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The role of intrinsic and extrinsic factors in shaping alternative migratory tactics and metabolic phenotypes in brown trout

Thesis presented by

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, at either University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Louise Clair Archer

Thesis summary

Variation among and within populations accounts for a considerable portion of phenotypic diversity produced in nature, and is instrumental to the structure and function of ecosystems. Understanding how, and why, intraspecific diversity persists is essential for predicting and managing the effects of global change, particularly because intraspecific variation may mediate diverse responses to changes in the environment. Alternative phenotypes – i.e. discrete phenotypic variation – can arise from a combination of proximate and ultimate mechanisms. Proximate mechanisms reflect how environmental factors shape phenotypic variation via intermediate physiological processes, which can themselves vary and be decomposed into genetic *versus* environmental components. Ultimate mechanisms concern the evolutionary function of a given phenotype. In this thesis, I explore how proximate and ultimate factors contribute to a particularly striking example of intraspecific diversity: alternative migratory tactics in brown trout *Salmo trutta*.

Brown trout are iconic for the variety of migratory life histories they exhibit; yet fundamental knowledge gaps remain regarding how environmental, physiological, and genetic factors integrate to underpin life history decisions among and within populations. In Chapter 2, I assessed how food restriction and population background influences the expression of migratory tactics in offspring from two populations that naturally differ in anadromy (i.e. sea-migration). Food restriction affected traits related to size and condition, and resulted in a higher frequency of anadromy in both populations, though populations varied in their responses according to the timing of food restriction treatments. While anadromy was overall more frequent in offspring from the naturally anadromous population, the expression of anadromous phenotypes in offspring from a non-anadromous population indicated that migratory tactics might emerge in response to unfavourable environmental conditions causing energetic limitation. In Chapter 3, I further considered proximate mechanisms by exploring how multiple environmental factors (food and temperature)

influence migration. Antagonistic effects of food restriction and increased temperature on condition and size-related traits were not translated at the level of migration tactics, where effects of food restriction and temperature were additive, but opposing (food restriction increased anadromy, whereas temperature decreased anadromy).

I explored how components of metabolic rate – a fundamental determinant of physiological status – varied according to food restriction and population background in Chapter 4. Standard metabolic rate (SMR) was lower in food-deprived fish, while SMR, maximum metabolic rate, and aerobic scope (AS) were higher in offspring from a naturally anadromous population compared to a non-anadromous population. Population-specific effects of food restriction on AS also emerged. I further addressed the causes and consequences of metabolic rate variation in Chapter 5, where I found metabolic traits varied according to both population background and temperature, with important consequences for growth rates (a key fitness-related trait that can shape life histories).

Collectively, these results contribute to our knowledge of how environmental and genetic factors underpin life-history diversity in terms of migratory tactics and physiology. Changes in environmental conditions will likely alter patterns of life-history diversity (mediated by changes in individual physiology) in ways that will also depend on population-specific factors. While predicting the impacts of multi-faceted environmental change will be complex, knowledge of the links between physiology, environment, and ultimately, life history, is crucial for conserving important biodiversity within brown trout, a species that is already in widespread decline due to pervasive global change.

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Thesis structure

The data chapters within this thesis are intended for publication in peer-reviewed journals and are thus written and formatted as standalone manuscripts. For ease of reading, figures and tables are embedded within the text and chapters are cross-referenced where appropriate, though each chapter can be read in isolation. I have indicated where chapters have already been submitted or published in peer-reviewed journals, and author contributions are listed at the beginning of each chapter.

Chapter 1

General Introduction

Much of the widespread diversity we observe in nature can be attributed to intra-specific phenotypic variation (Roff 1996). This intra-specific variation can be either continuous in kind, or discontinuous, i.e. discrete variation in morphological, behavioural or life history traits referred to as 'alternative phenotypes'. Alternative phenotypes are often underpinned by developmental plasticity as polyphenisms (Suzuki and Nijhout 2008), and have attracted considerable attention in evolutionary biology, *e.g.* in the context of speciation, or evolution by genetic accommodation (West-Eberhard 2003; Crispo 2007; Oliveira *et al.* 2008). Alternative phenotypes also play key roles in community ecology (Bolnick *et al.* 2011) and have applied relevance for conservation and ecosystem management (Naish and Hard 2008).

A fundamental goal of evolutionary ecology is to determine the relationship between ecologically relevant phenotypic variation and underlying genetic variation, and in doing so understand how and why divergent phenotypes and life histories arise and persist (Debat and David 2001). This challenge is especially important in the context of pervasive global change, where predicting the outcomes of environmental change on natural populations is made more complex by the occurrence of alternative phenotypes. Phenotypic variation among individuals and populations may mediate diverse responses to environmental change, and understanding the effects of phenotypic diversity is crucial to successful management and conservation of natural populations (Schindler *et al.* 2010, 2015).

The phenomenon of facultative migration

Considerable phenotypic diversity can stem from migration, a spectacular, yet relatively common phenomenon evident in all major animal taxa (Swingland and Greenwood 1984). Optimal breeding and feeding habitats are often

separated in space and time, and thus migration is a pivotal factor shaping the temporal and spatial distribution of animals, with important consequences for ecosystem function and structure (Polis *et al.* 2004; Janetski *et al.* 2009). In addition to considerable inter-specific variation in migratory patterns, encompassing obligate migratory and non-migratory lifestyles, strikingly different migratory phenotypes are also evident within species (Dingle and Drake 2007), with migration usually occurring at specific life stages to exploit alternative foraging opportunities or to avoid unfavourable abiotic conditions (Chapman *et al.* 2011a). Intraspecific variation in migratory tendency can span populations that may be entirely migratory, others that may be solely resident, and others again that may be "partially migratory" and comprise a mix of migratory and resident phenotypes (Lack 1943; Lundberg 1987; Kaitala *et al.* 1993; Chapman *et al.* 2011a). Alternative phenotypes among and within populations might be determined purely by genetics, plasticity, or reflect a mix of genetic and environmental factors, thus appearing somewhat "flexible". Facultative migration – where individuals show flexibility in tactic expression – is common among taxa (Chapman *et al.* 2011c, b), with well documented examples in birds (Lundberg 1988; Pulido *et al.* 1996; Newton 2008), ungulates (Ball *et al.* 2001; Cagnacci *et al.* 2011; Hebblewhite and Merrill 2011), zooplankton (Hansson and Hylander 2009), and fishes (Chapman *et al.* 2012; Dodson *et al.* 2013). However, despite the widespread occurrence of facultative migration, and its importance in ecosystem processes (Brodersen *et al.* 2008), the causes and consequences of the migration *versus* residency decision remain largely unresolved (Dingle and Drake 2007; Pulido 2011).

Proximate and ultimate drivers of migration

The origin and persistence of intra-specific variation in migratory behaviour can be understood in terms of proximate and ultimate factors. Ultimate factors refer to the evolutionary reasons for, or phylogenetic patterns underpinning, migration, whereas proximate factors reflect how migration is triggered in response to environmental cues and the ontogeny of its expression. The proximate mechanisms underpinning migration are themselves evolvable, and

may be subject to selection in response to environmental factors. As such, alternative migratory phenotypes are likely underpinned by a complex genotype to phenotype mapping, where similar phenotypes can arise from different genotypes, or the same genotype can produce dramatically different phenotypes, via plasticity mediated by environmental cues (Via *et al.* 1995).

Facultative migration is often considered as a threshold trait (Roff 1996). As such, environmentally-triggered alternative migratory "tactics" are produced by a conditional strategy, where the optimal tactic for a given environment is conditional on intrinsic or extrinsic cues. Under the framework of the "environmentally cued threshold model" (Tomkins and Hazel 2007), expression of alternative tactics is determined by the relationship between an environmentally-sensitive "status" trait (that may itself be influenced by genes *e.g.* physiological condition, or energy status) and a genetically variable threshold for said status trait. The inherited threshold can be thought of as a switch point. If an individual's status trait exceeds the switch point during an assessment period (or "decision window"), it remains resident and undergoes maturation. If the threshold for residency is not met, a migratory trajectory is adopted (though the timing of actual migration may be controlled by similar threshold mechanisms). Since environmental conditions are assumed to strongly influence potential status traits, environmentally-induced variation in the status trait may cause alternative migratory tactic to arise from similar genetic thresholds via life-history plasticity. The context-dependent status trait can thus be considered as an environmental "cue", related to the expression of alternative tactics by a "threshold reaction norm" (Tomkins and Hazel 2007; Piche *et al.* 2008; Pulido 2011; Buoro *et al.* 2012). Alternatively, since the underlying threshold is evolvable (*i.e.* an ultimate mechanism), population or individual-level genetic variation in the threshold may result in similar environments producing different migratory phenotypes, as shown in blackcaps *Sylvia atricapilla* (Pulido *et al.* 1996) and Atlantic salmon *Salmo salar* (Piche *et al.* 2008).

The environmentally cued threshold model provides a useful framework for understanding how the frequency of migration *versus* residency for a given population depends on both the distribution of the status trait, and the distribution of the threshold values in the population (Tomkins and Hazel 2007). The prevalence of a given migratory phenotype can vary in the short term in response to environmental conditions, and can shift over longer periods through evolution of the underlying threshold (Piche *et al.* 2008). In practice, however, it is not yet clear how environmental variation is translated into internal physiological signals, upon which individuals then base migratory decisions. Understanding the proximate determinants of phenotypic diversity i.e. how genetic and environmental factors interactively shape life histories via intermediary physiological processes, is thus the overarching theme of this thesis.

Overview of life history variation in salmonines

Fishes present many interesting examples of facultative migration, offering valuable opportunities to gain insight into the mechanisms underlying alternative migratory tactics (AMTs) (Jonsson and Jonsson 1993; Chapman *et al.* 2012). Salmonines (salmons, trouts, and charrs) in particular display a multitude of diverse migratory life histories that vary among populations and individuals in terms of migration propensity, migration distance, and migration destination (Dodson *et al.* 2013; Sloat *et al.* 2014; Kendall *et al.* 2014; Ferguson *et al.* 2019). Alternative reproductive tactics (ARTs) within male salmonines (*e.g.* the sneaker versus 'bourgeois' tactics) represent another interesting class of alternative phenotypes that are conceptually distinct from, but nonetheless related to, AMTs (Gross 1985, 1991; Hutchings and Myers 1994; Fleming 1996). While freshwater spawning is obligate, a continuum of migration tactics is seen in salmonines, encompassing individuals that remain in natal streams/lakes for their entire life cycles (residency), to those that migrate to larger rivers (fluvial-adfluvial migration), lakes (potomodromy), or to marine environments (anadromy) before returning to spawn in natal freshwater systems (Klemetsen *et al.* 2003; Ferguson *et al.* 2019; Nevoux *et al.* 2019). In several salmonines

(including brown trout *Salmo trutta*, the focal species of this thesis), populations may be dominated by either resident or migratory forms, but may also comprise a mixture of migratory phenotypes that can breed freely in sympatry (Chapman *et al.* 2012). Despite the considerable socioeconomic and cultural importance of various migratory phenotypes, (*e.g.* as a valued angling resource or a component of biodiversity), and their pivotal role in aquatic ecosystem dynamics (Naiman *et al.* 2002), the drivers of alternative life histories in salmonines remain largely unresolved (Harris and Milner 2008; Harris 2017). Such fundamental knowledge gaps limit our ability to manage and conserve these iconic species, which are in widespread decline due to anthropogenic pressures, most notably global change, in-stream barriers, and the development of aquaculture (Limburg and Waldman 2009).

The threshold reaction norm concept has been applied to understand migration decisions (or ARTs) in salmonines because their life histories appear largely compatible with the model framework (Hutchings and Myers 1994; Thorpe *et al.* 1998; Thériault *et al.* 2007). While offspring tend to show similar migratory tactics to their parents, either migratory phenotype can be produced from a given parental life history (Zimmerman and Reeves 2000; Berejikian *et al.* 2014). Since mounting evidence supports migration as a trait interactively determined by genotype and environment, i.e. under genotype-by-environmental control (Hutchings 2011), research has focused on identifying potential status traits, with inconclusive results. Previous studies have linked numerous aspects of physiological condition to migration tactics with equivocal support, including: body size (Thériault and Dodson 2003), body condition (Hecht *et al.* 2015), growth rates (Jonsson 1985), growth efficiency (Forseth *et al.* 1999; Morinville and Rasmussen 2003), metabolic rates (Sloat and Reeves 2014), and lipid stores (Jonsson and Jonsson 2005). Furthermore, the associations between these physiological traits and migration tendency have also been inconsistent. For example, fast growth and larger sizes have been both positively (Jonsson 1985; Acolas *et al.* 2012), and negatively (Morinville and Rasmussen 2003; McMillan *et al.* 2012) related to migration whilst others have

found no relationship (at a given age) (Thériault and Dodson 2003), or evidence for population-specific responses (Jonsson 1985).

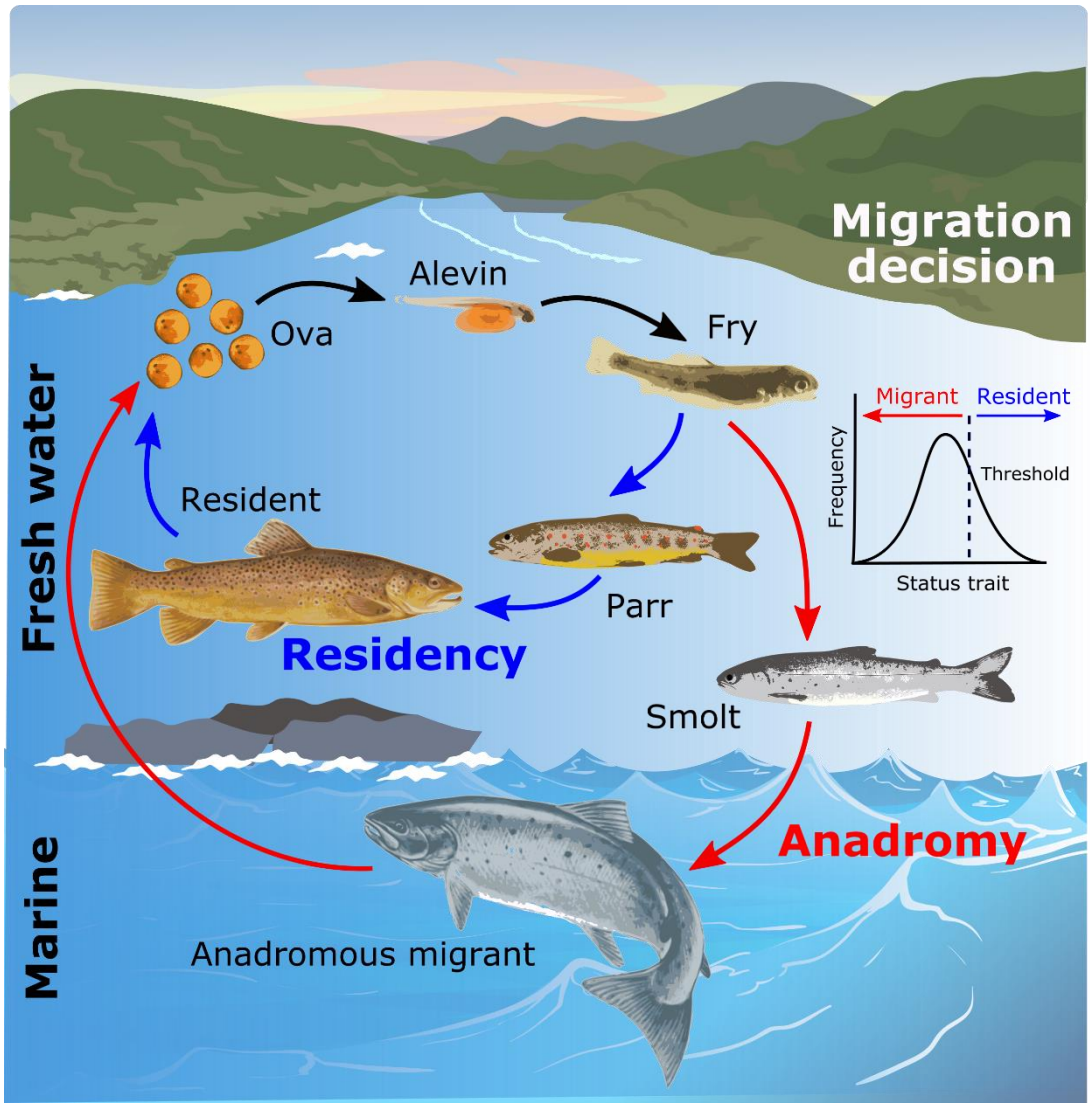

Figure 1: Overview of alternative migratory tactics in brown trout, with an andromous life history highlighted in red, and a resident life history in blue. The inset shows the threshold mechanism underpinning migration decisions. A normal distribution of status trait values in a given environment is shown for a hypothetical population that displays faculatative anadromy, with the mean threshold for residency represented by the dashed line.

Inconsistent results could occur if there are discrepancies in the timing of trait
155 measurements (often occurring around the time of migration) and when the

migration *versus* residency decision is actually made. Migration often requires considerable morphological and physiological adjustments (Tanguy *et al.* 1994), meaning the migration decision window likely occurs many months in advance of the actual migration event. For example, future migrants must reach sufficient sizes to successfully transition to the marine environment, where survival is size-dependent (Phillis *et al.* 2016; Armstrong *et al.* 2018). Thus, pre-migrants often display accelerated growth in advance of the migration period (Metcalfe *et al.* 1995), while residents redirect energy reserves towards gonadal development and sexual maturation (Tocher 2003; Jonsson and Jonsson 2005). Fewer studies have explored *when* the migratory decision might occur, but it is increasingly clear that size at migration may not reflect size (or other aspects of physiological condition) at the time when the decision was made. Indeed, there is evidence that migrants and residents can differ in potential status traits including growth rates (Beakes *et al.* 2010), metabolic costs (Morinville and Rasmussen 2003), and condition (Hecht *et al.* 2015) up to a full year before migration takes place (Thorpe *et al.* 1998; Thorpe and Metcalfe 1998; Satterthwaite *et al.* 2009). The timing and nature of the decision window is largely unknown in brown trout (Ferguson *et al.* 2019), but is fundamental to our understanding of the pathways by which alternative tactics develop (Thorpe *et al.* 1998)

While identification of underlying status traits has proved somewhat inconclusive, understanding the fitness costs and benefits of migration *versus* residency may help to clarify the factors that promote different migratory tactics. Since food abundance is often higher in the migratory destination (larger rivers, lakes or the sea) compared to food-limited natal streams (Imre *et al.* 2005), migration might be favoured as an optimal tactic if increased growth rates are achieved in the new habitat, translating into higher fecundity and ultimately fitness (Brönmark *et al.* 2013). Such a scenario is supported by latitudinal clines in the prevalence of anadromy, where at high latitudes, higher productivity of the marine environment relative to freshwaters has been linked to the evolution of the anadromous life history (Gross *et al.* 1988). However, any

feeding benefits must be traded off against the significant costs associated with migration, including considerable energetic expenditure (Stefansson *et al.* 2003), physiological stress (Peiman *et al.* 2017; Birnie-Gauvin *et al.* 2017), and an increased risk of predation (Dieperink *et al.* 2002).

The balance between migratory costs and benefits is not necessarily equal among individuals, and is particularly likely to vary between the sexes (Hendry *et al.* 2004). While larger females have larger eggs and are more fecund (Fleming 1996; Quinn 2018), male reproductive success is limited by access to mates (Fleming 1998), which is less size dependent because the success of ARTs (*e.g.* "sneaker" versus bourgeois, anadromous males) can be similar (Hutchings and Myers 1988; Foote *et al.* 1997; Young *et al.* 2013). Females thus often receive greater fitness benefits from migration (mediated by larger sizes at maturity) than males, reflected by general trends of female-biased migration (Nielsen *et al.* 2003; Rundio *et al.* 2012; Ohms *et al.* 2013; García-Vega *et al.* 2018; Kelson *et al.* 2019).

This balance of trade-offs may show further variation due to intraspecific differences in energetic uptake and output, with accumulating evidence to suggest that migration occurs in response to energetic limitation in the natal environment (Forseth *et al.* 1999). The acquisition and allocation of energy resources in juveniles is strongly influenced by extrinsic environmental conditions, with the resulting individual energetic status (indicated by various potential status traits) determining life history trajectories in juveniles (Jonsson and Jonsson 1993). In particular, factors determining food availability have been much linked to the frequency of migrants (O'Neal and Stanford 2011; Jones *et al.* 2015), *e.g.* increased competition at high population densities has been shown to influence adfluvial migration in brown trout (Olsson *et al.* 2006; Wysujack *et al.* 2009). Temperature appears to be an important abiotic factor affecting migration (Kendall *et al.* 2014), and cooler temperatures have been found to increase maturation *in lieu* of migration in steelhead trout, perhaps due to changes in lipid deposition (Sloat and Reeves 2014). However, we currently lack a synthetic understanding of how variable environmental

conditions such as food and temperature might mediate migratory tactics among populations and individuals through effects on physiological condition or energetic status.

The importance of energy metabolism

As the fundamental process governing an individual's energy budget, metabolism is likely to be profoundly linked to variation in migratory phenotypes. Standard metabolic rate (SMR) refers to the minimum energy required to sustain life, i.e. the costs of homeostasis and tissue maintenance in an inactive, unstressed, non-digestive organism (termed basal metabolic rate (BMR) in endotherms) (Chabot *et al.* 2016). Once size and age are accounted for, SMR can vary as much as three-fold among individuals of the same population (Burton *et al.* 2011; Konarzewski and Książek 2013). Such individual differences in SMR are integrally linked to variation in energy acquisition and allocation, making it a trait of particular interest in studies of life-history variation (Forseth *et al.* 1999; Burton *et al.* 2011; Metcalfe *et al.* 2016). Variation in SMR has been generally linked to differences in lifestyle among fishes (Killen *et al.* 2010), and intraspecific variation appears to be similarly linked to life-history differences. For example, in brown trout, juveniles with relatively higher baseline energetic demands subsequently adopted migratory tactics (Forseth *et al.* 1999). Migrating Atlantic salmon *S. salar* smolts were found to have higher SMR values than non-smolts deferring migration to a later age (Seppänen *et al.* 2010), and those with the highest recorded SMR also tended to migrate at younger ages (McCarthy 2000). Similarly, lower SMR was linked to a higher probability of freshwater maturation rather than migration in steelhead trout *Oncorhynchus mykiss* (Sloat and Reeves 2014).

While SMR defines the minimum energy demands, or metabolic "floor", maximum metabolic rate (MMR) defines the upper limits of metabolism as the highest rate of aerobic metabolism (oxygen transport and ATP production) that can be achieved (Norin and Clark 2016). An individual's aerobic scope (AS) is bounded by SMR and MMR, and reflects the potential energy that can be directed towards key competing functions (*e.g.* activity/locomotion, growth,

digestion) once baseline energy requirements have been met (Guderley and Pörtner 2010). MMR and AS appear to show similar levels of intraspecific variation to SMR (Metcalfe *et al.* 2016), but the reasons behind this variation are somewhat unclear. Previous studies in salmonines have linked variation in MMR and AS to swimming performance (Tudorache *et al.* 2007) and migration distance and effort (Eliason *et al.* 2011), supporting it as a trait of ecological relevance in facultatively migratory species. As such, MMR and AS may have implications for life histories, and could contribute to the emergence and persistence of alternative phenotypes. However, despite their relevance for life history and phenotypic diversity (Metcalfe *et al.* 2016; Biro *et al.* 2018), MMR and AS are relatively under-researched traits in relation to facultative migration. We know surprisingly little about how variation in, or indeed covariation between, these metabolic traits relates to patterns of alternative migratory tactics.

Since metabolic traits are strongly influenced by environmental conditions such as temperature (Clarke and Johnston 1999; Brown *et al.* 2016), it is crucial to also consider environmental factors when investigating the causes and consequences of metabolic rate variation in facultatively migratory species. For example, although empirical evidence suggests SMR is positively associated with migration because of its influence on energy status (McCarthy 2000), this relationship may be context-dependent (Burton *et al.* 2011), *i.e.* energetic limitation may only occur at warm temperatures, or if there is insufficient food to meet metabolic needs. Additionally, individuals or populations may also differ in their metabolic responses to changing extrinsic conditions (Metcalfe *et al.* 2016; Norin and Metcalfe 2019), further complicating the links between metabolism, environment, and life history. Moreover, since metabolic rates are partly determined by genetics (Pettersen *et al.* 2018) and are thus evolvable (Wone *et al.* 2015; Sadowska *et al.* 2015), metabolic trait variation between migratory phenotypes may arise due to the demands of a given life history. For example, in populations that have a high incidence of migration (where the thresholds for residency have presumably evolved to be relatively high),

selection may favour higher metabolic rates that improve migration performance (Dalziel *et al.* 2012a, b).

While metabolic components are assumed to have important implications for fitness, and thus shape life histories, empirical evidence linking metabolism to fitness-rated traits has provided mixed results. For example, higher metabolic rates have been positively (McCarthy 2000) and negatively linked to growth rates (Norin and Malte 2011), whereas others again have found the optimal metabolic rate to vary depending on food availability (Reid *et al.* 2011; Auer *et al.* 2015a, b) and habitat (Reid *et al.* 2012). Thus, it seems increasingly likely that the benefits of a given metabolic phenotype may be context-specific, and dependant on environmental conditions, or intrinsic factors related to population background and life history (Álvarez and Nicieza 2005; Robertsen *et al.* 2014). Further investigation into the links between metabolic trait variation, environmental conditions, and fitness will help to illuminate how these factors integrate to shape life histories.

Objectives and overview of the thesis

The overarching objective of this thesis was thus to explore the nexus between physiology, environment, and alternative life history tactics in a facultatively migratory species. In Chapter 2, I specifically aimed to investigate how expression of migratory tactics is mediated by interactions between food availability and population background, and is underpinned by various aspects of physiological condition. I also explored whether the timing of food restriction influences migration, in order to better clarify when in early life the migratory decision might be undertaken, and to assess if populations varied in their responses to the timing of food restriction.

In Chapter 3, I extend the study of the proximate drivers of life history variation by exploring how co-occurring environmental factors collectively influence migratory tactics. Here, I aimed to test if food restriction and increased temperature interactively influence traits associated with physiological

condition, and to investigate whether these effects scale up to affect migratory tactics in a similar fashion, in a facultatively migratory population.

Chapter 4 explores how intrinsic and extrinsic factors influence variation in metabolic traits in facultatively migratory populations. Specifically, I aimed to assess (i) how SMR, MMR, and AS vary in brown trout offspring from two populations (that naturally differ in migration tendency); and (ii) to test how metabolic traits respond to long-term conditions of food restriction. I also explored if metabolic responses to food restriction are dependent on population background and sex.

The objectives of Chapter 5 were to explore the causes and consequences of variation in metabolic traits in response to increased temperature. Specifically, I aimed to assess whether populations that differ in migratory tactics might also show variable metabolic responses to temperature increases. I then explored the implications of metabolic trait variation (in terms of differences between populations, and variation within populations driven by plastic responses to warming) for growth rates, a key-fitness related trait.

Chapter Six synthesises the results of the studies described above, and discusses their contribution towards developing an integrated understanding of the links between physiology, environment, and life history.

Additional reseach

In addition to the chapters presented in this thesis, I have also been involved in the following research during my studies:

Archer LC, Sohlström EH, Gallo B, Jochum M, Woodward G, Kordas RL, Rall BC, and O'Gorman EJ. 2019. Consistent temperature dependence of functional response parameters and their use in predicting population abundance. *Journal of Animal Ecology* **In Press**. https://doi.org/10.1111/1365-2656.13060.

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Chapter 2

The interplay between extrinsic and intrinsic factors in determining migration decisions in brown trout (*Salmo trutta*): An experimental study

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Abstract

Many species are capable of facultative migration, but the relative roles of extrinsic versus intrinsic factors in generating diverse migratory tactics remain unclear. Here we explore the proximate drivers of facultative migration in brown trout in an experimental laboratory setting. The effects of reduced food, as a putative environmental cue, were examined in two populations: one that exhibits high rates of anadromy (sea-migration) in nature, and one that does not exhibit anadromy in nature. Juveniles derived from wild-caught parents were reared for two years under four environmental treatments: low food in years 1 and 2 (Low-Low); high food in years 1 and 2 (High-High), low food in year 1 and high in year 2 (Low-High), and vice versa (High-Low). Food restriction had a significant effect on migratory tactics, with the frequency of smolts (juveniles choosing migration) highest in the Low-Low treatment in both populations. No individuals became smolts in the High-High treatment, and intermediate smolting rates were observed in the Low-High and High-Low treatments. Higher overall smolting rates in the naturally anadromous population suggested an inherited component to anadromy/migration decisions, but both populations showed variability in migratory tactics. Importantly, some fish from the naturally non-anadromous population became smolts in the experiment, implying the capacity for migration was lying 'dormant', but they exhibited lower hypo-osmoregulatory function than smolts from the naturally anadromous population. Tactic frequencies in the naturally anadromous population were more affected by food in the 2nd year, while food in the 1st year appeared more important for the naturally non-anadromous population. Migratory tactics were also related to sex, but underpinned in both sexes by growth in key periods, size and energetic state. Collectively these results reveal how migration decisions are shaped by a complex interplay between extrinsic and intrinsic factors, informing our ability to predict how facultatively migratory populations will respond to environmental change.

Introduction

Intraspecific phenotypic variation accounts for much of the diversity of form and function in nature (Roff 1996). Understanding the mechanisms generating and maintaining divergent phenotypes and life histories within and among populations is thus a fundamental goal of evolutionary ecology, with applied relevance to conservation and wildlife management (Naish and Hard 2008). A particularly striking example of alternative phenotypes is the phenomenon of facultative migration, whereby individuals within a population vary in their migratory tendencies. Facultatively migratory populations can comprise a mixture of migrant and resident individuals (sometimes called 'partial migration'), with migration at specific life stages occurring typically to take advantage of alternative foraging opportunities or avoid adverse abiotic (*e.g.* climatic) conditions (Chapman *et al.* 2011a). Despite its widespread occurrence across taxa and regions, fundamental gaps still exist in our understanding of proximate and ultimate drivers of facultative migration. In particular, there is a dearth of studies addressing how facultatively migratory species respond to environmental change (Doswald *et al.* 2009; Chapman *et al.* 2011b), limiting our ability to generalise about the impacts of anthropogenic factors on migratory species and to effectively manage their populations.

Polymorphisms such as facultative migration are potentially underpinned by a complex mapping between genotype and phenotype, i.e. phenotypic similarity can arise from different genotypes, or the same genotypes can produce dramatically different phenotypes through plasticity mediated by environmental cues (Roff 1996). As such, migration and residency have often been considered as environmentally-triggered alternative phenotypes/tactics produced by an evolvable conditional strategy, where optimal tactic choice in a given context is conditional on extrinsic or intrinsic cues (Chapman *et al.* 2011b). This interplay between proximate and ultimate drivers of conditional strategies has been formalised as the so-called 'environmentally cued threshold model' (Tomkins and Hazel 2007). Within this framework, alternative tactics are controlled by an environmentally-sensitive status trait (*e.g.* physiological

condition, energy state) and an inherited threshold, or 'switch point', which is assumed to be genetically variable. An individual assesses their status trait and, for example, adopts a resident tactic if it exceeds their inherited switch point, otherwise it switches to a migratory tactic. Individual physiological condition/energy state is strongly influenced by the environment, and so the assessed status trait can vary relative to the intrinsic threshold depending on external conditions; for this reason, the status trait can be thought of as an 'environmental cue' and the step function relating tactic expression to cue as a 'threshold reaction norm' (Tomkins and Hazel 2007; Piche *et al.* 2008; Pulido 2011; Buoro *et al.* 2012). There is some evidence for genetic variation in thresholds for alternative tactics, *e.g.*, in blackcaps *Sylvia atricapilla* (Pulido *et al.* 1996) and Atlantic salmon *Salmo salar* (Piche *et al.* 2008), but detailed understanding of how external environmental variation is translated into internal physiological signals, on which migratory decisions are then based, is lacking.

Salmonine fishes (salmons, trouts and charrs) are excellent models for disentangling causes of facultative migration as they display wide variation across a continuum of migratory strategies, coupled with obligate freshwater spawning (Klemetsen *et al.* 2003; Ferguson *et al.* 2019). Individuals can remain in freshwater post hatching for their entire life cycle, either staying in their natal stream or lake (residency tactic) or undertaking an adfluvial migration that takes them to a larger river or lake (potamodromous tactic) (Dodson *et al.* 2013; Ferguson *et al.* 2019). Facultative anadromy is an extreme form of this conditional migration strategy, where some individuals adopt the residency tactic whilst others from the same population undertake a marine migration (involving anywhere from tens to thousands of kilometres of directed movement between freshwater and saltwater). This is followed by a period of marine or estuarine feeding and growth (from months to years), before returning to spawn in natal streams (Jonsson and Jonsson 1993). Populations can contain both resident and migratory (anadromous or potamodromous) forms, or be dominated by one life history type (Chapman *et al.* 2012). Both

forms can breed freely in sympatry, and although offspring tend to track the tactics of their parents, either life history can be produced from a given migratory phenotype (Zimmerman and Reeves 2000; Berejikian *et al.* 2014). Such flexibility indicates an interplay between genetic predisposition and environmental conditions experienced i.e., genotype by environment interactions, underpinning facultative migration (Hutchings 2011).

The threshold reaction norm framework has been useful in understanding migratory decisions in salmonines (Hutchings and Myers 1994; Thorpe *et al.* 1998; Thériault *et al.* 2007). If during a key decision window, an individual's status trait exceeds their predetermined threshold, the fish adopts a residency tactic leading to maturation in freshwater; if not, maturation is deferred in favour of migration (Dodson *et al.* 2013; Kendall *et al.* 2014; Ferguson *et al.* 2017). However, the proximate factors on which individuals base the migration decision remain unclear. Previous studies have focused on a range of aspects of physiological state/energy status that may influence migratory tactics such as body size (Thériault and Dodson 2003), lipid reserves (Jonsson and Jonsson 2005), body condition (Hecht *et al.* 2015), growth (Jonsson 1985), growth efficiency (Forseth *et al.* 1999; Morinville and Rasmussen 2003), and metabolism (Sloat and Reeves 2014). While body size is often used as a surrogate for, or argued to itself be, the status trait triggering alternative migratory tactics, the associations here have been varied and inconclusive. Larger sizes and faster growth rates have been associated with early age at migration (Jonsson 1985), whereas others have found no size-based differences between migrants and non-migrants at a given age (Thériault and Dodson 2003), or conversely found larger sizes (and higher lipid reserves) to be associated with freshwater maturation *in lieu* of anadromy (McMillan *et al.* 2012). These inconsistencies could reflect species' specific responses, and thus require further exploration to establish potential status traits for a given species. Studies might also be inconclusive because size is typically measured sometime after the migratory decision itself, perhaps at the parr-to-smolt transformation stage, and size at migration may not accurately reflect size when the decision

was made. For example, residents may have meanwhile diverted energy into maturation and gonadal development at the expense of somatic growth (Tocher 2003), while migrants may undergo accelerated growth as the migration itself approaches (Metcalfe 1998).

Moreover, there may be at least two separate threshold decisions: an early one determining whether a fish will migrate *per se* or not, and a later one determining whether fish on a migratory trajectory actually migrate this year or defer migration to an older age (Ferguson *et al.* 2019). Size may be the cue used for the second decision, given that survival on entry to the sea or a lake is typically positively related to size (Klemetsen *et al.* 2003; Phillis *et al.* 2016). Yet, size at the migration point may be unrelated to, or inconsistently related to, the status trait triggering the initial migration decision, which could occur considerably earlier than the point at which migrants and resident become phenotypically distinguishable (Beakes *et al.* 2010). Identifying the key proximate drivers of migration is therefore complicated by the fact that the exact time windows for each of these putative decisions may not be known *a priori*, while correlations among physiological, energy status and growth traits may be variable across ontogeny or contexts. In the particular case of facultative anadromy, sea-migration requires a suite of adjustments in preparation for life in saltwater and therefore the physiological remodelling process, which includes changes in osmoregulation, colouration, and body shape (Tanguy *et al.* 1994), is likely to begin sometime in advance of the migratory period. The existence of early 'decision windows' that initiate divergent life-history trajectories in salmonine fishes (Thorpe *et al.* 1998; Thorpe and Metcalfe 1998) has some empirical support; for example, body condition of anadromous *Oncorhynchus. mykiss* was found to be significantly lower than resident counterparts within a year of hatching and a full 12 months prior to emigration (Hecht *et al.* 2015).

Although the proximate drivers of migration in salmonines are unresolved, there is some consensus that potamodromous or anadromous migratory tactics are promoted by energetic limitation in natal rivers, which prevents fish

reaching the inherited physiological threshold for maturation as residents (Kendall *et al.* 2014). Energetic limitation can arise through an interplay between environmental factors and intrinsic physiological state; for example, if freshwater food resources are insufficient to support growth rates or metabolic demands, then migration could be triggered that takes the fish to a better feeding environment such as the sea or a large lake (O'Neal and Stanford 2011; Sloat and Reeves 2014; Jones *et al.* 2015). Food limitation arising from competition at high population densities has also been shown to increase the proportion of adfluvial migratory brown trout, whereas low population densities have been associated with residency and maturation (Olsson *et al.* 2006; Wysujack *et al.* 2009). It remains largely unknown, however, during which ontogenetic stages food limitation is most important to migration decisions.

Brown trout (*Salmo trutta*) are an interesting model for understanding facultative migration as they exhibit highly variable strategies, with some individuals/populations remaining resident in their natal stream their entire lives, while others migrate to a larger river, a lake, an estuary, or the sea (Jonsson and Jonsson 1993; Klemetsen *et al.* 2003; Cucherousset *et al.* 2005; Ferguson *et al.* 2019). Here we present the results of an experimental laboratory study of brown trout that involved F1 progeny of wild-caught parents from two populations that exhibit divergent migratory life-histories in nature. Our primary aim was to explore the interaction between intrinsic proximal factors (which may encompass both inherited and non-inherited variation) and the extrinsic environment in generating alternative migratory tactics in brown trout. Specifically, we aimed to: (i) assess the relative importance of food availability and inherited differences between populations in determining alternative migratory tactics; (ii) determine whether food restriction was more important in the first year or second year of freshwater rearing; (iii) test for differences between our two populations in their response to food restriction and its timing, which may be indicative of genotype-by-environment interactions influencing tactic frequencies, and (iv) explore associations

between status traits (length, weight, condition factor) and migratory tactics. We expected that food restriction would increase the frequency of the migratory tactic overall. While we expected migratory tactic frequencies to vary overall between fish from our two population backgrounds, we also anticipated that the naturally non-anadromous stock might produce migratory phenotypes when subjected to reduced food, given that migration may only be expressed under certain environmental conditions (Roff 1996; Pulido 2011).

Materials and methods

Study populations

Wild-origin brown trout brood stock were obtained by seine netting from the Burrishoole (53° 57′ N: 09° 35′ W) and Erriff (53° 37′ 0.00" N: 09° 40′ 17.10" W) catchments in the west of Ireland in November 2015. Burrishoole brood stock were caught in Lough Bunaveela (46 ha, Figure S1) in the headwaters of the catchment. A local population of non-anadromous trout remain resident in Lough Bunaveela for most of their lifecycle, bar very short-distance directed movements (on the order of 10s to 100s of metres) between the lake and two spawning rivers (one inflowing to the lake, the other outflowing). No obvious genetic structure at neutral microsatellite markers is evident between these spawning rivers, implying trout from Lough Bunaveela comprise a single panmictic population (R. Finlay, *pers. comm.*). A large run of sea trout (typically 2000+ anadromous recruits annually) occurred in the Burrishoole catchment up to 30 years ago. The Burrishoole anadromous trout run collapsed in the late 1980s, coinciding with sea-lice outbreaks following the establishment of salmon aquaculture farms in the downstream estuary. The exact spawning locations of the historic anadromous individuals within the Burrishoole catchment remain uncertain, and we cannot exclude the potential for some anadromous fish having contributed to the Bunaveela population before the anadromous population collapse. Nevertheless, despite Bunaveela spawning streams being accessible to anadromous migrants, there is little to no evidence that the Bunaveela population produced anadromous trout historically or recently

(Poole *et al.* 2007; Magee 2017) and we thus consider it a population that rarely, if ever, expresses anadromy.

Erriff brood stock were caught in Tawnyard Lough, a small upland lake (56 ha) on the western side of the Erriff catchment (the National Salmonid Index catchment) that is fed by a primary inflowing stream, the Glendavoch River and a number of smaller tributaries (Figure S1). The vast majority of trout spawned in the Glendavoch River are believed to disperse as fry or parr to Tawnyard Lough (a distance of a few hundred metres to a few kilometres, depending on how far up the Glendavoch River spawning occurred), although a small fraction remain permanently resident in the natal stream (P. Gargan, *pers. comm.*). A large run of out-migrating anadromous juveniles (in the range of five hundred to three thousand smolts per year over the last 30 years) is enumerated annually in a trap at the outflow of Tawnyard Lough (Gargan *et al.* 2016). The remaining fish never go to sea but instead spend several years growing in the lake, before returning to spawn in the Glendavoch River and smaller tributaries once mature. Brood stock from the Tawnyard population used in this experiment putatively comprised a mix of anadromous and non-anadromous fish, assumed to represent naturally occurring frequencies of anadromous and non-anadromous tactics (see Table S1 for details of brood stock), with local expertise indicating that the Tawnyard population in general shows high rates of anadromy (P. Gargan, *pers comm*.). In summary, we consider the Tawnward population to have a strong migratory/anadromous background, and the Bunaveela population to have essentially no (recent) anadromous background and to exhibit only limited local movements. For ease of reading, juveniles derived from Tawnyard parents are hereafter referred to simply as the "anadromous-background" population and juveniles from Bunaveela parents as the "non-anadromous background" population.

Fish rearing

Females were stripped of eggs, and the eggs of each female were divided into two batches, each fertilised by the milt of a single male from the same source population (i.e. Tawnyard or Bunaveela; see Table S1 for full details on crossing).

Fertilised eggs were then incubated in standard Heath trays in a hatchery facility located within the Burrishoole catchment. Surviving unfed fry (two to three weeks prior to exogenous feeding) were transferred to a rearing facility at University College Cork (Aquaculture and Fisheries Development Centre). While transitioning to exogenous feeding, fry were held in 100L growth tanks on a recirculating aquaculture system (RAS) with bio filtration, and fed *ad libitum* to satiation using commercially available trout pellets (Skretting Ltd, Norway). The populations were kept separately in two 100L tanks during this initial rearing phase and maintained under a natural temperature regime regulated by a single conditioning unit. Once the fry had transitioned to exogenous feeding (June 2016), they were fed *ad libitum* with commercial trout pellets for a period of two months. All fish experienced the same constant photoperiod regime (12 hours of light and 12 of dark) during this initial rearing phase.

In September 2016, fish were randomly allocated into four 100L tanks in the same RAS as described above (two tanks for Tawnyard and two tanks for Bunaveela), at which point the experimental phase began and food manipulations were initiated (see next section for experimental treatments). A random subset of fish (n = 200 per population) were given individual identifier tags using unique colour combinations of visible implant elastomer tags (Northwest Marine Technology Ltd., USA). To facilitate growth, in December 2016 the fry were transferred (within their experimental groups) to 520L growth tanks in a larger RAS in the same aquaculture facility. Continuous through flow of water prevented any waste accumulation in tanks, with returning water passed to a central holding sump and treated via mechanical filtration, protein skimming, bio filtration, and ozone and UV sterilisation. Water quality in the system was monitored weekly, and levels of pH, nitrate, nitrite, and ammonia were within acceptable ranges for optimal fish health. During the experimental phase, the fish experienced a seasonally-changing photoperiod and temperature regime typical of the west of Ireland, simulated via an automated lighting system of LED lights (BioLumen, UK) above each tank and a single conditioning

unit. Negligible natural mortality occurred during the experimental phase but to maintain total biomass in the RAS at acceptable levels from a water quality perspective, fish were randomly culled (n = 120 in total across all tanks) over the course of the two years of tank rearing, with equal fish densities maintained between food treatments. Fish that were prematurely culled were excluded from all analyses. Full details on the stripping, crossing and rearing procedures are given in Supplementary Information.

Experimental design

The experimental phase ran for a 22 month period, from September 2016 to June 2018, with all fish humanely euthanized at the end of the experiment under licence (the study and all associated procedures were carried out with ethical approval from Health Products Regulatory Authority (HPRA) Ireland, under HPRA project license AE19130/P034, and HPRA individual licenses AE19130/1087, AE19130/I200, AE19130/I201 and AE19130/I202).

To investigate the relative importance of the extrinsic environment (food supply) and intrinsic inherited factors (population-of-origin) in determining migratory tactics, juveniles from the anadromous and non-anadromous background populations were divided evenly and allocated randomly across four tanks receiving water from the same recirculating source, each experiencing a different feeding regime over the experimental phase. Populations were kept separately for the duration of the study (n = 90 per feeding treatment per population, at the beginning of the experimental phase). Great care was taken to ensure that all measured variables other than feeding regime (fish densities, temperature, photoperiod, lux, flow rates) were constant across the tanks. The four feeding regime treatments were designed to test the effects of food restriction in the early versus late periods of this experimental phase, with each period corresponding to approximately 11 months (chosen because similar periods of *c.* 9 months have been reported to alter adfluvial migration rates in trout (Olsson *et al.* 2006)). These four food regimes were as follows: (i) *High-High* treatment: fish fed recommended daily pellet rations for optimal growth in both periods, calculated as a percentage of their body weight

and adjusted for seasonally-changing temperatures (Skretting Ltd, Norway); (ii) *Low-Low* treatment: fish fed 25% of recommended optimal rations in both periods; (iii) *High-Low* treatment: fish fed 100% of optimal daily rations in the first period and 25% of optimal daily ration in the second period; and (iv) *Low-High* treatment: fish fed 25% of optimal daily rations in the first period and 100% of optimal daily ration in the second period. A value of 25% of optimum levels was chosen for the *Low* feeding regime because similar reductions have previously been shown to reduce the frequency of the resident tactic in adfluvial brown trout (Wysujack *et al.* 2009). Rations were reduced down to 25% of optimal gradually over a four-week period, to minimise stress. Within each food treatment, absolute rations were adjusted according to manufacturer's instructions (see Table S2) on a monthly basis to account for changes in body mass and temperature (i.e. there was no variation in daily rations within months, within groups).

Life history determination and data collection

In the spring of 2017 and 2018 (March – June in year one and year two of the experimental phase of the study), fish were routinely assessed for morphological indicators of 'smoltification': the series of morphological, physiological and behavioural changes that is generally considered a precursor to downstream migration of juvenile salmonids (Tanguy *et al.* 1994). Here we use 'smolt' to simply mean a fish showing external morphological features consistent with preparing for a migration, and we used saltwater tolerance tests (see below) to further assess physiological aspects of smoltification. We visually assessed morphological smoltification (silvered flanks/loss of parr marks, pronounced lateral line, colourless fins and fusiform shape) according to Tanguy *et al.* (1994). No fish matched the morphological criteria of smolts in the spring of 2017, the very earliest point at which we expected any smoltification (Poole *et al.* 2007; Gargan *et al.* 2016). Individuals that matched the morphological criteria for smolts in spring 2018 were transferred to saltwater at 30 ppt for 24 hours to assess their hypo-osmoregulation as a further indicator of anadromy capacity. We used 30 ppt salinity [following Tanguy *et al.* (1994)] because trout

often spend large amounts of time in brackish water/estuaries when migrating, hence trout smolts are typically less saltwater tolerant than other salmonids *e.g.* Atlantic salmon (Urke *et al.* 2010). After the 24-hour immersion in saltwater, a period proposed to induce hypo-osmoregulation in euryhaline species (Schultz and McCormick 2012), fish were euthanised with an overdose of MS-222 and a blood sample was taken from the caudal vasculature using a 21G needle and a 2.6ml heparinised syringe. Blood samples were transferred to 2 ml epindorphs and centrifuged at 8000 rpm for 3 minutes. The plasma aliquot was then siphoned off and stored at -80 °C before being measured for plasma chloride concentration as an indicator of hypo-osmoregulatory ability.

All fish, whether identified morphologically as smolts or non-smolts, were dissected to visually determine sex and maturation status according to gonad development. Males were classed as sexually mature if they had enlarged white testes or had running milt. Males that had visible testes that were moderately enlarged but not running milt were classed as maturing. Females were classed as mature or maturing if the body cavity contained identifiable eggs. Fish with immature gonads, or that could not be identified as either male or female by visual inspection were classed as immature at the time of sampling, and their genotypic sex was later determined using a microsatellite sex marker (P. Prodöhl, unpublished). In the wild, the natural spawning period for these brown trout populations is in late autumn/early winter, and the migratory period is in the spring (Poole *et al.* 2007; Gargan *et al.* 2016). Fish showing signs of maturity in freshwater without having first gone to sea, were considered to be on a non-anadromous trajectory, while smolts migrating to sea in a given spring were all immature. Fish in our experiment were thus classed as smolts (migratory tactic) if they were morphologically assessed as smolts and were immature, and were classed as mature (freshwater maturation tactic) if they were mature or maturing at the time of sampling. Fish that were classed as immature, but did not have morphological indicators of smoltification, were considered to have an unknown life history tactic at the time of sampling. A small number of fish (n = 12) had significant skin/fin damage at the time of sampling, and were excluded

from the analysis. Whole body lipid content (%) was measured for all smolts, and for a random sample of mature fish (n = 111), using a SMART Trac 5 system (CEM GmnH, Kamp-Lintfort, Germany) of integrated microwave heating and nuclear resonance on homogenised samples.

Statistical analysis

To assess whether food treatment and population influenced life history tactics (Aims 1 and 2), we constructed generalized linear models (GLMs) with a logit link function and binary life-history response variables. One GLM was created to predict smolt status (binary response: 1 = smolt, 0 = non-smolt) using the *brglm* package in R (Kosmidis 2019) to account for separation in the data (no smolts recorded in the *High-High* treatment) (Heinze and Schemper 2002). A second GLM was created to predict maturation (binary response: 1 = mature or maturing, 0 = immature). Categorical explanatory variables in both of these GLMs included food treatment (*High-High, Low-High, Low-Low, High-Low)*, population (anadromous-background versus non-anadromous-background), and sex (male or female) as predictors. We constructed a third GLM to test for treatment/population effects on likelihood of being classed as "unassigned" (i.e. not having expressed a migratory/resident phenotype by the end of the study (binary response: 1 = unassigned, 0 = smolt or mature). We included an interaction term between food treatment and population to determine if life history responses in each population were similar under the different food regimes (Aim 3). To test whether food restriction was more important in the early or late rearing periods (Aim 2), we conducted Tukey post-hoc tests (with Bonferroni correction applied for multiple tests) of all possible pairwise comparisons among the levels of food treatment using the *emmeans* package in R (Lenth 2019). Overall, one expects the strongest difference in life-history tactics to be found between the *High-High* and *Low-Low* treatments. If the effects of food restriction are additive and the timing of food restriction does not mater, then one expects life-history tactics in the *Low-High* and *High-Low* treatments to be intermediate between the *High-High* and *Low-Low* treatments, and not significantly different from each other. Conversely, if food restriction is

more important in the first period, then one expects tactic frequencies in the *Low-High* treatment to be closer to those in the *Low-Low* treatment (and the *High-Low* treatment should be more similar to the *High-High* treatment), while if food restriction is more important in the second period, the *High-Low* treatment should be closer to the *Low-Low* treatment and the *Low-High* treatment to the *High-High*. To further explore factors influencing variation in saltwater tolerance (Aims 1-3) – a key component of life-history tactics – we constructed a linear model (normal errors) with plasma chloride concentration as the continuous response, and population, food treatment, sex, and an interaction between population and food treatment included as predictors.

