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Genetic basis and fitness consequences of migration in facultatively anadromous brown trout *Salmo trutta*

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for the degree of
Doctor of Philosophy

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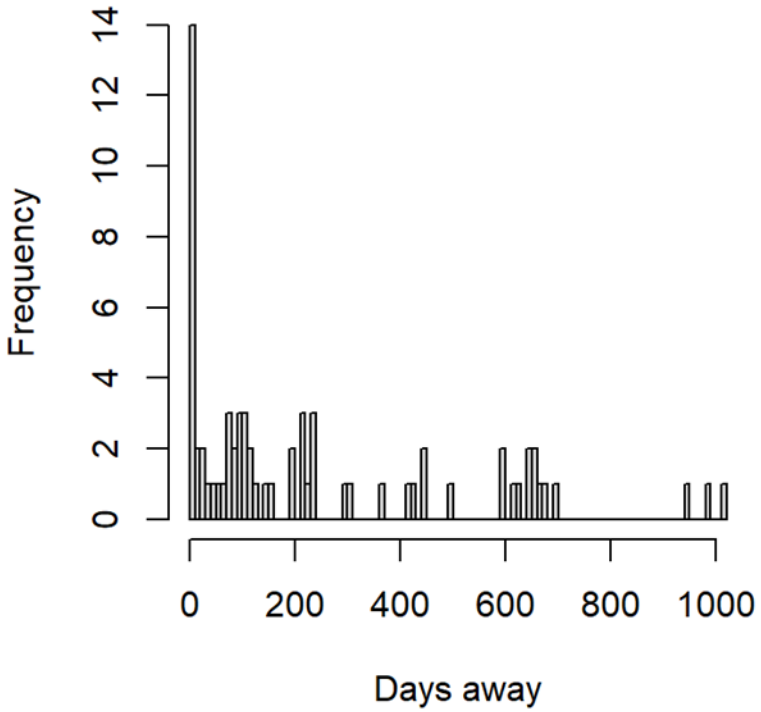
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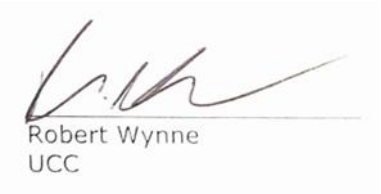
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Declaration

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.



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Robert Wynne

Abstract

Animal migration is a widespread, but complex phenomenon, that evolves in response to spatial, temporal or ontogenetic separation of optimal feeding and breeding habitats. Considerable diversity in migratory behaviours and associated physiological, morphological and life-history traits can occur even within a single species, making it challenging to understand the various evolutionary and ecological processes involved. This thesis focuses on brown trout (*Salmo trutta*) as an excellent model species to study the drivers and consequences of alternative migratory tactics, as they display a vast continuum of migratory life histories, even within one population. Until recently, relatively little was known about the genetic mechanisms and selective pressures shaping the huge variation in migratory life histories in brown trout and other salmonids. Advances in molecular biology (the 'omics revolution'), coupled with increasingly sophisticated telemetry techniques for studying movement behaviours in the wild, are now facilitating improved understanding of how migratory phenotypes are shaped by complex interactions between genes and environment. This, in turn, informs conservation and management of facultatively anadromous species in rapidly changing environments. The overarching aims of this thesis are to provide a deeper

understanding of the genetic/evolutionary basis of migration in brown trout, but also to use genetics as a tool to gain insights into basic aspects of brown trout biology.

The first data chapter (Chapter Two of the thesis) involved a common garden experiment undertaken in the Burrishoole catchment (northwest Ireland), which tested for heritable differences in migratory life history among geographically proximate, partially reproductively isolated, brown trout populations. Six different types of genetic crosses were made using wild-sourced broodstock. The progeny were then released into an experimental section of the Srahrevagh River (Burrishoole catchment) and their subsequent fates and movements tracked across three years using electrofishing surveys, trapping facilities and passive integrated transponder (PIT) telemetry, coupled with genetic parentage assignment (microsatellite markers) to assign sampled juveniles back to genetic cross types. The key findings of this chapter were that early movement behaviours (emigration from the river) and smolting rates (emigration to sea) differed among cross types, consistent with there being a quantifiable genetic basis to alternative migratory tactics. The contemporary sea trout run in the Burrishoole system is a remnant of what it once was, yet the numbers of resident/potamodromous brown trout remain apparently healthy. The results of Chapter Two imply that rapid evolutionary responses to anthropogenic environmental change could partially explain this switch away from anadromy towards residency.

Although it has long been known that migrants and residents can co-occur within the same trout population, the underlying molecular mechanisms driving alternative life histories has remained poorly understood. Therefore, in Chapter Three, I performed transcriptional profiling (RNA sequencing or “RNA-seq”) of brain and liver tissues collected from immature brown trout smolts (migrants) and mature residents, in the context of a tank-based laboratory experiment. The results stated in chapter three provide new insights into tissue - and sex-specific gene expression patterns and associated molecular processes (particularly metabolism-associated pathways within the liver) that underlie the production of alternate migratory life histories and physiologies.

In Chapter Four I then examined a different but related aspect of phenotypic diversity in brown trout, namely the long-recognised yet poorly understood phenomenon of

autumn versus spring outmigration (from the river into sea), using genetic identity analysis complemented with physical tagging (PIT telemetry) within a wild population of brown trout located in northwest Ireland (Burrishoole catchment). Both autumn and spring migrants exhibited a sex bias towards females, but was stronger in spring than in autumn outmigrants, implying that the fitness costs and benefits of adopting either strategy may differ between the sexes. Crucially, I also found that autumn outmigrants returned to freshwater at a similar rate and only slightly smaller size than spring outmigrants, despite the former category being much smaller on leaving freshwater and spending longer away overall than the latter category. These findings suggest that autumn outmigrants are not a demographic dead-end and may be key contributors to the overall gene pool. There is a brackish lagoon (Lough Furnace) at the freshwater-marine interface in this catchment that may support the maintenance of these evolutionarily important alternative migratory tactics. My results emphasise how autumn outmigrating trout, and the transitional habitats that support their existence, should not be overlooked in the context of evolutionarily enlightened fisheries management.

Collectively, the body of work presented in this thesis has shed new light on the molecular mechanisms and ecological/evolutionary processes involved in facultative animal migration and the maintenance of intraspecific phenotypic diversity in a culturally and economically important fish species. It is increasingly apparent that a "one size fits all" approach to management and conservation is suboptimal for species exhibiting complex life history patterns where a single population may express multiple life histories concurrently including for example as observed here partial migration and run time variation. Future genomics, transcriptomics and epigenomic work, coupled with detailed behavioural and physiological investigations, will aid progress towards a more holistic understanding of the evolutionary ecology of migration.

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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.

Additional Research

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

Wynne, R., & Wilding, C. (2018). Mitochondrial DNA haplotype diversity and origin of captive sand tiger sharks (*Carcharias taurus*). Journal of Zoo and Aquarium Research, 6(3), 74–78. https://doi.org/10.19227/jzar.v6i3.348

Colgan, T. J., Moran, P. A., Archer, L. C., Wynne, R., Hutton, S. A., McGinnity, P., & Reed, T. E. (2021). Evolution and Expression of the Immune System of a Facultatively Anadromous Salmonid. Frontiers in Immunology. https://doi.org/10.3389/fimmu.2021.568729

Perry, W. B., Kaufmann, J., Solberg, M. F., Brodie, C., Coral Medina, A. M., Pillay, K., Egerton, A., Harvey, A., Phillips, K. P., Coughlan, J., Egan, F., Grealis, R., Hutton, S., Leseur, F., Ryan, S., Poole, R., Rogan, G., Ryder, E., Schaal, P., Wynne, R., … Glover, K. A. (2021). Domestication-induced reduction in eye size revealed in multiple common garden experiments: The case of Atlantic salmon (*Salmo salar* L.). Evolutionary Applications, 14, 2319– 2332. https://doi.org/10.1111/eva.13297

Chapter One: General Introduction

Discrete phenotypic variation in nature

Phenotypic variation and associated life history diversity have been for many decades focal points of interest for both evolutionary biology and ecology. In the advent of recent technological advances in genomics and bioinformatics we are now able to gain more insight into the molecular mechanisms that drive phenotypic variation (Alvarez *et al.*, 2015; Todd *et al.*, 2016; Merlin and Liedvogel, 2019). Understanding the genetic underpinnings of phenotypic variation, as well as understanding how the local ecology selects for or against certain traits, is a key goal of contemporary evolutionary ecology (Hendry, 2016). Gaining further insight into the influences driving ecological and genetic factors and understanding how they interact may assist in understanding how phenotypic diversity arises and is maintained or eroded over time within a population.

Increased environmental instability associated with pervasive global change has made the need for further research into this field a top priority. Phenotypic variation in itself may be advantageous within a population, particularly when a population experiences periods of habitat instability or even rapid environmental change (Schindler *et al.*, 2010; Carlson and Satterthwaite, 2011). In natural populations, the occurrence of alternate phenotypes adds more complexity to the task of predicting trends and outcomes following environmental change (Stockwell *et al.*, 2003; Hendry, 2016). Ultimately, the ever-developing technologies at our disposal provide opportunities for the improved understanding of the biotic and abiotic mechanisms that drive phenotypic variations of individuals (Smith *et al.*, 2020). Gaining a further understanding of how genes and the environment interact to influence how individuals or populations respond to climate and habitat changes is becoming increasingly important when developing conservation strategies for specific species.

Phenotypic variation can be either continuous, where trait values vary along a quantitative continuum (e.g., human height), or discontinuous, where individuals fall into two or more discrete classes (e.g., winged versus wingless insect morphs). The latter type of variation is common in nature (Roff, 1996; Chapman *et al.*, 2012; Chevin

and Lande, 2013) and, like continuous variation, can arise as a consequence of genetic or environmental determination, or some interaction between the two (Roff, 1992, 1996; Tomkins and Hazel, 2007; Chevin and Lande, 2013). Discontinuous phenotypic variation can occur for morphological traits, such as horned versus hornless male morphs in beetles (Moczek, 2010) or seasonal polyphenisms in butterflies (Kingsolver and Wiernasz, 1991), but can also be apparent at the level of life history tactics, where two or more tactics are distinctly expressed within a species, or even within a population. This, in turn, can be associated with bimodal, or even multimodal, distributions of associated behavioural, physiological or morphological traits (Taborsky and Brockmann, 2010; Chevin and Lande, 2013).

Alternate migratory phenotypes

Migration is a common yet complex adaptation that has evolved across most animal taxa (Lennox *et al.*, 2016). Comparative studies suggest that migratory behaviour has rapidly and independently evolved in different lineages (Pulido, 2007). Whilst the ultimate drivers of animal migration have long been debated, contemporary literature across taxa continuously points to a series of primary drivers through which migratory life histories evolve (Roff, 1992; Lennox *et al.*, 2016; Gnanadesikan *et al.*, 2017; Shaw, 2020). These include varied seasonal conditions, ontogenetic separation of optimal feeding and/or breeding environments, mate availability, interspecific competition as well as intraspecific competition (Dingle and Drake, 2007). At the most basic level, a species expressing alternate migratory tactics can be split into two distinct groupings, migrants versus residents. It must be noted, however, that in some taxa, populations of the same species can harbour multiple different migratory phenotypes (Merlin and Liedvogel, 2019).

The details of migratory life history tactics become more nuanced when trying to understand why more than two migratory phenotypes are present. One possibility is that the migratory tendency is completely determined by genetics, therefore there is a migratory genetic type and a resident genetic type. In reality, this would be a gross and inaccurate simplification. In many systems, the decision to become a migrant or a resident is facultative, meaning the phenotype produced by a given genotype is

influenced by the environmental conditions. In some species, the level of environmental influence can make an individual more or less likely to adopt a migratory life history. Therefore, in reality, both genes and environment are important in determining migratory life history.

Alternate migratory phenotypes can differ with respect to the timing of the migration itself, the orientation of the migration, or even the distance travelled over the course of the migration (Shaw, 2020). However, no matter an individual's migratory trajectory, all migrations are characterised by a seasonal movement which allows an individual to escape suboptimal environmental conditions, substituting them for more favourable feeding and/or mating sites during the adverse seasons (Roff, 1992; Chapman *et al.*, 2011; Merlin and Liedvogel, 2019). Each migratory phenotype within a species has evolved following precise timings and orientations, and for a successful migration to occur, migrants will often have to be equipped with a collection of co-adapted sensory, morphological, physiological and behavioural traits (Newson *et al.*, 2009). These traits may well be genetically encoded in the organism, however, seasonal cues could trigger and turn on a subset of specific genes to begin the migratory journey, or even trigger a physiological metamorphosis to facilitate such a movement (Shaw and Couzin, 2013; Shaw, 2020). Given these environmental influences, as well as the fact that they must cope with transitions between very different habitats, it has been suggested that migratory species may be more vulnerable and susceptible to the effects of environmental change (Wilcove and Wikelski, 2008; Gnanadesikan *et al.*, 2017). This could be due to climate shifts, habitat change or destruction, and anthropogenic barriers to migration, which could directly affect migratory pathways (Wilcove and Wikelski, 2008). However, the converse has also been suggested, where migratory populations might show higher resilience and adaptability than non-migratory populations in the face of environmental change (Robison *et al.*, 2009).

Migration, particularly in species that exhibit facultative migratory life histories, is thought to be influenced by a combination of genes and environmental influences (Dingle, 1991; Thériault *et al.*, 2007; Liedvogel *et al.*, 2011; Chapman *et al.*, 2012). Facultative migratory life history has been described as a quantitative trait (Pulido, 2011, Ferguson et al., 2019), i.e., a complex phenotype controlled by multiple genetic

and environmental factors. However, the extent to which either genes or environments influence the migratory decision remains poorly understood (Phillis *et al.*, 2016; Nevoux *et al.*, 2019a; Rodger *et al.*, 2021). Nevertheless, evidence has been found, in the form of strong associations between migratory phenotypes and variants at particular genes, or groups of genes, which supports the involvement of genetics in the determination of migratory life history (Dodson *et al.*, 2013; Kendall *et al.*, 2015; Ferguson *et al.*, 2019).

Diversity of migration strategies in salmonids

Facultative migration has been observed across many taxa including; ungulates (Peters *et al.*, 2019), birds (Boyle, 2008; Jahn *et al.*, 2010; Sanz-Aguilar *et al.*, 2012), and insects (Attisano *et al.*, 2013; Odermatt *et al.*, 2017; Menz *et al.*, 2019). However, fishes make one of the most popular study models for investigating partial migration (Chapman *et al.*, 2012; Dodson *et al.*, 2013). Many fish species, with particular attention being paid to salmonids, have been used to examine and dissect variation in migration tendencies (Doctor *et al.*, 2014; Baerwald *et al.*, 2016; Lobón-Cerviá, 2017), both at the individual level and the population level (Chapman *et al.*, 2012; Ferguson *et al.*, 2017, 2019; Archer *et al.*, 2021).

Salmonids exhibit remarkable phenotypic diversity, with many species capable of displaying multiple migratory life histories (Jonsson and Jonsson, 1993; Birnie-Gauvin *et al.*, 2019). Furthermore, partial migration, when one fraction of the population is migratory and the other sedentary or resident, has been regularly observed in species of salmonids, such as brown trout and rainbow trout (Wysujack *et al.*, 2009; Ayer *et al.*, 2018; Nevoux *et al.*, 2019a). Such intra-population variation in migratory life history is in turn associated with variation in a suite of physiological, morphological, and behavioural traits (Chapman *et al.*, 2011). Variation among individuals in migratory life histories could entirely reflect genetic differences, entirely reflect environmental differences (phenotypic plasticity within a single genotype), or more likely a combination of, or interaction between, genetic and environmental influences.

This can be broadened still when looking at the habitats and environments between which fish migrate (Chapman *et al.*, 2012; Bond *et al.*, 2015; Birnie-Gauvin and Aarestrup, 2019). Salmonids have a particularly interesting evolutionary history, such that the taxonomic group boasts a wide variety of migratory life histories. Salmonids can exhibit short or large-scale anadromous migrations (fish that are born in fresh water, migrate to saltwater for growth, and return to fresh water to spawn), potamodromous migrations (regular migrations within large freshwater systems, including to and from lakes), or resident life histories, meaning they remain within their natal stream (Dodson *et al.*, 2013; Ferguson *et al.*, 2017, 2019; Birnie-Gauvin and Aarestrup, 2019).

Two species in particular – brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) – have been a focal point of recent studies on ultimate and proximate drivers of facultative migration (Jonsson *et al.*, 2001; Quinn and Myers, 2004; Chapman *et al.*, 2012; Birnie-Gauvin and Aarestrup, 2019; Nevoux *et al.*, 2019b). Both species exhibit considerable complexity in migratory life-histories, although “steelhead trout” (the anadromous form of *O. mykiss*) tend to exhibit much larger scale oceanic migrations compared to “sea trout” (the anadromous form of *S. trutta*), which tend to stay closer to coastlines, though Prodöhl *et al.*, (unpublished) report extensive migration in Irish waters of greater than 300Km. The molecular basis of (facultative) migration has been better studied in *O. mykiss* (e.g., Hecht *et al.*, 2012, 2013; Pearse *et al., 2014b,* Hale *et al.* 2018), although a few studies have also been conducted in *S. trutta* (Lemopoulos *et al.*, 2018; Lemopoulos *et al.*, 2019) and the underlying genetic architecture may indeed be different across the two species. Notwithstanding this growing interest, the finer details of how genes and environment interactively determine migratory life history via changes in gene expression remain poorly understood in salmonids generally.

