of local adaptation assumes stable local populations in which the phenotypic distribution approaches equilibrium (Hanski and Singer 2001). However local adaptation defined in this way is not a measure of migration load because populations are not being compared to an optimum they would achieve in the absence of migration (Lenormand 2002). Thus, increasing maladaptation is more likely to occur in heterogeneous fragmented habitats where the environment is highly variable in space and time, often fluctiating that is, but also where initial population sizes are small, genetic variation is reduced or limited and dispersal from neighbouring habitats is sufficiently strong to introduce maladapted variants.

### *0.2 The regional landscape context and its role in determining maladaptation*

Under natural selection individuals tend to adapt to their local environmental conditions (local adaptation), however local adaptation is hardly ever perfect and cannot be so dim to trade-offs, varying on a continuum from highly adapted to poorly adapted (Farkas et al. 2015), with some degree of maladaptation being common in nature (Crespi 2000). This spectrum of adaptive trait variation is particularly important and necessary to overcome the challenges imposed under environmental change. Theory and empirical evidence suggest that local adaptation is hard to attain and that maladaptation is widespread (Stearns and Sage 1980; Gillespie 1991; Lande and Shannon 1996; Garcıa-Ramos and Kirkpatrick 1997; Holt and Gomulkiewicz 1997; Kirkpatrick and Barton 1997; Crespi 2000; Thompson et al. 2002; Nesse 2005; Roy et al. 2005; Frank 2007; Garant et al. 2007). Local adaptation is expected under scenarios of low dispersal and strong selection (Brown and Pavlovic 1992; Day 2000; Kisdi 2002; Spichtig and Kawecki 2004), with fitness being maximized when the population's phenotypic distribution is found matching the optimum environmental value in a habitat. At this optimum, it is usually assumed that the adaptive value of a population is maximized and that fitness declines toward zero as the deviation of the actual from the optimum phenotype increases (MacArthur and Levins 1964). When selection is very strong however, fitness can fall off very quickly as the phenotypic distribution of a population deviates from this local optimum. Migration can also contribute to deviating populations from their optima for survival. This is because an increase in the connectivity of two habitats above some threshold results in a process of 'migrational meltdown' (Ronce and Kirkpatrick 2001), with the collapse of the total population size and the complete loss of one of the two habitats. Some other common constraints to local adaptation are related to differences in population sizes, genetic variation, as well as the spatio-temporal dynamic nature of the environment (e.g. fine vs. coarse-grained environments, stabilizing vs. fluctuating selection, spatial network structure and configuration). Together as a whole, these factors ensure that optima are rarely attained by local phenotypes and that maladapatation is prevalent and persistent (Hendry and Gonzalez 2008).

It is well known that observed patterns and their apparent relationships with response variables often depend upon the scale that is chosen for observation and the rules that are adopted for defining and mapping variables (Wiens 1989). Landscape context (**Fig. 1**) can thus contribute to reducing local adaptation in populations depending on the pattern of environmental heterogeneity, the spatial distance between habitats on landscapes, and the level of habitat connectivity (Hanski et al. 2010; Richardson et al. 2014; Farkas et al. 2015). These theoretical predictions are based on a wealth of empirical studies that have shown that local adaptation is contingent on a balance between selection, gene flow, and genetic drift operating within landscapes (Slatkin 1987; Lenormand 2002; Hendry et al. 2002; Nosil and Crespi 2004). Local adaptation often occurs in response to local selection gradients in fragmented landscapes with low connectivity (Endler 1986; Schluter 2000; Kawecki and Ebert 2004; Ghalambor et al. 2007; Hereford 2009). However, local adaptation can be further reduced depending on spatial landscape context because of (i) phenotypic plasticity, which can be highly adaptive (plasticity as an adaptive trait that can evolve without genetic change) that is favoured under conditions of high gene flow and spatial environmental heterogeneity (Sultan and Spencer 2002; Lind and Johansson 2007; Hollander 2008; Crispo 2008; Baythavong 2011; Lind et al. 2011), (ii) high genetic trait variance that may occur in the presence of low levels of gene flow from habitats with strong opposing selection (maladaptation: Urban 2006; Farkas et al. 2015), (iii) temporal variation in selection pressures and (iv) cost versus benefits of traits (trade-offs).

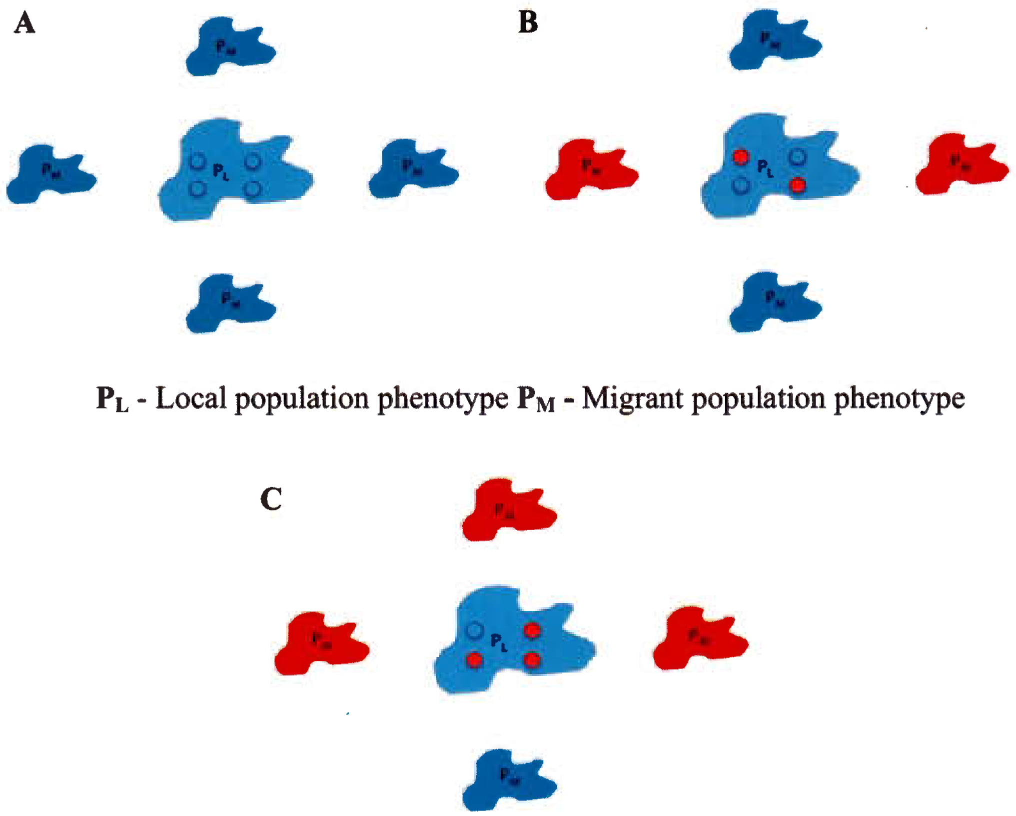


**Fig. 1** Theoretical predictions explaining the relationship between landscape context and local maladaptive trait divergence of the local population phenotype (**$P_L$**) subject to incoming migration from the migrant population (**$P_M$**) inhabiting in-close proximity habitats. **A.** Low local maladaptive trait variation in a homogenous landscape; **B.** Intermediate local maladaptive trait variation in a heterogeneous landscape; **C.** High local maladaptive trait variation in an asymmetric landscape.

Thus, the degree of local maladaptive trait variation can strongly be influenced by the characteristics of the landscape (**Fig. 1**), especially across landscapes that are spatially heterogeneous (**Fig. 1B and Fig. 1C**) and in habitats with contrasting environmental conditions that are found in-close proximity to each other.

The response of aquatic local food webs to altered environmental conditions thus depends on metacommunity responses – the balance of local filtering imposed by abiotic conditions and biotic interactions (Leibold et al. 2004), and effects of regional dispersal among habitats (Westley et al. 2010; Gray and Arnott 2012a). Vellend (2016) elegantly described the processes used to explain community patterns as going through a series of filters that are system-specific (dispersal, abiotic and biotic filters) that cause fitness differences among species in a metacommunity framework. While local environmental conditions and biotic interactions are selective agents, dispersal supplies a community with different species and genotypes on which the selective agents act (Cottenie and DeMeester 2004; Urban et al. 2008). These same concepts can be applied at the metapopulation-level, where in addition to local environmental filtering, local evolutionary processes may also occur (Urban et al. 2008). Thus abiotic variables (e.g. pH) can significantly alter ecosystem processes as well as act as selective agents of evolutionary diversification across landscape gradients. However, the influence of abiotic variables on interactions between ecology and evolution is largely unexplored. The possible consequences of fitness differences are relatively small in number (speciation, dispersal, drift, selection), but universally applicable (Vellend 2016).

Standing genetic variation for adaptive traits can be maintained among spatially heterogeneous habitats with divergent selection, and among-habitat dispersal may theoretically help (Bell and Gonzalez 2009; Gonzalez and Bell 2013) or hinder (Schiffers et al. 2013) local evolutionary responses to disturbance depending on the level of maladaptation of migrants. Reciprocal transplant experiments and common garden transplant experiments can be used to characterize the nature of phenotypic variation and adaptation among populations along environmental gradients, and the nature of selection on traits (Conover and Schultz 1995; Kawecki and Ebert 2004). A multitude of reciprocal transplant experiments have shown that adaptation is what causes local individuals to have higher fitness than foreign individuals (Leimu and Fischer 2008; Hereford 2009) enabling populations to persist through environmental change.

In the context of climate change it is important to keep in mind that, establishing that an observed phenotypic shift has a genetic basis as a necessary condition for inferring adaptive evolution in response to climate is not entirely sufficient because genetic changes might not be adaptive (Merilä and Hendry 2014). Phenotypic responses to selection could result from genetic differences (local adaptation), maternal effects, phenotypic plasticity, or a combination of these, and these sources of variation could potentially result in different evolutionary (Ghalambor et al. 2007) and ecological (Urban et al. 2008) outcomes. Ecological factors predicted to promote local adaptation include: low gene flow (i.e. low dispersal or strong habitat fidelity), strong selection against genotypes optimally adapted to other habitats but moderate selection against intermediate genotypes (most likely under moderate differences between habitats with respect to traits under selection), little temporal variation in the forces of selection, small differences between habitats in size and quality (e.g. the amount of resources), and costs of or constraints on adaptive plasticity (Kawecki and Ebert 2004). Local adaptation is expected to predominate in spatial heterogeneous but temporally stable environments among which dispersal is somewhat limited (Endler 1986; Schluter 2000; Kawecki and Ebert 2004; Ghalambor et al. 2007). However, local adaptation may also lead to trade-offs where adaptations to one environment can come at a cost of adaptation to another environment (Hereford 2009; Jones 2013). To name a few examples in nature of specialist phenotypes that incur adaptive fitness responses to selection at the local and the landscape level, includes; functional diversity among predators of the freshwater snail *Physa* creates an adaptive trade-off for the snail's shell morphology (De Witt et al. 1998), tadpoles of the pacific treefrog (*Pseudacris regilla*) survived best when exposed to the predator whose cues they were reared with, and worst when exposed to the other predator (Benard 2006), adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds (Muchhala 2007), *L. minutus* is highly adaptive to changes in environmental conditions with fitness trade-off in copepods with high acid tolerances at circumneutral pH (Derry and Arnott 2007; Negrín Dastis and Derry 2016) and butterfly flight activity affects reproductive performance and longevity relative to landscape structure (Gibbs and Van Dyck 2010). A trade-off in a fitness-determining trait can have a counterintuitive influence on the evolution of specialization (Egas et al. 2004). If maladaptation is persistent in natural systems, there must be weak trade-offs of adaptation associated with it (Hereford 2009). This would explain why predictions of theory relating evolution of specialization to costs of adaptation are frequently not met (Futuyma and Moreno 1988).

### *0.3 The role of abiotic and biotic ecological gradients*

The distribution and success of an organism is a function of abiotic constraints and biotic processes (Brönmark and Hansson 2010). In abiotic terms, pH, DOC (dissolved organic carbon) and temperature, among others, can act separately or in concert as potent ecological forces that can operate from the individual to the community level, affecting physiological tolerances and community interactions. Biotic factors such as predation can also have profound effects on aquatic food webs (Carpenter et al. 1985; Magnan 1988; Pace et al. 1999). Thus in combination, the abiotic and biotic factors provide the frame that determines the successful or unfavourable colonization of a species (**Fig. 2**).

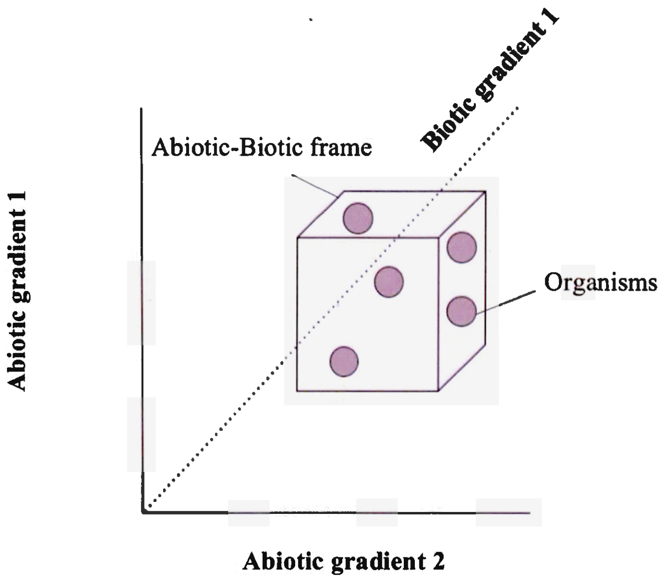


**Fig. 2** A graphical representation of abiotic and biotic environmental gradients. Spheres are species that have a niche that fits the Abiotic-Biotic frame and thus may colonize the specific lake (modified from Brönmark and Hansson 2010).

In regions, for example, were the bedrock is rich in bicarbonates, a combination of water and soils can have high acid neutralizing capacity (high alkalinity) and can thus buffer against acidity. In contrast, in other regions where bedrock is absent, pH can drop sharply. Thus the geological formation of a region can strongly affect the pH of a lake or pond, but processes such as respiration can also add carbon dioxide to the system via carbon fixation, which releases $H^+$ ions, leading to lower pH (Brönmark and Hansson 2010). Increased photosynthesis, on the other hand, which is essential for phytoplankton, may raise pH with carbonate ions precipitated as calcium carbonate, and represents the autotrophic proportion of basal production in the pelagic food web (Faithfull et al. 2011). In net-autotrophic systems photosynthesis rate exceeds respiration rate and oxygen is exported to the atmosphere, in contrast with net-heterotrophic systems where organisms are subsidized with carbon (dissolved organic carbon 'DOC' and/or particulate organic carbon 'POC'), and respiration rate becomes higher than photosynthesis rate leading to export of carbon dioxide to the atmosphere (Brönmark and Hansson 2010).

The relationship between pH and DOC is important, because pH can also be strongly influenced by the organic acids in DOC (Erlandsson et al. 2011). DOC can also thus reduce light penetration via shading, causing lower algal biomass (Ask et al. 2012), and potentially increase bacterial productivity and food web dependence on bacterially-derived carbon sources in place of phytoplankton (Sherr and Sherr 1987; Kankaala 1988; Jansson et al. 1999; Bergström and Jansson, 2000; Lennon and Pfaff 2005; Rautio and Warwick 2006; Lennon and Cottingham, 2008; Karlsson et al. 2009; Kankaala et al. 2010; Andersson et al. 2013; Forsström et al. 2013), affecting aquatic food webs (Faithfull et al. 2011). Carbon that is derived from pelagic bacteria has a lower nutritional value because it lacks the essential poly-unsaturated fatty acids (PUFAs) that are synthesized by algae (Wenzel et al. 2012). Therefore, DOC can have strong effects on basal resources such as phytoplankton and bacteria, which have very different nutritional value to higher consumers (Faithfull et al. 2011). Differences in DOC among aquatic habitats could therefore potentially modulate the availability of essential fatty acids to intermediate aquatic consumers such as zooplankton by stimulating or suppressing phytoplankton growth. There is evidence that increased terrestrial inputs of DOC to nutrient-poor aquatic ecosystems can lead to reduced production in higher trophic levels (Karlsson et al. 2009) and reduced overall net ecosystem production (Ask et al. 2012).

Landscape gradients in dissolved organic carbon (DOC) often co-occur with naturally derived acidity from humic sources (wetlands and peat) (Pastor et al. 2003). DOC, and its association with humic sources of acidity as mentioned earlier, is anticipated to potentially either increase or decrease in aquatic environments in regions that experience changes in precipitation and terrestrial runoff associated with climate change (Schindler et al. 1996; Zhang et al. 2010; Larsen et al. 2011). DOC can exert mild chemical stress on organisms, yet reduce iono-regulator disturbance from acidic waters, but still disrupt the types and quantities of basal resources available to higher consumers by inhibiting photosynthesis and algal growth and by promoting bacterial productivity (Steinberg et al. 2006). One avenue by which climate change is anticipated to impact the ecology of north-temperate freshwater ecosystems is through altered amounts of precipitation and associated allochthonous inputs of DOC to aquatic habitats (Larsen et al. 2011).

### *0.4 Zooplankton plasticity, adaptation and traits*

In its simplest definition, a trait is a surrogate of organismal performance, and evolutionists have used this meaning of the term for a long time (Violle et al. 2007). Zooplankton responses to selective pressures and traits are ecologically significant because they can affect survival (e.g. pH tolerance), maternal investment (e.g. egg number and size), and growth and reproduction (body size, lipid accumulation, larval survival, fecundity, and abundance). These traits are of special interest because they can act as potential sources of phenotypic variation along landscape environmental gradients and can thus directly affect performance and fitness along these gradients. These responses and traits can be particularly important because they can alter ecological processes (Matthews et al. 2011; Reznick 2013). Stockwell et al. (2003) identified three primary contexts in which humans drive trait changes: in situ anthropogenic disturbance, introduction of populations to new habitats, and introductions of new interacting species (e.g., hosts, pathogens, and predators) within the existing range of a species. Traits can range from the individual to the ecosystem level, including but not limited to the study of; morphological character developments, life-history changes and other demographic traits related to birth and death rates. According to Walsh et al. (2012), changes in the performance of these traits could have cascading effects at the gene, individual, population, community, and ecosystem levels, potentially impacting both top-down and bottom-up trophic interactions in food webs (Post et al. 2008; Palkovacs et al. 2008).

How phenotypic trait changes operate in nature is still not completely clear. How can we explain the causes that make certain trait changes more plastic than others and what properties do these traits contain that make them special in the sense that they provide species a certain evolutionary advantage is still unknown. One experimental tool, unrelated to the genetic mechanisms that underlie the adaptive evolution of a trait, has been particularly useful in addressing whether a specific trait has evolved in part by the process of adaptation is manipulation of the phenotype after Sinervo and Basolo (1996). Other experimental approaches, after (Pigliucci et al. 2006) have controlled for genotype within family variation, depicting genotypic reaction norms illustrating the concept of phenotypic plasticity taking into account that the slope of such reactions is a measure of the degree and pattern (positive or negative) of phenotypic plasticity. Thus, categorizing phenotypic traits in relation to their function is especially important when considering the potential for how evolution in these traits might have a feedback on ecology.

Generally, an adaptation is a phenotypic feature which is functionally designed by past natural selection, and which improves Darwinian fitness relative to alternative features (Williams 1966). However, plasticity and selection can jointly facilitate local adaptation (Torres-Dowdall et al. 2012). Local adaptation however may also lead to trade-offs, in which adaptation to one environment comes at a cost of adaptation to another environment, yet, many studies have not demonstrated local adaptation, suggesting that sometimes native populations are no better adapted than are genotypes from foreign environments (Hereford 2009). However, examples of locally adapted prey to fish exist, as is the case for *Daphnia* studies (De Meester 1996; Boriss et al. 1999; Declerck et al. 2001; Cousyn et al. 2001) for the most part. Boriss et al. (1999) showed that the observed changes in life-history characteristics might lead to differences in fitness under different predation regimes, such that populations from habitats with fish have highest fitness under fish predation regimes and populations without a fish background have higher fitness values under invertebrate predation regimes. Cousyn et al. (2001) also reported the case for rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes and found in this particular study concerning resting egg bank of the waterflea *Daphnia*, that the population studied experienced variable and welldocumented levels of fish predation over the past 30 years and shows correlated genetic changes in phototactic behavior, a predator-avoidance trait that is related to diel vertical migration and that these changes mainly involve an increased plasticity response upon exposure to predator kairomone, the direction of the changes being in agreement with the hypothesis of adaptive evolution. While adaptation to fish predation has been proven to be an important mechanism in *Daphnia*, in a different context, *Daphnia* exposed also to high cyanobacteria levels over long periods of time can adapt to being more tolerant of toxic cyanobacteria in their diet (Sarnelle and Wilson 2005).

### *0.5 The role of consumer nutrient dynamics (CND) and abiotic gradients*

Environmental gradients are key structuring agents that determine the structure of biodiversity across landscapes (Loreau et al. 2002; Hooper et al. 2005). However, much of the focus on understanding the spatial distribution of species richness and community compositional patterns in aquatic ecosystems has focused on abiotic factors such as pH, temperature, and nutrients (Pinel-Alloul 1995; Shurin et al. 2010; Greig et al. 2010; MacLennan et al. 2012; Shurin et al. 2012). Fish can also play an important role in supplying additional nutrients in the form of phosphorus (Schindler et al. 2001) and endogenous nitrogen to the ecosystem through excretory processes (Vanni 2002), where fish are able to affect ecosystem processes as much as bottom–up factors (El-Sabaawi et al. 2015). Fewer studies have addressed the role of biotic factors, especially top predators, in regulating the biodiversity and species composition of prey communities, especially at the landscape scale (Zarnetske et al. 2017). This is despite the known importance of fish predators in regulating prey communities in small local habitats (Estlander et al. 2017) as well as fish altering key ecosystem functions such as nutrient regeneration (Vanni 2002).

Salmonids are commercially important fish and are broadly distributed across their native range at northern latitudes (Scott and Crossman 1973). Salmonids can cause trophic cascades that result in increased algal biomass and production and are responsible for changes to energy and nutrient flux in both streams and lakes (Simon and Townsend 2003). Salmonid introductions in naturally fishless ponds and lakes can thus have varied effects that depend on both landscape characteristics of a region, such as geology and historical biogeography, as well as local habitat properties, such as water chemistry and local community assemblages of zooplankton.

Consumer-mediated nutrient dynamics (CND) are an indirect mechanism by which predators can alter prey organismal traits and have ecological consequences at the ecosystem-scale (reviewed in Atkinson et al. 2016). The importance of CND has been widely recognized, with pioneering work taking place in freshwater ecosystems (Kitchell et al. 1979). In aquatic ecosystems, animals have been recognized to play an integral role in the storage and remineralisation of elements (Vanni et al. 2006; Vaughn, 2010). Consumers thus play, an important functional role in biogeochemical processes (Capps et al. 2015). Studies that have measured the contribution of migrations of anadromous fish to nutrient dynamics in stream ecosystems (Holtgrieve and Schindler 2011; Tiegs et al. 2011) are a clear example of CND. Although there is empirical evidence to support CND effects from animals acting as sources of nutrients through excretion and nutrient sinks through growth, there are still many fundamental questions (Capps et al. 2015) involving CND that remain unclear (e.g. (1) the potential ecosystem-level effects of nutrient remineralisation, transport and storage between small aquatic and large aquatic and terrestrial animals, (2) the influence of immigration and emigration of consumers on nutrient dynamics, (3) context dependence of species effects on biogeochemical cycling (El-Sabaawi et al. 2012; Marshall et al. 2012), (4) the importance of homeostasis and ontogeny in driving excretion rates (Pilati and Vanni 2007; Small and Pringle 2010; Hossack et al. 2013) and (5) eco-evolutionary feedbacks in CND (Zandona et al. 2011; El-Sabaawi et al. 2015), among many others (Atkinson et al. 2016). Animals can be important mediators of biogeochemical cycles through "bottom-up" processes of storage (in tissues) and supply (through excretion and egestion) of nutrients (Elser and Urabe 1999; Sterner and Schulz 1998; Vanni 2002) (**Fig. 3**).

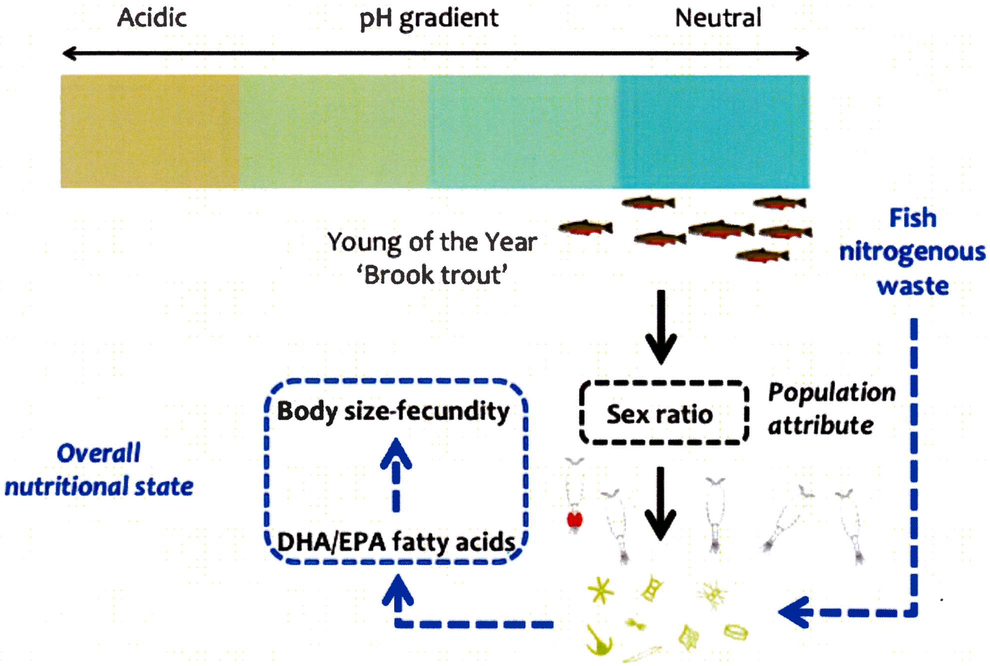


**Fig. 3** Conceptual representation of top-down (Predation) and bottom-up effects (Consumer Nutrient Dynamics) of fish on zooplankton abundance, traits and nutritional state. Feedbacks from algae back onto the zooplankton are also shown.

Abiotic factors (e.g. pH) can also strongly modulate the presence/absence of certain predators (**Fig. 4**). For example, the ecological tolerances of many fish species are exceeded in extremely saline lakes where large-bodied, predation-vulnerable but salinity-tolerant zooplankton can proliferate (Derry et al. 2003; Brucet et al. 2010). Therefore, it is important to consider both abiotic (e.g. pH) and biotic factors (e.g. top predators) when assessing the distribution of biodiversity in prey communities across landscapes.

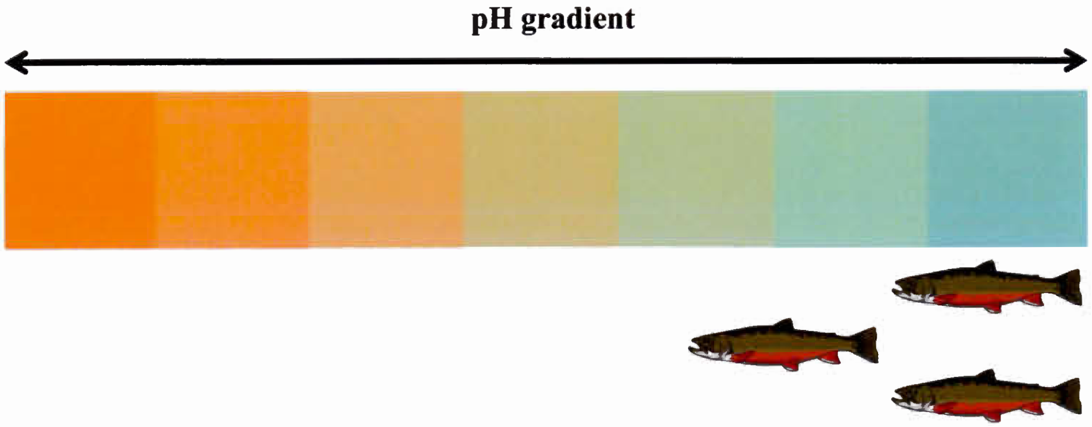


**Fig. 4** The absence/presence of fish determined along a pH environmental gradient. Due to fish physiological intolerances to acidic pH (orange area), fish are found closer to neutral conditions (emerald blue area).

### *0.6 Metapopulation*

Theoretical models for understanding mechanisms underlying the continuum between maladaptation and local adaptation in populations differ in assumptions concerning the landscape structure and the genetic determinism of traits underlying population-level performance traits. These theoretical models can be categorized as models of continuous landscapes with evolutionarily stable strategy models and single locus models (Brown and Pavlovic 1992; García Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997) versus models of patchy landscapes with multiple loci (Holt and Gaines 1992; Kawecki 1995; Holt 1996; Day 2000). Models that have blended these theoretical approaches are rarer (*but see* Kawecki (1995), Holt (1996), Holt and Gomulkiewicz (1997), Ronce and Kirkpatrick 2001, Hendry et al. 2001; Lenormand (2002); Hanski et al. 2010; Polechovà and Barton (2015). Ronce and Kirkpatrick (2001) two-patch quantitative genetic model showed how imbalances in population size between two habitat types connected by gene flow generated positive feedbacks at the population level, leading to specialization in the larger better-adapted population (migrational meltdown). Migrational meltdown with potentially alternative stable equilibria corresponds to the source-sink dynamics analyzed by Kawecki (1995), Holt (1996), Holt and Gomulkiewicz (1997), Lenormand (2002), in which gene flow prevents adaptation in the habitat with a small and poorly adapted population (sink population). As an extension of the two-patch model proposed by Ronce and Kirkpatrick (2001), Hanski et al. (2010) expanded this idea to a network of many populations that undergo local extinctions in which maladaptation was found to decrease population growth and increase the probability of local extinction.

In a large number of models however, symmetric selection operating between habitats occurring within a landscape, have been assumed for mathematical convenience when studying evolution of quantitative traits under stabilizing selection (Urban et al. 2013). This is despite that asymmetric fitness responses to selection are common (Hughes et al. 2007; Derry and Arnott 2007; Haak et al. 2011; Porter and Rice 2012; Negrín Dastis and Derry 2016). However it has been long thought that asymmetric selection is ubiquitous in nature (Schluter 1988), maintaining sub-optimal fitness traits through a bias of the equilibrium mean phenotype away from the individual fitness optimum (Parker and Smith 1990).

## *0.7 The importance of fatty acids in aquatic food webs*

Fatty acids are increasingly being incorporated in food web studies (Twining et al. 2016). Several studies have addressed environmental and community-level determinants of EFA composition in primary consumers such as crustacean zooplankton (Burns et al. 2011; Lau et al. 2012; Gladyshev et al. 2015). EFAs such as polyunsaturated fatty acids (PUFAs), and especially highly unsaturated fatty acids (HUFAs), can limit development, reproduction, and fitness of individual consumers at multiple trophic levels in aquatic food webs (Parrish 2009; Strandberg et al. 2015; Gearhart et al. 2017). In aquatic ecosystems, EFAs are synthesized by primary producers (Taipale et al. 2013), and are trophically-transferred from primary producers to higher consumers (Müller-Navara et al. 2003; Martin-Creuzburg et al. 2009; Taipale et al. 2011; Strandberg et al. 2015; Gearheart et al. 2017).

In aquatic ecosystems, fish consumer traits such as body size, tissue composition, growth rate, reproduction, life span, degree of homeostasis, and temperature preference and tolerance (Atkinson et al. 2016), have the potential to modulate or alter CND by boosting primary production that allows phytoplankton communities to thrive owing to the greater availability of nutrients due to fish excretory processes (Vanni et al. 2006; Wilson and Xenopoulos 2011). This in turn can directly affect organismal traits of prey (EFAs) through consumer-mediated trophic cascades. EFAs can then be potentially mediated to the population and/or community level (e.g. influencing competition for resources), with the potential to affect ecosystem structure and function.