To address Aim 4, we explored factors influencing variation in the length, weight and condition factor of fish at different measurement time points across the study period within a mixed-effects modelling framework [*nlme* package, (Pinheiro *et al.* 2019)]. Measurement time points were September and November in 2016, February, April, June, July, September, and December in 2017, and April 2018. Condition factor was calculated as Fulton's *K* where:

$$Condition\ (K) = \frac{mass\ (g)}{fork\ length\ (cm)^3} \times 100$$

For the subsequent analyses of status traits, we created a new categorical variable called 'life-history tactic' with two levels: migratory (i.e. immature smolts) or mature/maturing (hereafter simply called mature). Fish which were neither classified as migratory nor mature (unassigned fish) were not included in the status trait analyses, as it could not be determined which life history trajectory they might adopt (i.e. these fish could have displayed either migratory or mature tactics the following spring (a full three years after hatching), but the experiment was terminated the previous spring (two years after hatching)). In addition to life-history tactics, month (continuous variable), population (categorical variable with two levels), food treatment (categorical variable with four levels) and sex (categorical variable with two levels) were included as fixed effects, and individual identity was included as a random effect to account for multiple measurements on some individuals. We included an interaction

between life-history tactics and month (to test whether individuals on different life-history trajectories diverged through time in their length/weight/condition factor), an interaction between life-history tactics and population (to test whether average differences in length/weight/condition factor between the two tactics was similar across the two populations), and an interaction between population and food treatment (to test whether the effects of food regime were similar across populations). Temporal autocorrelation of the response variable was accounted for by modelling an autoregressive error structure as a first order lag function of month. Separate models were constructed each for length, weight and condition factor and normal errors were assumed in each case.

We also explored factors influencing variation in final length, *K* and whole body lipids (i.e. the final measurements for these status traits at the end of the study) in a mixed effects modelling framework, where life-history tactics, food treatment, population and sex were included as fixed effects, and date of terminal sample (categorical variable with 11 sampling dates) was modelled as a random effect. We included two interaction terms (life-history tactics × population, and food treatment × population), to explore whether the patterns for each population were similar across tactics and food treatments, respectively. Separate models were constructed each for length, *K* and whole body lipids and normal errors were assumed in each case. Marginal R^2 values for mixed effect models were calculated using the *MuMIn* package in R (Barton 2018).

For all of the above models, statistical significance at a 5% alpha level of predictor variables was assessed using likelihood ratio tests (LRT), and non-significant interaction terms were omitted so the main effects could be interpreted.

Finally, to assess whether variation in growth was associated with life-history tactics (Aim 4), we compared growth trajectories of migratory and mature fish by fitting three typical models of fish growth: the von-Bertelanffy growth curve, the Gompertz growth curve and a logistic growth curve. The logistic growth curve best described the data according to AIC (ΔAIC = 0), and was used for all

further growth trajectory analysis. The logistic growth equation models asymptotic growth as:

$$L = \frac{L_\infty}{1 + e^{(-g_i(T-I))}}$$

Where L is fork length, L_∞ is asymptotic fork length (cm), g_i is the growth rate (cm/day), T is time (days) and I is the inflection point. The logistic model was fitted using non-linear least squares to length data collected on individually-identifiable fish during the experiment, with separate models fitted for smolts and mature fish. As non-linear least squares regression is sensitive to starting values of parameters, the model was fitted using the *nls_multstart* function from the *nls.multstart* package in R (Padfield and Matheson 2018). This allowed for starting values for each parameter to be randomly selected from a bounded distribution over 1000 iterations of the model, with the best available model then selected by AIC. To determine the fit of the most parsimonious model to our data, we bootstrapped with replacement 10,000 times and constructed 95% confidence intervals from the bootstrapped fits.

All analysis was carried out in R version 3.5.3 (R Core Team 2019), and all statistical models were checked against assumptions of the given model (independence, non-normality of residuals, heteroscedasticity and multicollinearity).

Results

Life-history tactics

By the end of the experimental phase, a total of 567 fish had been categorised as either smolts, i.e. putatively migratory (n=36 females and n=18 males) or non-smolts (n=277 females and n=236 males). All of the smolts were by definition immature, and 15.52% of the non-smolt females and 28.39% of the non-smolt males were immature. See Table 1 for a full breakdown of life-history tactics by population background, food treatment and sex. The proportion of smolts varied according to food treatment and population (Figure 1). Highest proportions of smolts were seen in the *Low-Low* food treatment, in which

26.56% of the anadromous-background population, and 15.71% of the non-anadromous background population, were classified as smolts. The lowest rates of smolting were found in the *High-High* food treatment, in which no fish from either population were categorised as smolts. Intermediate smolting rates were observed in the other two treatments, with 6.45% of fish from the anadromous-background population and 13.75% of fish from the non-anadromous background population classified as smolts in the *Low-High* treatment, and 15.87% and 1.22% of fish from each population, respectively, classified as smolts in the *High-Low* treatment.

Table 1: Percentage of brown trout (n = 567, F1 offspring of wild trout from two population backgrounds) classed as smolts (i.e. migratory tactic) or non-smolts (mature or immature) after two years of experimental tank-rearing. Values correspond to percentages for each category, broken down by sex, of the total number of fish per tank (where each tank corresponds to a given population background by food treatment combination, i.e. a single row in the table). Sample size (n) given in brackets after the %.

Treatment	**Population background**	**% Smolts (n)**		**% Non-smolts (n)**			
				Mature		***Immature***	
		Female	*Male*	*Female*	*Male*	*Female*	*Male*
Low-Low	Anadromous	23.44 (15)	3.13 (2)	25.00 (16)	35.94 (23)	3.10 (2)	9.38 (6)
Low-High	Anadromous	4.84 (3)	1.61 (1)	50.00 (31)	32.26 (20)	1.61 (1)	9.68 (6)
High-Low	Anadromous	11.11 (7)	4.76 (3)	39.68 (25)	34.92 (22)	4.76 (3)	4.76 (3)
High-High	Anadromous	0.00 (0)	0.00 (0)	48.44 (31)	45.31 (29)	1.56 (1)	4.69 (3)
Low-Low	Non-anadromous	2.86 (2)	12.86 (9)	44.29 (31)	14.29 (10)	1.43 (1)	24.29 (17)
Low-High	Non-anadromous	10.00 (8)	3.75 (3)	42.50 (34)	28.75 (23)	7.50 (6)	7.50 (6)
High-Low	Non-anadromous	1.22 (1)	0.00 (0)	35.37 (29)	17.07 (14)	20.73 (17)	25.61 (21)
High-High	Non-anadromous	0.00 (0)	0.00 (0)	45.12 (37)	34.15 (28)	14.63 (12)	6.10 (5)

The probability of smolting was described by a GLM retaining food treatment (χ^2 = 44.57, df = 3, p < 0.001), population (χ^2 = 3.46, df = 1, p = 0.063), sex (χ^2 = 4.40, df = 1, p = 0.036), and an interaction between food treatment and population (LRT for the model with and without interaction term: χ^2 = 11.66, df = 3, p = 0.009). Overall across the two populations, there appeared to be an additive effect of food treatment on the probability of smolting – that is, the percentages of smolts in the *Low-High* and *High-Low* treatments were similar, and approximately intermediate to the percentages in the *Low-Low* and *High-High* treatments, when population was ignored (Figure 1). However, when population was taken into account, the life-history response to food treatment varied by population and appeared to be non-additive within each population (Table 2, Figure 1). Fish from the anadromous-background population exhibited a relatively high percentage of smolts (15.87%) under the *High-Low* treatment that was closer to the *Low-Low* treatment (26.56% smolts) than to the *High-High* treatment (0% smolts) and post-hoc comparisons of *High-Low* against *Low-Low* were not significant (p = 0.377). The opposite was true for the anadromous-background population in the *Low-High* treatment (6.45% smolts) with significant post-hoc comparisons of *Low-Low* and *Low-High* (p = 0.016). In contrast, fish from the non-anadromous-background population exhibited a relatively high percentage of smolts (13.75%) under the *Low-High* treatment that was closer to the *Low-Low* treatment (15.71% smolts) than to the *High-High* treatment (0% smolts) (post-hoc contrasts between *Low-High* and *Low-Low* were non-significant, p = 0.994), while the opposite was true for this population in the *High-Low* treatment (1.22% smolts) (post-hoc contrasts between *High-Low* and *Low-Low* were significant, p = 0.042). This implies that food restriction was more important in the second period for fish from the anadromous-background population, while food restriction in the first period was more important for the non-anadromous-background fish.

Maturation tactics in freshwater were also significantly affected by food treatment (χ^2 = 33.03, df = 3, p < 0.001), population (χ^2 = 12.14, df = 1, p < 0.001), sex (χ^2 = 4.54, df = 1, p = 0.033) but there was no significant interaction between

food treatment and population (LRT for the model with and without interaction term: χ^2 = 5.31, df = 3, p = 0.150). Food restriction had a negative effect on maturation probability, in direct contrast to food restriction effects on smolting rates. Fish in the *Low-Low* food treatment had the lowest probability of maturing, (p < 0.001, Table 2) and the highest rates of maturity were observed in the *High-High* food treatment, (p < 0.001, Table 2). Fish from the anadromous-background population were significantly more likely to mature than fish from the non-anadromous-background population in all food treatments (p = 0.001, Table 2). See Table 2 for all parameter estimates and associated standard errors. The probability of having been unassigned a life history showed similar patterns to maturation tactics, and was similarly significantly affected by food treatment (χ^2 = 16.95, df = 3, p = 0.001), population (χ^2 = 30.74, df = 1, p < 0.001) and sex (χ^2 = 16.21, df = 1, p < 0.001), see Table 2. The interaction between food treatment and population was marginally not significant (LRT for the model with and without interaction term: χ^2 = 7.75, df = 1, p = 0.052).

We found a significant effect of population on plasma chloride levels of fish classified as smolts (F = 9.47, df =1,48, p = 0.003), but the interaction term between population and food treatment was not significant (LRT for model with and without interaction term: F = 1.39, df = 2, p = 0.259). Fish from the anadromous-background population had significantly lower plasma chloride concentrations than non-anadromous-background fish (p = 0.003, Table 3, Figure 2). There was no significant effect of food treatment (F = 2.95, df = 2,48, p = 0.062) or sex (F = 0.01, df = 1,48, p = 0.991) on plasma chloride levels (Table 3).

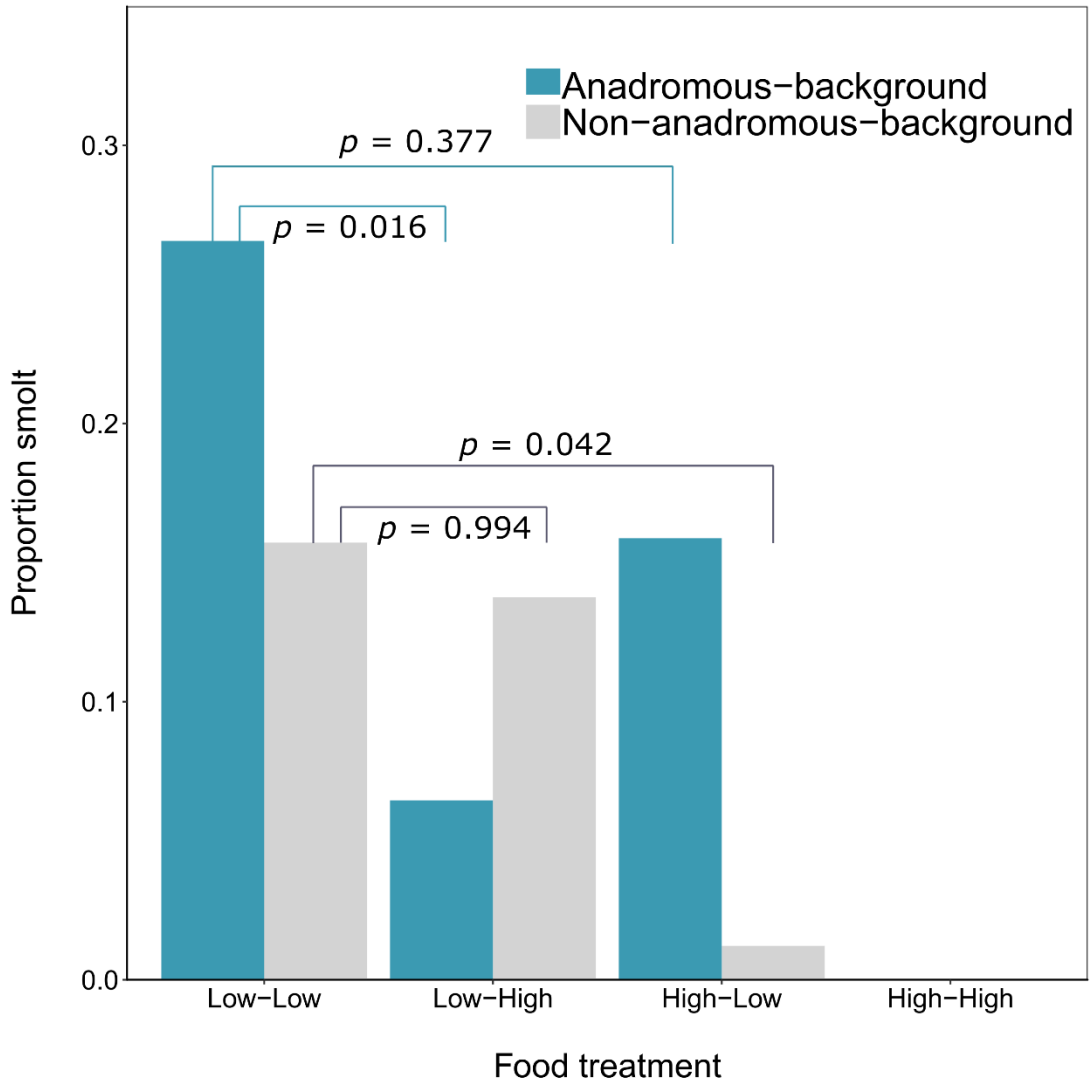

Figure 1: Proportion of brown trout (n = 567, F1 offspring of wild trout from two population backgrounds) classed as smolts after two years of tank rearing under varying food restriction treatments. Food treatment is denoted in the format "food in year one - food in year two", where "high" refers to optimal food rations and "low" refers to 25% of optimal rations. P–values shown are Tukey post-hoc pairwise comparisons across all levels of food treatment for each population.

565 **Table 2:** Parameter estimates with associated standard errors (SE) for two binomial generalised linear models (GLM) predicting smolt (migratory) probability (dummy coded: smolt = 1, non-smolt = 0) and freshwater maturation (dummy coded: mature/maturing = 1, immature = 0) in brown trout (n = 567). The reference level of each factor is in brackets, i.e. effects in both models were 570 contrasted against female fish from the anadromous-population background in the *Low-Low* food treatment. Statistical significance was assessed at $p < 0.05$.

Effect		**Estimate**	**SE**	**t-value**	***p*-value**
GLM of probability of smoltification:					
Intercept (Low-Low, female, Anadromous background)		-0.71	0.31	-2.28	0.022
Food:	Low-High	-1.61	0.57	-2.83	0.005
	High-Low	-0.66	0.44	-1.49	0.136
	High-High	-3.87	1.45	-2.66	0.008
Population: Non-anadromous background		-0.63	0.43	-1.47	0.142
Sex: Male		-0.63	0.31	-2.06	0.039
Low-High : Non-anadromous background		1.38	0.73	1.90	0.058
High-Low: Non-anadromous background		-1.75	0.99	-1.77	0.077
High-High: Non-anadromous background		0.33	2.06	0.16	0.873
GLM of probability of maturation:					
Intercept (Low-Low, female, Anadromous background)		0.97	0.24	4.12	< 0.001
Food:	Low-High	0.78	0.27	2.90	0.004
	High-Low	0.10	0.25	0.42	0.676
	High-High	1.43	0.30	4.78	< 0.001
Population: Non-anadromous background		-0.68	0.20	-3.43	0.001
Sex: Male		-0.41	0.19	-2.13	0.033
GLM of probability of "unassigned" phenotype:					
Intercept (Low-Low, female, Anadromous background)		-2.77	0.33	-8.29	< 0.001
Food:	Low-High	-0.44	0.34	-1.28	0.201
	High-Low	0.67	0.30	2.24	0.025
	High-High	-0.36	0.33	-1.08	0.279
Population: Non-anadromous background		1.32	0.25	5.18	< 0.001
Sex: Male		0.92	0.23	4.01	< 0.001

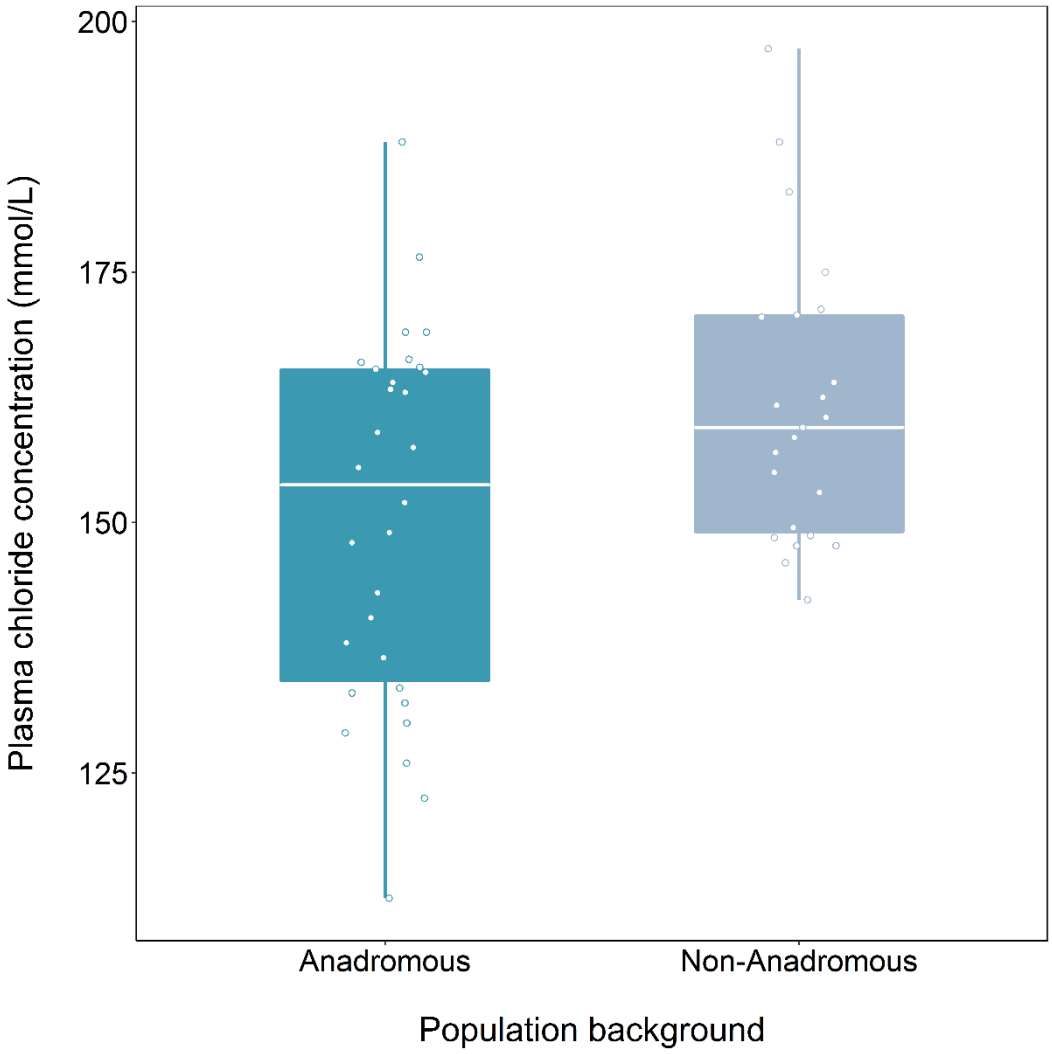

Figure 2: Plasma chloride concentration (mmol/L) after 24 hr saltwater immersion of brown trout smolts (migratory tactic, n = 54) derived from two population backgrounds. The median is represented by the white horizontal lines in each box.

Table 3: Parameter estimates with associated standard errors (SE) for the linear model testing effects of population, sex and food treatment on plasma chloride concentration (mmol/L) of brown trout classified as smolts (n = 54). The reference level of each factor is in brackets, i.e. effects were contrasted against female fish from the anadromous-population background in the *Low-Low* food treatment. Note that no individuals were classed as having adopted the anadromous tactic in the *High-High* food treatment, and this category was dropped for this analysis. Statistical significance was assessed at $p < 0.05$.

Effect	Estimate	SE	*t*-value	*p*-value
Intercept (Low-Low, female, Anadromous background)	148.81	3.85	38.68	< 0.001
Population	17.22	5.60	3.08	0.003
Food: Low-High	-9.99	5.85	-1.71	0.094
Food: High-Low	8.36	5.97	1.40	0.168
Sex: Male	0.06	5.15	0.01	0.991

Factors explaining variation in status traits at different time points

At the time at which the food treatments were first applied, fish from both populations were in similar condition ($F = 0.41$, df = 1,137, $p = 0.523$), however, anadromous-background fish were heavier ($F = 17.14$, df = 1,137, $p < 0.001$) and longer ($F = 16.31$, df = 1,137, $p < 0.001$) than non-anadromous-background fish. A mixed model analysis indicated further divergence in these status traits over the study period that was related to life-history tactics, food treatment, and population effects (Table 4, Figure 3). The models for length (marginal $R^2 = 0.77$), weight (marginal $R^2 = 0.62$), and *K* (marginal $R^2 = 0.35$) retained a significant interaction between food treatment and population, and a significant interaction between life-history tactics and month (Table 4). Sex did not have a significant effect on length ($\chi^2 = 0.024$, df = 1, $p = 0.877$), weight ($\chi^2 = 0.050$, df = 1, $p = 0.823$), or condition factor ($\chi^2 = 0.082$, df = 1, $p = 0.774$). After accounting for growth between measurement periods, (*i.e.* the fixed effect of measurement period), smolts tended to be shorter, lighter and have lower condition than mature fish (Table S3). The differences in length, weight and *K* were similar for both populations (an interaction between population and life-

history tactics was not retained in any of the final models, see Table 4). The significant interaction between food treatment and population indicated that fish from the anadromous-background were larger, and heavier (but in similar condition) than fish from the non-anadromous-background under both *High-Low* and *High-High* treatments (Table S3). However, in the *Low-Low* and *Low-High* treatments, there were negligible differences in length, weight and *K* between populations (Table S3). The significant interaction between month and life-history tactics indicated that changes in length, weight and *K* through time varied between smolts and mature fish. Mature fish tended to increase in length and weight quicker (Figure 3b, Table S3), while smolts tended to be in worse condition (lower *K*) earlier (Figure 3C Table S3). See Table S3 for all model outputs.

Factors explaining variation in final values for status traits

At the end of the study, fish differed in length, condition and lipid content according to food treatment, life-history tactics and population (Figure 4). The model describing length (marginal $R^2 = 0.50$) retained a significant interaction between food treatment and population (Table 5) but did not indicate a significant effect of life-history tactics ($\chi^2 = 2.83$, df = 1, $p = 0.093$), or sex ($\chi^2 = 0.005$, df = 1, $p = 0.947$). The models describing condition (marginal $R^2 = 0.56$) and whole body lipids (marginal $R^2 = 0.73$, Table 5) each retained an interaction between population and food treatment (Table 5), and included a significant effect of life-history tactics on condition ($\chi^2 = 64.58$, df = 1, $p < 0.001$), and whole body lipids ($\chi^2 = 7.71$, df = 1, $p = 0.005$). Sex did not have a significant effect on condition ($\chi^2 = 3.43$, df = 1, $p = 0.064$) or whole body lipids ($\chi^2 = 2.18$, df = 1, $p = 0.140$). Overall, smolts were of similar length to mature fish at the end of the study (Figure 4), but tended to be in poorer condition ($p < 0.001$, Table S4) and have slightly higher whole body lipids ($p = 0.008$, Table S4). We detected an interactive effect of food treatment and population, where fish from the anadromous-background population were larger than fish from the non-anadromous-background population, but similar under *Low-Low* food conditions (Table S4). However, non-anadromous-background fish were overall

in better condition (p = 0.011, Table S4) and had higher whole body lipids (p < 0.001, Table S4), and these differences between populations were strongest under conditions of *Low-Low* food (Table S4, Figure 4). The lack of significant interactions between life-history tactics and population in the models for length, K, and whole body lipids indicated that differences between populations were similar for both mature fish and smolts (Table 5). See Table S4 for all model outputs.

Growth rate differences

The somatic growth of fish during the experiment was well described by a logistic growth model. Initial model fitting indicated the most parsimonious model included separate growth parameters for smolts and mature fish. Mature fish had higher intrinsic growth rates (g_i = 0.0050, SE = 0.0006, p < 0.001), a smaller asymptotic size (L_∞= 25.44, SE = 0.86, p < 0.001), and a lower point of inflection (I = 172.7, SE = 13.8, p < 0.001) than smolts, where g_i = 0.0039 ± SE 0.0009 (p < 0.001), L_∞ = 27.31 ± SE 4.13 (p < 0.001) and I = 305.7 ± SE 89.9 (p = 0.001). Mature individuals were relatively larger earlier in life than smolts, and had faster overall growth (Figure 5).

Growth differences between the two populations were also identified, where fish from the anadromous-background population were relatively larger earlier in the study than fish from the non-anadromous-background population, and grew faster (Figure 6). Anadromous-background fish had higher intrinsic growth rates (g_i = 0.0045, SE = 0.0009, p < 0.001), similar asymptotic size (L_∞= 26.83, SE = 1.68, p < 0.001), and a lower point of inflection (I = 184.1, SE = 26.9, p < 0.001) than non-anadromous-background fish, where g_i = 0.0043 ± SE 0.0007 (p < 0.001), L_∞ = 26.45 ± SE 1.65 (p < 0.001), and I = 236.3 ± SE 32.9 (p < 0.001).

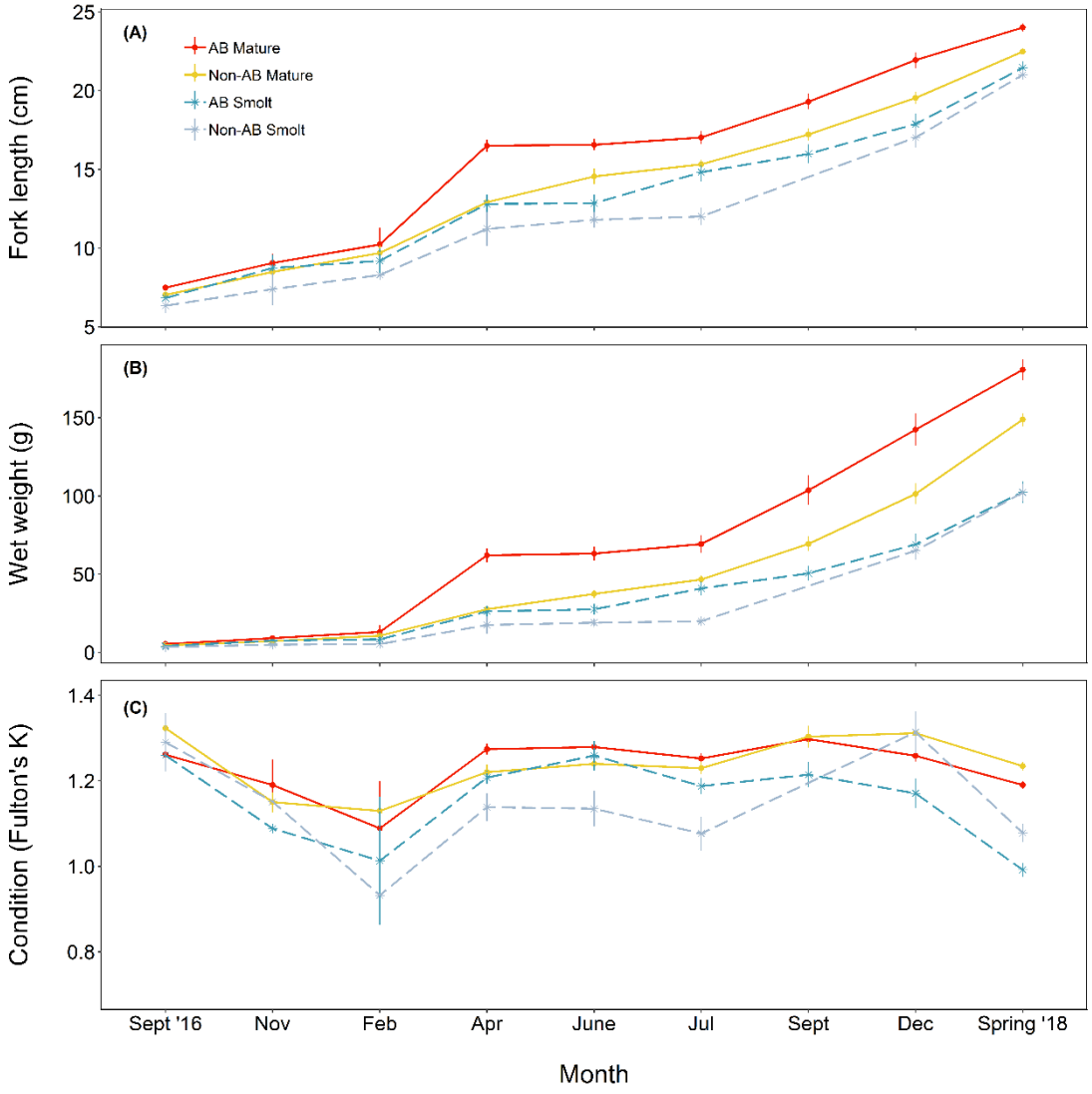

Figure 3: Trajectories of (a) length, (b) mass, and (c) condition factor (*K*) of brown trout offspring (derived from wild-caught parents from two populations) that were classed as either smolt (migratory tactic) or freshwater maturing (non-migratory/resident) tactic. AB = anadromous-background population; non-AB = non-anadromous-background population. Mean values (with associated standard errors) are shown for measurements taken at key time points over the course of two years of tank rearing.

Table 4: Results of the mixed effect model analysis for length, weight and condition factor (*K*) trajectories of brown trout in the experiment with life-history classed as either smolts (i.e. migratory) or freshwater mature across the study period. The results of the model selection procedure on interaction terms are given, and the selected model for each response is highlighted in bold. The models included a random effect of individual identity and a first-order autoregressive correlation structure with respect to month was also modelled.

Model	**df**	**AIC**	**logLik**	***L*-ratio**	***p*-value**
Length ~ month*life-history + population*life-history + population*food + sex	16	4277	-2122		
Length ~ month*life-history + population*food + sex	15	4276	-2123	1.52	0.218
Length ~ month + life-history + population*food + sex	14	4279	-2125	4.31	0.038
Length ~ month + life-history + population + food + sex	11	4306	-2142	33.31	< 0.001
Weight ~ month*life-history + population*life-history + population*food + sex	16	10229	-5099		
Weight ~ month*life-history + population*food + sex	15	10228	-5099	0.51	0.474
Weight ~ month + life-history + population*food + sex	14	10245	-5109	19.37	< 0.001
Weight ~ month + life-history + population + food + sex	11	10263	-5120	23.45	< 0.001
K ~ month*life-history + population*life-history + population*food + sex	16	-1524	778		
***K* ~ month* life-history + population*food + sex**	15	-1525	778	0.86	0.354
K ~ month + life-history + population*food + sex	14	-1514	771	12.77	< 0.001
K ~ month + life-history + population + food + sex	11	-1488	755	331.89	< 0.001

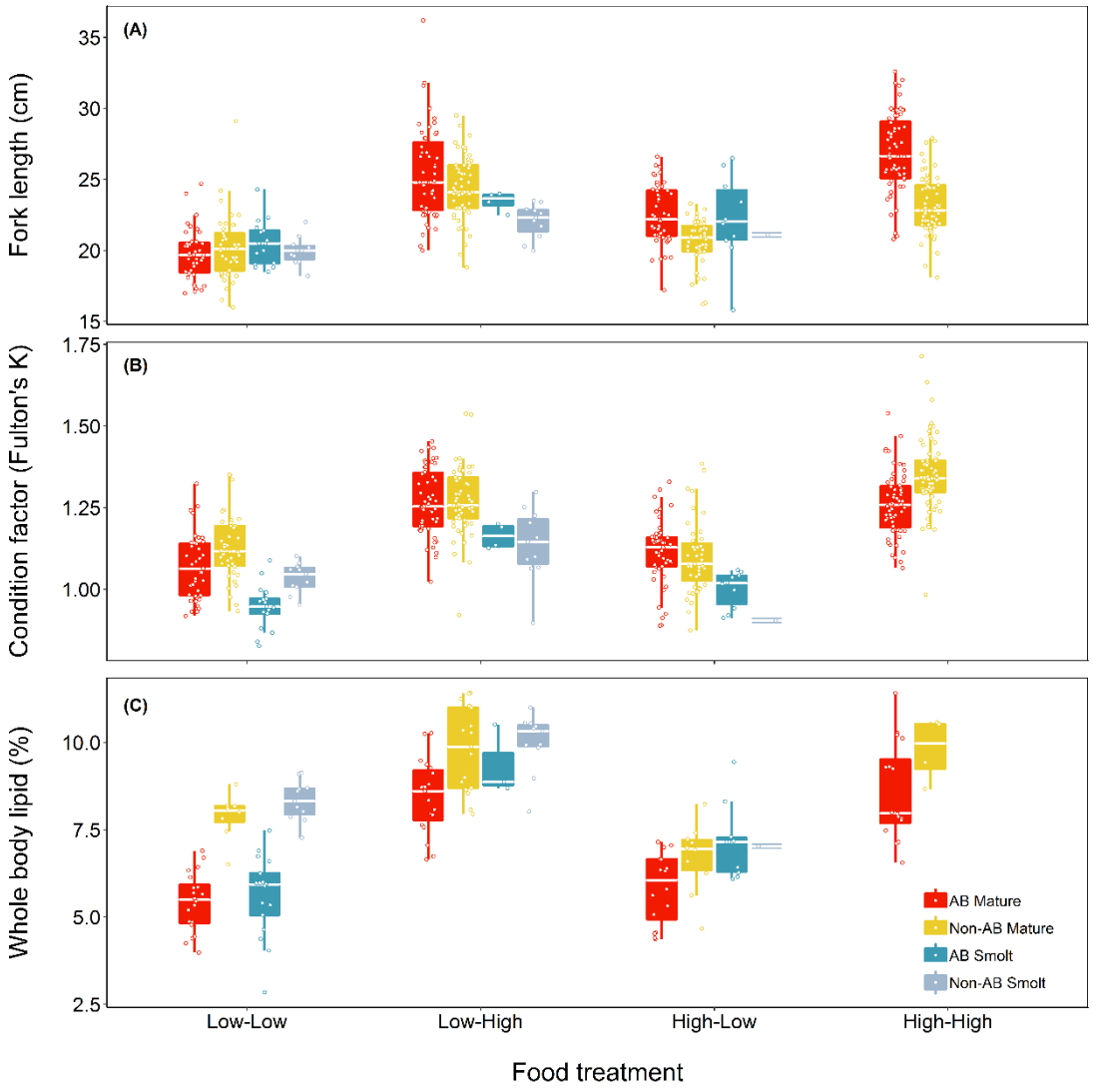

Figure 4: Effects of food treatment on final (a) length, (b) condition factor (*K*), and (c) whole body lipids at the end of the experimental study (Spring 2018) of brown trout offspring classed as either smolts (migratory) or freshwater maturing (non-migratory/resident). Offspring were derived from wild-caught parents from an anadromous-background population (AB) and a non-anadromous-background population (non-AB). The median is represented by the white horizontal lines in each box.

665 **Table 5:** Results of the mixed effect model analysis for length, condition factor (*K*), and whole body lipids of brown trout (life-history classed as either smolts or freshwater mature) at the end of the experimental study period. The results of the model selection procedure on interaction terms are given, and the selected model for each response is highlighted in bold. The models included a random effect of sample date.

Model	**df**	**AIC**	**logLik**	***L*-ratio**	***p*-value**
Length ~life-history*population + population*food + sex	13	2074	-1024		
Length ~ life-history + population*food + sex	12	2076	-1026	3.87	0.05
Length ~ life-history + population + food + sex	9	2093	-1037	22.98	< 0.001
K ~ life-history *population + population*food + sex	13	-798	411.91		
***K* ~ life-history + population*food + sex**	12	-800	411.90	0.01	0.922
K ~ life-history + population + food + sex	9	-786	402.11	19.59	< 0.001
Lipids ~life-history *population + population*food + sex	13	489	-231.4		
Lipids ~ life-history + population*food + sex	12	487	-231.6	0.46	0.500
Lipids ~ life-history + population + food + sex	9	503	-242.6	21.94	< 0.001

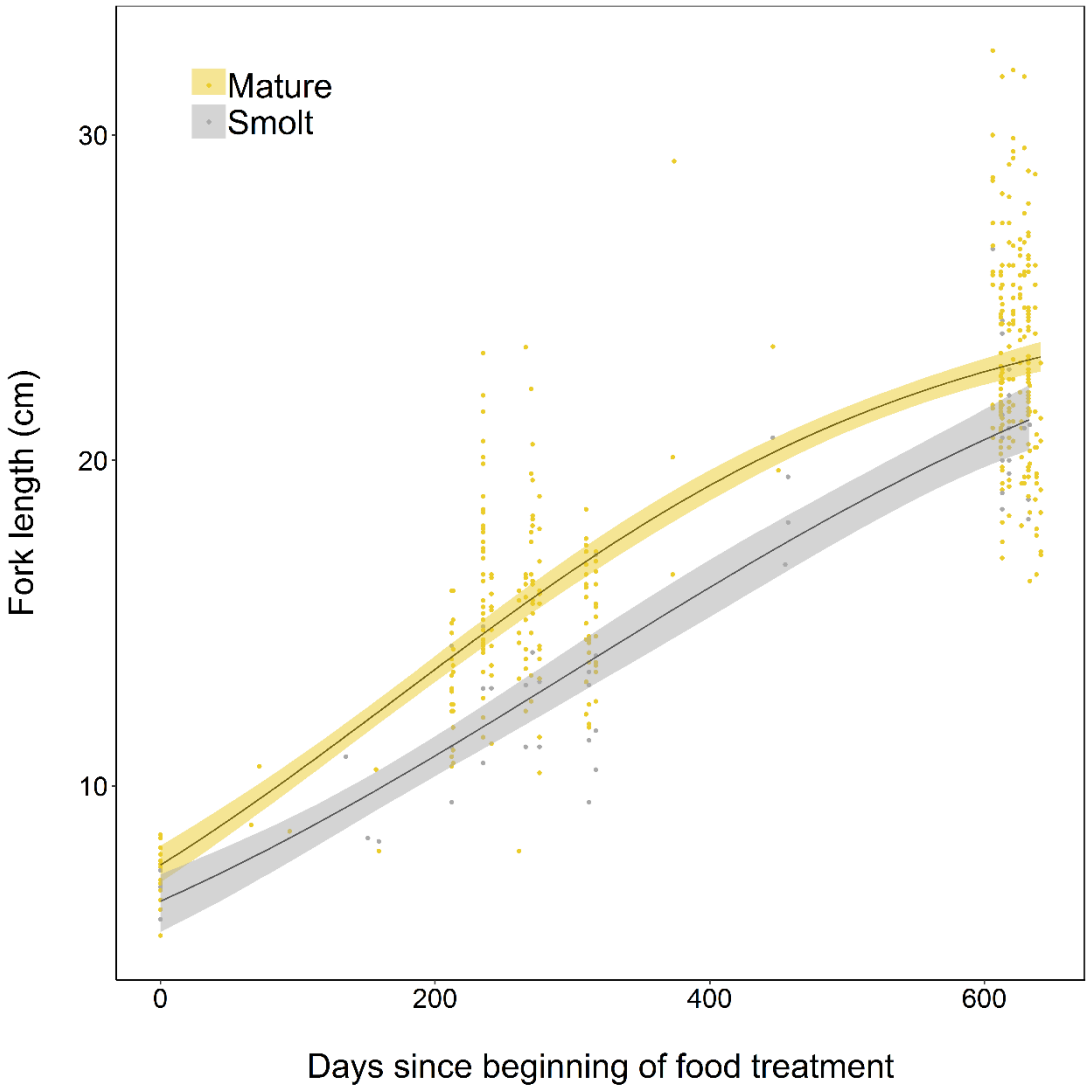

Figure 5: Growth curves, based on length measurements spanning two years of experimental tank-rearing, of brown trout classed as either smolt (migratory) or freshwater maturing (resident) in Spring 2018. Fitted lines are based on the best-fitting parameters from the logistic growth model, fitted using non-linear least squares regression. Shaded areas represent the 95% confidence intervals constructed by bootstrapping for 10,000 iterations.

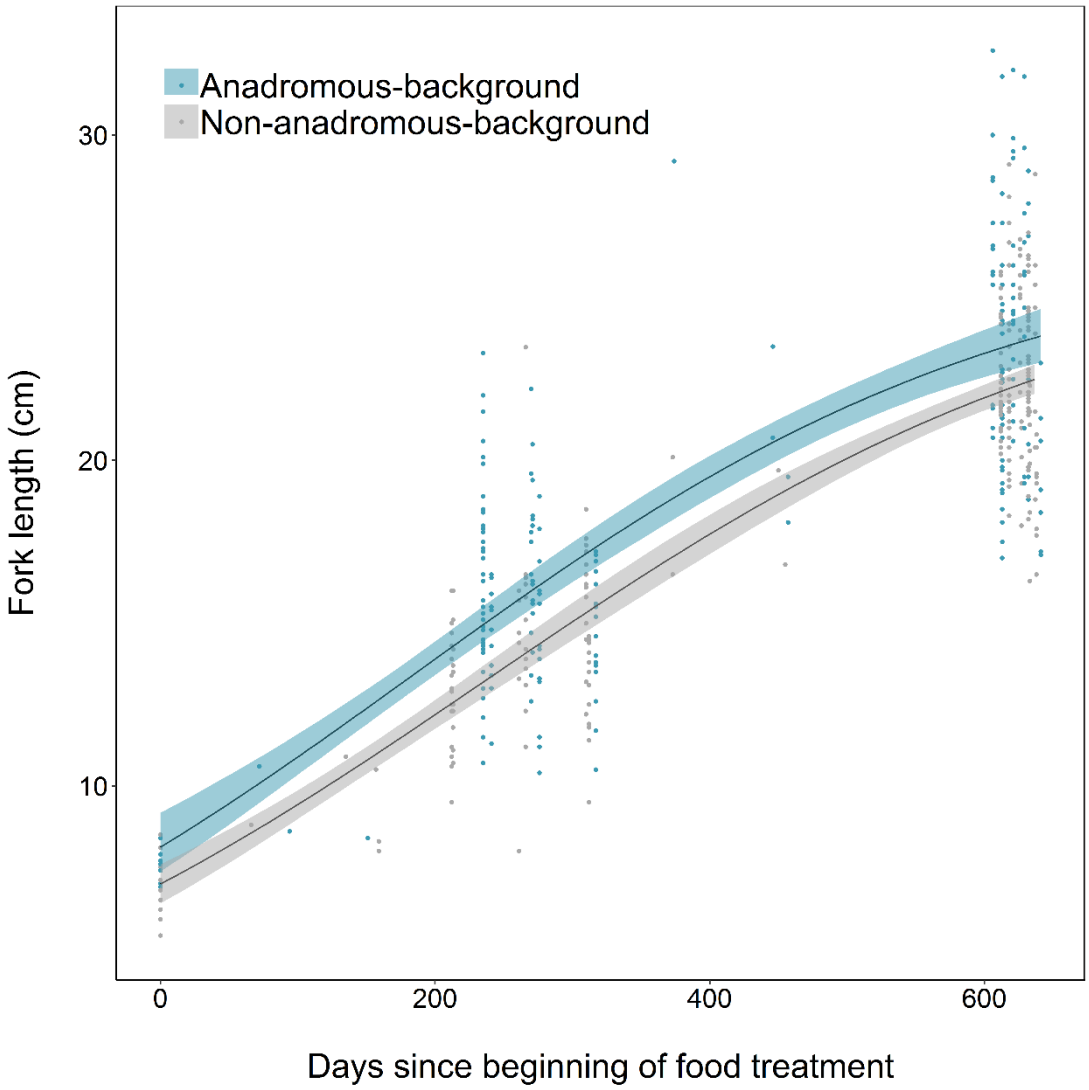

Figure 6: Growth curves, based on length measurements spanning two years of experimental tank rearing, of brown trout derived from two population backgrounds (anadromous or non-anadromous). Fitted lines are based on the best-fitting parameters from the logistic growth model, fitted using non-linear least squares regression. Shaded areas represent the 95% confidence intervals constructed by bootstrapping for 10,000 iterations.

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Discussion

Salmonine fishes exhibit some of the most striking examples of animal migration, but uncertainty still surrounds the mechanisms by which alternative migratory tactics can be expressed, or inhibited, across salmonine populations.

A principle aim of our study was to assess the importance of food availability at different time points during early ontogeny in determining migratory/life-history tactics in two populations of brown trout. Food reduction across almost two years led to increased rates of smolting (migratory tactic) in fish from both population backgrounds, whilst no fish were classed as having adopted the migratory tactic in either population after two years of experiencing high food, i.e. optimal rations (Figure 1). Migratory/life-history tactics were also influenced by population background, consistent with an inherited component to migratory/life-history decisions – fish derived from a naturally anadromous population were more often classed as smolts in our experiment, while offspring derived from a naturally non-anadromous population were more often classed as non-smolts, or having undergone freshwater maturation consistent with a residency tactic. Intriguingly, the populations responded differently to the timing of food restriction, with fish from an anadromous population background seemingly having been more affected by food restriction in their second year, whilst fish from a non-anadromous population background were more affected by food restriction in their first year. Females were more likely than males to become smolts under all food treatments. Collectively, these results indicate both extrinsic (food-driven) and intrinsic effects (related to population background, sex, and other individual-level attributes) on migratory/life-history tactics in brown trout, that may interact in complex ways and influence how populations respond in the wild to changing environmental conditions.

Differences in growth and body condition were apparent from an early stage between fish adopting different life-history/migratory tactics, and were maintained across the full (almost two-year) duration of the study. These differences were in turn also driven by both extrinsic and intrinsic effects. Extrinsic effects were evidenced by the fact that large differences in fork length, mass, body condition, and whole body lipids were apparent between fish reared under different food treatments, which in turn contributed to fish adopting different life-history tactics via phenotypic plasticity. Intrinsic differences

among individuals in 'status traits' clearly also contributed to migratory/life-history outcomes, given that differences in body size, condition, and lipids were apparent between populations, and between fish from each population that adopted different tactics *within* each food treatment – where the external environment was the same. Such intrinsic variation within and between populations could reflect inherited genetic effects, inherited non-genetic effects (*e.g.* parental effects, epigenetic inheritance), or non-inherited differences driven by early-life environmental influences that have a relatively long-lasting effect on phenotype (Burton and Metcalfe 2014). Expanding our approach to incorporate even earlier life stages (*e.g.* post-hatching/fry) could further illuminate how factors in early life influence life history.

Extrinsic factors

The observed increases in smolting in the face of food restriction, together with decreases in maturation, suggested that the reduction in food supply prevented individuals from meeting an intrinsic (*e.g.* genetically determined) threshold for residency and maturity in freshwater, which is in agreement with previous studies (Olsson *et al.* 2006; Wysujack *et al.* 2009; O'Neal and Stanford 2011; Jones *et al.* 2015). Indeed, the absence of any smolts under conditions of high food supply was surprising, particularly within fish from the Tawnyard population (anadromous-background), which has a naturally high frequency of anadromy in the wild (Gargan *et al.* 2016). This suggests that, in nature, a large number of fish in the Tawnyard system must typically experience relatively low food availability as freshwater juveniles, as otherwise anadromy rates (broadly estimated as 50 – 60% in the wild population) would be lower in the wild. Moreover, the balance of fitness cost and benefits of migration in the system must be such that natural selection has caused a relatively high threshold for residency to evolve (an ultimate mechanism; Hazel *et al.* 1990; Tomkins and Hazel 2007; Pulido 2011), meaning a minority of Tawnyard fish in the wild typically surpass their intrinsic freshwater maturation threshold and the anadromous tactic is more frequent.

Manipulation of the timing of food reduction revealed that life-history responses of a given population to environmental change might depend on the point during ontogeny at which the change is experienced. This could come about via two non-mutually exclusive mechanisms: populations could exhibit variation in sensitivity to cues experienced during given fixed "decision windows", and/or the timing of the decision windows themselves may vary across populations. In our study, food restriction in the first year (*Low-High* treatment) was a more important driver of smolting rates than food in the second year (*High-Low*) for fish from the non-anadromous-background population, whereas food in the second year was more important for the anadromous-background population. This was an intriguing outcome, and hints at a complex interplay between extrinsic environmental and intrinsic or population-specific factors. The apparently greater importance of food restriction in the first year for the non-anadromous-background population could perhaps be related to lower intrinsic growth rates in this population in the wild. Given their low potential growth rates, individuals in the non-anadromous-background population might be constrained to make a life-history decision (i.e. choose future migration or residency) early in life in order to divert energy intake towards meeting the associated demands of the chosen tactic. Because residents must accumulate sufficient lipid reserves to be converted into reproductive tissue before spawning (McMillan *et al.* 2012), in the wild, Bunaveela fish may have experienced selection for adopting a maturation trajectory relatively early in order to allow sufficient time for growth and energy accumulation, with early decision windows evolving as a consequence. In contrast, fish from the anadromous-background population with higher intrinsic growth potential may be less constrained in this regard, and may defer choice of migratory tactics to the second year of life, or indeed have flexibly reversible life-history trajectories where, for example, fish choosing residency based on high food in year one may switch to migratory tactics in response to low food in year two. There is some evidence for conditions in the second year of life being a key driver of migratory tactics in a

naturally facultatively anadromous brown trout population to support this (Cucherousset *et al.* 2005).

Coupled with a later "decision window"/higher sensitivity to conditions in year two, a naturally high intrinsic growth propensity in the anadromous-background population could have facilitated high levels of compensatory growth when receiving optimal food resources in year two in the *Low-High* treatment. If growth, or some aspect of energy usage related to growth such as body condition, is used as a cue for migratory tactic choice, this may then have translated into more individuals from this population meeting their threshold for maturation in the *Low-High* treatment. Strong compensatory responses after periods of food restriction have been observed in salmonids in general, and interestingly, the compensatory response has often appeared to be directed towards restoring body condition, rather than size. Nicieza and Metcalfe (1997) found food restricted fish recovered similar condition to controls within a year of food supply restoration, and Álvarez and Nicieza (2005) further found a compensatory response that resulted in restoration of condition and energy status rather than skeletal growth in brown trout post food restriction.