Genetics of salmonid migration

The extent to which salmonid life histories and migratory traits vary due to environmental versus genetic effects is still contested (Beckman *et al.*, 2003; Dodson *et al.*, 2013; Ferguson *et al.*, 2017, 2019; Nevoux *et al.*, 2019b). There is no doubt that

phenotypic plasticity in migration decisions occurs in response to environmental cues/constraints such as food availability and temperature (Olsson *et al.*, 2006; Jones *et al.*, 2015; Archer *et al.*, 2019; Archer *et al.*, 2020). However, such environmental influences do not rule out an additional role of genetic influences. Although it is possible for offspring to express a different migratory phenotype than that of their parents, many studies have shown that facultatively anadromous species such as brown trout exhibit a tendency to adopt their parental life history (Jonsson, 1985; Dębowski and Dobosz, 2017), implying a heritable basis to migration decisions on top of environmental effects. Moreover, different genotypes may respond differently to a given environment and hence exhibit differences in reactions norms across environments, i.e., genotype by environment (G x E) interactions. On top of direct genetic effects, maternal effects (Vøllestad and Lillehammer, 2000; Thorn and Morbey, 2018) and possibly even epigenetic inheritance (Wellband *et al.,* 2021) might additionally shape migratory phenotypes within the population.

Life history decisions in salmonids can be sequential in nature, entailing a series of dichotomous options (e.g. “migrate or not?”; “migrate to a lake or the sea?”, “migrate now or wait until the following year?”), each likely determined by a threshold control mechanism (Roff, 1996; Ferguson *et al.*, 2017, 2019). This threshold model is often interchangeably referred to in the literature as the environmental threshold model, or the genetic threshold model, sometimes changing based on the nature of the study (Pulido, 2011), but in reality both environmental and genetic aspects are involved. The threshold model states that the decision to adopt one or other life-history tactic is made by accounting for two components: a “liability” (typically modelled as a normally distributed trait relating to an individual’s condition or energetic status, itself controlled by a combination of environmental factors and genes), and a genetically-determined threshold (Caballero *et al.*, 2013; Phillis *et al.*, 2016; Ferguson *et al.*, 2019). In facultatively anadromous salmonid species, such as brown trout, if an individual’s energetic status or physiological condition exceeds the threshold value during a sensitivity period in early development, this individual will adopt a resident life history and sexually mature in the freshwater environment. Conversely, if an individual’s energetic status/physiological condition is lower than the threshold during the

sensitivity window, the individual will have to migrate to higher productivity environments (e.g. a lake, or the sea) to meet their energetic demand (Ferguson *et al.*, 2019; Archer, *et al.*, 2020; Archer *et al.*, 2021). Therefore, identical genotypes can produce different migratory traits based on variable environmental conditions (life history plasticity), whilst on the other hand, identical environmental conditions can also produce different phenotypic outcomes due to genetic variability between individuals in the threshold and/or the liability (Tomkins and Hazel, 2007). Similarly, the frequency of migration versus residency can vary across populations owing to genetic differences between populations or phenotypic plasticity in response to different environments.

The challenge for the researcher is to figure out how much of the phenotypic variation in migratory tactics or associated life-history traits is attributable to genes, environment, and their interaction. In particular, it can be challenging to control for environmental effects (Vøllestad and Lillehammer, 2000), which in aquatic species can have a considerable influence on life history (Bailey *et al.*, 2018; Archer *et al.*, 2019; Shry *et al.*, 2019). Common garden experiments (CGEs) are the standard method of assessing the genetic basis for phenotypic divergence among populations of the same species (McGinnity *et al.*, 2004; Piché *et al.*, 2008; Pulido and Berthold, 2010; de Villemereuil *et al.*, 2016; Hale *et al.*, 2018). The idea is simple: by exposing individuals from different populations/genetic backgrounds to the same environment (a common garden), environmental influences on an individual's life history are equalised and hence any resulting phenotypic differences among the groups can be attributed to heritable differences. This information can then be used to further understanding of the evolutionary processes involved, or to help interpret any changes in population composition, e.g., the extent to which phenotypic responses to environmental change or anthropogenic pressures might result from rapid evolution versus phenotypic plasticity. Common garden experiments are thus a valuable tool for gaining a deeper understanding of the genetic mechanisms involved in salmonid migratory life history (McGinnity *et al.*, 2004; Doctor *et al.*, 2014; O'Toole *et al.*, 2015; Lemopoulos *et al.*, 2019) and animal migration in general (Dingle 1991).

In recent years, increasing focus has been placed on finding quantitative trait loci (QTL) regions and genes underpinning facultative migration in salmonids

(Lemopoulos *et al.*, 2018). However, few genomic regions have been found that consistently differentiate migrants from residents across multiple populations or species, i.e. molecular signatures of parallel/convergent selection. In *Oncorhynchus mykiss* (steelhead/rainbow trout), several major migration QTLs have been found that cluster on the fifth chromosome (Omy05 region) (Hecht *et al.*, 2013; Pearse *et al.*, 2014b; Leitwein *et al.*, 2016; Arostegui *et al.*, 2019). However, Hale *et al* (2013) found that the Omy05 region did not consistently associate with migration phenotype (resident rainbows versus anadromous steelheads) across all *O. mykiss* populations considered. Studies on the Omy05 region have promoted further investigations across different salmonid species to find a master locus for migratory life histories, however to date relatively little evidence has been found for such loci (Narum *et al.*, 2011; Nichols *et al.*, 2016; Lemopoulos *et al.*, 2019). This does not imply that they do not exist -- rather, it suggests that the molecular mechanisms behind the migratory decision may vary between species, and possibly even between populations of the same species. However, it could also be that relatively few of the causative genes that influence the migratory decision have been discovered thus far.

Neutral genetic markers are not found typically to differentiate sympatric migratory and non-migratory individuals in brown trout populations (Ferguson *et al.*, 2017; Ferguson and Prodöhl, 2022). This implies that, in situations where migrants and residents co-occur in the same river or waterbody, they are freely interbreeding and do not constitute separate gene pools. This does not, however, mean that migration decisions are not influenced by genetics – indeed, certain functional genomic regions might be strongly differentiated between migrants and residents. Rather, it simply means that sympatric migrants and residents do not exhibit genome-wide differences and hence are not reproductively isolated (Ferguson *et al.*, 2019). Neutral markers would only be expected to differentiate allopatric migrant and resident populations, particularly in the presence of complete or partial barriers to gene flow (Lemopoulos *et al.*, 2018); e.g. where the residents occur above an impassable waterfalls and the migrants below.

Gene expression: linking genotype to phenotype.

Phenotypic differences among individuals or populations can reflect genetic differences or phenotypic plasticity, or some mix of the two (or G x E). This can also be understood at the level of gene expression, which can itself be thought of as a 'molecular phenotype' linking genotype to higher-level phenotypes. That is, gene expression differences among individuals or populations (which are in turn associated with phenotypic differences, e.g., in migratory life histories) can reflect sequence-level variation in regulatory loci, environmental effects on gene expression, or some mix of the two. A number of genes, and possibly some major effect loci, are likely involved in the initial decision to adopt a migratory instead of a residency tactic (Perrier *et al.*, 2011; Hecht *et al.*, 2012, 2013, 2015; Ferguson *et al.*, 2019). Complex regulation mechanisms, which may involve one or more master regulator genes, as well as a series of epigenetic modifications, are likely to control developmental and phenotypic divergence between migratory and resident individuals in salmonids (Aubin-Horth *et al.*, 2009; Baerwald *et al.*, 2016). More generally, it has been suggested that regulatory mechanisms facilitate developmental plasticity in species exhibiting polyphenisms by decoupling developmental pathways in each morph, thus, giving rise to morph-specific gene expression (Snell-Rood *et al.*, 2010, 2011). This would imply that differential gene expression between two or more morphs could begin earlier in ontogeny than the observed physical differences between the morphs. For example, in *O. mykiss*, differential gene expression between migrant and resident offspring is evident in the brain a full year before smoltification (McKinney *et al.*, 2015). It is not until smoltification approaches that migrants undergo phenotypic “remodelling”, which in itself will instigate transcriptomic changes to further divide the migratory and resident phenotypes (Sutherland *et al.*, 2014). These gene expression changes will result in observable characteristics as anadromous individuals undergo smoltification, including changes in body shape and colour, behavioural changes, and hormonal plus enzyme alterations (McCormick *et al.*, 1998; McCormick, 2012; Kendall *et al.*, 2015).

RNA sequencing (“RNA-seq”) and other transcriptomic technologies have been used to explore differential gene expression between different migratory life histories in salmonid fish. Particular attention has been given to both brown trout (Giger *et al.*,

2008; Rodger *et al.*, 2021; Wynne *et al.*, 2021) and rainbow trout (Hale *et al.*, 2014, 2016, 2018) as both species exhibit facultative anadromy as well as flexible freshwater migration strategies (Baerwald *et al.*, 2016; Birnie-Gauvin *et al.*, 2019; Ferguson *et al.*, 2019; Nevoux *et al.*, 2019). Many of these studies aim to uncover loci that display differential gene expression between migratory morphs and the associated molecular mechanisms involved (Giger *et al.*, 2008; Hale *et al.*, 2013; Hecht *et al.*, 2013; Houde *et al.*, 2019; Wynne *et al.*, 2021). Gene expression differences are ultimately controlled by regulatory loci, with the initial decision to adopt migration versus residency potentially controlled by a single "master control switch". The aforementioned migration-associated region on chromosome Omy5 in *O. mykiss* may be a good candidate region within which such a master switch is harboured (Nichols *et al.*, 2008; Leitwein *et al.*, 2016; Arostegui *et al.*, 2019; Kelson *et al.*, 2019; Pearse *et al.*, 2019).

Whilst recent transcriptomic studies have yielded interesting results (Giger *et al., 2008* Wynne *et al.,* 2021), most have focussed on a relatively narrow window of time in the life-history, which may be "downstream", developmentally-speaking, from the initial migratory decision. Furthermore, the often-destructive nature of sampling (e.g. of internal organs) prevents the study of within-individual changes in gene expression over time. Gene expression differences can manifest far earlier than visible changes distinguishing the migratory morphs (McKinney *et al.*, 2015). Thus it remains a considerable challenge to identify the key master genes and molecular mechanisms that mediate the actual migration decision itself, as opposed to genes that are differentially expressed later in ontogeny between migrants and residents as a consequence of the initial switch. Moreover, different organ systems (e.g. the brain versus the liver) might exhibit different gene expression profiles in migrants and residents, with these differences being amplified or diminished at different developmental time points.

The role of sex in salmonid migratory tendencies

In salmonids, females stand to gain more, in fitness terms, from migration to higher productivity environments than males do, and thus a female sex bias is to be expected and indeed is typically observed amongst migrants (Hendry and Stearns, 2004).

Female reproductive success is limited strongly by body size, given that larger females tend to produce both more eggs and larger eggs (Fleming, 1996; Einum and Fleming, 1999; Nielsen *et al.*, 2003). Male reproductive success, on the other hand, is often limited more by mate availability than by male body size (Fleming and Reynolds, 2004; Ferguson *et al.*, 2017, 2019). Freshwater environments are typically less productive than marine environments, particular at higher latitudes (Gross et al. 1988). Thus a larger body size can be attained, over a given growing period, at sea than in the river, and hence migration is particularly favoured in females. These growth-mediated fitness benefits are tempered, however, by potentially higher mortality risk at sea as well as energetic costs of migration itself, so the balance of costs and benefits for each sex will vary across particular contexts. Moreover, freshwater lakes and brackish lagoons can also be productive environments with potentially lower predation risk compared to the sea, and thus adfluvial migration to higher energy lake environments rather than the sea may similarly be favoured, again particularly in females.

In recent years, the extent to which sex-specific inheritance and selection patterns play a role in the dynamics of a population's migratory tendencies has been a focal point of salmonid research. Technological advancements have facilitated closer examinations of the molecular mechanisms that differ between females and males when it comes to migratory life history (Hale *et al.*, 2018; Shaw, 2020; Wynne *et al.*, 2021). Females and males often differ in their fitness optima for shared traits that have a shared genetic basis, leading to sexual conflict (Pearse *et al.*, 2019). In many species this is resolved via the evolution of distinct sex chromosomes, thus protecting any sexually antagonistic variations. However, in salmonid species that lack obviously differentiated sex chromosomes, the mechanisms by which sexual conflict is resolved remain largely unknown (Barson *et al.*, 2015; Pearse *et al.*, 2019).

Recent studies have looked into intra-locus sexual conflict in species like rainbow trout and brown trout where there is a presumed shared genetic basis between males and females for migratory tendency (Nevoux *et al.*, 2019a; Pearse *et al.*, 2019; Willis *et al.*, 2020). In rainbow trout, previous studies have highlighted a double inversion, and its associated genetic markers on Omy05 (Hecht *et al.*, 2012; Pearse *et al.*, 2014a; Arostegui *et al.*, 2019), with many studies linking the region with migratory tendency

in the species (Nichols *et al.*, 2008; Hecht *et al.*, 2012; Houde *et al.*, 2019). Pearse *et al.*, (2019) showed that the double inversion has sex-specific effects on migratory life history, suggesting that a large autosomal supergene acts as a mechanism for sexual conflict resolution, mediated by gene expression differences between males and females (Sutherland *at el.,* 2019). Recently, a putative master sex-determining gene sdY (a sexually dimorphic genomic region) was discovered and found to be conserved across 15 different salmonid species (Yano *et al.*, 2012, 2013; Sutherland, *et al.*, 2019), which may play a role in the dynamics of sexual conflict.

The variety of life history in brown trout

Salmonids can show great diversity in migratory phenotypes and it can be argued that brown trout (*Salmo trutta*) possess the largest life history diversity within the salmonid family (Klemetsen, 2013). Throughout history, brown trout migratory life history has all too often been grossly oversimplified to fit into a binary system of anadromy versus freshwater residency. However, many recent studies and reviews have helped develop a deeper understanding of the complexity of brown trout migratory life history (Ferguson, 2017; Birnie-Gauvin *et al.*, 2019; Ferguson *et al.*, 2019; Nevoux *et al.*, 2019b). In brown trout, two forms of resident life history (lake-resident, river-resident), as well as three main forms of migratory life history (fluvial-adfluvial potamodromy, lacustrine-adfluvial or allacustrine potamodromy, and anadromy) have been observed (Ferguson *et al.*, 2019) (Fig.1). However, these migratory forms can be further subdivided dependent on the destination of migration and other life-history attributes such as the age and seasonal timing of movements, and the scope for repeated migration and iteroparous spawning (Ferguson *et al.*, 2017, 2019; Huusko *et al.*, 2018).

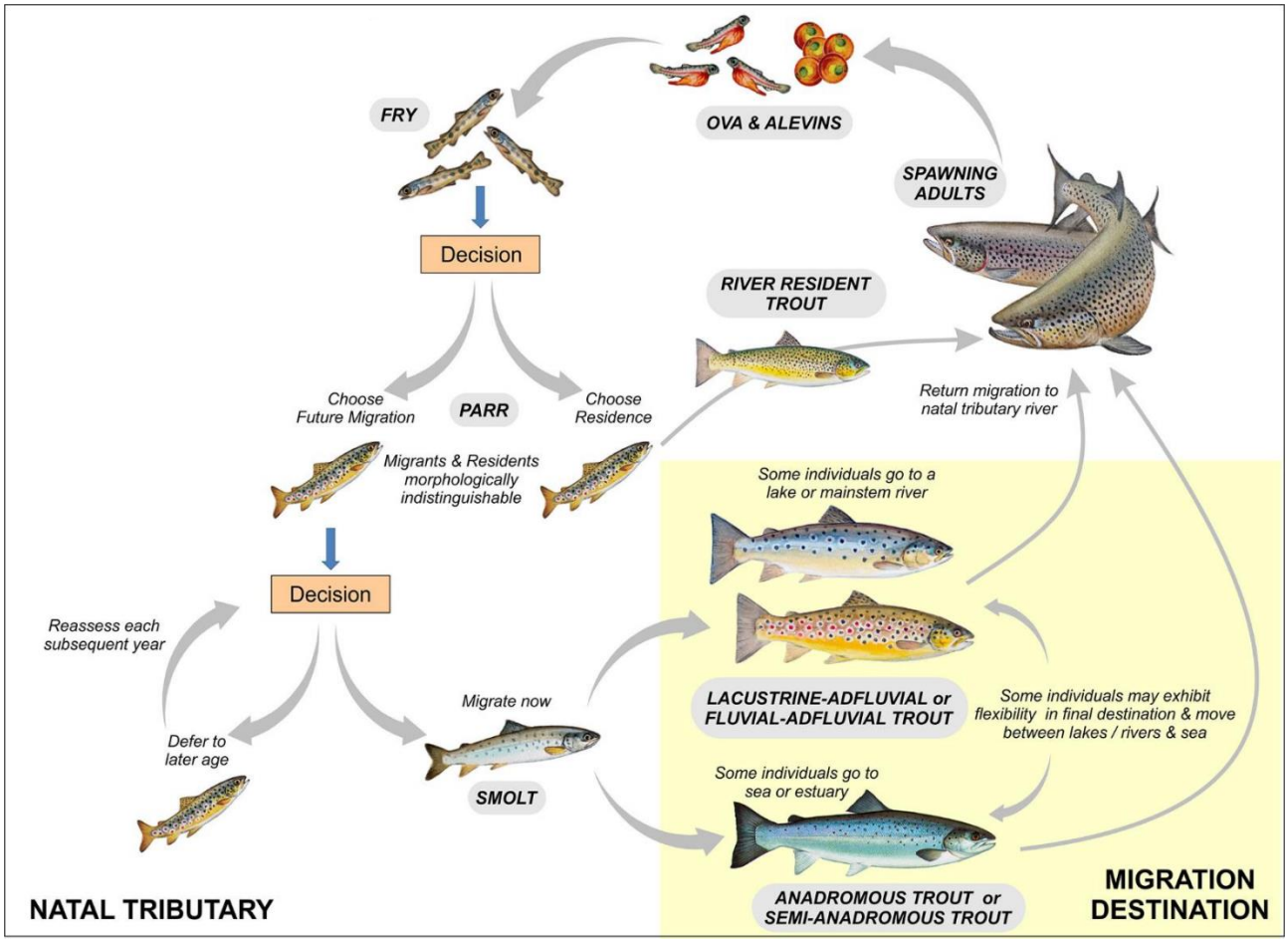


Figure 1: Life-cycle diversity of brown trout (*Salmo trutta*) showing all migratory options and variance from ova/alevins stage through to sexually mature adult stages (*modified from Ferguson et al., 2019*).