### *0.8 Study system*

For only **CHAPTER I** and in order to provide a comparison at the larger landscape level, I will also be working in various Canadian aquatic landscapes of north-eastern North America; (1) a homogeneous pH landscape (Laurentians, Qc) of exclusively circumneutral lakes (pH ≥ 6.0); (2) a heterogeneous pH landscape with a mixture of acidic and circumneutral lakes (Killarney, Ontario); and (3) a heterogeneous pH landscape in which relatively infrequent circumneutral ponds are embedded in a predominantly acidic landscape (Cape Race, NL). For the remaining chapters of my thesis (**CHAPTER II, CHAPTER III**), research will be centered on aquatic food webs of small ponds that span pH gradients on Cape Race (NL) (**Fig. 5**). There are many characteristics of the pond system on Cape Race that are attractive as a natural laboratory for investigating abiotic and biotic ecological gradients for adaptive and maladaptive population phenotypic variation in calanoid copepods: a multitude of small pond ecosystems, broad environmental gradients in DOC, pH, and salinity among ponds within close proximity of each other, simplified pond food webs dominated by calanoid copepods (*Leptodiaptomus minutus*), and adaptive variation among populations of the top predator, brook trout (*Salvelinus fontinalis*). These copepods are often the dominant primary consumers that transfer energy and essential compounds between basal resources and both invertebrate and fish predators in north-temperate aquatic food webs. The ponds are environmentally heterogeneous, ranging from highly acidic to circumneutral pH and tannic to clear waters. While copepods and predatory beetles occur across this spectrum of environmental conditions, the brook trout can naturally exist in circumneutral and mildly acidic ($5 \geq \text{pH} < 6$) ponds. In the ponds, zooplankton communities are often dominated by calanoid copepods (*Leptodiaptomus minutus*), predatory dytiscid (*Ilybius discedans*) and whirligig (*Dineutes discolour)* beetles, corixids, and occasionally brook trout (*Salvelinus fontinalis*). However, most brook trout on the Cape can be found as distinct populations in small streams that tumble off cliffs into the Atlantic Ocean, where they vary greatly in population size (Belmar-Lucero et al. 2012) and are locally adapted to overwintering habitat, with traits ranging from slow to fast growth and variation in size at maturity (Hutchings 1996).

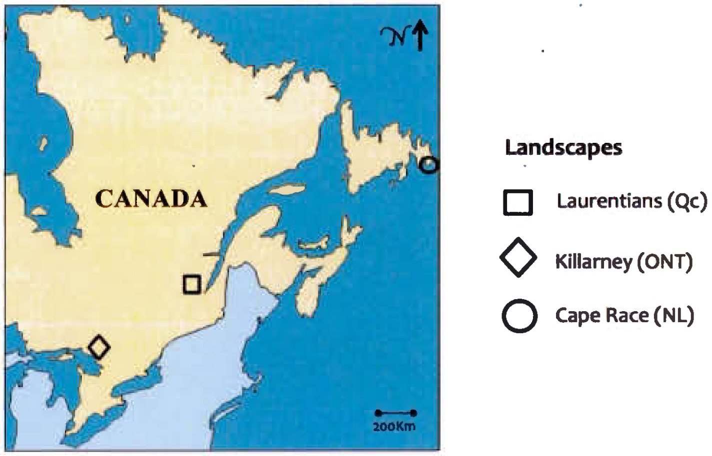


**Fig. 5** Location of the three study regions. Most of the detailed experimental work will be focused on small ponds in Cape Race (NL).

### *0.9 Thesis outline*

I will address the effects of acidity at the landscape level on ecologically important phenotypic traits that consider both bottom-up effects of resources and top-down impacts of predators in aquatic food webs. The focus will be on the role of phenotypic differences among *Leptodiaptomus minutus* calanoid copepod populations because these are often the dominant primary consumers that transfer energy and essential compounds between basal resources and invertebrate and fish predators in north-temperate aquatic food webs (Thorp and Covich 2001). A two-patch model was constructed as an extension of the heterogeneous two-patch model developed by Ronce and Kirkpatrick (2001) to explore how asymmetric versus symmetric single-trait fitness responses to selection and migration play a role in favoring the emergence of persistent maladaptation in a simple metapopulation. We additionally complemented this theoretical framework with a landscape level experiment that was conducted in the field. In my final chapter, I will also consider the effects of salmonid-driven nutrient dynamics (CND) at the whole-pond ecosystem level and measure its impact on fatty acid concentration at each trophic level of a simplified food web. The thesis (**Fig. 6**) is organized and divided into three core chapters that address via experiments conducted in the field and theoretical modelling approaches, the consequences of environmental, historical and spatial factors operating at various levels of ecological complexity, notably at the individual, population, community and ecosystem level.

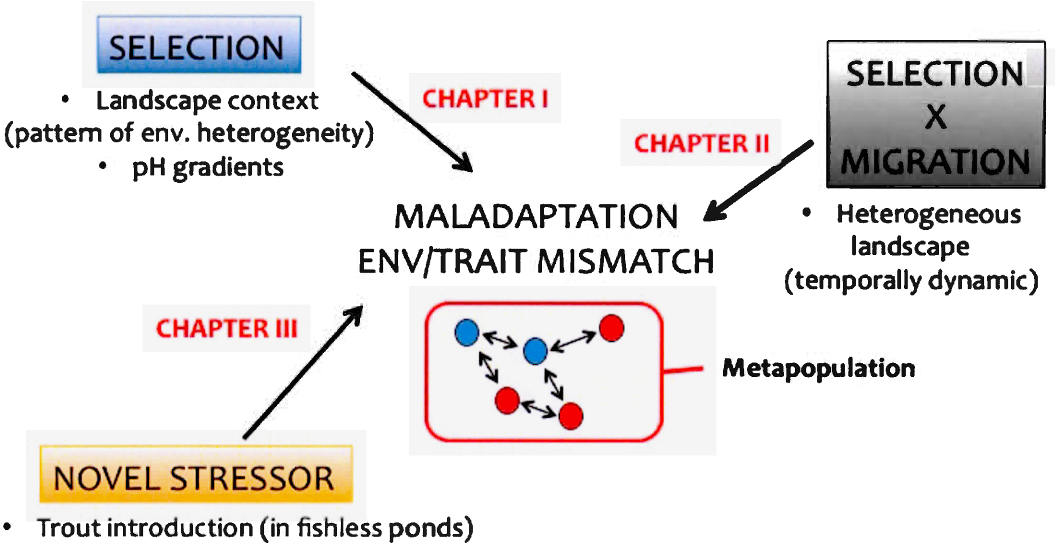


**Fig. 6** Unified and cohesive organization of chapters addressing through a combination of experimental field, laboratory manipulations and modelling, the central theme of the present thesis - maladaptation as environment-trait mismatch.

# CHAPITRE I

# THE IMPACT OF REGIONAL LANDSCAPE CONTEXT ON LOCAL MALADAPTIVE TRAIT DIVERGENCE: A FIELD TEST USING FRESHWATER COPEPOD ACID TOLERANCE

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), pp.841-859.

## Résumé

Theoretical work has shown that spatial landscape context can contribute to reducing local adaptation in populations depending on the spatial pattern of environmental heterogeneity, the spatial scale of distances between habitats on landscapes, and the level of habitat connectivity. However, only a handful of empirical studies have addressed the impact of regional landscape context on local trait divergence in natural populations. We tested if local adaptation in abiotic tolerance is diminished in landscapes with strong spatial heterogeneity and habitat proximity. We used a freshwater copepod (*Leptodiaptomus minutus*) that is known to show local adaptive divergence in acid tolerance as a study system to understand the effects of regional landscape-level spatial heterogeneity in lake/ pond pH on local trait divergence. We compared local divergence in copepod acid tolerance from three types of landscapes: (i) a homogeneous pH landscape of exclusively circumneutral lakes ($pH \geq 6.0$); (ii) a heterogeneous pH landscape with a mixture of acidic and circumneutral lakes; and (iii) a heterogeneous pH landscape in which relatively infrequent circumneutral ponds are embedded in a predominantly acidic landscape. We found that local adaptation to circumneutral lake/pond pH was most reduced in the pH-heterogeneous landscape dominated by acidic habitats, likely because of gene flow from surrounding nearby acidic ponds. Our study empirically confirms theoretical predictions that spatial landscape context is important for explaining regional differences in population environmental tolerances. These effects may become important for understanding regional differences in population fitness trade-offs when presented in combination with multiple stressors.

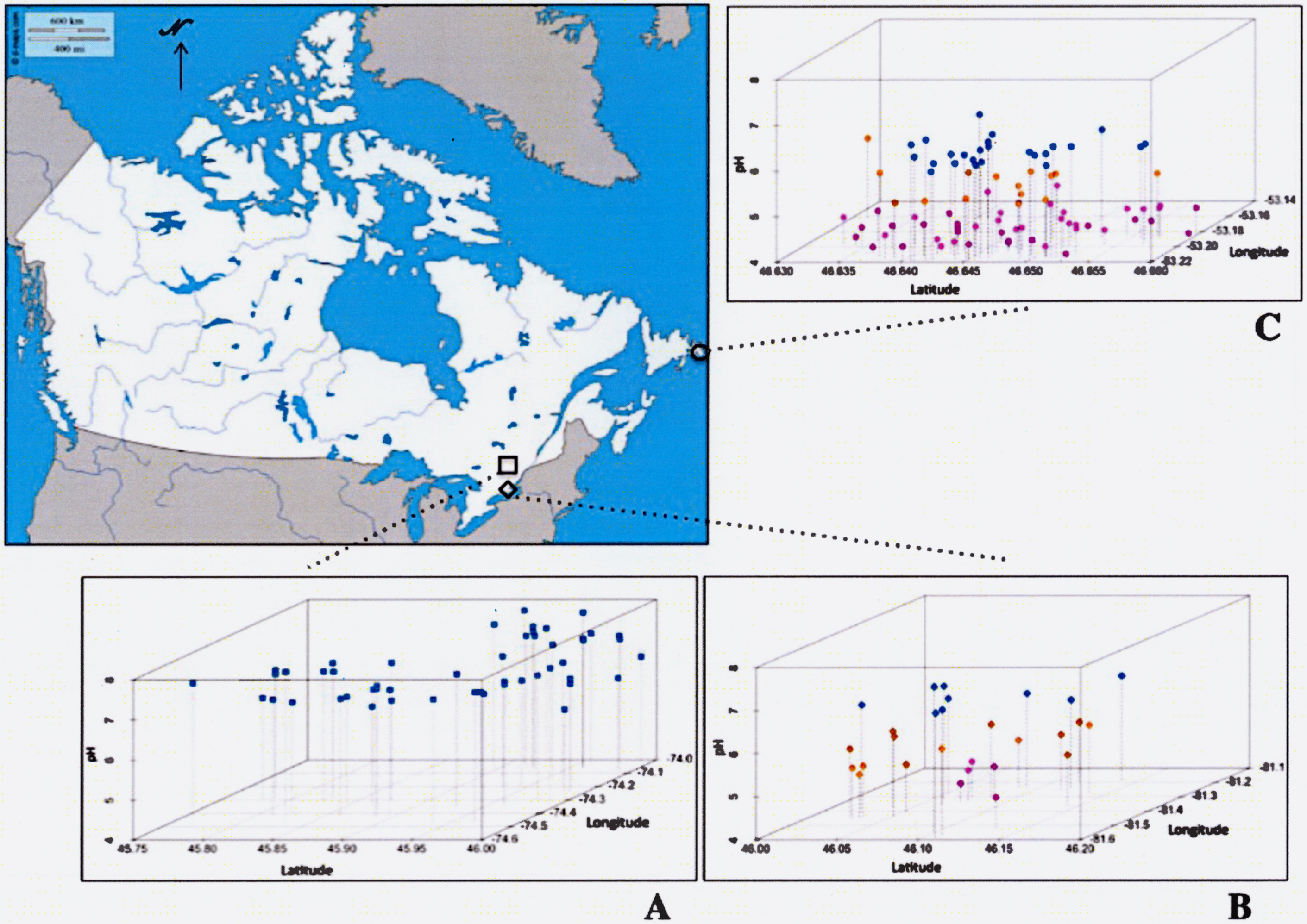
Keywords: Freshwater gradients, Landscape context, Maladaptation, Trait divergence, Zooplankton

## 1.1 Introduction

Metapopulation theory predicts that landscape context can contribute to reducing local adaptation (local adaptive trait divergence) in populations depending on the pattern of environmental heterogeneity, the spatial distance between habitats on landscapes, and the level of habitat connectivity (Hanski et al. 2010; Richardson et al. 2014; Farkas et al. 2015). These theoretical predictions are based on a wealth of empirical studies that have shown that local adaptation is contingent on a balance between selection, gene flow, and genetic drift operating within landscapes (Slatkin 1987; Lenormand 2002; Hendry et al. 2002; Nosil and Crespi 2004). Local adaptation often occurs in response to local selection gradients in fragmented landscapes with low connectivity (Endler 1986; Schluter 2000; Kawecki and Ebert 2004; Ghalambor et al. 2007; Hereford 2009). However, local adaptation can be reduced depending on spatial landscape context because of (i) phenotypic plasticity that is favoured under conditions of high gene flow and spatial environmental heterogeneity (Sultan and Spencer 2002; Lind and Johansson 2007; Hollander 2008; Baythavong 2011; Lind et al. 2011) or (ii) high genetic trait variance that may occur in the presence of low levels of gene flow from habitats with strong opposing selection (maladaptation: Urban 2006; Farkas et al. 2015). The aim of our study was to test if local adaptation in abiotic tolerance is diminished in landscapes with strong spatial heterogeneity and habitat proximity.

Ecologists have long recognized the importance of context dependence for ecological patterns and processes across environmental or spatiotemporal gradients, especially with respect to among-species interactions (Menge and Sutherland 1987; Cardinale et al. 2000; Shears et al. 2008; Clements et al. 2012). By contrast, the possibility that intraspecific differences in local adaptation of populations may also have a regional-context dependency has rarely been addressed by ecological or evolutionary empirical research (Tack et al. 2014). This is despite the growing recognition that intraspecific trait variation, and therefore local adaptation, can play an important role in ecological interactions (Bolnick et al. 2011; Violle et al. 2012), especially in ecosystems challenged by environmental change (Sgro et al. 2011; De Laender et al. 2014). In disturbed ecosystems, adaptive responses that occur within populations to one stressor may result in trade-offs for adaptive responses to another stressor (e.g. metal tolerance and sensitivity to ultraviolet radiation: Kashian et al. 2007; metal tolerance and inducible defenses to predators: Mirza and Pyle 2009); acid tolerance and inducible defenses to predators: Teplitsky et al. 2007). Therefore, the potential effects of regional landscape context on local population tolerance of a given stressor may become important for understanding regional differences in population fitness trade-offs when presented with multiple stressors.

We used a freshwater copepod (*Leptodiaptomus minutus*) that is known to show local adaptive divergence in acid tolerance (Derry and Arnott 2007) as a study system to test the effects of regional landscape-level spatial heterogeneity in lake/pond pH on local trait divergence. We compared local divergence in copepod acid tolerance from three types of landscapes (**Fig. 1**): (i) a pH-homogeneous landscape of exclusively circumneutral lakes (pH C 6.0); (ii) a pH-heterogeneous landscape with a mixture of acidic and circumneutral lakes; and (iii) a heterogeneous pH landscape in which relatively infrequent circumneutral ponds are embedded in a predominantly acidic landscape. We predicted that (i) circumneutral populations in the pH-homogeneous circumneutral landscape would have the least variable and lowest acid tolerance as a result of the least selection for acid tolerance both locally and at the landscape-level, (ii) circumneutral populations in the pH-heterogeneous landscape would have an intermediate acid tolerance and variability for this trait because of gene flow from across mixture of lake pH types, and (iii) circumneutral populations on the acid-dominated landscape would have the most variable and highest acid tolerance because of gene flow from the surrounding nearby ponds that are predominantly acidic, and hence would be most maladapted to circumneutral conditions.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45.80
45.85
45.90
45.95
46.00
-74.0
-74.1
-74.2
-74.3
-74.4
-74.5
-74.6
A
46.00
46.05
46.10
46.15
46.20
-81.1
-81.2
-81.3
-81.4
-81.5
-81.6
B

**Fig. 1** Geographic locations and spatial pattern of pH heterogeneity within each of the three regional landscape context types across central to eastern Canada: Laurentians, QC (open squares), Killarney, ON (open diamonds), and Cape Race, NL (open circles). Each panel shows a three-dimensional scatterplot for geographic locations and inter-annual pH of local lakes/ponds within each region: **A.** circumneutral, pH-homogeneous landscape (Laurentians, QC: 80 lakes sampled in 2010 and 2014 with pH: min 6.3; max 8.6; mean 7.3); **B.** pH-heterogeneous landscape with a mixture of lake pH types (Killarney ON: 32 lakes sampled in 2005 and 2011 with pH: min 4.4; max 7.8; mean 5.6); and **C.** pH-heterogeneous, acidic-dominated landscape (Cape Race NL: 108 ponds sampled in 2013, 2014 and 2015 with pH: min 3.6; max 7.4; median 4.8). Within each region, blue symbols indicate local habitats with temporally stable, circumneutral pH conditions ($pH \geq 6.0$), orange symbols indicate local habitats with intermediate acidity ($5.0 < pH < 6.0$), and pink symbols indicate local habitats with very acidic conditions ($4.0 < pH < 5.0$). Vertical dashed lines indicate latitude and longitude in degrees, minutes and seconds for each lake/pond.

We based our predictions on the following three assumptions: (i) Among-region differences in copepod population acid tolerances are a result of differences in genetic trait variance (local adaptation) rather than phenotypic plasticity. This assumption is supported on the pH-heterogenous landscape with the mixture of lake pH types (Derry and Arnott 2007; Derry et al. 2009; see discussion). (ii) There is a fitness trade-off for acid-tolerant genotypes at circumneutral pH (acid tolerance trades off with survival at pH 7, and neutral tolerance trades off with survival at acidic pH). This second assumption is supported for copepod populations on the pH-heterogeneous landscape with the mixture of lake pH types (Derry and Arnott 2007). We tested for a fitness tradeoff in acidic copepod populations from the predominantly acidic landscape at circumneutral pH in this study. (iii) The level of among-lake/pond gene flow is similar within each of the three study landscapes. The lakes/ponds in our study regions were largely disconnected and have low surface connectivity across all three landscapes. However, the spatial distance between lakes and ponds differed between the three regions. It is likely that passive dispersal and gene flow of copepods among lakes and ponds differ among the three landscapes as a result of differences in between-lake/pond proximity. Therefore, the spatial pattern of pH heterogeneity and the scale of among-habitat distances are non-independent influences that are considered to act on local trait divergence in our study.

## 1.2 Methods

### ***Study Organism***

The freshwater copepod, *L. minutus* Lilljeborg, is an excellent study system for studying broad-scale differences in local trait divergence to spatial pH heterogeneity because this species can be locally adapted in acid tolerance across fine spatial scales (Derry and Arnott 2007). *L. minutus* is regionally distributed across north-eastern North America where it is often a dominant species in crustacean zooplankton communities (Pinel-Alloul et al. 2013). *L. minutus* copepods are obligate sexual organisms, with at least two generations being produced per year in the spring and fall (Balcer et al. 1984). The mean life span of an adult *L. minutus* is 42 days, with 55–60 % of that time spent in a gravid condition for adult females (Chow-Fraser and Maly 1988).

This species shows no evidence of cryptic speciation across central-eastern North America (Thum and Derry 2008), and so we were confident that our morphological analysis unambiguously identified adults of this species across this geographic range. However, the post-glacial colonization of *L. minutus* in lakes and ponds of the three study regions have followed different historical patterns: while the first two regions share a common Great Lakes–St Lawrence River refugial source of colonists, the third acid-dominated region was colonized by a different refugium associated with the north Atlantic (Derry and Thum, unpublished data). Calanoid copepods such as *L. minutus* are considered to be dispersal-limited compared to other zooplankton taxa (Boileau and Hebert 1991; Bilton et al. 2001; Gray et al. 2012). Population genetic studies of calanoid copepods (*Eudiaptomus spp.)* in northern Europe have shown significant population structure on small spatial scales (Bohonak et al. 2006; Zeller et al. 2006). However, population genetic structure can differ between calanoid copepod species that do versus do not produce diapausing eggs: species that produce diapausing eggs are more likely to be dispersal limited over fine spatial scales (±100 km) (Zeller et al. 2006). Diapausing eggs produced by calanoid copepods are an important mode of dispersal over short distances (Zeller et al. 2006), which are carried by wind and mostly animal vectors such as fish (when waterways are connected) and birds between lakes/ponds (Cáceres and Soluk 2002; Green and Figuerola 2005; Gray and Arnott 2011). *L. minutus* is a species that produces diapausing resting eggs, and these diapausing eggs are an important source of recruitment to pelagic populations within lakes/ponds. Contemporary populations of *L. minutus* have been found to be spatially structured between lakes at a geographic scale of less than 10 km (average FST of 0.40 on the pH-heterogeneous landscape with mixed lake pH type; Derry et al. 2009). In combination with studies that have assessed local adaptation and trait divergence, there is evidence of restricted gene flow in *L. minutus* by means of short-range dispersal limitation and divergent selection in pH among lakes (Derry and Arnott 2007; Derry et al. 2010).

### *Study Regions*

We compared local divergence in copepod acid tolerance from three types of landscapes with contrasting spatial context in pH gradients (**Fig. 1**). All three of the landscape types are located in central-eastern Canada and are separated by hundreds of kilometers (**Fig. 1**): (i) a pH-homogeneous landscape of exclusively circumneutral lakes (pH C 6.0) in the Laurentians, Québec (QC); (ii) a pH-heterogeneous landscape with a mixture of acidic and circumneutral lakes at Killarney, Ontario (ON); and (iii) a heterogeneous pH landscape where relatively infrequent circumneutral ponds are embedded in a predominantly acidic landscape at Cape Race, Newfoundland (NL). The mean Euclidean distance between lakes/ ponds within the regions was 30.4 km for 80 lakes in circumneutral pH-homogeneous landscape, 6.9 km for 32 lakes in the pH-heterogeneous landscape with mixed lake pH types, and 1.2 km for 108 ponds in the acid-dominated landscape. Limnological characteristics of lakes/ponds for source copepod populations from these landscape types are described in **Supplementary**

**information, S.I.-1**.

The Laurentian hills just north of Montreal QC (45°55'N, 74°1'W), is a landscape that is comprised of lakes that are exclusively circumneutral pH (pH C 6.0) (pH: min 6.3; max 8.6; mean 7.3 across 80 lakes; Pinel-Alloul et al. 1990; Prairie et al. 2002; Robidoux et al. 2015; del Giorgio unpublished; Beisner unpublished; **Fig. 1A**). The spatial and temporal homogeneity in circumneutral lake pH in the Laurentians QC region is attributed to the Ca- and Mg-rich Morin anorthosite formation. This geological formation produced tills and other unconsolidated deposits in watersheds that have an almost unlimited buffering capacity and resistance to acid rain (Lachance et al. 1984; Houle et al. 2006).

The pH-heterogeneous landscape at Killarney Provincial Park, ON (46°01'N, 81°24'W) has a mixture of acidic and circumneutral lakes (pH: min 4.4; max 7.8; mean 5.6 across 32 lakes (Gray et al. 2012; **Fig. 1B**). Most Killarney lakes had historically circumneutral pH (Dixit et al. 2002), but many lakes acidified as a result of historical metal mining emissions (Sprules 1975) and poor geological buffering capacity of watersheds (Debicki 1982). Many of the acidified lakes have recovered or are in the process of recovering back to circumneutral pH (Keller et al. 2003; Holt and Yan 2003; Gray et al. 2012). For our study, local source populations were drawn from buffered, circumneutral lakes that never historically acidified because of the presence of limestone deposits within local watersheds. The temporal stability of pH in these specific source lakes is supported by paleolimnological data (Dixit et al. 2002) and extensive datasets in water chemistry dating from the period of peak acidification in 1975 to present (Sprules 1975; Keller et al. 2002; Holt and Yan 2003; Gray et al. 2012).

The acid-dominated landscape at Cape Race NL (46°39'N, 53°04'W), is a case where infrequent circumneutral ponds are embedded in an acidic landscape (pH: min 3.6; max 7.4; median 4.8 across 108 ponds; Charette and Derry 2016; **Fig. 1C**). This region is characterized by numerous ponds that are surrounded by an extensive carpet of heath moss (Rhacomitrium lanuginosum) and, where the drainage is low, by blanket and plateau bogs (Eastern Hyper-oceanic Barrens ecoregion) (Hoag and Price 1995; PAA 2008). At Cape Race NL, circumneutral source populations of copepods were chosen from ponds that showed no deviation from circumneutrality (pH C 6.0) over three summers with extreme climatic conditions that produced pH fluctuations in other ponds (see **Supplementary information, S.I.-2**).

### *Experiment 1: Is there a fitness cost to acid tolerance at circumneutral pH?*

We first tested one of our underlying assumptions, that there is a fitness cost to acid tolerance at circumneutral pH for copepods collected from the acid-dominated landscape at Cape Race NL. This fitness trade-off had already been detected for copepods from the pH-heterogeneous landscape with a mixture of pH types for lakes in a previous paper (Derry and Arnott 2007). For this study, we conducted a 21-day laboratory experiment with a 2 x 2 factorial design with two *L. minutus* population sources (one acidic pond, NRB2 vs. one circumneutral pond, Bella) and two levels of pH treatment (pH 3.6 vs. pH 7.0) (n = 3 replicates) in August 2014. The two copepod source ponds were chosen for the laboratory experiment because they had been included as part of a trial 5-day reciprocal field transplant experiment in the previous year (summer 2013). This prior experiment had confirmed that copepods from the circumneutral pond had low short-term survival in water from the acidic pond over 5 days (**Supplementary information, S.I.-3**).

Adult *L. minutus* were collected from two ponds at Cape Race NL at the end of July 2014: (i) a pH-stable acidic pond (NRB2: 45.65052N, -53.17754W; 2013–2015 inter-annual median pH of 4.41) and (ii) a pH-stable circumneutral pond (Bella: 46.64637N, -53.21115W; 2013–2015 inter-annual median pH of 6.59). Copepod densities in the source ponds ranged from 0.4 individuals $L^{-1}$ in Bella Pond to 2 individuals $L^{-1}$ in NRB2 pond. The copepods were transported at 0.5x pond densities in 1-L brown Nalgene bottles in chilled, dark coolers from Cape Race NL to Montreal QC over 3 days. The copepods were in good condition when they arrived at the laboratory at the Université du Québec à Montréal (UQAM). Within 24 h of arrival in the laboratory, copepods were placed on COMBO medium (Kilham et al. 1998) that had been pH-adjusted to the pH of their source pond (Bella copepods were placed on COMBO pH 7 and NRB2 source copepods were placed on COMBO pH 3.6). The copepods were incubated at densities that were equivalent to source pond densities and fed a 1:1 mix of *Cryptomonas sp.* (CPCC 336) and *Chlamydomonas reinhardtii* (CPCC 243) (Canadian Phycological Culture Centre (CPCC), University of Waterloo, Ontario, Canada). Acidification has not been found to alter the quality or usefulness of COMBO (Kilham et al. 1998).

Two levels of pH treatment were set from the COMBO medium pH of 8.0 by titration with H2SO4 and a Mettler Toledo FiveEasy TM FE20 pH meter (Mettler-Toledo AG Analytical, Schwerzenbach, Switzerland). Using a CKX41 microscope coupled to a DP21 digital color camera (Olympus, Tokyo, Japan), we placed an initial starting density of 24 adult *L. minutus* copepods in 200 mL of COMBO in a 250 mL glass beaker for each replicate. The COMBO growth medium with adjusted pH was changed in each of the beakers every 2–3 days. The pH drift between water changes was ± 0.2 pH units. Copepods were fed daily to ensure a continual source of healthy algal resources from the source phytoplankton cultures that were also grown on COMBO medium. Algal densities were monitored and maintained at 2–7 x $10^3$ cells $mL^{-1}$ with a 1:1 ratio of *Cryptomonas sp.*: *C. reinhardtii* throughout the duration of the experiment using a CH-2 model compound microscope (Olympus, Tokyo, Japan) with a slide hemocytometer under 40x total magnification. Nauplii were removed daily from the beakers. The beakers containing the copepods and the phytoplankton cultures were incubated in a Thermo Scientific Precision Model 818 Refrigerated Incubator (Thermo Scientific, Marietta, Ohio, USA) at 16 h: 8 h light: dark photoperiod conditions and a temperature of 19 °C ± 0.2. Adult copepods from each of the beakers were enumerated after the onset of the experiment on days 5, 9, 13, 17 and 21 with a high resolution CKX41 microscope coupled to a DP21 digital color camera (Olympus, Tokyo, Japan). Nauplii were removed from the beakers so that reproduction over the course of 21 days did not influence our survival measurements.

For the laboratory experiment, survival was measured as the absolute number of adult copepods at a given time since the initial density of copepods was constant among all experimental replicates.

### *Experiment 2: Does landscape context affect local trait divergence?*

We tested the effect of landscape context on local trait divergence in acid tolerance of copepods by applying a 3 x 3 factorial-design field transplant experiment in early to mid-summer of 2014. Regional landscape type (a pH-homogeneous landscape of exclusively circumneutral lakes (pH C 6.0) versus a pH-heterogeneous landscape with a mixture of acidic and circumneutral lakes versus a heterogeneous pH landscape with predominantly acidic pH conditions) and pH treatment (pH 7.0 vs. 4.6 vs. 3.6) were the main fixed factors. Adult copepod densities in the source lakes/ponds ranged from 0.3 to 10.3 individuals $L^{-1}$ in the circumneutral pH homogeneous region, from 0.5 to 4.5 individuals $L^{-1}$ in the pH- heterogeneous region with a mixture of pH types, and from 0.5 to 11.85 individuals $L^{-1}$ in the predominately acidic region. The duration of the experiment was 7 days and the response variable was short-term survival of adult copepods. An initial zooplankton sample of 2x ambient lake/pond density was taken from each copepod source pond at the onset of the experiment in order to estimate initial copepod densities (Ni) that were placed in the cubitainers. A final count of all adult copepods remaining in the experimental cubitainers was enumerated at the end of the experiment (Nf).

Within each regional landscape context type (**Fig. 1**), copepods were drawn from replicate within-region lake/pond population sources with temporally-stable circumneutral water pH (C6.0) and no history of acidification. The level of replication was unbalanced between landscape types because of differences in the relative frequency of circumneutral habitats within each region: Laurentians, QC: n = 9 source lakes; Killarney, ON: n = 6 source lakes; Cape Race, NL: n = 3 source ponds (total 18 source populations). Copepods were stocked at ambient epilimnetic lake/pond densities from each lake/pond population source into 20-L polyethylene cubitainers that contained pH-adjusted lake-water from Lac Croche QC (see below). As of a result of logistical constraints associated with sampling remote lakes and ponds in three geographically disparate regions, inoculations of copepods into the cubitainers occurred on different dates in the three regions: June 9–10th at the Laurentians, QC, June 21–28th at Killarney, ON, and July 17th at Cape Race, NL. However, given the relatively short time frame in early to mid-summer for the whole experiment, it is unlikely that seasonal variation in lake/pond-water pH or phytoplankton assemblages would have affected short-term toxicity responses of copepods to acid exposure. We confirmed that phytoplankton assemblages were similar between water collection dates for the depth at which we collected water from Lac Croche (top 1-m) (Beisner, unpublished data).

The pH treatment was set to three levels (pH 7.0, 4.6, and 3.6) in 20-L polyethylene cubitainers containing lakewater from a common source for all three regional landscape types (Lac Croche, Laurentians, Qc: 45.99174 N, -74.01405 W). The pH of Lac Croche was 7.0 in 2014, and so we did not titrate acid for the pH 7.0 set-point. The pH 4.6 and 3.6 set-points were done by acid titration with 2 mol $L^{-1}$ sulfuric acid ($H_2SO_4$) 1 day prior to copepod inoculation within each landscape type. Following titrations, the volume of all cubitainers was adjusted from 20- to 19-L to provide space for gas transfer across the air–water interface within these closed containers. The final pH of lake-water in each container was measured at the end of the 7-day experiment with a calibrated multi-parameter sonde (YSI 2009 Professional Pro Plus; YSI, Yellow Springs, Ohio, USA). All replicates were acidified to ±0.2 units of the target pH.