Alternatively, we cannot rule out the presence of multiple migration versus residency decision windows, that re-occur annually or more frequently, whereby an individual repeatedly re-assesses its status trait relative to its inherited freshwater maturation threshold and can remain 'undecided' at the first or even second windows, though there is little empirical evidence for this. A simpler explanatory model is that there is a single, initial decision determining migration versus residency, and then subsequent decision windows occur for fish on each trajectory (migrants and resident) related to the timing of expression of the adopted life-history tactic, where for example migrants must decide at what age to actually migrate (determined by pressures of size-dependent sea survival), or indeed *where* to migrate (Ferguson *et al.* 2019). Similarly, a resident individual must also decide *when* to mature (Thorpe *et al.* 1998; Thorpe and Metcalfe 1998), a decision shown to be affected by lipid reserves in Atlantic salmon (Rowe *et al.* 1991; Jonsson and Jonsson 1993, 2005)

and possibly triggered by similar threshold type mechanisms in brown trout. These timing decisions could be further influenced by extrinsic environmental conditions, giving rise to a temporal continuum of migration and maturation tactics. This may explain why some fish in our study were classified as having an undetermined life history (neither smolt nor mature) by the spring of year two: these individuals may simply have been delaying expression of a migratory or freshwater maturing phenotype until the following year. These *caveats* must be born in mind when interpreting our experimental results, as the life-history tactic frequencies we measured in year 2 could be indicative of age-specific tactic frequencies, rather than overall rates of migration versus residency across all ages. However, the basic conclusions were the same in the GLMs where the data were analysed as either smolt versus non-smolt, or immature versus mature, giving us confidence that the patterns reflect the migration decision *per se*.

Variation in status traits underpinning alternative tactics

Size-based differences between migrating individuals (those classified at the end of the study as smolts) and resident fish (those classified at the end of the study as mature) were established relatively early, with differences in weight, length, and condition that were maintained during the course of the study. The early divergence in physiological condition between migrants and residents supports the energy limitation scenario, where fish adopt migration as a result of failing to meet the necessary condition in early life to mature as residents in freshwater (Jonsson and Jonsson 1993). Maturing fish reached an apparent size asymptote earlier than migrating fish (i.e. had smaller inflection point in Figure 5, and were larger earlier in the study). Size appears to be a potential status trait that regulates, or correlates with factors regulating early sexual maturation, as has been documented in Atlantic salmon, where anadromous males are smaller than their counterparts that mature early in freshwater as so-called 'precocious parr' (Whalen and Parrish 1999; Garant *et al.* 2002). However, although body size has been suggested as a major component of the status (cueing) trait for anadromy in brook trout (Thériault *et al.* 2007), the divergence in mass and

condition we found in our study suggests that other factors beyond size also contribute to the maturation versus migration/anadromy decision. It seems increasingly likely that a suite of interlinked physiological components is assessed (*e.g.* overall energetic status or rate of change in energy), and no single trait controls the migratory/anadromy decision. Genetic covariance between life history traits such as growth, size, metabolism, and other morphological traits further suggests that migration decisions are associated with a suite of inter-linked phenotypic traits (Doctor *et al.* 2014; Hecht *et al.* 2015).

Fish on a migratory trajectory here appeared to maintain growth rates during the experiment (and had a higher inflection point), such that they were similar in length to mature fish by the end of our study. Constant, or even accelerated growth in pre-migratory fish (Metcalfe 1998) has been explained by size-dependent survival at sea (Klemetsen *et al.* 2003) due to better osmoregulation ability (Finstad and Ugedal 1998) and reduced predation of larger anadromous individuals (Dill 1983; Jonsson *et al.* 2017). Interestingly here, although skeletal growth (i.e. length) was maintained, migratory fish were considerably lighter and in worse condition than mature fish at the end of study, which suggests that once on a migratory trajectory, resources were primarily allocated to meeting a size-based threshold for surviving actual migration. The maintenance of growth rates in migrants as such does not contradict the energy limitation scenario, but rather suggests that migratory fish redirect what resources they obtain into becoming large enough to survive the migration, at a cost to their overall body condition.

The diminished body condition of migratory individuals was not, however, reflected in levels of whole-body lipids at the end of the study. Contrary to our expectations, migratory fish had marginally higher levels of whole body lipids than mature fish. Lipid storage has been identified previously as an important precursor of maturation in fish (Tocher 2003) and an indicator of a residency life history in salmonids (Sloat and Reeves 2014; Sloat *et al.* 2014 and references therein). The unexpected trend we observed in lipids may have been a consequence of measuring lipids during the smolt migration period, at which

stage fish that have initiated maturation might have already converted some of their energy stores into gonadal tissue, and hence show depleted lipids levels relative to migrants (Tocher 2003; Sloat and Reeves 2014). Alternatively, higher lipid levels in migrants could reflect accumulation of reserves, as either a bet-hedging strategy if resources in the migration destination are uncertain, or to fuel the migration journey itself (Stefansson *et al.* 2003). Pre-migratory "fattening" strategies are relatively common in migratory birds (Piersma *et al.* 2005) but less so in salmonines (Jonsson and Jonsson 2005).

Intrinsic factors

We had predicted that the two populations in our study would show variability in adopting migratory tactics across all food restriction scenarios and indeed, overall, the probability of smolting was higher in the anadromous background population than in the non-anadromous population. Moreover, higher hypo-osmoregulatory function (lower plasma chloride concentration) was documented in smolts from the former population relative to the latter, implying that smolts from the anadromous-background population were physiologically better prepared for transition to marine conditions. In contrast, although some fish from the non-anadromous-background population were classified as smolts in the experiment, these putative smolts exhibited relatively lower saltwater tolerance. A potential explanation for the reduced hypo-osmoregulatory function of non-anadromous-background smolts might be that they are poorly adapted to saltwater given their lack of (recent) evolutionary exposure to marine conditions. Relaxed selection leading to degradation of hypo-osmoregulation has similarly been observed in non-anadromous populations of landlocked Atlantic salmon (Nilsen *et al.* 2008; McCormick *et al.* 2019) and alewife *Alosa pseudoharengus* (Velotta *et al.* 2014, 2015). Alternatively, reduced saltwater tolerance could be evidence of an emerging migration continuum whereby putative smolts may have chosen a potamodromous (freshwater migratory) tactic and hence were unprepared physiologically for transitioning to saltwater. Nevertheless, the causal mechanisms underpinning anadromy and potamodromy are proposed to be

similar, *e.g.* reduced food availability has previously been reported to increase adfluvial migration in freshwater brown trout transplanted to streams of high population density (Olsson *et al.* 2006). All brown trout in Ireland presumably have anadromous ancestral origins, since they would have had to recolonise the island after the Last Glacial Maximum via the sea (Ferguson *et al.* 2019). It thus seems more likely that the capacity for anadromy (or at least migration), albeit somewhat deteriorated in terms of saltwater tolerance, lay dormant in the Bunaveela fish, with anadromy re-expressed under experimental conditions of energy limitation.

The putative re-emergence of an anadromous life history in our Bunaveela fish is of particular interest from a fisheries management perspective, as it suggests the capacity for anadromy (or at least migration) may lie dormant within apparently resident populations. Such populations may thus have the potential to contribute to the restoration of anadromous stocks that have experienced widespread reductions, as evidenced by Gargan *et al.* (2006) in two formerly anadromous populations that suffered collapses. Anadromous phenotypes arising from resident genotypes have similarly been documented in *O. mykiss* (Kelson *et al.* 2019), and from common garden experiments with lake resident *O. mykiss* which were formally anadromous but were prevented from migrating by impassable dams or waterfalls (Thrower *et al.* 2004). These findings make sense within the framework of the conditional threshold model (Tomkins and Hazel 2007), where environmental factors can affect life history tactic frequency by changing the distribution of the realised physiological state relative to inherited switch points (a proximate mechanism). Environmental factors could also drive longer term changes in tactic frequency via natural selection acting to shift the genotypic distribution of underlying switch points (an ultimate mechanism) (Hazel *et al.* 1990; Tomkins and Hazel 2007; Pulido 2011); for example, if survival or growth at sea is poor then migration may become less prevalent in the population if residents attain higher overall relative fitness than migrants. Within the Burrishoole system, the establishment of an Atlantic salmon farm in the estuary was implicated in the collapse of the

anadromous life history from this catchment over a period of 30 years due to high rates of sea lice transmission (Poole *et al.* 1996, 2007). Reduced marine survival rates may have imposed strong selection against anadromy, and hence caused the evolution of lower mean threshold values for freshwater maturation within the Burrishoole catchment as a whole. Our current results are consistent with this evolutionary explanation, in that we demonstrated heritable differences (or at least phenotypic differences among genetically divergent populations in a common garden experiment) – a pre-requisite for evolutionary responses. However, they also show that phenotypic plasticity can drive changes in migratory tactics, which may contribute to observed life-history changes in natural populations (Gargan *et al.* 2006; Sandlund and Jonsson 2016).

Early-life differences in length and mass between the two populations may proximately cause different anadromy propensities, as has been seen in brook trout, where size of juvenile fish was negatively related to probability of future residency (Thériault *et al.* 2007). Interestingly, though our populations differed in size early in the study (before food restriction), they were in similar condition at this time, suggesting that both populations had similar energy intake *versus* output, at least initially. Higher intrinsic growth rates in the anadromous background population may have increased the likelihood of eventual energetic limitation in freshwater, thus reducing relative condition and increasing anadromy propensity (exemplified in our *Low-Low* food treatment). Conversely, when food resources are in ample supply, high intrinsic growth rates could hasten freshwater maturity instead of anadromy in this population (*c.f.* the scenario of optimal food resources in our study). Such variability in migratory tactics is a feature of salmonines in general [*e.g.* "retirement" from anadromy in Dolly Varden *Salvelinus malma* (Bond *et al.* 2015)], which may buffer species from increasing anthropogenic pressures in the marine environment (Russell *et al.* 2012).

Conclusions

Collectively, the results of this study show that the adoption of migratory tactics in brown trout involves an interplay between inherited components and environmentally cued physiological condition, in line with previous salmonines studies (Chapman *et al.* 2012; Dodson *et al.* 2013; Kendall *et al.* 2014). The differences we observed in population responses to food restriction and its timing suggest a complex relationship between intrinsic and extrinsic factors that may allow for a continuum of migratory tactics to exist. These population differences, together with the fact that putative anadromy emerged within offspring of a naturally non-anadromous population, emphasise that a range of life-history outcomes are possible even within a single species, which can contribute to so-called portfolio effects that cushion the species as a whole from rapidly changing environmental conditions (Schindler *et al.* 2015). Although our study offers some important insight into how extrinsic and intrinsic factors interactively shape life-history tactics, we have only considered one element of the freshwater environment here. Future studies should expand to consider how other proximate drivers such as temperature, which influences a range of physiological and life-history traits in salmonines (Satterthwaite *et al.* 2010; McMillan *et al.* 2012; Sloat and Reeves 2014; Doctor *et al.* 2014; Kendall *et al.* 2014), govern migratory tactics in fish from different genetic backgrounds. Moreover, it is now important to expand this approach into natural systems using, for example, common garden or reciprocal transplant experiments, to assess whether these findings hold up under real world complexities.

Finally, our results have important implications for the conservation of facultatively migratory species, which are in global decline due to in-stream barriers, habitat degradation, climate change, overfishing and the expansion of aquaculture (Costello 2009; Limburg and Waldman 2009). Knowledge of how extrinsic and intrinsic factors affect fish migratory tactics may aid in successful management and restoration of facultatively migratory populations, and in doing so maintain important intraspecific biocomplexity, which offers increased resilience to effects of global change (Schindler *et al.* 2015).

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Supporting Information

The interplay between extrinsic and intrinsic factors in determining flexible decisions in brown trout (*Salmo trutta*): An experimental study

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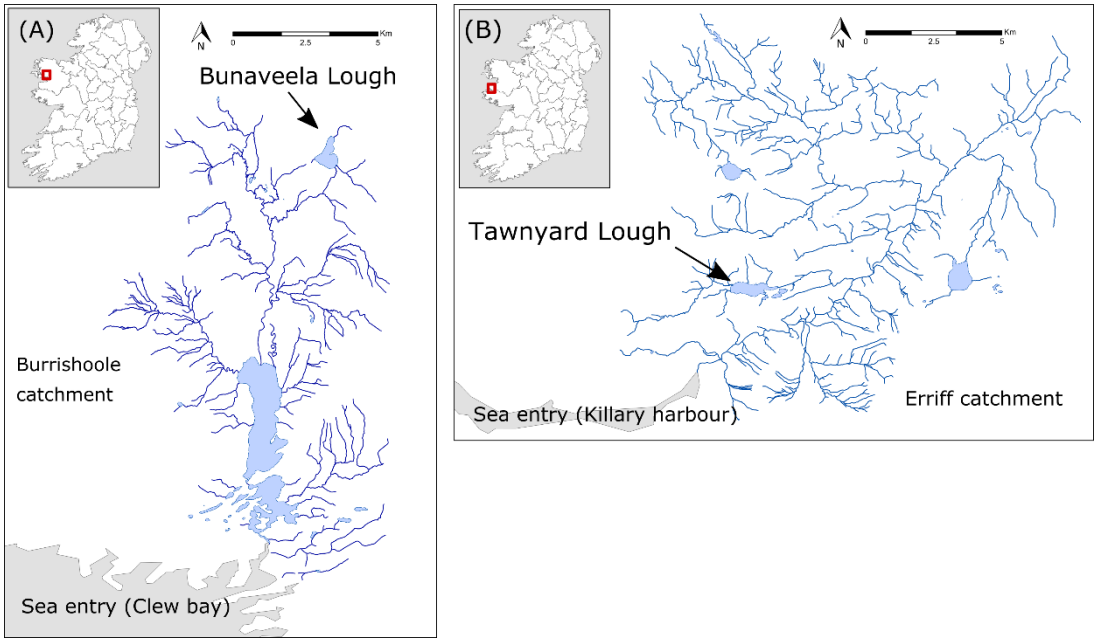

Figure S1: Locations in the west of Ireland of brown trout brood stock collected by seine netting in winter 2015, and used to produce F1 offspring for an experimental tank-rearing study. Fish with a non-anadromous-population background were offspring of brown trout collected from (a) Bunaveela Lough in the Burrishoole catchment (no natural occurrence of anadromy). Fish with an anadromous-population background were offspring of brood stock collected in (b) Tawnyard Lough in the Erriff catchment (a high natural frequency of anadromy).

Table S1: Brood stock crossing design for producing F1 offspring of wild-origin brown trout collected by seine netting from two populations (Bunaveela Lough and Tawnyard Lough) in the west of Ireland. Each female was stripped of eggs, which were then fertilised by the milt of two males from the same population and incubated in a hatchery facility within the Burrishoole catchment. Offspring produced from the Bunaveela population were considered to have a non-anadromous-population background, and offspring produced from the Tawnyard population were considered to have an anadromous-population background.

Stripping date	Population	Catchment	Sex	ID	Male cross #1	Male cross #2
22/12/2015	Bunaveela	Burrishoole	Female	BF1	BM1	BM2
22/12/2015	Bunaveela	Burrishoole	Female	BF2	BM1	BM2
22/12/2015	Bunaveela	Burrishoole	Female	BF3	BM3	BM4
22/12/2015	Bunaveela	Burrishoole	Female	BF4	BM3	BM4
22/12/2015	Bunaveela	Burrishoole	Female	BF5	BM5	BM6
22/12/2015	Bunaveela	Burrishoole	Female	BF6	BM5	BM6
22/12/2015	Bunaveela	Burrishoole	Female	BF7	BM7	BM8
22/12/2015	Bunaveela	Burrishoole	Female	BF8	BM7	BM8
22/12/2015	Bunaveela	Burrishoole	Female	BF9	BM9	BM10
22/12/2015	Bunaveela	Burrishoole	Female	BF10	BM9	BM10
22/12/2015	Bunaveela	Burrishoole	Female	BF11	BM11	BM12
22/12/2015	Bunaveela	Burrishoole	Female	BF12	BM11	BM12
22/12/2015	Bunaveela	Burrishoole	Female	BF13	BM13	BM15
22/12/2015	Bunaveela	Burrishoole	Female	BF14	BM13	BM15
22/12/2015	Bunaveela	Burrishoole	Female	BF15	BM17	BM18
22/12/2015	Bunaveela	Burrishoole	Female	BF16	BM17	BM18
22/12/2015	Bunaveela	Burrishoole	Female	BF17	BM19	BM20
22/12/2015	Bunaveela	Burrishoole	Female	BF18	BM19	BM20
22/12/2015	Bunaveela	Burrishoole	Female	BF19	BM22	BM23
22/12/2015	Bunaveela	Burrishoole	Female	BF20	BM22	BM23
22/12/2015	Bunaveela	Burrishoole	Female	BF21	BM25	BM26
27/11/2015	Tawnyard	Erriff	Female	TF4	TM6	TM7
27/11/2015	Tawnyard	Erriff	Female	TF5	TM6	TM7
27/11/2015	Tawnyard	Erriff	Female	TF6	TM9	TM10
10/12/2015	Tawnyard	Erriff	Female	TF7	TM12	TM13

Table S2: Feeding guidelines (as % body mass fed per day), used to adjust absolute daily feeding rations on a monthly basis according to body mass and rearing temperature of brown trout reared in a recirculating aquaculture system for 22 months. Type of feed (based on fish size) is listed in italics (Skretting, Norway).

Fish mass (g)	**Rearing temperature**							
	4 °C	**6 °C**	**8 °C**	**10 °C**	**12 °C**	**14 °C**	**16 °C**	**18 °C**
Nutra Parr								
2 - 6.5g	1.6	1.9	2.2	2.6	3	3.4	3.8	NA
6.5 - 8g	1.5	1.8	2.1	2.5	2.9	3.3	3.7	NA
Horizon								
8 - 12g	1.21	1.53	1.73	2.1	2.42	2.78	3.06	3.57
12 - 21g	1.21	1.36	1.57	1.87	2.16	2.55	2.84	3.34
Elite FR								
21 - 35g	1.22	1.47	1.71	1.95	2.2	2.57	2.93	3.42
35 - 80g	0.96	1.2	1.32	1.56	1.8	2.16	2.4	2.76
80 - 150g	0.68	0.88	1.08	1.28	1.48	1.68	1.88	2.08
150 - 300g	0.52	0.72	0.92	1.12	1.32	1.48	1.64	1.8
300 - 500g	0.46	0.61	0.76	0.94	1.1	1.24	1.38	1.52
500g +	0.34	0.48	0.62	0.76	0.89	1.02	1.16	1.3

Table S3: Parameter estimates with associated standard errors (SE) for mixed model analyses testing the effects of life-history tactics, population-background, food treatment, and sex on the fork length (cm), weight (g), and condition factor (*K*) trajectories of brown trout over two years of experimental tank rearing. Measurements were taken at key periods denoted by "month". Effects were contrasted against mature female fish from the anadromous-background in the *Low-Low* treatment. Statistical significance was assessed at $p < 0.05$.

Effect	Estimate	SE	t-value	*p*-value
Mixed model for length				
Intercept	7.103	0.387	18.336	< 0.001
Month	0.699	0.013	54.865	< 0.001
Life-history: Smolt	-2.310	0.596	-3.876	< 0.001
Population: Non-anadromous-background	-0.252	0.438	-0.576	0.565
Food: Low-High	3.322	0.429	7.736	< 0.001
High-Low	2.646	0.415	6.382	< 0.001
High-High	5.434	0.425	12.798	< 0.001
Sex: Male	-0.033	0.215	-0.154	0.878
Month: Smolt	0.067	0.032	2.068	0.039
Non-anadromous-background: Low-High	-0.774	0.600	-1.289	0.198
Non-anadromous-background: High-Low	-2.382	0.630	-3.781	< 0.001
Non-anadromous-background: High-High	-3.066	0.594	-5.160	< 0.001
Mixed model for weight				
Intercept	-73.06	8.11	-9.01	< 0.001
Month	9.47	0.28	34.24	< 0.001
Life-history: Smolt	22.61	12.69	1.78	0.075
Population: Non-anadromous-background	-1.36	9.07	-0.15	0.881
Food: Low-High	76.57	8.87	8.63	< 0.001
High-Low	37.75	8.56	4.41	< 0.001
High-High	114.86	8.78	13.09	< 0.001
Sex: Male	-0.99	4.45	-0.22	0.824
Month: Smolt	-3.08	0.70	-4.39	< 0.001
Non-anadromous-background: Low-High	-19.85	12.43	-1.60	0.111
Non-anadromous-background: High-Low	-38.59	13.05	-2.96	0.003
Non-anadromous-background: High-High	-57.67	12.32	-4.68	< 0.001
Mixed model for condition factor (Fulton's K)				
Intercept	1.193	0.016	76.858	< 0.001
Month	-0.004	0.001	-5.341	< 0.001
Life-history: Smolt	-0.001	0.027	-0.025	0.980
Population: Non-anadromous-background	0.023	0.016	1.430	< 0.001
Food: Low-High	0.123	0.016	7.923	< 0.001
High-Low	0.070	0.015	4.746	< 0.001
High-High	0.139	0.015	9.034	< 0.001

Sex: Male	0.002	0.008	0.285	0.776
Month: Smolt	-0.006	0.002	-3.564	< 0.001
Non-anadromous-background: Low-High	-0.036	0.022	-1.644	0.101
Non-anadromous-background: High-Low	-0.081	0.023	-3.461	0.001
Non-anadromous-background: High-High	0.041	0.022	1.838	0.067

Table S4: Parameter estimates with associated standard errors (SE) for mixed model analysis testing the effects of life-history tactics, population-background, food treatment, and sex on the final fork length (cm), condition factor (*K*) and whole body lipids (%) of brown trout after two years of experimental tank-rearing. Effects were contrasted against mature female fish from the anadromous-background in the *Low-Low* treatment. Statistical significance was assessed at $p < 0.05$.

Effect	df	Estimate	SE	t-value	*p*-value
Response: Length					
Intercept	437	20.00	0.41	48.58	< 0.001
Life-history: Smolt	437	-0.59	0.35	-1.66	0.097
Population: Non-anadromous-background	437	0.38	0.54	0.70	0.487
Food: Low-High	437	5.06	0.44	11.39	< 0.001
High-Low	437	2.41	0.55	4.42	< 0.001
High-High	437	6.83	0.54	12.56	< 0.001
Sex: Male	437	0.01	0.22	0.07	0.947
Non-anadromous-background: Low-High	437	-1.30	0.63	-2.05	0.040
Non-anadromous-background: High-Low	437	-2.14	0.75	-2.86	0.004
Non-anadromous-background: High-High	437	-3.90	0.76	-5.17	< 0.001
Response: Condition factor (Fulton's K)					
Intercept	437	1.079	0.018	60.095	< 0.001
Life-history: Smolt	437	-0.121	0.015	-7.948	< 0.001
Population: Non-anadromous-background	437	0.061	0.024	2.559	0.011
Food: Low-High	437	0.195	0.019	10.212	< 0.001
High-Low	437	0.048	0.024	2.007	0.045
High-High	437	0.192	0.024	8.063	< 0.001
Sex: Male	437	-0.017	0.009	-1.831	0.068
Non-anadromous-background: Low-High	437	-0.066	0.027	-2.439	0.015
Non-anadromous-background: High-Low	437	-0.078	0.032	-2.404	0.017
Non-anadromous-background: High-High	437	0.025	0.033	0.742	0.458
Response: Whole body lipids					
Intercept	144	5.38	0.21	26.21	< 0.001
Life-history: Smolt	144	0.51	0.19	2.69	0.008
Population: Non-anadromous-background	9	2.59	0.29	8.83	< 0.001
Food: Low-High	144	3.18	0.29	11.07	< 0.001

High-Low	144	0.84	0.27	3.17	0.002
High-High	144	3.31	0.32	10.29	< 0.001
Sex: Male	144	-0.25	0.17	-1.43	0.155
Non-anadromous-background: Low-High	144	-1.41	0.42	-3.39	0.001
Non-anadromous-background: High-Low	144	-2.09	0.48	-4.32	< 0.001
Non-anadromous-background: High-High	144	-1.36	0.65	-2.10	0.037

Chapter 3

Food and temperature stressors have opposing effects in determining flexible migration decisions in brown trout (*Salmo trutta*)

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Abstract

In an era of rapid global change, organisms in natural systems are exposed to a multitude of stressors that are likely to co-occur, with uncertain impacts. Here, we explore the individual and cumulative effects of co-occurring environmental stressors on the striking, yet poorly understood, phenomenon of facultative migration. We reared offspring of a brown trout population that naturally demonstrates facultative anadromy (sea-migration), under different environmental stressor treatments and measured life-history responses in terms of migratory tactics and freshwater maturation rates. Juvenile fish were exposed to reduced food availability, temperatures elevated to 1.8 °C above natural conditions, or both treatments in combination over 18 months of experimental tank rearing. When considered in isolation, reduced food had negative effects on the size, mass and condition of fish across the experiment, and we detected variable effects of warm temperatures (negative effects on size and mass, but positive effect on lipids). However, when combined with food restriction, temperature effects on these traits were less pronounced, implying antagonistic dual stressor effects on morphological traits. The two stressors combined additively, but had opposing effects on life-history tactics: migration increased and maturation rates were lower under low food conditions, whereas the opposite was true in the warm temperature treatment. Not all fish had expressed maturation or migration tactics by the end of the study, and the frequency of these "unassigned" fish was higher in food restriction treatments, but lower in warm treatments. Fish choosing migration over freshwater maturation tended to be smaller and in poorer condition (but were similarly sized to unassigned fish). We further detected effects of food restriction on hypo-osmoregulatory function of migrants that may influence the fitness benefits of the migratory tactic at sea. We also highlight that responses to multiple stressors may vary depending on the response considered. Collectively, our results indicate contrasting effects of environmental stressors on life-history trajectories in a facultatively migratory species.

Introduction

Against the backdrop of rapid global change, organisms are increasingly exposed to a variety of pressures stemming from anthropogenic activities (Sanderson *et al.* 2002). Temperature increases, habitat degradation, pollution, exploitation, and land use changes are examples of pressures, or "stressors", that contribute to recent patterns of population and biodiversity decline, and altered ecosystem functioning (Walther *et al.* 2002; Parmesan 2006). While much research has addressed the effects of individual stressors, in practice, stressors rarely occur in isolation, and it is imperative we also understand the combined effects of multiple stressors (Breitburg *et al.* 1998) in order to better forecast and manage species' responses to global change (Côté *et al.* 2016). This is not necessarily straightforward however, with co-occurring stressors – defined here as biotic or abiotic changes beyond the range typically experienced under natural conditions (Breitburg *et al.* 1999; Crain *et al.* 2008) – potentially acting additively, synergistically, or antagonistically (Folt *et al.* 1999; Crain *et al.* 2008).

Although a growing body of research is now expanding beyond single-stressor approaches, uncertainty still surrounds the net effects of co-occurring stressors, and empirical studies have provided mixed results. Meta-analyses suggest that synergistic effects dominate in the marine sphere (Crain *et al.* 2008; Harvey *et al.* 2013; Przeslawski *et al.* 2015), but antagonistic effects (Byrne and Przeslawski 2013) and additive effects (O'Gorman *et al.* 2012) have also been reported. In freshwater systems, which are particularly sensitive to multi-faceted change (Ormerod *et al.* 2010), antagonistic effects of multiple stressors are most prevalent (Jackson *et al.* 2016).

The effects of stressors may be highly context-specific. For example, increased temperature is a stressor likely to be experienced almost universally across natural systems, yet the impacts of warming at the individual level can range from positive to negative depending on whether optimum performance temperatures are exceeded (Huey and Stevenson 1979; Sinclair *et al.* 2016). Moreover, stressor effects can differ depending on the trait/response, or the level of organisation considered *e.g.* warming can increase individual metabolic

and feeding rates, but may reduce survival, cause population/species extinctions (Petchey *et al.* 1999; Fussmann *et al.* 2014) or alter community stability due to long-term changes in species' interaction strengths (Rall *et al.* 2010). Predicting stressor effects at multiple levels is likely to be additionally challenging when more than one stressor is involved (Galic *et al.* 2018).

An understudied aspect of multiple stressors is how effects at the individual level shape life-history trajectories, which in turn may mediate how stressors scale up to influence higher-level (*e.g.* population, community, ecosystem) processes. One fundamental decision that many animals face, which is associated with a broad range of life-history and eco-evolutionary consequences, is whether to migrate or not. Facultative migration – the phenomenon whereby individuals retain the capacity to adopt either a migratory or a non-migratory life-style – is common across many animal taxa (Lack 1943; Swingland and Greenwood 1984; Lundberg 1987; Kaitala *et al.* 1993; Chapman *et al.* 2011), with well documented examples in birds (Berthold and Querner 1982; Lundberg 1988; Pulido *et al.* 1996; Newton 2008), ungulates (Ball *et al.* 2001; Cagnacci *et al.* 2011; Hebblewhite and Merrill 2011), zooplankton (Hansson and Hylander 2009), and fishes (Northcote and Ward 1985; Jonsson 1985; Chapman *et al.* 2012; Dodson *et al.* 2013). Environmental stresses such as limited food or inclement temperatures often appear to play a role in driving individuals to migrate (Chapman *et al.* 2012). Alternative migratory phenotypes have often been considered within the framework of the "environmentally cued threshold model" (Tomkins and Hazel 2007; Piche *et al.* 2008; Pulido 2011; Buoro *et al.* 2012), in which tactic frequencies are controlled by the relationship between an environmentally-sensitive status trait (*e.g.* physiological condition or energy status) and an inherited threshold, assumed to be genetically variable. If the status trait exceeds the threshold, or "switch point", residency occurs; if not, migratory tactics are adopted. Environmental factors are likely to be important drivers of migratory tactics at both proximate and ultimate levels, yet few studies have addressed how facultatively migratory species respond to

pressures arising from environmental change (Doswald *et al.* 2009), either in isolation, or when stressors act in combination.

Salmonine fishes (trouts, salmons, and charrs) represent an excellent group to study multiple stressor effects (McGinnity *et al.* 2009; de Eyto *et al.* 2016). In facilitating obligate freshwater spawning, salmonines display a suite of migratory phenotypes, encompassing residents that remain in natal streams their entire lives, individuals that migrate to larger rivers or lakes (potomodromy), and others still that undertake a marine migration before returning to fresh water to spawn (anadromy) (Klemetsen *et al.* 2003; Dodson *et al.* 2013; Ferguson *et al.* 2019; Nevoux *et al.* 2019). The migration *versus* residency decision represents a trade-off, where the benefits of migration (avoiding abiotic or biotic stresses in the natal stream, exploiting better food resources in the new environment, which translate into higher growth and thus higher fecundity or mating success) must be balanced against the costs (energetic demands of migration, physiological stress of changing environments/habitats, a potentially increased risk of predation) (Kendall *et al.* 2014). Environmental conditions in natal fresh waters can interact with intrinsic physiological traits to determine alternative migratory tactics, *e.g.* if food resources cannot support growth or metabolism in early life, the resulting energetic deficit may promote migration. Food resources have been shown to directly (Davidsen *et al.* 2014; Jones *et al.* 2015; Archer *et al.* 2019) and indirectly influence migration (Olsson *et al.* 2006; Wysujack *et al.* 2009; O'Neal and Stanford 2011). Similarly, because temperature profoundly influences biological processes (Gillooly *et al.* 2001; Dell *et al.* 2011), temperature effects on physiological status traits/energetic balance likely make it an important environmental determinant of migratory decisions. Higher temperatures have been associated with increased anadromy *in lieu* of maturation in steelhead trout *Oncorhynchus mykiss* (Sloat and Reeves 2014), but under experimental conditions of constant food supply. Warming is likely to be accompanied by reductions in freshwater macroinvertebrate abundance (Durance and Ormerod

2007), with potentially synergistic effects if elevated metabolic demands induced by warming are compounded by low food availability.

Environmental stressors may also act to affect the performance of individuals once a migratory decision has been made. Although migration potentially confers many benefits on individuals, the ensuing costs mean that environmental conditions experienced prior to out-migration might further affect the future success of migrants, both during migration and in the subsequent environment (river, lake, or sea) (McCormick *et al.* 2009a). Sea-migration in particular requires substantial physiological remodelling for transitioning to salt water, and the changes in physiological regulation of ions, colouration, and body shape (collectively termed "smolting", (Tanguy *et al.* 1994)) necessitate an expensive investment by individuals that is likely to begin long before the migration is initiated, and hence may be affected by environmental stressors. Empirical evidence suggests that once the migratory decision is made, migrants divert resources towards accelerated growth (Metcalfe 1998). Smolt survival at sea is positively related to size (Ward and Slaney 1988) and, as such, favourable freshwater conditions may produce larger and more successful migrants, with associated fitness benefits altering the migration-residency trade off.

Here, we present the results of an experimental laboratory study of physiology, migration and maturation of brown trout, using the F1 progeny of wild-caught parents from a population that exhibits facultative migration in nature. We aimed to explore if, and how, life-history decisions are shaped by individual and interactive effects of two putative extrinsic environmental stressors. Specifically, we aimed to: (i) determine the effects of food restriction and elevated temperature on a range of physiological traits (size, mass, condition, lipids); (ii) assess the life-history consequences, in terms of migratory tactics and maturation decisions, of these stressors (both in isolation and combined); and (iii) explore how environmental stressors affect future migratory performance. We expected that food reduction and elevated temperature would each increase the prevalence of migratory tactics, with potentially synergistic

effects when the stressors were combined. We also expected stressors to negatively affect future migratory capacity of anadromous individuals by reducing potential for fast growth (and thus reducing smolt viability), or by inhibiting osmoregulation in salt water.

Materials and Methods

Study population and fish rearing

Brown trout brood stock from a wild population were caught by seine-netting in November 2015 in Tawnyard Lough, an upland lake of 56 ha in the Erriff catchment (National Salmonid Index Catchment) in the west of Ireland (53° 37′ 0.00" N: 09° 40′ 17.10" W). Tawnyard Lough is fed primarily by the Glendavoch river, and a number of smaller tributaries (Figure S1). Brown trout primarily spawn within the Glendavoch River and move downstream as fry or parr to Tawnyard Lough, a distance of a few hundred metres to kilometres, depending on where spawning occurred. Tawnyard Lough produces a large run of out-migrating anadromous juveniles (smolts), with annual estimates of 500 to 3000 smolts enumerated at the outflow of the Lough over the last 30 years (Gargan *et al.* 2016). An unknown proportion of the population remain within the lake, and undergo several years of freshwater growth before returning to the natal stream to spawn. While the migration phenotypes of the brood stock could not be determined unambiguously in this study (because external signs of prior migration are not completely unambiguous in this system), we assumed that the frequencies of migratory/resident phenotypes among our brood stock was broadly representative of naturally occurring frequencies, given that brood stock were obtained haphazardly. Moreover, our goals in this study were not to test explicitly for inherited variation in migration tactics, but rather to explore proximate (environmental) drivers. The *caveat* must be kept in mind, however, that any environmental effects we document are contingent on the genotypic composition of our sample.

Each ripe female (n = 7) was stripped of eggs, which were then split into two batches, and each batch was fertilised by the milt of a single male (n = 10) (*i.e.* each female was mated to two males, creating fourteen full-sib families). Due to

constraints in obtaining brood stock and variation in the timing of when females were ripe to be stripped, eggs were fertilised on three occasions in November and December 2015 (see Table S1 fertilisation/ brood stock crossing details). Fertilised eggs from each crossing were incubated separately in standard Heath trays in the nearby Burrishoole hatchery. Prior to exogenous feeding, fry from the late November/early December fertilisations (hereafter "Nov-Dec fertilisation group") were transferred to a rearing facility at University College Cork (Aquaculture and Fisheries Development Centre). Here, families were mixed, and fry were held in a 100 L growth tank on a recirculating aquaculture system (RAS), maintained by a conditioning unit at a seasonally varying temperature regime approximating that of the catchment from which the brood stock were obtained (i.e. the annual cycle in mean weekly temperatures in the nearby Burrishoole catchment for the preceding three years, see Figure S2). Note that in winter 2015, simulated water temperatures were higher than the source catchment due to initial logistical constraints in achieving low temperatures. Fry were fed *ad libitum* with commercially available trout pellets (Skretting Ltd., Norway) to facilitate their transition to exogenous feeding (by June 2016), and were then fed *ad libitum* until the experimental phase began. Fish experienced a constant photoperiod (12:12 hours of light: dark) during this initial rearing phase. Due to logistical constraints, an additional group of fry from the first fertilisation event (termed "Nov fertilisation group") was maintained at a natural temperature regime in a flow-through tank at the Burrishoole hatchery facility, where they transitioned to exogenous feeding via *ad libitum* feeding with the same trout pellets. Fry from the Nov group were transported to the UCC rearing facility in May 2016, and reared in a 100 L tank, in the same RAS and under the same conditions as the Nov-Dec fertilisation group. Due to size differences (Nov group fish were larger than Nov-Dec group fish by the beginning of the experiment) the two fertilisation groups were reared in separate tanks for the duration of the study to prevent emergence of feeding hierarchies.

Experimental treatments

The study and all associated procedures were carried out with ethical approval from Health Products Regulatory Authority (HPRA) Ireland, under HPRA project license AE19130/P034, and individual licenses AE19130/1087, AE19130/I200, AE19130/I201 and AE19130/I202). The experimental phase ran for an 18-month period from December 2016 to June 2018 with all fish humanely euthanized at the end of the experiment.

Juvenile brown trout were randomly allocated across eight 520L tanks at the end of November 2016 (initial n = 140 per tank for Nov-Dec group, and n = 35 per tank (filled to 203L) for Nov group), each assigned one of two temperature treatments and one of two food treatments. Water flowed continually through tanks to prevent the build-up of waste, returned to a central sump via mechanical filtration, and was treated with protein skimming, biofiltration and UV skimming. Weekly monitoring of water quality indicated that levels of pH, nitrate, nitrite, and ammonia were well within acceptable ranges for fish health. Mortality during the experimental phase was minimal (4%). To avoid compromising water quality with excessive biomass, fish were culled haphazardly (n = 229 in total across all treatments) over the two years of tank rearing, with equal densities in terms of fish numbers (fish per L) maintained across treatment groups and equal biomass densities (g per L) maintained between fertilisation groups. Fish culled in this manner were not included in the analyses.

To explore the individual and interactive effects of food restriction and temperature in determining migratory tactics, food and temperature treatments were applied in isolation and in combination for both fertilisation groups for the duration of the experimental phase. The two food treatments were: (i) high food treatment*:* fish fed recommended daily rations for optimal growth calculated as a percentage of their body mass and adjusted for seasonally-changing temperatures (Skretting Ltd., Norway); and (ii) low food treatment: fish fed 25% of optimal daily rations. A value of 25% of optimal daily rations was chosen for the low food treatment because similar restrictions have

previously been shown to reduce the frequency of residency in adfluvial brown trout (Wysujack *et al.* 2009). Food was dispensed during daylight hours via automatic feeders (Arvo-Tec Oy, Huutokoski, Finland) located above each tank. Feeders delivered regular pulses of food (lasting two seconds), with the frequency of pulses adjusted according to food treatment (i.e. fewer pulses for low food treatments). The two temperature regimes were achieved by passing water through one of two conditioning units that maintained two temperature treatments as follows : (i) cool temperature treatment: temperatures matching the natural, seasonally-varying, temperature regime for the Erriff catchment; and (ii) warm temperature treatment: temperatures elevated by 1.8 °C ± 0.55 (SD) above the cool temperature treatment. The cool treatment ranged from 5.9 - 16.4 °C (mean temperature = 10.9 °C ± 3.2 SD) and the warm treatment ranged from 7.5 - 18.2 °C (mean temperature = 12.7 °C ± 3.2 SD). An increase of 1.8 °C for the warm temperature treatment was chosen because this is in line with increases of 1 - 3 °C projected to occur under climate change scenarios (IPCC 2014). While both treatments remained within sub-lethal ranges for brown trout (Forseth *et al.* 2009; Jonsson and Jonsson 2009), the warm temperature treatment was considered "stressful" because the maximum temperatures in the warm treatment approached upper thermal growth limits for brown trout (18.7 °C). Optimal temperatures for growth have been estimated to be between 13.1 – 13.9 °C (Elliott and Hurley 2000; Hari *et al.* 2006; Elliott and Elliott 2010; Kovach *et al.* 2016). Temperatures in the warm treatment remained above this for twice as long as those in the cool treatment, which remained closer to optimal growth conditions. Food rations were reduced over four weeks and temperature was increased by 0.5 ºC per week to minimise stress. Within each treatment, absolute rations were adjusted on a monthly basis to account for changes in body mass and temperature.

Data collection and life-history determination

Within each food and temperature treatment combination, 25 – 30 fish per fertilisation group were lightly anaesthised with MS-222 and marked with unique colour combinations of visible implant elastomer (VIE) tags (Northwest

Marine Technology Ltd., Washington, USA), allowing for re-identification of individuals. Fork length and mass were measured at key periods throughout the study [Late-November in 2016 (prior to initiation of treatments), February, April, June, July, September, December in 2017, and April in 2018].

All fish were checked weekly for morphological indicators of smolting from March to June in each of 2017 and 2018, the period corresponding to the natural migratory period in the wild for this population (Gargan *et al.* 2016). Wild smolts typically migrate out of the Erriff system aged between 1+ and 4+, with the large majority doing so aged 2+ or 3+ (approximately equal numbers of each) (Gargan *et al.* 2016). Smolting is a precursor to downstream migration in several salmonines, and comprises a number of morphological, behavioural and physiological changes. We used the following morphological indicators to assess smolting [following Tanguy *et al.* 1994)]: silvering/loss of parr marks; pronounced lateral line (*i.e.* clearly visible and raised to the touch); transparent fins; and fusiform shape (pointed snout, slimmer body, and elongated caudal peduncle) (Riddell and Leggett 1981; Hard *et al.* 1999; Villar-Guerra *et al.* 2019). Fish that clearly matched three of these criteria were classed as smolts. In spring 2017, no fish matched the morphological criteria for smolts. In spring 2018, fish that matched the morphological criteria for smolts were transferred to salt water at 30 ppt for 24 hours to assess hypo-osmoregulatory function. Seawater "challenges" are used as an indicator of anadromy capacity, where the ability to regulate ion concentrations (*e.g.* to maintain plasma chloride concentration) in sea water is a measure of saltwater tolerance, or physiological "readiness" of smolts for seawater entry (Clarke 1982; McCormick *et al.* 1998; Schultz and McCormick 2012; McCormick 2012). Fish were monitored during this period to ensure that any fish showing signs of failing the challenge (loss of equilibrium) could be removed and euthanised (though no fish showed signs of failure in our study). After the 24-hour seawater challenge, fish were euthanised with an overdose of MS-222. The mass and fork length of each individual was recorded, and a blood sample was obtained from the caudal vasculature using a 21 gauge needle and a 2.6ml heparinised syringe. Blood samples were centrifuged at

8000 rpm for 3 minutes, and the plasma aliquot was siphoned off, stored at -80 °C and later measured for plasma chloride concentration as an indicator of osmoregulatory performance. Four to six gill filaments were placed in 100µl of ice-cold SEI buffer (150 mmol l^{-1} sucrose, 10 mmol l^{-1} EDTA, 50 mmol l^{-1} imidazole, pH 7.3) and frozen at -80 °C for later measurement of gill Na^+/K^+-ATPase (NKA) activity.

All fish (classed morphologically as smolts or non-smolts) were dissected in spring 2018 to visually determine sex and maturation status based on gonad development. Mature males had enlarged white testes or running milt. Maturing males had visible or moderately enlarged testes but no running milt. Mature females had visible eggs in the body cavity. Immature fish (unconfirmed sex at the time of sampling) were later genotyped to determine genotypic sex using a microsatellite sex marker. The natural spawning period for the wild population-of-origin is in late autumn/early winter, and the migratory period is in the spring (Gargan *et al.* 2016). Since freshwater maturation generally precludes migration in brown trout (Jonsson 1985; Dellefors and Faremo 1988; Dębowski and Dobosz 2016) any fish showing signs of maturing without having migrated to sea are considered to be on a non-anadromous trajectory, while smolts which undertake marine migrations are immature. We thus classed fish as smolts (migratory tactic) if they matched the morphological criteria for smolts and were immature. Fish were classed as mature (residency tactic) if they showed signs of maturation at the time of sampling. Fish that were immature and did not match the morphological criteria for smolts had an unknown life history at the time of sampling and were classed as "unassigned". Whole body lipid content (%) was measured for all smolts and a random sample of mature (n = 107) and unassigned (n =19) fish using a CEM Smart trac5 system of integrated heating and nuclear resonance (CEM Corporation, Matthews, NC, USA) on individual homogenised fish samples (Toussaint *et al.* 2002; Keeton *et al.* 2003; Nielsen *et al.* 2005). Plasma chloride concentration was measured by coulometric titration using a Jenway PCLM3 chloride meter (FishVet Group, Oranmore, Ireland) for all smolts and a random sample of non-smolts (n = 107

mature fish and n = 18 unassigned fish). Gill NKA activity was measured following McCormick *et al.* (2009) for a random sample of smolts and non-smolts (n = 25 smolts, n = 135 mature fish and n = 22 unassigned fish).

Statistical analysis

To test if food and temperature acted as stressors at the level of individual traits underpinning migration (Aim 1), we explored factors affecting fork length, mass and condition of fish across the study period within a mixed effects modelling framework (*nlme* package (Pinheiro *et al.* 2019)). We calculated condition factor as:

$$Condition\ factor\ = \frac{mass\ (g)}{fork\ length\ (cm)^{b}} \times 100$$

Where *b* is the slope estimated from the linear relationship between log (mass) and log(fork length) (Bolger and Connolly 1989). The mixed effects models included time (continuous variable representing weeks since start of experiment), a quadratic term for time (to account for non-linearity of traits through time), food treatment, temperature treatment, fertilisation group, and sex as fixed effects, and individual identity as a random effect to account for multiple measurements on some individuals. We included an interaction between food treatment and temperature treatment (to test for synergistic or antagonistic effects of food and temperature), and a three-way interaction (food treatment × temperature treatment × time) to test whether trajectories diverged through time according to treatment combination. To compare single stressor effects with combined stressor effects, we carried out pairwise comparisons across all levels of the food × temperature interaction using Tukey post-hoc tests (*emmeans* package (Lenth 2019)).

To test whether trait trajectories were similar in mature fish and smolts, we created additional mixed effects models with time, a quadratic term for time, migratory tactics (categorical variable, two levels: smolt/mature), sex, and fertilisation group as fixed effects, an interaction between time and migratory tactics, and a random effect of individual identity. We excluded unassigned fish

in this comparison of "status" traits, as we could not determine their life-history trajectory, *i.e.* some of the unassigned fish may have been on a migratory trajectory, but were deferring actual migration until a later age. For all of the above models, temporal autocorrelation of the response variable was accounted for by modelling an autoregressive error structure as a first order lag function of time. Separate models were constructed for z-standardised measures of length, mass and condition, and normal errors were assumed in each case.

We similarly used mixed effects models (normal errors) to explore factors influencing variation in final measurements of traits (z-standardised length, condition, and whole body lipids) at the end of the study. We included food treatment, temperature treatment, fertilisation group, sex, and an interaction between food treatment and temperature treatment as fixed effects, and date of terminal sample (categorical variable with 8 sampling dates) as a random effect. Additional mixed effects models tested for differences in final measurements of status traits according to migratory tactics (migratory tactic, sex and fertilisation group included as fixed effects, and terminal sample date as a random effect).

To test if food and temperature treatments affected life-history tactics (Aim 2), we built three generalized linear models (GLMs) with a logit-link function and binary life-history response variables. One GLM predicted smolt status (binary response: 1 = smolt, 0 = non-smolt), a second GLM predicted maturation (binary response: 1 = mature, 0 = immature), and a third GLM tested for differences in expression of any life-history tactics by the end of the study (*i.e.* by age 2+, second year of life) (binary response: 1 = unassigned, 0 = smolt or mature). All GLMs included the categorical variables: food treatment, temperature treatment, sex, fertilisation group, and an interaction between food and temperature treatments.

To explore variation in osmoregulatory performance, we first tested for differences in gill NKA activity (log transformed) and plasma chloride concentration between smolts and non-smolts using mixed effects models (normal errors). Each model included life-history tactic, fertilisation group, and

sex as fixed effects, and terminal sampling date as a random effect. We retained the "unassigned" life-history class in these analyses to determine if unassigned fish showed signs of hypo-osmoregulatory capacity in salt water relative to mature fish, suggesting that these unassigned fish were in fact on a smolting trajectory but were yet to express morphological indicators of smolting. Post-hoc pairwise comparisons between life-history tactics were carried out using the *emmeans* package (Lenth 2019).

We explored the mechanisms underpinning osmoregulatory capacity by fitting a GLM (normal errors) to gill NKA activity as a function of size-corrected plasma chloride concentration in smolts and non-smolts. Because hypo-osmoregulatory capacity generally increases with size in brown trout (Finstad and Ugedal 1998), we corrected for size in this analysis by using the residuals of the linear relationship between plasma chloride and fork length. Finally, we assessed how food and temperature treatments influenced osmoregulatory capacity of smolts (Aim 3) using mixed effects models. Separate models (normal errors) were constructed for z-standardised gill NKA activity and plasma chloride concentration, with food treatment, temperature treatment, sex, fertilisation group, fork length (a covariate to correct for body size effects), and a food × temperature interaction included as fixed effects, and terminal sample date as a random effect.

Marginal R^2 values for mixed effect models were calculated using the *MuMIn* package in R (Barton 2019). We used likelihood ratio tests (LRT) to assess statistical significance of predictor variables for all models at a 5% alpha level, and non-significant interaction terms were excluded to interpret main effects. Analysis was carried out in R version 3.5.3 (R Core Team 2019), and all models were checked against assumptions of the given model (independence, non-normality of residuals, heteroscedasticity and multicollinearity).

Results

At the termination of the experiment, 349 fish were assigned a life-history tactic (30 smolts and 319 mature fish) and 76 fish were classed as "unassigned" (Table

1). The frequency of smolting varied by food and temperature treatments. Overall, the proportion of smolts (averaged across fertilisation groups) was highest in the low food–cool temperature treatment (18.9 %), with the lowest proportion in the high food–warm temperature treatment (0.9 %), and intermediate proportions in low food–warm temperature (4.6 %) and high food–cool temperature treatments (3.8 %).

The proportion of mature fish (averaged across fertilisation groups) was highest in the high food–warm temperature treatment (92.5 %), followed by similar proportions in high food–cool temperature (75.2 %) and low food–warm temperature treatments (75.0 %). Maturation was lowest in the low food-cool temperature treatment (57.6 %).

Table 1: Percentage of brown trout (n = 425), F1 offspring of wild trout, classed as smolts (i.e. migratory tactic) or non-smolts (mature or unassigned) after two years of experimental tank-rearing. Offspring were derived from brood stock gametes fertilised in November and December 2015 (coded here as early November = "Nov" and late November/early December = "Nov-Dec"). Values correspond to percentages for each category (broken down by sex) of the total number of fish per tank (where each tank corresponds to a given food treatment by temperature regime combination, i.e. a single row in the table). Sample size (n) given in brackets after the %.