The term “freshwater resident” is often attributed to a fish that remains in freshwater its whole life. However, Vøllestad *et al.*, (2012) noted that not only do individuals make early localised movements within natal streams, but many may also disperse into neighbouring streams, some even moving into larger rivers or lakes to become potamodromous migrants. Similarly, the term “lake residents” has been frequently used to describe trout that are found in lakes, but these fish may have migrated from natal streams and rivers, and return to these streams on their spawning migrations, in which case they are more accurately called adfluvial potamodromous migrants (Ferguson *et al.,* 2019). Lake-spawning can also occur, however, in *Salmo trutta*, so some brown trout may truly live in lakes their whole lives, and the latter lake-resident

strategy may be more common than once thought (Näslund *et al.*, 1998; Saksgård and Hesthagen, 2004; Birnie-Gauvin *et al.*, 2019; Ferguson *et al.*, 2019). Therefore, “non-anadromy” is a catch-all term for fish that do not migrate to the sea, and may hide a diversity of stream/lake resident and freshwater migratory tactics. On top of migration to lakes, trout may migrate from small natal streams to larger rivers and back again i.e., *fluvial potamodromy* (Ferguson *et al.*, 2017, 2019). Moreover, seaward-migrating migrants can also show a range of tactics and behaviours: some may remain within brackish areas such as lagoons or estuaries (semi-anadromous “slob” trout, as they are known in Ireland and in parts of Britain), others may move into full seawater but remain close to the shore for the most part, whilst others again can exhibit much longer-range movements within the marine environment (Thorstad et al. 2016). The term “sea trout” is typically reserved for fish that spend some period of time in full seawater and return to freshwater silvered, but the lines blur here too as the fish become brownish again on their return spawning migration at different rates.

Ferguson *et al.,* (2017) noted that many authors will refer to the use of estuaries/lagoons as semi-anadromous or partial anadromous movements, which can add further ambiguity to discussions giving rise to the use of the term ‘partial migration’. Facultative migration is often a favoured term for brown trout over ‘partial migration’, as partial migration is a broader term simply referring to situations where some individuals in a population are migratory and others are non-migratory, without regard to whether this reflects a genetic polymorphism or phenotypic plasticity (or G x E). Furthermore, non-salmonid biologists might misinterpret the ‘partial’ to mean a measure of the distance travelled. Relatively few studies have focused on general aspects of both freshwater life histories and the nuances of anadromous life history in brown trout when compared to river residency and full anadromous movements, identifying a gap in the current literature. Within brown trout populations, multiple migratory life histories can occur concurrently, and within both migratory and non-migratory tactics there are often recorded sex biases (Piecuch et al., 2007; Ferguson et al., 2017, 2019; Nevoux et al., 2019a; Pearse et al., 2019), further adding to this complexity.

The concluding statements of the Ferguson *et al*. (2019) review highlighted the relatively low number of studies which try to gain deeper understanding of the genetic basis of migratory life history in brown trout, compared to other salmonid species. The review also notes that many studies that do explore the genetic basis of migration primarily focus on anadromy and do not provide a detailed account of other migratory life histories expressed by brown trout such as potamodromy. In recent years, it has become increasingly apparent that many species will be faced with potential habitat degradation in the face of a rapidly changing environment. With these environmental changes and anthropogenic threats to brown trout populations, the need to understand the molecular determinants of the full swathe of brown trout migratory life histories has become increasingly urgent, as this information is crucial to predicting evolutionary responses and the scope for rapid adaptation.

Thesis Overview

The overarching objective of this thesis was to explore the underlying genetic mechanisms by which alternative life history tactics in facultatively migratory brown trout are determined.

In Chapter Two, I specifically aimed to test for heritable differences in migratory life histories among neighbouring brown trout populations, between which gene flow is restricted. Furthermore, I also explored the phenotypic correlates of early movement behaviours by testing for differences in the length, weight, condition factor and sex ratio between these populations. Finally, the study reported in this chapter I sought to explore patterns in the timing of movements between progeny of experimental crosses and those progeny that were the offspring of local fish spawning naturally in the experimental river.

In Chapter Three, I performed transcriptional profiling of the brain and liver of male and female brown trout to understand the genes and processes that differentiate migratory and residency morphs and to investigate how these morphs may differ in gene expression between the sexes. I then examined whether putative genes associated with migratory or residency morphs demonstrated conserved or tissue-

specific expression profiles. Lastly, I investigated whether genes differentially expressed between migrants and residents were also differentially expressed between males and females, given their divergent phenotypic trajectories with respect to migration.

The objective of Chapter Four was to investigate the timing of migration, specifically the phenomenon of autumn migrating parr versus spring-migrating smolts in brown trout within a single population. This chapter aimed to further characterise these bimodal migration timing patterns in terms of age, size, and sex of juvenile outmigrants using genetic identity analysis together with information from passive integrated transponder tags to test for differences between spring outmigrants and autumn outmigrants with reference to their return rates, size and colour at return, time spent at sea or away from the catchment and specific growth rates with a view to understanding proximate and ultimate factors influencing these alternative migratory morphs.

Chapter Five synthesises the results of the studies described above and discusses their contribution towards developing an integrated understanding of the links between genetics, life history and ecology of brown trout.

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Chapter Two: A common garden experiment reveals heritable differences in migratory tendencies among geographically proximate brown trout populations

Abstract

Common garden experiments (CGEs) represent a classic approach to testing whether phenotypic divergence among populations of the same species has a genetic basis. This chapter reports on the results of a CGE undertaken in the Burrishoole catchment (Northwest Ireland) that aimed to test for heritable differences in migratory life histories among neighbouring brown trout populations, between which gene flow is restricted. Broodstock were obtained in winter 2016 from three locations: (1) below an impassable waterfall in the Rough River (Rough below-falls population) – a tributary in the Burrishoole system known to produce a mix of migratory (potamodromous and/or anadromous) and river-resident trout; (2) above a series of waterfalls in the Rough River (Rough Above population – presumed to be mostly river-resident); and (3) the Erriff system, a neighbouring fluvial dominated catchment known historically to produce large numbers of anadromous migrants. The progeny (unfed fry) were then introduced to an experimental section of the Rough River in spring 2017, bounded upstream by the impassable waterfalls and downstream by a trap that captures all downstream-moving individuals (Rough River Downstream trap; RRDT). Genetic parentage techniques were then used to assign juveniles sampled at different

stages/places back to experimental crossess. Based on the electrofishing data, no survival differences among offspring were observed with respect to maternal or paternal provenance. Offspring from Rough Above fathers were underrepresented amongst RRDT emigrants (‘movers’), whilst offspring with Erriff fathers were overrepresented, consistent with a genetic basis to early-life movement behaviours. RRDT juveniles were consistently larger but in poorer condition than juveniles sampled via electrofishing and were also more likely to be female. Crucially, the composition of age 2+ and 3+ smolts (n=23 in total) sampled at the sea-entry traps differed among experimental crossess, with an excess of progeny from Erriff mothers (83%) and a deficit from Rough Below mothers (17%) relative to the introduced fry percentages (54% and 46%, respectively). Whilst paternal provenance did not have a significant effect on smolt composition, the patterns mirrored those observed with the RRDT data, with 52% assigning to Erriff fathers (expected: 34%), 17% to Rough Above fathers (expected: 33%) and 30% to Rough Below fathers (expected: 33%).

1. Introduction

Migratory patterns vary greatly not only across species, but also among populations of the same species and even among individuals within the same population. This phenotypic diversity encompasses a range of physiological, behavioural and life-history traits that directly enable, or are indirectly associated with, migratory activity, collectively known as a 'migration syndrome' (Dingle and Drake, 2007). Understanding the extent to which intraspecific variation in migration syndromes is determined by genetic versus environmental factors remains an ongoing challenge in evolutionary ecology (Liedvogel *et al.*, 2011; Pulido, 2011; Charmantier and Gienapp, 2014). Demonstrating a heritable basis to migration propensity is a crucial first step in any evolutionary analysis that seeks to understand why migration rates are higher in some populations or individuals than others. Subsequent investigation might then seek to understand the relative roles of adaptive versus neutral processes, as well as the nature of the underlying genetic architecture (Merlin and Liedvogel, 2019; Shaw, 2020).

On top of these fundamental questions, understanding the genetics of migration has implications for conservation biology and wildlife/fisheries management. For example, if migratory traits are heritable, this can allow for rapid evolution in response to environmental or anthropogenic change (e.g., Pulido and Berthold, 2010; Phillis *et al.*, 2016), increasing the resilience of populations or population complexes (Stockwell *et al.*, 2003). Migratory populations might be particularly vulnerable to climate change, for example, given that migrants must cope with contrasting and highly variable abiotic and biotic conditions in different areas/habitats between which they move (Dingle and Drake, 2007; Orgeret *et al.*, 2022). In the specific context of fisheries, populations can undergo rapid changes in the frequency of migratory versus resident individuals (e.g., Poole, Dillane, Deeyto, *et al.*, 2007) , or in the timing of juvenile (e.g.,Otero *et al.*, 2014; Quinn *et al.*, 2015) or adult migration (e.g.,Quinn and Adams, 1996; Quinn *et al.*, 2006; Kovach *et al.*, 2013) and knowledge regarding the relative roles of rapid evolution versus phenotypic plasticity can inform adaptive management.

Common garden experiments (CGEs) represent a classical approach, with a long history in evolutionary biology, for testing whether phenotypic divergence among populations of the same species has a genetic basis (de Villemereuil *et al.*, 2016). The idea is simple: heritable variation can be revealed by growing individuals from different populations in a common environment, where they all experience similar conditions. If phenotypic differences persist among individuals from different population backgrounds, this points towards a genetic basis to population divergence, because environmental variation (phenotypic plasticity) is controlled for. Ideally the progeny of F1 or even F2 crosses between parental strains are introduced into the common garden, in order to minimise the influence of maternal effects or epigenetic inheritance (Mousseau and Fox, 1998) and limit the focus to direct genetic effects, but this is not always logistically feasible. Fishes are particularly well-suited to common garden experiments because broodstock (mature/maturing individuals used as parents in the baseline generation) can be relatively easily collected from wild environments, kept in captivity for some holding period and then stripped of their gametes to create experimental progeny under some desired crossing design. The progeny can then be reared from the egg stage in an artificial hatchery through until the point at which they are ready to be introduced into a 'common garden', which could involve a controlled laboratory environment (e.g., Archer *et al.*, 2019, 2020), semi-natural streams (e.g., Robertsen *et al.*, 2019; Solberg *et al.*, 2020), or fully natural wild aquatic environments (McGinnity *et al.*, 2004; Doctor *et al.*, 2014; O'Toole *et al.*, 2015).

Salmonid fishes (species belonging to the family Salmonidae) can express a wide diversity of life history and migratory tactics (Forseth *et al.*, 1999; Klemetsen *et al.*, 2003; Quinn and Myers, 2004; Dodson *et al.*, 2013; Ferguson *et al.*, 2017, 2019; Hutchings *et al.*, 2019). Typically, salmonids spawn in freshwater and juveniles rear in their natal streams/rivers for varying lengths of time, depending on the species or population. Various forms of migration occur, including anadromy (migration to saltwater for a period of growth followed by return migration to freshwater for spawning) and potamodromy (migrations within freshwater, e.g., from small streams to larger rivers, or from streams/rivers to lakes), with spatial and temporal aspects of migration varying hugely across and even within species (Klemetsen *et al.*, 2003;

Quinn, 2018; Ferguson *et al.*, 2019; Nevoux *et al.*, 2019a). Many salmonid populations also exhibit facultative migration, also sometimes called partial migration (Chapman, Hulthén, *et al.*, 2012; Chapman, Skov, *et al.*, 2012), wherein individuals are capable of adopting either a resident tactic (staying in or close to their natal stream throughout life) or a migratory tactic (moving downstream to a larger river, lake or the sea), with the ratio of residents to migrants varying both among populations (across space) and within populations (e.g., across years, or between males and females in any given year, or between different types of individuals of a given sex). Although often described in simple binary terms, in reality facultative/partial migration in salmonids can involve considerable complexity, with many life-history trajectories being possible (reviewed by Birnie-Gauvin *et al.*, 2019).This phenotypic diversity reflects both genetic differences, which have been shaped by past natural selection (ultimate factors) as well as neutral processes, and direct environmental effects on individuals (proximate factors), i.e., phenotypic plasticity, where the same genotype produces different phenotypes in response to environmental cues/constraints (Dodson *et al.*, 2013; Ferguson *et al.*, 2017, 2019; Birnie-Gauvin *et al.*, 2019).

In recent years, particular attention has been devoted to understanding the genetic basis of intraspecific divergence in migratory life histories in salmonids, both between and within populations (Hecht *et al.*, 2012; Hale *et al.*, 2016; Kelson *et al.*, 2019). Facultative migration has been considered as a classical threshold quantitative genetic trait, where qualitative (discontinuous) variation at the phenotypic level is controlled by multiple genes and environmental factors (Roff, 1992, 1996; Fleming and Reynolds, 2004; Ferguson *et al.*, 2019) The environmentally-cued threshold model (Tomkins and Hazel, 2007) has been particularly useful to conceptualise how alternative migratory tactics can emerge from an interplay between a genetically variable threshold and one or more environmentally-sensitive status traits (Buoro *et al.*, 2012; Lepais *et al.*, 2017; Ferguson *et al.*, 2019). Under this model, heritable but flexible migratory decisions are influenced by two components (Tomkins and Hazel, 2007; Pulido, 2011): a liability trait (or cue) such as some aspect of energetic status or physiological condition (which is environmentally sensitive but also potentially affected by genetic variation) and a genetically determined threshold. During a sensitive period in ontogeny (the “decision

window"), individuals whose status trait exceeds their inherited threshold value adopt a resident tactic, remaining within their natal river or stream where they will subsequently mature, whilst those whose status trait does not exceed their threshold adopt a migratory tactic (Ferguson *et al.*, 2019). This initial migration decision thus represents a key early "fork in the road", with subsequent decisions taken later in the life-history then determining the age at which migration is actually undertaken, the migration destination, the timing of return migration, etc (Birnie-Gauvin and Aarestrup, 2019; Ferguson *et al.*, 2019; Nevoux *et al.*, 2019b). Thus, identical genotypes can express different migratory tactics due to life history plasticity, which arises in the presence of environmental variability that alters the physical condition of an individual. Conversely, if the genetic threshold differs among populations, or among individuals within the same population, variation in migration tactics can result even if all individuals experience the same environmental conditions (Piché *et al.*, 2008).

The brown trout (*Salmo trutta*) is amongst the most phenotypically diverse salmonid species (Klemetsen, 2013; Ferguson *et al.*, 2019; Ferguson and Prodöhl, 2022) in which a broad range of migratory strategies are found including (facultative) river/lake residency, fluvial/adfluvial potamodromy, and anadromy (Ferguson *et al.*, 2017, 2019; Lobón-Cerviá, 2017; Birnie-Gauvin *et al.*, 2019; Nevoux *et al.*, 2019b). It has been suggested that brown trout may harbour different threshold values with regards to migratory life history (Jonsson and Jonsson, 2014), as marked differences in migratory life-histories are often evident, even over relatively short spatial scales (Vøllestad and Lillehammer, 2000; Birnie-Gauvin *et al.*, 2019; Lemopoulos *et al.*, 2019). While various lines of evidence suggest that genetic differences in part underpin this divergence in migration propensities (reviewed by Ferguson et al. 2017; 2019), there remains a paucity of direct evidence for heritable variation in migration-related traits in brown trout and other salmonids, e.g., via common garden experiments. As well as complex migratory life histories, brown trout can show particularly complex population structure, often even showing genetic differentiation within a single river catchment (Cross *et al.*, 1992; Nielsen *et al.*, 2003; Poole, *et al.*, 2007; Stelkens *et al.*, 2012; Gargan *et al.*, 2016; Magee, 2017). Adaptive genetic differentiation with respect to a range of morphological and life-history traits can also occur (Stelkens *et al.*, 2012), but

knowledge gaps remain regarding the extent and scale of local adaptation in salmonid fishes in general (Fraser *et al.*, 2011).