Environmental conditions, other than the main experimental treatments (regional landscape type of copepod source population and pH treatment), were controlled by using a common lake-water source for the incubation medium, and by exposing experimental cubitainers to similar temperature and UV conditions. Epilimnetic water from the one source lake (Lac Croche, Laurentians, QC: 45.99174N, -74.01405W) was 54-µm-filtered to remove crustacean zooplankton and most rotifers but retain algal and bacterial resources, and was used as an incubation medium for all experimental containers in the experiment. Lac Croche was not a copepod population source for the experiment. However, the distance from Lac Croche, QC to each of the three regional landscape types varied from ±24 h in the Laurentians, QC to a 3-day drive to Cape Race, NL. Therefore, to control for potential differences in phytoplankton degradation prior to the onset of the experiment between regional landscape types, we aged the filtered Lac Croche water in the 20-L cubitainers under cool, dark conditions for 10 days prior to the start of the experiment within each region. This ‘water aging‘ period also allowed for days necessary to collect copepods from remote lakes/ponds within each of the regions. In doing so, copepods from all three landscape types were exposed to similar initial water and resource conditions for the experiment: dissolved organic carbon (DOC) 6.8 mg $L^{-1}$, total phosphorous (TP) 5.0 µg $L^{-1}$, chlorophyll *a* (chl*a*) 1.0 µg $L^{-1}$. By transplanting the incubation water from a common source to the three regions rather than transplanting the copepods to a single common region, we also eliminated the effects of long-distance transport on copepod mortality before the onset of the experiment, and mitigated risk of transplanting copepod genotypes between regions.

Within each regional landscape, the 20-L cubitainers were incubated in situ at a common incubation lake or pond. This was done to mimic ambient natural temperature and UV conditions, as well as control for environmental variation among cubitainers within each region. We minimized between-region variation in incubation temperature and UV conditions by selecting one incubation lake/pond within each landscape type that had similar temperature and light characteristics between regions. These incubation lakes/pond were different from copepod population sources in the experiment and were comprised of the following: Lac Cromwell QC, 45.98846N, -74.00062W; Bell Lake ON, 46.13972N, -81.18944W; Pond BQ NL, 46.64897N, -53.21381W. The cubitainers were suspended from floating frames in the deeper incubation lakes (Lac Cromwell QC and Bell Lake ON), and were rolled to the deepest depth in the center of Pond BQ, NL. Weights tied to each container submerged the experiment at the desired depths in the incubation lakes/pond. Desired depths of incubation were calculated from 1 % UVB attenuation coefficients, which are based on DOC concentration (Morris et al. 1995), to eliminate mortality associated with exposure to UV radiation. The DOC and incubation depths of each incubation lake were the following: Lac Cromwell QC, DOC 5.7 mg $L^{-1}$, incubation depth 0.5 m; Bell Lake ON, DOC 6.3 mg $L^{-1}$, incubation depth 0.5 m; Pond BQ NL, DOC 12.4 mg $L^{-1}$, incubation depth 0.1 m. Continuous temperature readings were taken with a HOBO TidbiT v2 UTBI-001 TempLogger (Hoskin Scientific, Massachusetts, USA) in each incubation pond. These showed slightly different incubation temperatures among incubation lakes/pond at the desired depths: Lac Cromwell QC, mean 20.5 °C; Bell Lake ON, mean 21.5 °C; Pond BQ NL, mean 18.7 °C. However, these slight differences in incubation temperature were all within the range of thermal optima for this species and should not have affected short-term survival responses to pH treatment.

Final edible chlorophyll *a* concentration (chl*a*), which is an estimate of biomass of phytoplankton cells in the edible cell size range of <30 µm for zooplankton, was measured on day 7 at the end of the experiment. This was done to confirm that copepod mortality was a result of pH exposure rather than reduced food levels. This measurement was obtained by filtering a 1.0-L subsample of water from a 20-L cubitainer through a 30-micron mesh, and then collecting the phytoplankton from the filtrate on a 1.2-µm glass fiber filter. The filter was wrapped in foil and frozen at -20 °C until laboratory analysis. Extraction of chl*a* was done in hot ethanol and by measuring the chlorophyll spectrophotometrically at 665–750 nm on a Biochrom Ultrospec ® 2100 pro with a 10-cm quartz cuvette (Winterman and de Mots 1965; Sartory and Grobelaar 1984).

To estimate initial copepod densities (Ni) stocked into cubitainers, one epilimnetic zooplankton sample from each source lake/pond was collected at 2x ambient density, similar to the densities stocked into the containers, and preserved with 4 % sugar-formalin. Initial copepod densities from individual source lakes/ponds were based on a single replicate. A more accurate estimate of initial densities from individual source lakes/ponds would have required a higher level of replication than n = 1. However, for our experiment, the unit of replication for regional landscape type was at the between lake/pond-level rather than at the within lake/pond-level. The level of replication during the collection of initial samples followed this design. To estimate final copepod densities in the cubitainers at the end of the 7-day experiment (Nf), all 18 L of water from each cubitainer was filtered through a 54-lm screen, and the copepods were preserved with 4 % sugar-formalin. Adult *L. minutus* copepods were identified and counted under a SZX10 zoom stereomicroscope (Olympus, Tokyo, Japan) using the following keys: Smith and Fernando (1978); Witty (2004); Thorp and Covich (2010). The entire contents of lake/pond initial samples and containers were counted, and no subsamples were taken. Only well-preserved adult specimens showing no signs of body deterioration were counted in order to account for survival of acid tolerant adult copepods. Individuals that were not acid-tolerant usually died within 5 days at the levels of pH treatment that were applied, and showed substantial signs of morphological deterioration by day 7 (**Fig. 2**; Derry and Arnott 2007).

### *Statistical analyses*

For experiment 1, we conducted a 2 x 2 univariate Repeated Measures (RM)-ANOVA with copepod pond source (circumneutral Bella pond vs. acidic NRB2 pond) and pH (pH 7.0 and 3.6) as main fixed factors. The repeated unit was the glass beaker that held the copepods. The number of surviving adult copepods was the response variable (Nfinal), which was $\text{Log}_{10}$ (x+1)-transformed to $\text{Log}_{10}$ (Nfinal+1) to improve normality of the data distribution. The total duration of the experiment was 21 days and numbers of surviving copepods were counted every 4 days. Model assumptions of normality and equal variance were met with location and dispersion parameters, $\mu \simeq 0$ and $\sigma = 0.21$. Although this distribution was slightly skewed, skewness = 0.31, kurtosis ≃ 0 and variance = 0.04, it conformed to a normal distribution (Shapiro–Wilk W test; 0.96 Prob < W 0.09). Comparisons of significant main effects and their interactions were conducted using Tukey HSD post hoc tests.

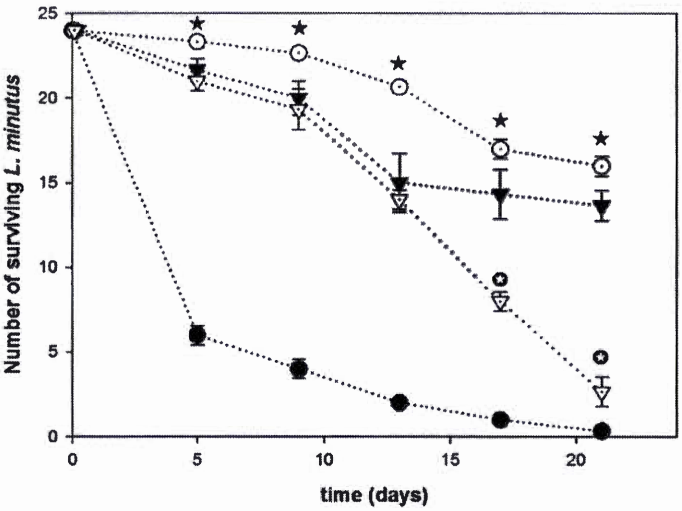


**Fig. 2** A 21-day 2 x 2 factorial laboratory experiment with adult *Leptodiaptomus minutus* from the acid-dominated landscape at Cape Race NL revealed a reciprocal fitness trade-off in survival (mean ± standard error) between acid-tolerant copepods at circumneutral pH 7.0 and acid-sensitive copepods at acidic pH 3.6. Circles indicate copepods from a circumneutral pond source (Bella pond) and triangles indicate copepods from an acidic pond source (NRB2 pond). Solid symbols indicate the copepod pond source treatment at pH 3.6, and open symbols indicate the copepod pond source treatment at pH 7.0. While solid stars indicate pairwise differences in survival that were detected for circumneutral pond-source copepods between levels of pH treatment, white stars with black circle background indicate pairwise differences in survival that were detected for acidic pond-source copepods between levels of pH treatment following RM-ANOVA (Tukey HSD tests, $P < 0.05$).

For experiment 2, we conducted a 3 x 3 factorial mixed model with landscape context type and pH treatment as fixed factors, and lake/pond copepod source as a random blocking factor nested within each regional landscape context type. A mixed model with unbounded variance components was chosen because it provides the flexibility of fitting models with various fixed and random elements (McLean et al. 1991). To evaluate the effects of landscape context and pH exposure on copepod acid tolerance, initial adult copepod abundance (Ninitial) was a covariate, and final adult copepod abundance (Nfinal) was the response variable in the mixed model. Initial copepod abundance (Ninitial) respected the necessary condition of a covariate by not being affected by the factors levels of the experiment and reflected prior environmental conditions of the observational units (Freund and Wilson 2003). Both initial copepod abundance and final adult copepod abundance were Log-transformed to $\text{Log}_{10}$ (Ninitial+1) and $\text{Log}_{10}$ (Nfinal+1) to normalize data distributions and improve homogeneity of variance. The assumption of normality was met because model residuals conformed to a normal distribution (Shapiro–Wilk W test; 0.98 Prob < W 0.90) with location and dispersion parameters, $\mu = 0.01$ and $\sigma = 0.29$. This distribution was almost perfectly symmetric with skewness = -0.05, kurtosis = -0.5 and variance = 0.8. Tukey HSD tests were used to test for specific pairwise differences at a significance level of $P < 0.05$).

A 3 x 3 factorial mixed model was also applied to evaluate final algal biomass in each of the treatment combinations at the end of experiment 2. In this case, regional landscape context type and pH were fixed factors and copepod pond source was as a random blocking factor. The response variable was final chlorophyll a concentration on day 7 in the cubitainers (Nfinal [chl*a*]). (Nfinal [chl*a*]) was $\text{Log}_{10}$ (x + 1) transformed to $\text{Log}_{10}$ Nfinal [chl*a* + 1]. Residuals conformed to a 3-parameter Weibull distribution (Cramer-von Misses $W^2$ test; 0.04 Prob < $W^2$ 0.25) with a coefficient of skewness approaching zero with shape, scale and threshold parameter of $\beta = 3.99$, $\alpha = 0.22$ and $\vartheta = -0.20$, which is found very close to the normal distribution. Post hoc Tukey HSD tests were conducted where significant effects were detected for main factors and their interactions. All statistical analysis were conducted using JMP 12 ® 2015 by SAS Institute Inc., Cary, NC, USA.

## 1.3 Results

### ***Experiment 1: Is there a fitness cost to acid tolerance at circumneutral pH?***

Acid-tolerant copepods from the acidic source pond had an overall higher survival across both levels of pH treatment (RM-ANOVA: copepod population source in effect; P < 0.0001, **Table 1**). Copepod survival across the two population sources was lower in the acidified pH treatment (RM-ANOVA: pH main effect, P < 0.0001, **Table 1**). There was high survival for acid-tolerant copepods from the acidic-source pond at pH 3.6 and for acid-sensitive copepods from the circumneutral-source pond at pH 7.0 throughout the experiment (**Fig. 2**). However, the response of copepods to pH treatment depended on their population source (RM-ANOVA: copepod population source 9 pH interaction, P < 0.0001, **Table 1**). Consistent with the trial reciprocal transplant experiment (**Supplementary Information, S.I.-3**), the survival of circumneutral pond source copepods was negatively affected by pH 3.6 at day 5 of the experiment (Tukey HSD post hoc tests, P < 0.0001; circle symbols, **Fig. 2**). The survival of acidic pond source copepods was negatively affected by pH 7.0 by day 17 of the experiment (Tukey HSD post hoc tests, P < 0.0001; triangle symbols, **Fig. 2**). This was evidence for a fitness tradeoff associated with pH tolerance because copepod acid tolerance traded off with survival at pH 7 following 3 weeks, and neutral tolerance in copepods traded off with short-term survival at acidic pH over 3–5 days.

### *Experiment 2: Does regional landscape context affect local trait divergence?*

Landscape context did influence local trait divergence of copepod acid tolerance in circumneutral lakes and ponds across central to eastern North America (mixed model: regional landscape context type 9 pH interaction, P < 0.05, **Table 2**; **Fig. 3**).

**Table 1** Statistical results of the laboratory transplant experiment (experiment 1) as revealed by univariate RM-ANOVA that show adult copepod survival in response to copepod pond source and pH treatments over 21 days.

| Summary | DF | F | Prob>F |
|---|---|---|---|
| **Copepod pond source** | 1 | 64.88 | **< 0.0001** |
| **pH** | 1 | 108.08 | **< 0.0001** |
| **Copepod pond source x pH** | 1 | 232.77 | **< 0.0001** |
| **Time** | 4 | 24.94 | **< 0.0001** |

Bolded P values are significant at P < 0.0001

**Table 2** Statistical results of the 7-day field transplant experiment (experiment 2) as revealed by a factorial mixed model, with initial copepod abundance [Log10 (Ninitial + 1)] as a co-variate, and final adult copepod survival [Log10 (Nfinal + 1)] as the response variable

| Summary | DF | F | Prob>F |
|---|---|---|---|
| **Log10 (Ninitial +1)** | 1 | 135.46 | **< 0.0001** |
| **Regional landscape context** | 2 | 16.99 | **0.0005** |
| **pH** | 2 | 56.05 | **< 0.0001** |
| **Regional landscape context x pH** | 4 | 2.82 | **0.0428** |

Main effects were regional landscape context of copepod source and pH, and lake/pond copepod source was used as a random blocking factor nested within each regional landscape context type.

Bolded P values are significant at $P < 0.05$.

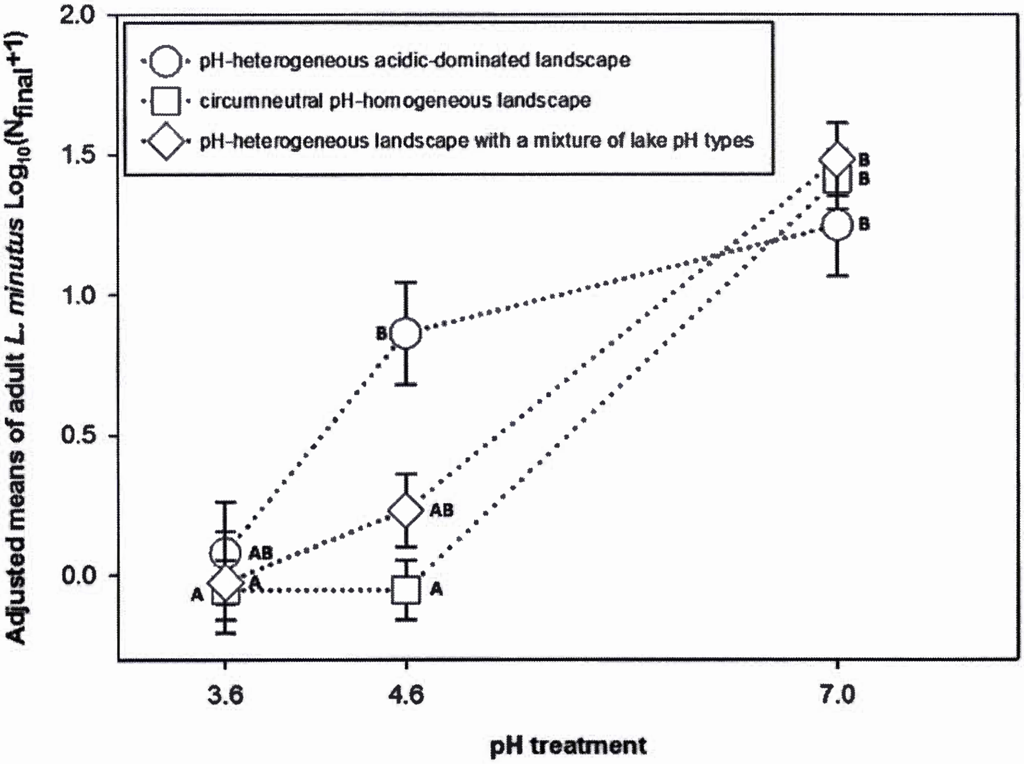


**Fig. 3** A 7-day 3 x 3 factorial field transplant experiment indicated that regional landscape context was important for modifying the mean acid tolerance of freshwater copepods from pH-stable, circumneutral lakes/ponds within each region (adjusted means in final copepod survival ± standard error, with initial pond source copepod abundance as a covariate). Regional landscape context is indicated by symbols: circumneutral, pH-homogeneous landscape (squares Laurentians QC), pH-heterogeneous landscape with a mixture of lake pH types (diamonds Killarney ON), and pH-heterogeneous, acidic-dominated landscape (circles Cape Race NL). Letters indicate pairwise differences in adult copepod survival detected between regional landscape context types within each level of pH treatment following a factorial mixed model ($P < 0.05$, Tukey HSD tests).

Copepods from the acid dominated landscape were more acid tolerant and had higher mean short-term survival across the three pH treatments compared to copepods from the other two regions (mixed model: regional landscape context main effect, $P = 0.0005$, **Table 2**; **Fig. 3**). Acidic pH generally depressed copepod survival across landscape types (mixed model: pH main effect, $P < 0.0001$, **Table 2**; **Fig. 3**), but the response was dependent on regional landscape context. As predicted, copepods from the circumneutral pH homogenous region (Laurentians, QC) were least acid tolerant and had the lowest survival at pH 4.6 compared to the acid-dominated pond landscape at Cape Race NL ($P < 0.05$ Tukey HSD post hoc test; **Fig. 3**). Copepods from the pH-heterogeneous landscape with a mixture of lake pH types had an intermediate survival and acid tolerance compared to the circumneutral pH-homogeneous landscape and the acid-dominated landscape at pH 4.6 ($P < 0.05$ Tukey HSD post hoc test; **Fig. 3**). At pH 3.6, copepods from all three regional landscape context types had low mean acid tolerance. Differences in variance for acid tolerance between the circumneutral pH-homogeneous region and the pH-heterogeneous region were not distinguishable at pH 4.6 and pH 3.6. However, at pH 3.6, copepods from the acid-dominated landscape at Cape Race NL had a more variable response (based on error bars; **Fig. 3**).

Final edible chl*a* concentrations (algal biomass) were lower in cubitainers with copepods from the acid-dominated landscape (mixed model; regional copepod landscape source type main effect, $P = 0.0006$, **Table 3**) and with acidified lake-water pH (mixed model; pH main effect, $P < 0.0001$, **Table 3**) (**Fig. 4**). A significant interaction was detected between regional copepod source landscape context and pH ($P = 0.0314$; **Table 3**). Final edible chl*a* concentrations in cubitainers with copepods from the circumneutral pH-homogenous region and with copepods from the pH-heterogeneous landscape with mixed lake pH types had similar algal biomass concentrations at pH 7.0 and pH 4.6. By contrast, algal concentrations in cubitainers with copepods from the acid-dominated landscape were lower than in the cubitainers with copepods from the other two landscape context types at pH 7.0 and pH 4.6 ($P < 0.05$ Tukey HSD post hoc test; **Fig. 4**). The lowest concentrations of algal biomass occurred at pH 3.6 for all regional copepod landscape source types (**Fig. 4**).

**Table 3** Statistical results of the 7-day field transplant experiment (experiment 2) as revealed by a factorial mixed model that show final chlorophyll *a* concentration (Log10 Nfinal [chl *a* + 1]; µg $L^{-1}$) in response to treatments of regional landscape context of copepod source and pH

| Summary | DF | F | Prob>F |
|---|---|---|---|
| **Regional landscape context** | 2 | 14.08 | **0.0006** |
| **pH** | 2 | 33.72 | **< 0.0001** |
| **Regional landscape context x pH** | 4 | 3.13 | **0.0314** |

Lake/pond copepod source was used as a random blocking factor within each regional landscape context type

Bolded P values are significant at $P < 0.05$.

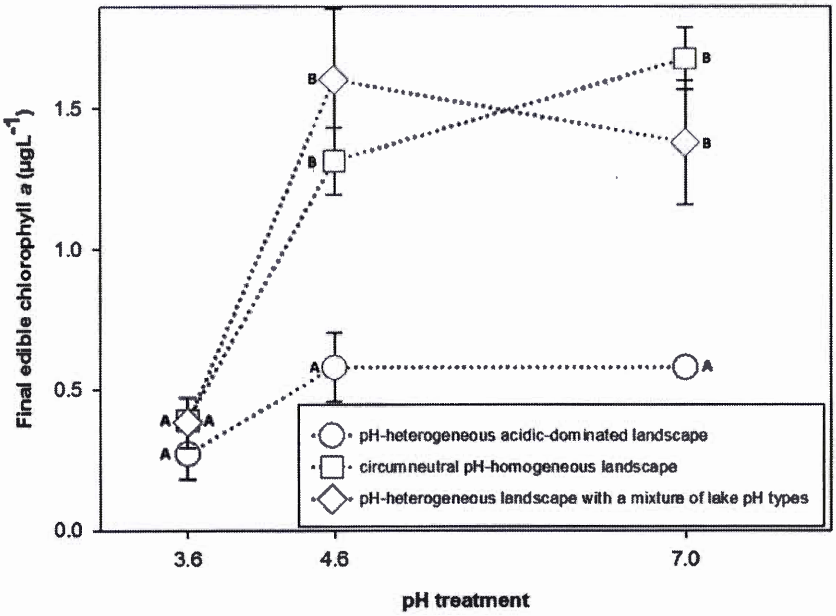


**Fig. 4** Final edible chlorophyll *a* concentrations (µg L$^{-1}$) in the experimental cubitainers at the end of the 7-day 3 x 3 factorial field transplant experiment. Water and basal phytoplankton + bacterial resources were sourced from a single lake for the entire experiment (Lac Croche QC). Regional landscape context of copepod population source is indicated by symbols: circumneutral, pH-homogeneous landscape (squares Laurentians QC), pH-heterogeneous landscape with a mixture of lake pH types (diamonds Killarney ON), and pH-heterogeneous, acidic-dominated landscape (circles Cape Race NL). Letters indicate pairwise differences detected between regional landscape context types of copepod source within each level of pH treatment following a factorial mixed model ($P < 0.05$, Tukey HSD tests).

## 1.4 Discussion

Regional landscape context impacted local trait divergence in acid tolerance of freshwater copepods. Consistent with our predictions, we found that copepods from circumneutral lakes/ponds (i) in the circumneutral pH-homogeneous landscape were the least acid tolerant, (ii) in the pH-heterogeneous landscape with a mixture of lake pH types had an intermediate level of acid tolerance, and (iii) in the acid-dominated landscape had the greatest acid tolerance. We had anticipated that copepods from circumneutral lakes/ponds in the circumneutral pH-homogeneous landscape would have the least variable acid tolerance and that copepods from the acid-dominated landscape would have the most variable acid tolerance. This prediction held, but only for copepods from the acid-dominated landscape at pH 3.6. Several mechanisms can be invoked to explain these findings: (i) the regional pattern and extremity of landscape pH heterogeneity, (ii) the spatial scale of distances between lakes/ponds in the different landscapes, and (iii) regional differences in post-glacial colonisation history that may have resulted in different genetic combinations across northeastern North America. Regardless of the relative influence of each of these mechanisms on local trait divergence, which may differ between regions, our study underscores that regional landscape context as a whole (environmental + spatial + historical factors) was important for determining differences in local patterns of adaptive trait divergence across a broad-scale. Few other empirical studies have addressed broad-scale differences in local adaptive trait divergence of populations in relation to landscape context (Tack and Roslin 2010; Tack et al. 2014). This is despite the growing recognition that within-species variation in abiotic tolerances can play an important role in ecological interactions (Bolnick et al. 2011; Violle et al. 2012), especially in ecosystems challenged by environmental change (Sgro et al. 2011; De Laender et al. 2014).

Local lake/pond-water pH can be a selective agent in the evolution of acid tolerance for a variety of animals (Räsänen et al. 2003; Derry and Arnott 2007; Hangartner et al. 2012). In a previous study (Derry and Arnott 2007) as well as in this study (**Fig. 2**), we found evidence for a fitness tradeoff in survival associated with acid tolerant copepods in water with circumneutral pH. This fitness tradeoff was present in acid-tolerant copepods from both pH-heterogeneous study landscapes at pH 6.0–7.0 (Derry and Arnott 2007; **Fig. 2**). For acid-sensitive copepods from circumneutral habitats, mortality to acid exposure was detected after 5–7 days in water at pH 4.6 (**S.I.-2**; **Figs. 2, 3**). We interpreted the presence of acid-tolerant copepods in circumneutral lakes/ponds in the pH-heterogeneous landscapes to be maladaptive at the local lake/pond level because of a reciprocal fitness tradeoff for copepods along lake/pond pH gradients (Derry and Arnott 2007; **Fig. 2**). However, it is possible that maladaptive trait divergence at the local level may be adaptive at the landscape-level depending on landscape context. Theoretical work predicts hat low cost of local maladaptation leads to network adaptation of a generalist and high cost of local maladaptation leads to network adaptation of habitat specialists at the landscape-level (Hanski et al. 2010).

The handful of other empirical studies that tested the effect of landscape context on adaptation and trait variation have shown that landscape context influences the strength and sign of local adaptation such that the higher the fraction of immigrants in a population, the higher the level of maladapation (Tack and Roslin 2010). These patterns can be modified by spatial scale and the configuration of spatial environmental heterogeneity (Tack et al. 2014). In our study, we also found that a combination of environmental (spatial pattern and extremity of landscape pH heterogeneity) and spatial (distance between lakes/ponds) factors likely played a primary role in determining the regional variation in local maladaptive trait divergence. There were more acid-tolerant copepods from circumneutral ponds in the acid-dominated landscape compared to the pH-heterogeneous landscape with a mixture of lake pH types (Derry and Arnott 2007; **Fig. 3**). This elevated level of local maladaptive trait variation in circumneutral ponds likely occurred because of gene flow from neighbouring acidic ponds with opposing selection that dominate the landscape at Cape Race NL (**Fig. 1c**). The spatial distance between ponds is less on the acid-dominated landscape (scale of 1 km) compared to the two other regions (scale of 10 km) (**Fig. 1**). This may have facilitated a higher level of passive dispersal (on the part of copepods, by wind and birds) and gene flow between ponds at Cape Race NL compared to between lakes in the other two regions. The gradient in acidity is also more extreme in the acid-dominated landscape (pH: min 3.6; max 7.4; median 4.8) compared to the pH-heterogeneous landscape with a mixture of lake pH types (pH: min 4.4; max 7.8; mean 5.6) (**Fig. 1**). The extremity of pH gradients on the acid-dominated landscape may also explain the higher acid tolerance of copepods from circumneutral ponds (little mortality at pH 4.6) compared to the pH-heterogeneous landscape with a mixture of lake pH types (high mortality at pH 4.6) (**Fig. 3**). Both environmental and spatial attributes of landscape context therefore may have contributed to a higher level of local maladaptive trait divergence in freshwater copepod populations on the acid-dominated landscape.

On the pH-heterogeneous landscape with a mixture of lake pH types, the relative influence of landscape pH gradients and spatial factors is less clear because the pattern and extremity of landscape pH gradients, as well as spatial distances between lakes, are all intermediate in relation to the two other landscapes considered. However, previous research has shown that copepods populations are locally adapted to pH among lakes in the pH-heterogeneous region with a mixture of lake pH types (Killarney ON: Derry and Arnott 2007; Derry et al. 2009). This suggests that divergent selection from landscape pH gradients is strong enough to produce adaptive divergence, and/or gene flow is low enough to not homogenize trait differences among lake populations (average FST of 0.40; Derry et al. 2009).

We did not distinguish between genetic, plastic, and maternal effects in copepod acid tolerance, and it is possible that differences in regional landscape context may produce different evolutionary outcomes. For example, the evolution of phenotypic plasticity in acid tolerance has unlikely occurred on the pH-homogeneous circumneutral landscape where there is an absence of selective gradients for this trait. We did not explicitly test for phenotypic plasticity copepod acid tolerance on the acid-dominated landscape. However, the environmental and spatial characteristics of pond system on the acid-dominated landscape at Cape Race, NL encapsulates the conditions that are anticipated to favour the evolution of phenotypic plasticity: high spatial environmental heterogeneity and close habitat proximity (Sultan and Spencer 2002; Lind and Johansson 2007; Hollander 2008; Baythavong 2011; Lind et al. 2011). Future research could integrate regional comparisons of quantitative genetics to understand the role of landscape context in determining broad-scale patterns in genetic and environmental sources of phenotypic variation for the same trait as seen in studies of plant pathogens (Tack et al. 2014).

Historical colonization patterns following the retreat of glaciers in north-eastern North America may have played a role in the differences in local trait divergence in copepod acid tolerance that we observed. The acid-dominated landscape at Cape Race NL is located on the island of Newfoundland where most of the original fauna and flora were eradicated during the last glaciation, but taxon richness remains impoverished compared to the mainland because of isolation (South 1983). There is evidence that *L. minutus* copepod populations across the Eastern Maritimes were colonized from a different glacial refugial source than *L. minutus* populations in the Great Lakes–St. Lawrence River watershed (circumneutral pH-homogeneous landscape at Laurentians QC and the pH-heterogeneous landscape with mixed lake pH types at Killarney ON) (Thum and Derry 2008). These historical differences in post-glacial colonization could have resulted in a different genetic basis of the species in the different regions, especially in Newfoundland. For example, it is possible that an acid-tolerant genotype could have colonized the acid-dominated region at Cape Race NL, and that the other two regions were colonized by a more diverse assemblage of genotypes that was subsequently selected upon by contemporary pH gradients. We cannot exclude this possibility, but suggest that post-glacial adaptive divergence in copepod acid tolerance may have occurred at both Cape Race NL and Killarney ON as a result of contemporary pH gradients among spatially-fragmented lakes/ponds.

The differences in copepod acid tolerance trait divergence that we observed among regional landscape context types were a direct effect of acid toxicity rather than food limitation or quality. This is because the lowest concentrations of final algal biomass (chl*a*) were detected in cubitainers with the highest copepod survival (copepods from the acid-dominated landscape at Cape Race, NL) at the end of the experiment (**Fig. 4**). Also, a trial reciprocal transplant experiment in 2013 (Supplementary Information, **S.I.-3**) revealed a high mortality of copepods from a circumneutral pond on natural water from a neighbouring acidic pond where basal resources were not limiting compared to home basal resources (chl*a* in the acidic water = 0.767 μg $L^{-1}$ vs. chl*a* in the circumneutral water = 0.562 μg $L^{-1}$). Moreover, we observed a reciprocal tradeoff in fitness between acid-tolerant copepods at pH 7.0 and acid-sensitive copepods at pH 3.6 in a laboratory experiment where basal resources were controlled for in terms of both quantity and quality by providing all replicates with equal concentrations and types of phytoplankton (**Fig. 2**). Therefore, the copepod survival responses that we measured were a direct result of pH toxicity rather than differences in food limitation resulting from the pH manipulation.

We contribute a rare study that relates regional landscape context in the pattern, extremity, and scale of environmental heterogeneity to local population trait divergence of abiotic tolerance. Understanding landscape context-dependent contingencies of population abiotic tolerance could have an important role in disentangling how fitness trade-offs to multiple stressors may play out across broad spatial scales (Steinberg 2012).