Food, Temperature	**Fertilisation Group**	**% Smolts (n)**		**% Mature (n)**		**% Unassigned (n)**	
		Female	*Male*	*Female*	*Male*	*Female*	*Male*
Low, Cool	Nov	6.1 (2)	3.0 (1)	48.5 (16)	6.1 (2)	6.1 (2)	30.3 (10)
Low, Cool	Nov-Dec	15.1 (11)	8.2 (6)	19.2 (14)	39.7 (29)	8.2 (6)	9.6 (7)
Low, Warm	Nov	0 (0)	0 (0)	51.5 (17)	36.4 (12)	3.0 (1)	9.9 (3)
Low, Warm	Nov-Dec	2.7 (2)	4.0 (3)	41.3 (31)	28.0 (21)	2.7 (2)	21.3 (16)
High, Cool	Nov	2.9 (1)	0 (0)	32.4 (11)	32.4 (11)	5.9 (2)	26.5 (9)
High, Cool	Nov-Dec	2.8 (2)	1.4 (1)	43.7 (31)	36.6 (26)	2.8 (2)	12.7 (9)
High, Warm	Nov	0 (0)	0 (0)	61.8 (21)	23.5 (8)	2.9 (1)	11.8 (4)
High, Warm	Nov-Dec	1.4 (1)	0 (0)	48.6 (35)	47.2 (34)	0 (0)	2.8 (2)

Morphological trait trajectories

Physiological trait trajectories diverged through time in response to food treatment, temperature treatment, and fertilisation group (Figure 1A, Table 2). The models for length (marginal $R^2 = 0.68$) and condition factor (marginal $R^2 = 0.33$) retained significant interactions between food treatment and temperature treatment, food treatment and time, and temperature treatment and time (Table 2). The model for mass (marginal $R^2 = 0.61$) retained a significant time × food × temperature interaction (Table 2). Fertilisation group had a significant effect on length ($\chi^2 = 57.17$, df = 1, $p < 0.001$), mass ($\chi^2 = 24.49$, df = 1, $p < 0.001$), and condition factor ($\chi^2 = 8.73$, df = 1, $p = 0.003$), with fish in the Nov fertilisation group tending to be larger and heavier than those in the Nov-Dec group, and in marginally lower condition. There was no significant effect of sex on length ($\chi^2 = 0.73$, df = 1, $p = 0.394$), mass ($\chi^2 = 2.01$, df = 1, $p = 0.156$) or condition factor ($\chi^2 = 0.29$, df = 1, $p = 0.591$) across the study. When food and temperature stressors were experienced in isolation (*i.e.* a single treatment applied) fish receiving the low food treatment were smaller (post hoc comparison of low food–cool temperature *versus* high food-cool temperature treatment: t-value = 12.06, $p < 0.001$), lighter (t-value = 13.26, $p < 0.001$) and in poorer condition (t-value = 10.74, $p < 0.001$). Fish in the warm temperature treatment were also smaller (warm temperature-high food *versus* cool temperature-high food treatment: t-value = 3.23, $p = 0.007$), lighter (t-value = 3.66, $p = 0.002$), but in similar condition (t-value = 1.41, $p = 0.495$) (Figure 2A, B, C). The positive interaction term between food treatment and temperature treatment indicated that effects of combined stressor treatments on length, mass, and *K* were less than we might expect based off their effects in isolation.

Fish also varied in length (marginal $R^2 = 0.55$), mass (marginal $R^2 = 0.36$) and condition factor (marginal $R^2 = 0.16$) trajectories according to migratory tactics, with smolts tending to be smaller than mature fish across the duration of the study period ($\chi^2 = 15.55$, df = 1, $p < 0.001$). The significant interaction between migratory tactics and time for mass and condition factor (Figure 1B, Table 3)

indicated smolts gained less mass, with lower condition trajectories (Figure 2D,E,F).

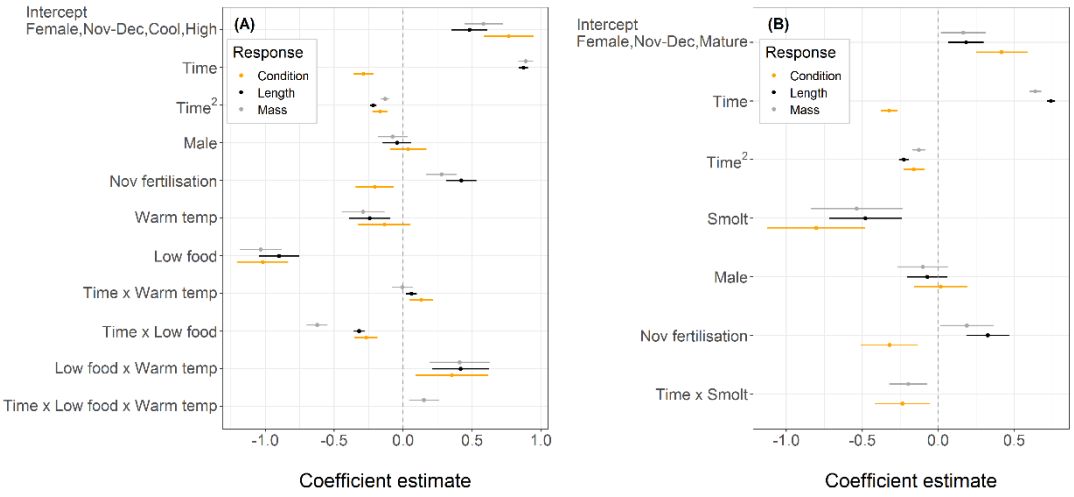

Figure 1: Coefficient estimates (± associated standard errors) of mixed effects models describing z-standardised length, mass and condition trajectories of brown trout offspring (derived from wild-caught parents from a facultatively anadromous population) that were (A) exposed to food and temperature stressors and (B) classed as adopting either smolt (migratory tactic) or freshwater maturing (non-migratory/resident) tactics after 18 months of tank rearing.

Table 2: Results of the mixed effect model analysis for length, mass and condition trajectories of brown trout exposed to food and temperature stressors. The results of the model selection procedure on interaction terms are given, and the selected model for each response is highlighted in bold. The models included a random effect of individual identity and a first-order autoregressive correlation structure with respect to time (weeks of experimental treatment) was also modelled.

Model	**df**	**AIC**	**logLik**	***L*-ratio**	***p*-value**
Length ~ time*food*temperature + time^2 + fertilisation + sex	14	1222.8	-597.4		
Length ~ time*food + time*temperature + time^2 + food*temperature + fertilisation + sex	13	1222.5	-598.3	1.8	0.186
Length ~ time*food + time^2 + food*temperature + fertilisation + sex	12	1229.7	-602.8	9.1	0.003
Length ~ time*temperature + time^2 +food*temperature + time^2 + fertilisation + sex	12	1422.2	-699.1	201.6	< 0.001
Length ~ time*food + time*temperature + time^2 + fertilisation + sex	12	1236.1	-606.1	15.6	< 0.001
Mass ~ time*food*temperature + time^2 + fertilisation + sex	14	1667.1	-819.6		
Mass ~ time*food + time*temperature + time^2 + food*temperature + fertilisation + sex	13	1672.6	-823.3	7.5	0.006
Condition ~ time*food*temperature + time^2 + fertilisation + sex	14	3023.4	-1497.7		
Condition ~ time*food + time*temperature + time^2 + food*temperature + fertilisation + sex	13	3022.4	-1498.2	0.9	0.337
Condition ~ time*food + time^2 + food*temperature + fertilisation + sex	12	3029.8	-1502.9	9.4	0.002
Condition ~ time*temperature + time^2 +food*temperature + time^2 + fertilisation + sex	12	3059.6	-1517.8	39.3	< 0.001
Condition ~ time*food + time*temperature + time^2 + fertilisation + sex	12	3027.3	-1501.7	7.0	0.008

Table 3: Results of the mixed effect model analysis for length, mass and condition trajectories of brown trout in the experiment with life-history classed as either smolts (i.e. migratory) or freshwater mature across the study period. The results of the model selection procedure on interaction terms are given, and the selected model for each response is highlighted in bold. The models included a random effect of individual identity and a first-order autoregressive correlation structure with respect to time (weeks of experimental treatment) was also modelled.

Model	**df**	**AIC**	**logLik**	**L-ratio**	**p-value**
Length ~ time*life-history + time^2 + sex + fertilisation	10	1300.6	-640.3		
Length ~ time + life- history + sex + fertilisation	9	1298.9	-640.4	0.2	0.637
Mass ~ time*life-history + time^2 + sex + fertilisation	10	1836.0	-908.0		
Mass ~ time + life- history + time^2 + sex + fertilisation	9	1843.6	-912.8	9.7	0.002
Condition ~ time*life-history + time^2 + sex + fertilisation	10	2674.4	-1327.2		
Condition ~ time + life- history + time^2 sex + fertilisation	9	2678.8	-1330.4	6.4	0.011

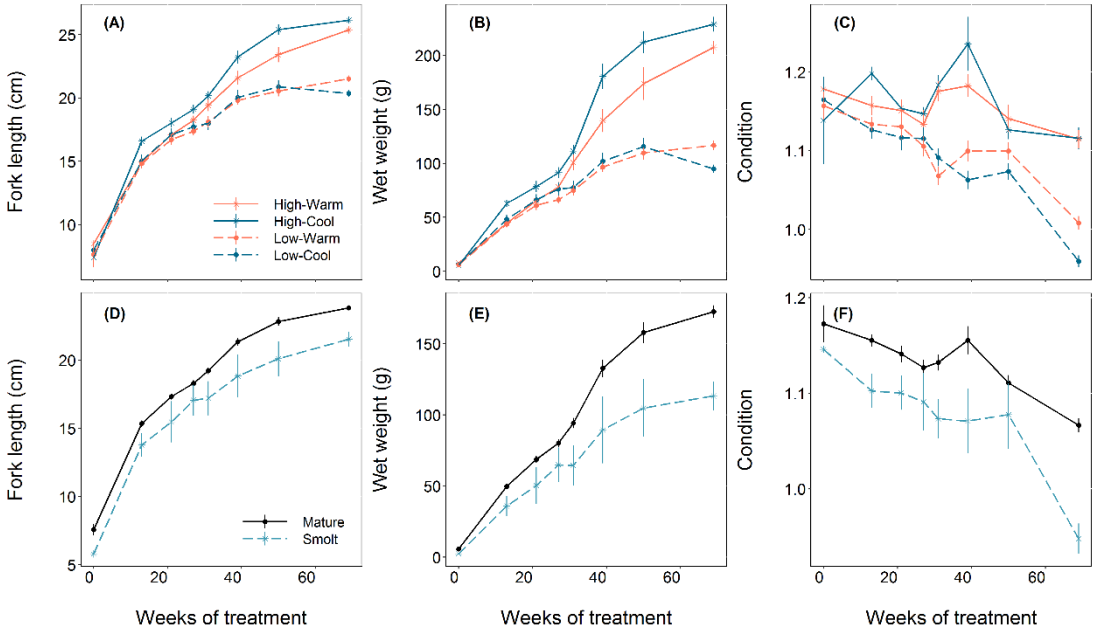

Figure 2: Trajectories of fork length, mass, and condition of brown trout offspring (derived from wild-caught parents from a facultatively anadromous population) under different food treatments and temperature treatments (A, B, C), and classed according to life-history tactics (D, E, and F). Food and temperature treatments are denoted in the format "Food-Temperature" (High or Low food, and Warm or Cool temperature) and life histories were classed as either smolt (migratory tactics) or mature (non-migratory). Week 0 = end of November 2015, when fish were 10 to 11 months old (Nov-Dec and Nov group, respectively).

Morphological traits at the end of the study

At the end of the experiment, fish varied in length, condition and whole body lipids depending on food treatment, temperature treatment, life-history tactics and fertilisation group (Figure 3A, B). The models describing final length (marginal R^2 = 0.48) and condition (marginal R^2 = 0.38) each retained a significant interaction between food treatment and temperature treatment but the model describing whole body lipids (marginal R^2 = 0.41) did not (Table 4). We detected significant negative main effects of food treatment (χ^2 = 63.44, df = 1, p < 0.001) but positive effects of temperature treatment (χ^2 = 3.91, df = 1, p

= 0.048) on lipid levels (Figure 3A). The significant positive interaction term (Figure 3A) indicated an antagonistic effect of food and temperature treatments on length and condition (Figure 3C, D, and E). Fertilisation group significantly affected length (χ^2 = 4.56, df = 1, p = 0.033) and condition (χ^2 = 5.15, df = 1, p = 0.023). Fish in the Nov fertilisation group tended to be larger but in poorer condition, with similar lipid levels (χ^2 = 0.02, df = 1, p = 0.880) to those in the Nov-Dec group. There was no significant effect of sex on length (χ^2 = 0.14 df = 1, p = 0.712), condition (χ^2 = 2.60, df = 1, p = 0.107) or lipids (χ^2 = 1.91, df = 1, p = 0.167).

Life-history tactics significantly affected final length (χ^2 = 4.80, df = 1, p = 0.036), final condition (χ^2 = 19.62, df = 1, p < 0.001), and final lipids (χ^2 = 13.87, df = 1, p = 0.002). Overall, smolts were smaller than mature fish, with lower condition values, and higher lipid levels (Figure 3B, C, D, E). Smolts and unassigned fish were similarly sized (χ^2 = 0.35, df = 1, p = 0.554), with similar lipid levels (χ^2 = 1.49, df = 1, p = 0.222), though smolts had lower condition values (χ^2 = 07.48, df = 1, p = 0.006) (Figure S3).

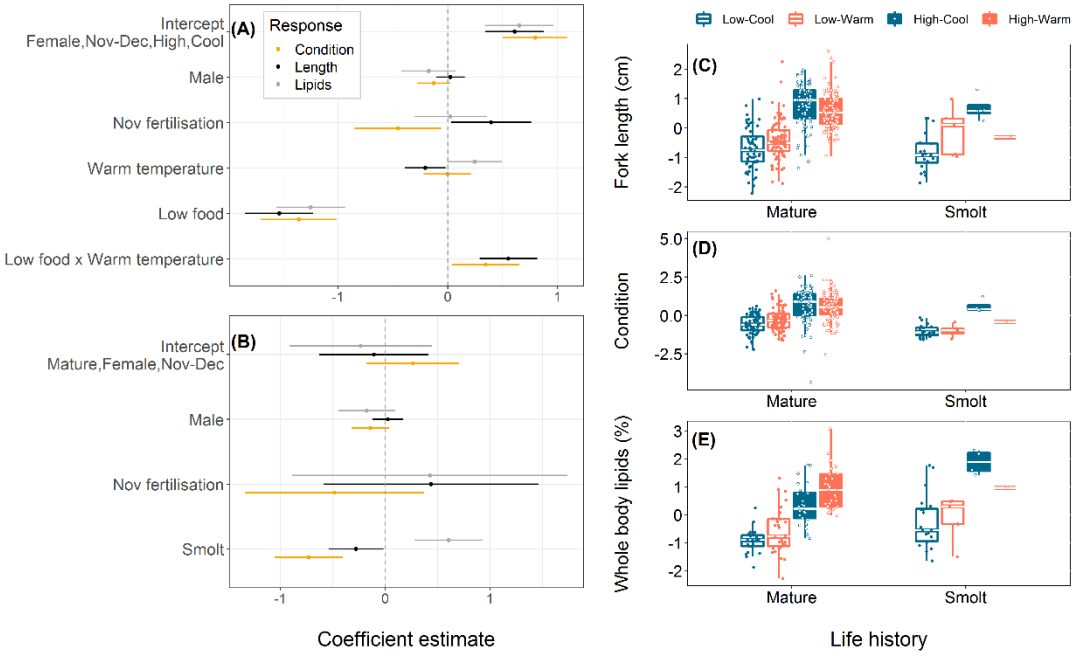

Figure 3: Coefficient estimates (± standard errors) from mixed effect models describing effects of (A) food treatment and temperature treatment and (B) migratory tactics on z-standardised final measures of length, condition, and whole body lipids of brown trout offspring classed as either smolts (migratory) or freshwater mature (non-migratory/resident) at the end of the experimental study (Spring 2018). Median values of (C) length, (D) condition, and (E) whole body lipids are represented by the white horizontal lines in each box in (C), (D), and (E). Food and temperature treatments are denoted in the format "Food-Temperature" (High or Low food, and Warm or Cool temperature). Note that only one smolt was recorded in the High-Warm treatment, and thus there is no corresponding white line for the median in the High-Warm treatment.

Table 4: Results of the mixed effect model analysis for length, condition, and whole body lipids of brown trout exposed to food and temperature stressors at the end of the experimental study period. The results of the model selection procedure on interaction terms are given, and the selected model for each response is highlighted in bold. The models included a random effect of terminal sample date.

Model	**df**	**AIC**	**logLik**	***L*-ratio**	***p*-value**
Length ~ food*temperature + fertilisation + sex	8	901.5	-422.8		
Length ~ food + temperature + fertilisation + sex	7	916.5	-451.3	16.98	< 0.001
Condition ~ food*temperature + fertilisation + sex	8	1034.0	-509.0		
Condition ~ food + temperature + fertilisation + sex	7	1036.9	-511.5	4.94	0.026
Lipids ~ food*temperature + fertilisation + sex	8	375.5	-179.8		
Lipids ~ food + temperature + fertilisation + sex	7	375.7	-180.8	2.18	0.140

Migratory tactics

The model describing the probability of smolting had significant effects of food treatment ($\chi^2 = 16.50$, df = 1, $p < 0.001$), temperature treatment ($\chi^2 = 14.08$, df = 1, $p < 0.001$), fertilisation group ($\chi^2 = 7.09$, df = 1, $p = 0.008$) and sex ($\chi^2 = 4.34$, df = 1, $p = 0.037$). The interaction between food treatment and temperature treatment was not significant (LRT for model with and without interaction term: $\chi^2 = 0.02$, df = 1, $p = 0.882$). Food restriction increased the probability of smolting whereas the warm temperature treatment decreased the probability of smolting (Figure 4A, B). Males were less likely to smolt than females, and fish in the Nov fertilisation group were less likely to smolt than those in the Nov-Dec group (Figure 4A, B).

The model describing the probability of maturing also had significant effects of food treatment ($\chi^2 = 19.13$, df = 1, $p < 0.001$), temperature treatment ($\chi^2 = 17.49$, df = 1, $p < 0.001$), sex ($\chi^2 = 15.90$, df = 1, $p < 0.001$), but the effect of fertilisation group was not significant ($\chi^2 = 1.04$, df = 1, $p = 0.308$). The interaction between food treatment and temperature treatment was not significant (LRT for model with and without interaction term: $\chi^2 = 0.99$, df = 1, $p = 0.319$). In contrast to effects on smolting, the high food treatment increased the probability of maturing, as did the warm temperature treatment (Figure 4C, D). Males were less likely to mature than females in all treatments (Figure 4C, D).

The model describing the probability of being unassigned a life-history tactic included significant effects of food treatment ($\chi^2 = 5.62$, df = 1, $p = 0.018$), temperature treatment ($\chi^2 = 4.91$, df = 1, $p = 0.027$), sex ($\chi^2 = 34.05$, df = 1, $p < 0.001$) and fertilisation group ($\chi^2 = 7.69$ df = 1, $p = 0.006$). The interaction between food treatment and temperature treatment was not significant (LRT for model with and without interaction term: $\chi^2 = 3.31$, df = 1, $p = 0.069$). Fish were significantly more likely to be unassigned a life history in either the low food or cool temperature treatments, as were males, and fish from the Nov fertilisation group (Table 5).

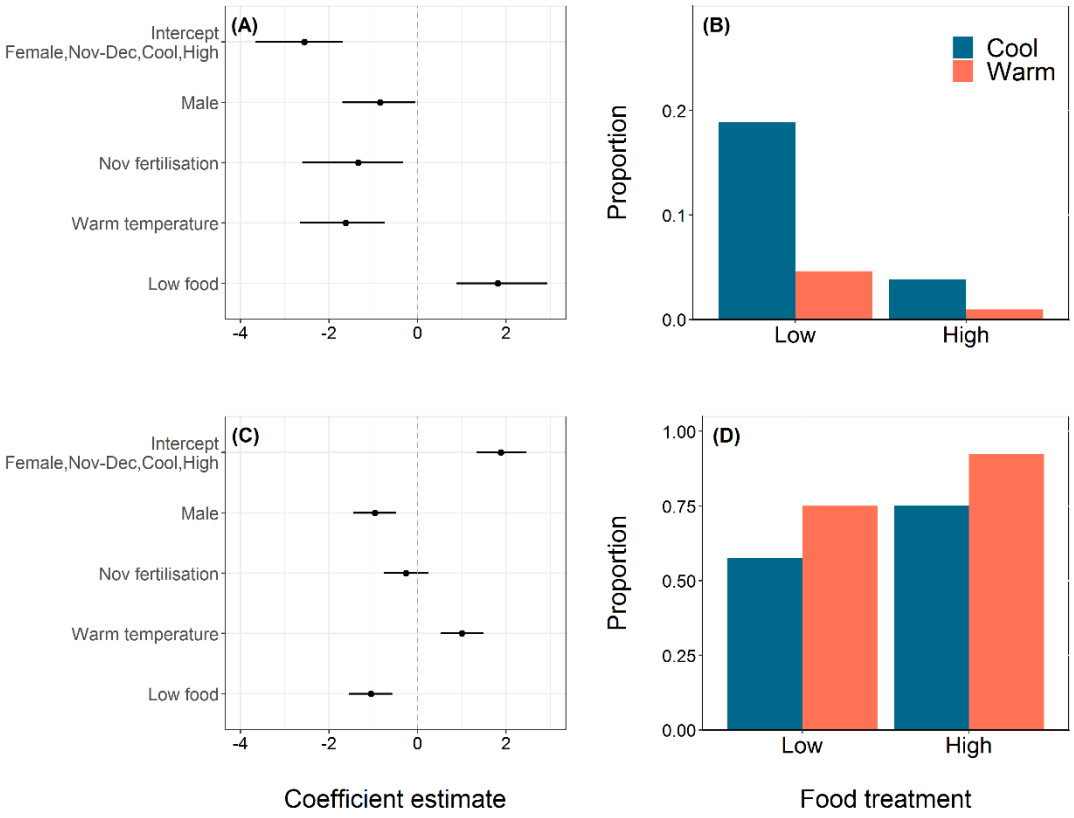

Figure 4: Co-efficient estimates (± 95% confidence intervals) of GLMs describing probability of adopting (A, B) migratory and (C, D) maturation tactics in brown trout (n = 425, F1 offspring of wild trout from naturally facultatively anadromous population). Fish were classed as smolts or maturing after 18 months of tank rearing under varying food restriction and temperature treatments.

Table 5: Parameter estimates with associated standard errors (SE) for three binomial generalised linear models (GLM) predicting smolt (migratory) probability (dummy coded: smolt = 1, non-smolt = 0), freshwater maturation (dummy coded: mature/maturing = 1, immature = 0), and "unassigned" life-history tactics (dummy coded: unassigned = 1, smolt/mature = 0) in brown trout (n = 425). The reference level of each factor is in brackets, i.e. effects in all models were contrasted against female fish from the Nov-Dec fertilisation in the High food and Cool temperature treatment. Statistical significance was assessed at $p < 0.05$.

Effect	**Estimate**	**SE**	**t-value**	***p*-value**
GLM of probability of smolting:				
Intercept (High-Cool, Female, Nov-Dec fertilisation)	-2.559	0.494	-5.176	< 0.001
Food: Low	1.811	0.513	3.533	< 0.001
Temperature: Warm	-1.621	0.481	-3.372	0.001
Fertilisation group: Nov	-1.341	0.569	-2.358	0.018
Sex: Male	-0.849	0.417	-2.037	0.042
GLM of probability of maturation:				
Intercept (High-Cool, Female, Nov-Dec fertilisation)	1.879	0.284	6.625	< 0.001
Food: Low	-1.054	0.248	-4.242	< 0.001
Temperature: Warm	1.004	0.247	4.073	< 0.001
Fertilisation group: Nov	-0.261	0.255	-1.023	0.306
Sex: Male	-0.963	0.248	-3.888	< 0.001
GLM of probability of being unassigned a life history:				
Intercept (High-Cool, Female, Nov-Dec fertilisation)	-2.923	0.366	-7.986	< 0.001
Food: Low	0.644	0.276	2.337	0.019
Temperature: Warm	-0.601	0.274	-2.192	0.028
Fertilisation group: Nov	0.784	0.281	2.789	0.005
Sex: Male	1.655	0.311	5.320	< 0.001

Osmoregulatory performance

Gill NKA activity varied according to life history (χ^2 = 56.74, df = 2, p < 0.001), but was not affected by sex (χ^2 = 1.28., df = 1, p = 0.258) or fertilisation group (χ^2 = 0.72, df = 1, p = 0.397). Post hoc testing showed smolts had significantly higher NKA activity than mature fish (t = -7.41, df = 172, p < 0.001) and unassigned fish (t = 5.15, df = 172, p < 0.001; Figure 5A). Similarly, plasma chloride concentration was significantly affected by life history (χ^2 = 52.14, df = 2, p < 0.001), with no significant effect of sex (χ^2 = 2.75, df = 1, p = 0.097) or fertilisation group (χ^2 = 2.03, df = 1, p = 0.154). Smolts had significantly lower plasma chloride concentration after saltwater exposure than mature fish (t = 5.56, df = 144, p < 0.001) and unassigned fish (t = -6.77, df = 144, p < 0.001; see Figure 5B). Size-corrected plasma chloride concentration decreased significantly with gill NKA activity in smolts (χ^2 = 14.18, df = 1, p < 0.001, Figure 5C), however there was no significant relationship between size-corrected plasma chloride concentration and gill NKA activity in non-smolts (χ^2 = 1.79, df = 1, p = 0.180, Figure 5D).

After accounting for the significant effect of body size (χ^2 = 5.97, df = 1, p = 0.015), the model describing plasma chloride concentration in smolts (marginal R^2 = 0.49) did not retain a significant food × temperature treatment interaction (LRT: χ^2 = 0.26, df = 1, p = 0.610). We detected a significant main effect of food treatment on plasma chloride concentration (χ^2 = 5.29, df = 1, p = 0.021), where the high food treatment was associated with lower chloride values (Figure 6A, B). There was no significant effect of temperature treatment (χ^2 = 2.26, df = 1, p = 0.133), sex (χ^2 = 1.60, df = 1, p = 0.205) or fertilisation group (χ^2 = 2.77, df = 1, p = 0.096) on chloride concentrations. Mixed model analysis indicated non-significant effects of fork length (χ^2 = 0.06, df = 1, p = 0.814), food treatment (χ^2 = 0.03, df = 1, p = 0.862), temperature treatment (χ^2 = 0.85, df = 1, p = 0.358), sex (χ^2 = 2.47, df = 1, p = 0.116) and fertilisation group (χ^2 = 3.53, df = 1, p = 0.060) on gill NKA activity in smolts (marginal R^2 = 0.20, Figure 6A). Overall, this indicates positive direct effects food treatment (independent of size) on saltwater tolerance of smolts, which were not reflected in gill NKA activity. See Tables S6 and S7 for parameter estimates from the mixed effect models.

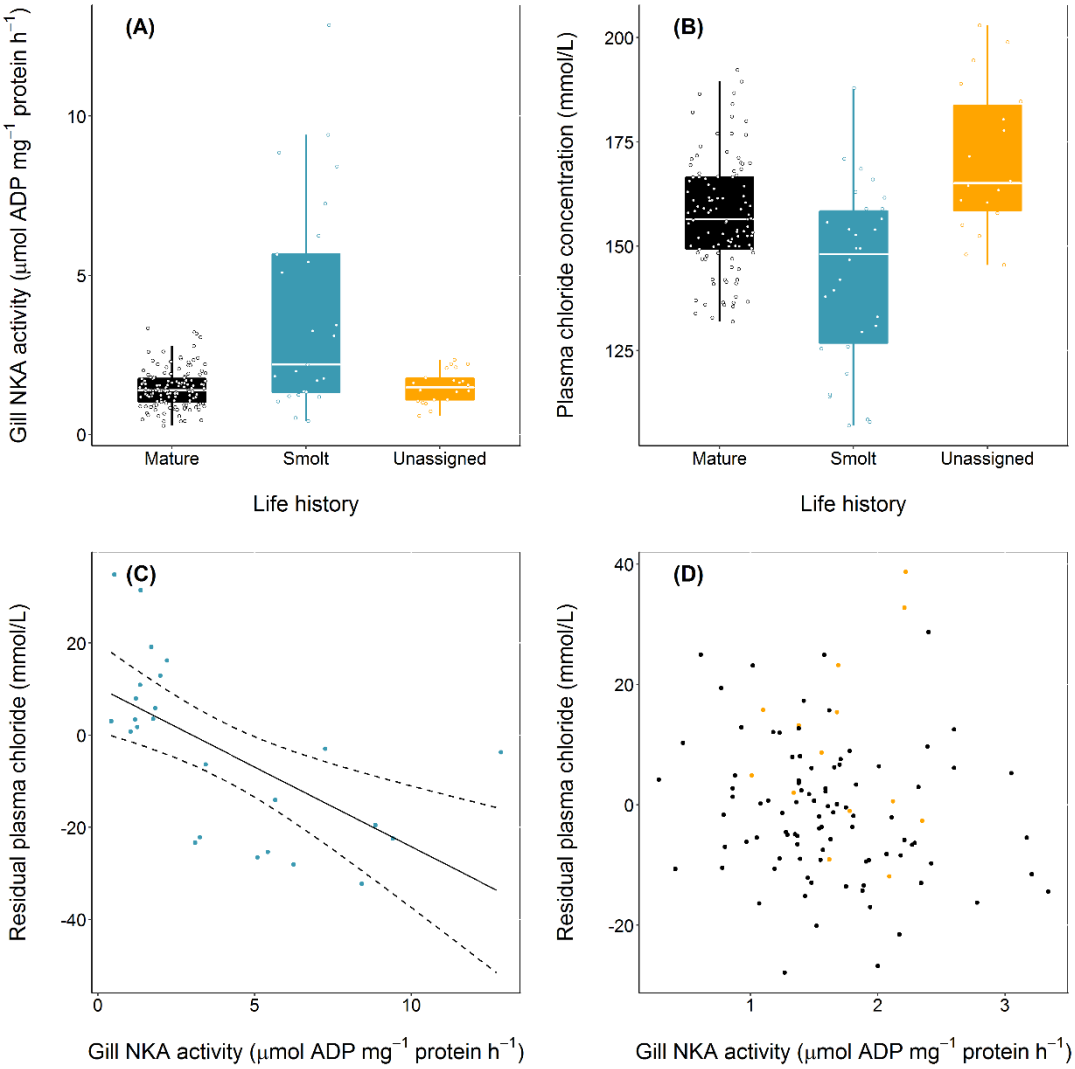

Figure 5: Variation in (A) gill NKA activity, and (B) plasma chloride concentration of brown trout – classed as smolts (migratory tactics) and mature (non-migratory), or unassigned a life-history tactic – after 24 hours in salt water (30 ppt salinity). Size-corrected plasma chloride concentration was negatively related to Gill NKA activity in (C) smolts (dashed line = 95% confidence interval) but there was no relationship in (D) non-smolts (mature and unassigned fish).

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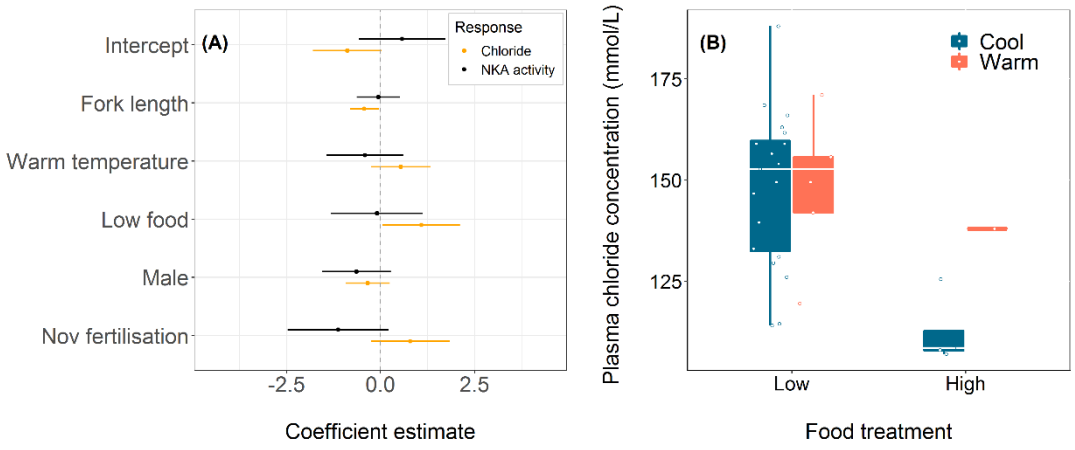

Figure 6: Coefficient estimates (± 95% confidence intervals) from the mixed effects models describing z-standardised (A) gill NKA activity and plasma chloride concentration of brown trout smolts after a 24 hours in salt water (30 ppt salinity). Variation in plasma chloride concentration of smolts according to food treatment is shown in (B), where the white lines in each box represent the median. Note that only one smolt was recorded in the High-Warm treatment, and thus there is no corresponding white line for the median in the High-Warm treatment.

Discussion

Accelerating global change is exposing ecosystems to a multitude of co-occurring stressors, the implications of which are uncertain, particularly for migratory populations. Here we showed that food restriction increased the occurrence of a migratory phenotype (smolts), but conversely elevated temperature reduced smolting rates in favour of increased freshwater maturation (a phenotype consistent with a residency tactic). The observed effects on life-history were underpinned by complex, interactive effects of these putative stressors on underlying status traits associated with migratory decisions.

Effects of multiple stressors on underlying morphological traits

While stressors applied in isolation generally appeared to have negative effects on morphological traits, the effects of warming were less pronounced than food restriction, and varied depending on the response considered. Though fish at higher temperatures were smaller than their counterparts in the cool treatment, they maintained similar condition trajectories, and indeed had higher lipid stores at the end of the study, suggesting temperature may alter patterns of energy allocation in different ways to food restriction. Smaller sizes and higher lipid stores might arise if investment into gonadal development is prioritised over somatic growth earlier when environmental conditions appear favourable for early growth (*i.e.* the warm temperature treatments) (Jonsson *et al.* 2013), supported by the high prevalence of mature fish in warm treatments. Interestingly, the cumulative effects of food restriction and temperature were less than expected, based on their effects in isolation, suggesting complex antagonistic interactions between the stressors, whereby increased temperatures reduced body size and mass at high, but not low, food levels. The dampened response to temperature when combined with food restriction could perhaps be explained by metabolic rate depression under low food conditions, which has previously been documented in food-limited brown trout (Auer *et al.* 2015, 2016). This, together with overall reduced consumption rates, may simply have swamped any effects of temperature on growth in the low food treatments. Indeed, bioenergetics modelling of stream-dwelling rainbow trout growth has indicated growth may be more affected by factors influencing food intake rates (such as reduced overall food availability) than by direct effects of temperature, particularly during warmer summer months (Railsback and Rose 1999).

Effects of multiple stressors on migration

The antagonistic effects of food restriction and higher temperatures on physiological traits were not apparent at the level of migratory tactics. Indeed, opposing (additive) effects of these putative stressors on migratory phenotypes seemed initially to be counterintuitive. While an increase in the migratory tactic in response to food limitation is in line with previous work (Olsson *et al.* 2006;

Wysujack *et al.* 2009; O'Neal and Stanford 2011; Jones *et al.* 2015; Archer *et al.* 2019), surprisingly, a temperature increase of 1.8 °C above the natural temperature regime of the source catchment reduced smolting rates. An energy limitation scenario (where an environmental stressor may act to prevent individuals from reaching genetically determined maturation thresholds) was supported in our results at the level of the status traits. Future migrants (*i.e.* smolts) were consistently smaller than fish that matured in fresh water (*i.e.* residents) and differences in mass and condition trajectories indicated migrants were energetically deficient (relative to mature fish). Energetic limitation appeared to be associated with low food availability, but less so with warmer temperatures.

Warmer temperatures have been proposed to generally increase the frequency of migrants through energetic limitation, if associated elevated metabolic demands are not offset by increased energetic intake (Sloat and Reeves 2014; Kendall *et al.* 2014). However, warming can have a range of context-dependent impacts on patterns of energy acquisition and allocation in salmonines, which in turn may lead to a diversity of effects on life histories. For example, warmer temperatures are associated with higher levels of growth up until some thermal optimum (Jensen 1990; Forseth and Jonsson 1994; Elliott *et al.* 1995; Ojanguren *et al.* 2001; Jonsson *et al.* 2013), but bioenergetics modelling shows that optimal temperatures for growth are negatively related to daily ration amount and body size (Beauchamp 2009). Thus, higher temperatures could either increase or decrease average somatic growth, depending on food supply, the current distribution of fish sizes, and proximity to thermal growth optima. High somatic growth, along with high body condition and lipids, has been linked to increased freshwater maturation in facultatively migratory salmonines (Jonsson and Jonsson 1993; Dodson *et al.* 2013; Hecht *et al.* 2015), but other studies have found that faster growing juveniles may be more likely to migrate at earlier ages and smaller sizes because they are more energetically constrained by limited food availability (owing to much higher metabolic costs) than slower growers (Forseth *et al.* 1999). Moreover, migration tendency is linked to the relative

productivity of marine and freshwater habitats, with anadromy more prevalent in areas where the marine environment offers better opportunities for feeding and growth (*e.g.* in higher latitudes) (Gross *et al.* 1988). Although the temperature stressor we simulated in our study is in line with projected climate warming scenarios of 1–3 °C (IPCC 2014), our warm temperature treatment remained largely within the optimal temperature range for growth in brown trout of 13–17 °C (Elliott *et al.* 1995; Elliott and Hurley 2000; Ojanguren *et al.* 2001) (maximum temperature in the warm treatments was 18.2 °C). It is therefore likely that warmer temperatures did not tip most individuals into an energetic deficit, thus fish were more likely to mature, rather than to smolt, in the warm treatments.

Bioenergetic modelling of migratory variation in steelhead trout has suggested that reductions in food resources can be mediated or exacerbated by water temperatures to alter expression of life histories (Benjamin *et al.* 2013). Few studies have empirically tested the cumulative effects of food supply and temperature on migratory tactics, but from our study, it appears these two environmental stressors may act additively, rather than synergistically, at least for populations that are well within their thermal limits. Moreover, the positive effect of temperature on maturation, coupled with negative temperature effects on the frequencies of smolts and unassigned fish, indicates here that warming acts to hasten the expression of life histories, driving earlier maturation instead of migration. Similar changes in life-history dynamics have been predicted in partially migratory masu salmon (*O. masou*), where favourable early growth conditions associated with warming promoted maturation over migration and caused an overall decline in life-history diversity (Morita *et al.* 2014).

Antagonistic effects of temperature and food on physiological traits (presumed to underpin migratory decisions) were not translated at the level of migratory tactics (where the putative stressors combined additively). Our results provide additional evidence that multiple stressors can alter ecological responses in unexpected ways, sometimes termed “ecological surprises” (Paine *et al.* 1998). This suggests that effects of stressors can vary depending on the level of

organisation, or indeed the response, that is measured (Galic *et al.* 2018). It also underscores how environmental factors may affect migratory decisions directly, and not solely through environmentally-induced changes in putative cueing traits. For example, temperature can affect gene expression with long-lasting consequences for future behaviour and life history (Jonsson and Jonsson 2019). There is some evidence to support that warming, in particular, can directly alter life-history tactics *e.g.* temperature hastened maturation at smaller sizes in nine-spined sticklebacks (*Pungitius pungitius*) independently of temperature-mediated growth (Kuparinen *et al.* 2011). Changes in somatic growth or energy allocation due to antagonistic effects of stressors therefore adds an additional layer of complexity to our ability to infer future migratory tactics from patterns of juvenile growth.

Early life conditions affect migration propensity

The effects of fertilisation group on migratory propensity was an unexpected outcome of our study. While the relatively small numbers of brood stock used makes it difficult to draw conclusions regarding differences between fertilisation groups, which most likely stem from inherited genetic effects or epigenetic/parental effects, differences might nonetheless reflect non-inherited variation arising from early-life environment that has knock-on effects for future phenotype (Burton and Metcalfe 2014). Moreover, differences in the timing of readiness for reproduction/fertilisation also indicates differences among brood stock (*e.g.* spawning site in the wild) that may have translated into genetic or parental effects, rather than an effect of fertilisation date *per se*. Although both fertilisation groups experienced the same food restriction and temperature treatments, phenotypic differences that were established before the application of treatments continued throughout the experimental phase of the study (*e.g.* fish from the Nov fertilisation group, whilst larger, tended to be in poorer condition), supporting genetic/ parental effects as factors which may be equally as important as downstream environmental conditions. Interestingly, individuals in the earlier fertilisation group were more likely to be classed as "unassigned" by the end of the study. Delayed phenotypic expression

of migratory tactics can be reconciled with the existence of multiple decision windows, where an initial window determines the overall migration *versus* residency decision but then subsequent windows determine the actual age at which migrants become smolts, and residents mature (Ferguson *et al.* 2019). Age at smolting, and age at maturation, may involve similar threshold mechanisms as the overarching migration decision but perhaps with different status traits playing the role of 'trigger'; *e.g.* size or growth rates may determine age at smolting in Atlantic salmon but lipid levels may determine age at first maturation (Rowe *et al.* 1991; Jonsson and Jonsson 1993, 2005). Complex environmental stressor effects on these various cues could then increase or decrease overall life-history diversity through temporal variation in migration or maturation patterns. In the Erriff system from which our brood stock was obtained, seaward-migration of wild fish is typically undertaken by smolts at two or three years after hatching (Gargan *et al.* 2016), with potentially up to half of smolts migrating at age 3+. Similarly, although the age distribution of mature residents is unknown for the Erriff system, maturation at ages of 3+ and older is likely. As such, the patterns we observed might have reflected stressor effects on age at migration or age at maturity, in addition to effects on tactic choice *per se*. At least in relation to food restriction, we have no reason to expect that the increased smolting rates we observed in our low food treatments at age 2+ would have been counter-balanced by increased maturation rates at age 3+ of the remaining unassigned fish; if anything, these were likely to have been simply delaying actual smolting until an older age, and therefore larger size (given that larger smolts are more likely to survive the critical transition to the marine environment). In other words, smolting rate differences measured across ages 2+ and 3+ combined, if the experiment had been continued for an additional year, were likely to have been even more pronounced between food treatments. It is less obvious whether the same can be said for temperature effects, but we have no *a priori* reason to expect that age 3+ smolting rates would respond in the opposite direction to sustained higher temperatures than age 2+ smolting rates.

Stressor effects on future migratory capacity

A final aim of our study was to assess if exposure to multiple stressors influenced the capacity of migratory individuals to successfully transition to salt water (an indicator of future migratory success). Smolts generally showed heightened hypo-osmoregulatory performance relative to non-smolts (for a given fish size as indexed by reduced plasma chloride levels following saltwater exposure), which was associated with increased Na^+, K^+-ATPase activity, a key enzyme involved in ion regulation. This is in agreement with many previous studies in salmonines that have described high NKA activity in smolts, which is related directly to ability to maintain homeostasis in seawater (McCormick *et al.* 1998, 2009b, 2013; Nilsen *et al.* 2007). We had expected the addition of stressors might further influence the hypo-osmoregulatory performance of smolts through negative effects on size, irrespective of enzyme activity, and indeed, we did detect a size dependency in plasma chloride levels, which was not reflected in gill NKA activity. Size-dependent increases in salinity tolerance that are independent of the size-dependent smolt decision have also been previously established for salmonines (McCormick *et al.* 1998). Thus, though the overall frequency of smolting was lower in optimal growth conditions (*i.e.* high food regimes in our study), the small number of smolts that were produced under these good growing conditions were larger and in better condition than their counterparts exposed to less favourable conditions. Intriguingly, we detected an additional negative effect of food restriction on hypo-osmoregulatory performance that was independent of size. Food limitation could potentially contribute to competitive interactions between individuals, emergence of dominance hierarchies, and generally heightened physiological stress, with implications for seawater tolerance and survival of brown trout (Pickering 1989; Sigholt and Finstad 1990; Liebert and Schreck 2006; Midwood *et al.* 2014). Collectively, these results suggest that food restriction may act as a stressor to migrants, which may have negative impacts for survival at sea (Ward and Slaney 1988), and possibly reduce the overall fitness of the migratory life history.

Implications and considerations

Considerable uncertainty still surrounds how environment and genetics integrate, via mediating physiological traits, to influence complex life-history patterns of facultatively migratory species (Crozier *et al.* 2008). Here, we focused on two stressors that are likely to occur in synchrony based on projections of global change, with important implications for an experimentally reared population of trout that can be extrapolated to wild populations, though some caveats should be noted. Whilst macroinvertebrate abundance and size are indeed projected to shrink with rising temperatures across the range of brown trout distribution (Durance and Ormerod 2007), in natural systems trout have been shown to track shifts in prey community assemblies (Woodward *et al.* 2010) or even become more selective in diet as temperatures increase (O'Gorman *et al.* 2016). Furthermore, given that our study population originated from a relatively cool catchment, it is possible that temperature increases in similar systems will primarily serve to increase fish growth/energetic status and promote residency, although this very much depends on how other key factors such as food supply, flow rates, biotic factors also respond to climate change. Any reductions in anadromy would likely alter the transfer of nutrients between freshwater and marine systems, with consequences for wider ecosystem processes (Doughty *et al.* 2016). If warming results in overall decreases in life-history diversity within populations (*e.g.* Benjamin *et al.* 2013; Morita *et al.* 2014) that are coherent over broad spatial scales, this could lead to a reduction in the "portfolio effect" in salmonines, whereby maintaining a range of phenotypic diversity buffers aggregations of populations and even entire species from changing environmental conditions (Schindler *et al.* 2015).

Expanding our approach to additional populations, including those that are closer to their thermal limits, *e.g.* in southern Europe, may alter the patterns we observed here (Almodóvar *et al.* 2012). There is some evidence for genotype by temperature interactions in key phenotypic traits in salmonines (Doctor *et al.* 2014) but the role of intrinsic factors relative to environmental context requires

further exploration (Ferguson *et al.* 2017). Common garden or reciprocal transplant style experiments in the wild would help to elucidate the mechanisms underpinning responses to multiple stressors in migratory species, whilst also incorporating the complexities of natural systems, such as changes in prey community structure, or abiotic correlates of warming (*e.g.* reduced oxygen/flow) (Clews *et al.* 2010). Nevertheless, our study has important implications for the development of management strategies to conserve facultatively migratory salmonines, a culturally iconic group in global decline due to aquaculture expansion, habitat degradation, and climate change (Costello 2009; Limburg and Waldman 2009).

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Supporting Information

Food and temperature stressors have opposing effects in determining flexible migration decisions in brown trout (*Salmo trutta*)

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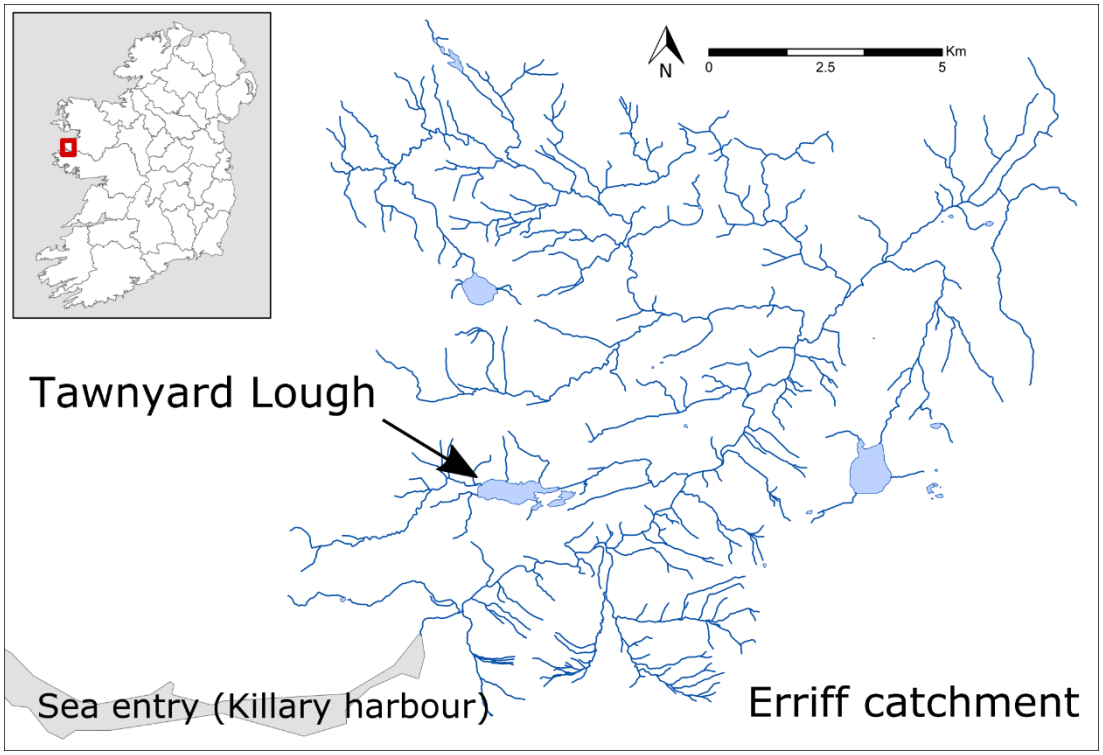

Figure S1: Location in the west of Ireland of brown trout brood stock collected by seine netting in winter 2015, and used to produce F1 offspring for an experimental tank-rearing study. Fish used in the study were offspring of brood stock collected in Tawnyard Lough in the Erriff catchment (a population with a high natural frequency of anadromy).

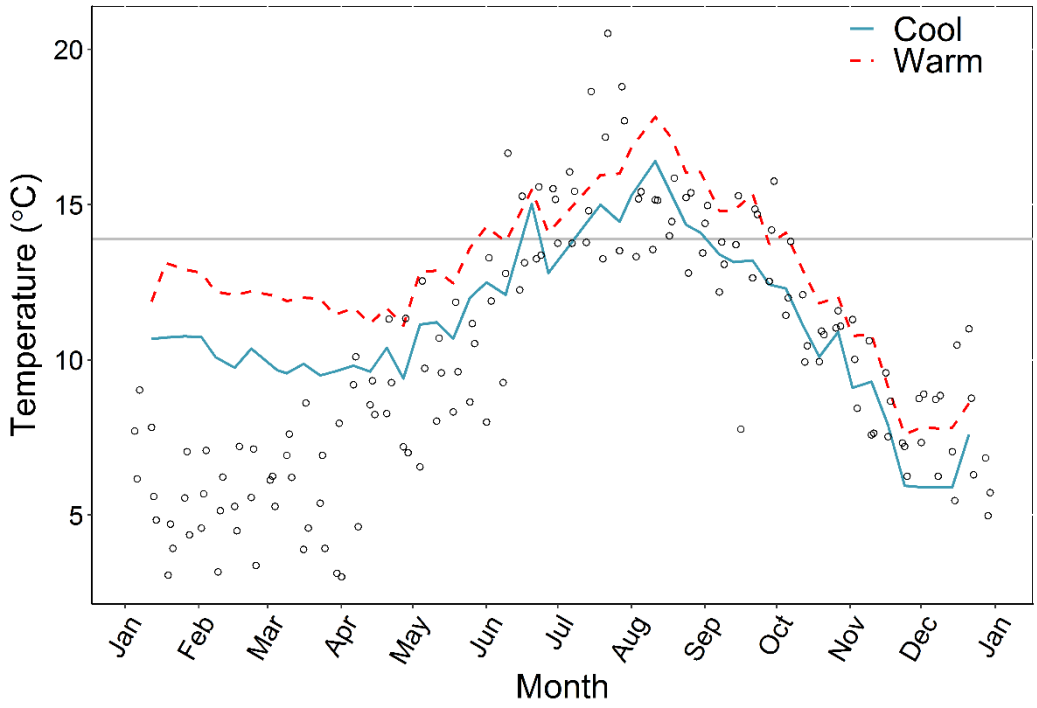

Figure S2: Weekly temperatures (open circles) for the three years preceding the study, as recorded at the Marine Institute long-term monitoring station at Newport, Mayo (west of Ireland), close to the Erriff catchment where brood stock used in the experiment originated. Red and blue lines are the mean weekly temperatures (averaged across tanks) for the cool and warm treatments in the laboratory experiment. The grey line represents the thermal growth optimum for trout (13.9 °C) (Elliott and Hurley 2000).