The primary aim of this chapter was to test the hypothesis that migration propensity differs among brown trout from different genetic backgrounds in a wild CGE, and the secondary aim was to test for survival differences among cross-types consistent with local adaptation. Broodstock for the CGE were collected in winter 2016 from three locations. The first location was below an impassable waterfall in the Rough River (Rough below-falls population, RB). The Rough River is a key tributary in the Burrishoole system known to produce a mix of migratory (potamodromous and/or anadromous) and river-resident trout. However, the second location, above the waterfall in the Rough River (Rough Above population, RA) is thought to harbour a mostly river-resident population. The third broodstock collection location was the Erriff system (Erriff population, ER), a neighbouring catchment known to produce sea trout. Six genetic cross-types were produced from these broodstock. The F1 progeny were then introduced as unfed fry to an experimental section of the Rough River in spring 2017, bounded upstream by the impassable waterfalls and downstream by a trap that captures all downstream-moving individuals (Rough River Downstream trap; RRDT).

The general prediction was that offspring with two Erriff parents should be more likely to go to sea (as silvered smolts) than offspring with two Burrishoole (Rough Below or Rough Above) parents, assuming a stronger inherited tendency towards anadromy in the Erriff than the Burrishoole system, while hybrid offspring (one ER parent, the other RB or RA) should exhibit intermediate anadromy rates, assuming additive gene action. Furthermore, offspring sired by RA males should exhibit lower migration propensity than offspring sired by RB or ER males, if above-falls populations have evolved towards stream residency (given that migration should be selected against in above-falls populations, (Phillis *et al.*, 2016). Finally, fish with one or two ER parents might be less likely to survive to the smolt stage than fish with two Burrishoole (RB or RA) parents, under a local adaptation scenario where “locals” outperform “foreigners” in the home environment of the locals. Any such differential mortality among provenances must then be taken into account when interpreting observed numbers of migrants/smolts among cross types.

These overarching aims and hypotheses in turn involved three specific objectives:

(1) To test for differential movement of experimental progeny out of the Rough River at early life stages (age 0+, 1+ and 2+) with respect to maternal and paternal provenance (while accounting for potential differential in-river survival), which would indicate heritable differences in early downstream movement behaviours.

(2) To explore phenotypic correlates of early movement behaviours by testing for differences in the length, weight, condition factor and sex ratio of juveniles caught in the RRDT movers (defined as trout that are actively moving out of the experimental river) versus via electrofishing stayers (defined as trout that are residing within the experimental river at the point of capture, these fish may choose to migrate at a later point), while accounting for changes in each variable over time and potential differences with respect to maternal or paternal provenance.

(3) To test whether progeny from certain experimental cross-types were over- or under-represented at the smolt stage, using trap captures and automated PIT-detections at the sea-entry traps, which would indicate heritable differences in anadromy rates.

Materials and Methods

2.1 Background

In November 2016 wild brown trout broodstock were captured via electrofishing and seine-netting from both the Burrishoole (53° 57′ N: 09° 35′ W) and the Erriff (53° 37′ 0.00″ N: 09° 40′ 17.10″ W) catchments, Northwest Ireland (Fig. 1b).

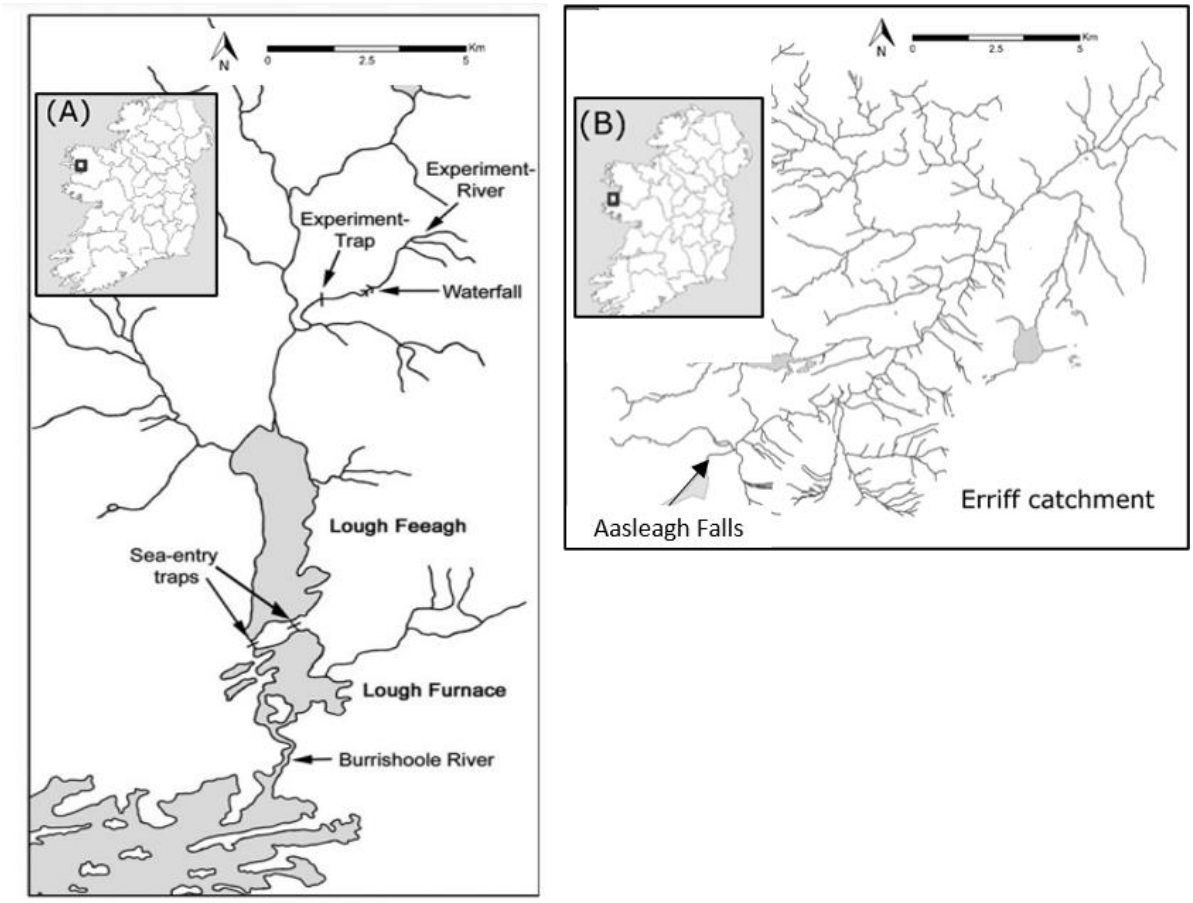


Figure 1: Locations in the west of Ireland from which *Salmo trutta* broodstock were collected in the winter of 2016 to generate the experimental crosses.

Historically, the Burrishoole catchment was known to have a large run of sea trout, with typically 2000+ anadromous migrants recorded moving downstream out of the system (through the sea-entry traps) annually. However, in the late 1980s/early 1990s the anadromous trout run of the Burrishoole collapsed, coincident with increasing Atlantic salmon aquaculture in coastal regions utilised/transited through by Burrishoole sea trout and associated increases in sea-lice parasitism (Poole *et al.*, 2007). The Rough River is believed to have been a major contributor to past sea trout runs in the Burrishoole system, but other tributaries likely also contributed (J. Magee, unpublished PhD thesis).

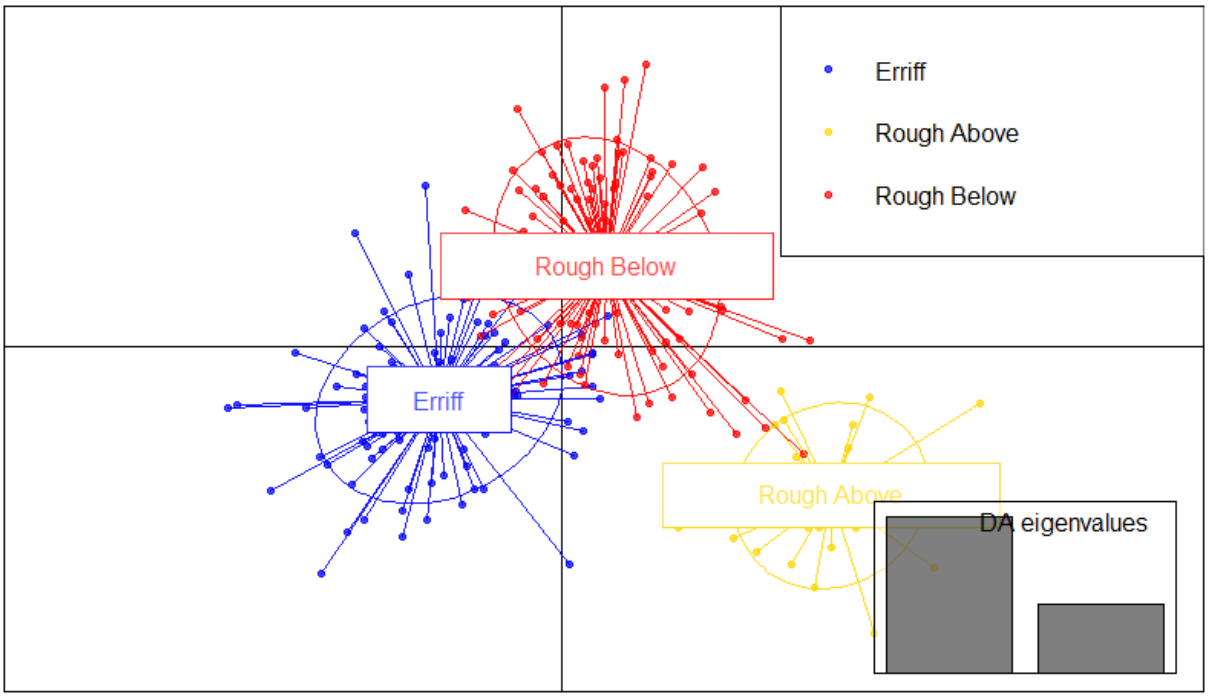


Figure 2: Distinct genetic profiles between Erriff (blue; n = 98), Rough Above falls (yellow; n = 26) and Rough Below falls (red; n = 114) broodstock based on 16 microsatellite loci. Scatterplots displaying the results of a discriminate analysis of principle components (DAPC) for the 238 individuals which comprised the brood stock of the experiment.

Broodstock from the Burrishoole catchment were caught via electrofishing in the experimental river, hereby the Rough River and as they moved in (downstream trap) or out (upstream trap) of the Rough River. The Rough River (also known as the Srahrevagh River) has a series of waterfalls towards the most easternly point of the river that are impassable to upstream migrating trout. Trout can conceivably pass downstream over the falls, and many such fish might survive the ordeal and possibly reproduce below the falls; however, returning migrants cannot get back up above the falls, so any gene flow is one-directional (from above to below). Thus, the waterfalls have over time acted as a geological barrier that has resulted in the above-falls and Rough below-falls populations having distinct genetic profiles (Figure 2). The waterfalls resulted from isostatic lift some 5,000 years before present following the retreat of the ice sheets at the end of the last glacial period; colonising trout, as a result, having been isolated there since that time (P. McGinnity *pers. comm*.).

Migration is likely strongly selected against in the above-falls population, by virtue of the fact that any migratory individuals that move downstream over the falls would not be able to get back up above them for spawning. Thus, the above-falls population is here assumed to have a much stronger tendency towards residency than the other two populations.

Unfortunately, it was not possible to include a pure Rough Above group in the experiment, as the fecundity of Rough Above females is so low (given their diminutive size), no more than 40 or 50 eggs per female, that an unfeasibly large number of dams would have been required to yield sufficient surviving progeny.

The Rough below-falls population in the Rough River contains multiple migratory life histories, with a mix of river residents and migratory individuals. The timing of trout movement out of the Rough River shows a bimodal seasonal pattern (Figure 3). Some of the migrants from the Rough below-falls population might exhibit adfluvial potamodromy, meaning they move down into Lough Feeagh for a period of feeding and growth prior to return to the Rough River for spawning. Others might exhibit pure anadromy, where they migrate to sea without first spending much time in Lough Feeagh. Others again might exhibit a mix of these migratory tactics, spending a period of time feeding in the lake and then subsequently moving downstream into brackish Lough Furnace and possibly beyond into saltwater proper (Clew Bay and other coastal regions). The Burrishoole population complex experienced a crash in sea trout numbers in the late 1980s/early 1990s, associated with sea-lice problems linked to Atlantic salmon farming (Poole et al. 2007), and while there has been some recovery, the current sea trout run is much smaller than the historical run size.

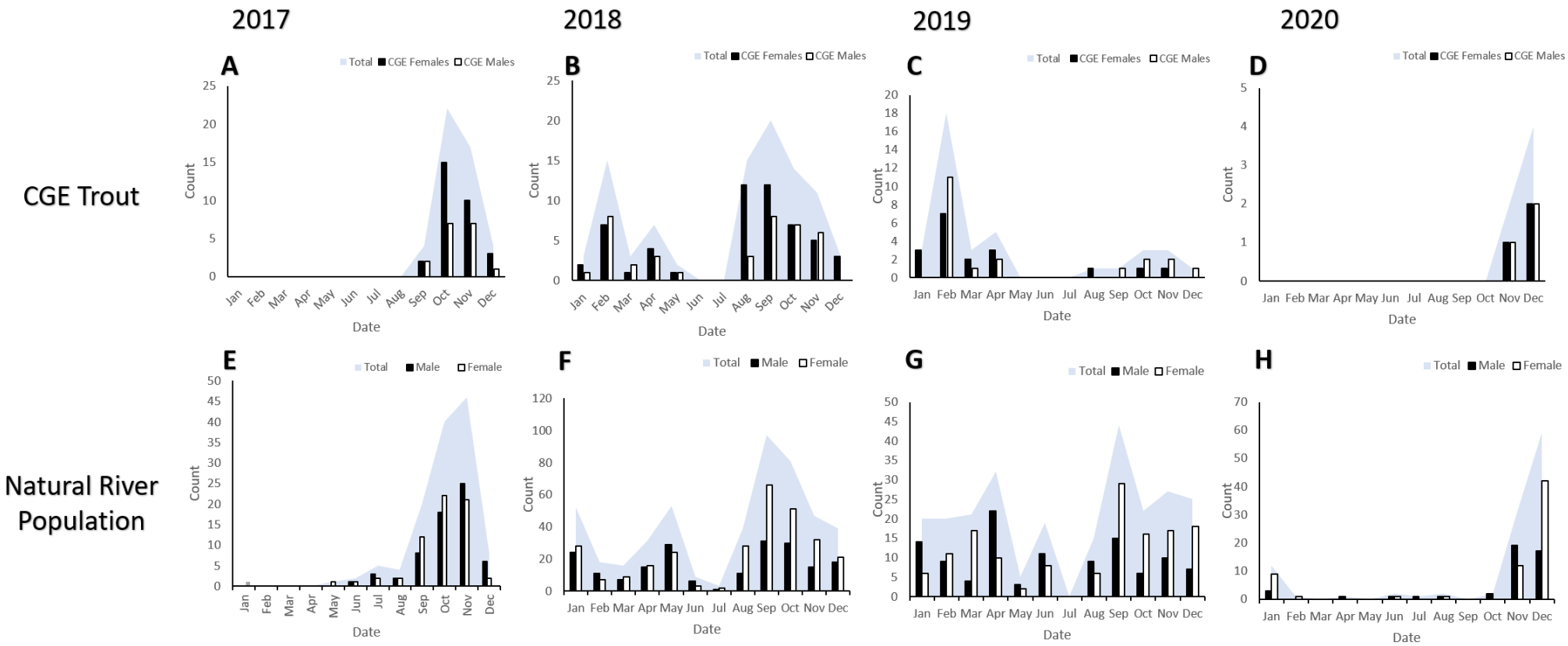


Figure 3(A-H): The timing of female and male brown trout movements out of the RRDT across the duration of the experiment (2017-2020), with A-D showing the movements for the experimental fish (CGE trout) and E-H showing the movement of naturally-produced, non-experimental, fish inferred to be from the same natal cohort (hatched in 2017) as our experimentally introduced groups.

The Erriff brood stock were caught by electrofishing and seine-netting at various sites along the system. Historically, the Erriff catchment had a large run (between 500-3000 smolts) of anadromous outmigrants enumerated annually (Gargan *et al.*, 2016). Thus, it is a population with a strong tendency towards anadromy, but recent years have witnessed declines in marine survival and growth rates of sea trout returning to the system, again linked with sea-lice problems (Gargan *et al.*, 2016). It was not possible in the current study to determine whether each individual Erriff broodstock had been to sea or not, as scale samples were not taken and body colour close to spawning time is an unreliable indicator of migratory status. The hypothesis was simply that Erriff trout, on the average, should have a higher genetic tendency towards anadromy than the Rough Above population, with the Rough below-falls population having perhaps an intermediate anadromy tendency relative to the other two, given the more dramatic recent declines in sea trout in the Burrishoole system – which might reflect contemporary evolution towards lower anadromy rates (Poole et al. 2007).