# CHAPITRE II

# ASYMMETRY IN SELECTION AND THE EFFECT OF CONNECTIVITY ON MALADAPTIVE PHENOTYPE-ENVIRONMENT MISMATCH AMONG COPEPOD POPULATIONS IN STABLE AND FLUCTUATING ENVIRONMENTS

Negrín Dastis J.O., Milne R., Guichard F., Derry, A.M., 2018. Asymmetry in selection and the effect of connectivity on maladaptive phenotype-environment mismatch among copepod populations in stable and fluctuating environments. *In prep.*

## Résumé

Phenotype-environment mismatch is prevalent in natural populations and has been proposed as an important biological mechanism influencing the likelihood of local adaptation and/or maladaptation at various spatial scales. Our study is a first to explicitly highlight the importance of asymmetry in selection and dispersal in determining the persistence of maladaptation in natural metapopulations. We first conducted a reciprocal transplant field experiment under common garden conditions in a natural heterogeneous landscape, to empirically test relative maladaptation of the freshwater copepod *Leptodiaptomus minutus* to pond acidity. We then used an extension of the heterogeneous two-patch migrational meltdown model to explore how an asymmetry in the strength of selection interacts with symmetric dispersal to favour the emergence of regional maladaptation and heterogeneity in population sizes at the metapopulation level. We additionally compared the influence of the asymmetry in stable versus uncorrelated environments that independently fluctuate relative to each other. In environments that were temporally stable, we found no difference between symmetric and asymmetric selection on regional level maladaptation. Asymmetric selection played a major role in maintaining metapopulation heterogeneity in population size. In environments that fluctuate on the other hand, we found again that symmetric and asymmetric selection did not influence regional levels of maladaptation. Interestingly, we found that in environments that fluctuate, heterogeneity in population sizes was maintained under symmetric and asymmetric selection, with a greater cost for the latter. In congruence with the model, maladaptation in calanoid copepod populations resulted from an interaction between asymmetry in selection and the landscape context. Using empirical evidence, we provide the first model that has investigated how asymmetry in selection interacts with symmetrical dispersal to predict the magnitude of maladaptation and heterogeneity in population sizes at the regional metapopulation level.

Keywords: Asymmetric selection, Dispersal, Heterogeneous landscapes, Maladaptation, Metapopulation, Phenotype-environment mismatch

## 2.1 Introduction

Phenotype-environment mismatches have been proposed to influence levels of local adaptation and maladaptation in natural systems (Bilton et al. 2002; Nosil et al. 2005; Schluter 2009). DeWitt (1998) was the first to introduce the term 'phenotype-environment mismatch', to describe the reduction in fitness incurred when an organism that is specialized to one environment finds itself in an alternative environment. This phenotype-environment mismatch can act as a biological barrier to dispersal whereby it reduces population connectivity because of immigrant inviability arising from local adaptation (Hendry 2004; Nosil et al. 2005). Phenotype-environment mismatches can thus act as a post-colonization barrier to dispersal (Marshall et al. 2010), and are especially interesting to explore in natural systems with spatially discrete populations inhabiting heterogeneous fragmented landscapes.

Freshwater zooplankton has several life history characteristics that could promote rapid dispersal (Havel and Shurin 2004) over short spatial scales. At the regional level however and in copepods in particular, biogeographic patterns in deglaciated regions suggest restricted dispersal, were those species that used glacial refugia have not expanded their range very far since the last glaciation (Hebert and Hann 1986) and have generally followed historical hydrologic pathways (Stemberger 1995). There is genetic evidence, high degrees of differentiation in allozyme gene frequencies even over small spatial scales, to support that copepods passively disperse and are restricted in their distribution because of strong divergence among local populations (Hebert and Moran 1980; Boileau and Hebert 1988).

The scale of patchy environments represent a unique spatial context to study maladaptation because they represent a spatial structure that is a sensible metaphor for many fragmented landscapes and is important because at the metapopulation scale, the strong changes at a local scale can have weak effects on the population size or on the traits distribution at a metapopulation scale (Legrand et al. 2017). This is typical of open populations where population persistence is determined by demographic processes operating at the metapopulation level, rather than by reproduction and survival within local populations alone (Marshall 2010). In heterogeneous landscapes, the source-sink concept (Dias 1996a; Diffendorfer 1998; Thomas and Kunin 1999) naturally emerges where a local demographic surplus arising in good quality habitats (source), and a local demographic deficit occurs in habitats of poor quality (sink) (Dias 1996a). Therefore maladaptation in a source-sink system can be theoretically maintained through a combination of weak selection for traits that are sub-optimal in sinks, and migration between habitats with strong opposing selection (Urban 2006;

Farkas et al. 2015).

Sub-optimal traits at the landscape level can be so numerous compared to source habitats that a source-sink inversion can occur, where sources can become sinks (Ronce and Kirkpatrick 2001). Adaptation to the former sink habitat could thus evolve and spread in the landscape, such that former sinks become sources (Dias 1996a). In source-sink metapopulations, and under a constant environment, maladapted individuals may persist provided they receive a flow of immigrants from elsewhere (Holt and Gomulkiewicz 1997; Gonzalez and Holt 2002). Under stochastic environmental variability on the other hand, maladaptation can further increase its persistence by inflating the abundance of maladapted sink populations (Gonzalez and Holt 2002), creating metapopulations that are entirely composed of sinks and where sources are rare. Whether the use of fitness versus traits define maladaptation, and whether maladaptation should be considered in a relative or absolute sense (Hendry and Gonzalez 2008), lead us to (1) conduct an inter-annual experiment in the field where relative maladaptation and acute short term fitness responses (survival) to pH abiotic conditions was empirically tested in a reciprocal transplant experiment conducted in nature for two consecutive years under identical experimental conditions and (2), build a mathematical model that explicitly takes into account the mechanisms of selection and dispersal (**fig.1**), and uses traits, or distinct population-level phenotypic signatures, to predict absolute maladaptation in natural populations. For (1), we specifically tested, as in another study (Negrín Dastis and Derry 2016), maladaptation in a metapopulation of freshwater copepods in which there is an asymmetric functional fitness response of survival to pond pH at the landscape level. This is because while maladaptation would persist in copepod populations where the fitness response to selection from pond pH was weaker, because of high survival of acidic pH-adapted phenotypes to circumneutral pH, maladaptation would be absent in pond populations were selection against maladapted phenotypes was strong, because of low survival of neutral pH-adapted phenotypes to acidic pH.

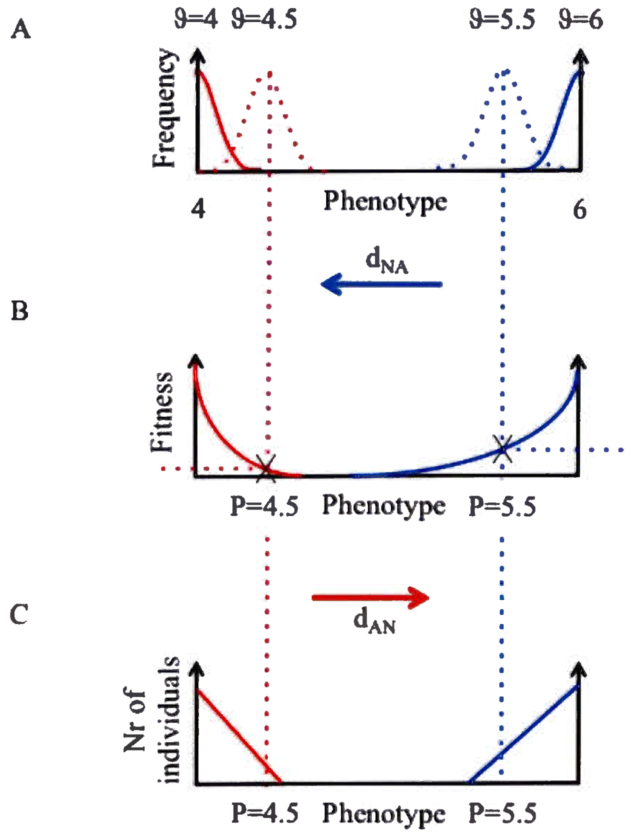


**Fig. 1** Conceptual figure introducing, **A.** the frequency distribution of two spatially structured discrete populations with two characteristic phenotypes, **B.** the influence of the asymmetry on fitness, and C. the number of individuals in the population. Phenotype (P), dispersal; Acid>Neutral ($d_{AN}$), Neutral>Acid ($d_{NA}$), (d), environmental optimum ($\vartheta$).

For (2), we developed a two-patch metapopulation model with inherent source-sink characteristics including stabilizing selection, dispersal operating between habitats, and an symmetric/asymmetric selection function that acts additively on the change in phenotype and multiplicatively on the change in population size. We developed an extension of the two -patch model developed by Ronce and Kirkpatrick (2001) to consider these influences operate on the dynamic maintenance of population maladaptation, mean phenotype and population size at the local and larger regional metapopulation level. Our response metric was different from Ronce and

Kirkpatrick (2001) whose focus was to measure the final dynamic equilibrium generated by the effects of habitat heterogeneity, stabilizing selection and movement rate on population maladaptation and population size. Instead, we focus first on how asymmetric selection interacts with dispersal to maintain persistent maladaptation in a metapopulation. This is because of a weak population fitness response to selection that results in the persistence of higher levels of maladaptation rather than a strong population fitness response to selection which acts to maintain populations close to the optimum phenotypic value for survival (Gomulkiewicz and Holt 1995; Crespi 2000; Brady and Richardson 2017). This can be especially important when incoming maladaptive dispersal overwhelms local selection.

On-going adaptive evolution is thought to be important in metapopulations where it culls maladapted migrants that would otherwise accumulate through migrational load in subpopulations in a heterogeneous landscape (Kinnison and Hairston 2007). Population size, genetic variation, the spatial dynamic nature of the environment (e.g. network topology and connectivity: Seymour et al. 2015; Shtilerman and Stone 2015), and the temporal dynamic of phenotypic selection (Siepielski et al. 2009) therefore all act in a continuum in space and time to constrain local adaptation, and maintain maladaptation persistent.

## 2.2 Methods

### *Empirical field experiment*

We conducted a field transplant experiment with populations of freshwater copepods (*Leptodiaptomus minutus* Lilljeborg) that have a known asymmetric fitness response in survival to pH gradients (Derry and Arnott 2007; Negrín Dastis and Derry 2016). We used inter-annual differences in average regional pond pH to test for the influence of landscape context in selection on local maladaptation of copepods in ponds with stable and fluctuating pH, assuming random and bidirectional levels of passive dispersal of copepods between ponds (Boileau and Hebert 1991; Zeller et al. 2006). The experiment was done in a predominantly acidic, yet pH-heterogeneous, landscape at Cape Race, Newfoundland (NL), Canada (Negrín Dastis and Derry 2016) over two consecutive years (2013 to 2014). The experiment had a 3 x 2 x 2 factorial design with copepod population source (3 levels: temporally-stable circumneutral, temporally-stable acidic and fluctuating pH, pH treatment (2 levels: pH 6 vs. pH 3.6), and temporal landscape pH context that occurred on two successive years (2 levels: 2013 - acidic landscape (mean pH 4.76±0.07 at landscape level; n=108 ponds) vs. 2014 - circumneutral landscape (mean pH 5.36±0.09 at landscape level; n=108 ponds)) as main factors. The level of replication was n=3 for each treatment combination with however an imbalanced design between the two years because more observations were made in 2014 (N=44) compared to 2013 (N=12). The experiment was done with 20-L closed cubitainer bottles containing the water of a common pond (Pond BQ NL, 46.64897N, -53.21381W), and the cubitainers were all incubated at the deepest point of this pond (1 m) for one week. Copepods as a biological study system and the methods for the experiment are described in detail in Negrín Dastis and Derry (2016). Whereas Negrín Dastis and Derry (2016) focused only on temporarily-stable circumneutral ponds at Cape Race NL and in two other regions with contrasting landscape structure, the present study focuses on the level of maladaptation in copepod populations from a diversity of pH pond types within the same metapopulation at Cape Race NL: populations in ponds with temporally-stable circumneutral pH and ponds with temporally-stable acidic pH. The response metric was adult copepod survival and the full factorial model were analysed with a linear mixed factorial model (LMM) with the lme4 package in R (R Core team, 2016).

### *Model description*

We present a deterministic model that is an extension of Ronce and Kirkpatrick (2001) based as well on discrete populations but inhabiting a heterogeneous freshwater pond landscape with a strong abiotic pH gradient. We quantify the asymmetric and symmetric consequences of phenotype-environment mismatch for maladaptation and population demography under varying values of selection and dispersal strength. As in Ronce and Kirkpatrick (2001), immigrants in our system have phenotypes that reflect the populations from which they originate and second, the rate of establishment of new populations depends on the match between the phenotype of the immigrants and the local environmental conditions. We consider the simplest case of a constant environment as defined by Lande and Shannon (1996), with stabilizing selection acting within generations, where the mean phenotype evolves to the optimum. As in other models (Lande 1976; Falconer and McKay1996), the rate of evolution in the mean phenotype in response to selection on a single quantitative character is proportional to the product of the additive genetic variance (relaxed assumption in our model because we only use phenotypic variance $\sigma p^2$) in the character and the intensity of directional selection. Thus within any generation, the additive phenotypic variance contributes to the *genetic load* an amount $\frac{\gamma}{2}\,\sigma p^2$ due to stabilizing selection, increasing the average distance of individual phenotypes from the optimum. The *evolutionary load* caused by the deviation of the population mean phenotype from the optimum phenotype, $\frac{\gamma}{2}\,(P-\vartheta)^2$, is determined both by the pattern of environmental change and by the response of the mean phenotype to selection (Lande and Shannon 1996). Dispersal from neighboring habitats with contrasting selection can however introduce individuals with differing phenotypes that contribute to the overall maladaptation state in a local habitat. Thus we make the fitness of an individual in a habitat directly depend on the local strength of selection, the strength of dispersal, and the asymmetry in selection. We additionally added noise around the deterministic environmental value within each patch to better understand the role of phenotypic variation in adaptation and population persistence in a changing environment. We did this by simply generating random numbers from the normal distribution with specific mean and variance, then, adding each random number generated at every time step to the mean deterministic environmental value (**fig.2**). We explored independent uncorrelated environmental noise across ponds with different random value for both ponds.

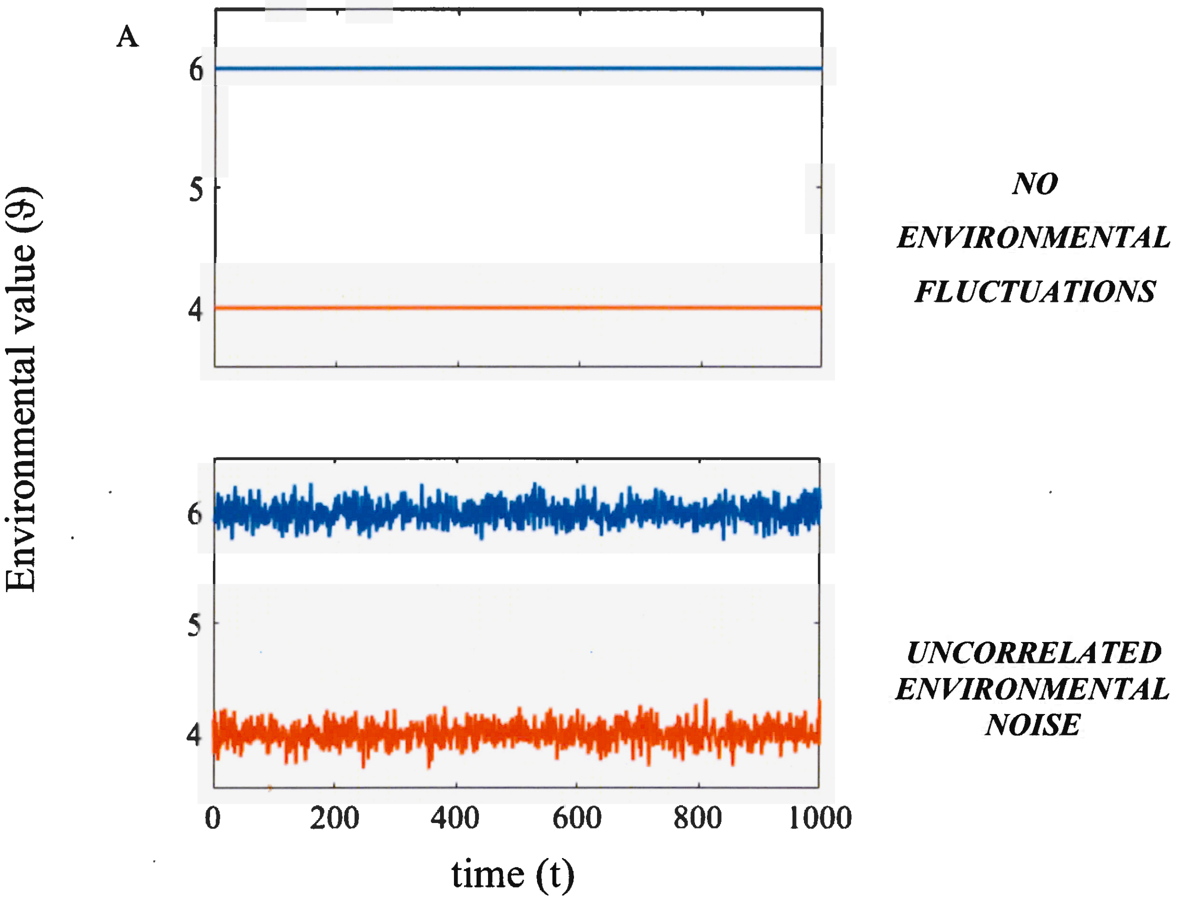


**Fig. 2** Independent uncorrelated environmental noise generated within each patch, fluctuating around the mean deterministic environmental value of $\vartheta=4$ and $\vartheta=6$ for the acid (red) and neutral (blue) patch respectively. The fluctuations were estimated from a sample of random numbers drawn from the normal distribution with mean 0 and variance 1. **A.** standard deviation of 0, **B.** standard deviation of 0.2.

### *Model intrinsic mechanics*

The environmental optimum in any given habitat is expressed by the parameter $\vartheta$, thus population growth is predicted to be at its optimum when phenotypes match their phenotypic optimum $(\mathrm{P} = \vartheta)$, which is expected in local habitats where this species is locally adapted. On the other hand, selection coded in the model as Ɣ, measures the intensity of stabilizing selection around this optimum. The strength of dispersal, or said otherwise the number of individual's dispersing in or out of any given habitat at any point in space and time, corresponds to the parameter $(d)$ in the model. We make dispersal symmetrical with the same immigration and emigration rates between two exchanging habitats in terms of number of individuals' N. Dispersal affects local population dynamics and shifts the mean phenotypic value potentially decreasing local adaptation due to immigrants originating from another habitat with a different phenotype than residents. In the model, dispersal operates passively between two habitat patches with contrasting environmental optima ($\vartheta = 4$ vs. $\vartheta = 6$), with a symmetrical and constant influx fraction of migrants from both habitats at every time step of the simulation. We assume that each habitat carrying capacity is equal and that there are no intrinsic differences in quality between the two. In the model, we implement an asymmetric functional fitness response to selection that is combined with selection to represent an additive cost contributing to total stabilizing selection $(\boldsymbol{eq.1})$. A list of model parameters and value thresholds are provided in **table 1**. The expected Malthusian fitness per generation of an individual with phenotype P at time t in a population of density N in is approximated by the following individual fitness function:

$$\mathbf{rt+1} = \mathrm{ro}\left(1-\frac{\mathrm{Nt}}{\mathrm{K}}\right) - \gamma\left(\frac{\sigma \mathrm{p}}{2}\right) - \gamma\left(\frac{(\mathrm{Pt}-\vartheta \mathrm{t})^2}{2}\right) + \alpha\gamma\left(\frac{(\mathrm{Pt}-\vartheta \mathrm{t})^2}{2}\right) * (\mathrm{Pt}-\vartheta \mathrm{t}+\alpha) * (\vartheta \mathrm{t}-\mathrm{Pt}-\alpha), \boldsymbol{eq.1}$$

The first term in the right hand side of $(\boldsymbol{eq.}\,\mathbf{1})$ describes the effect of density dependence, which here has a logistic form. The effect of this density dependence decreases in time with the number of individuals $N$ found in any given habitat. ro describes the fitness at low density of an individual with the optimal phenotype and K the maximal number of perfectly adapted individuals. The second term represents the *demographic load* due to stabilizing selection Ɣ acting on the variance σp around the phenotype P. Based on Lande and Shannon (1996), the third term represents the *evolutionary load*, which is the difference between the optimum and the phenotype and describes mortality caused by stabilizing selection on the phenotype P. The fourth and last term represents asymmetric selection, i.e. as there is some maladaptation α =0.5 necessarily leads to lower growth compared to α =0). In terms of model behaviour, fitness and population size incur an extra penalty cost due to the asymmetry in selection (**supplementary material table S.I.I**).

**Table 1** Two-patch model parameters, brief description and values used during simulations.

| Parameters | Description | Values |
|---|---|---|
| ***Local habitat dynamics:*** | | |
| $\vartheta$ | Environmental optimum | (4,6) |
| P | Local phenotype | (4,6) |
| N | Initial number of individuals in the population | (100) |
| K | Carrying capacity | (1000) |
| ***Spatial component:*** | | |
| d | Dispersal strength | (0, 0.1, 0.2, 0.3, 0.4) |
| ***Local adaptation:*** | | |
| $\gamma$ | Selection | (0, 0.1, 0.2, 0.3, 0.4) |
| $\alpha$ | Degree of asymmetry | (0, 0.5) |
| $\sigma p^2$ | Phenotypic variance | (0.01) |

Within any habitat and at every time step, the asymmetry imposes an extra load on the new population size N $(\boldsymbol{eq.2a})$ **(supplementary material table S.I.II)** and phenotype P $(\boldsymbol{eq.2b})$ **(supplementary material table S.I.III)**. We assume that phenotypes and breeding values are distributed normally within each population and that the phenotypic variance $\sigma p^2$ is constant and identical in the two habitats (Ronce and Kirkpatrick 2001). Therefore, the rate of evolution in the mean phenotype in response to selection on a single quantitative character is proportional to the product of the additive genetic variance in the character and the intensity of directional selection $(\frac{\gamma}{2})\ \sigma p^2$(Lande 1976; Falconer and McKay 1996). $\Delta N$ and $\Delta P$ represent population size and phenotypic differences between the two patches respectively. In the model and under the influence of dispersal, the joint changes in population size N and phenotype P are expressed by the two following equations:

$$\mathbf{Nt+1} = \mathrm{Nt} + \mathrm{Nt} * (\mathrm{ro} * \left(1 - \frac{\mathrm{Nt}}{\mathrm{K}}\right) - \gamma\left(\frac{\sigma \mathrm{p}}{2}\right) - (-1-\alpha) * \gamma * \left(\frac{(\mathrm{Pt}-\vartheta \mathrm{t})^2}{2}\right)$$
$$* (\mathrm{Pt} - \vartheta\mathrm{t} + \alpha) * (\vartheta\mathrm{t} - \mathrm{Pt} - \alpha) + (\mathrm{m} * (\Delta \mathrm{N}), \boldsymbol{eq.2a}$$

$$\mathbf{Pt+1} = \mathrm{Pt} - (1-\alpha) * \gamma * (\vartheta\mathrm{t} - \mathrm{Pt}) * \frac{(-1 + (\alpha * (\mathrm{Pt} - \vartheta\mathrm{t} + \alpha) * (\vartheta\mathrm{t} - \mathrm{Pt} - \alpha)}{1+\alpha}$$
$$+ \mathrm{m} * \left(\frac{\mathrm{N_A}}{\mathrm{N_N}}\right) * (\Delta \mathrm{P}), \boldsymbol{eq.2b}$$

As in Ronce and Kirkpatrick (2001) we provide a relative measure of maladaptation by the dynamic variable Z, which measures the number of phenotypic standard deviations that separates the phenotype from the environmental optimum in that habitat.

$$\mathbf{Zt+1} = \frac{\mathrm{Pt} - \vartheta\mathrm{t}}{\sigma \mathrm{p}}, \boldsymbol{eq.3}$$

Therefore, in the model, maladaptation is directly proportional to the relative difference between the observed phenotype of the standing population and the environmental condition divided by the phenotypic variance. For simplicity, the variance around the mean phenotype was kept constant for all simulations, with an evolutionary load created in the population because we manipulated 1) the strength of selection and the degree of asymmetry, and 2) the strength of dispersal. To estimate the joint influences of selection and dispersal on growth and the overall consequences of selection and dispersal on maladaptation, simulations were run for enough generations (t= 1000) to allow the system to reach equilibrium. All model simulations were run using Matlab 2016a by MathWorks Inc., Natick, Massachusetts, USA.

## 2.3 Results

### *A. Empirical field experiment*

Copepods from the stable acidic pond pH population source had overall higher survival across both levels of pH treatment (acidic pH 3.6 and circumneutral pH 6) than copepods from ponds with temporally stable circumneutral pH (LMM: copepod population source main effect; $P < 0.05$, **fig.3**). For pH treatment, copepod survival was lower at acidified pH 3.6 treatment than at circumneutral pH 6 (LMM: pH main effect, $P < 0.05$, **fig.3**). Annual landscape context also explained copepod survival across population sources and pH treatment (LMM: annual landscape pH context main effect, $P < 0.05$). Copepod survival response to pH was dependant on both population source (LMM: copepod population source x pH treatment interaction, $P < 0.05$, **fig.3**, **supplementary material table S.I.IV**) and on annual landscape pH context (LMM: pH x annual landscape pH context main effect, $P < 0.05$). We also detected an interaction between copepod population source and annual landscape pH context (LMM: Copepod population source x annual landscape pH context two-way interaction, $P < 0.05$, **fig.3, supplementary material table S.I.IV**). We did not however detect a population source x pH x annual landscape pH context three-way interaction. Copepods from circumneutral-pH source ponds in a less acidic landscape context had low survival at pH 3.6 compared to pH 6 (**fig.3**; Tukey HSD post hoc tests, $P<0.05$) and copepods from acidic-pH source ponds in a more acidic landscape had similarly high survival at pH 3.6 compared to pH 6 (**fig.3**).

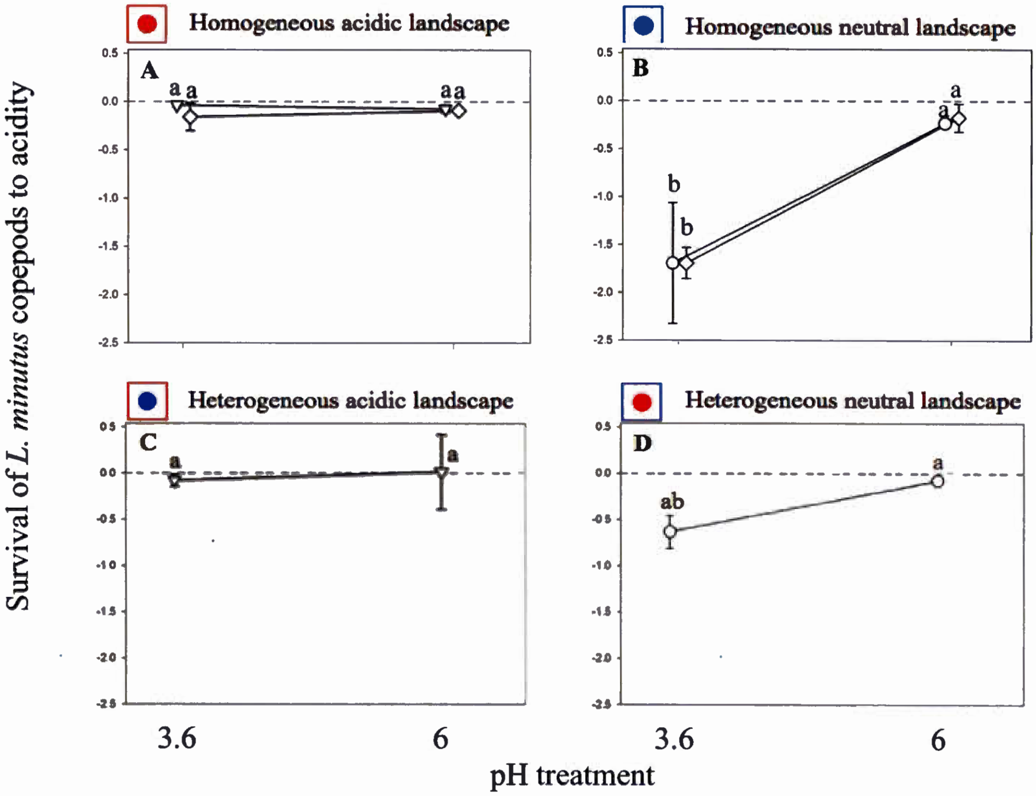


**Fig. 3** Results of the reciprocal transplant experiment conducted during two consecutive years at Cape Race (NL, Canada). **A.** An acid-adapted (lower triangles) and a fluctuating pH (diamonds) population embedded in an acidic landscape, **B.** a circumneutral-adapted population and a fluctuating pH (diamonds) population embedded in a circumneutral landscape, **C.** an acid-adapted population embedded in a circumneutral landscape, **D.** a circumneutral-adapted population embedded in an acidic landscape. Adult *L. minutus* survival to acidity was measured as $\text{Log}_{10}(N_{\text{final}+1}) - \text{Log}_{10}(N_{\text{initial}+1})$. Tukey HSD pairwise differences (alpha =0.05) represent pairwise differences for the entire model.

### *B. Two-patch Metapopulation Model*

#### *No environmental fluctuations*

At the metapopulation level and under symmetric and asymmetric conditions, there was no absolute change in maladaptation at the regional level (**fig.4A, fig.4B**). In terms of population size heterogeneity however we found that population sizes differences were most important under the strength of asymmetric selection (**fig.4D**) and symmetric selection (**fig.4C**). Overall, the stability of a constant environment operating around the deterministic value in each patch still enabled weak heterogeneity in maladaptation at the larger regional level (**fig.4A, fig.4B**).

#### *Independent uncorrelated environmental noise within each patch*

At the metapopulation level and under symmetric and asymmetric conditions, heterogeneity in maladaptation remained weak (**fig.5A, fig.5B**), as in the previous section with no fluctuations. Under symmetric selection however, and in contrast with the previous section, regional maladaptation (**fig.5C**) became important and especially under intermediate levels of dispersal and strong selection. Under asymmetric selection, heterogeneity in population sizes was less important due to influence of the fluctuations within each patch operating around the deterministic value (**fig.5D**). The fluctuations had a greater impact on the population size differences between the two patches than on the resulting maladaptation at the regional level.

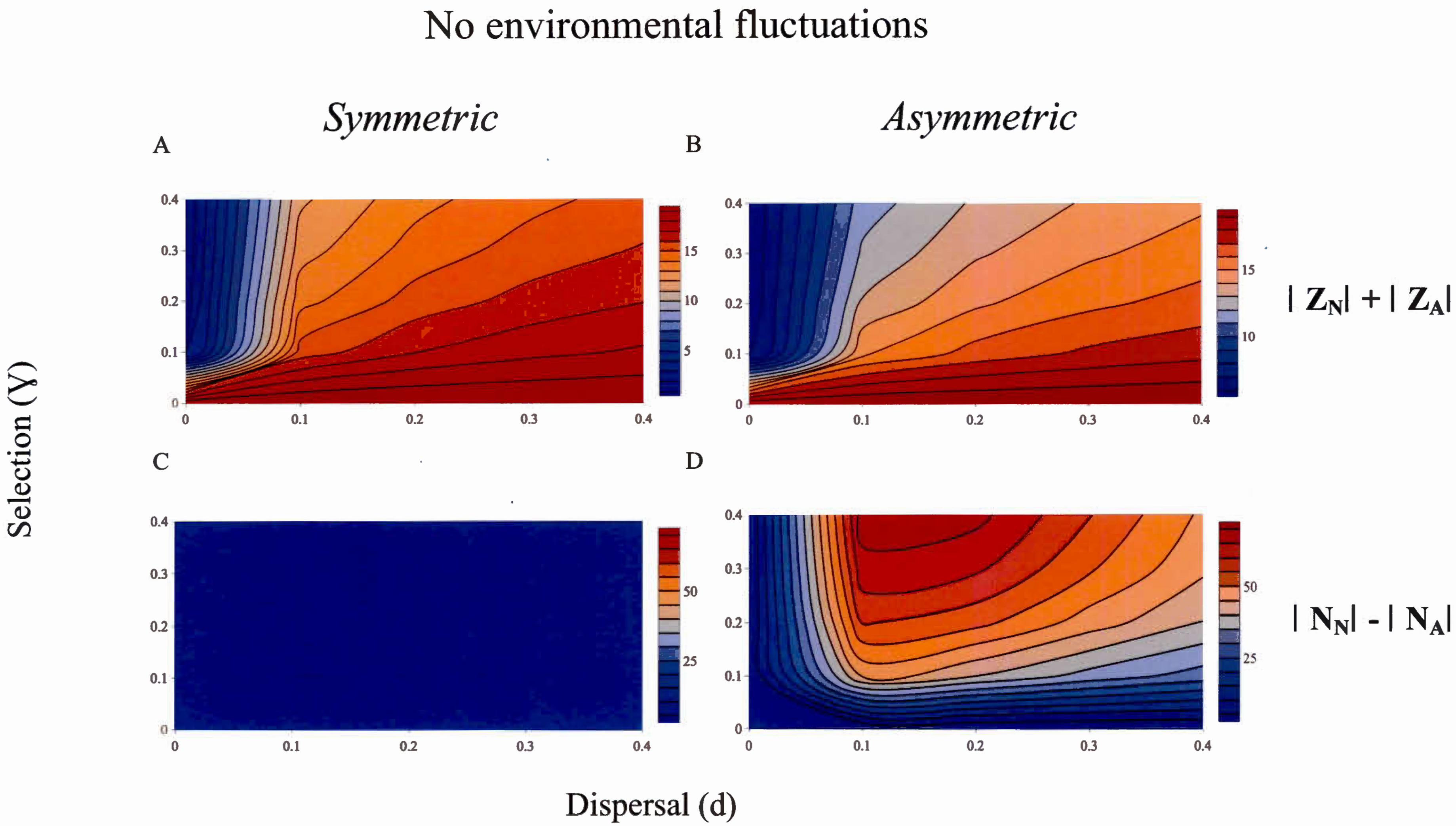


**Fig. 4** Total regional metapopulation contour plots (A and B) at t=1000 in stable environments under the influence of within patch local selection and between patch dispersal and metapopulation heterogeneity in population size (C and D).