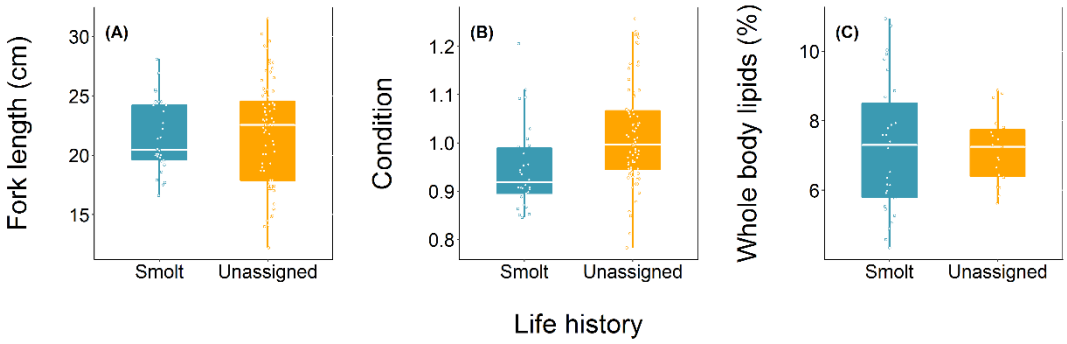

Figure S3: Final measures of (A) length, (B) condition, and (C) whole body lipids of brown trout offspring classed as either smolts (migratory) or unassigned a life-history tactic at the end of the experimental study. Median values of length, condition, and whole body lipids are represented by the white horizontal lines in each box in (A), (B), and (C).

Table S1: Brood stock crossing design for producing F1 offspring of wild-origin brown trout collected by seine netting from a population in Tawnyard Lough in the west of Ireland. Each female was stripped of eggs, which were then fertilised by the milt of two males from the same population and incubated in a hatchery facility within the Burrishoole catchment. Fish were subsequently reared in two separate groups according to fertilisation date, where "November fertilisation group" refers to offspring produced from crosses made on 12th November 2015, and "Nov-Dec fertilisation group" refers to offspring produced from crosses on 27th November and 10th December 2015.

Stripping date	Fertilisation Group	Catchment	Sex	ID	Male cross #1	Male cross #2
12/11/2015	Nov	Erriff (Tawnyard)	Female	TF1	TM1	TM2
12/11/2015	Nov	Erriff (Tawnyard	Female	TF2	TM1	TM2
12/11/2015	Nov	Erriff (Tawnyard)	Female	TF3	TM3	TM4
27/11/2015	Nov-Dec	Erriff (Tawnyard)	Female	TF4	TM5	TM6
27/11/2015	Nov-Dec	Erriff (Tawnyard)	Female	TF5	TM5	TM6
27/11/2015	Nov-Dec	Erriff (Tawnyard)	Female	TF6	TM7	TM8
10/12/2015	Nov-Dec	Erriff (Tawnyard)	Female	TF7	TM9	TM10

Table S2: Parameter estimates with associated standard errors (SE) for mixed model analyses testing the effects of food treatment, temperature treatment, fertilisation group, and sex on the fork length (cm), mass (g), and condition trajectories of brown trout over 18 months of experimental tank rearing. Measurements were taken at key periods denoted by "time". Effects were contrasted against female fish from the Nov-Dec fertilisation group in the high food and cool temperature treatment. Responses are z-standardised and statistical significance was assessed at $p < 0.05$.

Effect	Estimate	SE	t-value	*p*-value
Mixed model for length				
Intercept (Female, Nov-Dec, Cool, High)	0.48	0.07	7.28	< 0.001
Time	0.87	0.02	51.23	< 0.001
Time^2	-0.22	0.01	-17.45	< 0.001
Sex: Male	-0.04	0.05	-0.85	0.397
Fertilisation group: Nov	0.42	0.06	7.53	< 0.001
Temperature: Warm	-0.24	0.08	-3.23	0.001
Food: Low	-0.90	0.07	-12.06	< 0.001
Time × Warm temperature	0.06	0.02	3.02	0.003
Time × Low food	-0.32	0.02	-16.08	< 0.001
Low food × Warm temperature	0.42	0.11	3.96	< 0.001
Mixed model for mass				
Intercept (Female, Nov-Dec, Cool, High)	0.58	0.07	8.18	< 0.001
Time	0.89	0.03	32.55	< 0.001
Time^2	-0.13	0.02	-8.09	< 0.001
Sex: Male	-0.08	0.05	-1.41	0.159
Fertilisation group: Nov	0.28	0.06	4.93	< 0.001
Temperature: Warm	-0.29	0.08	-3.66	< 0.001
Food: Low	-1.03	0.08	-13.26	< 0.001
Time × Warm temperature	-0.01	0.04	-0.15	0.880
Time × Low food	-0.62	0.04	-16.05	< 0.001
Low food × Warm temperature	0.41	0.11	3.69	< 0.001
Time × Low food × Warm temperature	0.15	0.06	2.72	0.007
Mixed model for condition				
Intercept (Female, Nov-Dec, Cool, High)	0.76	0.09	8.36	< 0.001
Time	-0.29	0.04	-7.88	< 0.001
Time^2	-0.17	0.03	-5.83	< 0.001
Sex: Male	0.04	0.07	0.54	0.593
Fertilisation group: Nov	-0.21	0.07	-2.94	0.003
Temperature: Warm	-0.14	0.10	-1.41	0.160
Food: Low	-1.02	0.09	-10.74	< 0.001
Time × Warm temperature	0.13	0.04	3.08	0.002
Time × Low food	-0.27	0.04	-6.31	< 0.001
Low food × Warm temperature	0.35	0.13	2.64	0.009

Table S3: Parameter estimates with associated standard errors (SE) for mixed model analyses testing the effects of life-history tactics, fertilisation group, and sex on the fork length (cm), weight (g), and condition trajectories of brown trout classed as smolts (migratory) or mature (non-migratory) over 18 months of experimental tank rearing. Measurements were taken at key periods denoted by "time". Effects were contrasted against mature female fish from the Nov-Dec fertilisation group. Responses are z-standardised and statistical significance was assessed at $p < 0.05$.

Effect	**Estimate**	**SE**	**t-value**	***p*-value**
Mixed model for length				
Intercept (Female, Nov-Dec, Mature)	0.18	0.06	3.06	0.002
Time	0.74	0.01	52.94	< 0.001
Time^2	-0.23	0.02	-13.91	< 0.001
Life-history: Smolt	-0.48	0.12	-3.93	< 0.001
Sex: Male	-0.07	0.07	-1.06	0.290
Fertilisation group: Nov	0.33	0.07	4.48	< 0.001
Mixed model for mass				
Intercept (Female, Nov-Dec, Mature)	0.16	0.08	2.18	0.029
Time	0.64	0.02	32.27	< 0.001
Time^2	-0.13	0.02	-5.95	< 0.001
Life-history: Smolt	-0.54	0.15	-3.47	0.001
Sex: Male	-0.10	0.08	-1.19	0.234
Fertilisation group: Nov	0.19	0.09	2.11	0.036
Time × Smolt	-0.20	0.06	-3.10	0.002
Mixed model for condition				
Intercept (Female, Nov-Dec, Mature)	0.42	0.09	4.81	< 0.001
Time	-0.32	0.03	-11.99	< 0.001
Time^2	-0.16	0.03	-4.63	< 0.001
Life-history: Smolt	-0.80	0.16	-4.87	< 0.001
Sex: Male	0.02	0.09	0.18	0.859
Fertilisation group: Nov	-0.32	0.10	-3.37	0.001
Time × Smolt	-0.24	0.09	-2.53	0.012

Table S4: Parameter estimates with associated standard errors (SE) for mixed model analysis testing the effects of food treatment, temperature treatment, fertilisation group, and sex on the final fork length (cm), condition, and whole body lipids (%) of brown trout after 18 months of experimental tank-rearing. Effects were contrasted against female fish from the Nov-Dec fertilisation group in the high food and cool temperature treatment. Responses are z-standardised and statistical significance was assessed at $p < 0.05$.

Effect	**Estimate**	**SE**	**t-value**	***p*-value**
Mixed model for length				
Intercept (Female, Nov-Dec, High, Cool)	0.61	0.14	4.50	< 0.001
Sex: Male	0.02	0.07	0.37	0.714
Fertilisation group: Nov	0.40	0.19	2.12	0.078
Temperature: Warm	-0.20	0.09	-2.16	0.031
Food: Low	-1.53	0.16	-9.68	< 0.001
Low food x Warm temperature	0.55	0.13	4.13	< 0.001
Mixed model for condition				
Intercept (Female, Nov-Dec, High, Cool)	0.80	0.15	5.31	< 0.001
Sex: Male	-0.12	0.08	-1.60	0.110
Fertilisation group: Nov	-0.45	0.20	-2.25	0.065
Temperature: Warm	-0.01	0.11	-0.02	0.987
Food: Low	-1.36	0.18	-7.65	< 0.001
Low food x Warm temperature	0.35	0.16	2.21	0.027
Mixed model for whole body lipids (%)				
Intercept (Female, Nov-Dec, High, Cool)	0.65	0.16	4.08	< 0.001
Sex: Male	-0.17	0.13	-1.36	0.177
Fertilisation group: Nov	0.03	0.17	0.15	0.887
Temperature: Warm	0.25	0.13	1.95	0.054
Food: Low	-1.25	0.16	-7.84	< 0.001

Table S5: Parameter estimates with associated standard errors (SE) for mixed model analysis testing the effects of life-history tactics, fertilisation group, and sex on the final fork length (cm), condition and whole body lipids (%) of brown trout after 18 months of experimental tank-rearing. Effects were contrasted against mature female fish from the Nov-Dec fertilisation group. Responses are z-standardised and statistical significance was assessed at $p < 0.05$.

Effect	**Estimate**	**SE**	**t-value**	***p*-value**
Mixed model for length				
Intercept (Mature, Female, Nov-Dec)	-0.11	0.27	-0.40	0.688
Life-history: Smolt	-0.28	0.13	-2.08	0.038
Sex: Male	0.03	0.07	-0.35	0.726
Fertilisation group: Nov	0.44	0.52	0.84	0.435
Mixed model for condition				
Intercept (Mature, Female, Nov-Dec)	0.27	0.23	1.17	0.241
Life-history: Smolt	-0.73	0.17	-4.40	< 0.001
Sex: Male	-0.14	0.09	-1.51	0.133
Fertilisation group: Nov	-0.48	0.43	-1.11	0.309
Mixed model for whole body lipids (%)				
Intercept (Mature, Female, Nov-Dec)	-0.23	0.35	-0.67	0.504
Life-history: Smolt	0.61	0.17	3.67	< 0.001
Sex: Male	-0.17	0.14	-1.26	0.209
Fertilisation group: Nov	0.43	0.67	0.64	0.547

Table S6: Parameter estimates with associated standard errors (SE) for mixed model analysis testing the differences in the gill NKA activity (log transformed) and plasma chloride concentration of brown trout classed as smolts (migratory tactics), mature or unassigned (non-migratory tactics) after 24 saltwater immersion. Effects were contrasted against mature female fish from the Nov-Dec fertilisation group. Statistical significance was assessed at $p < 0.05$.

Effect	**Estimate**	**SE**	**t-value**	***p*-value**
Mixed model for gill NKA activity (log transformed)				
Intercept	0.12	0.16	0.77	0.445
Life-history: Smolt	0.81	0.11	7.41	< 0.001
Life-history: Unassigned	0.02	0.12	0.15	0.879
Sex: Male	-0.09	0.08	-1.12	0.266
Fertilisation group: Nov	0.23	0.27	0.84	0.442
Mixed model for plasma chloride concentration				
Intercept	161.61	3.59	45.00	< 0.001
Life-history: Smolt	-16.24	2.92	-5.56	< 0.001
Life-history: Unassigned	13.68	3.69	3.71	< 0.001
Sex: Male	3.84	2.36	1.63	0.105
Fertilisation group: Nov	-9.04	6.45	-1.40	0.210

Table S7: Parameter estimates with associated standard errors (SE) for mixed model analysis testing the effects of fork length, food treatment, temperature treatment, fertilisation group, and sex on the gill NKA activity and plasma chloride concentration after 24 saltwater immersion of brown trout smolts after 18 months of experimental tank-rearing. Effects were contrasted against female fish from the Nov-Dec fertilisation group in the high food and cool temperature treatment. Responses are z-standardised and statistical significance was assessed at $p < 0.05$.

Effect	**Estimate**	**SE**	**t-value**	***p*-value**
Mixed model for gill NKA activity				
Intercept	0.57	0.59	0.98	0.342
Fork length	-0.06	0.29	-0.21	0.840
Food: Low	-0.09	0.62	-0.15	0.889
Temperature: Warm	-0.42	0.52	-0.80	0.435
Sex: Male	-0.64	0.47	-1.37	0.189
Fertilisation group: Nov	-1.12	0.69	-1.64	0.200
Mixed model for plasma chloride concentration				
Intercept	-0.88	0.47	-1.90	0.072
Fork length	-0.43	0.20	-2.18	0.041
Food: Low	1.08	0.53	2.06	0.109
Temperature: Warm	0.54	0.40	1.34	0.194
Sex: Male	-0.34	0.30	-1.13	0.271
Fertilisation group: Nov	0.79	0.53	1.49	0.211

Chapter 4

Metabolic traits in brown trout (*Salmo trutta*) vary in response to food restriction and population background

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Key words: climate change, partial migration, anadromy, aquatic, brown trout, metabolism, phenotypic flexibility, plasticity

Contributions: LA, TR, and PMcG conceived the study. LA, SH, and LH collected data and contributed to experimental design. LA conducted statistical analysis and wrote the manuscript. All authors contributed to interpretation of results and revisions of the manuscript.

Abstract

Metabolic rates vary hugely within and between populations of the same species, yet we know relatively little about the factors causing this intraspecific variation. Given that metabolic rate determines the energetic cost of life, uncovering these sources of variation is important to understand and forecast population responses to environmental change. Moreover, few studies have examined factors causing intraspecific variation in metabolic flexibility. Here, we explore if, and how, extrinsic environmental conditions and population-specific factors contribute to variation in metabolic traits in brown trout, an iconic species that is capable of extreme variation in migratory life-history tactics. We measured metabolic traits in offspring from two wild populations of brown trout that naturally show divergent migratory tactics (one anadromous i.e. sea-migratory, and one non-anadromous) that had been reared under experimental conditions of long-term food restriction. Both populations showed decreased standard metabolic rates (SMR – baseline energy requirements) under low food conditions. The non-anadromous population had an overall lower SMR relative to the anadromous population, but maximum metabolic rate (MMR), and consequently, aerobic scope (AS), were both higher in the anadromous population, and were higher overall in males compared to females. Intriguingly, the anadromous population had a higher AS under low food conditions compared to optimal food conditions, indicating population-specific effects of food restriction on AS. Collectively, our results suggest the different components of metabolic rate can vary in their response to extrinsic environmental conditions, and can also vary according to intrinsic (i.e. population background/sex) effects. Moreover, populations can further differ in their flexibility of metabolic traits, potentially due to population-specific factors related to life history (*e.g.* migratory tactics). Overall, our study suggests that responses to environmental change may be population specific, but incorporating an understanding of variation in metabolic traits, and their flexibility, will improve our ability to conserve populations.

Introduction

Metabolic rate represents the fundamental energetic cost of living that underpins organism performance in variable and changing environments. Since metabolism has profound implications for fitness (Pettersen *et al.* 2016, 2018), relatively higher or lower metabolic rates have in turn been linked to variation in fitness components such as growth rates (Auer *et al.* 2015c; Zeng *et al.* 2017), and survival (Bochdansky *et al.* 2005) in ways that often depend on environmental context (Burton *et al.* 2011; Auer *et al.* 2015b, c). The minimum energy expenditure required for tissue maintenance and homeostasis is termed standard metabolic rate (SMR) in ectotherms (basal metabolic rate (BMR) in endotherms within the thermoneutral range). SMR occurs when an organism is inactive, unstressed, and not digesting (Chabot *et al.* 2016). Maximum metabolic rate (MMR) sets the upper bounds of energy expenditure as the highest rate of aerobic metabolism (transport of oxygen and production of ATP) that can be achieved (Norin and Metcalfe 2019). Together, SMR and MMR define an organism's aerobic scope (AS), a trait that determines the amount of energy that can be directed towards key functions including digestion, movement, growth, and reproduction through increased metabolism, once baseline energy requirements (i.e. SMR) are met (Guderley and Pörtner 2010).

Large variation in both SMR/BMR and MMR (and consequently AS) exists among species, populations, and individuals (Burton *et al.* 2011; Konarzewski and Książek 2013; Hillman *et al.* 2013; Norin and Clark 2016), with variation linked to differences in lifestyle (Killen *et al.* 2010), geographic distribution (Angilletta 2001; Naya and Bozinovic 2012), thermal regime (Álvarez *et al.* 2006; Eliason *et al.* 2011; Sandblom *et al.* 2016), and behavioural differences (Metcalfe *et al.* 2016). In aquatic ectotherms, factors related to life-history tactics appear to underpin many inter-individual and intra-individual differences in metabolic traits. Along with a 16-fold variation in MMR reported across fish species that occupy different ecological niches (Norin and Clark 2016), metabolic rates can still show *c*. 3-fold inter-individual variation after accounting for age and size differences (Metcalfe *et al.* 2016). Such variation

likely arises because the optimal combination of the various components of metabolic phenotype is context-specific (Auer *et al.* 2015b, c), or because populations (or types of individuals within populations) experience different selection pressures due to life-history differences, or extrinsic/intrinsic factors. For example, sockeye salmon *Oncorhynchus nerka* populations that undertake longer, or more challenging migrations have higher AS (Eliason *et al.* 2011), and higher metabolic rates have also been documented in males *versus* females, *e.g.* higher AS in male pink salmon *O. gorbuscha* (Clark *et al.* 2011).

On top of variation *per se*, patterns of covariation in metabolic phenotypes can also be different across and within species. SMR and MMR have been proposed to be tightly linked because of the "increased intake hypothesis", whereby a high SMR requires investment in metabolic machinery that also facilitates a high MMR, with associated fitness benefits (Biro and Stamps 2010; Burton *et al.* 2011). While SMR and MMR generally do appear to be correlated within species (Auer *et al.* 2017), the traits can vary in their response to different environmental factors, and the coupling of metabolic traits can be context dependent (Killen *et al.* 2013; Norin *et al.* 2016). Moreover, a decoupling of SMR and MMR can occur over time because each is under individual selection pressures (Norin and Metcalfe 2019), which often operate in parallel but may also act independently (*e.g.* Wone *et al.* 2015; Barceló *et al.* 2016). Thus, even if SMR and MMR are somewhat functionally linked, ecologically significant variation in overarching AS can arise due to differences in the sensitivities of each metabolic trait to environmental conditions. Thus, within-individual variation in response to variation in the environment may account to some extent for intraspecific patterns of variation and covariation in metabolic traits.

The ability of a single genotype to display different physiological, morphological, or behavioural phenotypes in response to variation in environmental factors is called phenotypic plasticity. Phenotypic 'flexibility' has been defined as a particular type of plasticity in which within-individual changes are reversible (Piersma and Drent 2003), as distinct from developmental plasticity, where phenotypic responses to early developmental

conditions remain relatively fixed for the rest of life (West-Eberhard 2003). Phenotypic flexibility is an important attribute that facilitates individuals in coping with changing conditions (Seebacher *et al.* 2015), with life-history consequences that may scale up to affect higher levels of organisation, for example, population persistence, community stability, and ecosystem processes (Bolnick *et al.* 2011). Flexibility in metabolic rate is likely to be an important component here, and indeed, there is a growing body of evidence supporting metabolic plasticity as a widespread response to environmental change (Hofmann and Todgham 2010). Factors including temperature (Seebacher *et al.* 2015; Sandblom *et al.* 2016), food availability (Auer *et al.* 2015c, 2016a; Zeng *et al.* 2018), food quality (Naya *et al.* 2007), oxygen availability (Hochachka *et al.* 1996; Norin *et al.* 2016) and salinity (Allan *et al.* 2006) have all been shown to induce short term and longer term (i.e. acclimation) changes in metabolic rates of organisms.

In ectotherms, SMR generally appears to be more flexible in the extent of its response to extrinsic factors than MMR (Norin and Metcalfe 2019). For example, increased temperatures were shown to cause reduction of SMR in European perch *Perca fluviatilis*, a thermal compensation response that was not apparent in MMR (Sandblom *et al.* 2016). Similar flexibility in BMR relative to MMR (or cold-induced maximum aerobic metabolism) has been demonstrated in endotherms in response to temperature (Nespolo *et al.* 2001; van de Ven *et al.* 2013; Dubois *et al.* 2016). Food availability has also been frequently shown to induce flexibility in SMR (and BMR) (Naya *et al.* 2007; Auer *et al.* 2015c, 2016a; Langer *et al.* 2018), suggesting reductions in baseline metabolism, rather than MMR, tends to underpin overall metabolic flexibility in response to food restriction (Zeng *et al.* 2018).

Although there is a significant number of studies detailing inter and intra specific variation in metabolic responses, we know considerably less about factors giving rise to differences in metabolic flexibility (Norin and Metcalfe 2019). Variation in metabolic rate flexibility between populations has been described primarily as changes in SMR or BMR, and particularly in response to

distribution or temperature factors *e.g.* cane toads *Rhinela marina* at high latitudes show more plastic resting metabolic rates in response to temperature than their counterparts at low latitudes (Winwood-Smith *et al.* 2015; McCann *et al.* 2018). Similarly, rufous-collared sparrow *Zonotrichia capensis* populations from seasonally-variable or temperate environments show more flexible BMRs in response to temperature than those from arid desert systems, though desert populations conversely showed more BMR flexibility at low food conditions, a finding that highlights the context-dependency of optimal metabolic phenotypes (Cavieres and Sabat 2008; Maldonado *et al.* 2012). Given that the optimal metabolic phenotype in a given context can show considerable variability depending on the population background, incorporating population-specific (or life history) factors into the investigation of metabolic variation and flexibility is likely to have important implications for managing and conserving species experiencing environmental change, yet few studies have addressed this.

Salmonine fishes (salmons, trouts, and charrs) represent an excellent group to study variation in metabolic phenotypes. As obligate freshwater spawning species, salmonines display a multitude of life-history strategies that incorporate a wide variety of migratory tactics (Klemetsen *et al.* 2003). Some individuals remain resident in natal freshwaters for their entire life cycles, whilst others migrate to more productive feeding grounds such as larger rivers and lakes (termed "potomodromy"), or even undertake dramatic migrations to the sea (termed "anadromy") (Ferguson *et al.* 2019). Migration generally facilitates high levels of growth in the new habitat, with migrants typically returning to spawn in freshwater at larger sizes than non-migratory "residents". Facultative migration – where individuals can adopt either a migratory or a non-migratory lifestyle - is common in salmonines, and populations can be primarily resident, migratory, or comprise a mix of both tactics (Chapman *et al.* 2012). Such alternative migratory phenotypes can be understood using the framework of the "environmentally cued threshold model" (Tomkins and Hazel 2007; Piche *et al.* 2008; Pulido 2011; Buoro *et al.* 2012), whereby tactic frequencies are

155 controlled by the relationship between an environmentally-sensitive trait (*e.g.* physiological condition or energetic status) and a genetically-variable threshold. Migration is triggered depending on whether or not an individual's "status" trait exceeds the threshold condition for residency. Energetic limitation in natal freshwaters is proposed to be a strong determinant of migration 160 (Forseth *et al.* 1999). As such, variation in migratory tactics is likely to be linked to variation in metabolic rates *e.g.* steelhead trout *O. mykiss* that matured in freshwater *in lieu* of migrating tended to have lower SMR values (Sloat and Reeves 2014); and juvenile Atlantic salmon *Salmo salar* with higher SMR early in life were more likely to subsequently migrate (McCarthy 2000).

165 Moreover, once the migration decision has been made, different energetic demands are associated with the alternative tactics. For example, migrants must have sufficiently high aerobic capacity (i.e. MMR/ AS) to sustain swimming performance during the migration itself (which can cover distances of tens to several thousand kilometres), and to facilitate high growth in the new 170 environment. In contrast, residents typically have lower energetic requirements, but must cope with access to fewer food resources in the freshwater environment (relative to lacustrine or marine resources) (Gross *et al.* 1988). Populations that display predominantly one migratory phenotype over another are thus likely to experience different selection pressures on metabolic 175 traits, whereby a migratory life-style might favour increases in the upper bounds of metabolisms (MMR), and residency might promote decreases in baseline energetic requirements (SMR), each with implications for overall AS and energy balance. This has some empirical support, for example, migratory three-spined stickleback *Gasterosteus aculeatus* had higher SMR, active metabolic rate, and 180 AS than non-migratory morphs (Tudorache *et al.* 2007), and anadromous (sea-migratory) juvenile Atlantic salmon *S. salar* had higher SMR than non-migrants (Seppänen *et al.* 2010). Less is known about how differences in migratory lifestyle might interact with environmental conditions to cause variation in metabolic traits.

Here, we explore the effects of extrinsic (food supply) *versus* intrinsic (population/sex) factors on metabolic rates in experimental F1 offspring derived from two wild populations of brown trout that differ in their migratory tactics. Specifically, we aimed to (i) asses how a period of long-term food restriction alters SMR, MMR and AS (ii) test if populations that naturally vary in their life histories also vary in SMR, MMR, and AS and (iii) explore if populations show variation in their metabolic responses to conditions of food restriction. We expected that food restriction would overall have a greater effect on SMR compared to MMR. We also expected that offspring derived from the naturally anadromous (i.e. migratory) population would show relatively higher MMR or AS, and those from the non-anadromous population would show relatively lower SMR, but the populations might vary in their ability to adjust their metabolic traits.

Methods

Study populations and fish rearing

Brown trout brood stock from two wild populations were caught by seine netting in November 2015 in the Erriff (53° 37′ 0.00" N: 09° 40′ 17.10" W) and the Burrishoole (53° 57′ N: 09° 35′ W) catchments in the west of Ireland (Figure S1). Erriff brood stock were caught in the Tawnyard Lough, an upland lake of 56 ha which is fed primarily by the Glendavoch river and a number of smaller tributaries. The Tawnyard Lough population spawn mainly in the Glendavoch River, and move downstream as fry or parr to Tawnyard Lough (a distance of a few hundred metres to kilometres, depending on where spawning occurred). Tawnyard Lough produces a large run of out-migrating anadromous juveniles (smolts), with annual estimates of 500 to 3000 smolts enumerated at the outflow of the Lough over the last 30 years (Gargan *et al.* 2016). An unknown proportion of the population remain within the lake, and undergo several years of freshwater growth before returning to the natal stream to spawn, with local experitise indicating that the Tawnyard population in general has a strong anadromous component (broadly estimated as 50 – 60% of population expressing anadromy) (P. Gargan, *pers comm*).

Burrishoole brood stock were caught in Lough Bunaveela (46 ha) in the headwaters of the catchment. A population of non-anadromous trout remain resident in Lough Bunaveela for most of their lifecycle, undertaking only short-distance, directed movements (10 – 100s of metres) between the lake and inflowing/outflowing spawning streams. Although the anadromous life history is present in the larger Burrishoole catchment, the development of aquaculture in Clew Bay is believed to have caused the anadromous trout run to decline severely in Burrishoole in the late 1980s. Despite Bunaveela spawning streams being accessible to anadromous fish, there is no evidence that the Bunaveela population has ever produced anadromous fish, either historically, or recently (Poole *et al.* 2007; Magee 2017). In summary, we consider offspring derived from the Tawnyard brood stock to have a strong anadromous background (hereafter termed the "anadromous background population"), and offspring from the Bunaveela brood stock to have no recent anadromous background (termed the "non-anadromous background population").

See Archer *et al.* (2019) for detailed description of crossing, fertilisation, and rearing procedures, which we describe here in brief. Each ripe female was mated to two males from the same source population. Fertilised eggs were incubated at a hatchery in the Burrishoole catchment. Post-hatching, fry were transferred to a rearing facility at University College Cork (Aquaculture and Fisheries Development Centre). Here, fry were initially held in two 100L growth tanks on a recirculating aquaculture system (RAS) maintained at natural temperature regime for the west of Ireland, and moved to 520L tanks on a larger RAS to facilitate growth in December 2016. Populations were reared separately for the entirety of the study to prevent emergence of dominance hierarchies. Fry were fed *ad libitum* with commercially available trout pellets (Skretting Ltd., Norway) until experimental food treatments (see below) began in September 2016. During the experimental phase, a programmed lighting system of LED lights above each tank mimicked the photoperiod of the source catchments. Water in the RAS was treated with mechanical filtration, bio filtration and UV skimming, and water quality (checked weekly) consistently remained within acceptable

levels for fish health. Great care was taken to ensure that all measured variables other than feeding regime (fish densities, temperature, photoperiod, lux, flow rates) were constant across the tanks.

Food restriction treatments

Fish in this study experienced experimental food restriction treatments from September 2016 to June 2018. The study, and all associated procedures, were carried out with ethical approval from Health Products Regulatory Authority (HPRA) Ireland, under HPRA project license AE19130/P034, and individual licenses AE19130/1087, AE19130/I200, AE19130/I201 and AE19130/I202 with all fish humanely euthanized under licence in June 2018 as part of a parallel experiment (Archer *et al.* 2019).

To explore the effects of extrinsic environment (food restriction) and intrinsic factors on metabolism, juvenile brown trout from each population were randomly allocated one of four food treatments in September 2016 (n = 90 per feeding treatment per population, at the beginning of the experimental phase). The food treatments were as follows: (i) High-High food: fish fed recommended daily pellet rations for optimal growth, calculated as a percentage of their body weight and adjusted for seasonally-changing temperatures (Skretting Ltd, Norway); (ii) Low-Low: fish fed 25% of recommended optimal rations. (iii) High-Low: fish switched from optimal rations to 25% optimal ration (i.e. from High to Low) in June 2017; and (iv) Low-High: fish switched from 25% of optimal rations to 100% optimal rations in June 2017 (i.e. from Low to High). The reductions to 25% of optimal food rations took place gradually over a four-week period to minimise stress. Within each tank, absolute rations were adjusted on a monthly basis to account for changes in body mass and temperature.

Measurement of metabolic traits

Eight to twelve individuals of each population in each food treatment were measured for SMR and MMR in February 2018 in a controlled-temperature (CT) chamber at 8 °C (mean temperature 7.99 °C ± 0.26 SD, matching the natural temperature in the wild for these populations).

Measurement of MMR

Whole-animal oxygen consumption (MO_2) in animals operating at their maximum aerobic metabolic rate was used as proxy for MMR (Norin and Metcalfe 2019) following best practices outlined in Norin and Clark (2016). We used an exhaustive chase protocol following Norin and Clark (2016) to elicit MMR in the same individuals that we measured for SMR. Prior to SMR measurements, each individual fish due to be measured for SMR that day was first placed in an aerated 50L tank and manually chased by hand until exhaustion, determined to occur when the fish were unresponsive (i.e. did not elicit burst swimming) to tactile stimulus (typically after 2 to 3 minutes of sustained chasing). Once exhausted, the fish was immediately transferred to a respirometry chamber in the same system used to measure SMR, the chamber was sealed, and oxygen decline within the closed chamber loop (recirculation pump operational) was recorded for a 60s measurement period. The time taken to transfer fish to chambers and begin recording oxygen measurements never exceeded 20s, ensuring that minimal recovery from the exhaustive chase procedure occurred before recording oxygen consumption.

Measurement of SMR

The SMR of individual fish was determined overnight in a darkened CT chamber using intermittent-flow respirometry, following best practices outlined in Svendsen *et al.* (2016). The respirometry system consisted of four acrylic respirometry chambers (1200 ml) (Loligo Systems, Viborg, Denmark), submerged in a water bath, flushed with de-chlorinated water bubbled to 100% oxygen saturation by an air stone. PVC tubing (10mm diameter, non-permeable to oxygen) connected each individual chamber to two pumps (Eheim Ltd., Deizisau, Germany): the "flush" pump flushed fully oxygenated water through the chambers. A second "recirculation" pump recirculated water in a closed loop through the chamber, whereby water exiting the chamber was passed through a 10mm flow through oxygen cell (PreSens Ltd., Regensburg, Germany) that continually measured dissolved oxygen concentration, before being recirculated back to the same respirometry chamber via PVC tubing. Thus,

individual oxygen consumption (MO_2, used as a proxy for SMR in fasted, non-active animals) was measured in repeated cycles that consisted of a flush period (flush pump operational) and a measurement period (recirculation pump operational) where oxygen level in each chamber was recorded at one second intervals to estimate oxygen decline (i.e. oxygen uptake). Each cycle consisted of 330s of flushing, and a measurement period of 200 - 300s (to ensure sufficient O_2 depletion for calculating MO_2 in different-sized fish). We also allowed a 30s buffer period before recording oxygen uptake once the flush pump was switched off, to allow the chamber water and flush water to mix completely and reach an equilibrium oxygen saturation .

Fish were fasted for 28 h prior to being placed into individual respirometry chambers to ensure individuals were in a post absorptive state (Cutts *et al.* 2002). Fish entered the chambers between 11:00 and 12:00 each day, and were left to acclimatise for five hours, with chambers continually flushed with oxygen-saturated water during this acclimation period. SMR measurements began between 16:00 and 17:00, and ended between 09:00 and 10:00 the following morning, allowing for a minimum of 100 measurements of oxygen uptake per individual. Fish were not disturbed during this ~20-hr SMR measurement period. Once SMR measurements had finished, each fish was removed from the chamber, lightly anaesethised with MS-222, blotted dry, and mass and fork length were recorded. Each fish was then given an individual identifier tag using unique colour combinations of visible implant elastomer tags (Northwest Marine Technology Ltd., USA). To limit bacterial growth in the system, the entire respirometry set-up was rinsed with bleach after each overnight SMR respirometry trial. We also measured background (i.e., bacterial) respiration rates in each chamber on a daily basis by recording oxygen decline in empty chambers for one measurement cycle before fish entered the respirometry system, and again for one measurement cycle the following morning (once the respirometry measurements had ended and fish were removed from the chambers).

Determination of sex

To determine sex, we euthanised fish that had been measured for SMR and MMR via an overdose of MS-222 in April 2018 (approximately 2 months after respirometry measurements due to involvement in an ongoing parallel study, see Archer *et al.* (2019)). If sex could not be determined anatomically, genotypic sex was later assigned using a microsatellite sex marker (P. Prodöhl, *unpublished*). We were unable to re-identify six individuals due to tag loss, leaving n = 55 fish successfully assigned for sex. Sex ratios were approximately similar across food treatment groups and across population backgrounds.

Statistical analysis

To estimate SMR (mg O_2 h^{-1}), we first calculated MO_2 values for each repeated measurement of oxygen uptake recorded during the overnight SMR respirometry trials. MO_2 (mg O_2 h^{-1}) was calculated as the most consistent linear decline in oxygen recorded during each measurement cycle, estimated by rolling regression in the *respR* package in R (Harianto and Carey 2019). All measurements of MO_2 were visually inspected to assess regression fit, and only MO_2 values with an acceptable fit (associated R^2 values > 0.90, unless a clear linear trend was determined upon visual inspection of fit) were included in subsequent SMR calculations. To account for any background respiration included in these MO_2 values, we estimated background respiration by calculating MO_2 values for the oxygen uptake measurements in empty chambers, both before and after each overnight SMR respirometry trial (as described above). Because background MO_2 rates were assumed to increase linearly through time over the course of the experiment (due to bacterial growth), we allowed for a dynamic background correction value (i.e. that increased overnight), calculated as:

$$MO_{2\_bg} = bg_0 + (t \times bg)$$

Where MO_{2_bg} is background MO_2, at a given measurement time point t, the time elapsed since initiating overnight SMR measurements, bg_0 and bg are parameters (the intercept and slope respectively) estimated from the matrix

regression of the background oxygen uptake before, and background oxygen uptake after, as a function of time elapsed. We then used MO_{2_bg} to account for background respiration by subtracting MO_{2_bg} from each value of $M\mathrm{O}_2$ as calculated for an individual fish at successive time points during the overnight SMR respirometry trials. $M\mathrm{O}_{2\_bg}$ never exceeded more than 2% of total $M\mathrm{O}_2$ in all cases, confirming that background respiration rates remained low throughout the study.

SMR for each individual fish was calculated by taking the mean of the lowest 10th percentile of background-corrected $M\mathrm{O}_2$ values recorded over the 20-h SMR measurement period, then excluding outliers (values more than two standard deviations from this mean).

We estimated individual MMR (mg O_2 h^{-1}) using the *respR* package (Harianto and Carey 2019) by calculating $M\mathrm{O}_2$ as the linear decline in oxygen in each individual respirometry chamber in the 60s measurement period immediately after the exhaustive chase protocol (i.e. extracting slopes from the linear regression of oxygen concentration against time over a 60s period). Oxygen sensor probe and equipment malfunctions resulted in respirometry measurements for 6 fish being discarded, leaving a total of n = 61 individuals measured for SMR and MMR. Absolute aerobic scope (AS) for each fish was calculated as the difference (mg O_2 h^{-1}) between MMR and SMR.

Since metabolic responses to food restriction are well-documented to be reversible in salmonines (metabolic rates are restored to pre food restriction levels once standard food rations are reinstated (O'Connor *et al.* 2000)), we first assessed if any potential metabolic responses to food restriction had been reversed/offset in the Low-High groups by February 2018 (when we measured metabolic traits). No differences in SMR (ANOVA: $\chi^2 = 0.23$, df = 1, $P = 0.633$), MMR ($\chi^2 = 0.40$, df = 1, $P = 0.528$), or AS ($\chi^2 = 0.51$, df = 1, $P = 0.476$) existed between the High-High food treatment and Low-High treatment. Similarly, we tested whether potential metabolic responses to food restriction were affected by the length of the food restriction period *i.e.* did Low-Low (17 months

restriction) differ from High-Low (7 months restriction). No differences existed in SMR ($\chi^2 = 0.04$, df = 1, $P = 0.836$), MMR ($\chi^2 = 0.44$, df = 1, $P = 0.509$), or AS ($\chi^2 = 0.47$, df = 1, $P = 0.494$) between the Low-Low food treatment and the High-Low treatment. Since our primary interest was simply in the overarching effects of food restriction on metabolism (and not the effects of switching food treatments *per se*), we combined the High-High and Low-High treatments into a single "High Food" treatment group, and combined the Low-Low and the High-Low treatments into a single "Low Food" treatment group. We present analyses using the "High Food" and "Low Food" groups here, with the *caveat* that "High" or "Low" refers specifically to the food treatment experienced in the ~7-month period prior to metabolic measurements in year 2 of life (a timescale over which metabolic rates have been shown to be consistent in salmonines (Seppänen *et al.* 2010)). Moreover, pilot SMR measurements collected from our populations in April and May 2017 (following similar respirometry protocols to those described above) showed similar effects of High/Low food treatments as the results described below (which focus solely on the 2018 measurements of SMR, MMR, and AS). This indicates that responses to food treatments were: (i) consistent though time (or at least between years); and (ii) most likely as a result of phenotypic plasticity rather than random variation.

To avoid the pitfalls associated with solely using *P*-values (Halsey *et al.* 2015; Halsey 2019), we first tested for factors influencing mass-independent measures of SMR, MMR, and AS through estimation statistics (i.e. estimating effect sizes) using the *dabestr* package (Ho *et al.* 2019). We used the residuals of the linear relationships between $\log_{10}$ body mass, and SMR, MMR, and AS (all $\log_{10}$ transformed) to correct for body size in these analyses. Residuals generated from these analyses (rSMR, rMMR, and rAS) gave mass-independent estimates of metabolic rates (individuals with positive residuals have a higher than expected metabolic rate for a given fish size, whereas negative residuals indicate a lower than expected rate). Effect sizes for mean differences in rSMR, rMMR, and rAS were computed for all pairwise comparisons between all levels of food treatment (high or low) and population background (anadromous or non-

anadromous) factors, and 95% confidence intervals (CIs) were constructed by bootstrapped resampling for 5,000 resamples. An additional set of analyses tested for sex-based differences in metabolic traits, whereby we similarly estimated effect sizes for pairwise comparisons of rSMR, rMMR, and rAS between the two levels of sex. Similar analyses were also run using an alternative ANCOVA approach, which tested for variation in the relationships between body mass and SMR, MMR, and AS according to population, food treatment, and sex factors using general linear models (GLMs) (see Supporting Information). The results (shown in the SI) were qualitatively similar, suggesting that the findings based on estimation statistics that we present here are robust.

Finally, to explore whether population background and food treatments affected the size-independent relationships between different aspects of metabolism, we created three GLMs (normal errors). The first GLM included rMMR as a response variable, and rSMR, food treatment and population treatment as explanatory variables, interactions between rSMR and food treatment, and between rSMR and population, along with a three-way interaction term (rSMR × food × population). The second GLM included rAS as the response variable, and similarly included rSMR, food treatment, population treatment, and interaction terms for rSMR × food, rSMR × population and rSMR × food × population. We constructed a third GLM with rAS as the response variable, and rMMR, food treatment, population treatment as predictors, along with interaction terms for rMMR × food, rMMR × population and rMMR × food × population.

For the estimation statistics approach, we considered an estimated difference in means between groups to exist (i.e. was significant) if the 95% CI of the effect size did not include zero. We used likelihood ratio tests (LRT) to assess statistical significance of predictor variables for all of the GLM models at a 5% alpha level, and non-significant interaction terms were excluded to interpret main effects. All analysis was carried out in R version 3.6.0 (R Core Team 2019) and all models were checked against assumptions of the given model

(independence, non-normality of residuals, heteroscedasticity and multicollinearity).

Results

Effects of population and food restriction on metabolic rate

Overall, whole-animal SMR, MMR and AS varied with food treatments and across populations, with higher mean SMR, MMR and AS in the anadromous population (see Table 1 for mean values and SD by population and treatment combinations).

Table 1: Mean values and associated standard deviations (SD) for the length (mm), mass (g), standard metabolic rate (SMR) (mg O_2 hr^{-1}), maximum metabolic rate (MMR) (mg O_2 hr^{-1}), and aerobic scope (AS) (mg O_2 hr^{-1}) of brown trout offspring derived from two wild populations (AB = anadromous background population, non-AB = non anadromous background population). Offspring experimentally reared under two food treatments (High = optimal rations, Low = 25% of optimal rations).

Food, Population	**Length** (mean ± SD)	**Mass** (mean ± SD)	**SMR** (mean ± SD)	**MMR** (mean ± SD)	**AS** (mean ± SD)
High, AB	201.6 ± 18.8	109.65 ± 30.40	5.39 ± 1.71	40.52 ±14.47	35.13 ± 13.38
Low, AB	200.6 ± 9.2	101.76 ± 16.38	4.51 ± 0.71	43.04 ± 8.94	38.52 ± 8.79
High, Non-AB	199.7 ± 15.5	110.83 ± 27.59	5.18 ± 1.49	31.87 ± 10.79	26.69 ± 9.64
Low, Non-AB	199.0 ± 17.7	101.37 ± 27.30	4.21 ± 1.54	30.31 ± 11.73	26.09 ± 11.13

Fish from the low food treatments had lower mass-independent SMR (lower rSMR) than those in the high food treatment (Figure 1A), and this difference in mean rSMR was evident in both populations (Figure 1A, Table 2). Fish from the anadromous background population had a marginally higher rSMR than those from the non-anadromous background population in both food treatments, however the 95% CIs for the mean difference in rSMR between populations overlapped zero (Figure 1B, Table 2).

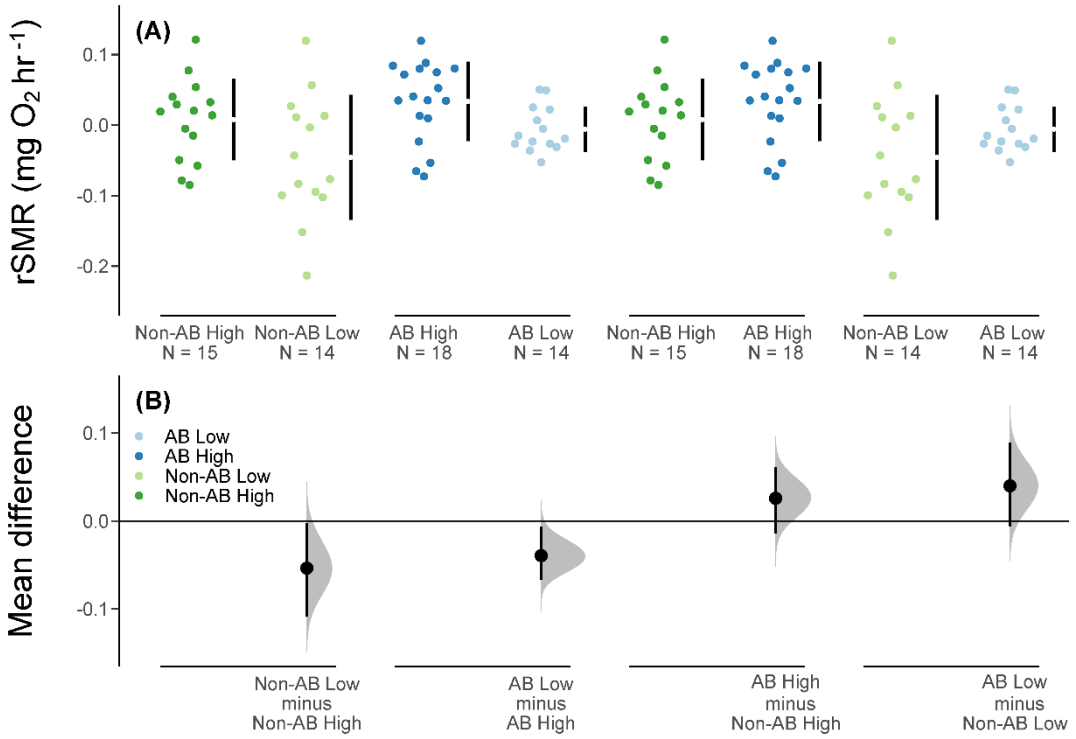

Figure 1: (A) Residual standard metabolic rate (rSMR) values (body mass corrected) for brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish were reared under two experimental food treatments: optimal food rations (High); and 25% of optimal daily rations (Low). Black vertical bars represent the standard deviation around the mean (shown as a gap in the bars), and sample size is shown as "N =". (B) Cumming estimation plots for each population background and food treatment combination.with effect sizes shown as black dots (i.e. the mean differences in rSMR among the groups), the distributions (shaded curves) and 95% confidence intervals (back bars) of the effect sizes obtained from non-parametric bootstrap resampling (5,000 resamples).

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Table 2: Effect sizes (Δ) and associated 95% confidence intervals (CIs) for differences in mean residual standard metabolic rate (rSMR) (mg O_2 hr^{-1}), residual maximum metabolic rate (rMMR) (mg O_2 hr^{-1}), and residual aerobic scope (rAS) (mg O_2 hr^{-1}) of brown trout offspring derived from two wild populations (AB = anadromous background population, non-AB = non anadromous background population), exposed to two food treatments (High = optimal rations, Low = 25% of optimal rations). CIs were constructed by non-parametric bootstrap resampling (5,000 resamples).

Mean difference (Δ)	**Δ rSMR** (95% CI)	**Δ rMMR** (95% CI)	**Δ rAS** (95% CI)
Low AB – High AB	-0.039 (-0.067; -0.007)	0.060 (-0.003; 0.129)	0.075 (0.004; 0.155)
Low non-AB – High non-AB	-0.054 (-0.109; -0.002)	0.006 (-0.066; 0.078)	0.013 (-0.071; 0.101)
High AB – High non-AB	0.026 (-0.014; 0.061)	0.111 (0.039; 0.175)	0.125 (0.039; 0.200)
Low AB – Low non-AB	0.040 (-0.006; 0.089)	0.165 (0.091; 0.236)	0.187 (0.101; 0.271)
Female – Male	-0.019 (-0.051; 0.020)	-0.155 (-0.203;-0.104)	-0.178 (-0.236; -0.118)

There was no effect of food on rMMR in either population (95% CIs for the mean difference in rMMR overlapped zero, Figure 2A, B). Fish from the anadromous background population had a higher rMMR than those from the non-anadromous background population in both food treatments (Figure 2A, B, Table 2).

Similarly, fish from the anadromous background population had a higher rAS than the non-anadromous background population under both food treatments (Figure 3A, B, Table 2). We also detected population-specific effects of food treatment on rAS, whereby fish in the anadromous population in the low food treatment had a marginally higher rAS than those in the high food treatment (Figure 3B, Table 2). This food treatment effect on rAS was not apparent in the non-anadromous population (Figure 3B).

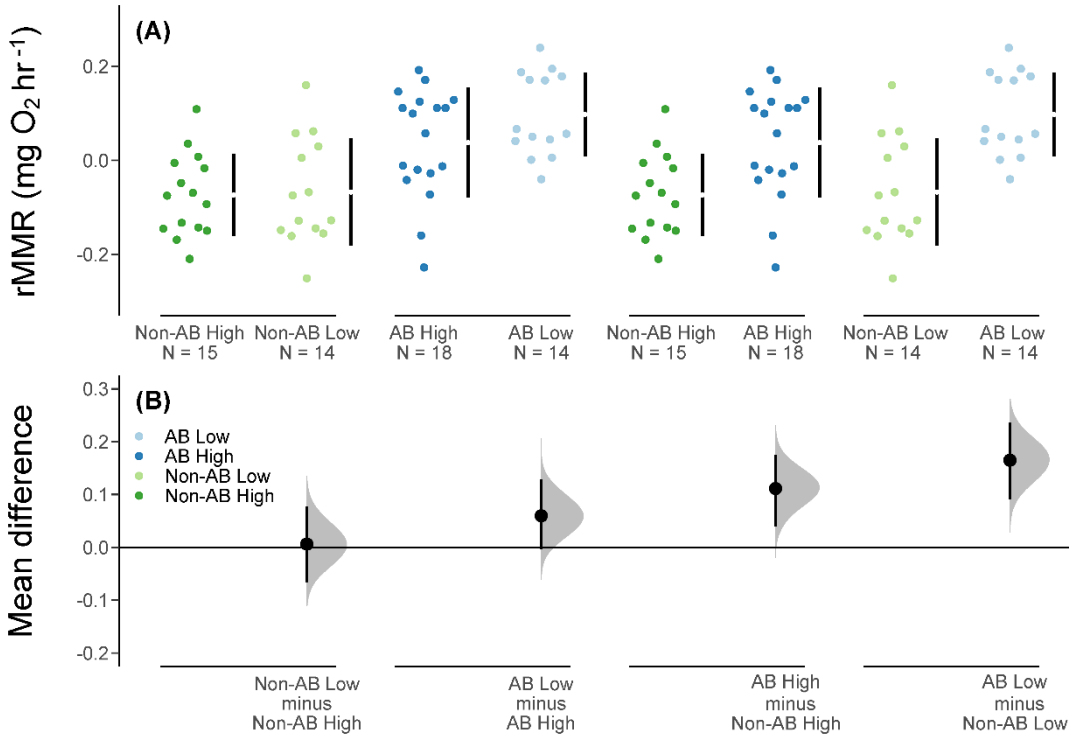

Figure 2: (A) Residual maximum metabolic rate (rMMR) values (body mass corrected) for brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish were reared under two experimental food treatments: optimal food rations (High); and 25% of optimal daily rations (Low). Black vertical bars represent the standard deviation around the mean (shown as a gap in the bars), and sample size is shown as "N =". (B) Cumming estimation plots for each population background and food treatment combination.with effect sizes shown as black dots (i.e. the mean differences in rMMR among the groups), the distributions (shaded curves) and 95% confidence intervals (back bars) of the effect sizes obtained from non-parametric bootstrap resampling (5,000 resamples).