2.2 Stripping

Across four dates in December 2016 (December 6th, 14th, 15th and 21st), 95 adult female brown trout (n=41 Erriff females; n=54 Rough Below females) and 143 adult male brown trout (n=58 Erriff males; n=22 Rough Above males; n=60 Rough Below males, Table 2.1) were captured from the wild to form potential broodstock for this experiment. On the day of stripping (see Table 2.1), all fish were weighed, measured and photographed, and then stripped of their gametes (Table 2.1). Additionally, a volumetric egg count was recorded for each female. Some males were stripped on multiple dates (Table 2.1). Not all captured males were stripped: of the 143 individuals captured, 121 individuals (see Table 2.1 for details) ended up producing milt.

Table 2.1 Stripping dates and number of fish stripped on each date from each broodstock type used.

			Stripping Date			
	Number of Individuals Captured	Number of Individuals Whose Gametes Were Used	6th December 2016	14th December 2016	15th December 2016	21st December 2016
Erriff Female	41	41	9	14	6	12
Erriff Male[1]	58	44	16	30	6	25
Rough Above Male[2]	25	22	22	19	8	8
Rough Below Female	54	53	30	17	0	6

Rough Below Male[3]	60	55	28	20	7	10
Total	**238**	**216**	105	101	27	61

[1]19 Erriff males were stripped on both the 14th and the 21st of December

[2] Of the 22 Rough above males, 19 were stripped on the 6th and 14th of December; Eight individuals were stripped on the 6th, 14th, and 15th of December; and six individuals were stripped on all four dates

[3] Four Rough below males were stripped on the 6th and 15th of December; and three individuals were stripped on the 14th and 15th of December

2.3 Creating the genetic crosses

Following the successful stripping of 216 individuals, the gametes were sorted into a series of holding pots where an individual's gametes could be held separately before the families were created. Initially there were 94 batches of eggs, where each batch corresponds to a separate female (except for one batch, in which the eggs of two Rough Below females were mixed). Each batch was then split into sub-batches (90 x 3 sub-batches plus 4 x 2 sub-batches), and each sub-batch was fertilised by the milt of a different male. In total, 94 females (41 Erriff and 53 Rough Below) were each crossed to three males (one Erriff male, one Rough above male, one Rough below male), whilst the remaining four females were each crossed to two males (one Erriff male, one Rough Below male). Some males that produced lots of milt were mated to multiple females: there were 121 males used in total (44 Erriff, 22 Rough Above, 55 Rough Below), with 29 males mated to a single female, 66 males each mated to two females, seven males mated to three females, nine males mated to four females, three males mated to five females, three males mated to six females, three males mated to seven females, and one male mated to nine females. Overall, 18,590 fertilized eggs were counted at the start of the experiment.

Table 2.2 Numbers of eggs stripped from each maternal provenance and estimates of remaining eggs after the mechanical shocking per maternal provenance.

Maternal Provenance	Number of Females	Total Eggs Stripped	Average Number of Eggs Per Female	Number of Eggs Fertilized by Erriff Males (n = 44)	Number of Eggs Fertilized by Above falls Males (n = 22)	Number of Eggs Fertilized byBelow fall Males (n = 55)	Total Mortality	Percentage Mortality	Estimate of Remaining Eggs
Erriff Females	41	10428	254	3400	3608	3420	1597	15%	8831
Rough Below Females	53	8162	154	2842	2469	2851	1487	18%	6675
Total	94	18590	198	6242	6077	6271	3084	34%	15506

2.4 Incubation

The 18,590 fertilised eggs were then incubated at an experimental hatchery facility operated by the Marine Institute Newport, in the Burrishoole catchment, supplied with water piped directly from Lough Feeagh. This consisted of four flumes, each subdivided into three trays (with only one tray used on flume #2). Each tray was further subdivided into separate sections, with each section receiving the fertilised eggs from a single female (except for one section, which received the eggs from the two RB females that had been mixed prior to fertilisation). Thus, sub-batches within females (corresponding to maternal half-sibs) were mixed post-fertilisation and incubated together in a single section. Egg mortalities were counted daily per section during this initial incubation phase (Table 2.2). After around five weeks on the 24th and 26th of January 2017 (dependent on the flume tray), all eggs were physically shocked (by mechanically agitating the eggs) so that the infertile and dead eggs turned white and could be easily separated from the fertile healthy ones. This causes the chorionic membrane of infertile and dead eggs to rupture. This, in turn exposes the yolk to water which causes the yolk to coagulate, turning them pale or white such that these unviable eggs can be identified, counted and removed.

2.5 Mixing

Approximately 2-3 weeks after the eggs were shocked, there were 15,506 eggs remaining (Table 2.2). All of these remaining viable eggs were transferred to a new set of two flumes (one top flume with three trays, one bottom flume with two trays). At this stage, 84 of the original 94 egg batches had sufficient remaining viable eggs to be transferred across to the new flumes. These comprised 37 batches from original flumes 1 and 2, which were all mixed together and split into two approximately even groups – with one group then transferred to Top Flume Tray 1 of the new flumes, the other group transferred to Bottom Flume Tray 1. The next set of 35 egg batches from original Flume 3 (Trays 1, 2 and 3) and original Flume 4 (Tray 1) were then all mixed together, and similarly split into two approximately even-sized groups: with one group transferred to Top Flume Tray 2 and the other to Bottom Flume Tray 2 of the new flumes. All remaining eggs were then incubated in these new flumes and the alevins allowed to hatch out, with all mortalities counted daily. When the yolk-sacs of the

alevins are nearly used up (~10% yolk-sac remaining), they are now called ‘unfed fry’ – i.e., fry close to transitioning to exogenous feeding. Surviving unfed fry from Top Flume Trays 1 and 2 and Bottom Flume Trays 1 and 2 were then introduced (stocked out) to the experimental river over two separate dates. The unfed fry from Top Flume Tray 1 and Bottom Flume Tray 1 were stocked out on the 10th of March 2017, whist the unfed fry from Top Flume Tray 2 and Bottom Flume Tray 2 were stocked out on the 23rd of March 2017.

2.6 Estimating number of unfed fry per experimental crosses introduced into the Rough River

Accurate counts of the number of eggs per full-sib family (combination of a given dam and sire) were only available at the initial fertilised egg stage, because it was not possible for logistic reasons to separately hold and incubate eggs from all 281 full-sib families through until the unfed-fry stage. Prior to mixing, the eggs of each female were incubated separately, and it was assumed that any pre-mixing egg mortality within these maternal half-sibling families was random with respect to paternal identity (sire). Thus, by the end of the pre-mixing period, an exact count was available of the number of eggs per maternal half-sib family, but only an estimate of the number of eggs per full-sib family. After the eggs from different females had been mixed, it was no longer possible to keep track of egg/alevin mortality rates at the maternal half-sibling level, but records were kept of which half-sibling families went into which flumes/trays. Post-mixing mortality was assumed to be random with respect to both maternal and paternal identity within a given flume compartment, so an estimate of the number of eggs surviving to the unfed fry stage per full-sib family was arrived at by multiplying the estimated number of initial eggs per full-sib family in a given flume compartment by the overall post-mixing survival rate of that specific flume compartment. These estimates at the full-sib level were then aggregated by cross type to give an overall estimate of the number of unfed fry stocked out for each of the six cross-types. These numbers could in turn then be aggregated by maternal provenance, i.e., numbers stocked out per Erriff mother and Rough Below mother, and by paternal provenance, i.e., numbers stocked out per Erriff father, Rough Below father, and Rough Above father.

2.7 Fry introduction and data collection

Unfed fry from the 281 families were introduced to the experiment river in late March of 2017. A total of 12,833 unfed fry from 94 different females were estimated to have been introduced (Table 2.3). On top of these experimental fish, naturally-spawned (non-experimental) fish from a range of natal cohorts were present in the river, as wild adults were not prevented from accessing spawning sites within the Rough River. The run of naturally spawning female trout into the Rough River generally varies annually between 100 and 150 individuals and with a fecundity of approximately 250 eggs, one might estimate that naturally spawned offspring in the river coincident with the introduced experimental fry in the region of 25,000 to 37,500 fry (P. McGinnity pers. comm.).

Table 2.3 Estimates of number of offspring introduced into the experimental river broken down into maternal provenance, paternal provenance and by genetic cross group.

Maternal Provenance	Estimate of Number of each Maternal Provenance Offspring Released	Paternal Provenance	Estimate of Number of each Paternal Provenance Offspring Released	Genetic Cross Group	Estimate of Offspring Released	Total Estimate of Offspring Released
Erriff	6885	Erriff	4305	Erriff Female x Erriff Male	2227	12833
				Erriff Female x Rough Above Falls Male	2429	
		Rough Above Falls	4242	Erriff Female x Rough Below Falls Male	2229	
				Rough Below Falls Female x Erriff Male	2078	
Rough Below Falls	5948	Rough Below Falls	4286	Rough Below Falls Female x Rough Above Falls Male	1813	
				Rough Below Falls Female x Rough Below Falls Male	2057	

Monitoring of downstream movements out of the experiment river via the RRDT began on the day of introduction and continued daily (sometimes multiple times in one day in more intense migratory periods) for the duration of the experiment. All trout captured were measured for their length (mm) and weight (g) and genetic samples were taken in the form of a tail fin clip, which were preserved in 99% molecular grade ethanol for subsequent DNA work. Later on in the experiment, when the fish were transitioning from 0+ to 1+, the trout were PIT tagged if they were 65mm or larger. This was done to track the movements of individuals and compare patterns among the experimental crossess, as well as to explore movement patterns of non-experimental (naturally

produced) juveniles. In addition to fish captures via the RRDT, the experimental river was electrofished a total of 29 times from March 2017 to September 2020. Only 13 of these 29 electrofishing events involved the capture of fish that subsequently assigned to one of our six experimental crossess (i.e., only naturally produced fish caught in the other 16 events), so the focus here is on these 13 sampling occasions. For the analyses of survival (representation of progeny of each experimental crosses in the river at different time points), the focus is on the larger electrofishing (EF) events (when 20 trout were caught across the six experimental crossess), but for the analyses of size-at-age and sex ratios, data from all electrofishing events (including the smaller ones) are included.

To investigate trout movements over the duration of the experiment, as well as the degree of movement between the upper and lower sections of the experimental river either side of the RRDT, a network of swim-through, cross-channel HDX PIT antennae readers were installed in these river sections. These antennae, which were active and maintained for the duration of the study period, use a continuous electromagnetic field that wirelessly powers any nearby HDX 4005 PIT tag, causing the tag to transmit a unique 12-digit identification number that is subsequently received and recorded by the antenna reader along with the date and time. Antennae were strategically placed at multiple sites throughout the catchment in order to collect data at each stage of a trout's migratory journey. Antennae were installed upstream of the RRDT in the upper part of the experimental river, as well as below (downstream of) the RRDT in the lower section of the experimental river. There was also an antenna installed in the Black River, a higher order river downstream of the experimental river through which migratory trout would have to pass to reach Lough Feeagh, or to migrate from there towards the sea. At the two sea entry traps (Salmon Leap Downstream Trap, SLDT; and Mill Race Downstream Trap; MRDT) there were fixed antenna at the inlet of each trap as well as handheld scanners which were used by personnel from the Marine Institute and/or the FishEyE team of University College Cork to identify tagged experimental fish (see Figure 1 for details). The fixed antennae were designed to span the entire channel width and depth at their respective locations during all but exceptionally high flow conditions. The performance of each antenna was regularly

checked with a test tag every 10 to 14 days and shortly after each flood or storm events. If damaged, repairs were generally completed within 48 hours of antenna damage to ensure that antennae remained operational throughout the majority of the study period.

Size and sex information on out-migrating brown trout obtained from September 2017 to December 2020 during which tissue samples for genetic analysis were also taken downstream migrating trout captured at trapping facilities situated on two short rivers that join L. Feeagh to L. Furnace (Mill Race and Salmon Leap sea-entry traps; Figure 1). An individual's multi-locus genotype was then used as a 'genetic tag' to match individuals to the same trout which may have been previously sampled out-migrating at the RRDT, or even to match an individual (augmented with PIT tag matches), thus allowing for comparisons of the relative performance between the genetic groups. The sea-entry traps were monitored daily during periods of low migration, and twice daily during periods of peak migration. PIT-tagged fish (RFID HDX, Biomark) were detected in downstream and upstream directions by fixed antenna (Biomark, with IS1001 Readers) and hand scanners (HPRPlus, Biomark) at the Salmon Leap traps and hand scanners (HRPlus and HPRLite, Biomark) at the Mill Race traps.

2.8 DNA extraction

Genomic DNA was extracted from all broodstock (parents) and all juveniles sampled in the wild over the course of the experiment (potential progeny of experimental parents) using the Promega Wizard® SV 96 Genomic DNA Purification System. DNA quality and quantity was assessed on agarose gels by comparison with a Quick-Load® Purple 100 bp DNA Ladder (New England Biolabs). Concentration of DNA for PCR was adjusted to 2-10ng/µl.

2.9 PCR and genotyping

Multiplex PCR was used to amplify 16 loci in two reactions (panels). Fluorescent labels were added to the forward primers by Applied Biosystems (ABI) and standard dye sets to enable visualisation on ABI genetic analysers and reverse primers included a GTTT 'pig tail' to minimise stuttering. These two panels facilitated the amplification of one

microsatellite loci and a locus (sdy) for genetic sex determination (Prodöhl et al. unpublished). In panel 1, a single set of primers (One102) amplified two distinct and apparently unlinked loci which could be scored effectively. Primer sequences were taken from relevant publications as follows; *Ssa*197 & *Ssa*85 (O'Reilly *et al.*, 1996); *Ssa*D71 (King *et al.*, 2005); *Ssa*410, *Ssa*416 (Cairney *et al.*, 2000); CAO48828, CAO53293 & CAO60177 (Vasemägi *et al.*, 2005); One102, One103 & One108 (Olsen *et al.*, 2000); pp*Str*2 & pp*Str*3 (Keenan *et al.*, 2013); *Cocl*-lav-4 (Rogers *et al.*, 2004); *Sasa*TAP2 (Grimholt *et al.*, 2002); One9Asc (Scribner *et al.*, 1996). All PCRs were performed in a total volume of 3.5µl, including 2-10ng of genomic DNA and 1.75µl Plain Combi PPP Master Mix (TopBio). Primer concentrations (same for forward and reverse primers for each locus) and fluorescent label employed in each panel were as follows:

Panel 1; SalmoYF (VIC) – 0.03µM, Ssa85(NED) – 0.04µM, Oneu9ASC (VIC) – 0.05µM, Ssa416UOS (FAM) – 0.06µM, One102 (NED) – 0.08µM, CAO48828 (VIC) – 0.08µM, One103 (FAM) – 0.1µM, CAO53293 (PET) – 0.15µM, SsaD48 (FAM) – 0.2µM, CoclLav4 (VIC) – 0.2µM, One108 (VIC) – 0.25µM

Panel 2; Ssa197 (VIC) – 0.04µM, SsaD71 (NED) – 0.05µM, ppStr3 (FAM) – 0.06µM, SasaTAP2 (NED) – 0.08µM, CAO60177 (FAM) – 0.2µM, Ssa410UOS (PET) – 0.25µM.

Cycling conditions were as follows: Initial denaturation at 95°C for 15 minutes, followed by 25 cycles of 94°C for 30 seconds, 55°C for 60 seconds and 72°C for 90 seconds. A final extension step of 30 minutes at 60°C was performed. Electrophoresis was performed on an ABI3500xl (UCC) or ABI7500xl (QUB) DNA analyser using POP-7™ Polymer. Each sample was prepared in Hi-Di™ Formamide with GeneScan™ 600 LIZ™ Dye Size Standard (ThermoFisher Scientific) as a size ladder with which to compare allele size. Allele calling was conducted in GeneMarker (SoftGenetics).

2.10 Genetic parentage analysis

Parental assignment was used to link offspring sampled at various times and locations over the course of the study back to their original parents (i.e., the broodstock used to make the experimental crosses), and hence determine the experimental crosses to which the progeny belonged. Fish that did not assign to any of the experimental broodstock were assumed to have been produced by unknown (i.e., unsampled) parents spawning naturally in the wild. These assignments were carried out with the Family Assignment Program (FAP; Taggart 2007). FAP is particularly useful to estimate exclusion-based family assignment probabilities within family mixtures where all parental genotypes are known (O'Toole *et al.*, 2015). When running parentage assignment (i.e., assignment analysis mode within FAP), a minimum number of 'scored' loci of 10 loci was set (i.e., fish that were successfully genotyped at 10 or more loci based on a robust power analysis, see supplementary Table 2.1). When running FAP the 'allele size tolerance' parameter was set to zero while the 'allele mismatch tolerance' parameter was set to two, which allowed for an empirical evaluation of potential genotype scoring errors. Therefore, in cases where mismatches were observed for one or two of the full set of marker loci, and taking the likelihood of full matches for the remaining loci (from the results of the power analysis), it was possible to account and to correct for mismatches resulting from scoring errors.

2.11 Data analysis

Aim 1: Is there a genetic basis of early life stage movement out in brown trout?

To test whether overall movement rates out of the experimental stream differed among the experimental crosses, the total number of trout caught in the RRDT across the years 2017, 2018 and 2019 was summed for each assignment group, and compared against the initial (estimated) number of introduced fry per group. Fish caught in the RRDT in 2020 were excluded, as some of these (age 3+) might have been mature fish (here the focus was on juvenile emigration only). A chi-squared analysis was performed to test whether the observed proportions of juvenile emigrants assigning to each maternal provenance (Erriff mothers versus Rough Below mothers) differed from the corresponding proportions in the introduced fry. Similarly, the proportions

assigning to each paternal provenance (Erriff fathers versus Rough Above fathers versus Rough Below fathers) was compared against expected proportion.