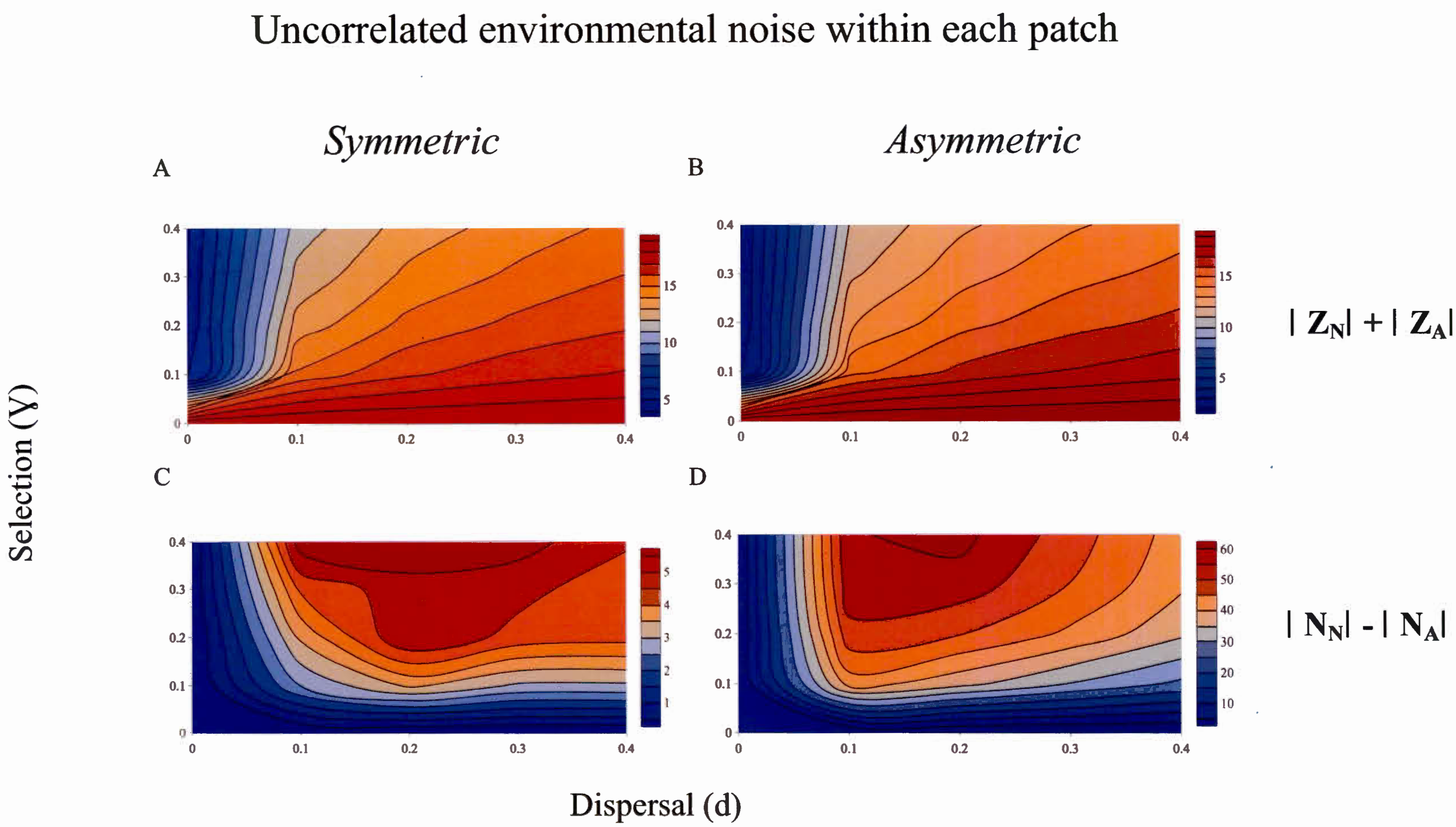


**Fig. 5** Total regional metapopulation contour plots (A and B) at t=1000 in stable environments under the influence of within patch local selection and between patch dispersal and metapopulation heterogeneity in population size (C and D).

## 2.4 Discussion

Our empirical field experiment provided a real example in nature of how asymmetric survival responses to pH determine whether locally selected versus regionally selected phenotypes can dominate in local habitat patches. Our modelling results have broader implications for better getting a handle of how much or in what way the role of connectivity between habitats assists or hinders population-level adaptation and/or maladaptation. Just like local selection, we use empirical evidence for it to implement it in a two-patch metapopulation model and understand how it interacts with spatial and temporal structure.

Source-sink dynamics can alter competitive outcomes in both sources and sinks via dispersal which act to alter per capita demographic rates (Fox 2007), and have thus been used more widely to explain competitive coexistence (Amarasekare 2004; Amarasekare and Nisbet 2001). While community models study how flows of individuals sustained in one habitat can persist locally in other habitats, despite a trend towards exclusion from resident species (mass effects) due to negative interactions such as competition and predation (Holt et al. 2003), source-sink models consider how sub-optimal populations in sinks are able to persist under dispersal from source habitats. Thus, sink habitats are usually dominated by a large number of individuals with phenotypes that are maladapted to their habitat, sub-optimal that is, because of the regular influx of migrants from source habitats. While dispersal has already been given much attention as an important mechanism affecting the strength of selection, another important understudied mechanism that can generate source-sink structures and maintain maladaptation persistent in heterogeneous environments is symmetry in the strength selection. This simple situation explored in the not so recent (Pulliam 1988; Holt 1993) and more recent theoretical and modelling literature (Ronce and Kirkpatrick 2001; Holt et al. 2003; Drake and Griffen 2013; Blanquart 2014; Holt and Barfield 2015), can explain the existence and persistence of local maladaptation, where the degree of maladaptation (Dias 1996a) of the immigrant source has the potential to influence the adaptive evolution within a source-sink structure (Holt 1993; Holt 1996). This is because while non-random dispersal maintains the stability of a source-sink system, especially when sink habitats represent a large proportion of the landscape, passive dispersal can depress density in the source population leading ultimately to its extinction (Holt 1993). In models of population dynamics that include density-dependence, where demographic parameters in sources become dependant on the demography is sink habitats (Gundersen et al. 2001), equilibrium levels of populations cannot be simply interpreted as 'carrying capacities' of the environment (Lebreton and Gonzalez Davila 1993), because the abundance observed in a sink reflects local and regional processes operating jointly (Holt 1985; Holt and Gaines 1992).

We analyzed the evolutionary dynamics of asymmetric selection as an extension of Ronce and Kirpatrick (2001) migrational meltdown two-patch metapopulation model and related these to the emergence of maladaptation at the regional level. We found that maladaptation arose as an emerging property in heterogeneous habitats, and that its signature was largely determined by an interaction between the asymmetry in selection, dispersal and environmental fluctuations. In the model, population growth of maladapted individuals usually occurred under an interaction between weak selection and weak dispersal, however selection was more efficient at downplaying the role of dispersal in maintaining the average phenotype close to the environmental optimum. This would be expected in animals that passively disperse rather than actively migrate, as in the second case individuals are able to avoid the effects of selection by migrating to other habitats where they are released from its direct influence (Legrand et al. 2017), but not the former.

Overall, evolutionary effects in terms of regional maladaptation where stronger under high connectivity and ecological effects in terms of heterogeneity in population sizes were stronger under low to intermediate connectivity. Symmetric and asymmetric selection impacted population size heterogeneity under fluctuating conditions but not under stable conditions. In contrast, asymmetric selection strongly affected heterogeneity in population size under stable and fluctuating conditions. Under both assymetric and symmetric selection, weak selection and weak dispersal balanced each other to maintain some level of maladaptation. Counter-intuitively and under asymmetric selection and environmental fluctuations, we did not observe a decline in maladaptation at the regional level with increasing dispersal, but persistent maladaptation rather. In conclusion, a combination of asymmetry in selection and intermediate dispersal and environmental fluctuations, can maintain heterogeneity in population sizes and maladaptation persistent at the metapopulation level.

# CHAPITRE III

# A FISH-MEDIATED TROPHIC CASCADE ON FRESHWATER CALANOID COPEPOD ABUNDANCE IS CONCEALED BY FOOD WEB FATTY ACID AVAILABILITY, FUNCTIONAL TRAITS AND POPULATION SEX RATIO

Negrín Dastis J.O., Yates, M., Fraser, D.J., Derry, A.M., 2018. A fish-mediated trophic cascade on freshwater calanoid copepod abundance
is concealed by food web fatty acid availability, functional traits and population sex ratio. Journal of Plankton Research (*Accepted with minor revisions)*

## Résumé

We applied essential fatty acids as a biomarker to experimentally test the effects of a fish-mediated trophic cascade on calanoid copepod nutritional state and functional traits in north-temperate freshwater ponds. A whole-pond experiment was conducted where young-of-the-year brook trout (*Salvelinus fontinalis*) were introduced into three natural, fishless ponds with no previous history of fish introduction, and compared with four similar naturally-fishless ponds. We unexpectedly found no effect of the fish-mediated trophic cascade on copepod population nutritional state and abundance, despite enhanced phytoplankton resources in fish-stocked ponds. While salmonids increased individual female copepod fecundity, the fish also altered copepod population sex ratios in favour of males. Fish-mediated bottom-up effects from enhanced phytoplankton explained the increased copepod fecundity. However, the fish did not sufficiently enhance the EFA-content of phytoplankton communities to completely over-ride their negative effects on copepod nutritional state and abundance. Negative fish effects on copepods likely included expenditure of key lipids in male copepods through increased reproductive activity, and top-down selective predation on gravid female copepods. Therefore, fish-mediated trophic cascades on zooplankton abundance can be concealed by the balance of their effects on food web EFA availability, zooplankton functional traits, and zooplankton population sex ratios.

Keywords: Body size, Crustacean zooplankton, Fatty acids, Food web, Sex ratio

## 3.1 Introduction

In aquatic food webs, trophic cascades are simultaneously determined by both consumer predation (top-down processes) and consumer resource control on primary production (bottom-up processes) (Carpenter et al. 1987). In freshwater ecosystems, several meta-analyses have consistently shown evidence for strong top-down effects of fish on zooplankton (Shurin et al. 2002; Borer et al. 2005), that are stronger compared to bottom-up resources, and moderate effects of fish on phytoplankton biomass (Brett and Goldman 1997; Gruner et al. 2008). However, given that the influence of fish on aquatic communities through bottom-up processes can be variable among ecosystems, there is a need to understand how consumer-driven nutrient dynamics can potentially alter other understudied properties of food webs (Atkinson et al. 2016), such as organismal nutritional state (e.g., Twining et al. 2016) and functional groups and traits (Hulot et al. 2014). We present a first study to apply essential fatty acids (EFAs) as a biomarker to experimentally test the effects of freshwater fish on zooplankton nutritional state and functional traits through the combined influence of top-down and bottom-up influences in a trophic cascade.

Essential fatty acids (EFAs) are an emerging biomolecular tool for understanding trophic interactions in food webs (reviewed in Twining et al. 2016). In aquatic ecosystems, EFAs are synthesized by primary producers, which are primarily phytoplankton in lentic systems such as lakes and ponds (Taipale et al. 2013). Subsequently, trophic transfer of EFAs occurs from the phytoplankton to primary consumers, such as crustacean zooplankton (Taipale et al. 2011), and higher consumers, such as fish (Strandberg et al. 2015; Gearheart et al. 2017). The EFA content of phytoplankton is strongly linked with the identity of phytoplankton taxa. While certain phytoplankton groups are enriched with EFAs known as polyunsaturated fatty acids (PUFAs) (e.g., diatoms and mixotrophic cryptophytes, chrysophytes, and dinoflagellates), other phytoplankton groups are biochemically depauperate in EFAs and nutritionally-poor for zooplankton (e.g., chlorophytes and cyanobacteria) (Taipale et al. 2013; Galloway et al. 2015). A subgroup of PUFAs known as highly unsaturated fatty acids (HUFAs) are linked to key fitness-related functional traits in crustacean zooplankton, such as body size and fecundity (Müller-Navara et al. 2004; Charette and Derry 2016). However, the degree to which zooplankton FA content tracks their diet can depend on environmental factors such as season and temperature (McMeans et al. 2015). While several studies have addressed environmental and community-level determinants of EFA composition in crustacean zooplankton (e.g., Burns et al. 2011; Lau et al. 2012; Gladyshev et al. 2015), no research has tested if fish-mediated trophic cascades can alter the trophic transfer of EFAs to zooplankton and influence their functional traits.

We conducted a whole-pond experiment where young-of-the-year (YOY) brook trout (Salvelinus fontinalis) were introduced into three north temperate, natural fishless ponds. Four fishless reference ponds were concurrently monitored to quantify seasonal effects in relation to fish impacts on phytoplankton and zooplankton. Copepod nutritional state was inferred through ω3:ω6 FA ratios because low ω3:ω6 FA ratios have been shown to indicate poor nutritional state in other crustacean zooplankton such as Daphnia (Taipale et al. 2015a). We predicted that bottom-up nutrient enrichment from trout excretion would stimulate phytoplankton communities, and enhance ω3:ω6 FA ratios as well as body size-fecundity relationships of herbivorous copepods in fish-stocked ponds. Other research has shown that top-down fish predation on calanoid copepods in freshwater and marine ecosystems can selectively reduce the abundance of egg-bearing females compared to males (Hairston et al. 1983; Hirst et al. 2010). We therefore predicted that we would detect a signal of top-down predation by the brook trout through altered sex ratios of the calanoid copepod populations in fish-stocked ponds by end of summer. The influence of brook trout on calanoid copepod population nutritional state and abundance was expected to be a net outcome of the effects of bottom-up stimulation of algal resources and top-down predation by fish in selectively removing gravid females.

## 3.2 Methods

*Study system.* Our study was conducted in seven naturally fishless freshwater ponds on Cape Race, Newfoundland (NL), Canada (46°38'33.35''N, 53°12'02.27''W) over the summer of 2015. Of these ponds, three ponds were stocked with YOY trout, and four control ponds remained fishless throughout the field experiment. Responses in the dominant zooplankton grazer, a calanoid copepod (*Leptodiaptomus minutus*), were measured for between-pond population differences in fatty acid composition, functional traits (body length-fecundity relationships), sex ratios, and abundance. These responses were compared between the fish-stocked ponds and fishless ponds at end of summer, following two months of fish incubation in the fish-stocked ponds.

We also measured phytoplankton community composition and seston FA content, zooplankton community composition, and physico-chemical pond characteristics. Two time points of data collection corresponded to early summer immediately prior to fish stocking (30 June 2015) and late summer, following two months of fish

**Table I:** Table of geographical coordinates, physico-chemical, and basal resource characteristics of the 7 study ponds in late June 2015 and in early September in 2015 at Cape Race, NL, Canada. The three ponds stocked with YOY brook trout are indicated by #. Except for latitude (Lat), longitude (Long), surface area (SA) and depth (Depth), white column shading corresponds to late June sampling prior to fish stocking and grey column shading corresponds to early September sampling following two-months of fish exposure in the fish-stocked ponds.

| Ponds | Lat | Long | SA ($m^2$) | Depth (m) | Temp (°C) | | DO (mg $L^{-1}$) | | pH | | SPC (µS $cm^{-1}$) | | TP (µM) | | TN (µM) | | DOC (mg $L^{-1}$) | | Chl*a* (µg $L^{-1}$) | | BP (µgC $L^{-1}$ $day^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P2# | 46.6458821712 | -53.2157588912 | 140 | 0.38 | 17.0 | 18.5 | 6.0 | 6.4 | 5.2 | 5.5 | 176.9 | 139.5 | 0.181 | 0.197 | 26.06 | 39.27 | 4.4 | 8 | 0.2 | 0.6 | 3.2 | 1.2 |
| NR2 | 46.6512026509 | -53.2026676716 | 1622 | 0.3 | 13.8 | 17.0 | 8.2 | 8.0 | 5.5 | 5 | 78 | 74.1 | 0.245 | 0.216 | 23.27 | 17.35 | 2.5 | 2.2 | 0.2 | 0.7 | 3.4 | 1.2 |
| P1# | 46.6528478720 | -53.2091634723 | 126 | 1 | 19.5 | 16.5 | 6.1 | 6.1 | 5.2 | 5 | 59.7 | 53.7 | 0.378 | 0.375 | 36.63 | 53.47 | 9.6 | 10.1 | 1.1 | 2.2 | 7.8 | 1.9 |
| OB6 | 46.657389 | -53.1880 | 2084 | 0.28 | 14.2 | 15.2 | 7.7 | 7.5 | 4.9 | 4.8 | 59.1 | 55.4 | 0.142 | 0.407 | 43.19 | 69.36 | 8.9 | 5 | 0.3 | 0.7 | 3.3 | 1.9 |
| OS9 | 46.6493449182 | -53.1950941681 | 850 | 0.5 | 15.4 | 16.9 | 7.4 | 8.0 | 5.2 | 5.3 | 68.7 | 52.7 | 0.152 | 0.242 | 20.06 | 34.55 | 8.5 | 11.8 | 0.7 | 0.4 | 9 | 1.1 |
| P3# | 46.6435800000 | -53.2188370000 | 234 | 0.2 | 19.9 | 18.1 | 7.4 | 7.7 | 6 | 6.3 | 267.1 | 218 | 0.843 | 2.102 | 48.33 | 122.12 | 16 | 25.2 | 0.4 | 2.5 | 8.4 | 2.5 |
| WR1 | 46.6410825238 | -53.1639735006 | 691 | 0.4 | 18.3 | 13.9 | 8.2 | 8.8 | 4.8 | 4.5 | 84.1 | 71.1 | 0.194 | 0.310 | 21.03 | 41.52 | 5.1 | 7.4 | 0.7 | 0.9 | 6.4 | 4.7 |

**(#) Ponds stocked with brook trout YOY:**

**Pond P2: YOY (N=93)**
**Pond P1: YOY (N=87)**
**Pond P3: YOY (N=146)**

exposure in the fish-stocked ponds (01 September 2015). The region is dominated by a single fish species, brook trout (Bernos and Fraser, 2016), which was the rationale for working with this particular fish species for our experiment. All study ponds were located within a small spatial scale (0.52 $km^2$), and were isolated from stream connections. The fish-stocked ponds had pH $\geq$ 5.2 and oxygen concentrations $\geq$ 6.0 mg $L^{-1}$, and low variation in nutrients and DOC at the onset of the experiment (Table I). The fishless ponds had similar initial early summer phytoplankton community composition (Fig. 1S) despite some variation in physico-chemical characteristics: total phosphorus (TP) 0.142 to 0.245 μM, total nitrogen (TN) 20.06 to 43.19 μM, dissolved organic carbon (DOC) 2.5 to 8.9 mg $L^{-1}$, and pH 4.9 to 5.5 (Table I). The YOY that were placed in the fish-stocked ponds originated from two stream populations: Cripple Cove River, CC (46° 38.854' N, 53° 06.269' W) and Freshwater River, FW (46° 38.914' N, 53° 13.301' W) (Supplementary material, Table 1S).

*Fish stocking.* We stocked three fishless ponds with no previous history of fish introduction with YOY trout at equivalent densities and at equivalent size distributions of fish in late June 2015 (Supplementary material, Figure 2S). We were unable to stock more than 3 ponds in this region because of the pond selection criteria that we applied for fish stocking, to prevent the escape and spread of YOY trout to other neighbouring ponds, as well as to ensure that we would be able to fully recapture the fish at the end of the experiment (Supplementary material, Methods 1S). Since there was variation in the surface area of the fish-stocked ponds (Table I), different absolute numbers of fish were introduced into each fish-stocked pond to maintain the same fish stocking density. Depths of the fish-stocked ponds were similar (0.2-1.0 m, Table I) and so surface area provided a good estimate for standardising fish stocking densities. Fish stocking occurred over two days: June 29 in ponds P1 and P2 and June 30 in pond P3. We recaptured stocked fish on Sept. 1, 2015; ponds were repeatedly fished using a combination of backpack electrofishing, gill nets, and seining. Each pond was fished on multiple consecutive occasions until

**Table II** Final mass (grams, mean ± standard error of the mean (SEM)) of YOY brook trout stocked into three fishless ponds in late June and recovered in early September 2015 for the fish-stocking pond experiment in 2015 at Cape Race NL, Canada.

| **Pond** | **Latitude** | **Longitude** | **LATE JUNE Weight (g)** | **EARLY SEPT Weight (g)** |
|---|---|---|---|---|
| P1 | 46.6528478720 | -53.2091634723 | 0.32 ± 0.04 | 6.80 ± 0.40 |
| P2 | 46.6458821712 | -53.2157588912 | 0.28 ± 0.03 | 7.02 ± 0.66 |
| P3 | 46.6435800000 | -53.2188370000 | 0.51 ± 0.03 | 2.70 ± 0.06 |

no fish were subsequently recaptured. Each recaptured fish was weighed to estimate mean total fish biomass per pond (Table II).

*Physico-chemical variables.* High-resolution, continuous reading temperature TidbiT v2 UTBI-001 TempLoggers (Hoskin Scientific, Massachusetts, USA) were placed in the mid water column of study ponds (1 datalogger per pond) to quantify the temperature differences between ponds over the duration of the fish stocking experiment from late June to early Sept 2015. The following physico-chemical explanatory variables were measured from each pond at the two time points in early and late summer: surface area ($m^2$), maximum depth (cm), temperature (°C), pH, dissolved oxygen (DO, mg $L^{-1}$), specific conductivity (SPC, $\mu S\ cm^{-1}$), total phosphorus (TP, μM), total nitrogen (TN, μM), dissolved organic carbon (DOC, mg $L^{-1}$), tannic acid (TA, mg $L^{-1}$), algal biomass in the edible feeding size range for copepod *L. minutus* (<30 μm, edible chlorophyll *a*, μg $L^{-1}$), and bacterial production (BP, μgC $L^{-1}$ $day^{-1}$). Relative maximum depth was measured at the approximate pond center without disturbing surface sediments with an inflatable boat or by reaching from shore for the smallest, most shallow ponds. Surface area ($m^2$) of each pond was determined with Google Earth Pro© 2013 by Google, Mountain View, CA, USA. Temperature (°C), specific conductivity ($\mu S\ cm^{-1}$), pH, and dissolved oxygen (mg $L^{-1}$) were measured with an YSI ProPlus multiparameter probe (YSI model 10102030; Yellow Springs Inc.). For analyses of water chemistry, two replicate samples were collected from the pond litoral zone and subsequently passed through an 80μm size to remove debris, large particle sizes and other sedimentary particles suspended in the water. Upon collection, water chemistry samples were refrigerated and express-shipped within a week of collection to an analytical laboratory at UQAM (Montréal, Québec). Analytical methods are in Supplementary material (Methods 1S).

*Fatty acids (FA).* For seston FA collection, we filtered 50 L of pond water through a 30 μm GF-C filter to obtain 2-3 mg of sample dry weight per pond. For copepod FA collection, live *L. minutus* copepods were manually picked from zooplankton communities with a 10 ml-graduated pipette under a stereo-microscope

(Olympus SZ61 Olympus Canada Inc., Richmond Hill, ON, Canada). FA samples were placed in 1.5 mL cryogenic vials and frozen in an insulated liquid nitrogen Thermo Scientific double walled vacuum vessel (Model 813) at −80°C, which was then transported to UQAM and placed in a −80°C freezer until analyses. Fatty acids were extracted from seston and copepod samples using a chloroform-methanol-water (4:2:1) wash cycle, which was then converted to µg of lipid per mg dry weight by gravimetry. The extracted lipids were methylated using toluene and $H_2SO_4$-methanol. Fatty acids (C14–C24) were identified as Fatty Acid Methyl Esters (FAME) using a gas chromatograph (7890A, Agilent, Agilent Technologies Canada Inc., Mississauga ON, Canada). Total FA fatty acid concentrations were calculated with an internal standard, C19:0 (nonadecanoic acid) using calibration curves based on known standard concentrations reported as µg FAME $mg^{-1}$ total dry weight. Phytoplankton source FA biomarkers (Taipale et al. 2013; McMeans et al. 2015; Taipale et al. 2015b; Grosbois 2017) were selected from the total suite of FAs analysed (Supplementary material, Tables 2S, 3S) for our data analyses.

*Phytoplankton and zooplankton.* Methods for phytoplankton and crustacean zooplankton collection, identification, and enumeration are described in Supplementary material (Methods 1S). All crustacean zooplankton individuals were counted in our samples, and we enumerated the number of male and female *L. minutus* copepods. We estimated individual body size of gravid female *L. minutus* copepods (total length; µm) with a high-resolution dissecting microscope (SZ2-IL-ST, Olympus SZ, Japan), and copepod length did not include the caudal setae as described by McCauley (1984). Individual female fecundity was estimated by counting the number of eggs per individual gravid copepod female. No distinction was made between diapausing and subitaneous eggs.

*Statistical analyses*

We conducted Wilcoxon signed rank matched paired two-tailed tests, the non-parametric analogue to the paired t-test which allows for differences between pairs that are non-normally distributed because of skewed data, paired by individual pond to measure within pond changes for each univariate environmental parameter (TP, TN, DOC, Chl *a*, and BP) between early and late summer within the fish-stocked ponds (n=3) and within the fishless reference ponds (n=4). We then conducted a series of non-metric multidimensional scaling (NMDS) analyses based on Hellinger distance dissimilarity matrices (Legendre and Legendre, 2012) to track shifts in the copepod FA composition (*L. minutus*), phytoplankton communities (biovolume (mg $m^{-3}$), seston FA composition, and crustacean zooplankton communities (relative species abundance) between early and late summer, which corresponded with pre-stocking and post-stocking in the fish-stocked ponds. Stress tests were performed to test for good model fit in 2D space (Kruskal, 1964), using 1000 iterations. NMDS ordination plots were constructed using the metaMDS function{vegan} and a constrained ordination function cca{ade4}in R (R Core Team, 2016). This was followed with Permutational MANOVA (PERMANOVA) test for differences between stocked and fishless ponds using PRIMER v. 6.1.11 (Clarke and Gorley, 2006) with PERMANOVA + 1.0.1 add-on package (Anderson *et al.* 2008). We used a sum of squares sequential type I with 999 permutations of the residuals under the full model with fish stocking coded as a fixed effect and season as a random effect. On the basis of the Hellinger distance ressemblance measure, we equally tested for the homogeneity of multivariate dispersions within groups with the PERMDISP function in PRIMER v. 6.1.11 (Clarke and Gorley, 2006) and statistical assumptions regarding deviations from centroids were verified with 999 permutations.

We tested the influence of fish-stocking and season (early summer versus late summer) on body length-fecundity relationships using linear regression. Wilcoxon signed rank matched paired two-tailed tests were also applied to explain differences in copepod population abundance and total crustacean zooplankton abundance. To test for differences in copepod nutritional state (ω3:ω6 FA ratios) and female and male copepod abundance (sex ratios) responses to fish-stocking and season, we applied a linear mixed model (LMM) with the lme4 package in R (R Core Team, 2016), with fish stocking coded as a fixed effect and season coded as a random effect for each model response respectively. Normality and equal variance was confirmed for copepod nutritional state (Shapiro-Wilk W test; 0.90 Prob < W 0.15), and for male and female copepod responses to stocking through verification of model conditional residuals (Shapiro-Wilk W test; 0.88 Prob < W 0.07) for the restricted maximum likelihood (REML) for mixed models. Significant main effects were confirmed using Tukey HSD pairwise comparisons and a Wald p-value was generated from the covariance parameter estimates to test for the significance of the season random effect for each model.

## 3.3 Results

### Fish-stocking treatment

Differences in fish recapture and therefore fish mortality between the fish-stocked ponds produced variation in the level to which fish enhanced pond nutrients and phytoplankton. The number of fish recaptures per fish-stocked ponds was: Pond P1, n=2 CC fish, 6.8 g ± 0.4; Pond P2, n=14 CC fish, 7.02 g ± 0.66; Pond P3, n=99 FW fish, 2.70g ± 0.06 (Table II). This variation in fish mortality and individual fish growth rates produced a gradient of nutrient enrichment between fish-stocked ponds (n=3) that could not be detected statistically in comparison with fishless ponds (n=4): TP, *P*=0.109; DOC, *P*=0.296 (Wilcoxon signed rank matched paired tests, before vs. after fish stocking). However, edible algal biomass flourished from the period of initial fish stocking to the period of fish recapture in the fish-stocked ponds (mean response difference pre and post stocking +1.2 μg $L^{-1}$) compared to fishless ponds (mean response difference pre and post control fishless ponds +0.2 μg $L^{-1}$) (*P*=0.046; Fig. 1A, Wilcoxon signed rank matched paired test). Total nitrogen (TN) increased in the fish stocked ponds (mean response difference pre and post stocking +34.61 μM) compared to the fishless ponds (mean response difference pre and post control

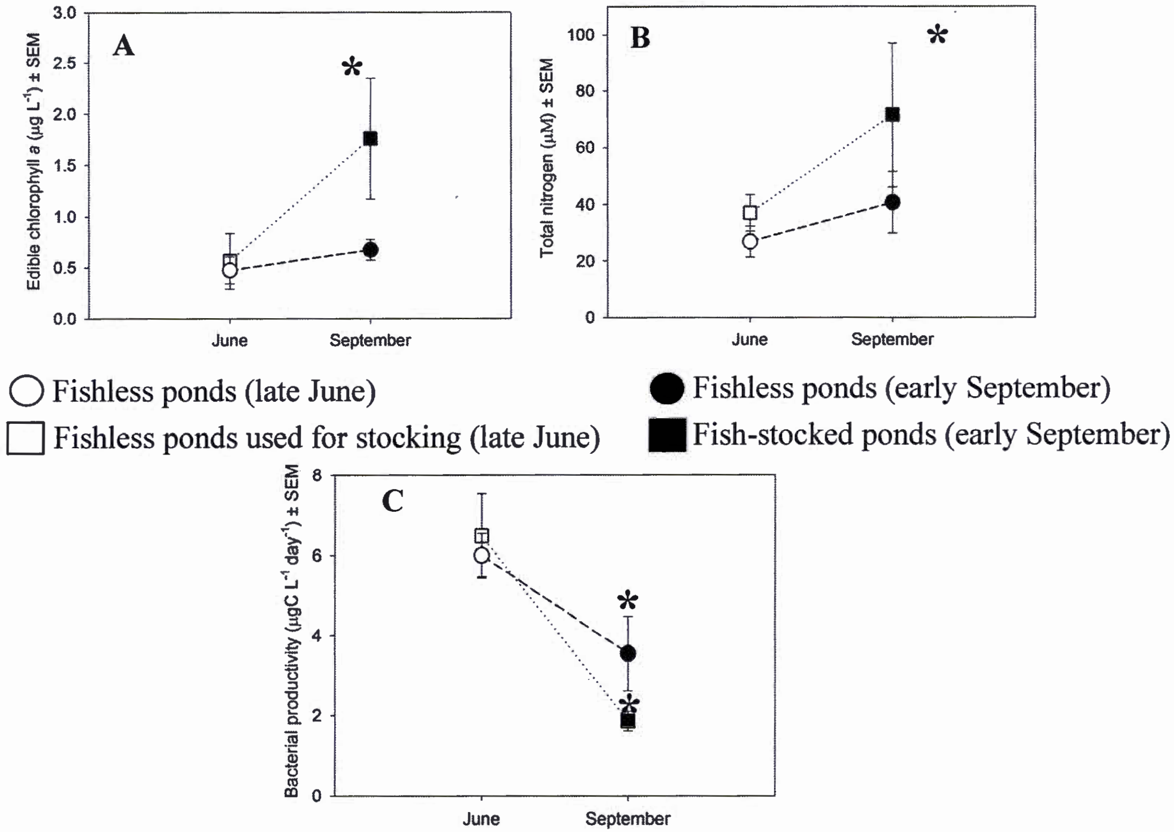


**Fig. 1** Pond water chemical and biotic characteristics resources in the three fish-stocked ponds and seven fishless reference study ponds at Cape Race, NL, Canada. Square symbols represent fish-stocked ponds and circle symbols represent fishless ponds; within these symbols, open symbols represent time of initial stocking in early summer and solid symbols are after two months of brook trout exposure at the time of fish recapture in late summer. **A.** edible chlorophyll *a* (edible chl *a*; µg L$^{-1}$), **B.** total nitrogen (µM), **C.** bacterial productivity (BP; µg C L$^{-1}$ day$^{-1}$). Asterisks (*) represent significant differences between either initial stocking and post-stocking in the fish-stocked ponds or between early summer and late summer in the fishless reference ponds as revealed by matching paired tests ($P<0.05$).

fishless ponds +13.80 μM) (*P*=0.031; Fig. 1B, Wilcoxon signed rank matched paired test). Bacterial productivity declined in all ponds, fish stocked (mean response difference pre and post stocking -4.6 μgC $L^{-1}$ $day^{-1}$) and fishless (mean response difference pre and post control fishless ponds -3.3 μgC $L^{-1}$ $day^{-1}$), from early summer to late summer, reflecting a seasonal effect (Fig. 1C; *P*=0.015, Wilcoxon signed rank matched paired test).