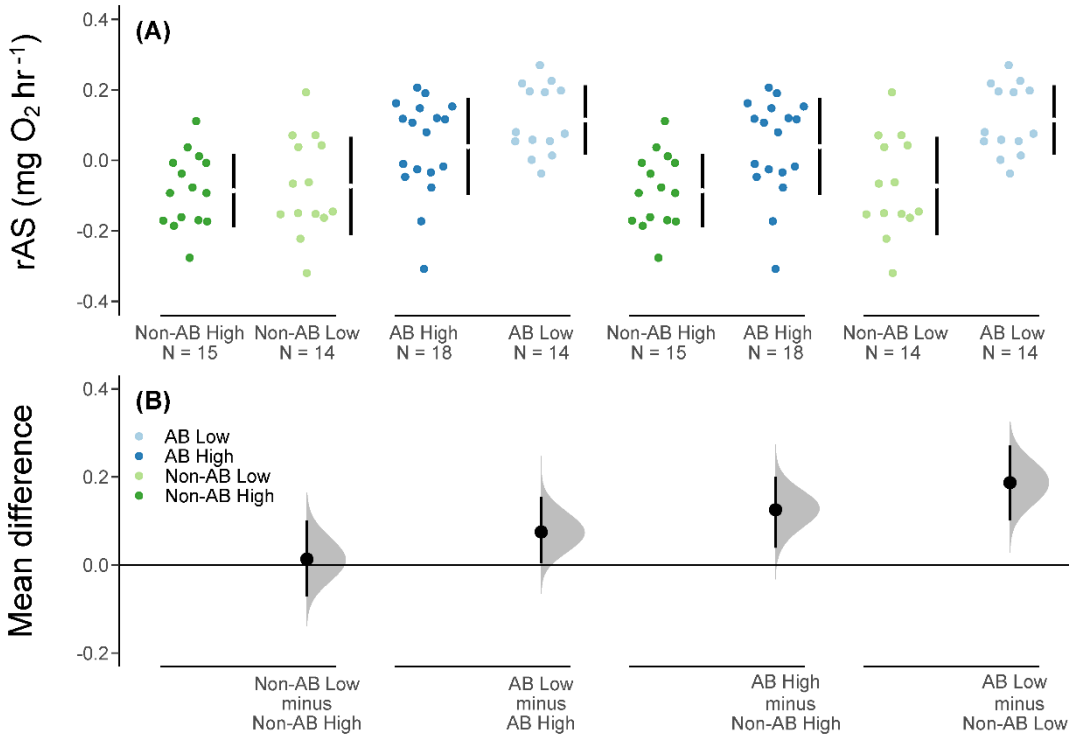

Figure 3: (A) Residual aerobic scope (rAS) values (body mass corrected) for brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish were reared under two experimental food treatments: optimal food rations (High); and 25% of optimal daily rations (Low). Black vertical bars represent the standard deviation around the mean (shown as a gap in the bars), and sample size is shown as "N =". (B) Cumming estimation plots for each population background and food treatment combination.with effect sizes shown as black dots (i.e. the mean differences in rAS among the groups), the distributions (shaded curves) and 95% confidence intervals (back bars) of the effect sizes obtained from non-parametric bootstrap resampling (5,000 resamples).

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Coupling of metabolic traits

When considering size-independent effects of rSMR on rMMR, the interaction terms for rSMR × food × population (χ^2 = 0.23, df = 1, P = 0.633), rSMR × food (χ^2 = 1.45, df = 1, P = 0.229), rSMR × population (χ^2 = 0.90, df = 1, P = 0.344), and food × population (χ^2 = 2.66, df = 1, P = 0.103) were all non-significant. The main effects of rSMR (χ^2 = 0.66, df = 1, P = 0.417) and food (χ^2 = 2.21, df = 1, P = 0.137) were also non-significant. We detected a significant main effect of population background (χ^2 = 22.35, df = 1, P < 0.001), whereby the anadromous background population had a higher rMMR for a given rSMR (Figure 4A).

Effects of rSMR on rAS were similar, where we detected non-significant effects of rSMR × food × population (χ^2 = 0.24, df = 1, P = 0.624), rSMR × food (χ^2 = 1.39, df = 1, P = 0.239), rSMR × population (χ^2 = 0.92, df = 1, P = 0.337), and food × population (χ^2 = 2.69, df = 1, P = 0.101), and non-significant main effects of rSMR (χ^2 = 0.004, df = 1, P = 0.952) and food (χ^2 = 1.86, df = 1, P = 0.173). The anadromous population had a significantly higher rAS for a given rSMR (effect of population background: χ^2 = 21.98, df = 1, P < 0.001; Figure 4B).

We detected a significant positive relationship between rMMR and rAS (χ^2 = 4689.8, df = 1, P < 0.001; Figure 4C), but interactions between rMMR × food × population (χ^2 = 1.16, df = 1, P = 0.201), rMMR × food (χ^2 = 0.2, df = 1, P = 0.673), rMMR × population (χ^2 = 2.3, df = 1, P = 0.1297), and food × population (χ^2 = 0.1, df = 1, P = 0.768) were all non-significant. The main effects of food (χ^2 = 2.4, df = 1, P = 0.123) and population (χ^2 = 1.9, df = 1, P = 0.163) were also non-significant.

See Supporting Information for coefficient estimates for all of the above models.

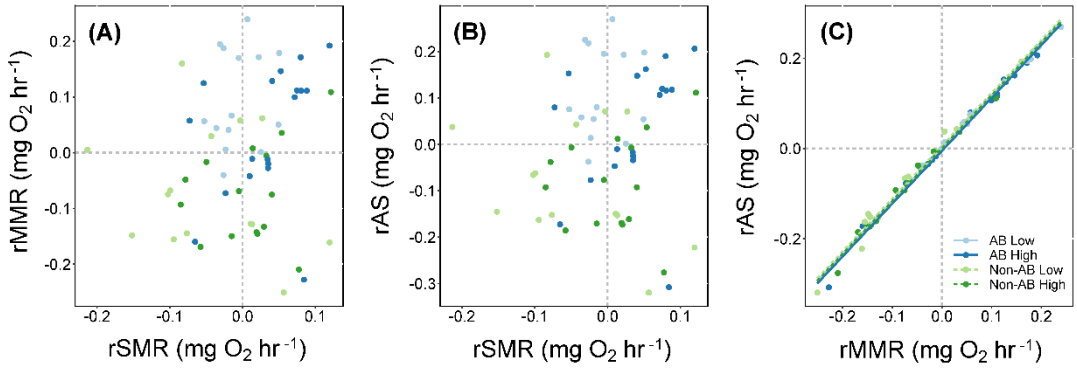

Figure 4: Size-independent relationships between: (A) residual standard metabolic rate (rSMR) and residual maximum metabolic rate (rMMR); (B) rSMR and residual aerobic scope (rAS); and (C) rMMR and rAS for brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish experienced two food reduction treatments: optimal food rations (High) and 25% of optimal rations (Low).

Effects of sex on metabolism

There were no sex-based differences in rSMR between , with similar rSMR in males and females (Figure 5A, Table 2). However, male fish had higher rMMR than female fish (Figure 5B, Table 2), and similar sex-based differences were detected in rAS (Figure 5C, Table 2).

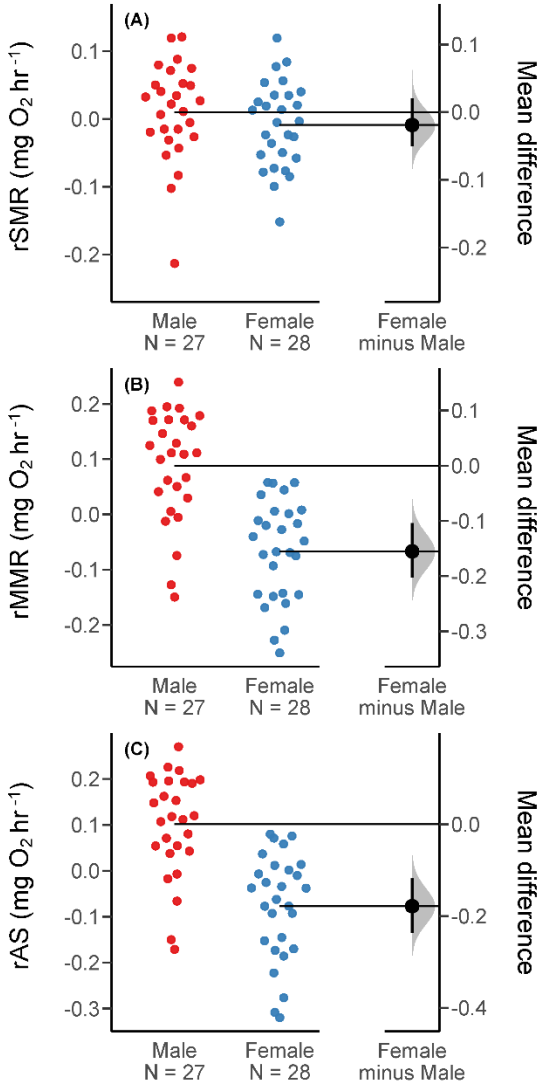

Figure 5: Gardner-Altman estimation plots for: (A) standard metabolic rate (SMR); (B) maximum metabolic rate (MMR); and (C) aerobic scope (AS) of brown trout classed as female or male after two years of experimental tank rearing. The Gardner-Altman estimation plots show the residual (body mass corrected) SMR/MMR/AS on the left axes and the effect size (mean difference between females and males) is represented by the black dot on the right axes, along with the distribution (shaded curve) and 95% confidence interval (black bars) of the effect size, obtained via non-parametric bootstrap resampling (5,000 resamples).

Discussion

Intra-specific variation in metabolic rates is widespread across species, yet there are still gaps in our understanding of how intrinsic and extrinsic environmental factors can interact to influence the various components of an individual's metabolism. Here, we exposed brown trout offspring from two population backgrounds (one anadromous/sea-migratory, one non-anadromous) to long-term food restriction to determine if, and how, intrinsic factors (i.e. population/sex specific effects) *versus* extrinsic factors (food resources) affect metabolic rates. Fish from both populations had lower SMR under low food conditions, with slight differences in overall SMR between populations. Fish from the anadromous population had higher MMR, and consequently, higher AS than the non-anadromous population under all food regimes. Intriguingly, fish from the anadromous-background also had a higher AS at low food compared to high food conditions, suggesting this population was more flexible in maximum metabolic traits than the non-anadromous population. We also found differences in MMR and AS linked to sex, which were not apparent in SMR. Collectively, our results suggest the various components of metabolism are differentially affected by intrinsic and extrinsic factors. Moreover, populations may vary in their capacity to flexibly adjust metabolic traits in response to environmental conditions, with consequences for population resilience to global change.

Effects of extrinsic environment on metabolic traits

The lower SMR we observed in response to long-term food restriction (> 7 months) is in line with previous work showing SMR (or BMR) to be strongly sensitive to food availability, typically without corresponding changes in MMR (Metcalfe *et al.* 2016). SMR has been found to show similar flexible decreases in food-poor environments (Naya *et al.* 2007; Auer *et al.* 2015c, 2016a; Zeng *et al.* 2018; Langer *et al.* 2018) or, conversely, to increase at high food availability (Van Leeuwen *et al.* 2011, 2012). Reductions in SMR are assumed to be optimal when food is scarce because the overall energetic cost of living is similarly reduced, thus facilitating higher growth (and consequently, fitness) (Auer *et al.* 2015c).

Such flexibility in SMR will likely have positive implications for species experiencing rapid environmental change, with temperature-induced plasticity in SMR linked to increased resilience to climate change (Magozzi and Calosi 2015). However, the adaptiveness of a given flexible response will depend upon both the predictability of the environmental change (i.e. the pattern of fluctuations in the environment), and the speed at which organisms can flexibly adjust their phenotypes to match these changing conditions (Reed *et al.* 2010). Moreover, it is unclear whether SMR flexibility translates into overall fitness benefits in scenarios of multi-faceted environmental change. This is particularly pertinent for aquatic ectotherms such as salmonines, which are likely to experience reductions in invertebrate prey size and abundance alongside warming (Durance and Ormerod 2007). It remains to be seen whether such populations have the capacity to sufficiently reduce SMR in response to combined stressors of food restriction and warming, though a study in common carp *Cyprinus carpio* indicates that the benefits of food-induced SMR plasticity may be temperature dependent. (Zeng *et al.* 2018).

Effects of intrinsic factors on metabolic traits

We detected overall variation in SMR, MMR, and AS according to population factors, with higher metabolic traits observed for the anadromous population. Population-level variation in metabolic traits could arise either though plasticity/flexibility/acclimation, or reflect genetic differences (which could include genetic variation in plasticity itself, *e.g.* variation among genotypes in their extent of flexibility). Metabolic rates are evolvable (Pettersen *et al.* 2018) and have been shown to respond to selection across relatively short time frames, *e.g.* BMR increased within 11 generations of selection in bank voles *Myodes glareolus* (Sadowska *et al.* 2015). The differences in SMR (for a given fish size) between populations was in agreement with our expectations, with the non-anadromous population having a lower mass-adjusted SMR than the anadromous population. However, the effect size of population on SMR was small in both food treatments, with 95% CIs that included zero, suggesting that differences between populations were marginal. Nevertheless, any differences

in SMR could be indicative of life-history differences between the two populations, where lower SMR in the non-anadromous population has arisen as an evolutionary or plastic response to lower levels of productivity in freshwaters relative to marine (Gross *et al.* 1988). Since we observed population-level differences in SMR in both high and low food treatments, it seems more likely that the differences in SMR between populations are due to inherited genetic differences rather than plastic responses (i.e. the non-anadromous population had lower SMR even at optimal food levels). Such inherited differences could arise from standard inherited allelic variation, or from inherited environmental influences (*e.g.* maternal effects) that could include epigenetic inheritance. Regardless of the inheritance mechanism, the resulting fixed phenotypic differences between the populations could be adaptive. For example, the non-anadromous population may have experienced stronger selection for reduced SMR in order to minimise their baseline energy requirements (Gross *et al.* 1988), whereas selection on SMR may have been in the opposite direction in the naturally anadromous population, whereby higher SMR (and indeed MMR or AS) could facilitate rapid somatic growth in order to reach target smolt sizes to successfully migrate (McCarthy 2000).

The relatively strong differences we observed between populations in MMR (and consequently AS) suggests the upper bounds of metabolism may be more affected by population-specific factors than the lower bounds. We had expected the anadromous population to show comparatively higher MMR than the non-anadromous population, and indeed this was the case, a finding that reflects higher MMR and AS previously described in migratory versus non-migratory ecotypes of three-spined sticklebacks (Tudorache *et al.* 2007). A genetic basis to MMR has been proposed to underpin metabolic variation between migratory forms of three-spined stickleback, where differences in MMR between anadromous and non-anadromous populations have been explained within the context of relaxed selection on swimming performance in stream-resident populations, mediated by reductions in MMR (Dalziel *et al.* 2012b). In contrast, anadromous populations that undertake more arduous/lengthy migrations

tend to have higher swimming/cardiac performances, and higher MMR (Lee *et al.* 2003; Eliason *et al.* 2011; Dalziel *et al.* 2012a), indicating migration effort may further underpin consistent differences in MMR between migratory and non-migratory individuals or populations (Seppänen *et al.* 2010). A higher MMR in the anadromous population may also confer fitness benefits by facilitating high levels of growth, either through direct selection on MMR in the freshwater environment (where fast growth increased migration success), and indeed, differences in intrinsic freshwater growth rates have previously been described for our study populations (Archer *et al.* 2019). Indirect selection on MMR in juveniles might also occur because of a positive genetic correlation with MMR expressed in the marine environment, where high growth rates are translated into increased fecundity, with rank-order MMR in fish generally repeatable through time (Norin and Clark 2016).

Sex-based differences in MMR (and AS) that were not evident in SMR further suggests that MMR is more strongly influenced by intrinsic rather than environmental factors. We observed a higher MMR in males, suggesting that while males and females had similar basic energetic requirements, males had more scope to increase their metabolism and divert resources into processes such as growth, or aggressive interactions underpinning competition. Salmonines generally show patterns of sex-specific aggression, with differences in aggression developing early, *e.g.* juvenile *O. mykiss* display more aggressive behaviour than females (Johnsson and Åkerman 1998), a trait likely genetically correlated to sex-based differences in competitive ability as adults (Johnsson *et al.* 2001). On a broader scale, our finding corroborates evidence for sex-specific differences in AS described in pink salmon *O. gorbuscha* (Clark *et al.* 2011), and in cardiovascular performance of migrating sockeye salmon *O. nerka* (Sandblom *et al.* 2009). Collectively, these studies suggest that the relatively lower AS of female salmonines could make them more susceptible to effects of global change.

Differences in flexibility of metabolic traits

That we detected stronger effects of food restriction on SMR compared to MMR or AS lends further support to the proposal that the "ceiling", which constrains upper limits of metabolism, is less flexible than the metabolic "floor" (i.e. SMR) (Sandblom *et al.* 2016). Nonetheless, we did detect population-specific flexibility in AS, where the anadromous population had marginally higher AS at low rather than high food conditions, a difference that was not apparent in the non-anadromous population. The AS flexibility in the anadromous population appeared to be somewhat underpinned by decreased SMR at low food conditions (i.e. similar effect sizes for food treatment effects on SMR in both populations, but higher/positive effects sizes of low food on AS were only seen in the anadromous population). The few studies that have explored the effects of food restriction on MMR or AS have found little evidence for food-induced flexibility in these traits (Van Leeuwen *et al.* 2011; Killen 2014; Auer *et al.* 2016b; Zeng *et al.* 2018). The population-specific increase in AS in response to food restriction that we observed is initially counter-intuitive, but can interpreted as further evidence for the optimal combination of metabolic traits being context-dependent. For example, context-dependency of flexibility in MMR and AS have previously been described in barramundi *Lates calcarifer* that showed variable plasticity to hypoxia, salinity, and temperature changes (Norin *et al.* 2016). It is less clear why a higher AS might be optimal in a low food environment. The ability to flexibly increase AS may perhaps be a consequence of the migratory background of this population, particularly if the conditions that promote a migratory life-history in this population also tend to promote flexibility in SMR, MMR, or AS (*e.g.* fluctuations in food resources/quality in the catchment-of-origin drive patterns of migration and also flexibility in AS).

Plasticity in MMR or AS in the anadromous population may be an adaptive response to conditions of low food, given that food restriction increases the frequency of migratory tactics in brown trout in general (Ferguson *et al.* 2019 and references therein) and has been shown to increase the prevalence of migrants in this population specifically (Archer *et al.* 2019). If low food

environments promote migration, individuals that can increase their MMR are likely to have higher fitness, since high aerobic capacity (i.e. MMR or AS) is required to fuel swimming performance of migrating fish (Claireaux *et al.* 2005; Eliason *et al.* 2011). A flexible MMR in low conditions could be thus be a population-level adaptive response to a migratory background, a response that only emerges at low food availability when it potentially facilitates improved migration performance. Such a response is comparable to studies documenting increased whole animal oxygen consumption of high latitude (i.e. cold-acclimated) killifish *Fundulus heteroclitus* compared to low-latitude fish, with differences only evident at cold extremes (Fangue *et al.* 2009; Dhillon and Schulte 2011).

Higher AS (but not SMR) have been previously associated with competitive performance (Killen *et al.* 2014), and increased food intake (Auer *et al.* 2015a). Selection for higher MMR in low food environments has recently been shown in juvenile Atlantic salmon *S. salar* (Auer *et al.* 2018a), with higher MMR explained in relation to increased competitive ability. As such, a flexible maximum metabolic rate may represent an alternative metabolic strategy to lowering SMR, in order to maintain food consumption rates (and growth) in response to long-term food restriction. The benefits of such an alternative metabolic strategy become clear in the context of long-term food restriction scenarios because SMR depression is associated with the accumulation of harmful mitochondrial reactive oxygen species (ROS) that can impose long-term costs on life-history traits (Salin *et al.* 2018). Alternatively, we cannot rule out that food reduction may have induced higher MMR as a by-product of increased numbers of aggressive interactions between individuals (Seebacher *et al.* 2013), with anadromous brown trout tending to show more aggressive behaviour than non-anadromous forms (Lahti *et al.* 2001).

Implications and considerations

Overall, the importance of population-specific factors evident here is consistent with a role for natural selection in the evolution of MMR and SMR, though SMR appears to also respond in a more flexible/plastic manner to environmental

conditions. Intriguingly, because we observed metabolic trait variation in populations that naturally express different life histories (in terms of migratory tactics) such population-level differences may be further influenced by proximate and ultimate mechanisms underpinning alternative migration (Hazel *et al.* 1990; Tomkins and Hazel 2007; Pulido 2011). For example, if primarily non-migratory populations (or individuals) tend to have lower SMR, this may increase the frequency of fish that meet their residency threshold/"switch point", meaning the population shifts further towards residency and undergoes further selection for lower SMR up to some limit. Conversely, if a migratory population (or individual) tends to have higher SMR, this increases the likelihood that individuals become energetically constrained in freshwater, (i.e. do not meet their residency threshold), and thus more fish become migrants (and the population as a whole experiences positive selection on SMR). Subsequently, migrants with a higher MMR will likely perform better during migration and at sea, achieving higher levels of fitness, and thus selection will favour a higher MMR in such populations. Environmental conditions could also affect evolutionary processes, if factors such as low food or inclement temperatures interact with metabolic traits and further alter the costs and benefits of migration *versus* residency tactics. A tight evolutionary coupling has recently been described for metabolic rate and pace of life history in guppies *Poecilia reticulate* (Auer *et al.* 2018b), and similar mechanisms could be at play in species with alternative migratory life histories. Because metabolism and life-history traits are both influenced by environmental factors, reciprocal translocation or "common garden" experiments in nature (*e.g.* Auer *et al.* (2018b)) can help to disentangle interactions between physiology, environment, and genetics as both the causes and consequences of alternative migratory tactics (i.e. whether covariation between metabolic traits and life history is caused by phenotypic flexibility or fixed inherited differences).

It is important also to note that here we only considered metabolic variation at a single life stage, and it is likely that metabolism (and its flexibility) can vary though ontogeny (Pettersen *et al.* 2016), depending on energy requirements

associated with developmental stage (Beaman *et al.* 2016; Burggren 2018), or seasonal changes (Versteegh *et al.* 2012; Petit *et al.* 2013). Expanding our approach to incorporate repeated measurements on individuals throughout their lives (or indeed on individuals at different stages of ontogeny) would further illuminate when and how metabolic flexibility develops, and is most beneficial. Nonetheless, our results indicate that metabolic traits can respond differently to extrinsic and intrinsic factors, and metabolic responses can further vary according to population-specific factors. That maximum metabolism was more fixed than minimum metabolism suggests that trout may be more constrained in their capacity to adjust AS in response to extrinsic factors, with important conservation implications for a species that is already in global decline due to anthropogenic activities (Limburg and Waldman 2009). Moreover, the variation in metabolic traits that we observed in our two brown trout populations indicates that responses to environmental change are unlikely to be universal; and developing effective management strategies is not necessarily a straightforward task. Nevertheless, greater plasticity is linked to higher resilience (Magozzi and Calosi 2015; Seebacher *et al.* 2015) if environmental changes are predictable (Reed *et al.* 2010) and understanding the capacity of species and populations to flexibly adjust their metabolic traits is essential for predicting and mitigating the effects of progressively changing environmental conditions in natural systems.

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Supporting Information

Metabolic traits in brown trout (*Salmo trutta*) vary in response to food restriction and population background

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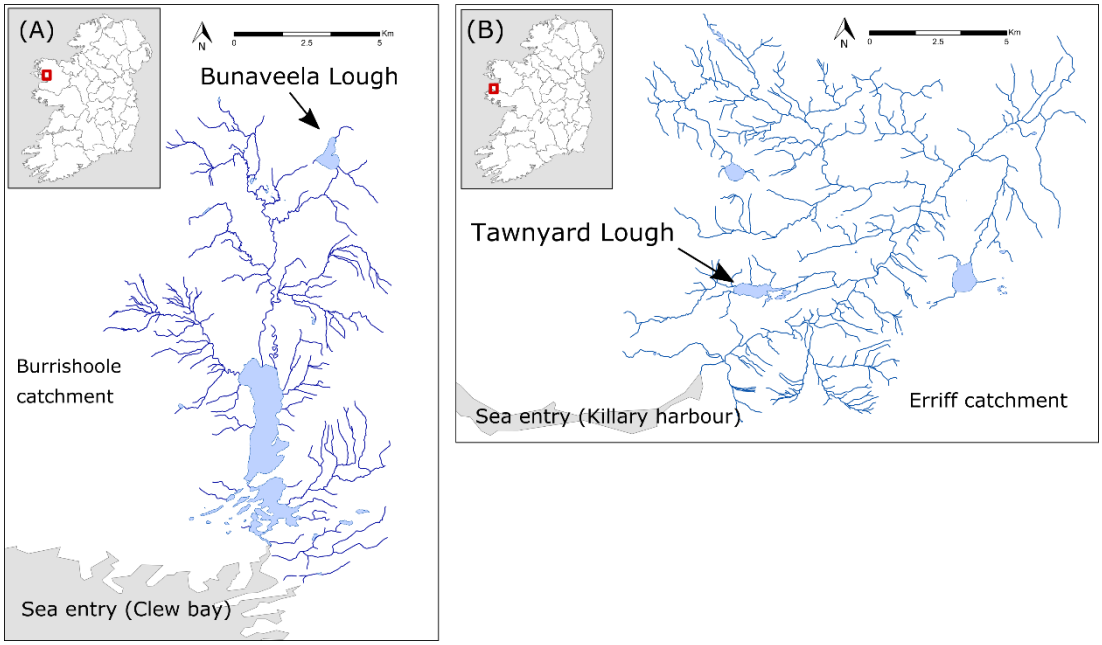

Figure S1: (A) Bunaveela Lough in the Burrishoole catchment, where brown trout brood stock were collected by seine netting in November 2015, used to produce F1 offspring for the experimental study. The wild Bunaveela population does not express the anadromous life history, and experimental offspring from Bunaveela brood stock were considered to have non-anadromous population background. (B) Tawnyard Lough in the Erriff catchment, site of brown trout brood stock collections (via seine netting) in November 2015, used to produce F1 offspring for the experimental study. The wild Tawnyard Lough population has a strong anadromous component, and experimental offspring from Tawnyard brood stock were considered to have an anadromous population background.

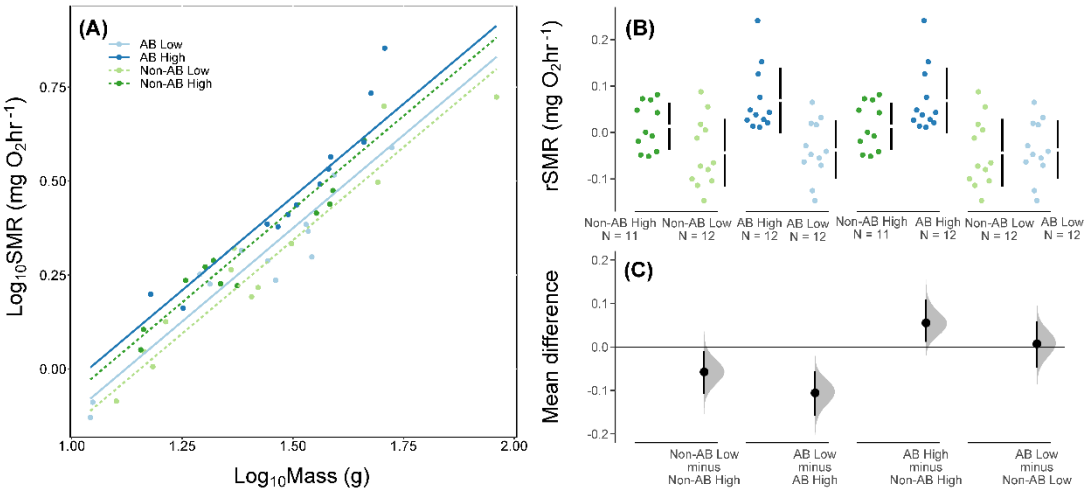

Figure S2: (A) Body mass scaling of standard metabolic rate (SMR) measured in April and May 2017 in brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish were reared under two experimental food treatments: optimal food rations (High); and 25% of optimal daily rations (Low). (B) Residual SMR values (body mass corrected) for each population background and food treatment combination. Black vertical bars represent the standard deviation around the mean (shown as a gap in the bars), and sample size is shown as "N =". (C) Cumming estimation plots with effect sizes shown as black dots (i.e. the mean differences in rSMR among the groups), the distributions (shaded curves) and 95% confidence intervals (back bars) of the effect sizes obtained from non-parametric bootstrap resampling (5,000 resamples).

Table S1: Parameter estimates and associated standard errors (SE), *t*-values, and *P*-values from a mixed effect model (marginal R^2 = 0.92) testing the effects of food treatment (high or low) and population background (anadromous or non-anadromous) on standard metabolic rate (SMR) measured in brown trout in April and May 2017. SMR was log_{10}-transformed, and body mass (log_{10}-transformed) was included as a covariate. Date of SMR measurement was included as a random effect. Significance was assessed at a 5% alpha level. Effects are contrasted against fish from the non-anadromous population background in the high food treatment.

Response	Parameter	Estimate	SE	*t*-value	*P*-value
log_{10} SMR	Intercept	-1.06	0.07	-15.19	< 0.001
	Food: Low	-0.08	0.02	-3.69	0.005
	Population: Anadromous	0.03	0.02	1.40	0.196
	log_{10} Body mass	0.99	0.05	21.11	< 0.001

Table S2: Parameter estimates, with associated standard errors (SE), *t*-values, and *P*-values from the linear model describing log_{10}-transformed metabolic rates (mg O_2 hr^{-1}) as a function of log_{10}-transformed body mass in n = 61 brown trout (SMR = standard metabolic rate, MMR = maximum metabolic rate, and AS = aerobic scope).

Response	Parameter	Estimate	SE	*t*-value	*P*-value
log_{10} SMR	Intercept	-1.54	0.16	-9.50	< 0.001
	log_{10} Body mass	1.10	0.08	13.64	< 0.001
log_{10} MMR	Intercept	-0.26	0.30	-0.85	0.397
	log_{10} Body mass	0.89	0.15	5.99	< 0.001
log_{10} AS	Intercept	-0.24	0.35	-0.68	0.497
	log_{10} Body mass	0.85	0.17	4.89	< 0.001

Table S3: Parameter estimates and associated standard errors (SE), *t*-values, and *P*-values from the general linear models testing the effects of food treatment (high or low) and population background (anadromous or non-anadromous) on relationships between residual (i.e. body mass corrected) standard metabolic rate (rSMR) and residual maximum metabolic rate (rMMR), rSMR and residual aerobic scope (rAS), and rMMR and rAS in brown trout. Significance was assessed at a 5% alpha level. Effects are contrasted against fish from the non-anadromous population background in the high food treatment.

Response	Parameter	Estimate	SE	*t*-value	*P*-value
rMMR	Intercept	-0.09	0.02	-3.79	< 0.001
	rSMR	0.18	0.22	0.81	0.420
	Food: Low	0.04	0.03	1.49	0.142
	Population: Anadromous	0.13	0.03	4.73	< 0.001
rAS	Intercept	-0.10	0.03	-3.70	< 0.001
	rSMR	0.02	0.26	0.06	0.952
	Food: Low	0.05	0.03	1.36	0.178
	Population: Anadromous	0.15	0.03	4.69	< 0.001
rAS	Intercept	0.001	0.003	0.17	0.868
	rMMR	1.172	0.017	68.48	< 0.001
	Food: Low	0.005	0.004	1.54	0.128
	Population: Anadromous	-0.006	0.004	-1.39	0.169

Supplementary ANCOVA analyses

As additional analyses to acertain the robustness of our estimation statistics approach, we also tested for variation in the relationships between body mass and SMR, MMR, and AS according to population and food treatment factors using general linear models (GLMs). Body mass and metabolic rates were log_{10} transformed to normalise and linearise the data. The GLMs included either log_{10} SMR, log_{10} MMR, or log_{10} AS as response variables, and the models included log10 body mass, food treatment (high or low) and population background (anadromous or non-anadromous) as explanatory variables. We also included an interaction between body mass and food treatment, an interaction between mass and population, and a food × population interaction to test if responses to food reductions were similar for both populations. We created an additional set of GLMs to test for sex-based differences in log_{10}-transformed SMR, MMR, and AS, where sex (male or female) and log_{10} body mass were included as response variables. For all of the above GLMs, separate models were constructed for SMR, MMR and AS (log_{10}-transformed) and we assumed normal errors in each case.

Supplementary ANCOVA results

Log_{10} SMR increased with log_{10} body mass ($\chi2 = 205.26$, df = 1, $P < 0.001$), and the food treatment × population interaction term was non-significant (LRT for model with and without interaction term: $\chi2 = 0.22$, df = 1, $P = 0.643$). Log_{10} SMR was significantly affected by food treatment ($\chi2 = 8.72$, df = 1, $P = 0.003$) and population background ($\chi2 = 4.20$, df = 1, $P = 0.040$). For a given size, fish in the low food treatment had lower SMR values than those in the high food treatment, and fish from the non-anadromous population also had a lower SMR than those from the anadromous population (Figure S3A).

Log_{10} MMR increased with log_{10} body mass ($\chi2 = 51.70$, df = 1, $P < 0.001$), with a non-significant interaction between food treatment and population ($\chi2 = 0.99$, df = 1, $P = 0.320$), and no significant differences between food treatments ($\chi2 = 1.63$, df = 1, $P = 0.202$). There was a significant effect of population ($\chi2 = 25.88$, df = 1, $P < 0.001$), where for a given size, fish from the anadromous population

had a higher MMR than those from the non-anadromous population (Figure S3B).

Log_{10} AS similarly varied with log_{10} body mass ($\chi2 = 34.33$, df = 1, $P < 0.001$), with no significant interaction between food treatment and population ($\chi2 = 0.94$, df = 1, P = 0.333), and a non-significant effect of food treatment ($\chi2 = 2.08$, df = 1, P = 0.149). Fish from the anadromous population had a higher AS (for a given fish size) than those from the non-anadromous background (Figure S3C: $\chi2 = 23.74$, df = 1, $P < 0.001$).

The positive relationship between log_{10} SMR and log_{10} body mass ($\chi2 = 141.01$, df = 1, $P < 0.001$) was similar for female and male fish (Figure S4A: $\chi2 = 1.08$, df = 1, P = 0.299). However, males of a given size had a higher MMR than females (Figure S4B: $\chi2 = 36.28$, df = 1, $P < 0.001$) and sex-based differences were similarly detected in AS for a given fish size (Figure S4C: $\chi2 = 33.90$, df = 1, $P < 0.001$). See Table S4 and Table S5 for coefficients from all above GLMs.

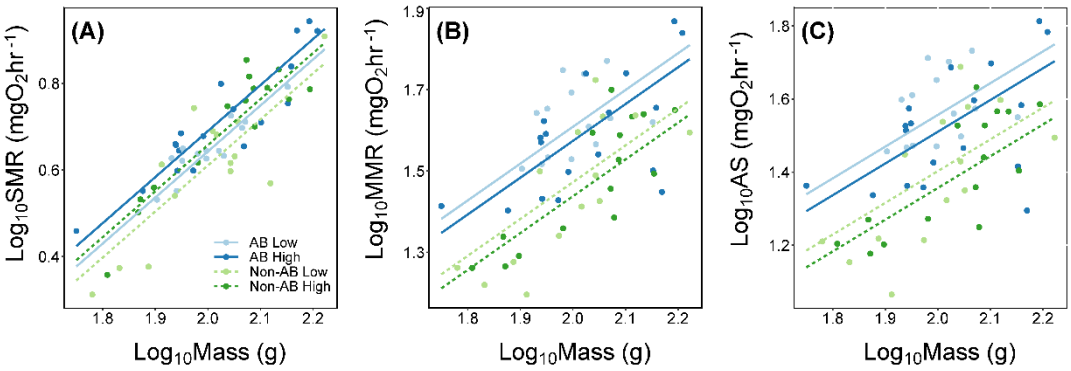

Figure S3: Body mass scaling of (A) standard metabolic rate (SMR), (B) maximum metabolic rate (MMR), and (C) aerobic scope (AS) of brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish were reared under two experimental food treatments: optimal food rations (High); and 25% of optimal daily rations (Low).

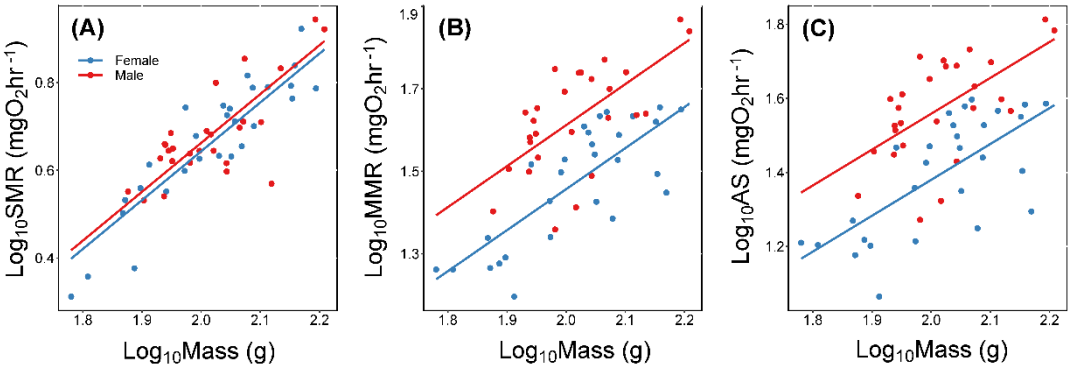

Figure S4: Body mass scaling of (A) standard metabolic rate (SMR); (B) maximum metabolic rate (MMR); and (C) aerobic scope (AS) of brown trout classed as female or male after two years of experimental tank rearing.

Table S4: Parameter estimates and associated standard errors (SE), *t*-values, and *P*-values from the general linear models testing the effects of food treatment (high or low) and population background (anadromous or non-anadromous) on standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) in brown trout. SMR, MMR, and AS were $\log_{10}$-transformed, and body mass ($\log_{10}$-transformed) was included as a covariate. Significance was assessed at a 5% alpha level. Effects are contrasted against fish from the non-anadromous population background in the high food treatment.

Response	Parameter	Estimate	SE	*t*-value	*P*-value
$\log_{10}$ SMR	Intercept	-1.47	0.15	-9.73	< 0.001
	Food: Low	-0.05	0.02	-2.95	0.005
	Population: Anadromous	0.03	0.02	2.05	0.045
	$\log_{10}$ Body mass	1.06	0.07	14.33	< 0.001
$\log_{10}$ MMR	Intercept	-0.37	0.26	-1.45	0.152
	Food: Low	0.03	0.03	1.28	0.207
	Population: Anadromous	0.14	0.03	5.09	< 0.001
	$\log_{10}$ Body mass	0.90	0.13	7.19	< 0.001
$\log_{10}$ AS	Intercept	-0.38	0.30	-1.27	0.211
	Food: Low	0.05	0.03	1.44	0.155
	Population: Anadromous	0.15	0.03	4.87	< 0.001
	$\log_{10}$ Body mass	0.87	0.15	5.86	< 0.001

Table S5: Parameter estimates and associated standard errors (SE), *t*-values, and *P*-values from the general linear models testing the effect of sex on standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS) of brown trout (n = 55). SMR, MMR, and AS were $\log_{10}$-transformed, and $\log_{10}$ body mass was included as a covariate in all models. Significance was assessed at a 5% alpha level. Effects are contrasted against female fish.

Response	Parameter	Estimate	SE	*t*-value	*P*-value
$\log_{10}$ RMR	Intercept	-1.58	0.19	-8.36	< 0.001
	Sex: Male	0.02	0.02	1.04	0.304
	$\log_{10}$ Body mass	1.11	0.09	11.87	< 0.001
$\log_{10}$ MMR	Intercept	-0.53	0.27	-2.00	0.051
	Sex: Male	0.16	0.03	6.02	< 0.001
	$\log_{10}$ Body mass	0.99	0.13	7.56	< 0.001
$\log_{10}$ AS	Intercept	-0.56	0.32	-1.76	0.084
	Sex: Male	0.18	0.03	5.82	< 0.001
	$\log_{10}$ Body mass	0.97	0.16	6.18	< 0.001

Chapter 5

Developmental temperature and population factors affect variation and covariation of metabolic traits, with complex effects on growth rates in brown trout (*Salmo trutta*)

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Contributions: LA, TR, and PMG conceived the study. LA, SH, and LH collected data and contributed to experimental design. LA conducted statistical analysis and wrote the mansucript. All authors contributed to interpretation of results and revisions of the manuscript.

Abstract

Metabolism defines the energetic cost of life, yet we still know relatively little about how intraspecific variation in standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) arises and persists. Inconsistent associations between metabolic traits and fitness/performance metrics indicates that the optimal metabolic phenotype, or combination of metabolic traits, may be context-dependent. Here, we use a tank-rearing experiment to epxlore how environmental and population-specific factors influence the links between growth rates (a key performance trait) and metabolism in brown trout, a species that demonstrates considerable variation in migratory life histories. Growth rates and metabolic traits were measured in offspring from two populations that naturally vary in migratory tactics (one anadromous i.e. sea migratory, one non-anadromous), exposed to ~15 months of warming at 1.8 °C above natural temperatures. The anadromous population had overall higher SMR, MMR, and AS compared to the non-anadromous population, but both populations showed lower SMR in the warm treatment. We observed lower MMR and AS in the anadromous population in the warm temperature treatment, but not in the non-anadromous population. The variation in metabolic traits had complex implications for growth rates across the study that were dependent on population background and temperature. Lower SMR was associated with higher growth in the warm treatment, suggesting SMR reduction was an acclimation response to minimise maintenance costs under warm conditions. Higher MMR and AS were linked to higher growth rates in the anadromous population, potentially due to behavioural or life-history differences. A strong coupling between SMR and MMR or AS in this population further suggested that metabolic trait covariation was underpinned by the demands of fuelling a high-powered metabolism. Collectively, our study indicates that populations vary in their capacity to acclimate different metabolic traits in response to chronic warming and that the links between metabolic traits and growth rates are also population specific. In the wild, the effects of a given metabolic phenotype on growth rates and overall fitness are likely to depend on both environmental context and population background, and we

should consider both factors when forecasting population responses to warming.

Introduction

As the fundamental biological rate determining resource use and energy balance (Brown *et al.* 2016), metabolism underlies organism performance, and ultimately, fitness (Burton *et al.* 2011). Understanding the links between metabolism, performance, and environmental conditions is widely recognised as being crucial to forecasting species' and population responses to global change (Seebacher *et al.* 2015), yet this is not necessarily straightforward. Despite considerable variation in metabolic traits apparent within and between populations, uncertainty still surrounds the drivers of such variation, or its consequences for fitness in different environmental contexts (Pettersen *et al.* 2018). It is imperative that we uncover the sources of metabolic variation, and assess how such variation can influence the performance of individuals, and hence the ability of populations to cope with global change.

The baseline energetic demands of ectotherms are defined by their standard metabolic rate (SMR), termed basal metabolic rate (BMR) in endotherms within their thermoneutral zone. SMR represents the minimum energetic costs of maintaining tissues and homeostasis in an organism that is inactive, unstressed, and non-digestive (Chabot *et al.* 2016). The upper bounds of metabolism are set by the maximum metabolic rate (MMR), which is the highest rate of aerobic metabolism (i.e. oxygen transport and ATP production) that can be achieved (Norin and Metcalfe 2019). Aerobic scope (AS) – the difference between an organism's SMR and MMR – determines the potential energy that can be allocated towards important functions including digestion, activity, growth and reproduction (Guderley and Pörtner 2010). SMR, the most frequently measured metabolic trait, shows significant inter-individual variation, with up to threefold differences in SMR even among similarly sized and aged individuals from the same population (Burton *et al.* 2011; Konarzewski and Książek 2013). Although MMR (and consequently AS) is measured less often, the level of variation appears similar to that of SMR, once age and size are accounted for

(Hillman *et al.* 2013; Norin and Clark 2016). While SMR and MMR are often correlated within species (Auer *et al.* 2017; Swanson *et al.* 2017), the relationship between metabolic traits can also vary considerably (Cutts *et al.* 2002; Chappell *et al.* 2007; Wone *et al.* 2015; Norin *et al.* 2016; Barceló *et al.* 2016). This substantial variation and covariation in metabolic phenotypes can be understood in terms of ultimate mechanisms (evolved differences due to past natural selection), or proximate mechanisms (differences due to developmental variation or plastic responses to variable environments) (Tinbergen 1963; Bateson and Laland 2013).

Given the likely importance of metabolism for fitness, the reason behind the persistence of such variation is somewhat unclear, but one possibility is that the optimum metabolic phenotype is context-dependent (Burton *et al.* 2011). Variation in optimum phenotypes may occur across populations, *e.g.* due to spatial variation in selection pressures such as temperature, food supply, or habitat quality (Thomas *et al.* 2001; Angilletta *et al.* 2002). Within populations, variation in optimal phenotypes might emerge through time via fluctuating selection arising from temporal variation in environmental conditions (Schulte 2015). Moreover, at any given time, the optimum phenotype may vary between different types of individuals within and among populations. For example, within a population of three-spined sticklebacks *Gasterosteus aculeatus*, migrants had higher standard and active metabolic rates than residents (Tudorache *et al.* 2007), with similar differences among populations linked to reduced selection on swimming performance in stream-resident populations (Dalziel *et al.* 2012a, b). Variation in optimal phenotypes might also emerge between different behavioural types *e.g.* shy *versus* bold individuals (Dingemanse *et al.* 2004; Boon *et al.* 2007; Careau *et al.* 2008). Collectively, the above ultimate mechanisms can lead to evolved (i.e. genetically based) variation in metabolic traits being maintained within and across populations. Over time, a decoupling of metabolic traits can also occur because each trait is subject to subtly distinct selection pressures (Norin and Metcalfe 2019), which often act

in parallel but may also operate independently (Wone *et al.* 2015; Barceló *et al.* 2016).

Environmental factors are also instrumental in controlling metabolic rate variation from a proximate perspective. Dramatic changes in metabolic components have been reported in response to temperature (Fry 1971; Pörtner and Knust 2007; Clark *et al.* 2013), hypoxia (Dupont-Prinet *et al.* 2013; Norin *et al.* 2016), food availability (O'Connor *et al.* 2000; Van Leeuwen *et al.* 2011, 2012; Auer *et al.* 2015c), and habitat structure (Finstad *et al.* 2004; Millidine *et al.* 2006). Since aerobic metabolism is dependent on factors influencing oxygen demand and uptake (Fry 1971), temperature profoundly determines metabolic rates (Gillooly *et al.* 2001; Angilletta *et al.* 2002; Pörtner and Knust 2007; Brown *et al.* 2016; Pettersen *et al.* 2018). However, the magnitude of any temperature-induced change in metabolism tends to decrease with exposure time as the animal becomes acclimated to the new temperature (Seebacher *et al.* 2015). Acute effects of temperature on metabolism are reasonably well researched, but we know less about the effects of chronic temperature exposure (i.e. shifts in thermal regime lasting months to years) on different metabolic traits, despite such time scales being highly relevant in the context of climate change (Clark *et al.* 2013).

Predicting the effects of temperature is made more complicated because the various components of metabolism can show different sensitivities to environmental conditions. There is some evidence for more plastic metabolic "floors" (SMR) than "ceilings" (MMR) in response to temperature, as documented in European perch *Perca fluviatilis* that reduced SMR after long-term warming with no compensatory adjustments seen in MMR (Sandblom *et al.* 2016). Indeed, many fish species appear to show minimal temperature-induced changes in AS (Lefevre 2016; Jutfelt *et al.* 2018), though this is not always the case (Norin *et al.* 2014). Similar divergence in the relative responses of BMR and MMR (or cold-induced maximum metabolism) to temperature is apparent in endotherms (Nespolo *et al.* 2001; van de Ven *et al.* 2013; Dubois *et al.* 2016), indicating that decoupling of metabolic traits may be widespread.

While a positive relationship between SMR and MMR has been proposed under the "increased intake" hypothesis – where a higher SMR maintains the metabolic machinery that fuels high MMR (Biro and Stamps 2010; Burton *et al.* 2011) – covariation between metabolic components may be stronger, weaker, or non-existent depending on environmental context (Killen *et al.* 2013; Norin *et al.* 2016), and can vary between individuals (Norin and Malte 2011). It seems that SMR and MMR might thus be subject to subtly different proximate (or ultimate) constraints that might be revealed or masked by a given set of environmental conditions (Killen *et al.* 2013; Norin and Metcalfe 2019).

While the fitness implications of metabolism are presumed to be considerable because it shapes life histories (Stearns 1992), there is equivocal evidence for effects of metabolic traits on various fitness metrics (Burton *et al.* 2011). Positive relationships between SMR/RMR and growth (Yamamoto *et al.* 1998; McCarthy 2000), reproduction (Sadowska *et al.* 2013), and survival (Jackson *et al.* 2001) imply fitness benefits of higher SMR that are in line with the "increased intake" hypothesis. Yet SMR/BMR has also been negatively linked to growth (Álvarez and Nicieza 2005; Norin and Malte 2011), reproduction (Blackmer *et al.* 2005) and survival (Álvarez and Nicieza 2005; Larivée *et al.* 2010), supporting an alternative "compensation" hypothesis, whereby a lower SMR is advantageous for energy-saving purposes (Burton *et al.* 2011). Surprisingly little attention has been paid to the associations between MMR or AS and fitness (Metcalfe *et al.* 2016), even though AS has been proposed as the overarching physiological trait governing fitness-related functions (Brown *et al.* 1993; Pörtner and Knust 2007; Pörtner and Farrell 2008). Nonetheless, MMR appears to show similarly inconsistent relationships with fitness (Hayes and O'Connor 1999; Boratyński and Koteja 2009; Zub *et al.* 2014). Such inconsistencies are likely explained by context-dependent fitness benefits of various metabolic trait phenotypes. For example, higher SMR (or MMR) may only be beneficial when resources are plentiful (Reid *et al.* 2011, 2012; Auer *et al.* 2015c; Killen *et al.* 2016) or predictable (Hoogenboom *et al.* 2013), and have been found to have negative (Bochdansky *et al.* 2005), or no effects (Álvarez and Nicieza 2005) when

resources are limited. Moreover, the fitness consequences of metabolic traits may depend on complex links between the larger metabolic phenotype (*e.g.* the coupling of SMR and MMR), and the environmental conditions encountered (Auer *et al.* 2015b).

Since the optimal metabolic phenotype (or combination of traits) varies across space/among populations as the environmental context changes, metabolic variation might be further associated with population-specific (*e.g.* evolved) differences in life history, such as migration tendency, yet relatively few studies have investigated these links. Salmonine fishes (salmon, trout, and charr) offer great potential for exploration of the proximate and ultimate drivers of metabolic trait variation and covariation. In facilitating obligate freshwater spawning, salmonines exhibit diverse life-histories that encompass a continuum of migratory tactics, from individuals that remain resident in natal freshwater streams for their entire life cycle, to those that undertake spectacular migrations to larger rivers, lakes, or the sea (Klemetsen *et al.* 2003). Facultative migration, where individuals in a population can display either migratory or non-migratory (resident) tactics (Chapman *et al.* 2011a), is proposed to be triggered by the relationship between an environmentally-sensitive trait and a genetically-variable threshold (Tomkins and Hazel 2007; Piche *et al.* 2008; Pulido 2011; Buoro *et al.* 2012). While the exact trait controlling migration tactics remains unresolved (Ferguson *et al.* 2019), migration is often expressed as a consequence of energetic limitation in natal streams (Forseth *et al.* 1999). Variation in migratory life histories among individuals and populations is thus likely to be linked to variation in metabolic traits, *e.g.* anadromous (sea-migratory) Atlantic salmon *Salmo salar* had higher SMR than non-migrants across three populations (Seppänen *et al.* 2010), and future migrants within a population had higher SMR than individuals that delayed migration (McCarthy 2000).