If the composition of juvenile emigrants (RRDT captures) differed with respect to provenance, this could reflect differential movement propensities, but equally it could reflect differential survival in the river. For example, if the offspring of two Erriff parents survive less well in the Rough River than the offspring of two Rough Below parents, we expect correspondingly fewer of the former than the latter to be caught in the RRDT, even absent any genetic differences in movement propensities. To test whether progeny from different experimental crosses exhibited differential survival within the experimental river, a series of chi-squared tests were performed that compared the observed proportions of progeny at each major electrofishing event assigning to each maternal provenance to the corresponding expected proportions based on the initial fry introduction. The same was done with respect to paternal provenance.

To further explore whether the composition of trout captured in the RRDT differed with respect to maternal provenance and paternal provenance at specific life stages/times of year, the RRDT data was split into five six-month emigration periods: Late 2017 (age 0+), Early 2018 (age 1+), Late 2018 (age 1+), Early 2019 (age 2+) and Late 2019 (age 2+). Again, chi-squared tests were used to test whether the proportions assigning to each maternal provenance and paternal provenance in each emigration period differed from expected proportions based on the initial fry introduction. Additional chi-squared tests were performed to see if the observed proportions for each six-month period of downstream movement differed with respect to the proportions observed in the river at that time, using the most recent electrofishing event.

Aim 2: An investigation into the phenotypic correlates of juvenile brown trout movement

To explore phenotypic correlates of juvenile movement behaviours at the individual level, a series of generalised additive models (GAMs) were constructed for four different responses variables: (1) fish length; (2) fish mass; (3) fish condition factor

(measured as Fulton's K = weight/(length^3)), and (4) sex ratio. The first three response variables were continuous and so normal errors were assumed, whilst the fourth was measured as a binary variable (1=female, 0=male) and so binomial errors were assumed. The data used in each case comprised all progeny assigning to experimental crossess (i.e., unassigned fish assumed to be produced by naturally spawning parents were excluded) from all electrofishing events and all RRDT samples from 2017 (when experimental fish were aged 0+), 2018 (aged 1+) and 2019 (aged 2+) combined. Data were available from 2020 also, but these were excluded here as the numbers were low at that point for the experimental crossess, plus some of the RRDT captures in 2020 might have involved age 3+ fish that were sexually mature and could have been moving downstream after spawning (i.e., kelts – the focus here is purely on downstream movement of immature juveniles).

Each GAM included a smoothed term for date (converted to a numeric variable, *Julian Day*) to account for changes over time due to fish growth and/or selective mortality. The *mgcv* package (Wood 2015) in R was used to fit the GAMs, with the restricted maximum likelihood (REML) method chosen to avoid overfitting. To test the hypothesis that the relevant phenotypic attribute (length, weight, condition, sex) differed consistently between 'movers' versus 'stayers', a categorical variable was included in each model with two levels corresponding to capture method: RRDT (movers) versus electrofishing (current stayers, i.e., fish currently resident in the experimental river; note that some fraction of these fish might move downstream at some subsequent time point, so they are not necessarily all permanent residents). To test whether any differences between movers and stayers varied over time, separate smoothing terms for the date effect were fitted for fish caught in the RRDT versus by electrofishing. To test whether progeny from different experimental crossess differed consistently in their phenotypic attributes (length, weight, condition, sex ratio), maternal provenance (two-level factor: Erriff mothers versus Rough Below mothers) and paternal provenance (three-level factor: Erriff fathers versus Rough Above fathers versus Rough Below fathers) were included as categorical variables in each model. Separate smoothing terms for the date effect were fitted for each maternal provenance level and each paternal provenance level, to test whether the temporal patterns varied across

provenances. Backwards model selection was performed for each GAM, removing non-significant (P>0.05) terms in sequential order starting with the highest P values, until all terms remaining in the model were significant.

Aim 3:

Chi-squared tests were used to test whether the composition of smolts (trout sampled at the sea-entry traps on their departure from freshwater; i.e., anadromous migrants) was non-random with respect to the six experimental cross types, using the original proportions in the introduced fry as the baseline. This was further broken down by maternal provenance and paternal provenance, to test whether any observed compositional differences at the smolt stage were driven more by the provenance of the mother or the father.

Additional findings on the patterns of movement of the experimental river's natural brown trout population

To explore patterns in the timing of fish movements through the Rough River, into Lough Furnace and from there to the sea-entry traps, detection data on PIT-tagged individuals from fixed and hand-held PIT-tag detectors at each sea entry trap were summarised into the same six-month time periods as for the RRDT data (see above) and also broken down according to maternal and paternal provenance, for progeny assigning to experimental crossess. In addition, data on an additional set of n=29 non-experimental fish (juveniles produced by natural spawners) with PIT tags was considered, which were detected at both the RRDT (using hand-held scanners) and then also subsequently at the sea-entry traps (using hand-held scanners and/or the fixed antenna arrays). These migratory individuals are of interest here because they are known to have definitely migrated to Lough Furnace (the tidal lagoon in the lower part of the catchment) and from there possibly to the sea proper. To do so, they must first pass through the freshwater Lough Feeagh, but individuals might vary in the extent of time spent there (or in riverine habitat downstream of the RRDT and upstream of Lough Feeagh, e.g., the Black River). To test the hypothesis that size/age at departure from the Rough River affects the time spent in intervening freshwater

(riverine or lacustrine) habitats, a GAM was run in which the response variable was the number of days between detection in the RRDT and detection at the sea-entry traps, and the length of the fish on departure from the Rough River was the sole continuous (smoothed term) explanatory variable.

All analyses were carried out in R version 4.1.3 (R Core Team, 2022).

3. Results

3.1 Aim 1: Is there a genetic basis of early life stage movement out in brown trout?

Overall, 186 trout belonging to the experiment (i.e., progeny assigning to one of the six experimental crosses) were captured in the RRDT, i.e., emigrating from the experimental river, across the years 2017-2019. An additional six (age 3+) trout were captured in the RRDT in the year 2020, which might have been mature individuals. Considering only putative juvenile emigrants (2017-2019 RRDT captures), the proportions assigning to Erriff mothers (61%; n=113) versus Rough Below mothers (39%; n=73) was different than expected ($X^2 = 6.534$, df = 1, P = 0.011) based on the introduced fry proportions (54% versus 46%, respectively; Fig.4, Table 2.4). There was no effect of paternal provenance on the composition of these juvenile emigrants ($X^2 = 4.130$, df = 2, P = 0.127), but the effects were in the expected direction, with 42% assigning to Erriff sires (expected = 34%), 23% assigning to Rough Above sires (expected = 33%) and 35% assigning to Rough Below sires (expected = 33%) (Fig.4, Table 2.4).

The proportions of offspring assigning to Erriff mothers versus Rough Below mothers did not differ from the introduced fry proportions for any of the electrofishing events or RRDT emigration periods (Table 2.4). Furthermore, these proportions did not change substantially throughout the experiment when comparing each electrofishing event to the immediately preceding electrofishing event, when comparing each RRDT sampling period to the nearest electrofishing event, or when comparing each RRDT sampling period to the immediately preceding RRDT sampling period (P> 0.05 for the effect of maternal provenance in all cases; Fig 4; Table 2.4). The proportions of offspring assigning to each paternal provenance for each major electrofishing event also did not differ significantly from expected proportions based on the introduced fry (P>0.05 for the effect of paternal provenance in all cases; Fig 4; Table 2.4).

The composition of the offspring moving through the RRDT in late 2017 differed with respect to paternal provenance, relative to the composition in the introduced fry ($X^2 = 10.640$, df = 2, P = 0.005) (Fig 4; Table 2.4). This significant effect of paternal

provenance was driven by a deficit of progeny from Rough Above sires (X^2 = 8.934, df = 1, P = 0.002) and an excess of progeny from Erriff sires (X^2 = 9.329, df = 1, P = 0.002), with the number of progeny from Rough Below sires not differing from expectation (P = 0.139).

Similarly, the composition of the offspring moving through the RRDT in late 2018 differed with respect to paternal provenance, relative to the composition in the introduced fry (X^2= 7.461, df = 2, P = 0.023) (Fig 4; Table 2.4). Again, this significant effect of paternal provenance was driven by a deficit of progeny from Rough Above sires (X^2 = 6.210, df = 1, P = 0.012), with the number of progeny from Erriff sires (P= 0.322) and Rough Below sires (P=0.267) not differing from expectation. The only other chi-square test that revealed a statistically significant effect of paternal provenance was between fish moving out of the RRDT in late 2018 and fish captured via electrofishing in late 2018 (August and November events combined; X^2= 7.015, df = 2, P = 0.029).

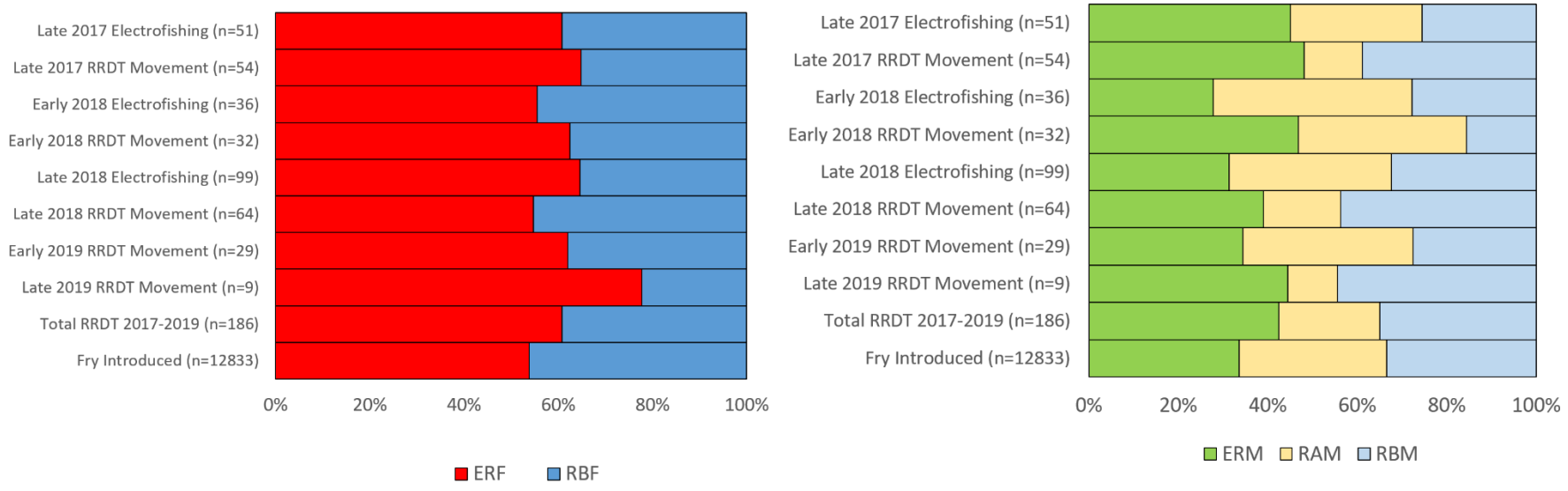


Fig 4: Representation of progeny assigning to each maternal provenance (A) and paternal provenance (B) at each sampling stage for trout caught at specific electrofishing events and trout caught in the RRDT where the latter is broken down into Early and Late six-month movement periods). Total counts for each sampling event are given to the left of the bars. ERF = Erriff females, RBF= Rough Below females; ERM = Erriff males, RAM= Rough Above males, RBM = Rough Below Males.

Table 2.4. Chi-square results table testing whether proportions of each parental grouping changed over the course of the experiment. Significant results indicated by the use of * P= <0.05, P**= 0.005.

	Proportion Comparison	X-squared statistic	df	P-Value
Maternal Provenance	RRDT Early 2017 vs Introduced Fry	0.361	1	0.548
	RRDT Late 2017 vs Introduced Fry	2.184	1	0.139
	RRDT Late 2017 vs November 2017 Electrofishing	0.051	1	0.822
	RRDT Early 2018 vs Introduced Fry	0.646	1	0.421
	RRDT Early 2018 vs April 2018 Electrofishing	0.001	1	0.999
	RRDT Late 2018 vs Introduced Fry	0.287	1	0.592
	RRDT Late 2018 vs Late 2018 Electrofishing	1.226	1	0.268
	RRDT Early 2019 vs Introduced Fry	0.492	1	0.482
	RRDT Early 2019 vs Late 2018 Electrofishing	0.001	1	0.972
	RRDT Late 2019 vs Introduced Fry	1.222	1	0.268
	RRDT Late 2019 vs Late 2018 Electrofishing	0.182	1	0.668
	Total RRDT 2017-2019	3.255	1	0.071
Paternal Provenance	RRDT Early 2017 vs Introduced Fry	0.484	2	0.784
	RRDT Late 2017 vs Introduced Fry	10.64	2	0.005**
	RRDT Late 2017 vs November 2017 Electrofishing	4.893	2	0.086
	RRDT Early 2018 vs Introduced Fry	4.901	2	0.086
	RRDT Early 2018 vs April 2018 Electrofishing	2.229	2	0.229
	RRDT Late 2018 vs Introduced Fry	7.461	2	0.023*
	RRDT Late 2018 vs Late 2018 Electrofishing	7.015	2	0.029*
	RRDT Early 2019 vs Introduced Fry	0.511	2	0.774
	RRDT Early 2019 vs Late 2018 Electrofishing	0.246	2	0.884
	RRDT Late 2019 vs Introduced Fry	1.954	2	0.376
	RRDT Late 2019 vs Late 2018 Electrofishing	2.338	2	0.311
	Total RRDT 2017-2019	10.542	2	0.005**

3.2 Aim 2: An investigation into the phenotypic correlates of juvenile brown trout movement

When fish length was the response variable, the best GAM was one in which the smoother term for the date effect was different (P=0.031) for movers (fish caught in the RRDT; estimated degrees of freedom (*edf*) for smooth term = 5.585) versus stayers (fish caught by electrofishing; *edf* = 1.863). Fish length gradually increased over time, with periods of more rapid increase in the summer growing season and periods of less rapid increase in the winter months, as expected (Fig. 5A). Movers were consistently longer than stayers, with the differences between them being slightly less pronounced in the winter months (Fig. 5A). There were no effects of the experimental crosses to which the progeny belonged on fish length (P>0.05 for all terms in the GAM involving maternal or paternal provenance).

Similar patterns were found for fish weight (Fig.5B), except the smoother terms for the date effect were not significantly different (P=0.372) between movers and stayers. The overall smooth effect of date (*edf* = 6.280) was statistically significantly different (P<0.001) from zero, indicating that fish gained weight over time in a nonlinear fashion (Fig. 5B). The average weight of movers was not different from that of stayers, although it was close to being statistically significant (P=0.07). There were no effects of the experimental crosses to which the progeny belonged on fish weight (P>0.05 for all terms in the GAM involving maternal or paternal provenance).

When condition factor was the response variable, the best GAM was one in which the smoother term for the date effect was different (P=0.003) for movers and stayers. The condition of fish caught in the RRDT (movers) was relatively constant over time, while the condition of fish caught by electrofishing (stayers) was relatively constant in 2017 and 2018 and then increased in 2019 (Fig. 5C). The smoother term for the date effect also varied with respect to paternal provenance, driven by a decrease in condition of progeny of Rough Above males in 2019 (smoother term for this group was different (*edf* = 7.036; P=0.001) from that of the other two groups, which did not differ from each other (P=0.790; *edf* = 1.00)). There was no overall difference (P=0.864) in mean condition of fish with respect to paternal provenance. The overall main effect of movers

versus stayers was statistically significant (P=0.037). There were no effects of maternal provenance on condition factor (P>0.05 for all terms in the GAM involving maternal provenance).

When sex was the binary response variable, the best model was one in which there was only a main effect of movers versus stayers (fish caught in the RRDT were more likely to be females; P=0.015). The smoother term for date was close to being significantly different from zero (*edf* = 1.229. P=0.077), with the sex ratio being overall slightly more male-biased in 2019 versus in 2018 or 2017 (Fig.5D). There were no effects of the experimental crosses to which the progeny belonged on fish sex (P>0.05 for all terms in the GAM involving maternal or paternal provenance).