***Calanoid copepods – fatty acids, traits, and abundance***

Copepod FA composition was altered by trout in fish-stocked ponds (Fig. 2A; PERMANOVA, 999 permutations and type I SS: fish stocking main effect, df=1, *Pseudo-F*=3.52, *P*=0.018; Supplementary material Table 2S). However, this shift in copepod FA composition was driven by enrichment with ω-6 PUFA Ɣ-linolenic acid (C18:3n6c) (*GLA*) in the most fish-impacted pond, P3, and not by ω-3 HUFA-enrichment as we had predicted. In fishless ponds, season had a neglible effect on copepod fatty acid composition (Fig. 2A). Neither fish stocking (Fig. 2B; LMM, fish stocking main effect, *F*=0.651, *P*=0.436) nor season (Fig. 2B; LMM, season random effect, Wald p-value 0.842) significantly influenced the nutritional state of copepods (ω3:ω6 FA ratios).

There was a positive relationship between female copepod body length and fecundity across all study ponds in early summer (Fig. 3A; linear regression: n=33; *P*<0.0001; $r^2_{adj}$=0.56). However, by late summer, the positive relationship between gravid female copepod body length and fecundity was enhanced in fish-stocked ponds (Fig 3B linear regressions; fish-stocked ponds: n=8; *P*=0.013) compared to the fishless ponds ($r^2_{adj}$=0.61; fishless ponds: n=5; *P*=0.134; $r^2_{adj}$=0.44). Also as predicted, the relative abundance of male copepods compared to female copepods increased in the fish-stocked ponds, but not in the fishless ponds between early and late summer (Fig. 3C; LMM, fish stocking main effect, *F*=7.206, *P*=0.021). Season (Fig. 3C; LMM, season random effect, Wald p-value 0.660), did not explain any significant

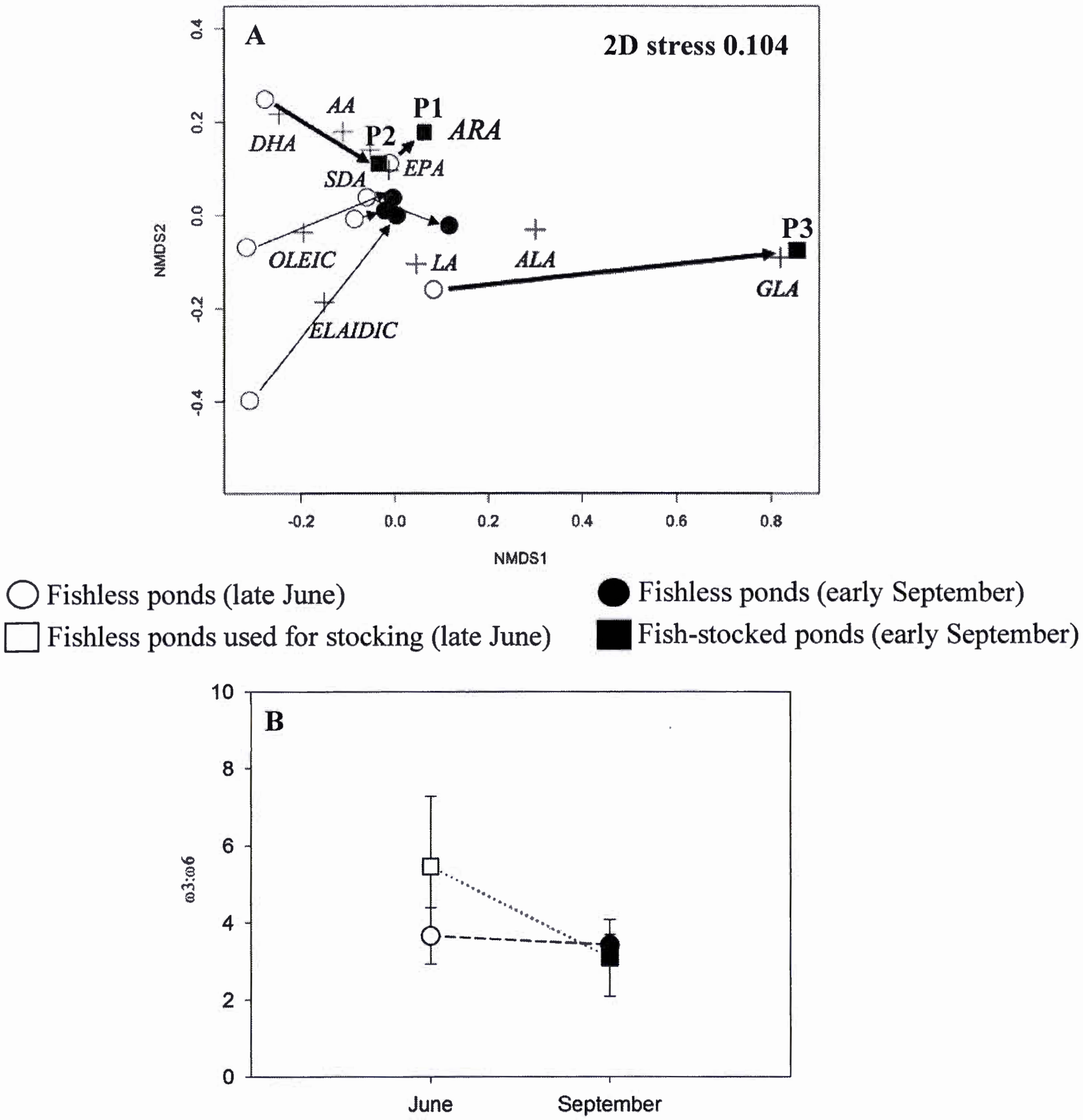


**Fig. 2** Effects of YOY brook trout stocking and season on copepod fatty acids in fish-stocked and fishless ponds. Square symbols represent fish-stocked ponds and circle symbols represent fishless ponds; within these symbols, open symbols represent early summer, which was the time of initial fish stocking in fish-stocked ponds, and solid symbols represent late summer, which was after two months of brook trout exposure at the time of fish re-capture in the fish-stocked ponds. **A.** Temporal shifts in phytoplankton indicator fatty acid composition in calanoid copepods as revealed by NMDS. Arrows represent trajectories of plankton communities between early and late summer. Please refer to Supplementary Table 2S for corresponding information on the multiple and specific phytoplankton fatty acid biomarker abbreviations detected in copepod fatty tissue. **B.** Nutritional state of calanoid copepods as revealed by ω3:ω6 ratios in fish-stocked and fishless ponds between early and late summer.

variation in sex ratio. Total copepod abundance remained at similar densities between early and late summer across all ponds (Fig. 3D; matched paired test: Wilcoxon signed rank two-tailed test, Prob >|S| 0.937). Neither season nor fish stocking had a detectable effect on the taxon dominance of crustacean zooplankton communities (PERMANOVA: fish stocking main effect, *P*=0.945; season main effect, *P*=0.598; Supplementary material, Figure 3S-A), or on total crustacean zooplankton abundance (matched paired test: Wilcoxon signed rank two-tailed test, Prob >|S| 0.218; Fig. 3S-B).

### ***Phytoplankton communities and seston fatty acids***

Fish-stocking generated changes in phytoplankton community composition that exceeded the range of seasonal variation in fishless ponds (PERMANOVA, 999 permutations and type I SS: fish-stocking main effect, df=1, *Pseudo-F*=3.02, *P*=0.015; Fig. 4A). Although we lost an initial pre-stocking sample for seston fatty acids from the most fish-impacted pond (P3), which was subsequently discarded from the statistical analysis to avoid overfitting of the model response, fish-stocking in ponds P1 and P2 alone caused changes in seston FAs that exceeded the range of seasonal variation in the fishless ponds (PERMANOVA, 999 permutations and type I SS: fish stocking main effect, df=1, *Pseudo-F*=3.95, *P*=0.012; Fig. 4B). It was evident that the seston in pond P3 became enriched with ω-6 PUFA arachidonic acid (*ARA*) relative to all other ponds by end of summer (Fig. 4B). There were no detectable effects of season on the composition of phytoplankton communities (Fig. 4A) and seston FAs (Fig. 4B; Supplementary material Table 3S). In fish-stocked ponds, cryptophytes (*P*=0.156) and especially dinoflagellates (*P*=0.003) increased between early and late summer (Wilcoxon signed rank matched paired test, Fig. 4C), and did not undergo similar increases in abundance in fishless ponds (Fig. 4D). Together, these two mixotrophic groups were >50% of the total phytoplankton biovolume by the end of the summer in the fish-stocked ponds: cryptophytes (38%) and dinoflagellates (16%). By comparison, cryptophytes and dinoflagellates comprised <1% of total

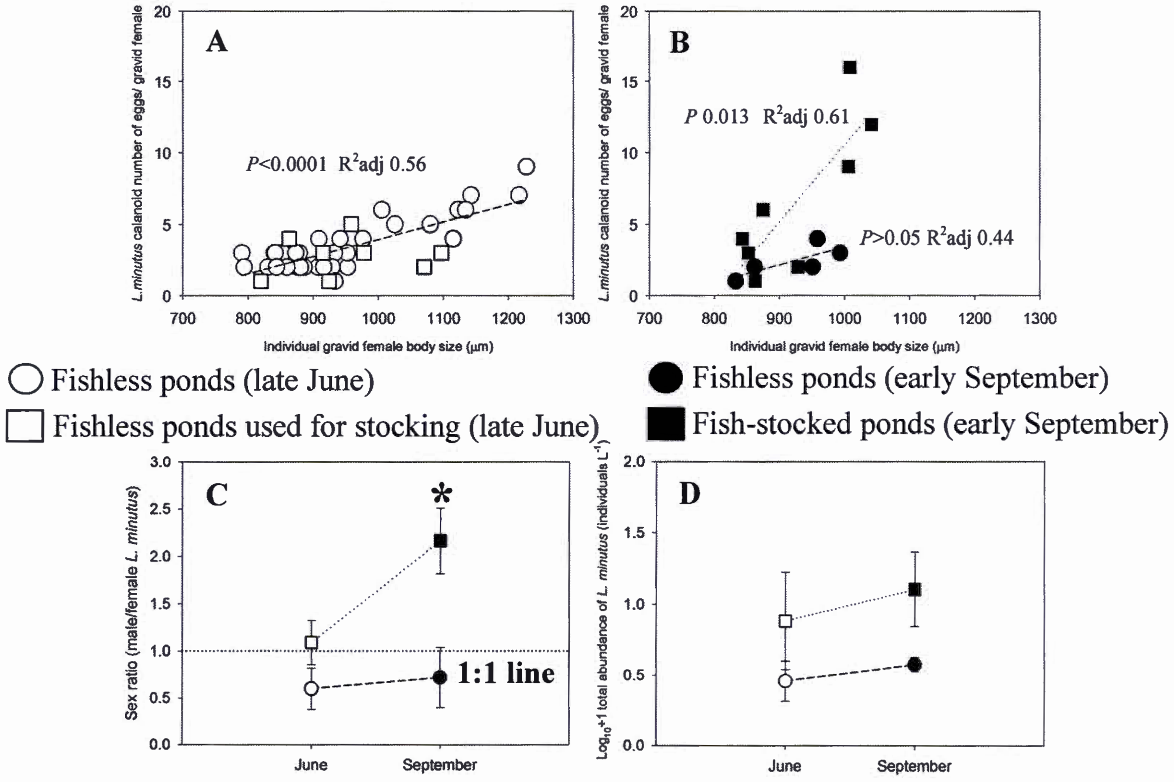


**Fig. 3** Relationships between copepod body length-fecundity relationships, copepod population abundance and sex ratios, and total crustacean zooplankton abundance in response to fish-stocking and season. Square symbols represent fish-stocked ponds and circle symbols represent fishless ponds; within these symbols, open symbols represent time of initial stocking in early summer and solid symbols are after two months of brook trout exposure at the time of fish re-capture in late summer. Simple linear regressions between individual gravid female calanoid copepod body size (μm) and fecundity (number of eggs / individual gravid female) in **A.** fishless, reference ponds prior to fish stocking in early summer and **B.** in fish-stocked and fishless reference ponds at end of summer. **C.** Ratios of male individual copepod abundance to individual female copepod abundance per $L^{-1}$. **D.** Differences population abundance of calanoid copepods in response to fish stocking and season. Asterisks (*) represent significant differences between either initial stocking and post-stocking in the fish-stocked ponds or between early summer and late summer in the fishless reference ponds as revealed by matching paired tests ($P<0.05$).

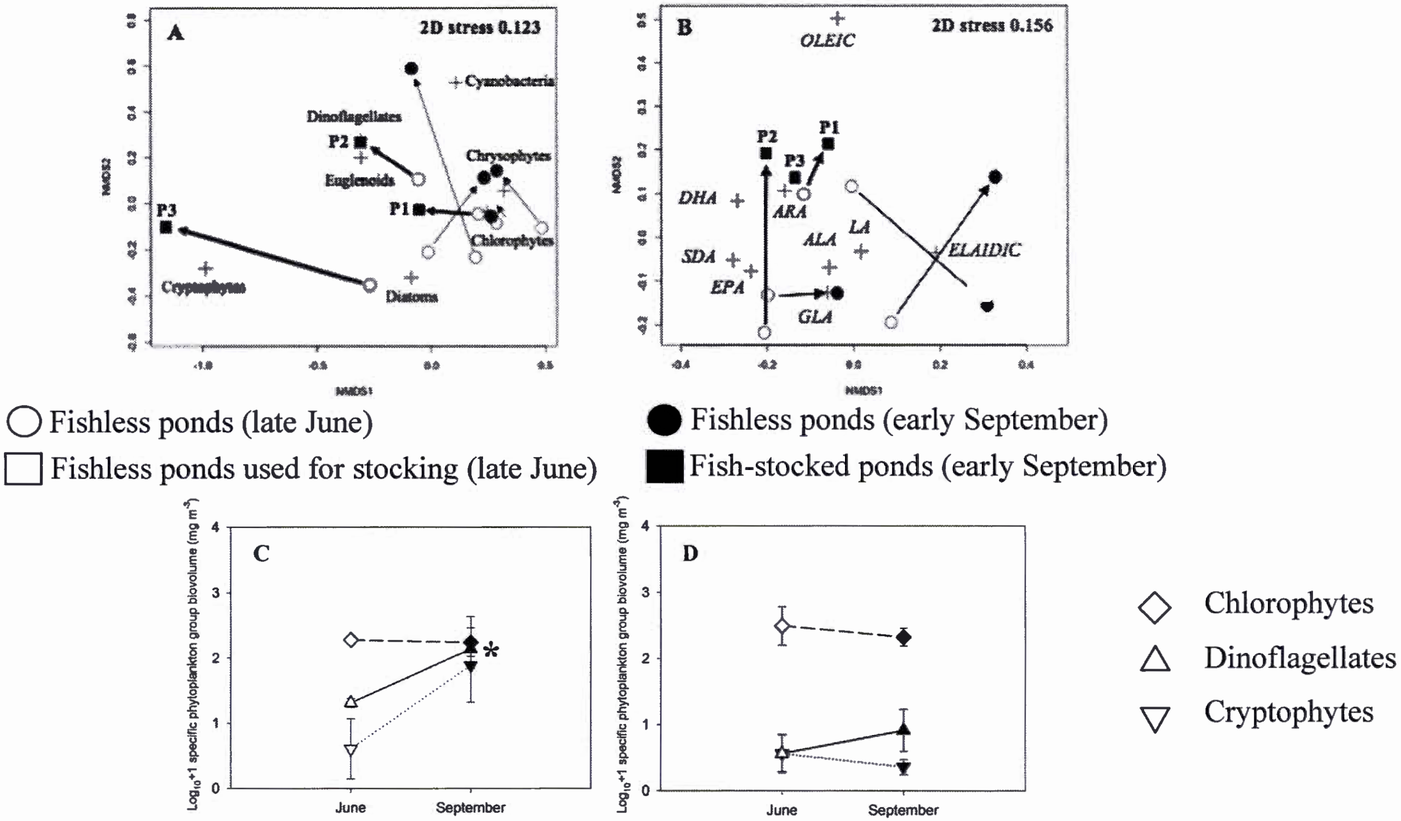


**Fig. 4 A)** NMDS of phytoplankton community composition (Hellinger-tranformed relative taxon biovolume; mg m$^{-3}$) and **B)** NMDS of seston phytoplankton biomarker fatty acid composition. In panels **A.** and **B.**, square symbols represent fish-stocked ponds and circle symbols represent fishless ponds. Arrows on the NMDS biplots in represent trajectories of phytoplankton communities between these two periods. The biovolume (mg m$^{-3}$) of three major phytoplankton groups (cryptophytes, dinoflagellates, and chlorophytes) are shown in **C.** fish-stocked ponds and in **D.** fishless ponds. In panels **C.** and **D.**, we used the following symbols to designate key phytoplankton groups: downward triangles, crytophytes; upward triangles, dinoflagellates; diamonds, chlorophytes. Asterisks (*) represent significant differences between initial stocking and post-stocking in the fish-stocked ponds ($P<0.05$).

phytoplankton biovolume by end of summer in the fishless ponds. Chlorophyte biovolume was relatively high in all ponds at end of summer: 35% total biovolume in fish-stocked ponds and 81% total biovolume in fishless ponds.

## 3.4 Discussion

We present a first study to apply EFAs to quantify the effects of a fish-mediated trophic cascade on herbivorous crustacean zooplankton nutritional state in aquatic ecosystems. Despite enhanced quantity (Fig. 1A) and nutritional quality (Fig 4) of phytoplankton communities in fish-stocked ponds, the presence of YOY trout neither increased the nutritional state (Fig. 2B) nor the population abundance (Fig. 3D) of the calanoid copepods. However, YOY trout enhanced body size-fecundity relationships of individual female copepods (Fig. 3A, B), likely because of stimulated phytoplankton resources (Fig. 1A, 4). Copepod population sex ratios became skewed towards a higher abundance of males relative to females by end of summer (Fig. 3C). Male-dominated sex ratios of calanid copepods have also been detected in other freshwater and marine ecosystems where fish selectively prey on visually-conspicous gravid female copepods (Hairston et al. 1983; Hirst et al. 2010). Positive effects of enhanced individual female copepod fecundity on copepod population abundance through fish-mediated bottom-up processes on phytoplankton were likely offset by selective fish predation effects on copepod population sex ratio. Therefore, the fish did not sufficiently enhance the EFA-content of phytoplankton communities to completely over-ride their negative effects on copepod population nutritional state and abundance through predation.

We detected trophic cascades that transmitted fish effects to the phytoplankton (biomass, Fig. 1A; taxonomic community composition, Fig. 4A; FA composition of seston, Fig 4B), likely through enhanced nutrients (Fig. 1B) from fish excretion. More specifically, the presence of salmonids in fish-stocked ponds increased the total phytoplankton biomass (Fig. 1A), the abundance of nutritious phytoplankton groups (cryptophytes and dinoflagellates; Fig. 4A), as well as the PUFA content of seston (Fig. 4B) that were available as a resource for crustacean zooplankton. In the fish-stocked ponds, mixotrophic dinoflagellates and cryptophytes comprised >50% of total phytoplankton biovolume by the end of summer (Fig. 4C) compared to <1% in fishless ponds (Fig. 4D). Dinoflagellates and cryptophytes are algal groups that are known to be enriched in ω3 PUFAs such as DHA (Taipale et al. 2013; Galloway et al. 2014; 2015). However, ω-6 PUFAs, not ω-3 PUFAs, were enriched in the seston of fish-stocked ponds (Fig. 4B). Chlorophytes, enriched with ω-6 PUFAs, were the dominant phytoplankton taxa across all ponds (Fig. 4C, D), and are not considered highly nutritious for zooplankton because this algal group does not contain ω3 PUFAs (Taipale et al. 2013; Galloway et al. 2014; 2015). Herbivorous copepods can selectively harvest nutritious groups of phytoplankton (Paffenhöfer and Lewis 1990; Burns et al. 2011), but were likely also feeding on chlorophytes and other algal groups. A dominance of phytoplankton communities by less nutritious chlorophytes across all ponds may have contributed to why fish-mediated shifts in the compositional quality of phytoplankton did not result in enhanced nutritional state of copepod populations in fish-stocked ponds by end of summer (Fig. 2B).

Many studies have shown evidence for strong top-down effects of fish predators in aquatic ecosystems (Brett and Goldman 1997; Shurin et al. 2002; Borer et al. 2005; Gruner et al. 2008). There was likely also a strong top-down effect of fish predation by YOY trout at the end of summer in the fish-stocked ponds in our study. First, trout predation on crustacean zooplankon may have been focused on copepods compared to other taxa in the zooplankton community. Copepods tend to preferentially accumulate the HUFA, DHA (docosahexaenoic acid), compared to other zooplankton such as cladocerans (Burns et al. 2011; Lau et al. 2012; Gladyshev et al. 2015), and can provide a source of DHA to higher consumers such as fish in aquatic food webs (Strandberg et al. 2015). This may have especially been the case in our pond system where large-bodied cladocerans and copepods were absent, and the crustacean community was species-poor (6 species; Supplementary material, Fig 3S-A). Second, although we detected enhanced female copepod fecundity in the fish-stocked ponds (Fig. 3A, B), there was no changes in copepod population abundance between early and late summer in all ponds (Fig. 3D), despite enhanced fecundity in fish-stocked ponds. A possible explanation is that the fish were selectively preying on gravid females. Indeed, copepod population sex ratios became skewed towards male abundance in the fish-stocked ponds but not in fishless ponds by end of summer (Fig. 3C). Sex-specific fish predation on more visually-conspicuous egg-bearing females can reduce female copepod abundance disproportionately more than male copepod abundance (Hairston et al. 1983; Hirst et al. 2010).

Dietary quality is an important determinant of the fatty acid content of calanoid copepod eggs, and of the hatching and survival of larval nauplii (Guisande and Harris 1995). We did not measure the fatty acid content of the calanoid copepod eggs, and so we cannot conclude if the selective removal of gravid female copepods by fish predation reduced the overall nutritional state of copepod populations in fish-stocked ponds. It is possible that female copepods channelled FA resources to egg production by mobilising accumulated reserves that had not been consumed in recent diet but had been accumulated over the summer (Syväranta and Rautio, 2010). However, enhanced female fecundity may have been accompanied by a loss of important FAs in the male copepods as a result of increased reproductive activity; increased reproductive activity has been linked with reduced lipid content in marine calanoid copepods (Sargent and Falk-Peterson 1988). Therefore, although the trout enhanced individual female copepod fecundity (Fig. 3A, B) via bottom-up effects on enhanced quantity and nutritious quality of phytoplankton (Fig. 4), the overall nutritional state of the copepod populations showed a tendancy towards reduced nutritonal state rather than increased nutritional state (Fig 2B), likely because of a combination of poor HUFA availability in the phytoplankton community (Fig. 4A), increased reproductive activity of males in response to the females, and selective top-down fish predation on gravid females. Together, simultaneous bottom-up and top-down processes mediated by the fish-induced trophic cascade may have cancelled positive effects of enhanced phytoplankton resources on total copepod population nutritional state and abundance.

## 3.5 Conclusion

We present a first study to apply EFAs to detect fish-mediated trophic cascades on zooplankton in aquatic ecosystems. There was unexpectedly no detectable effect of the fish-mediated trophic cascade on copepod population nutritional state and abundance, despite enhanced phytoplankton resources in fish-stocked ponds. A combination of HUFA-poor phytoplankton communities, increased male copepod reproductive activity, and top-down selective fish predation on gravid female copepods likely offset gains in copepod population abundance through enhanced female copepod fecundity. Therefore, fish-mediated trophic cascades on zooplankton abundance can be concealed by the balance of their effects on food web EFA availability, zooplankton functional traits, and zooplankton population sex ratios.

# CONCLUSIONS

Populations are often locally adapted or show ecological specialization to their home environments depending on genetic drift and gene flow (Crispo 2008; Hereford 2009), but also selection, with abiotic environments being thus strong selective drivers of adaptive specialization. Previous research has shown evidence for ecological specialization in *L. minutus* copepods to different pH environments in absence of biotic interactions, where acid tolerances of calanoid copepod populations are a function of the pH of habitat source (Derry and Arnott 2007).

# CONTRIBUTIONS TO SCIENTIFIC KNOWLEDGE

## CHAPTER I

In **CHAPTER I**, I was able to test the effect of gene flow on local adaptation. I did this by testing experimentally local adaptation to pH in calanoid copepods (*L. minutus*) along a landscape gradient in acidity in a previously unstudied area (Cape Race, NL). This was based on the assumption of maladaptation to neutral conditions, whereby populations that were adapted to acidic conditions incurred a fitness cost in circumneutral environments, and not plasticity or the evolution of broad pH tolerance.

The basic predictions for trait divergence are that (1) circumneutral populations in a circumneutral landscape should be least acid tolerant and (2) those in an embedded landscape should be generally more acid tolerant with the assumption that gene flow from the surrounding acidic ponds should result in local " maladaptation " (i.e. higher acid tolerance also in circumneutral ponds).

For the mixed landscape case (3) I predicted that spatial heterogeneity would act to favour phenotypic plasticity or high genetic trait variance in presence of gene flow or, alternatively, small scale local adaptation if gene flow is restricted either due to low dispersal or due to strong divergent selection. Again, these predictions depended on a trade-off in survival responses between acid and neutral tolerance that was evident in the reciprocal transplant experiment conducted in the laboratory.

The key finding is that copepods from circumneutral ponds within the acidic landscape (scenario 3) have highest acid tolerance (i.e. highest survival at pH 4.6), indicating that these circumneutral populations are maladapted to the local environment. Furthermore, it should be considered that what is adaptive, is in fact determined by the landscape context, this is because natural selection may be acting on the landscape level, whereby what is considered "adaptive" may be determined by selection acting on metapopulations - rather than local populations. This is especially the case in organisms (e.g. zooplankton) that undergo metapopulation dynamics, whereby local populations may be maladapted due to high gene flow from contrasting environments.

In the present study system, in a landscape where ponds generally are circumneutral, I would then maybe expect low acid tolerance (no selection for increased acid tolerance). In a landscape where neutral ponds are embedded in an acidic pH landscape, gene flow or repeated colonizations from acid ponds (which are more abundant) should result in higher acid tolerance also in the neutral ponds. In a landscape that consists of a mix of different pH ponds, the predictions are maybe less clear-cut as they may depend on the scale at which selection acts (i.e. locally or on landscape level) as well as the scale of dispersal. Within each of these specific landscapes, and in terms of pH local habitat heterogeneity, I found a strong asymmetry in the predominant acidic landscape that occurred in response to selection for acid tolerance that was non-existent in the homogenous landscape.

## CHAPTER II

In **CHAPTER II**, I decided to build upon this previous asymmetry in selection in terms of responses to pH acidity to carry out a reciprocal transplant experiment designed to determine locally selected versus selected phenotypes in local habitat patches and used this empirical problem to construct a two-patch model that aimed at understanding how maladaptation is maintained through this asymmetric fitness response to selection. I found that maladaptation in abiotic tolerance at the regional level were persistent through an interaction between weak asymmetric selection, intermediate dispersal and environmental fluctuations. Structural features of the landscape (e.g. pond pH inter-annual variation), had emergent effects on maladaptation.

# CHAPTER III

Adaptation involves coping not only with the physical abiotic environment, but also with the complex biotic environment (intra- and inter-specific competition for resources with other organisms but also predation). In **CHAPTER III**, I quantified sources of phenotypic variation in *L. minutus* copepods that are associated with landscape gradients in pH (body size, fecundity, and the trophic accumulation of lipids and essential fatty acids (PUFAs)). Essential compounds that are synthesized the base of the aquatic food web by phytoplankton include essential polyunsaturated fatty acids (PUFAs), which are critical for growth, development, and reproduction, and are transferred to higher trophic levels through dietary accumulation. Any environmental parameter that affects the distribution of PUFAs at the base of the food chain could alter the transfer of these compounds to consumers in aquatic ecosystems (Fuschino et al. 2011; Taipale et al. 2011). For example, dissolved organic carbon (DOC) can have strong effects on basal resources such as phytoplankton and bacteria, which have very different nutritional value to higher consumers (Faithfull et al. 2011). Therefore carbon that is derived from pelagic bacteria has a lower nutritional value because it lacks the essential poly-unsaturated fatty acids (PUFAs) that are synthesized by algae.

In our study system, the one environmental parameter that has the potential to limit the transfer of essential fatty acids from the base of the food chain to higher trophic levels is pH. In aquatic environments, pH can control the availability of nutritious resources such as algae that contain essential trophically transferred fatty acids. Therefore, it is also possible that population-level differences in the nutritional state of primary consumers (zooplankton) may arise across landscape gradients in pH. This latter trait has potential to have a strong influence on eco-evolutionary feedbacks because zooplankton are the principal pathway for the trophic transfer of essential fatty acids from algae to higher consumers. Interestingly, I found that not only abiotic differences in terms of pH but also Consumer-driven Nutrient Dynamics (CND) of *S. fontinalis* among aquatic habitats, could therefore potentially modulate the availability of essential fatty acids to intermediate aquatic consumers such as zooplankton by stimulating or suppressing phytoplankton growth.

# RELEVANCE

## CHAPTER I

Overall, **CHAPTER I** of this thesis has empirically tested metapopulation theory involving predictions of how landscape context can contribute to increasing or reducing local adaptation in populations depending on the pattern of environmental heterogeneity, the spatial distance between habitats on landscapes, and the level of habitat connectivity (Hanski et al. 2010; Richardson et al. 2014; Farkas et al. 2015). Our study empirically confirms that spatial landscape context is important for explaining regional differences in population environmental tolerances. This is one of one of the only empirical studies to test theoretical predictions about landscape context in the pattern, extremity, and scale of environmental heterogeneity to local population trait divergence.

## CHAPTER II

In addition, in **CHAPTER II**, I developed a theoretical model that is based on another two-patch metapopulation model (Ronce and Kirkpatrick 2001), which aimed at testing the influence of maladaptation maintained by asymmetric selection and dispersal, on maladaptation at the regional level and heterogeneity in population sizes. Unexpectedly, I found that under both assymetric and symmetric selection, weak selection and weak dispersal balanced each other to maintain some level of maladaptation. Counter-intuitively and under asymmetric selection, we did not observe a decline in maladaptation at the regional level, but persistent maladaptation.