While variation in metabolic traits could have proximate/causal effects i.e. contribute to the emergence of migratory tactics among populations via the threshold model, metabolic variation might persist as a compensatory adaptation in migratory or resident populations (via ultimate mechanisms) that

increases the benefits of migration/residency relative to costs (Hendry *et al.* 2004). For example, while larger body sizes and higher growth are associated with increased migration propensity (Jonsson 1985; Forseth *et al.* 1999; Morinville and Rasmussen 2003), survival-at-sea also increases with size (Armstrong *et al.* 2018). We might thus expect higher SMR, MMR, or AS to confer growth-mediated fitness benefits in migratory populations. In contrast, non-migratory populations must cope with unpredictable/patchy temperatures (Elliott 2000; Rutherford *et al.* 2004) and food availability typical of freshwater streams (Martin-Smith and Armstrong 2002), which might favour a lower SMR that minimises energy requirements.

Here, we explore the effects of temperature and population factors on metabolic traits and growth (a key fitness-associated trait) in experimentally reared F1 offspring from two wild trout populations that naturally differ in migratory tactics. Specifically, we aimed to: (i) assess how long-term temperature elevation and population background affects metabolic traits (SMR, MMR, and AS); (ii) test if populations of variable migratory propensity show variation in metabolic responses to warming; (iii) test whether variation in metabolic traits influences growth performance; and (iv) investigate how relationships between metabolic traits and growth vary according to temperature and population background. We expected that increased temperature would result in compensatory responses in metabolic rates (lower SMR, and/or higher MMR and AS) and increased growth rates but that the migratory population would overall show higher metabolic rates and growth rates. We also expected that SMR, MMR, and AS would be positively related to growth, but the strength or direction of this relationship could vary between populations, and be magnified by warmer temperatures.

Methods

Study populations and fish rearing

We obtained brown trout brood stock from two wild populations in November 2015 by seine netting in the Erriff (53° 37′ 0.00" N: 09° 40′ 17.10" W) and the Burrishoole (53° 57′ N: 09° 35′ W) catchments in the west of Ireland (Figure S1).

Erriff broodstock were caught in the Tawnyard Lough (56 ha), an upland lake fed by the Glendavoch river and a number of smaller tributaries. Trout in the Tawnyard Lough primarily spawn in the Glenadavoch River, moving downstream as fry or parr to the Lough (covering several hundred metres to kilometres, depending on spawning location). A large run of out-migrating anadromous juveniles (smolts) are produced from Tawnyard Lough each year, with estimates of 500 to 3000 smolts enumerated annually at the outflow of the Lough (Gargan *et al.* 2016). An undetermined proportion of the population remain in the Lough, undergoing several years of freshwater growth before spawning in their natal stream, with local expertise indicating that the Tawnyard population overall has a strong anadromous component (broadly estimated as 50 – 60% of the wild population) (P. Gargan, *pers comm.*).

The Burrishoole brood stock were caught in the Srahrevagh River (*ca.* 7250 m^2 of salmonid habitat), a tributary of the Black River in the headwaters of the catchment that drains into Lough Feeagh, and ultimately Clew Bay. Although an anadromous life history occurred in the Burrishoole catchment up to 30 years ago (annual estimates of up to 2000 anadromous recruits), the anadromous trout run collapsed in the 1980s, coinciding with sea lice outbreaks from a salmon aquaculture facility established in the downstream estuary (Poole *et al.* 2007). Recent annual runs of up-migrating sea trout are typically in the low hundreds. Although the exact spawning locations of historic or contemporary anadromous individuals are uncertain, brood stock used in our study showed no signs of having undertaken a marine migration, i.e. were all non-anadromous fish In summary, we consider offspring derived from the Tawnyard brood stock to have a strong anadromous background (hereafter termed the "anadromous background population"), and offspring from the Srahrevagh brood stock to have no recent anadromous background (termed the "non-anadromous background population").

See Archer *et al.* (2019) and Chapter 3 for detailed description of crossing, fertilisation, and rearing procedures, described here in brief. Eggs from each female were fertilised by 1-2 males from the same source population and

incubated at the Burrishoole hatchery facility. Post-hatching, fry were transferred to a rearing facility at University College Cork (Aquaculture and Fisheries Development Centre), Ireland. Here, they were held in two 100L growth tanks (one per population) on a recirculating aquaculture system (RAS), maintained at a natural temperature regime typical of the west of Ireland under a constant photoperiod (12:12 hours of light: dark), until experimental treatments began. During this initial rearing period, fish were fed *ad libitum* with commercially available trout pellets (Skretting Ltd., Norway). In December 2016, 140 fry were allocated to one of four 203L capacity tanks in a larger experimental RAS (two tanks per population, initial n = 35 per tank). Within the experimental RAS, LED lights above each tank mimicked the natural photoperiod of the source catchments. Water flowed continually through tanks, was treated with mechanical filtration, biofiltration, protein and UV skimming, and was mixed in a central sump tank. Mixed water from the sump was passed through one of two conditioning units, one that maintained a seasonal temperature regime typical of the west of Ireland, or one that was elevated to 1.8 °C (± 0.55 SD) above the natural temperature regime. Thus, each tank received water from the same recirculating source, heated to one of two temperatures. Fish were fed recommended daily pellet rations for optimal growth calculated as a percentage of their body mass, with absolute rations adjusted monthly to account for changes in temperatures and body mass (Skretting Ltd., Norway). Water quality in the RAS (checked weekly) was consistently within acceptable levels for fish health, and great care was taken to ensure that all measured variables other than temperature regime (fish densities, feeding, photoperiod, lux, flow rates) were constant across tanks. Mortality was negligible during the experimental period, but fish were haphazardly culled (n = 20) over the course of tank rearing for inclusion in parallel studies.

The study and all associated procedures were carried out with ethical approval from Health Products Regulatory Authority (HPRA) Ireland, under HPRA project license AE19130/P034, and individual licenses AE19130/1087,

AE19130/I200, AE19130/I201 and AE19130/I202 with all fish humanely euthanized under licence in April 2018.

Temperature treatments

To explore the effects of temperature and population factors on growth and metabolism, juvenile brown trout from each population were randomly allocated to one of two temperature regimes in January 2016, with the populations reared separately to prevent emergence of dominance hierarchies. The two temperature regimes were: (i) cool temperature treatment: temperatures matched the natural, seasonally-varying temperatures of the source catchments; and (ii) warm temperature treatment: temperature elevated to 1.8 °C ± 0.55 (SD) above the cool treatment. The cool treatment ranged from 5.9 - 16.4 °C (mean temperature = 10.8 °C ± 3.3 SD) and the warm treatment ranged from 7.5 - 18.2 °C (mean temperature = 12.6 °C ± 3.4 SD). The 1.8 °C elevation in the warm treatment was chosen to reflect increases of 1 - 3 °C projected under climate change scenarios (IPCC 2014), but is within sub-lethal ranges for brown trout (Forseth *et al.* 2009; Jonsson and Jonsson 2009). Temperature was increased by 0.5 ºC per week when initiating treatments to minimise stress. Within each temperature treatment, 24-26 fish of each population were lightly anaesethised with MS-222, and marked with a unique colour combination of visible implant elastomer (VIE) tags (Northwest Marine Technology., USA) to allow for re-identification. Six individuals lost VIE tags over the course of the experiment, leaving n = 96 individually identifiable fish.

Data collection

To calculate growth rates of VIE tagged individuals across the study period, the fork length (mm) and mass (g) of lightly anaesethised fish was recorded in April, June, July, August, September, and December 2017. In February 2018 (approximately 12 months after temperature treatments were established), eight fish from each population and temperature treatment combination underwent respirometry trials to measure metabolic traits (n = 32 individuals in total).

Measurement of MMR

Whole-animal oxygen consumption (MO_2) in animals operating at their maximum aerobic metabolic rate was used as proxy for MMR (Norin and Metcalfe 2019), following best practices outlined in Norin and Clark (2016). We used an exhaustive chase protocol following Norin and Clark (2016) to elicit MMR in the same individuals that we measured for SMR. Prior to SMR measurements, each individual fish due to be measured for SMR later that day was first placed in an aerated 50L tank in a controlled-temperature (CT) chamber at 7.9 °C ± 0.1 SD, and manually chased by hand until exhaustion. We determined exhaustion to occur when the fish were unresponsive (i.e. did not exhibit burst swimming) to tactile stimulus (typically after 2 to 3 minutes of sustained chasing). Once exhausted, the fish was immediately transferred to a respirometry chamber in the same system used to measure SMR, the chamber was sealed, and oxygen decline within the closed chamber loop (recirculation pump operational) was recorded for a 60s measurement period. The time taken to transfer fish to the chamber and begin recording oxygen measurements never exceeded 20s, ensuring that minimal recovery from the exhaustive chase procedure occurred before recording oxygen consumption.

Measurement of SMR

The SMR of individual fish was determined using intermittent-flow respirometry, following best practices outlined in Svendsen *et al.* (2016). SMR measurements took place overnight in a darkened CT chamber maintained at 7.9 °C ± 0.1 SD (the mid-point between the cool and warm temperature treatments at the time of measurements). The respirometry system consisted of four acrylic respirometry chambers (1200 ml) (Loligo Systems, Viborg, Denmark), submerged in a water bath, flushed with de-chlorinated water bubbled to 100% oxygen saturation by an air stone. PVC tubing (10mm diameter, non-permeable to oxygen) connected each individual chamber to two pumps (Eheim Ltd., Deizisau, Germany). A “flush” pump flushed fully oxygenated water through the chambers. A second “recirculation” pump recirculated water in a closed loop through the chamber, whereby water exiting

the chamber was passed through a 10mm flow through oxygen cell (PreSens Ltd., Regensburg, Germany) that continually measured dissolved oxygen concentration, before being recirculated back to the same respirometry chamber via PVC tubing. Thus, individual oxygen consumption (MO_2, used as a proxy for SMR in fasted, inactive animals) was measured in repeated cycles that consisted of a flush period (flush pump operational) and a measurement period (recirculation pump operational) where oxygen level in each chamber was recorded at one second intervals to estimate oxygen decline (i.e. oxygen uptake). Each cycle consisted of 330s of flushing, and a measurement period of 200 - 300s (to ensure sufficient O_2 depletion for calculating MO_2 in different-sized fish). We also allowed a 30s buffer period before recording oxygen uptake once the flush pump was switched off, to allow the chamber water and flush water to mix completely and reach an equilibrium oxygen saturation.

Fish were fasted for 28h prior to respirometry measurements to ensure individuals were in a post absorptive state (Cutts *et al.* 2002). Fish were placed in individual respirometry chambers between 11:00 and 12:00 each day, and were left to acclimatise for five hours, with chambers continually flushed with oxygen-saturated water during this acclimation period. SMR measurements began between 16:00 and 17:00, and ended between 09:00 and 10:00 the following morning, allowing for a minimum of 100 measurements of oxygen uptake per individual over a ~20-h period, during which time fish were not disturbed. Once SMR measurements had finished, fish were returned to their rearing tanks. To limit bacterial growth in the system, the entire respirometry set-up was washed with bleach after each overnight SMR respirometry trial. We also measured background (i.e. bacterial) respiration rates in each chamber on a daily basis by recording oxygen decline in empty chambers for one measurement cycle before fish entered the respirometry system, and again for one measurement cycle once the respirometry measurements had ended and fish were removed from the chambers.

Measurement of MMR

Whole-animal oxygen consumption (MO_2) in animals operating at their maximum aerobic metabolic rate was used as proxy for MMR (Norin and Metcalfe 2019), following best practices outlined in Norin and Clark (2016). We used an exhaustive chase protocol following Norin and Clark (2016) to elicit MMR in the same individuals that we measured for SMR. Prior to SMR measurements, each individual fish due to be measured for SMR later that day was first placed in an aerated 50L tank and manually chased by hand until exhaustion. We determined exhaustion to occur when the fish were unresponsive (i.e. did not exhibit burst swimming) to tactile stimulus (typically after 2 to 3 minutes of sustained chasing). Once exhausted, the fish was immediately transferred to a respirometry chamber in the same system used to measure SMR, the chamber was sealed, and oxygen decline within the closed chamber loop (recirculation pump operational) was recorded for a 60s measurement period. The time taken to transfer fish to the chamber and begin recording oxygen measurements never exceeded 20s, ensuring that minimal recovery from the exhaustive chase procedure occurred before recording oxygen consumption.

Statistical Analysis

We estimated SMR (mg O_2 h^{-1}) by first calculating an individual's MO_2 values (mg O_2 h^{-1}) for each repeated measurement of oxygen uptake recorded during the overnight SMR respirometry trials. MO_2 was calculated as the most consistent linear decline in oxygen recorded during each measurement loop, estimated by rolling regression using the *respR* package (Harianto and Carey 2019). We inspected all MO_2 measurements to assess regression fit, and only included MO_2 values with an acceptable fit (R^2 values > 0.90) in subsequent SMR calculations, unless a clear linear trend was determined upon visual inspection of fit. Any background respiration in MO_2 values was accounted for by subtracting the MO_2 values calculated in empty chambers before and after each trial. We assumed background MO_2 rates increased linearly over the course of each trial (due to bacterial growth), and therefore allowed for a

dynamic background correction value (i.e. background MO_2 increased overnight), calculated as:

$$MO_{2_{bg}} = bg_0 + (t \times bg)$$

where $MO_{2_{bg}}$ is background MO_2, at a given measurement time point t, the time elapsed since initiating overnight SMR measurements, bg_0 and bg are parameters (the intercept and slope respectively) estimated from the matrix regression of the background oxygen uptake before and after experiments, as a function of time elapsed. We then subtracted $MO_{2_{bg}}$ from each value of MO_2, as calculated for an individual fish at successive time points during the overnight SMR respirometry trials. $MO_{2_{bg}}$ never exceeded more than 2% of total MO_2 in all cases, confirming that background respiration rates remained low throughout the study. Individual SMR was then calculated by talking the mean of the lowest 10th percentile of background-corrected MO_2 values recorded over the 20-h SMR measurement period, then excluding outliers (values more than two SD from this mean).

We estimated individual MMR (mg O_2 h^{-1}) using the *respR* package (Harianto and Carey 2019) by calculating MO_2 as the linear decline in oxygen in each individual respirometry chamber in the 60s measurement period immediately after the exhaustive chase protocol (i.e. extracting slopes from the linear regression of oxygen concentration against time over a 60s period). We calculated individual absolute aerobic scope (AS) (mg O_2 h^{-1}) as the difference between MMR and SMR.

We explored variation in metabolic traits by first testing if the relationships between body mass and SMR, MMR, or AS were affected by temperature and population factors (Aims 1 and 2) using three general linear models (GLMs). Body mass and metabolic rates were $\log_{10}$ transformed to normalise and linearise the data. Each GLM (normal errors) included either $\log_{10}$ SMR, $\log_{10}$ MMR, or $\log_{10}$ AS as the response variable, and $\log_{10}$ body mass, temperature treatment and population background as explanatory variables. To test for variation in the scaling of metabolic rates, each model included interactions

between $\log_{10}$ body mass and temperature, and $\log_{10}$ body mass and population, along with an interaction between temperature and population, and a three-way interaction term ($\log_{10}$ body mass × temperature × population).

To explore how factors affected metabolic rate independent of mass, and to avoid the pitfalls associated with solely using *P*-values (Halsey *et al.* 2015; Halsey 2019), we next tested for differences in mass-independent measures of SMR, MMR, and AS by estimation statistics (i.e. calculating effect sizes) using the *dabestr* package (Ho *et al.* 2019). The residuals of the linear relationship between $\log_{10}$ body mass and SMR, MMR, and AS (all $\log_{10}$ transformed) were used to correct for body mass in these analyses (see Table S1 for model summaries). Residual values of metabolic rates (rSMR, rMMR, rAS) gave mass-independent estimates of metabolic rates (individuals with positive residuals have a higher than expected metabolic rate for a given fish size, whereas negative residuals indicate a lower than expected rate). Effect sizes for mean differences in rSMR, rMMR, and rAS were computed for all pairwise comparisons between all levels of temperature treatment and population background, and 95% confidence intervals (CIs) were constructed by bootstrapped resampling for 5,000 resamples.

To assess whether temperature treatment and population background influenced the relationship between metabolic traits, we constructed three GLMs (normal errors). We used residual metabolic rates in these analyses to give mass-independent estimates of metabolic rates. The first GLM included rMMR as a response variable, with rSMR, temperature treatment, and population background included as explanatory variables. A second GLM included rAS as the response, and rSMR, temperature treatment, and population background were similarly included as predictors. We included two-way interaction terms for rSMR × temperature, and rSMR × population, along with a three-way rSMR × temperature × population interaction to test for differences in covariation between MMR or AS with SMR. The third GLM modelled rAS as function as rMMR, temperature treatment, and population

background, and similarly included the interaction terms rMMR × temperature, rMMR × population, and rMMR × temperature × population.

Finally, we explored how metabolic rates, population and temperature treatments influence growth rates across the study period (Aims 3 and 4) within a mixed effects modelling framework using the *nlme* package (Pinheiro *et al.* 2019). We estimated growth rates as the specific growth rate (% day^{-1}) in terms of fork length (G_L) between measurement periods according to:

$$G_L = 100 \times (\ln S_t - \ln S_i)/d$$

where S_t is the fork length at time t, S_i is the initial fork length, and d is the time elapsed (in days) between S_i and S_t (Hopkins 1992). We built three mixed effects models (normal errors) to examine how rSMR, rMMR and rAS influenced growth rates in fish that underwent respirometry trials. Each model included time (continuous variable corresponding to months since start of experiment), a third order polynomial term for time (to account for non-linearity through time), temperature treatment, population background, and metabolic rate (either rSMR, rMMR, or rAS) as fixed effects, and individual identity as a random effect to account for multiple growth rate measurements of individuals. We included two-way interactions between metabolic rate and temperature, metabolic rate and population, and temperature and population, and a three-way interaction (metabolic rate × temperature × population). Since growth rate is size dependent (Nicieza and Álvarez 2009; Killen 2014), we included initial fork length as a covariate in the models, and we accounted for temporal autocorrelation of growth rates by modelling an autoregressive error structure as a first order lag function of time. To test if metabolic rate effects were consistent across the experiment, we constructed three additional mixed effect models as described above, but included an additional interaction between metabolic rate and time.

We used likelihood ratio tests (LRT) to assess statistical significance of predictor variables for all models at a 5% alpha level. Non-significant interaction terms were excluded to interpret main effects. For the estimation statistics approach,

we considered an estimated difference in means between groups to exist (i.e. was significant) if the 95% CI of the effect size did not include zero. Marginal R^2 values for mixed effect models were calculated using the *MuMIn* package (Barton 2019). Analysis was carried out in R version 3.6.0 (R Core Team 2019) and all models were checked against assumptions of the given model (independence, non-normality of residuals, heteroscedasticity and multicollinearity).

Results

Variation in metabolism

Whole-animal SMR, MMR, and AS varied between temperature treatments and population background (see Table 1 for a summary of mean values of metabolic rates for each temperature and population combination).

As expected, $\log_{10}$ SMR increased significantly with $\log_{10}$ body mass ($\chi^2 = 31.79$, df = 1, $P < 0.001$, Table S2), but we did not find any significant interactions between body mass, temperature, and population ($\chi^2 = 0.31$, df = 1, $P = 0.580$), between body mass and temperature ($\chi^2 = 0.11$, df = 1, $P = 0.735$), or between body mass and population ($\chi^2 = 0.13$, df = 1, $P = 0.716$). The temperature × population interaction was also non-significant ($\chi^2 = 0.01$, df = 1, $P = 0.985$), but the main effects of temperature treatment ($\chi^2 = 9.55$, df = 1, $P = 0.002$), and population background ($\chi^2 = 5.05$, df = 1, $P = 0.025$) were significant. For a given size, fish in the warm temperature treatment had lower SMR values, as did fish from the non-anadromous population (Figure 1A, Table S2). Similarly, estimation statistics showed fish in the warm temperature treatment had lower mass-independent SMR (lower rSMR) than those from the cool treatment (Figure 1B, Table 2). The non-anadromous population also tended to have lower rSMR at both temperatures, although this difference was less apparent in the warm temperature treatment, where the 95% CIs for the mean difference in rSMR between populations overlapped zero (Figure 1C, Table 2).

Table 1: Mean values and associated standard deviations (SD) for the length (mm), mass (g), standard metabolic rate (SMR) (mg O_2 hr^{-1}), maximum

metabolic rate (MMR) (mg O_2 hr^{-1}), and aerobic scope (AS) (mg O_2 hr^{-1}) of brown trout (n = 32) offspring derived from two wild populations (AB = Anadromous background, Non-AB = Non-anadromous background). Fish were measured for metabolic traits in February 2018 after ~12 months of experimental rearing at one of two temperature treatments (Cool = natural temperature regime, Warm = 1.8 °C above natural temperature regime).

Temperature, Population	**Length** (mean ± SD)	**Mass** (mean ± SD)	**SMR** (mean ± SD)	**MMR** (mean ± SD)	**AS** (mean ± SD)
Cool, AB	218.50 ± 11.30	119.00 ± 23.00	5.97 ± 1.32	54.00 ± 9.60	48.04 ± 8.65
Warm, AB	222.88 ± 6.90	133.59 ± 17.13	5.57 ± 1.34	45.57 ± 10.89	40.00 ± 10.29
Cool, Non-AB	201.75 ± 8.22	108.58 ± 14.66	4.80 ± 1.02	36.83 ± 8.32	32.04 ± 8.01
Warm, Non-AB	202.75 ± 19.95	108.10 ± 29.88	3.95 ± 1.03	35.16 ± 8.79	31.21 ± 8.08

After accounting for the effects of individual variation in $\log_{10}$ body mass on $\log_{10}$ MMR (χ^2 = 11.13, df = 1, P = 0.001, Table S2), there were no significant interactions between body mass, temperature, and population (χ^2 = 0.01, df = 1, P = 0.992), between body mass and temperature (χ^2 = 0.06, df = 1, P = 0.810), between body mass and population (χ^2 = 0.87, df = 1, P = 0.352), or between temperature and population (χ^2 = 1.15, df = 1, P = 0.284). We detected significant main effects of temperature treatment (χ^2 = 4.10, df = 1, P = 0.043) and population background (χ^2 = 10.59, df = 1, P = 0.001) on $\log_{10}$ MMR. Overall, MMR (for a given size) was lower in the warm treatment, and in the non-anadromous background population (Figure 2A, Table S2). When considering size-independent MMR (rMMR), a lower rMMR in the warm treatment was evident only fish from the anadromous background population (Figure 2B, C, Table 2), and population differences in rMMR were seen in the cool treatment only (Figure 2B, C).

Table 2: Effect sizes (Δ) and associated 95% confidence intervals (CIs) for differences in mean residual standard metabolic rate (rSMR) (mg O_2 hr^{-1}), residual maximum metabolic rate (rMMR) (mg O_2 hr^{-1}), and residual aerobic scope (rAS) (mg O_2 hr^{-1}) of brown trout (n = 32) offspring derived from two wild populations (AB = anadromous background population, non-AB = non

anadromous background population), exposed to two temperature treatments (Cool = natural temperature regime, Warm = 1.8 °C above natural temperature regime). CIs were constructed by non-parametric bootstrap resampling (5,000 resamples).

Mean difference (Δ)	**Δ rSMR** (95% CI)	**Δ rMMR** (95% CI)	**Δ rAS** (95% CI)
Warm AB – Cool AB	-0.084 (-0.153; -0.020)	-0.116 (-0.192; -0.029)	-0.121 (-0.206; -0.031)
Warm non-AB – Cool non-AB	-0.075 (-0.134; -0.005)	-0.012 (-0.073; 0.057)	-0.002 (-0.073; 0.076)
Cool non-AB – Cool AB	-0.060 (-0.118; -0.005)	-0.144 (-0.217; -0.068)	-0.157 (-0.241; -0.073)
Warm non-AB – Warm AB	-0.050 (-0.125; 0.023)	-0.040 (-0.122; 0.026)	-0.038 (-0.124; 0.037)

We detected similar trends in AS, with non-significant interactions between body mass, temperature, and population (χ^2 = 0.01, df = 1, P = 0.942), between body mass and temperature (χ^2 = 0.04, df = 1, P = 0.834), between body mass and population (χ^2 = 0.93, df = 1, P = 0.336), and between temperature and population (χ^2 = 1.27, df = 1, P = 0.260). After accounting for the effects of $\log_{10}$ body mass on $\log_{10}$ AS (χ^2 = 7.81, df = 1, P = 0.005, Table S2), the main effect of temperature treatment was also non-significant (χ^2 = 2.98, df = 1, P = 0.084). Population background significantly affected $\log_{10}$ AS (χ^2 = 9.51, df = 1, P = 0.002), whereby AS (for a given size) was lower in the non-anadromous background population (Figure 3A, Table S2). However, rAS tended to be lower in the warm treatment in the anadromous background population (Figure 3B, C, Table 2), but not in the non-anadromous population. Population differences in rAS were strongest in the cool treatment (Figure 3C).

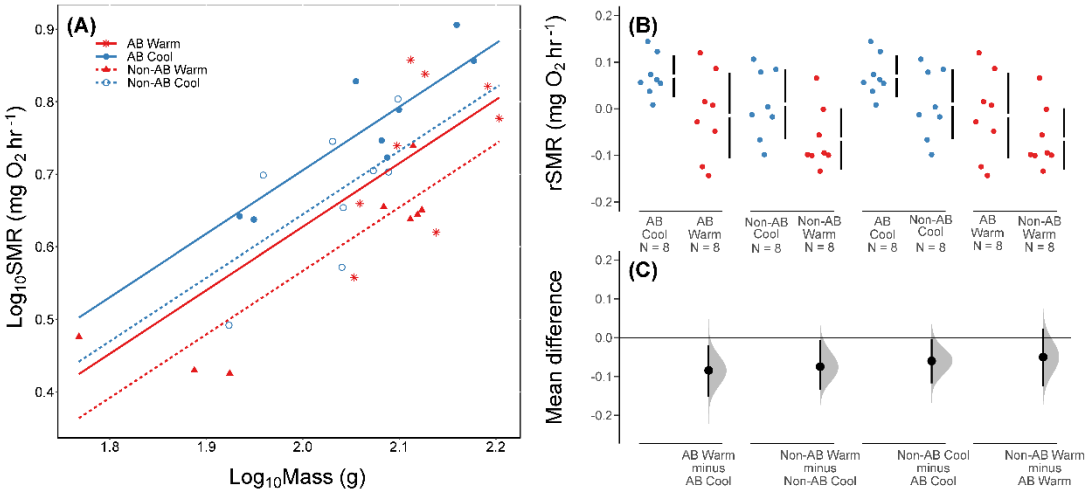

Figure 1: (A) Body mass scaling of standard metabolic rate (SMR) of brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish were reared under two experimental temperature treatments: a natural temperature regime (Cool); and temperature elevated 1.8°C above the cool regime (Warm). (B) Residual SMR values (body mass corrected) for each population background and temperature treatment combination. Black vertical bars represent the standard deviation around the mean (shown as a gap in the bars), and sample size is shown as "N =". (C) Cumming estimation plots with effect sizes shown as black dots (i.e. the mean differences in rSMR among the groups), the distributions (shaded curves) and 95% confidence intervals (back bars) of the effect sizes obtained from non-parametric bootstrap resampling (5,000 resamples).

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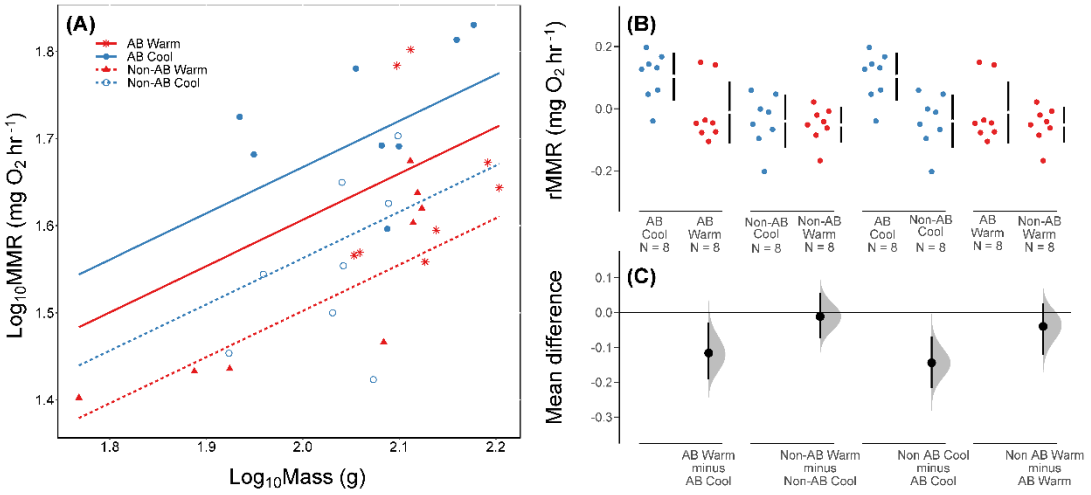

Figure 2: (A) Body mass scaling of maximum metabolic rate (MMR) of brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish were reared under two experimental temperature treatments: a natural temperature regime (Cool); and temperatures elevated 1.8°C above the Cool regime (Warm). (B) Residual MMR values (body mass corrected) for each population background and temperature treatment combination. Black vertical bars represent the standard deviation around the mean (shown as a gap in the bars), and sample size is shown as "N =". (C) Cumming estimation plots with effect sizes shown as black dots (i.e. the mean differences in rMMR among the groups), the distributions (shaded curves) and 95% confidence intervals (back bars) of the effect sizes obtained from non-parametric bootstrap resampling (5,000 resamples).

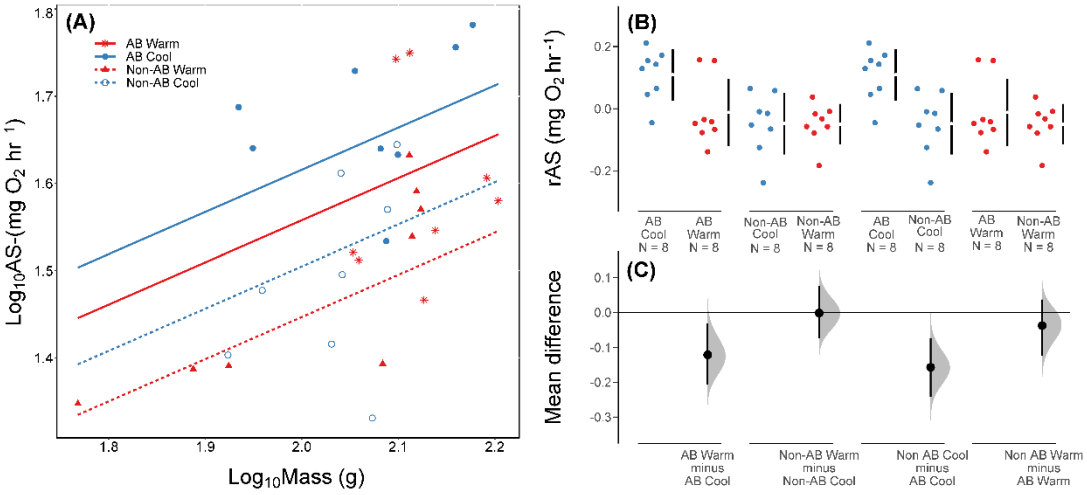

Figure 3: (A) Body mass scaling of aerobic scope (AS) of brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish were reared under two experimental temperature treatments: a natural temperature regime (Cool); and temperatures elevated 1.8°C above the cool regime (Warm). (B) Residual AS values (body mass corrected) for each population background and temperature treatment combination. Black vertical bars represent the standard deviation around the mean (shown as a gap in the bars), and sample size is shown as "N =". (C) Cumming estimation plots with effect sizes shown as black dots (i.e. the mean differences in rAS among the groups), the distributions (shaded curves) and 95% confidence intervals (back bars) of the effect sizes obtained from non-parametric bootstrap resampling (5,000 resamples).

Relationships between metabolic traits

After adjusting for body mass, rMMR was positively related to rSMR, with a significant interaction between rSMR and population ($\chi^2 = 5.16$, df = 1, $P = 0.023$) whereby the anadromous population had a higher rMMR for a given rSMR (Figure 4A, Table S3). The two-way interactions between temperature and rSMR ($\chi^2 = 0.63$, df = 1, $P = 0.429$), and between temperature and population ($\chi^2 = 0.25$, df = 1, $P = 0.618$), and the three-way interaction between temperature, population, and rSMR ($\chi^2 = 0.64$, df = 1, $P = 0.425$) were all non-significant. We did not detect a significant main effect of temperature ($\chi^2 = 1.22$, df = 1, $P = 0.270$).

The positive relationship between rAS and rSMR varied according to a significant rSMR × population interaction ($\chi^2 = 5.02$, df = 1, $P = 0.025$), whereby the anadromous population had a higher rAS for a given rSMR (Figure 4B, Table S3). The rAS × temperature interaction ($\chi^2 = 0.66$, df = 1, $P = 0.420$), the temperature × population interaction ($\chi^2 = 0.28$, df = 1, $P = 0.597$), and the rSMR × temperature × population interaction ($\chi^2 = 0.66$, df = 1, $P = 0.418$) were all non-significant. The main effect of temperature was also non-significant ($\chi^2 = 1.17$, df = 1, $P = 0.276$).

There was a strong positive relationship between rAS and rMMR (Figure 4C, $\chi^2 = 2490.9$, df = 1, $P < 0.001$). We did not detect significant interactions between rMMR and temperature ($\chi^2 = 0.50$, df = 1, $P = 0.479$), between rMMR and population ($\chi^2 = 1.41$, df = 1, $P = 0.234$), or between rMMR, temperature, and population ($\chi^2 = 0.26$, df = 1, $P = 0.613$). The temperature × population interaction was also non-significant ($\chi^2 = 0.47$, df = 1, $P = 0.493$). While the main effect of population was non-significant ($\chi^2 = 1.46$, df = 1, $P = 0.227$), the main effect of temperature was significant ($\chi^2 = 6.23$, df = 1, $P = 0.013$), whereby fish in the warm treatment had a marginally higher rAS for a given rMMR (Figure 4C, Table S3).

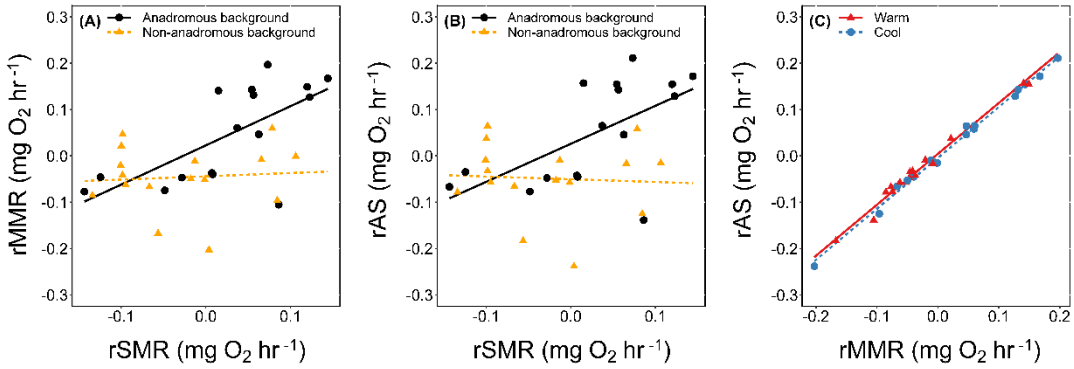

Figure 4: Relationships between: (A) Residual standard metabolic rate (rSMR) and residual maximum metabolic rate (rMMR); (B) rSMR and residual aerobic scope (rAS); and (C) rMMR and rAS for brown trout offspring derived from an anadromous background population (AB) and a non-anadromous background population (non-AB). Fish experienced two temperature treatments: a natural temperature regime (Cool), or temperatures elevated 1.8°C above the cool regime (Warm). Residual (size-independent) values for metabolic rates were estimated from the linear relationship between each metabolic rate (SMR, MMR, or AS) as a function of body mass (both $\log_{10}$-transformed).

Metabolic traits and growth

Specific growth rates of fish included in respirometry trials varied non-linearly through time (polynomial term for time: χ^2 = 134.32, df =3, P < 0.001; Figure 5A). After controlling for significant negative effects of initial body size on growth (χ^2 = 14.23, df =1, P < 0.001; Table S4), the mixed effects model describing the effects of rSMR on specific growth rate (marginal R^2 = 0.69) retained significant two-way interactions between rSMR and temperature, and between temperature and population (Table 3). Overall, the negative rSMR × temperature term indicated that in the warm treatment, higher rSMR were associated with lower growth rates (Figure 5B, Table S4). The negative interaction between temperature and population indicated that non-anadromous population had marginally lower growth rates in the warm treatment (Figure 5A, B). Neither the interaction term for rSMR × temperature

× population, nor the term for rSMR × population was significant in the model (Table 3).

The mixed effects model describing the effects of rMMR on specific growth rate (marginal $R^2 = 0.67$) retained a significant two-way interaction between rMMR and population (Table 3), once the effects of initial size and time were accounted for (effect of time: $\chi^2 = 133.15$, df = 3, $P < 0.001$; and of initial size: $\chi^2 = 14.50$, df =1, $P < 0.001$, Table S4). The anadromous background population showed a weak, positive effect of rMMR on growth, but the relationship was negative in the non-anadromous population (Figure 5A, C, and Table S4). Interaction terms for rMMR × temperature × population, for rMMR × temperature, and for temperature × population were not significant in the model describing growth rate as a function of rMMR (Table 3).

The model describing the effects of rAS on specific growth rate (marginal $R^2 = 0.69$) similarly retained a significant two-way interaction between rAS and population (Table 3), once the effects of initial size and time were accounted for (effect of time: $\chi^2 = 132.99$, df = 3, $P < 0.001$; and of initial size: $\chi^2 = 12.52$, df =1, $P < 0.001$ Table S4). The effect of rAS on growth was positive in the anadromous population but negative in the non-anadromous population (Figure 5A, D, Table S4). The model did not include significant interaction terms for rAS × temperature × population, for rAS × temperature, or for temperature × population (Table 3).

The additional set of mixed effect model analyses indicated that relationships between metabolic rates and growth were variable through time (Figure S2, Table S5) (LRT: rSMR × polynomial term for time: $\chi^2 = 38.60$, df = 3, $P < 0.001$; rMMR × polynomial term for time: $\chi^2 = 10.45$, df = 3, $P = 0.015$; rAS × polynomial term for time: $\chi^2 = 8.80$, df = 3, $P = 0.032$).

See Supporting Information for summaries of all GLM and mixed effects model coefficients.

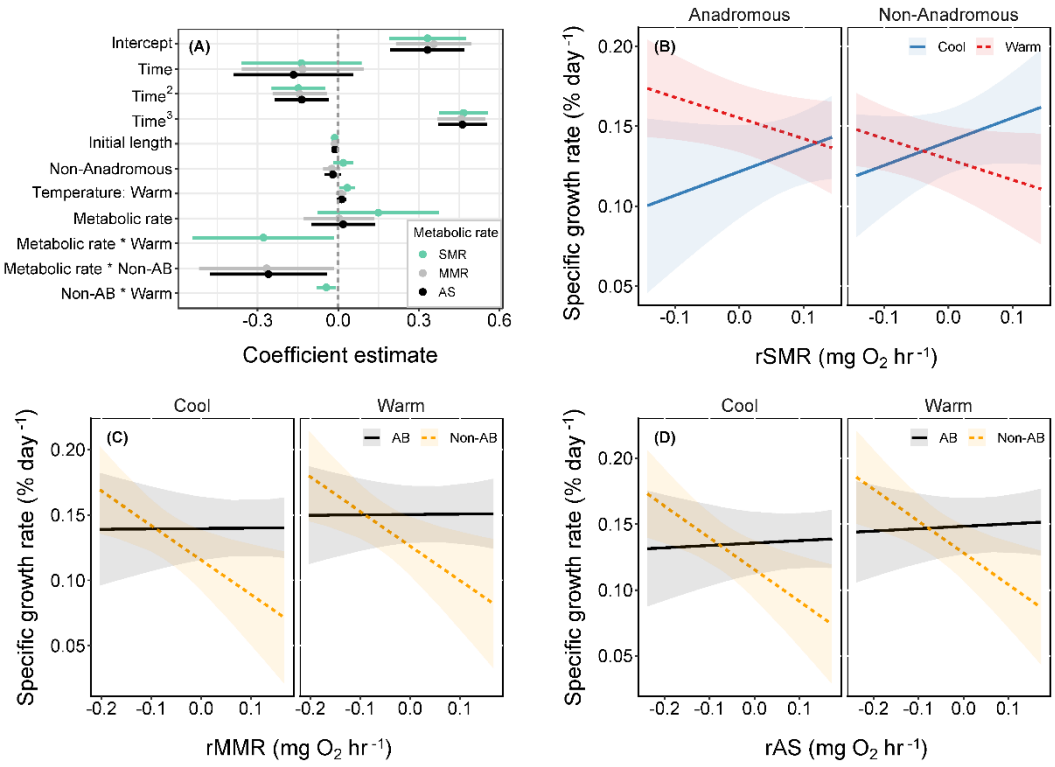

Figure 5: (A) Coefficient estimates (± 95% confidence intervals) from the mixed effect models describing the effects of residual metabolic rate components (SMR = standard metabolic rate, MMR = maximum metabolic rate, and AS = aerobic scope, temperature treatment (Cool and Warm), and population background (AB = anadromous background, Non-AB = non-anadromous background) on specific growth rates of brown trout obtained from repeated measurements across the study ("Time" = months since initiating treatments). The mixed models were used to predict specific growth rates of brown trout in response to marginal effects of (B) rSMR, temperature treatment, and popualtion background, (C) marginal effects of rMMR, temperature treatment, and popualtion background and (D) marginal effects of rAS, temperature treatment, and population background. Shaded regions in (B), (C), and (D) show the 95% confidence intervals for the predictions. Growth rates were predicted at mean values for the remaining explanatory variables in the models.

Table 3: Results of the mixed effect model analysis for specific growth rate trajectories (% day^{-1}) of brown trout that were measured for standard metabolic rate (SMR), maximum metabolic rate (MMR) and aerobic scope (AS). The results of the model selection procedure on interaction terms are given (significance assessed at $P < 0.05$), and the selected model for specific growth rate as a function of a given metabolic component is highlighted in bold. The models included a random effect of individual identity and a first-order autoregressive correlation structure with respect to time was modelled ("Time" = months since beginning of treatment).

Model terms	**df**	**AIC**	**logLik**	***L*-ratio**	***P*-value**
rSMR*Temperature*Population + poly(Time, 3) + Length	15	-487.46	258.73		
rSMR*Temperature + rSMR*Population + Temperature*Population + poly(Time, 3) + Length	14	-486.41	257.20	3.05	0.081
rSMR*Population + Temperature*Population + poly(Time, 3) + Length	13	-483.75	254.88	4.66	0.031
rSMR*Temperature + Temperature*Population + poly(Time, 3) + Length	13	-487.76	256.88	0.64	0.422
rSMR*Temperature + rSMR*Population + poly(Time, 3) + Length	13	-482.70	254.35	5.71	0.017
rMMR*Temperature*Population + poly(Time, 3) + Length	15	-486.17	258.09		
rMMR*Temperature + rMMR*Population + Temperature*Population + poly(Time, 3) + Length	14	-488.12	258.06	0.05	0.818
rMMR*Population + Temperature*Population + poly(Time, 3) + Length	13	-489.95	257.97	0.17	0.678
rMMR*Temperature + Temperature*Population + poly(Time, 3) + Length	13	-484.62	255.31	5.50	0.019
rMMR*Temperature + rMMR*Population + poly(Time, 3) + Length	13	-488.36	257.18	1.76	0.184
rMMR*Population + Temperature + poly(Time, 3) + Length	12	-488.00	256.00	4.13	0.127
rAS*Temperature*Population + poly(Time, 3) + Length	15	-487.10	258.55		
rAS*Temperature + rAS*Population + Temperature*Population + poly(Time, 3) + Length	14	-489.10	258.55	0.01	0.982
rAS*Population + Temperature*Population + poly(Time, 3) + Length	13	-490.67	258.33	0.43	0.513
rAS*Temperature + Temperature*Population + poly(Time, 3) + Length	13	-485.32	255.66	5.78	0.016
rAS*Temperature + rAS*Population + poly(Time, 3) + Length	13	-489.73	257.87	1.36	0.243
rAS*Population + Temperature + poly(Time, 3) + Length	12	-488.70	256.35	4.40	0.111

Discussion

Metabolism defines the energetic balance of organisms, with considerable implications for performance, and ultimately, fitness. Yet despite widespread variation in metabolic traits, we know surprisingly little about how or why such variation persists, or how metabolic variation affects individual performance. Here, we explored the intrinsic and extrinsic causes of variation in metabolism in facultatively migratory brown trout by experimentally rearing two populations (that naturally differ in anadromy tactics) under a temperature regime that simulated a 1.8°C increase above natural temperatures, in line with climate change projections. Both populations had lower SMR in the warm temperature treatment, with lower MMR and AS also seen in the anadromous population in the warm temperature treatment, a difference that was not evident in the non-anadromous population. The variation in metabolic traits had complex implications for growth rates across the study that varied by population background and temperature. Lower SMR were associated with higher growth in the warm treatment, whereas lower MMR and AS were linked to higher growth rates in the non-anadromous population. Overall, our study indicates that populations may show variable responses to changing environmental conditions in terms of metabolic traits, with potential fitness consequences in a warming world.

Effects of temperature on metabolism

The lower SMR displayed by both populations in the warm treatment suggests that adjustment of this key physiological trait is a plastic, or acclimation, response to chronic warming. While acute warming is well known to cause an initial increase in ectotherm metabolic rates (Clarke and Johnston 1999; Angilletta *et al.* 2002), exposure over longer time scales (i.e. those comparable to the 15 months of warming in our study) tends to reduce the magnitude of the response because acclimation occurs (Seebacher *et al.* 2015). The reduction in SMR we observed supports the potential for thermal compensation by way of the "plastic floors" hypothesis, where a lower SMR at warmer temperatures is beneficial because it reduces maintenance costs (Sandblom *et al.* 2016). Such

acclimation responses in fish can be considerable, *e.g.* Atlantic halibut (*Hippoglossus hippoglosssus*) acclimated at warmer temperatures (ranging from 5-18°C) for 14 weeks showed stable SMR from 10-16°C (Gräns *et al.* 2014). Acclimation capacity has been generally linked to increased resilience to environmental change (Magozzi and Calosi 2015; Seebacher *et al.* 2015). However, while temperatures are broadly projected to increase, more extreme and frequent warming events are also forecast (Meehl and Tebaldi 2004; Seneviratne *et al.* 2014). The adaptiveness of a given flexible response will thus greatly depend on both the pattern of fluctuations in temperatures, and the speed at which individuals can alter their phenotype (Reed *et al.* 2010).

Population effects on metabolism

In addition to temperature-induced changes, we observed variation in SMR between our study populations that matched our expectations of lower baseline energetic requirements in the non-anadromous population. Metabolic traits are evolvable (Rønning *et al.* 2007; Nilsson *et al.* 2009; Pettersen *et al.* 2018) and can respond to selection over relatively short periods (Sadowska *et al.* 2015). As such, population-level variation could reflect genetic differences or plastic/acclimation responses related to life-history differences between populations. For example, a lower SMR could be optimal for the non-anadromous population because freshwater systems have relatively low productivity (Gross *et al.* 1988), whereas higher SMR in the anadromous population might facilitate rapid growth to reach sufficient smolt size to successfully migrate (Ward and Slaney 1988), particularly if SMR and MMR are linked under the "increased intake" hypothesis (Burton *et al.* 2011). The higher MMR and AS we observed in the anadromous population might similarly reflect evolutionary change or plasticity driven by the demands of migration. MMR and AS is higher in anadromous sockeye salmon *Oncorhynchus nerka* populations that undertake more challenging migrations (Lee *et al.* 2003; Eliason *et al.* 2011), whereas relatively lower MMR and AS has been reported in resident *versus* migratory three-spine stickleback populations, linked to weak selection on swimming performance (Dalziel *et al.* 2012a, b). Higher SMR and MMR might

subsequently be favoured in the marine environment by facilitating predator avoidance (*e.g.* locomotor capacity) (Plaut 2001; Killen *et al.* 2015; Eliason and Farrell 2016). We also cannot rule out that metabolic traits may be reflective of environmental constraints specific to each population, *e.g.* counter gradient selection driven by poor/fluctuating resources experienced by the anadromous population, whereby a higher AS maximises consumption and growth when opportunities arise (Álvarez *et al.* 2006).

Intriguingly, while both of our study populations showed similar reductions in SMR in the warm temperature treatment, we detected variable responses in size-independent measures of MMR and AS with warming. Similar MMR and AS in the non-anadromous populations in both warm and cool treatments suggested that aerobic performance in this population was either unaffected by warming, or else showed a heightened acclimation response to the temperature increase than the anadromous population (which had lower MMR and AS in the warm *versus* cool treatment). Relatively little is known about the response of MMR or AS to chronic temperature increases (Schulte *et al.* 2011; Clark *et al.* 2013), but the population-level variation we observed supports mounting evidence that effects of long-term warming may vary considerably between and potentially within species. For example, thermal compensation in AS by way of acclimation in MMR has previously been seen in Atlantic halibut (Gräns *et al.* 2014). However, tropical barramundi *Lates calcarifer* showed reduced MMR and AS after acclimation to high temperatures, despite observed increases in MMR after acute exposure to warming (Norin *et al.* 2014). Similarly, a small decline in MMR was observed in shorthorn sculpin *Myoxocephalus scorpius* after eight weeks of warming, even though a reduction in SMR partially restored AS by the end of the acclimation period (Sandblom *et al.* 2014).

Since a high AS is generally considered to increase individual performance and fitness (Pörtner and Farrell 2008; Biro and Stamps 2010), and is a trait linked with migration effort (Eliason *et al.* 2011; Clark *et al.* 2011) it is initially unclear why the anadromous population did not appear capable of maintaining MMR. This implies there might be costs associated with maintaining a high MMR that

are specific to the anadromous population, in other words, temperature-induced flexibility in MMR may not be adaptive in this population *e.g.* if environmental fluctuations are rapid/extreme and the speed of acclimation is slow, the benefits of plasticity may be limited (Reed *et al.* 2010). Alternatively, the reduction in SMR may have caused an inevitable reduction in MMR, which is in line with the "increased intake" hypothesis underpinning energetic capacity in the anadromous population (Burton *et al.* 2011). This seems likely given the strong coupling we observed between SMR and MMR, which was absent in the non-anadromous population.