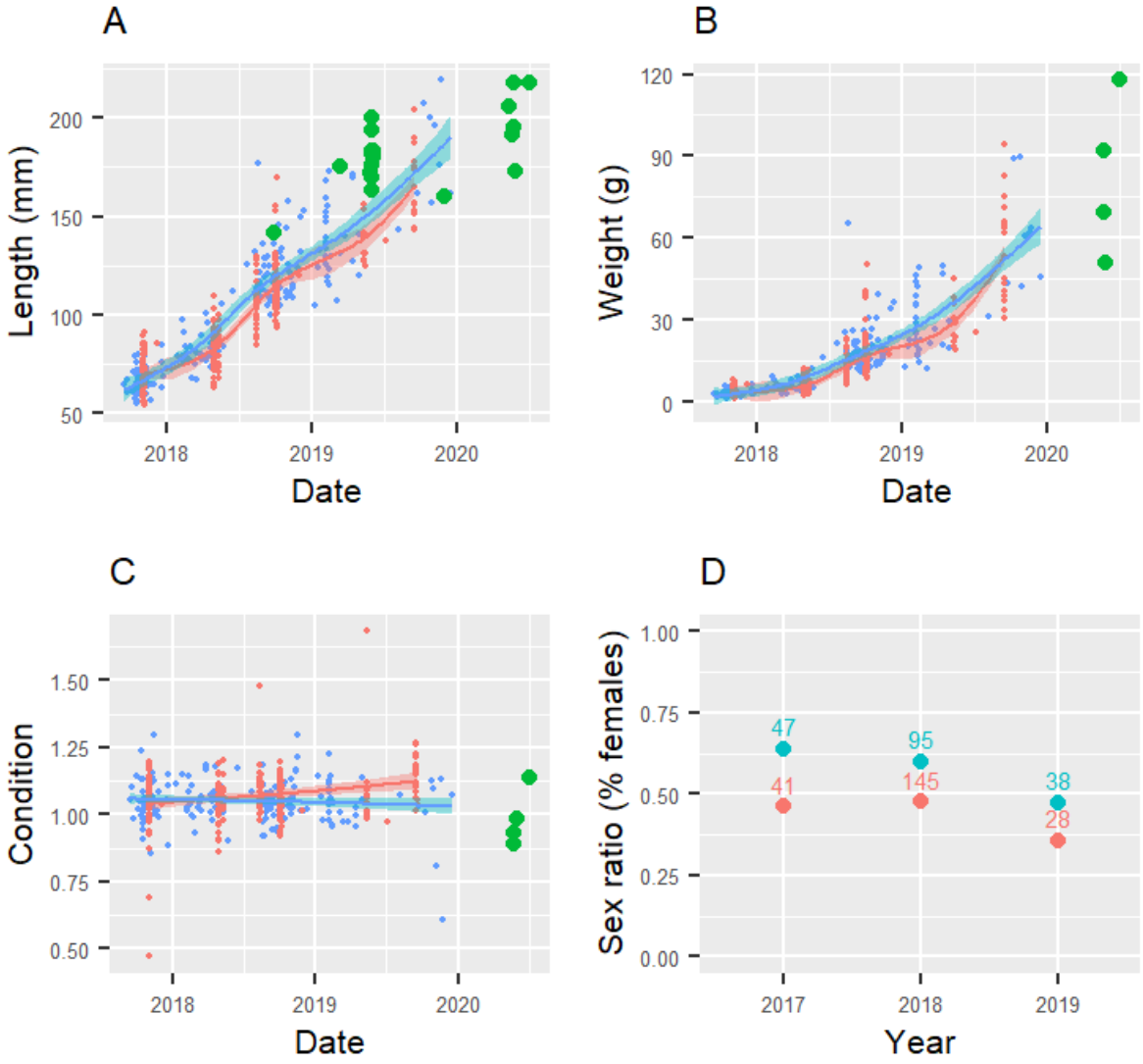


Fig.5: Phenotypic attributes differing between stayers (red) and movers (blue). Length (A), weight (B) and condition factor (C) of 0+ progeny (2017), 1+ progeny (2018) and 2+ progeny (2019) of all experimental crosses combined are plotted as a function of date in the first three panels, with blue dots corresponding to fish caught in the RRDT (movers) and red dots corresponding to fish caught by electrofishing (stayers). Fitted curves in each case correspond to GAM smoothers for the date effect (blue = RRDT; red = electrofishing, ribbons = 95% confidence intervals). Green dots in panels A-C are sea-entry downstream trap captures (n=19 smolts with length data, four of which also had weight, and therefore condition, information), included here for comparative purposes but not included in the GAM fits. Panel D shows the average sex ratio (%

females) for each year, with blue dots again corresponding to RRDT samples and red dots to electrofishing samples (numbers above dots are sample sizes).

3.3 Aim 3

In total, 19 smolts sampled at the sea-entry traps via hand-capture (i.e., fin-clipped for genetics) assigned to one of the six experimental crosses. Thirteen of these fish had PIT tags, eleven of which were detected via the fixed arrays and/or hand-held detectors at the sea-entry traps. An additional four fish carrying PIT tags were detected at the sea-entry traps by the fixed antenna/hand-held detectors, but were not re-sampled for genetics as their provenance was already known (based on their first capture event in the Rough River or RRDT, during which the PIT tag was inserted). Thus, a total of 23 smolts belonging to the experiment were determined to have migrated through the sea-entry traps in the downstream direction, with one of these smolts outmigrating in 2018 (age 1+), 13 outmigrating in 2019 (age 2+), seven in 2020 (age 3+), and two in 2021 (age 4+).

Overall, the composition of experimental progeny sampled at the sea-entry traps (via either hand-capture or antenna detection) differed significantly (X^2 =22.499, df = 5, p < 0.001) among the six cross-types, relative to the expected composition based on the initial introduced fry. There was a statistically significant difference in the proportions of trout at the sea entry traps from each maternal grouping (83%; n = 19) assigned to Erriff mothers, and 17% (n = 4) to Rough Below mothers) relative to the initial proportions of unfed fry introduced to the experimental river (X^2 = 6.534, df = 1, p = 0.011) (Fig 6B). There was no statistically significant difference in the proportions of trout at the sea entry traps from each paternal grouping, with 53% (n = 12) assigning to Erriff sires; 17% (n = 4) to Rough Above sires and 30% (n = 7) to Rough Below sires, relative to the initial proportions of unfed fry introduced to the experimental river (X^2 = 6.715, df = 1, p < 0.001; Fig 6C). The timing of seaward migration was significantly biased towards early (January- June) migrations (n = 21) compared to late (July – December) migrations (n = 2) (X^2 =19.593, df = 1, P < 0.001). A genetic sex was established for 18 of the 23 trout observed at the sea entry traps. Overall, the sex ratio did not significantly deviate from parity within this sample (P > 0.05). However,

there was an observed female sex bias among those that migrated through the sea-entry traps early in the year (January-June (X^2 = 4.7647, df = 1, P = 0.029). There was no significant difference between the sexes when compared with parity in late (July-December) outward migrations at the sea entry traps (P = >0.05).

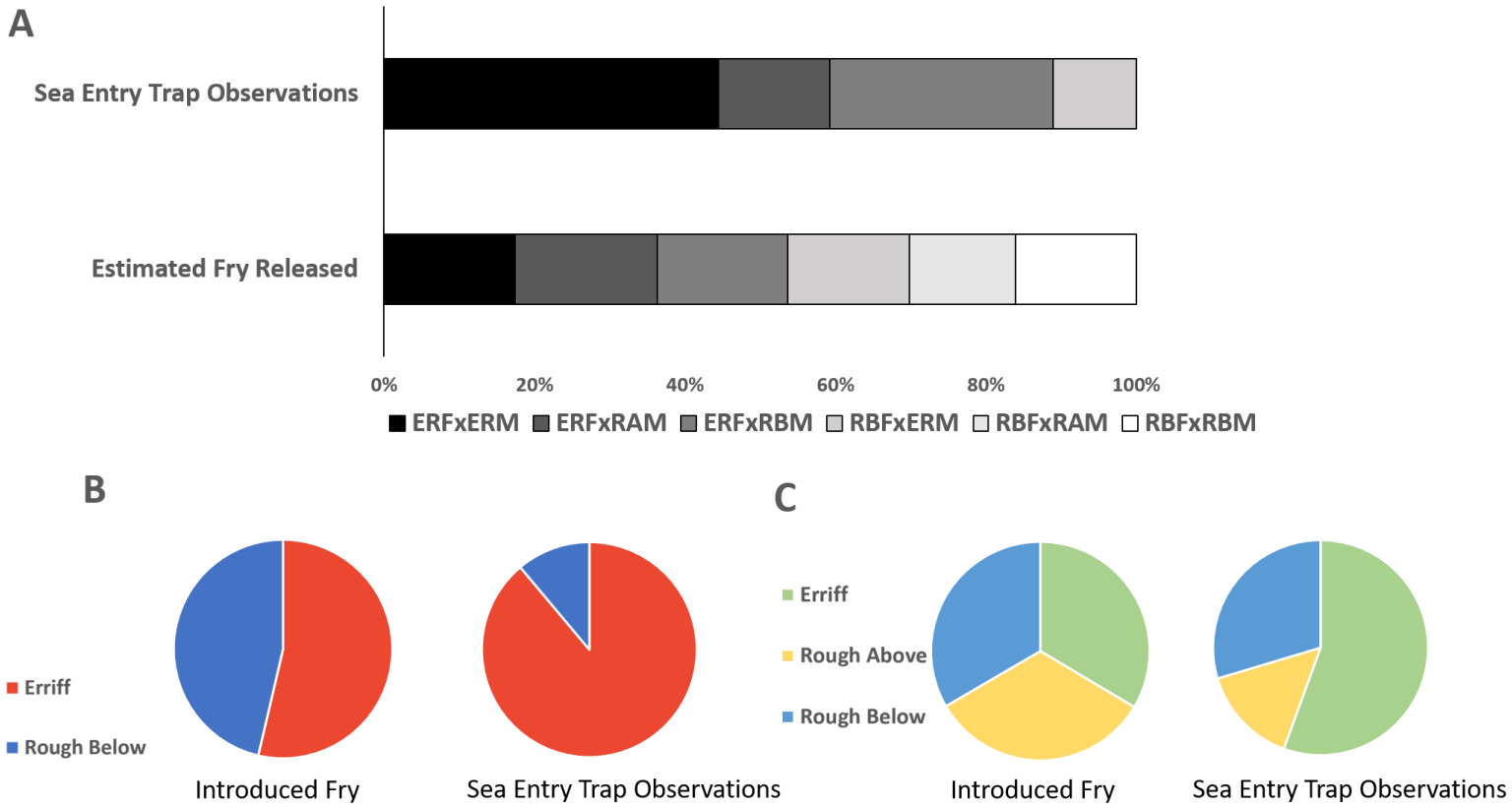


Fig 6(A-C): The change in proportions between the initially introduced experimental fry (ERF = Erriff females, ERM = Erriff males, RAM = Rough above falls Males, RBF = Rough below falls female, and RBM = Rough below falls male) and the sea entry trap

observations (either via fixed trap antenna or via handheld scanning and genetic sampling) over the course of the experiment (2017-2021). Comparisons are made for (A) the six genetic cross types, (B) maternal provenance and (C) paternal provenance.

3.4 Additional findings on the patterns of movement of the experimental rivers natural brown trout population

The RRDT antenna data showed that offspring of Erriff females and Rough Below females moved past the RRDT at similar times of year, although the number of detections was typically higher for the offspring of Erriff females when compared to the offspring of Rough Below females (Fig. 7A). Offspring of Erriff females were detected in higher numbers in early and late 2018 when compared to the offspring of Rough Below females, which were not detected in comparable numbers until early 2019. The offspring of Rough Below females were also detected in the Black River at relativity high volume in late 2020 when the trout were aged 3+.

The offspring of Erriff males, Rough Above males and Rough Below males all moved passed the RRDT antenna at similar times of year. However, the offspring of Rough Above males were detected at lower numbers when compared to the other paternal groups (Fig 7B). Furthermore, all paternal provenances were detected by the Black River antenna at similar rates, although Fig 7B shows that the offspring of Rough Above males were apparently underrepresented within this section of the river. Offspring of Rough Below males were also detected moving through the Black River in late 2020.

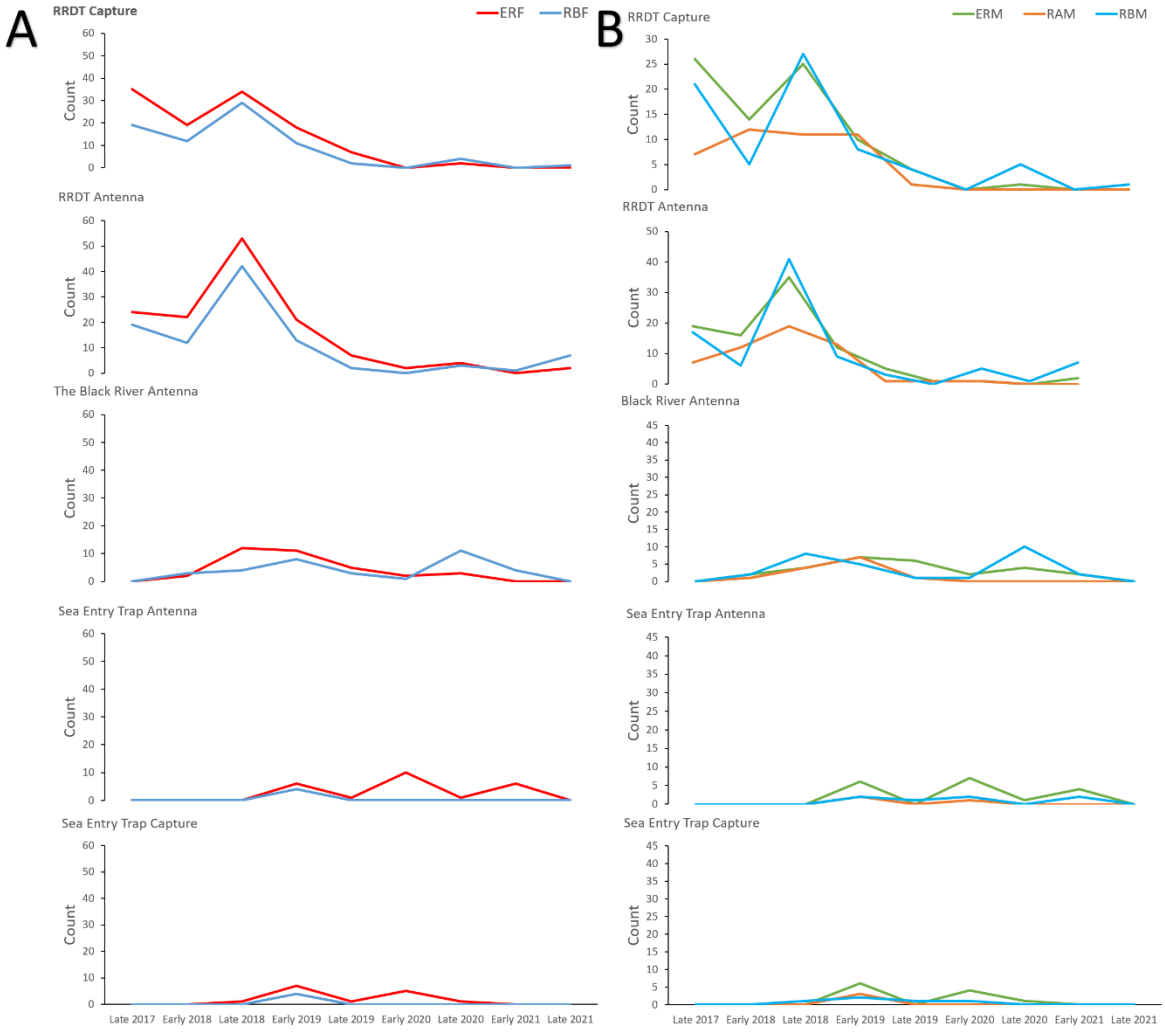


Fig 7. Antenna detections over the duration of the experiment.

A set of n=29 non-experimental fish with PIT tags were identified as having migrated out of the Rough River (caught in the RRDT, n=16 in 2017 and n=13 in 2018) and from there to the sea-entry downstream traps (i.e., left the freshwater portion of the catchment). The distribution of their lengths on capture in the RRDT was approximately bimodal (Fig.8A), with the lower modal group presumably corresponding to age 1+ fish and the upper modal group to age 2+ or age 3+ fish. There was a general negative relationship between the number of days it took these fish to migrate from the RRDT to the sea-entry traps, which was nonlinear (Fig. 8B) and well approximated by a GAM fit (edf = 2.806, P=0.003).