CHAPTER III

With **CHAPTER III**, I was able to detect salmonid-mediated CND on altered concentrations of polyunsaturated fatty acids (PUFAs) and body size-fecundity traits in calanoid copepods via shifts in the phytoplankton community. Additionally, I found that CND-mediated transfer of fatty acids from phytoplankton communities to crustacean zooplankton were altered by pH when considered at the landscape scale.

In conclusion, with this thesis I have been able to better understand in a spatial context the ecological and evolutionary processes that take place along landscape gradients in acidity at the individual, population, community and larger ecosystem trophic levels. However it remains to be tested if in other heterogeneous landscapes, either gene flow constrains divergence - and hence resulting in maladapted phenotypes in a given landscape - vs. selection favouring maintenance of genetically based trait variation or phenotypic plasticity. Although the study tests for an effect of the landscape on trait divergence in the context of putative gene flow, alternative explanations to gene flow and landscape context could be due to; the rather large distances among the 3 regions, different genetic basis of the species (e.g. historical colonization patterns) or some other regional differences that are not related to pH variation (e.g. temperature). Also, maladaptive trait variation may also have other causes than gene flow (e.g. trade-offs with other adaptive traits).

# LIMITATIONS AND AVENUES FOR FUTURE RESEARCH

Future studies could for example further develop some of the ideas and experiment developed and designed in **CHAPTER I** by looking at how individuals, populations and communities respond to multiple stressors, and their impact on freshwater ecosystems from an ecological and evolutionary standpoint. Although freshwater science and management are replete with mutiple-stressor problems, few studies have been designed explicitly to untangle their effects (Ormerod et al. 2010). As an example, experiments designed to detect adaptive or maladaptive responses to these multiple stressors within and between generations could potentially inform us of the impact of these stressors in combination, especially under various climate change scenarios. Last but not least, assuming the impact of differentially adapted copepods on algal consumption is due to the population origin of the algae, this could provide an interesting path from evolution affecting ecology (algal abundance), especially from an eco-evolutionary dynamics perspective (Hendry 2016). It is a bit speculative, but maladaptation could arise from higher numbers of acid tolerant copepods - or in addition some differences in traits or ability to consume resources.

In order to avoid oversimplifying models, but rather getting a better handle of ecological and evolutionary interactions and developing further on **CHAPTER II**, more models need to also consider temporally correlated positive and negative noise, in order to tease apart the magnitude of effects when comparing dispersal, drift and adaptation/ maladaptation. As a future direction to this model, it would be interesting to further explore and characterize the importance of feedback loops (positive vs. negative) in generating functional connectivity between a larger network of patches. It is predicted that a *positive feedback loop* would select for specialized phenotypes to better disperse in response to increase patch isolation, on the contrary a *negative feedback loop* will occur when patch isolation selects for residency (high dispersal costs) (Legrand et al. 2017). This chapter in conclusion is one of the only metapopulation modelling studies that have integrated asymmetry in selection, which is prevalent in natural populations to abiotic gradients, in understanding landscape-level maintenance of maladaptive trait variation in populations.

Finally, and building on **CHAPTER III**, further experimental studies could be designed to look at how top predators with different genetically-based intraspecific variation of specific traits influence directly (e.g. nutrient excretion rates) and/or indirectly (e.g. decomposition rates) both community structure and ecosystem function in aquatic systems. For example, from an evolutionary perspective, it would be interesting to further explore the factors that affect the stoichiometric plasticity of consumers (e.g. body size, feeding history and ontogeny) and its impact on nutrient dynamics. Also, how the abiotic characteristics of an ecosystem affect the net impact of consumers on ecosystem processes (e.g. feeding and excretion rates) have been largely unexplored. This is the first study to apply fatty acids for understanding top-down and bottom-up effects of freshwater fish on crustacean zooplankton nutritional state.

Overall the thesis made no attempts to distinguish between genetically-based local adaptation and environmental sources of trait divergence such as maternal effects and transgenerational plasticity. These would be areas of research that could be followed up on to further extend the research. For instance, for copepods Vehmaa et al. (2012) found that maternal effects may act as an adaptation mechanism for copepods facing pH and temperature changes.

Under rapid and unpredictable condition changes, when food resources become overexploited or food and environmental quality deteriorate, maternal allocation of resources can have profound implication for the production of offspring appropriate for the conditions that the progeny are expected to encounter (Gorbi et al.

2011). This implies prey potential for predicting environmental cues in rapidly changing environments. Some few examples include, effects of fish predation on mean size and body length and weakened ability to produce resting eggs for cladoceran populations (Langeland 1982), genetically based trait changes in *Daphnia* population co-existing with fish (Fisk et al. 2007) and life-history evolution in *Daphnia* populations after variation in migratory behavior and feeding morphology of a fish predator (Walsh et al. 2012).

Kazantseva and Alekseev (2007) also traced maternal effects by studying the bank of winter resting eggs as a proxy for seasonal adaptations, showing that the transfer of information from one parent generation to the other (transgenerational) concerning day length in subsequent *Daphnia* generations is more important for population seasonal adaptations than the transfer of information about food conditions. Factors associated with maternal effects in copepods, such as offspring size, have been so far associated with food conditions and seasonal changes. We can then hypothesize then that induced changes in maternal effects due to different pressures will have important consequences for the adaptation of populations.

Future work should aim to identify the conditions under which maternal effects are likely to play a role in evolution, as well as explicitly test the contribution of maternal effects to evolutionary responses (Räsänen and Kruuk 2007), and more especially so under fluctutating environmental conditions are non-optimal, it is better to produce a few larger, high quality and fast developing offspring, whereas the number of offspring should be maximized in extremely favorable conditions because all perform well, regardless of size (Parker and Begon 1986). Maternal effects, which can be transgenerational and are often adaptive (Galloway and Etterson 2007; Marshall 2008), are expected to be important in heterogenous environments when environmental cues are predictable (Räsänen and Kruuk 2007).

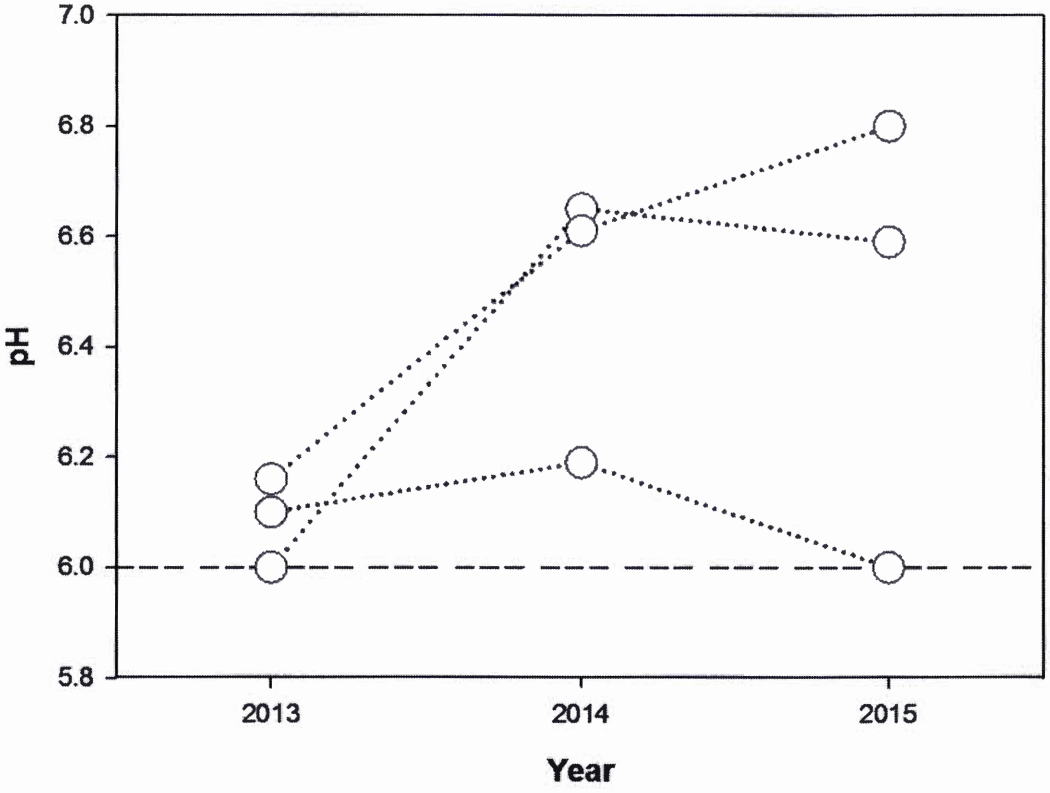
# ANNEXE CHAPITRE I

**S.I.-1.** Geographical coordinates and summary of water chemistry (pH, dissolved organic carbon (DOC), total phosphorus (TP), and final edible chlorophyll *a* concentration (chl*a*) for copepod source lakes/ponds from each regional landscape context types: Laurentians QC, Killarney ON and Cape Race NL. With the exception of lakes indicated by * for which pH was measured in 2011 (Arnott unpub., Queen`s University) and for other water chemistry variables in 2005 (Gray et al. 2012), all other water chemistry measurements are from summer 2014.

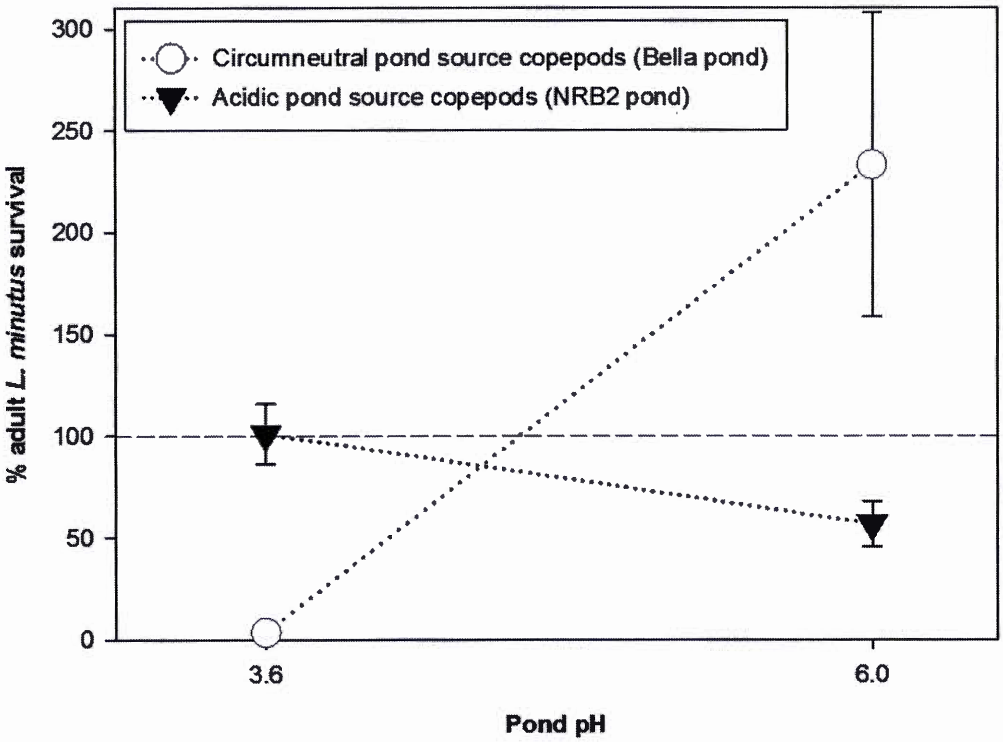
| Regional source | pH | Lake/Pond | Latitude | Longitude | DOC mgL$^{-1}$ | TP µgL$^{-1}$ | chl*a* µgL$^{-1}$ |
|---|---|---|---|---|---|---|---|
| Laurentians, Qc | 6.97 | Long | 45.98947 | -74.08201 | 5.68 | 5.40 | 2.02 |
| | 7.0 | Croche | 45.99174 | -74.01405 | 6.77 | 5.05 | 1.00 |
| | 6.99 | Pin Rouge | 45.9614 | -74.0403 | 8.06 | 9.15 | 1.76 |
| | 6.8 | Cromwell | 45.98937 | -74.000754 | 5.74 | 9.64 | 1.99 |
| | 6.94 | Thibault | 45.9776 | -74.02069 | 7.56 | 11.59 | 3.26 |
| | 7.3 | Castor | 46.01817 | -74.03975 | 8.59 | 9.29 | 1.53 |
| | 7.45 | Vingt Sous | 45.95136 | -74.37196 | 2.80 | 6.51 | 0.92 |
| | 7.5 | Noir | 45.87306 | -74.3023 | 5.51 | 8.18 | 2.76 |
| | 7.28 | A l'ours | 45.95937 | -74.05638 | 8.92 | 12.01 | 2.20 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 7.36 | Teardrop | 46.0425 | -81.413333 | 1.48 | *na* | 0.31 |
| | 7.47 | Howry | 46.15277 | -81.474167 | 5.64 | 10.29 | 1.92 |
| Killarney, ON | 7.3 | Gem | 46.15833 | -81.4375 | 6.42 | 3.96 | 2.42 |
| | 6.79 | Ishmael* | 46.10916 | -81.5925 | 4.10 | 5.80 | 1.60 |
| | 6.68 | Helen* | 46.10694 | -81.5625 | 4.10 | 7.00 | 1.06 |
| | 7.08 | Low* | 46.10166 | -81.560556 | 3.00 | 3.40 | 0.79 |
| | 6.65 | Bella | 46.64637 | -53.211151 | 7.09 | 7.33 | 0.79 |
| Cape Race, NL | 6.61 | BS11 | 46.64588 | -53.215758 | 13.45 | 9.95 | 1.15 |
| | 6.19 | Star | 46.64420 | -53.218880 | 19.93 | 45.66 | 1.23 |

**S.I.-2.** Inter-annual pH variation in the three circumneutral copepod source ponds i the acidic dominated landscape at Cape Race NL from 2013 to 2015. All three stud ponds remained above circumneutral pH (pH≥6; indicated by dashed line) (Negrín Dastis and Derry, unpublished data).



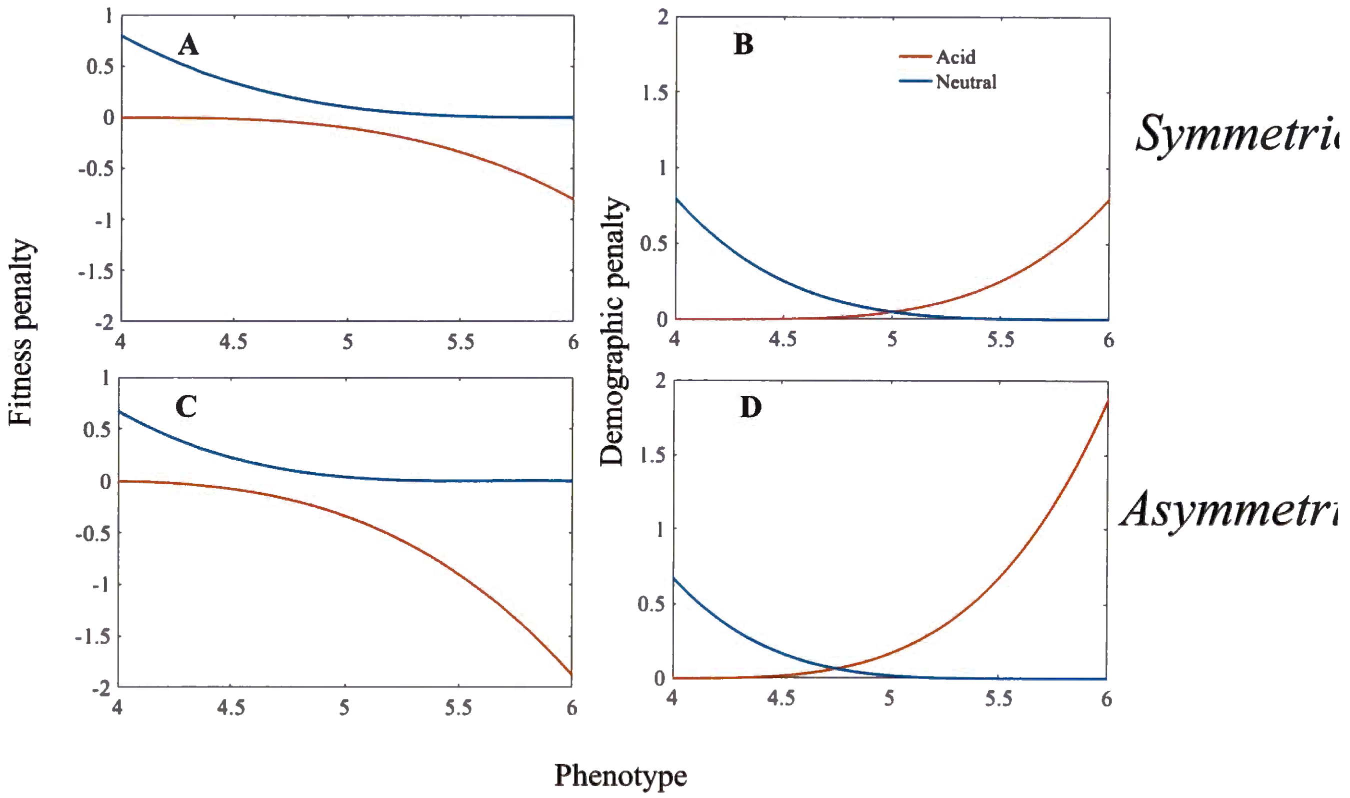
**S.I.-3.** Trial reciprocal transplant experiment involving *L. minutus* copepods that were exchanged between a circumneutral pond (Bella pond; pH 6.0 in 2013; Supplementary Information, **S.I.-1 and S.I.-2**) and an acidic pond (NRB2 pond; pH 3.6 in 2013) on the acidic-dominated landscape at Cape Race NL. Circumneutral pond source copepods are indicated by circle symbols and acidic pond source copepods are indicated by triangle symbols.



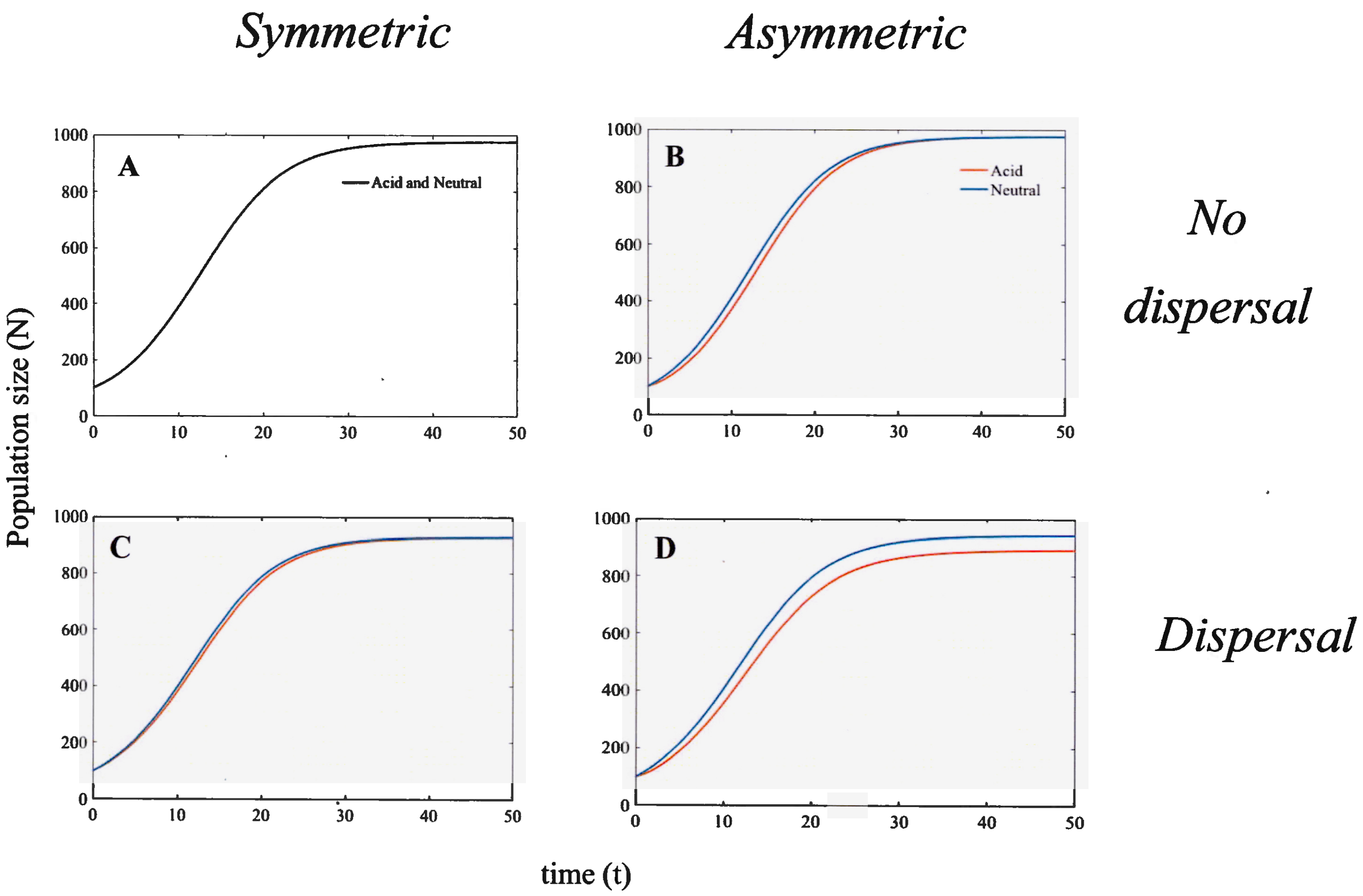
***Methods:*** Copepods were reciprocally transplanted between two ponds on Cape Race NL: circumneutral Bella pond (pH 6.0, see Supplementary information, **S.I.-1**, **S.I.-2.)** and acidic NRB2 pond (pH 3.6; 46.65052, −53.17754; DOC: 15.75 $\mathrm{mgL^{-1}}$, TP: 14.1 $\mathrm{\mu gL^{-1}}$; chl*a*: 1.1 $\mathrm{\mu gL^{-1}}$). Ambient copepod source densities were placed in 20-L closed polyethylene cubitainers with each of the two pond water types for 5 days in July 2013. The experimental design was a balanced 2 x 2 factorial (n=6 replicates) with copepod population source and pond water pH as the main factors. Initial copepod counts ($N_i$) collected directly from each source pond, was used a covariate and transformed to $\mathrm{Log_{10}}$(Ni+1). Final copepod counts transformed to $\mathrm{Log_{10}}$(Nf+1), were used as the response variable and were counted in each cubitainer at the end of the experiment. All normality and equal variance conditions were met following logarithmic transformation (Shapiro Wilk W test; 0.94 Prob < W 0.23), and results were analysed by ANCOVA using JMP 12 ® 2015 by SAS Institute Inc., Cary, NC, USA.

***Results:*** *Leptodiaptomus minutus* copepods were exchanged between a circumneutral pond (Bella, pH 6.0) and an acidic pond (NRB2, pH 3.6) on natural pond water. Circumneutral pond source copepod survival was reduced on acidic pond water compared to at circumneutral pH 6.0, as well as compared to acidic source copepods at acidic pH (ANCOVA, copepod pond source x pond: df=1, $F$=71.26, $P$<0.0001; Tukey HSD tests, $P$<0.050). Survival is expressed as % final copepod abundance relative to initial pond source abundance following 5 days of incubation. A survival rate in excess of 100% was observed for circumneutral pond source copepods at circumneutral pH. This was likely because of developmental maturation of copepodids (juvenile copepods), which had not been included in the count of adult individuals at the start of the experiment but became adults by the end of the experiment.

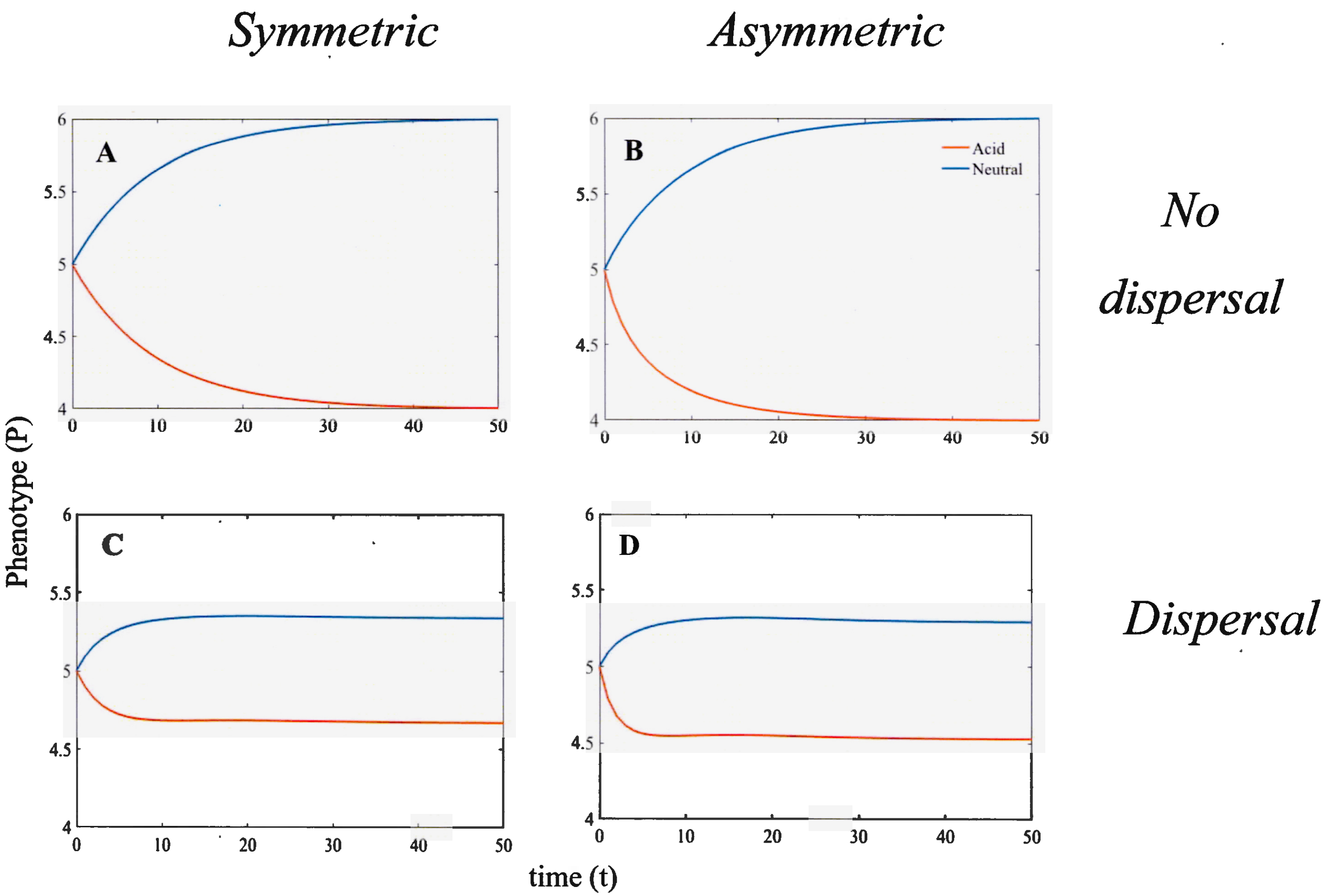
# ANNEXE CHAPITRE II



**S.I.I** Model behavior in terms of penalty incurred for values of fitness (A, C) and population size in each habitat (B, D) under weak selection ($\gamma$=0.1) and the influence of symmetric ($\alpha$=0) and asymmetric ($\alpha$=0.5) selection without dispersal.



**S.I.II** The influence of local selection and dispersal on the resulting population size (N) at t=50 for two discrete populations (Acid and Neutral) under symmetric (A-C) (alpha=0) and asymmetric (B-D) (alpha=0.5) selection.



**S.I.III** The influence of local selection and dispersal on the resulting phenotype (P) at t=50 for two discrete populations (Acid and Neutral) under symmetric (A-C) (alpha=0) and asymmetric (B-D) (alpha=0.5) selection.

| Summary | Nparm | F Ratio | **Prob>F** |
|---|---|---|---|
| pH (3.6 vs. 6.0) | 1 | 33.68 | **<0.0001** |
| Copepod population source (Acidic vs. Circumneutral vs. Fluctuating) | 2 | 14.14 | **0.0013** |
| Annual landscape context (Acidic vs. Circumneutral) | 1 | 11.60 | **0.0020** |
| pH x Annual landscape context | 1 | 7.68 | **0.0086** |
| Copepod population source X pH | 2 | 7.53 | **0.0018** |
| Copepod population source x Annual landscape context | 2 | 3.40 | **0.0474** |
| Copepod population source X pH X Annual landscape context | 2 | 1.52 | 0.2305 |

**S.I.IV** Linear mixed model (LMM) statistical results of the reciprocal field experiments conducted in the field during two consecutive years (2013 and 2014) at Cape Race (NL, Canada). Survival to acidity [Log10 (Nfinal + 1)- Log10 (Ninitial + 1)]] was used as the response variable and pond was used as a random variable in the model and all tests were run at a significant value of $\alpha$=0.05. Based on the response variable residuals and a goodness of fit test, the data is from the Glog distribution (AICc -3.13; Shapiro–Wilk W test; 0.96 Prob < W 0.11). In the Fluctuating pond pH category, pH alternated between circumneutral and acidic pH between years. In the other Pond pH categories, pond pH did not cross the threshold between acidic (pH<6.0) and circumneutral (pH>6) pH conditions.

# ANNEXE CHAPITRE III

**Methods 1S.** Supplementary methods: physico-chemical and habitat characteristics of brook trout source streams, pond selection criteria for brook trout stocking, analytical methods for water chemistry, algal biomass, bacterial production, and the field collection and community analyses of phytoplankton and crustacean zooplankton.

## *A.* Field Methods

### *Physico-chemical and habitat characteristics of brook trout source streams*

For temperature (Temp; $^{O}$C), values were calculated from readings measured continuously (every 01h30min) between late June 2015 and early September in 2015 (n=511) in each of Cripple Cove River and Freshwater River. One temperature data logger was placed at the center of each stream from late June to early September 2015 to capture seasonal variation in the temperatures to which brook trout are naturally exposed over the duration of the pond experiment. pH, depth (cm) and specific conductivity (SPC; $\mu$S cm$^{-1}$) were measured during the month of June 2015 (Cripple n=35; Freshwater n=61). Dissolved oxygen (DO; mg L$^{-1}$) was only measured in Freshwater river during the month of June 2015 (Freshwater n=13). Vegetation of stream bed covered (Veg; %) and silt (Silt; %) were also measured for each river habitat. N.D. indicates were no data were collected. pH and dissolved oxygen were measured between 15 – 19 June 2015 along 34 transects in CC River (between 46° 38.854' N, 53° 06.269' W and 46° 39.733' N, 53° 06.756' W) and along 61 transects in FW River (between 46° 38.914' N, 53° 13.301' W and 46° 40.128' N, 53° 12.437' W).

### *Pond selection criteria for brook trout stocking*

Ponds for stocking were selected based on the following: 1) isolation of surface and ground water from other bodies of water to prevent escape and spread of the fish during the experiment, 2) shallow depths (0.5-1m) and small surface areas (149-250 m$^{-2}$) to enable re-capture of the fish by electrofishing at the end of the experiment, and 3) physico-chemical conditions (pH: 5.17-6 and mid-day summer temperatures:17-19.9°C that would allow survival of brook trout based on what is known about the ecological tolerances of this species at Cape Race NL (Wood and Fraser 2015; Bernos and Fraser, 2016).

## B. Laboratory methods

### *Analystical methods for water chemistry, algal biomass (chla), and bacterial production*

In the laboratory, total phosphorus was measured spectrophotometrically with a Biochrom Utlrospec®2100 pro by the molybdenum method after persulfate digestion (Griesbach and Peters, 1991). Dissolved organic carbon concentrations were measured with 0.45 μm-filtered samples (surfactant-free membrane filters) after acidification (phosphoric acid 5%) followed by sodium persulfate oxidation using a 1010 TOC analyzer (O.I. Analytical, College Station, TX, USA). Tannic acid equivalents were also measured spectrophotometrically by the absorption coefficients of water samples at 440nm, and then converting these values to phenolic equivalents (Cuthbert and del Giorgio 1992). Total nitrogen (TN; μg $L^{-1}$) was analyzed with a continuous flow analyzer (ALPKEM Flow Solution IV ©) using an alkaline persulfate digestion method, coupled with a cadmium reactor, following a standard protocol (Patton and Kryskalla, 2003).