Consequences of metabolic rate variation for growth

The variation in metabolic traits we observed had consequences for growth, which depended largely on temperature treatment in the case of SMR. Lower SMR were associated with higher growth in warm, but not in cool treatments, supporting a context-dependency to the fitness consequences of a given SMR (Burton *et al.* 2011). However, it is important to note that growth rate is just one component of fitness, and might not always map positively or linearly onto fitness (Arendt 1997; Blanckenhorn 1998). Nonetheless, in this case, a lower SMR under warm conditions was likely beneficial for growth because maintenance energy costs were reduced, analogous to reductions in SMR facilitating higher growth or energy storage when food is limited (Naya *et al.* 2007; Auer *et al.* 2015c, 2016; Zeng *et al.* 2017). However, we also detected a complex interaction between SMR and time, indicating that the relationship between SMR and growth can further vary according to temporal factors. For example, the negative association between warm temperatures and SMR on growth may have been strongest during periods when temperatures exceeded the optimal ranges for growth in trout *e.g.* during the summer months (Elliott *et al.* 1995; Ojanguren *et al.* 2001). Moreover, while relative metabolic rates of salmonines tend to be stable through time (Nespolo and Franco 2007; Seppänen *et al.* 2010), absolute metabolic rates can vary considerably depending on a suite of environmental factors (Metcalfe *et al.* 2016; Norin and Clark 2016). As such, the link between SMR, growth, and temperature may also

depend on additional factors, *e.g.* food supply (Zeng *et al.* 2018), which in turn show fluctuations in time and space (Álvarez and Nicieza 2005; Robertsen *et al.* 2014).

A similarly complex mapping emerged between both MMR and AS and growth rate, which depended on population background and varied through time. Higher MMR and AS were associated with slightly higher growth rates in the anadromous population (independent of temperature), but this relationship was reversed in the non-anadromous population. Food intake (Auer *et al.* 2015a), and digestive/assimilation capacity (Alsop and Wood 1997) increase with MMR and AS, which presumably underlies the positive correlation between MMR, AS, and growth in the anadromous population. Higher growth (relative to a non-anadromous population) has previously been described in fish originating from the same source catchment as the anadromous-background population in the present study (Archer *et al.* 2019). Fast juvenile growth has been associated with the anadromy lifestyle in general (Forseth *et al.* 1999), appearing to be partially underpinned here by a higher metabolic scope. This implies that while a high metabolic scope is optimal in migratory populations, (perhaps because MMR and AS are linked to swimming performance (Dalziel *et al.* 2012b), and migration effort/performance (Eliason *et al.* 2011; Clark *et al.* 2011)), growth rates in non-anadromous populations are not limited by aerobic capacity.

Observed differences in the strength of the association between both MMR and AS and growth, and its variability through time, reflect inconsistent outcomes among studies that have previously assessed the relationship between metabolism and fitness-related traits (Metcalfe *et al.* 2016). In this case, behavioural differences between populations may have altered the magnitude of the association between MMR and AS and growth. For example, migratory fish populations (and individuals) typically show higher levels of activity, boldness, and aggression (Metcalfe *et al.* 1995; Lahti *et al.* 2001; Chapman *et al.* 2011b), which may in turn cause fish from anadromous populations to operate closer to MMR more frequently. Thus, a higher MMR and AS is likely be more

advantageous in terms of maximising growth (Burton *et al.* 2011), but the association may be weaker, or absent in the non-anadromous population, which potentially approaches MMR infrequently. There is a dearth of knowledge regarding how often individuals approach their MMR, and whether this varies among individuals or populations, though existing studies suggest considerable variation is likely (Murchie *et al.* 2011; Seebacher *et al.* 2013; Killen *et al.* 2014). The negative association between MMR and growth in the non-anadromous population implies a stronger influence of SMR on growth, supporting the "compensation" hypothesis as a factor underpinning metabolic variation in this population (Burton *et al.* 2011) (particularly when considered alongside the weaker correlation between SMR and MMR in the non-anadromous population). Alternatively, variability in the relationship between MMR and AS and growth between populations, might reflect differences in the allocation of surplus resources (fuelled by AS) towards other competing functions besides somatic growth, *e.g.* lipid accumulation and gonadal development (Jonsson *et al.* 2013), or competitive/aggressive interactions (Killen *et al.* 2014). This could also explain temporal variability in the MMR and AS – growth relationship, where the benefits of a higher MMR and AS may be enhanced when growing conditions are optimal *e.g.* at times of high food availability, and growth benefits reduce when food is scarce (Auer *et al.* 2015b).

Implications and considerations

Variation in the relationship between growth, population, and MMR and AS underscores that considering additional biotic and abiotic factors that vary through time or space might further disentangle the links between environment, metabolism, and fitness components (Pettersen *et al.* 2016). Here, we measured metabolic traits only after a long-term period of temperature acclimation and growth. Although relative metabolic rates in salmonines are consistent over this time scale (Seppänen *et al.* 2010), repeated measurements of metabolic traits could further illuminate how metabolic phenotype (or associations between metabolic traits and fitness) can vary according to fluctuating extrinsic and intrinsic conditions (Versteegh *et al.* 2012; Biro *et al.*

2018), or developmental stage (Beaman *et al.* 2016; Burggren 2018). Moreover, metabolic measurements at a finer temporal scale would allow exploration of variation in acclimation time, a trait that has received relatively little attention but likely has important implications for ectotherms experiencing both chronic and variable temperature changes (Sandblom *et al.* 2014). Additionally, we did not consider acute responses to warming, which may preclude any acclimation response. For example, if acute warming initially increases SMR before thermal compensation occurs, the resulting reduction in AS may limit capacity for essential functions such as feeding or predator avoidance (Clark *et al.* 2011; Sandblom *et al.* 2014). Extending this study to include more realistic/natural conditions (*e.g.* co-occurring abiotic or biotic stressors), coupled with tracking of individual reproductive success would give further insight into how optimal combinations of metabolic traits and life history are shaped by environmental context.

Nonetheless, the variation (and covariation) we detected in metabolic traits arising from long-term temperature exposure and population factors has implications for the performance of populations experiencing rapid climate change (Parmesan 2006). Studies have most often focused on the link between SMR or BMR and fitness components, but we add to a growing body of research suggesting that MMR (and consequently, AS) can influence fitness components (Boratyński and Koteja 2009; Clark *et al.* 2013; Zub *et al.* 2014; Auer *et al.* 2015b). In our case, links between MMR and growth rates – a key fitness-related trait – were mediated by population-specific factors. Moreover, population-based variation in the correlation between SMR and MMR and AS hints at the intriguing possibility of fundamental links between these traits that may vary according to extrinsic or intrinsic factors, thus limiting or enhancing capacity to respond to environmental change (Metcalfe *et al.* 2016; Norin *et al.* 2016). Though metabolic acclimation capacity is linked to increased resilience (Seebacher *et al.* 2015) – at least when environmental change is predictable (Reed *et al.* 2010) – such population-level variation underscores that responses to change are likely to be highly variable, even within species. Understanding

the context-dependency of associations between metabolic phenotypes and fitness, and how these can vary depending on population-specific factors, is required for successful management and conservation of aquatic species that are already in widespread decline due to progressive warming and global change (Pörtner and Knust 2007; Limburg and Waldman 2009; Martins *et al.* 2011).

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Supporting Information

Developmental temperature and population factors affect variation and covariation of metabolic traits, with complex effects on growth rates in brown trout (*Salmo trutta*)

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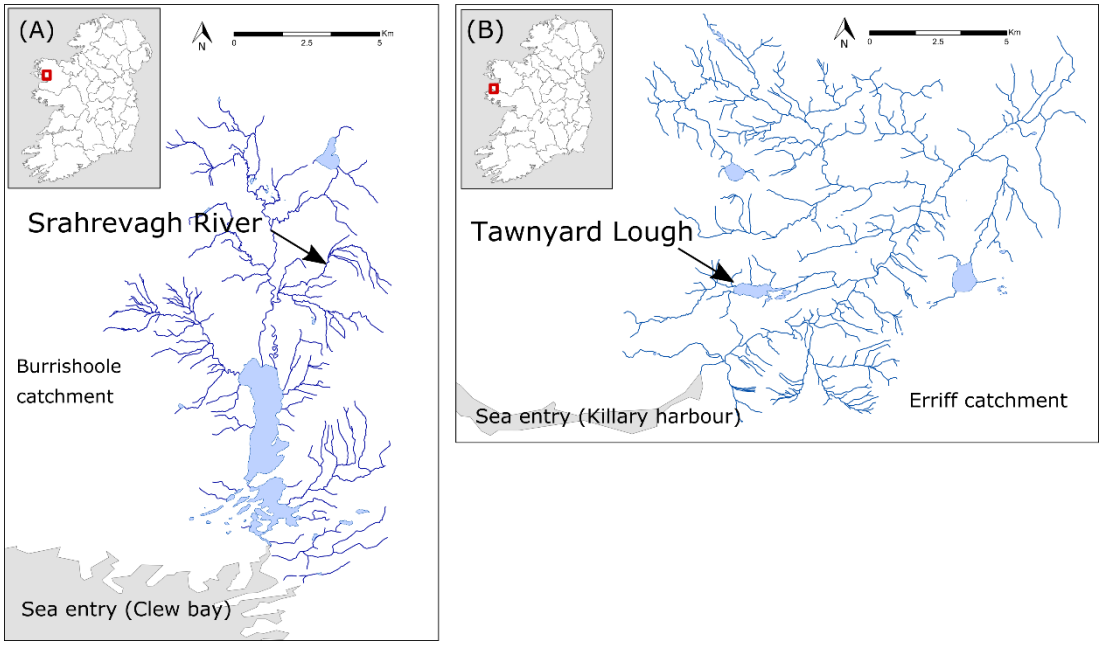

Figure S1: (A) Srahrevagh River in the Burrishoole catchment, where brown trout brood stock were collected by seine netting in November 2015, used to produce F1 offspring for the experimental study. The wild Srahrevagh population does not express the anadromous life history, and experimental offspring from Srahrevagh brood stock were considered to have non-anadromous population background. (B) Tawnyard Lough in the Erriff catchment, site of brown trout brood stock collections (via seine netting) in November 2015, used to produce F1 offspring for the experimental study. The wild Tawnyard Lough population has a strong anadromous component, and experimental offspring from Tawnyard brood stock were considered to have an anadromous population background.

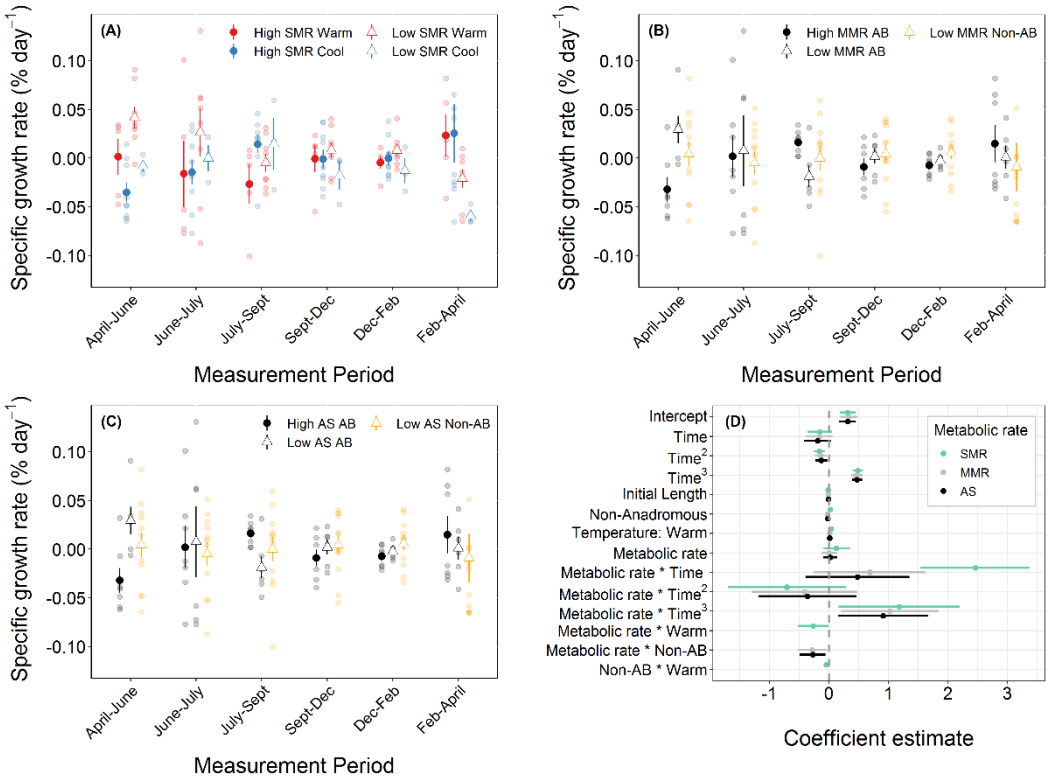

Figure S2: Mean specific growth rate trajectories (residual values corrected for initial body size) (with associated standard errors) of brown trout classed as having: (A) High or low standard metabolic rate (SMR); (B) High or low maximum metabolic rate (MMR); and (C) High or low aerobic scope (AS) according to residual SMR/MMR/AS values after accounting for variation in body mass ("High" metabolic phenotype = rSMR/rMMR/rAS > 0 and "Low" = rSMR/rMMR/rAS < 0). Fish were offspring from two population backgrounds (AB = Anadromous background, Non-AB = Non-Anadromous background), reared under two temperature treatments (Cool = natural temperature regime, Warm = elevated 1.8°C above Cool). (D) Coefficient estimates (± 95% confidence intervals) from mixed effect models testing interactive effects of time (months since experiment start) and metabolic rate (rSMR/rMMR/rAS), along with effects of temperature treatment, and population background on specific growth rate trajectories of brown trout.

Table S1: Parameter estimates, with associated standard errors (SE), *t*-values, and *P*-values from the linear model describing log_{10}-transformed metabolic rates (mg O_2 hr^{-1}) as a function of log_{10}-transformed body mass in n = 32 brown trout (SMR = standard metabolic rate, MMR = maximum metabolic rate, and AS = aerobic scope).

Response	Parameter	Estimate	SE	t-value	*P*-value
log_{10} SMR	Intercept	-1.28	0.33	-3.89	0.001
	log_{10} Body mass	0.95	0.16	6.00	< 0.001
log_{10} MMR	Intercept	0.16	0.39	0.42	0.676
	log_{10} Body mass	0.71	0.19	3.74	0.001
log_{10} AS	Intercept	0.18	0.42	0.42	0.680
	log_{10} Body mass	0.67	0.21	3.27	0.003

Table S2: Parameter estimates and associated standard errors (SE), *t*-values, and *P*-values from the general linear models testing the effects of temperature treatment (cool or warm) and population background (anadromous or non-anadromous) on standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) in brown trout. SMR, MMR, and AS were $\log_{10}$-transformed, and body mass ($\log_{10}$-transformed) was included as a covariate. Significance was assessed at $P < 0.05$. Effects are contrasted against fish from the anadromous population background in the cool temperature treatment.

Response	Parameter	Estimate	SE	t-value	*P*-value
$\log_{10}$ SMR	Intercept	-1.045	0.300	-3.479	0.002
	Population: Non-anadromous	-0.061	0.027	-2.246	0.033
	Temperature: Warm	-0.078	0.025	-3.090	0.004
	$\log_{10}$ Body mass	0.875	0.144	6.092	< 0.001
$\log_{10}$ MMR	Intercept	0.605	0.356	1.698	0.101
	Population: Non-anadromous	-0.104	0.032	-3.255	0.003
	Temperature: Warm	-0.061	0.030	-2.024	0.053
	$\log_{10}$ Body mass	0.531	0.170	3.117	0.004
$\log_{10}$ AS	Intercept	0.649	0.399	1.626	0.115
	Population: Non-anadromous	-0.111	0.036	-3.084	0.005
	Temperature: Warm	-0.058	0.034	-1.727	0.095
	$\log_{10}$ Body mass	0.483	0.191	2.532	0.017

Table S3: Parameter estimates and associated standard errors (SE), *t*-values, and *P*-values from the general linear models testing the effects of temperature treatment (cool or warm) and population background (anadromous or non-anadromous) on relationships between size-corrected standard metabolic rate (SMR) and maximum metabolic rate (MMR), SMR and aerobic scope (AS), and MMR and AS in brown trout. SMR, MMR, and AS were corrected for body mass by taking the residuals of the linear relationship between metabolic rate and body mass to give rSMR, rMMR, and rAS. Significance was assessed at $P < 0.05$. Effects are contrasted against fish from the anadromous population background in the cool temperature treatment.

Response	Parameter	Estimate	SE	t-value	*P*-value
rMMR	Intercept	0.043	0.027	1.572	0.128
	rSMR	0.731	0.261	2.798	0.009
	Population: Non-anadromous	-0.073	0.029	-2.499	0.019
	Temperature: Warm	-0.035	0.031	-1.103	0.280
	rSMR × Non-anadromous	-0.774	0.349	-2.221	0.035
rAS	Intercept	0.049	0.032	1.561	0.130
	rSMR	0.690	0.301	2.292	0.030
	Population: Non-anadromous	-0.084	0.034	-2.482	0.020
	Temperature: Warm	-0.039	0.036	-1.089	0.286
	rSMR × Non-anadromous	-0.878	0.402	-2.187	0.038
rAS	Intercept	-0.007	0.004	-2.053	0.050
	rMMR	1.113	0.022	49.909	< 0.001
	Population: Non-anadromous	0.005	0.004	1.208	0.237
	Temperature: Warm	0.010	0.004	2.495	0.019

Table S4: Parameter estimates and associated standard errors (SE), *t*-values, and *P*-values from three mixed effects models testing the effects of standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS) on specific growth rate trajectories of brown trout from two population backgrounds. Specific growth rate was calculated from repeated fork length measurements in fish experiencing either natural (Cool) or elevated (Warm) temperature regimes. SMR, MMR, and AS were corrected for body mass by taking the residuals of the relationship between body mass and metabolic rate to give rSMR, rMMR, and rAS. Initial fork length was included as a covariate in all models. Significance was assessed at $P < 0.05$. Effects are contrasted against fish from the anadromous population in the cool treatment.

Response	Effect	Estimate	SE	*t*-value	*P*-value
Specific growth rate	Intercept	0.332	0.073	4.559	< 0.001
	Non-Anadromous	0.019	0.019	0.996	0.332
	Time	-0.136	0.114	-1.200	0.233
	$Time^2$	-0.148	0.051	-2.939	0.004
	$Time^3$	0.466	0.046	10.081	< 0.001
	Initial length	-0.013	0.004	-3.644	< 0.001
	rSMR	0.149	0.115	1.294	0.211
	Temperature: Warm	0.033	0.014	2.335	0.031
	rSMR × Warm	-0.278	0.134	-2.071	0.052
	Warm × Non- Anadromous	-0.044	0.018	-2.433	0.025
Specific growth rate	Intercept	0.356	0.072	4.955	< 0.001
	Non-Anadromous	-0.024	0.016	-1.513	0.146
	Time	-0.133	0.115	-1.152	0.252
	$Time^2$	-0.143	0.051	-2.794	0.006
	$Time^3$	0.458	0.046	9.997	< 0.001
	Initial length	-0.013	0.004	-3.692	< 0.001
	rMMR	0.003	0.067	0.046	0.964
	Temperature: Warm	0.011	0.010	1.125	0.274
	rMMR × Non-Anadromous	-0.267	0.128	-2.094	0.049
Specific growth rate	Intercept	0.331	0.071	4.695	< 0.001
	Non-Anadromous	-0.020	0.015	-1.335	0.197
	Time	-0.166	0.114	-1.465	0.146
	$Time^2$	-0.135	0.051	-2.633	0.010
	$Time^3$	0.462	0.046	10.083	< 0.001
	Initial length	-0.012	0.004	-3.431	0.001
	rAS	0.018	0.060	0.303	0.765
	Temperature: Warm	0.013	0.009	1.375	0.184
	rAS × Non-Anadromous	-0.259	0.111	-2.345	0.029

Table S5: Parameter estimates and associated standard errors (SE), *t*-values, and *P*-values from the mixed effects models testing the for interactive effects of time (weeks since start of experiment) and standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) on specific growth rate trajectories of brown trout. Fish were from two population backgrounds (AB = anadromous and non-AB = non-anadromous). Specific growth rate was calculated from repeated fork length measurements in fish experiencing either natural (Cool) or elevated (Warm) temperature regimes. SMR, MMR, and AS were corrected for body mass by taking the residuals of the relationship between body mass and metabolic rate to give rSMR, rMMR, and rAS. Initial fork length was included as a covariate in all models. Significance was assessed at $P < 0.05$. Effects are contrasted against fish from the anadromous population in the cool treatment.

Response	Effect	Estimate	SE	*t*-value	*P*-value
Specific growth rate	Intercept	0.316	0.071	4.456	< 0.001
	Non-Anadromous	0.020	0.019	1.049	0.307
	Time	-0.155	0.108	-1.437	0.153
	$Time^2$	-0.162	0.047	-3.479	0.001
	$Time^3$	0.487	0.045	10.924	< 0.001
	Initial length	-0.012	0.003	-3.549	0.001
	rSMR	0.128	0.116	1.106	0.283
	Temperature: Warm	0.036	0.014	2.513	0.021
	rSMR × Warm	-0.263	0.134	-1.955	0.065
	Warm × Non-AB	-0.046	0.018	-2.512	0.021
	rSMR × Time	2.459	0.469	5.244	< 0.001
	rSMR × $Time^2$	-0.708	0.506	-1.401	0.164
	rSMR × $Time^3$	1.181	0.521	2.268	0.025
Specific growth rate	Intercept	0.331	0.072	4.585	< 0.001
	Non-Anadromous	-0.021	0.016	-1.317	0.203
	Time	-0.164	0.115	-1.424	0.157
	$Time^2$	-0.136	0.051	-2.654	0.009
	$Time^3$	0.469	0.045	10.421	< 0.001
	Initial length	-0.012	0.004	-3.324	0.001
	rMMR	0.004	0.066	0.057	0.955
	Temperature: Warm	0.011	0.009	1.145	0.266
	rMMR × Non-AB	-0.280	0.127	-2.209	0.039
	rMMR × Time	0.686	0.479	1.432	0.155
	rMMR × $Time^2$	-0.409	0.455	-0.899	0.370
	rMMR × $Time^3$	1.023	0.421	2.428	0.017

Specific growth rate	Intercept	0.314	0.071	4.399	< 0.001
	Non-Anadromous	-0.018	0.015	-1.215	0.239
	Time	-0.189	0.114	-1.658	0.100
	$Time^2$	-0.129	0.052	-2.501	0.014
	$Time^3$	0.472	0.045	10.423	< 0.001
	Initial length	-0.011	0.004	-3.142	0.002
	rAS	0.019	0.060	0.320	0.752
	Temperature: Warm	0.013	0.009	1.399	0.177
	rAS × Non-AB	-0.272	0.110	-2.472	0.023
	rAS × Time	0.480	0.445	1.077	0.284
	rAS × $Time^2$	-0.362	0.422	-0.858	0.392
	rAS × $Time^3$	0.911	0.388	2.350	0.020

Chapter 6

General Discussion

Enormous phenotypic diversity is evident between populations and among individuals within populations (Roff 1996), with pivotal roles in ecosystem structure and function (Bolnick *et al.* 2011; Des Roches *et al.* 2018). Understanding why, and how, such intraspecific diversity arises and persists is necessary to inform management and conservation of species in a changing world (Naish and Hard 2008), not least because different phenotypes may show variable responses to environmental change. Mechanisms underpinning intraspecific diversity can be understood from proximate and ultimate perspectives (Tinbergen 1963), where proximate mechanisms relate to the environmental or ontogenetic factors influencing phenotypic expression, and ultimate mechanisms concern evolutionary function and phylogenetic control of phenotypes (Laland *et al.* 2011; Bateson and Laland 2013). With this in mind, the overarching objective of this thesis was to advance the understanding of how proximate and ultimate factors (with more emphasis on the former) contribute to shaping phenotypic diversity in brown trout, a culturally and economically important species that is iconic for the impressive array of life histories it displays (Klemetsen *et al.* 2003; Ferguson *et al.* 2019; Nevoux *et al.* 2019).

Overview of each chapter

My overall approach has been to address the causes and consequences of life history diversity in brown trout by exploring the interplays between physiology, environment, and phenotypic diversity, focusing on alternative migratory tactics. In Chapter 2 I aimed to explore how extrinsic (i.e. environmental) and intrinsic (i.e. population) factors interactively determine expression of migratory *versus* non-migratory tactics. In experimental offspring from two populations that naturally differ in migration frequency, I measured rates of anadromy, maturation, and various traits associated with physiological

condition, in response to long-term food restriction treatments. To clarify when in early life the migratory decision might occur I also investigated how the timing of food restriction influences migration, which proved to be variable between the two populations. Although anadromy rates tended to be higher in the offspring from the anadromous population, anadromous phenotypes emerged at lower frequencies among offspring from the non-migratory population, with implications for conservation and restoration of the anadromous life history among brown trout populations.

In Chapter 3, I aimed to extend the study of the proximate drivers of life history variation by exploring how co-occurring environmental factors collectively influence migratory tactics. I reared offspring from an anadromous-background population under conditions of low food and warm temperature treatments that simulated potential climate change scenarios. The food and temperature stressor treatments had additive, but opposing effects on migration tactics, whereby food restriction increased migration rates, but warm temperatures increased maturation rates *in lieu* of migration. The combined stressors had antagonistic effects on size-associated traits and underlying physiological condition, suggesting that responses to cumulative effects of global change will not be straightforward, and may depend upon the response considered.

Chapter 4 explored how metabolic traits vary according to intrinsic and extrinsic factors. Specifically, I measured SMR, MMR, and AS in brown trout offspring from two populations that differ in migration tendency, after exposure to long-term conditions of food restriction. Variation in SMR and MMR was related to population background and food restriction treatments, and I detected population-specific variation in the ability to adjust AS in response to food restriction. The results in Chapter 4 contribute to our understanding of how populations can respond to fluctuating food resources via phenotypic plasticity in metabolic rate, with potential consequences for population persistence if food resources are limited or temporally variable.

I further explored causes and consequences of metabolic rate variation in Chapter 5 by assessing how temperature influences variation in metabolic traits

among populations, and the consequences of metabolic variation for a fitness-related trait. I measured SMR, MMR, AS, and individual growth rates in offspring from two populations with divergent migratory tactics that were reared under long-term warming conditions. Metabolic traits showed both population-level variation and plastic responses to warming. The relationship between metabolic traits and growth depended on either temperature (for SMR) or population background (for MMR and AS), which suggests that future climate warming effects on performance in wild populations are likely to be context dependent.

In this chapter, I discuss how these studies contribute to our understanding of the interaction between genes and environment underpinning phenotypic diversity in terms of: (i) life-history tactics, and (ii) physiology. In synthesising the results of this thesis with existing studies, I highlight the broader implications of the findings presented here. I also address some considerations faced while undertaking this research, and identify areas for future research to build upon this body of work.

Proximate drivers of facultative migration

Support for the threshold model

Much of our understanding of facultative migration has been aided by applying the environmentally cued threshold model (Tomkins and Hazel 2007). Under this framework, alternative tactics are conditional upon the relationship between a "status" trait (which is cued by the environment, but also probably influenced by genetic factors), and an inherited threshold for the status trait (Tomkins and Hazel 2007; Piche *et al.* 2008; Pulido 2011; Buoro *et al.* 2012). It follows that divergent phenotypes can emerge under similar environmental conditions, but also that similar phenotypes might be produced from genetic dissimilarity. Based on the threshold reaction norm concept, I had expected that manipulations of environmental conditions (i.e. food treatments in Chapter 2, along with food and temperature treatments in Chapter 3) would result in different frequencies of migratory phenotypes emerging in the various treatments. The relatively higher rates of migratory phenotypes observed in

food restriction treatments in Chapters 2 and 3 were compatible with the threshold model, as was the higher frequency of the mature phenotype in warm treatments in Chapter 3 (although the expression of migratory tactics in this case was in the opposite direction to expectations). Moreover, the emergence of anadromous phenotypes in fish derived from the Bunaveela population (that naturally does not show any anadromy in the wild) suggests that experimental food restriction prevented some individuals within this population from meeting their threshold for residency. Nonetheless, despite the apparent re-expression of anadromous phenotypes within the Bunaveela under low food conditions, anadromy was comparatively more frequent among fish from the naturally anadromous Erriff population, indicating a genetic component to anadromy. This offers some insight into ultimate mechanisms underlying facultative migration, whereby the mean threshold values have potentially evolved to differ between the two populations (Piche *et al.* 2008).

An important *caveat* here is that smolting was measured in year two of life, meaning total anadromy rates across all potential smolt ages were not quantified. Outcomes could potentially differ if the probability of smolting in year three varies by population. Within the Erriff catchment, the majority of smolts migrate in the second (2+) or third (3+) year of life, with approximately equal frequencies of 2+ and 3+ smolts (Gargan *et al.* 2016). Since the Bunaveela population does not express anadromy in nature, we have no definitive information on the distribution of smolts among different age classes. However, smolts historically produced in the wider Burrishoole catchment (pre-collapse of the anadromous stocks) exhibited approximately equal ratios of 2+ to 3+ smolts (Poole *et al.* 1996), and more recent records indicate a bias towards 2+ smolts (Poole *et al.* 2007). We thus have no compelling reason to suspect that the population-level patterns of anadromy might vary if the study had been extended for an additional year.

Evidence for potential status traits

The environmentally cued threshold model proposes that individuals undertaking migratory decisions assess a "status" trait, yet studies have not

conclusively identified any one trait that controls migratory decisions in salmonines (Kendall *et al.* 2014; Ferguson *et al.* 2019). The results described in Chapters 2 and 3 add to a growing body of evidence supporting a multitude of potential status traits as being instrumental in migratory decisions. In light of previous studies describing both positive and negative relationships between migration tactics and proposed status traits *e.g.* size (Morinville and Rasmussen 2003; Acolas *et al.* 2012), mass (Winter *et al.* 2016), condition (Boel *et al.* 2014; Hecht *et al.* 2015), growth (Jonsson 1985; Morinville and Rasmussen 2003; Acolas *et al.* 2012), energetic demands (Forseth *et al.* 1999), and lipid deposition (McMillan *et al.* 2012; Sloat and Reeves 2014), it seem increasingly apparent that it is more likely that a suite of interlinked physiological (or morphological) traits combine to influence migration tactics. In Chapters 2 and 3, future migrants and non-migrants showed divergent trajectories in traits such as condition, body size, and mass, far in advance of the natural migration period. Though we cannot definitively say if these traits had causal effects in the migratory decision-making process, or were consequences of adopting a chosen tactic, nonetheless, the results in Chapters 2 and 3 do suggest that a number of size/physiologically-associated traits are integral to the migratory phenotype. Such traits may reflect some underlying energetic state that is assessed, or their association with migration tactics might arise from genetic or environmental covariation between these traits and other physiological traits that influence migration (*e.g.* rate of change in energy balance or lipid deposition) (Doctor *et al.* 2014).

Relationships between migratory tactics and different physiological components or potential "status" traits may be further mediated by intrinsic or extrinsic conditions, making it more difficult to characterise clear associations, particularly if traits vary in environmental sensitivities. For example, antagonistic effects of environmental stressors on traits in Chapter 3 did not translate at the level of migratory tactics, perhaps due to variable effects of co-occurring environmental factors on key underlying traits (Galic *et al.* 2018). Moreover, links between the underlying traits themselves may be variable, for

example, associations between metabolic traits and growth (two strong candidates for status traits) varied according to environmental factors (*i.e.* temperature) and population background in Chapter 5, in addition to showing temporal sensitivity. When considered alongside population-level variation in metabolic traits in offspring from populations of different migratory backgrounds (Chapters 4 and 5), the divergence in physiological and morphological traits implies fundamental differences in energy uptake and expenditure between migrants and residents (Forseth *et al.* 1999; Sloat and Reeves 2014), or at least between predominantly migratory and non-migratory populations. Individual-level tracking of relationships among metabolic traits and other surrogate status traits (size/physiologically-associated traits) across an extended period during development would help to clarify how these traits collectively form an "energetic phenotype" that influences migratory tactics.

Environmental factors influencing migration

Understanding how environmental factors mediate the expression of life-history tactics via underlying traits was a key goal of this thesis, and was specifically addressed in Chapters 2 and 3. The higher rates of anadromy in response to food limitation (Chapters 2 and 3) is in agreement with studies that have found direct and indirect manipulations of food availability to alter patterns of adfluvial/marine migration and freshwater maturation (Olsson *et al.* 2006; Wysujack *et al.* 2009; O'Neal and Stanford 2011; Jonsson *et al.* 2012; Jones *et al.* 2015). An interesting result emerging from Chapter 2 was that the timing of food limitation can alter patterns of migration *versus* residency in a population-specific manner. This could be explained by variable "decision windows" between populations, or differential sensitivity between populations to annually recurring decision windows. While we could not distinguish in Chapter 2 if populations showed temporally distinct decisions windows, or if the migration decision is revisited annually, threshold-type models used to understand life-history trajectories in Atlantic salmon *S. salar* (Thorpe *et al.* 1998) and steelhead *O. mykiss* (Satterthwaite *et al.* 2009) propose existence of multiple distinct decision windows. Moreover, each window might relate to

separate maturation or emigration decisions. A similar series of decisions (or "switches") could exist for brown trout, each of which is independently controlled by a combination of environmental and genetic factors (Ferguson *et al.* 2019).

The prevalence of individuals that could not be assigned a life-history phenotype in Chapters 2 and 3 further supports the existence of additional life-history decisions that may be more related to the timing of phenotypic expression. Further exploration is required to determine whether similar threshold mechanisms are at play here. Existence of additional decision windows could perhaps explain why associations between potential status traits and migration tactics have generally proved to be inconsistent (Kendall *et al.* 2014; Ferguson *et al.* 2019), particularly if different traits hold varying degrees of importance for various decisions related to migration and residency. Condition (and/or other traits related to energy status) might form the basis for the initial migration *versus* residency decision, whereas size, lipid stores, or growth might be more important in decisions regarding *when* to migrate or mature, respectively (Thorpe *et al.* 1998; Thorpe and Metcalfe 1998). Similarly, migration destination might be influenced by factors related to, but nonetheless distinct from, the initial migratory decision. For example, metabolic rate has been implicated in the decision of brown trout to migrate from natal streams, but subsequent growth rates (Cucherousset *et al.* 2005), or rate of lipid depletion (Boel *et al.* 2014) might dictate whether trout terminate migration in the larger river stem or lake, or continue to the sea. It seems increasingly obvious that we should reject the notion of life-history tactics as a simple dichotomy of migration *versus* residency, which belies the complex mosaic of life-history decisions in trout (Birnie-Gauvin *et al.* 2019). Instead, consideration of myriad trajectories – encompassing freshwater maturation at a variety of ages and migration/anadromy at a variety of ages – is required, with each trajectory controlled by a combination of inherited and environmental factors mediated by interlinked physiological traits.

While the overarching effects of food availability in Chapters 2 and 3 were in line with expectations, the negative relationship between temperature and migratory tactics in Chapter 3 was somewhat unexpected given the fundamental role of temperature in defining energetic balance in ectotherms (Fry 1971; Clarke and Johnston 1999; Gillooly *et al.* 2001). Since cooler water temperatures are associated with increased lipid deposition (Kammerer and Heppell 2013), which is linked to maturation in salmonines (McMillan *et al.* 2012; Sloat and Reeves 2014), higher temperatures have generally been postulated to increase the frequency of migratory phenotypes (Sogard *et al.* 2012; Sloat and Reeves 2014; Sloat *et al.* 2014; Kendall *et al.* 2014). However, the results in Chapter 3 suggest that warming acted in this case to increase early freshwater maturation. While the mechanisms behind warming-induced maturation are not immediately obvious, it seems that an acclimation response to temperature (*e.g.* in minimum energy requirements, similar to SMR reductions in Chapter 5) resulted in more individuals reaching their threshold for residency. Any such reduction in baseline energy costs would thus allow for accumulation of energy stores/body condition necessary for maturation. Temperature effects on maturation might thus have been mediated via effects on condition (or energy allocation) at key times (even though effects on size/mass were negative, we detected a neutral or positive relationship between temperature and condition that varied through time in Chapter 3). Positive relationships between body condition and temperatures up to 20 °C have been observed for Atlantic salmon within aquaculture settings (Tromp *et al.* 2018), and have been linked to more frequent and earlier maturation in the species (Good and Davidson 2016; Debes *et al.* 2019).

The unexpected results in Chapter 3 underscore that temperature changes, or indeed, any environmental changes, are likely to depend on the pre-existing conditions in a given environment. Changes in thermal regimes (or other freshwater conditions) that remain within the optimal range for growth may promote residency if physiological condition can be maintained, a scenario particularly likely in relatively cool systems that undergo warming (Benjamin *et*

al. 2013). This is in agreement with predictions from state-dependent models indicating that dramatic temperature changes (> 3 °C) would be required to alter the balance of life-history expression in *O. mykiss* inhabiting cool streams (Satterthwaite *et al.* 2010). Nevertheless, the results in Chapters 2 and 3 collectively indicate that changes in the freshwater environment may still alter the frequency of the anadromous and resident life-history tactics relatively quickly. As implied by this thesis, such changes could occur at the population level via changes in individual energy uptake/allocation in response to abiotic conditions or fluctuations in local patterns of resource availability. However, from these results we can infer changes that may occur on a broader scale. The prevalence of anadromy generally increases with latitude, reflecting the gradient in the balance between freshwater and marine productivity (Gross *et al.* 1988). As such, observed and predicted decreases in macroinvertebrate prey (*e.g.* Durance and Ormerod 2007) might drive more marine migration in some areas, whereas increased eutrophication may promote freshwater residency in others (Gross 1987). Predicted increases in temperature (IPCC 2014) might overwhelmingly act to tip the balance from anadromy to residency across much of the species' distribution in northern Europe, if effects of warming on growth rates are largely positive and within thermal optima (*e.g.* Chapters 3 and 5; Benjamin *et al.* 2013). Thus, changes in the balance of anadromy *versus* residency may vary depending on geographic location, and proximity to physiological optima, either enhancing or reducing life history diversity. A shift towards uniformity in life histories will have negative implications for the "portfolio effect" in salmonines, where high levels of life-history diversity can buffer species as a whole from environmental change (Schindler *et al.* 2010, 2015). Changes in the relative frequency of alternative life histories might also have consequences for the broader ecosystem. For example, changes in marine nutrients supplied by returning anadromous fish can alter freshwater community structure and ecosystem function (Naiman *et al.* 2002; Janetski *et al.* 2009; Doughty *et al.* 2016), or even result in eco-evolutionary feedback dynamics (Auer *et al.* 2018).

Proximate and ultimate factors influencing metabolic traits

While the importance of proximate factors in driving migratory tactics was the primary aim of Chapters 2 and 3, in Chapters 4 and 5 I explored how interactions between environmental and intrinsic (population) factors mediate physiology at a more fundamental level: metabolic rate variation. Despite considerable intraspecific variation evident in minimum metabolic rates (Biro and Stamps 2010; Burton *et al.* 2011), the reasons underpinning the persistence of such variation are somewhat unclear. Lower SMR in offspring from non-anadromous populations (Chapters 4 and 5), and in conditions of food restriction (Chapter 4) and a long-term temperature increase (Chapter 5) collectively support SMR variation arising from both environmental and population factors (Norin and Metcalfe 2019). Variation in maximum aerobic metabolism is less-often studied but purportedly similar, and likely to be of great ecological relevance (Metcalfe *et al.* 2016; Norin and Clark 2016). That MMR and AS tended overall to be higher in the anadromous-background populations hints at a genetic component to these traits. Moreover, population-specific effects of food restriction (Chapter 4) and temperature (Chapter 5) point towards potential genotype-by-environment effects on MMR and AS.

Reductions in SMR in warm temperatures/low food conditions presumably occurred as plastic or acclimation responses to energetically challenging environments. Such responses are in line with the principle of "plastic metabolic floors" (a reduction in baseline energetic demands) as a key compensation mechanism in coping with inclement conditions (Sandblom *et al.* 2016). However, MMR, and consequently, AS, appear to be somewhat more immutable and to be largely determined by population-specific factors. While capacity for physiological plasticity can improve resilience to predictable effects of warming (Seebacher *et al.* 2015), constraints in the flexibility of MMR and AS may mean that compensation responses to environmental change are insufficient (Sandblom *et al.* 2016).

The population-level differences observed in SMR, MMR, and AS might be indicative of divergent plastic responses by populations, and also raises the

intriguing possibility of ultimate mechanisms underlying metabolic trait variation, *e.g.* selection within the anadromous background population favouring higher SMR, MMR, or AS facilitating fast growth (McCarthy 2000) or increased swimming performance (Eliason *et al.* 2011). Interestingly, though AS was marginally higher in food-restricted fish from the anadromous-background population (Chapter 4), AS was lower in fish originating from the same population after long-term warming (Chapter 5). While these variable responses may simply be related to food/temperature induced shifts in the patterns of future life-history trajectories (as seen in Chapter 2 and 3), it nonetheless raises questions about the future performance of this population with climate warming (Pörtner and Farrell 2008; Sandblom *et al.* 2016). Will constraints in the upper boundaries of aerobic metabolism limit the capacity of migratory populations to respond to change? Some population-specific responses to warming have been predicted in salmonines (Martins *et al.* 2011), but the viability of many migratory populations is in doubt in a rapidly warming world (Crozier *et al.* 2008; Jonsson and Jonsson 2009; Farrell 2009).

Implications of metabolic trait variation for fitness

Collectively, results from Chapters 4 and 5 indicate that while metabolism is generally assumed pivotal in shaping life histories though effects on fitness (Brown *et al.* 1993; Ricklefs and Wikelski 2002; Guderley and Pörtner 2010), this association is mediated by environmental and genetic factors. The negative relationship between SMR and growth under warm conditions in Chapter 5 adds further support to studies describing context-dependent fitness benefits of a given metabolic phenotype (Norin and Malte 2011; Auer *et al.* 2015a, b). Although growth is a key fitness-related trait, it is important to note here that improved growth performance may not necessarily translate into improved fitness (Mangel and Stamps 2001), and growth is itself a trait likely to show context-dependent fitness benefits. For example, faster growth in populations that tend to migrate might confer additional fitness benefits in terms of survival-at-sea (Kendall *et al.* 2014; Armstrong *et al.* 2018) but could be less important for non-migratory populations, and may thus have contributed to the

population-specific variation in the relationship between MMR and AS and growth observed in Chapter 5. Examination of additional fitness-related traits (*e.g.* lipid stores) may reveal population-specific differences in how surplus energy (fuelled by MMR and AS) is allocated and traded off between competing functions.

On a broader scale, the ability to adjust physiological components in response to environmental factors has been positively associated with species' resilience to global change (Seebacher *et al.* 2015). However, the advantages of phenotypic plasticity for population persistence will depend upon both the speed at which the phenotype can change, but also the nature of the environmental change, i.e. whether fluctuations are predictable, and how accurately the current environment predicts future conditions (Reed *et al.* 2010). It is also important to note that in Chapters 4 and 5, fish were exposed to long-term changes in food and temperature but acute responses/short-term changes in environmental conditions were not considered, which could have altered the relationship between metabolic and fitness-related traits. This is particularly relevant since more frequent extreme weather events are forecasted with a changing climate (Meehl and Tebaldi 2004; Seneviratne *et al.* 2014), particularly if some acute effects (*e.g.* those affecting locomotion/survival) may be more important for fitness than longer term ones (*e.g.* growth). Nevertheless, understanding how facultatively migratory species such as brown trout respond to global change in terms of energetics may potentially contribute some mechanistic insights into changes that might scale up and partially underpin any changes in life histories. For example, though I did not directly test the association between metabolic rates and life-history expression at the individual level, the capacity of fish to lower SMR over a long timescale may shed some light on why relatively high rates of maturation were still possible in the low food conditions and warm temperature treatments in Chapters 2 and 3. Building on the knowledge of how various metabolic components relate to life history, and how this nexus is mediated by environmental factors will provide further insight into the ecological significance of metabolic variation.

Considerations and limitations of the research

Experimental approach

The results presented here help to reveal how environmental factors can combine with intrinsic factors to influence phenotypic diversity, but it is important to consider them within the context of the experimental tank-based approach that formed the basis for this research. While experimental tank rearing is a powerful approach that allows clean manipulation of key environmental variables of interest, while holding other factors constant, these advantages must be traded off against the inevitable loss of natural complexity in an artificial setting. Inferring the applicability of findings from controlled experiments to wild populations is somewhat difficult, and effects described here may vary in nature depending on the location or population considered. For example, while I explored combined environmental factors in Chapter 2, responses in wild populations may be swamped by additional stressors or other confounding factors (Merilä and Hendry 2014; de Eyto *et al.* 2016; Galic *et al.* 2018). Extending this common-garden approach to more variable natural systems, or reciprocal transplant experiments in the wild between populations of divergent migratory tactics would complement the tank-based manipulations described here and give insight on whether these findings hold up in more realistic and ecologically relevant contexts.

An additional drawback of this experimental approach is the considerable spatial and logistical constraints associated with the housing and husbandry of large numbers of fish under artificial conditions. A recurring limitation arising from spatial constraints is replication of experimental manipulations at the tank-level, which would allow any potential inter-tank variation/noise to be estimated and accounted for. Although I attempted to minimise any such nuisance variation related to tank effects (*e.g.* by standardising all conditions besides manipulated variables, analysing the data at the individual level and fitting random effects where appropriate), additional tank-level replication would make any future experimental studies more robust. Nonetheless, despite any potential nuisance variation related to tank effects, the results I describe

here are broadly in line with theoretical predictions, suggesting that conclusions drawn with respect to population and environmental effects are more parsimonious than simple tank-related effects. Likewise, logistics of tank rearing also limited our capacity for replicating treatments at the population level. While the use of two populations in Chapters 2, 4, and 5 is an advance on a single population approach - a common limitation among studies of facultatively migratory species (Olsson *et al.* 2006; Wysujack *et al.* 2009; Chapman *et al.* 2011) – inclusion of additional populations would determine the general applicability and robustness of my findings described here. This is particularly desirable given population-level variation is a key outcome of this thesis (Chapters 2, 4, and 5).

The importance of population background for traits related to both migration and metabolism throughout much of this thesis is clear. However, less obvious is whether such population-background effects can be attributed to life-history differences between populations, or are simply indicative of the different catchments of origin (i.e. the various populations having evolved in distinct river systems, possibly originating from different lineages). For example, brood stock from each population were of unknown life history, but were assumed to represent the naturally occurring balance of migration *versus* residency for each population (ranging from non-anadromous, to strongly anadromous). Using parents of a known life history (*e.g.* via stable isotope analysis) would help to parse out factors related specifically to life history from those related to various other population-level differences. This would be particularly powerful if representatives of each possible life-history phenotype could be obtained as brood stock from populations, however, this is not always possible (*e.g.* in wholly resident populations). The presence of reciprocal hybrids from each population/life history would also be desirable to control for parental effects, which can be considerable (Taborsky 2006; Burton *et al.* 2013; Moore *et al.* 2019). Nevertheless, life history is intricately linked to population-specific factors, being both proximately, and ultimately (via selective forces) determined by such factors (*e.g.* growth opportunity in the local environment), and is thus

likely to be representative of any major differences between populations. On a more philosophical note, disentangling whether divergent phenotypic responses among populations to environmental manipulations represents an adaptive genetic basis to the trait of interest, or implies other population-specific explanations (*e.g.* genetic drift or gene flow) is a fundamental challenge of the common-garden style approach employed in this thesis (Merilä and Hendry 2014). While the results in this thesis are largely consistent with a genetic basis to migration and metabolic traits, other potential population-specific explanations cannot be excluded completely.

Conclusions and future directions

A recurring theme throughout this thesis is the decomposition of drivers underpinning phenotypic diversity into genetic and environmental components. The results described here offer some insight into how the culturally and economically important brown trout (and other salmonine species) will respond to changing environmental conditions, with implications for conservation and management strategies. The potential for (re-)emergence of migration in resident populations (Chapter 2; Thrower *et al.* 2004) offers some positive perspective on the restoration of anadromous salmonine populations that have drastically declined by as much as 80% in recent years (Limburg and Waldman 2009). Similar experiments using salmonine populations where migration has ceased due to impassable barriers/ dams would help to clarify further whether freshwater resident populations maintain the capacity to produce migratory individuals.

The general flexibility in life-history expression displayed by my focal populations offers some encouraging evidence that brown trout have the capacity to maintain a diverse suite of life histories among populations, which may help to cushion the entire species from global change (Schindler *et al.* 2015). However, the ecological and evolutionary impacts of any environmental changes on physiology and life-history expression will depend on a multitude of factors, not least geographic location and population-specific proximity to optimal growth. Some populations may show increased diversity, whereas

others may become more uniform. The consequences of altered intraspecific diversity require further investigation (Bálint *et al.* 2011; Ceballos *et al.* 2017), but are somewhat analogous to biotic homogenisation at the community level in response to climate change, with negative impacts on community stability (O'Gorman *et al.* 2012, 2019). The logical next step is to consider environmental change across the broader range of brown trout distribution, in populations that may be closer to their thermal limits or those experiencing additional pressures from interspecific dynamics or disease. Consideration of multiple, co-occurring environmental factors (*e.g.* Chapter 2) is essential in future studies in order to make realistic predictions regarding environmental change (Côté *et al.* 2016). A novel approach would be to take advantage of natural environmental gradients, which allow for *in situ* manipulation of environmental conditions. So-called "natural laboratories" such as geothermally-heated systems (Woodward *et al.* 2010; O'Gorman *et al.* 2014), or areas warmed as a by-product of nuclear energy production (Sandblom *et al.* 2016; Huss *et al.* 2019) offer unique opportunities to explore the effects of warming/biotic interactions on metabolic traits and life-histories (*e.g.* O'Gorman *et al.* 2016; Pilakouta *et al.* 2019). By combining natural complexity with semi-controlled conditions, these could prove to be an exciting extension to the common-garden or translocation experiments that are invaluable to disentangling environmentally-induced/plastic responses from long-term evolutionary change.

While much of this thesis has focused on direct effects of proximate factors on phenotypic diversity, the existence of an underlying genetic component to migration-associated traits is clear. Consideration of the evolutionary effects of environmental forces, or how these may indirectly alter life histories via ultimate mechanisms is equally important. For example, directly testing how changes in freshwater conditions can alter the future fitness of various phenotypes (*e.g.* reduced salinity tolerance in Chapter 3 and growth in Chapter 5) will indicate how ultimate mechanisms give rise to phenotypic change within, and divergence across, populations. Beyond effects in the natal freshwaters (explored in this thesis), environmental changes on the migration

journey, and in the destination environment, will alter the costs and benefits of each tactic, and ultimately the relative fitness of migrants *versus* residents. Direct stress from various factors – changes in pH, oxygen levels, temperatures – on the migration route (Eliason *et al.* 2011; Peiman *et al.* 2017), or large-scale shifts in freshwater or marine food webs will likely impose strong selection on facultatively migratory species (Crozier and Hutchings 2014). Such environmental changes could act as forces of selection on underlying migration thresholds, or on the genes influencing physiological condition or energetic/metabolic traits (Phillis *et al.* 2016) with consequences for migration propensity, or perhaps, migration destination. Future studies on (i) the heritability of metabolic phenotypes, energy budgets, and migratory tactics, and (ii) their relative fitness in different contexts are fundamental to understanding the evolutionary basis to alternative life histories, along with the physiological underpinnings.

In an era of rapid global change and biodiversity loss, there is a pressing need to understand how species with strikingly diverse and complex life histories might respond. Can facultatively migratory species display sufficient flexibility to cope with changes to key elements of the environment? The results of this thesis suggest that brown trout have considerable capacity to respond in terms of physiology and life history, but outstanding questions still remain on the future of migratory species (Lennox *et al.* 2019). Developing further knowledge of how genetic and environmental forces interactively shape physiology, and ultimately, life histories is essential to anticipating and managing the consequences of anthropogenic change for declining salmonine populations.

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