Extraction of chlorophyll *a* was done in hot ethanol by measuring the photosynthetic pigment spectrophotometrically at 665–750 nm on a Biochrom Ultrospec 2100 pro with a 10-cm quartz cuvette (Winterman and de Mots 1965; Sartory and Grobelaar 1984). Edible algal biomass (<30 μm; Chl *a*, μg $L^{-1}$) was measured by the same methods as described for total chlorophyll *a* in the spatial survey except that we used a Nitex filter of 30 μm mesh size to first remove the larger size classes of seston from the samples.

Rates of bacterial production (BP, μgC $L^{-1}$ $day^{-1}$), which give a measure of bacterial protein synthesis (Smith and Azam 1992; Kirchman 2001), were determined by incorporation of 20 μ $L^{-1}$ tritiated leucine (specific activity 54.1 Ci $mmol^{-1}$) added to 1.5 ml of pond water.

### *Plankton collection, identification, and enumeration*

***Phytoplankton.*** Water samples for phytoplankton community biovolume analyses were collected from the centre of the 10 ponds and preserved with Lugol`s solution. Phytoplankton community composition was enumerated with an inverted microscope (Olympus 1×71) to genus level following U.S. EPA (2012) at 400–600x magnificantion in a Utermöhl microscope slide with ultra thin glass bottom plate. Cell dimensions were measured 10 times for each algal group via a camera-mounted microscope and converted to biovolume (Hillebrand et al. 1999). A minimum of 400 identified algal units was identified following Wehr and Sheath (2003).

***Crustacean zooplankton***. We gently filtered 45 L of water per pond source from all 7-study ponds with a Bilge pump (Model Tsunami T500 MPH) which was then concentrated on 54 μm Nitex, after which the zooplankton were anaesthetized with carbon dioxide exposure and preserved in a buffered 4% sugar-formalin. Crustacean zooplankton were identified to species level for all taxa and species abundances per L of filtered water were enumerated using a high-resolution dissecting microscope (SZ2-IL-ST, Olympus SZ, Japan). Identification of crustacean zooplankton was completed with the following taxonomic keys: Witty (2004), Thorp and Covich (2010), Haney et al. (2013), Smith and Fernando (1978) for copepods and, De Melo and Herbert (1994) for Bosminidae.

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**Figure 1S.** Principal Component Analysis (PCA) of late June main phytoplankton groups (Hellinger-transformed biovolumes) sampled in the 3 ponds used for fish-stocking (#) and the 4 fishless ponds used as reference fishless ponds for the entire duration of the experiment.

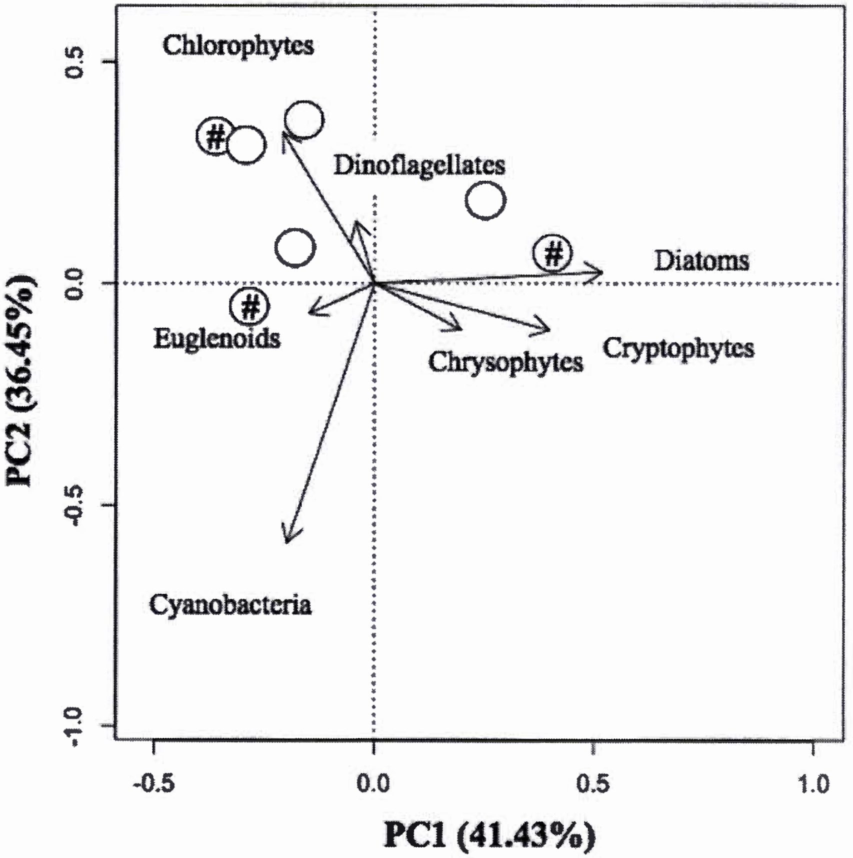
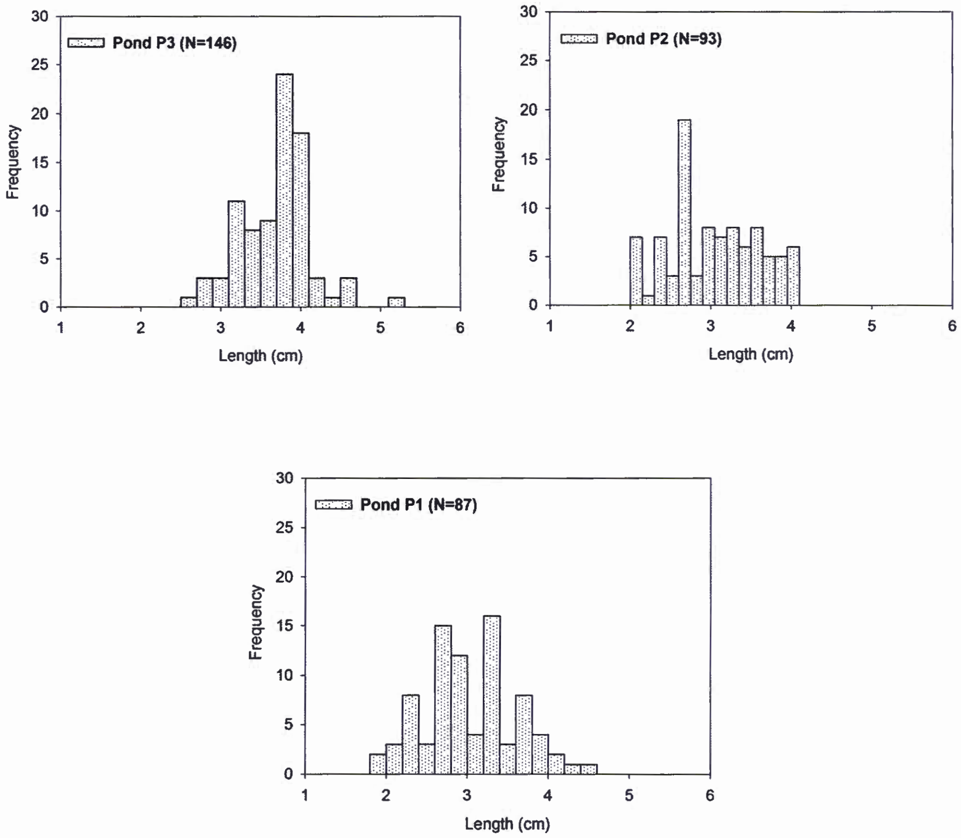


**Figure 2S.** Frequency and length (cm) of YOY brook trout from two nearby stream populations introduced into three naturally fishless ponds at Cape Race NL for two months in summer 2015 (0.6 fish $m^{-2}$ per stocked pond; 01 July to 01 September 2015).



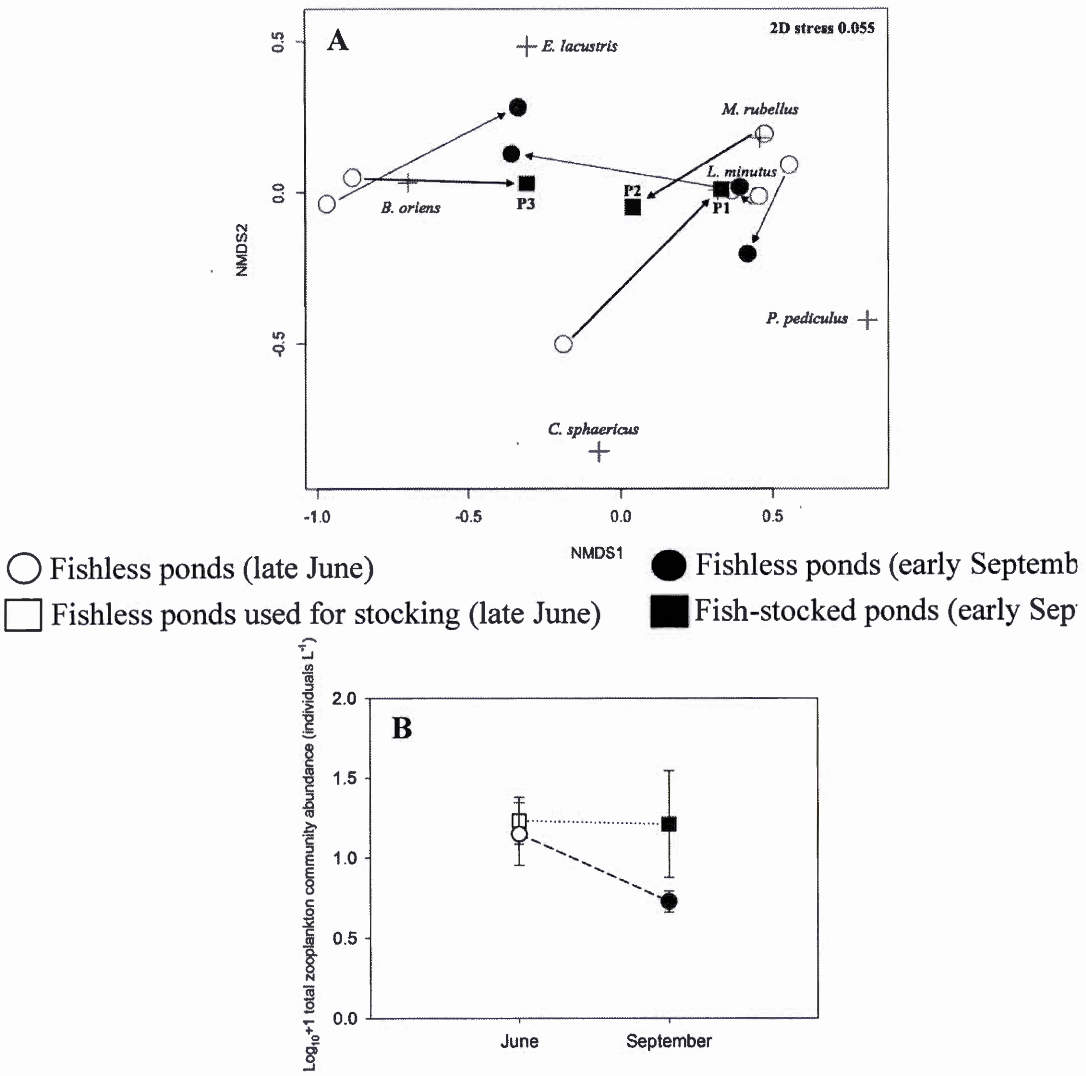


**Figure 3S. A)** NMDS of crustacean zooplankton communities (Hellinger-transformed relative taxon abundance; number of individuals L$^{-1}$) showing effects c YOY brook trout stocking and season (early summer to late summer) on temporal shifts in crustacean zooplankton community composition (n=3 fish stocked ponds; n=4 fishless reference ponds). Square symbols represent fish-stocked ponds and circle symbols represent fishless ponds; within these symbols, open symbols represent time of initial stocking in early summer and solid symbols are after two months of brook trout exposure at the time of fish re-capture in late summer. Arrow on the NMDS biplots represent trajectories of plankton communities between these two periods. **B)** Total community abundance (number of individuals of all crustace zooplankton species detected L$^{-1}$) between early and late summer.

**Table 1S.** Habitat characteristics (mean ± standard error of the mean (SEM)) of the two streams that were used as population sources of brook trout (*Salvelinus fontinalis*) for the fish-stocking pond experiment in 2015 (Cripple Cove River and Freshwater River, Cape Race NL, Canada). Latitude (Lat), longitude (Long), temperature (Temp; $^{0}$C), dissolved oxygen (DO, mg $L^{-1}$) and specific conductivity (SPC, µS $cm^{-1}$). Please refer to field methods for detailed measurements of physico-chemical and habitat characteristics of brook trout source streams (Supplementary material, Methods 1S). Values include ± standard error of the mean (SEM).

| **Source river** | **Lat** | **Long** | **Temp** | **YOY e-fished** | **pH** | **DO** | **Depth** | **Vegetation** | **Silt** |
|---|---|---|---|---|---|---|---|---|---|
| **Cripple** | 46.648990 | -53.106279 | 17.0 ± 0.1 | 180 | 6.2±0.03 | n.a. | 16.5±1.2 | 51.7±5.2 | 13.8±2.9 |
| **Freshwater** | 46.652100 | -53.218482 | 16.9 ± 0.3 | 146 | 6.5±0.04 | 9.7±0.3 | 16.0±0.7 | 61.6±3.6 | 4.2±1.7 |

**Table 2S.** Total fatty acid composition of calanoid copepods (*Leptodiaptomus minutus*; µg mg$^{-1}$) from the 7 study ponds in late June 2015 and in early September in 2015 at Cape Race NL (Canada). The three ponds that were stocked with YOY brook trout are indicated by #. White shading corresponds to late June sampling prior to fish stocking and grey shading corresponds to early September sampling. Specific copepod fatty acids selected for statistical analyses are indicated ($), selected based on a synthesis of the fatty acid literature by Grosbois (2017).

| PONDS | B | | BR4 | | P2# | | NR2 | | P1# | | OB6 | | OS6 | | OS9 | | P3# | | WR1 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| COPEPOD FATTY ACIDS | | | | | | | | | | | | | | | | | | | | |
| Myristic acid (C14:0) | 0 | 4.478 | 2.512 | 0 | 2.674 | 7.677 | 4.153 | 10.373 | 8.959 | 7.817 | 4.656 | 8.739 | 0 | 3.658 | 0 | 3.048 | 2.558 | 0.053 | 3.328 | 3.768 |
| Pentadecanoic Acid (C15 :0) | 0 | 0.294 | 0.189 | 0 | 0.16 | 0.787 | 0.353 | 0.654 | 0.399 | 0.345 | 0.163 | 0.622 | 0.128 | 0.227 | 0.156 | 0.318 | 0.235 | 0 | 0.091 | 0.315 |
| Palmitic Acid (C16:0) $ | 0 | 5.788 | 6.352 | 0 | 5.734 | 9.516 | 8.447 | 15.735 | 7.669 | 9.827 | 8.827 | 10.947 | 5.509 | 4.692 | 5.73 | 11.194 | 6.95 | 0 | 6.937 | 7.822 |
| Palmitoleic Acid (C16:1n7) | 0 | 1.547 | 1.959 | 0 | 1.569 | 5.671 | 2.923 | 3.529 | 5.208 | 1.665 | 1.307 | 4.271 | 1.409 | 2.133 | 1.771 | 1.274 | 1.801 | 0 | 1.427 | 2.606 |
| Heptadecanoic Acid (C17:0) | 0 | 0.263 | 0.063 | 0 | 0 | 0.344 | 0.118 | 0.202 | 0.005 | 0.218 | 0.055 | 0.219 | 0 | 0.098 | 0 | 0 | 0.129 | 0 | 0.055 | 0 |
| Cis-10-Heptadecenoic Acid (C17:1n7c) | 0 | 0.037 | 0 | 0 | 0 | 0.103 | 0.015 | 0.101 | 0.093 | 0.064 | 0 | 0.031 | 0 | 0.049 | 0 | 0 | 0.022 | 0 | 0.018 | 0 |
| Stearic Acid (C18:0) | 3.899 | 2.401 | 1.207 | 0.893 | 2.737 | 3.012 | 2.256 | 4.005 | 1.897 | 2.627 | 1.862 | 2.226 | 1.223 | 0.901 | 2.356 | 4.004 | 1.552 | 0.803 | 1.141 | 2.383 |
| Elaidic Acid (C18:1n9t) $ | 0.433 | 2.419 | 2.699 | 0 | 1.835 | 5.66 | 6.47 | 5.676 | 4.838 | 3.09 | 6.132 | 3.134 | 2.317 | 2.75 | 1.518 | 1.801 | 2.974 | 0.054 | 6.529 | 1.905 |
| Oleic Acid (C18:1n9c) $ | 0 | 0.573 | 0.445 | 0 | 0.418 | 1.586 | 0.727 | 1.013 | 2.293 | 1.249 | 0 | 0.909 | 0 | 0.54 | 0.628 | 0 | 0.69 | 0 | 0.92 | 0.476 |
| Linolelaidic Acid (C18:2n6t) | 0 | 0.009 | 0 | 0 | 0 | 0.011 | 0 | 0 | 0.075 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Linoleic Acid (C18:2n6c) $ | 0 | 1.376 | 0.825 | 0 | 0.354 | 3.435 | 1.914 | 1.925 | 3.077 | 3.256 | 0.493 | 1.754 | 1.158 | 1.26 | 0.576 | 0.88 | 2.198 | 0.054 | 1.232 | 0.873 |
| Γ-Linoleic Acid (C18:3n6) $ | 1.082 | 0.249 | 0.063 | 0.992 | 0 | 0.666 | 0.193 | 0.101 | 0.761 | 0.618 | 0.055 | 0.282 | 0 | 0.065 | 0.052 | 0.08 | 0.108 | 0.214 | 0.018 | 0.079 |
| Linolenic Acid (C18:3n3) $ | 0.433 | 1.502 | 1.206 | 0.099 | 0.225 | 3.33 | 2.417 | 3.14 | 3.761 | 6.24 | 0.328 | 1.879 | 1.029 | 1.93 | 0.575 | 1.2 | 1.788 | 0.267 | 0.331 | 0.714 |
| Arachidic Acid (C20:0) | 0 | 0.09 | 0.032 | 0 | 0.032 | 0.225 | 0.149 | 0.102 | 0.244 | 0.103 | 0 | 0.094 | 0 | 0.049 | 0 | 0 | 0.022 | 0 | 0 | 0 |
| Cis-9-Eicosenoic Acid (C20:1n11c) | 0 | 0.047 | 0 | 0 | 0 | 0.132 | 0.193 | 0.051 | 0.122 | 0.064 | 0 | 0 | 0 | 0.049 | 0 | 0 | 0.022 | 0 | 0 | 0 |
| Arachidonic Acid (C20:4n6) $ | 0 | 1.108 | 0.191 | 0 | 0.355 | 1.914 | 0.506 | 0.763 | 1.106 | 0.672 | 0.165 | 0.566 | 0.065 | 0.23 | 0.263 | 0.321 | 0.346 | 0 | 0.129 | 0.239 |
| Cis-11,14,17-Eicosatrienoic Acid (C20:3n3c) | 0 | 0.014 | 0 | 0 | 0 | 0.023 | 0.015 | 0 | 0.066 | 0.039 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cis-5,8,11,14,17-Eicosapentaenoic Acid (C20:5n3c) $ | 0.217 | 3.431 | 1.051 | 0 | 2.777 | 5.332 | 2.65 | 2.186 | 3.781 | 2.079 | 1.153 | 2.043 | 0.581 | 0.92 | 0.84 | 0.683 | 1.881 | 0.054 | 0.553 | 0.796 |
| Behenic Acid (C22:0) | 0 | 0.024 | 0 | 0 | 0 | 0.07 | 0.045 | 0 | 0.301 | 0.013 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Erucic Acid (C22:1n9) | 0 | 0.009 | 0 | 0 | 0 | 0.029 | 0.015 | 0 | 0.033 | 0.013 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lignoceric Acid (C24:0) | 0 | 0 | 0 | 0 | 0 | 0 | 0.015 | 0 | 0.005 | 0 | 0 | 0.032 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cis-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6n3c) $ | 0 | 6.49 | 0.959 | 0 | 3.176 | 7.689 | 3.242 | 1.99 | 8.737 | 7.493 | 1.544 | 1.862 | 0.583 | 0.61 | 1.107 | 0.403 | 2.952 | 0 | 0.87 | 0.64 |
| Nervonic Acid (C24:1n9) | 0 | 0.071 | 0 | 0 | 0 | 0.081 | 0 | 0 | 0.071 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12-Methyltetradecanoate (a-15:0) | 0 | 0.135 | 0.158 | 0 | 0.064 | 0.006 | 0.133 | 0.352 | 0.148 | 0 | 0.054 | 0.187 | 0.255 | 0.162 | 0.156 | 0.278 | 0.107 | 0 | 0.055 | 0.158 |
| 14-Methylpentadecanoate (i-16:0) | 0 | 0.028 | 0.032 | 0 | 0 | 0.051 | 0.03 | 0.05 | 0.098 | 0.128 | 0 | 0.031 | 0 | 0.049 | 0 | 0.08 | 0.043 | 0 | 0.018 | 0 |
| 15-Methylhexadecanoate (i-17:0) | 0 | 0.056 | 0 | 0 | 0.032 | 0.115 | 0.03 | 0.101 | 0.163 | 0.103 | 0 | 0.063 | 0 | 0.049 | 0 | 0.04 | 0.043 | 0 | 0.018 | 0 |
| 2-Hydroxytetradecanoate (2-OH 14:0) | 0 | 0.042 | 0 | 0 | 0 | 0.12 | 0 | 0 | 0.088 | 0.038 | 0 | 0.062 | 0 | 0 | 0 | 0.04 | 0.086 | 0 | 0 | 0 |
| Stearidonic Acid (C18:4n3) $ | 0.649 | 2.328 | 0.983 | 0.099 | 0.193 | 4.885 | 1.645 | 3.898 | 7.119 | 5.62 | 0.656 | 3.882 | 1.543 | 2.371 | 1.046 | 1.32 | 1.227 | 0 | 0.533 | 1.586 |
| 2-Hydroxyhexadecanoate (2-OH 16:0) | 0 | 0 | 0.063 | 0 | 0 | 0 | 0 | 0.354 | 0 | 0 | 0.055 | 0.282 | 0.129 | 0.229 | 0.105 | 0.12 | 0 | 0 | 0.037 | 0.159 |

**Table 3S.** Total fatty acid composition of seston (µg mg$^{-1}$) from the 7 ponds involved in the fish-stocking pond experiment study in late June 2015 and in early September in 2015 at Cape Race NL (Canada). The three ponds that were stocked with YOY brook trout are indicated by #. White shading corresponds to late June sampling prior to fish stocking and grey shading corresponds to early September sampling, following two-months of fish exposure in the stocked ponds. Specific seston fatty acids selected for the statistical analysis of the fish stocking experiment are indicated ($), selected based on a synthesis of the fatty acid literature by Grosbois (2017). Fatty acid concentrations that were below analytical detection limits are indicated by n.a.

| **PONDS** | **B** | | **BR4** | | **P2#** | | **NR2** | | **P1#** | | **OB6** | | **OS6** | | **OS9** | | **P3#** | | **WR1** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SESTON FATTY ACIDS** | | | | | | | | | | | | | | | | | | | | |
| Myristic acid (C14:0) | n.a | 0.052 | 1.225 | 0.744 | 2.020 | 2.158 | 1.237 | 0.837 | 4.085 | 1.125 | 0.000 | 0.349 | 1.922 | n.a | 0.838 | 3.049 | n.a | 0.057 | 4.058 | 0.585 |
| Pentadecanoic Acid (C15 :0) | n.a | 0.000 | 0.199 | 0.280 | 0.218 | 0.427 | 0.370 | 0.232 | 0.171 | 0.186 | 0.000 | 0.047 | 0.255 | n.a | 0.246 | 1.804 | n.a | 0.018 | 0.165 | 0.210 |
| Palmitic Acid (C16:0) $ | n.a | 0.000 | 5.490 | 3.796 | 3.576 | 7.157 | 6.204 | 4.292 | 3.777 | 3.219 | 1.131 | 1.233 | 4.617 | n.a | 2.930 | 27.027 | n.a | 0.306 | 4.048 | 2.722 |
| Palmitoleic Acid (C16:1n7) | n.a | 3.203 | 2.388 | 0.961 | 1.157 | 1.620 | 1.507 | 1.153 | 1.661 | 0.945 | 2.262 | 0.578 | 1.412 | n.a | 0.967 | 3.382 | n.a | 0.245 | 1.739 | 0.967 |
| Heptadecanoic Acid (C17:0) | n.a | 0.000 | 0.076 | 0.094 | 0.000 | 0.000 | 0.000 | 0.100 | 0.052 | 0.000 | 0.000 | 0.010 | 0.095 | n.a | 0.000 | 0.631 | n.a | 0.000 | 0.059 | 0.074 |
| Cis-10-Heptadecenoic Acid (C17:1n7c) | n.a | 0.000 | 0.019 | 0.009 | 0.000 | 0.000 | 0.022 | 0.033 | 0.026 | 0.021 | 0.000 | 0.000 | 0.000 | n.a | 0.000 | 0.000 | n.a | 0.000 | 0.012 | 0.011 |
| Stearic Acid (C18:0) | n.a | 0.131 | 0.820 | 1.583 | 1.047 | 3.074 | 1.120 | 1.783 | 0.585 | 0.814 | 0.379 | 0.248 | 1.220 | n.a | 1.058 | 11.745 | n.a | 0.093 | 0.904 | 0.792 |
| Elaidic Acid (C18:1n9t) $ | n.a | 0.707 | 3.592 | 2.215 | 1.719 | 2.059 | 2.788 | 7.521 | 1.558 | 1.377 | 0.433 | 0.829 | 2.277 | n.a | 1.057 | 14.073 | n.a | 0.136 | 3.045 | 1.331 |
| Oleic Acid (C18:1n9c) $ | n.a | 0.000 | 0.562 | 0.265 | 0.427 | 0.768 | 0.000 | 0.680 | 0.000 | 0.970 | 0.000 | 0.000 | 0.391 | n.a | 0.286 | 0.000 | n.a | 0.064 | 0.000 | 0.000 |
| Linolelaidic Acid (C18:2n6t) | n.a | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | n.a | 0.000 | 0.000 | n.a | 0.000 | 0.000 | 0.000 |
| Linoleic Acid (C18:2n6c) $ | n.a | 0.000 | 0.829 | 0.419 | 0.659 | 0.584 | 0.549 | 1.058 | 0.637 | 0.542 | 0.000 | 0.152 | 0.724 | n.a | 0.276 | 1.897 | n.a | 0.039 | 1.034 | 0.443 |
| Γ-Linoleic Acid (C18:3n6) $ | n.a | 0.026 | 0.019 | 0.009 | 0.052 | 0.000 | 0.066 | 0.011 | 0.034 | 0.052 | 0.054 | 0.029 | 0.029 | n.a | 0.038 | 0.119 | n.a | 0.000 | 0.048 | 0.042 |
| Linolenic Acid (C18:3n3) $ | n.a | 0.000 | 1.980 | 0.436 | 0.233 | 0.215 | 0.877 | 0.122 | 0.430 | 0.907 | 0.072 | 0.162 | 1.000 | n.a | 0.114 | 1.067 | n.a | 0.043 | 0.487 | 0.201 |
| Arachidic Acid (C20:0) | n.a | 0.000 | 0.440 | 0.112 | 0.052 | 0.123 | 0.088 | 0.045 | 0.026 | 0.073 | 0.000 | 0.000 | 0.144 | n.a | 0.057 | 0.199 | n.a | 0.007 | 0.048 | 0.042 |
| Cis-9-Eicosenoic Acid (C20:1n11c) | n.a | 0.000 | 0.095 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.021 | 0.000 | 0.000 | 0.038 | n.a | 0.000 | 0.000 | n.a | 0.000 | 0.000 | 0.000 |
| Arachidonic Acid (C20:4n6) $ | n.a | 0.000 | 0.019 | 0.009 | 0.026 | 0.093 | 0.000 | 0.022 | 0.026 | 0.031 | 0.000 | 0.000 | 0.048 | n.a | 0.019 | 0.040 | n.a | 0.000 | 0.048 | 0.021 |
| Cis-11,14,17-Eicosatrienoic Acid (C20:3n3c) | n.a | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | n.a | 0.000 | 0.000 | n.a | 0.000 | 0.000 | 0.000 |
| Cis-5,8,11,14,17-Eicosapentaenoic Acid (C20:5n3c) $ | n.a | 0.000 | 0.086 | 0.026 | 0.091 | 0.092 | 0.044 | 0.022 | 0.121 | 0.031 | 0.000 | 0.038 | 0.153 | n.a | 0.038 | 0.040 | n.a | 0.007 | 0.322 | 0.053 |
| Behenic Acid (C22:0) | n.a | 0.000 | 0.653 | 0.233 | 0.013 | 0.062 | 0.044 | 0.045 | 0.000 | 0.074 | 0.000 | 0.000 | 0.067 | n.a | 0.029 | 0.120 | n.a | 0.007 | 0.012 | 0.032 |
| Erucic Acid (C22:1n9) | n.a | 0.000 | 0.086 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | n.a | 0.000 | 0.000 | n.a | 0.000 | 0.000 | 0.000 |
| Lignoceric Acid (C24:0) | n.a | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | n.a | 0.000 | 0.000 | n.a | 0.000 | 0.000 | 0.000 |
| Cis-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6n3c) $ | n.a | 0.000 | 0.038 | 0.017 | 0.052 | 0.062 | 0.022 | 0.022 | 0.000 | 0.063 | 0.000 | 0.010 | 0.125 | n.a | 0.010 | 0.000 | n.a | 0.004 | 0.287 | 0.021 |
| Nervonic Acid (C24:1n9) | n.a | 0.000 | 0.000 | 0.000 | 0.013 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.010 | n.a | 0.000 | 0.000 | n.a | 0.000 | 0.012 | 0.000 |
| 12-Methyltetradecanoate (a-15:0) | n.a | 0.000 | 0.057 | 0.068 | 0.026 | 0.122 | 0.044 | 0.044 | 0.085 | 0.041 | 0.286 | 0.019 | 0.047 | n.a | 0.047 | 0.353 | n.a | 0.014 | 0.000 | 0.052 |
| 14-Methylpentadecanoate (i-16:0) | n.a | 0.000 | 0.076 | 0.119 | 0.064 | 0.122 | 0.109 | 0.100 | 0.069 | 0.114 | 0.000 | 0.009 | 0.066 | n.a | 0.085 | 0.708 | n.a | 0.007 | 0.047 | 0.095 |
| 15-Methylhexadecanoate (i-17:0) | n.a | 0.000 | 0.010 | 0.017 | 0.013 | 0.000 | 0.000 | 0.011 | 0.009 | 0.021 | 0.000 | 0.000 | 0.010 | n.a | 0.010 | 0.039 | n.a | 0.004 | 0.012 | 0.011 |
| 2-Hydroxytetradecanoate (2-OH 14:0) | n.a | 0.000 | 0.000 | 0.008 | 0.000 | 0.031 | 0.000 | 0.011 | 0.009 | 0.010 | 0.000 | 0.000 | 0.009 | n.a | 0.009 | 0.039 | n.a | 0.000 | 0.000 | 0.010 |
| Stearidonic Acid (C18:4n3) $ | n.a | 0.000 | 0.847 | 0.290 | 0.607 | 0.645 | 0.263 | 0.189 | 1.419 | 0.636 | 0.000 | 0.152 | 1.066 | n.a | 0.181 | 0.197 | n.a | 0.029 | 1.628 | 0.264 |
| 2-Hydroxyhexadecanoate (2-OH 16:0) | n.a | 0.000 | 0.086 | 0.026 | 0.065 | 0.061 | 0.022 | 0.011 | 0.146 | 0.052 | 0.000 | 0.019 | 0.105 | n.a | 0.019 | 0.039 | n.a | 0.000 | 0.166 | 0.032 |

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