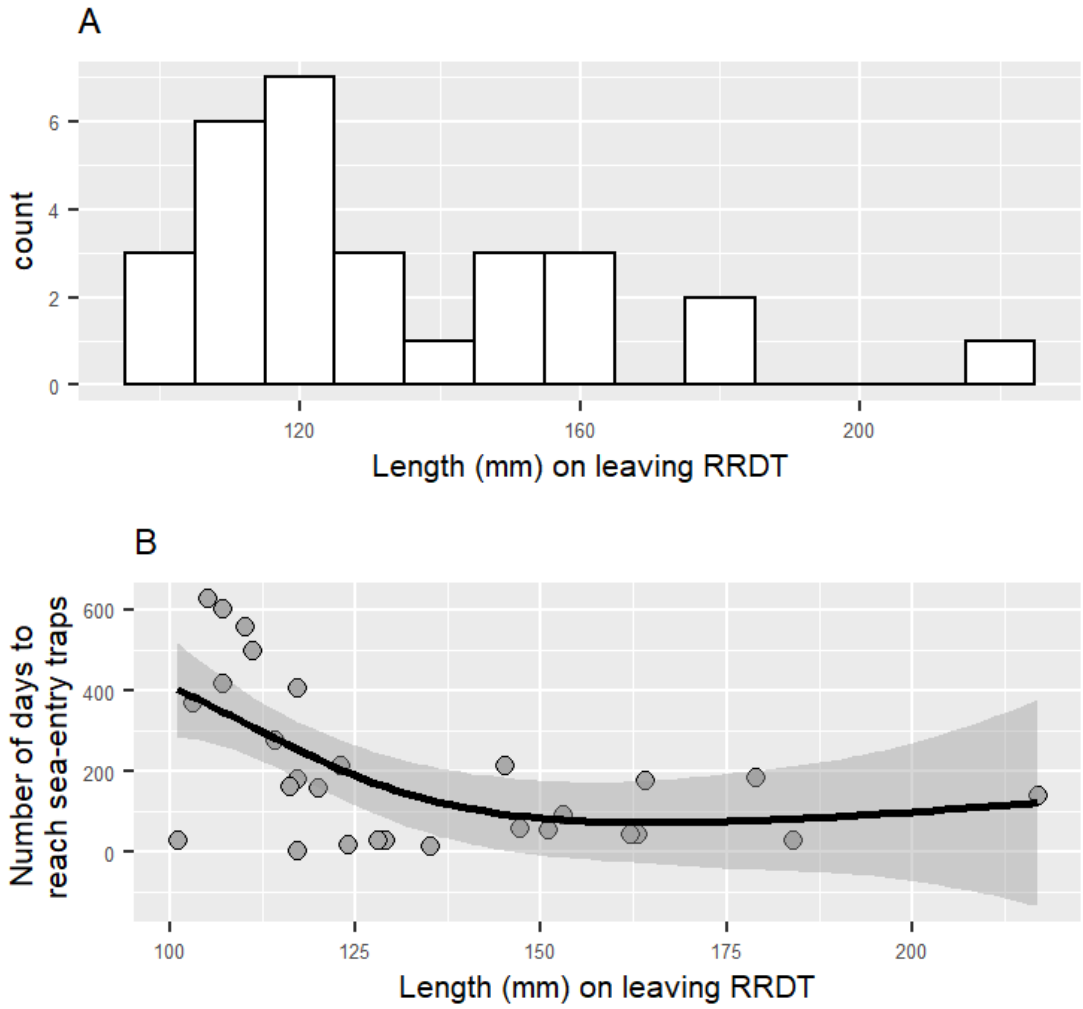


Fig. 8: The distribution of lengths (A) for n=29 non-experimental fish migrating out of the RRDT in 2017 and 2018, and (B) the number of days it takes these fish to migrate from the RRDT to the sea-entry downstream traps, plotted as a function of their length on leaving the Rough River. The black curve corresponds to a GAM fit (ribbons = 95% confidence intervals).

4. Discussion

The primary aim of this chapter was to exploit a common garden experimental set-up to test the hypothesis that trout deriving from populations that naturally exhibit divergent migratory tactics (extent of sea trout production) in their home environments should maintain these differences when reared under shared environmental conditions – in this case, the home environment of the Rough below-falls population.

The developmental environment will still influence the phenotype of any given individual, but differences in mean phenotype among groups should reflect inherited tendencies (effects of genes or nongenetic factors transmitted from parents to offspring), given that each group experiences the same early-life environment on the average. The results largely bore out these basic predictions, in that anadromy rates (number of seaward-migrating smolts, relative to initial number of introduced fry) differed among our six cross-types. Specifically, there was an excess of progeny from Erriff (high anadromy background population) mothers and a deficit of progeny of Rough Below (low anadromy background) mothers at the sea-entry traps. Although not significant statistically, the effects were in the expected direction for the fathers too: approximately equal numbers of smolts per paternal provenance were expected under the null hypothesis of no heritable differences in anadromy propensity, yet 52% assigned to Erriff fathers and 17% to Rough Above (resident background) fathers. On top of these core findings, group-specific differences in movement rates out of the nursery stream (Rough River) were observed via the RRDT data during certain key periods, with offspring of Erriff fathers over-represented and those of Rough Above fathers under-represented. The freshwater antennae data (PIT tag detections in the Rough and Black rivers) were too patchy to draw strong conclusions on finer-scale behavioural patterns or potamodromous migrations, but they largely mirrored the patterns observed via the RRDT captures in terms of movement timing and group differences. Finally, analysis of movement data from non-experimental fish (naturally produced in the Rough River) showed interesting size/age-related variation in time spent in Lough Feeagh.

Previous work on brown trout from the Burrishoole and Erriff catchments (Archer *et al.*, 2019, 2020) has explored similar questions, but utilising laboratory experiments undertaken in an indoor recirculating aquaculture system. Such experiments have the advantage that environmental conditions (e.g. temperature, water chemistry, food) and other parameters such as fish density and composition can be tightly controlled or manipulated to desired levels, but the downside is that results might not translate across to wild environments, especially if traits of interest are subject to strong genotype by environment (G x E) interactions (Hutchings, 2011). The new results

presented in this chapter therefore represent a substantial advance that build on, and indeed corroborate, these earlier findings. Archer et al. (2019) reared offspring of pure crosses between broodstock obtained from Lough Bunaveela (a putative lake-resident population in the Burrishoole system) and from Tawnyard Lough (a putative anadromous population in the Erriff system). Anadromy rates, assessed as the percentage of fish per tank that underwent an obvious parr-to-smolt transformation, were higher overall for trout from the Erriff population background, but also responded differently in each population to food deprivation manipulations, implying G x E interactions (Archer et al. 2019). Interestingly, some smolts were produced by the Bunaveela fish under low food treatments, implying that migration capacity can lie dormant in populations that are (currently) non-migratory in the wild, consistent with threshold models of migration (Tomkins and Hazel, 2007; Pulido, 2011). In the current study, we found lower (albeit not statistically significant) smolting rates among fish with Rough Above fathers compared to the other two paternal provenances. Among the four (out of 23 in total) smolts sampled at the sea-entry traps that assigned to Rough Above fathers, all had Erriff mothers, again consistent with a high predisposition towards anadromy in the Erriff population. A pure Rough Above group would have been expected to produce even fewer, if any, smolts, compared to hybrid groups involving Rough Above sires. Even if resident populations retain the ability to produce smolts under the right environmental circumstances, the physiological capacity to cope with the freshwater to marine transition might have degraded over the generations under relaxed selection in such populations. Consistent with this, Archer et al. (2019) documented lower hypo-osmoregulatory abilities (higher plasma chloride levels following 24-hr exposure to 30ppt saltwater) in Bunaveela smolts (defined on morphological grounds) compared to Erriff smolts. Testing for such osmoregulatory differences in a wild CGE is an obvious next step for future work.

In addition to allowing for exploration of the heritable basis of life history differences among populations, common garden experiments also allow testing for signatures of local adaptation, where local-origin individuals are expected to perform better than foreign-origin individuals in terms of their total fitness, or components thereof (e.g. stage-specific survival), in the “home” environment of the locals. For example, in a

previous CGE in the Burrishoole system with Atlantic salmon *Salmo salar*, the overall lifetime success of “foreigners” (offspring of pure crosses between broodstock collected from the Owenmore River, a neighbouring catchment whose mouth is approximately 50km in coastal distance from that of the Burrishoole system) was estimated to be only 31% that of “locals”, when both were introduced as eyed-eggs to the Rough River (O’Toole *et al.*, 2015). Additional common garden experiments in the Burrishoole system showed substantially lower fitness of farm-origin Atlantic salmon compared to local, wild fish, with hybrids exhibiting intermediate fitness (McGinnity *et al.*, 1997, 2009). The farmed fish in these experiments originally derived from Norway, where selective pressures are likely quite different from the west of Ireland, but they also differed from the wild fish in terms of having a history of domestication and selective breeding. Other work in the Burrishoole system has documented reduced fitness of local-origin ranched Atlantic salmon (with a history of recent domestication) relative to wild-origin salmon (McGinnity *et al.*, 2009; de Eyto *et al.*, 2016; O’Sullivan *et al.*, 2020). Collectively, these studies of Atlantic salmon – a sister species to brown trout – indicate that adaptive divergence in respect of different wild environments, or wild versus captive environments, can be strong across a range of spatial and temporal scales.

In contrast, the current CGE with brown trout found no evidence for survival differences in the Rough River between offspring from different population genetic backgrounds, based on their relative representation in the electrofishing samples. The selective environments in the Burrishoole and Erriff systems might not be different enough, at least at the freshwater stage, to have produced substantial adaptive divergence among Erriff and Rough River trout, and the selective environments in the above- and below-falls sections of the Rough River are presumably very similar in abiotic and biotic terms. Migration should be strongly selected against in the above-falls population, given that migrants cannot get back above the impassable barrier on their return spawning migration. However, unless migratory propensity loci are linked to loci influencing freshwater survival, we would not expect (and indeed did not observe) offspring of matings between Rough Below dams and Rough Above sires to survive worse than those of matings between Rough Below dams and Rough Below

sires, when both are reared below the falls. Interestingly, a large component of the overall lifetime fitness differences observed in the Atlantic salmon CGEs was related to marine survival differences (McGinnity *et al.*, 1997, 2004; O'Toole *et al.*, 2015). Successful transitioning from freshwater to marine environments, coupled with possible differences in homing abilities on the return migration, thus seem to play a large role in the local adaptation of anadromous salmonids (Fraser *et al.*, 2011), whereas these factors might be less evident in brown trout populations where not all (or even no) individuals undertake anadromous migrations. Nevertheless, other studies of brown trout have documented molecular or quantitative genetic evidence for local adaptation at regional (Meier *et al.*, 2011) or more local scales (Westley *et al.*, 2013). It must also be noted that "gold standard" evidence for local adaptation comes from reciprocal transplant experiments, preferably with replication at the population level (Kawecki and Ebert, 2004; Fraser *et al.*, 2011). Such experiments have been performed with brown trout (e.g. Stelkens *et al.*, 2012; Westley *et al.*, 2013; Labonne *et al.*, 2020) but with mixed results, emphasising the complexity of factors determining the extent and scale of local adaptation.

Group-specific differences in early movement behaviours were observed in our current CGE study, but these were only apparent in the second halves of 2017 (0+ fry) and 2018 (1+ parr), and the patterns were driven by maternal but not paternal provenance effects. There was a significant deficit of "movers" in both periods among progeny of Rough Above sires, consistent with a hypothesised genetic tendency towards residency in above-falls populations (Thrower et al., 2004; Thrower & Hard, 2009, Ferguson et al 2016). Moreover, there was a significant excess of offspring of Erriff sires moving through the RRDT during these same periods. In general, trout move downstream at the fry and parr life stages to exploit better feeding opportunities in downstream riverine or lacustrine habitats, or to avoid excessive competition within the natal stream (Nevoux *et al.*, 2019). Some might even migrate to estuarine or brackish habitats at these young ages (see Chapter four), but in the current study only one out of 23 smolts assigning to the CGE was sampled at the sea-entry traps in 2018 at age 1+ (a fish with an Erriff mother and a Rough Below father). All other smolts were captured/detected at the sea-entry traps at ages 2+ (2019) or 3+ (2020). Thus, the

differential rates of movement out of the Rough River likely reflect heritable differences in the tendency to perform active potamodromous migrations, or in the tendency to simply move gradually downstream as the fish age, prior to any anadromous migration (which might then only occur in certain individuals). Indeed, what constitutes “active migration” versus simple movement is debatable and a matter of spatial and temporal scale; the key inference here is simply that movement/migration behaviours can be heritable and under divergent selection in different populations.

The Erriff system has much less lacustrine habitat than the Burrishoole system, so adfluvial potamodromy (migration from natal streams to lakes) is a less viable option in the former, but fluvial potamodromy (migration from natal streams to larger rivers) is presumably still an option in the Erriff system. Both fluvial and adfluvial potamodromy are viable options for brown trout below the falls in the Rough River, given the existence of suitable rearing habitat in the Black River (into which the Rough River flows) and Lough Feeagh (into which the Black River flows). It is therefore unclear why relatively more of the fish moving through the RRDT in late 2017 and late 2018 assigned to Erriff sires compared to Rough Below sires. It may be that residency within natal streams is more disfavoured in the Erriff than in the Burrishoole system, e.g. if food or feeding territories are more limiting. The antenna detections data in the current study were unfortunately insufficient to be able to fully resolve differences in the continuum of possible migration life-histories, from stream-residency to potamodromy to semi-anadromy (migration to brackish waters only) and anadromy (migration to fully marine habitats). It is noteworthy, however, that naturally produced (i.e. not experimentally introduced) brown trout originating from the Rough River during our study period differed substantially in the amount of time they spent in Lough Feeagh before being detected as smolts at the sea-entry traps (Fig. 8). Individuals that left the Rough River at younger ages/smaller sizes subsequently spent longer in Feeagh, perhaps because they needed more time in productive lacustrine habitat to reach critical sizes necessary to survive the smolting transition. Recent studies have drawn attention to the importance of lakes in the life histories of many salmonids, and to variation within and among anadromous populations in patterns of lake use prior to possible seaward migration (Klemetsen *et al.*, 2003; Klemetsen, 2013; Arostegui *et*

al., 2019; Birnie-Gauvin and Aarestrup, 2019; Lennox *et al.*, 2020). Lakes may provide greater feeding resources with reduced competition between conspecifics, but might also contain a great diversity or abundance of gape-limited predators, so the benefits and costs of migrating into lakes, and the duration of lake sojourns, likely depend on trout body size or energetic status. It would be very interesting in future studies to use telemetry coupled with individual-level growth and survival data to characterise the drivers and consequences of lake use and resolve the various possible life history pathways.

When group-specific phenotypic differences are found in common garden experiments, this points towards *heritable* variation, but on top of direct genetic inheritance (i.e., DNA sequence variants that are passed from parents to offspring), epigenetic inheritance is also a possibility. Epigenetic alterations to the structure of DNA, such as methylation patterns or histone modifications, can in theory be passed from parent to offspring via sperm or egg cells, such that the environment experienced by the parents can indirectly influence the phenotypes of their offspring via transmissible changes in gene regulation. Although the idea of epigenetic inheritance remains controversial and poorly understood empirically (Nestler 2016; Jablonka 2017), recent work with salmonid fishes hints at the possibility of trans-generational inheritance of DNA methylation patterns (Wellband *et al.*, 2021; Venney *et al.*, 2022). Such epigenomic modifications may even have sex-specific effects (Venney *et al.*, 2022), such that differences in the environments experienced by mothers versus fathers are then transmitted epigenetically to their progeny, with resulting effects on offspring life-history traits, an intriguing possibility that warrants future study. Thus, in addition to maternal effects (see below), epigenetic effects must be taken into account when interpreting the results of common garden experiments, and isolating true (i.e. DNA sequence based) genetic effects might require breeding the organisms under common environmental conditions for several generations prior to testing for phenotypic differences, in order to purge/equalise epigenetic/maternal effects among groups. This is logistically challenging in large and relatively long-lived species such as salmonids and could also lead to rapid genetic adaptation to the shared

environment, which is obviously undesirable if the goal is to understand adaptive divergence among natural populations.

One intriguing aspect of the results in this chapter was that paternal, but not maternal, provenance influenced early-life movement rates out of the Rough River, but the patterns were reversed at the smolt (sea-entry traps) stage, with significant representation differences found with respect to maternal, but not paternal, provenance. The lack of paternal provenance effects at the smolt stage could have been a statistical power issue, as the total number of smolts (across the three paternal groupings) was only 19, plus the effects were in the expected direction (more smolts from Erriff sires, fewer smolts from Rough Above sires). Statistical power was likely less of an issue with the RRDT data, however, as the sample sizes were larger, though still not overwhelming. Fathers pass only genetic material on to their offspring, whereas mothers can additionally influence their offspring via so-called “maternal effects”, where maternal phenotype affects offspring phenotype over-and-above the effects of shared genes (note that maternal effects could themselves involve epigenetic mechanisms). In salmonids, for example, larger females can produce larger (as well as more) eggs, and egg size can then influence the traits and survival prospects of the offspring, with these egg-size mediated maternal effects waning as the offspring age (Einum and Fleming, 1999; Heath *et al.*, 1999; Garant *et al.*, 2003; O’Toole *et al.*, 2015). The eggs of Erriff dams were on average 34% larger than those of Rough Below dams used for our experiment, but this difference was not statistically significant (P=0.077). In any case, egg size differences would not explain why there was an effect of paternal but not maternal provenance on early-life downstream movement rates, unless a higher genetic predisposition towards migration in the Erriff population was countered by maternal effects in the opposite direction (promoting residency). It remains possible, however, that egg size or other traits associated with maternal life history influence offspring life histories on top of direct genetic effects (Van Leeuwen *et al.*, 2017; Hughes *et al.*, 2018). Further data at the family or individual level would be required to test these ideas.

Another possible explanation for the differential effects of maternal versus paternal provenance on migration probabilities of the offspring could be sex linkage, where life

history genes are clustered on sex chromosomes or in autosomal regions involved in sex determination. In mammals, for example, Y-linked genes are only passed from father to son, whilst sons only inherit a single copy (from their mother) of X-linked genes. The opposite occurs in birds with ZW sex determination systems, where females are the heterogametic sex. Brown trout do not possess obvious sex chromosomes, but a male-specific sex-determining locus (*sdy*) locus has been identified that is conserved across salmonids (Yano *et al.*, 2013). In theory, genes affecting migration tendencies or other sexually dimorphic traits could be inherited by males only if they are physically linked with the *sdy* locus, but this remains speculative.

Regardless of the provenance of the parents, there was a notable sex bias amongst the migrating offspring, where fish moving through the RRDT and the sea-entry were skewed towards females, whilst no sex bias was found within putative stayers captured via electrofishing in the Rough River. This is consistent with the general observation that females are more likely to undertake potamodromous or anadromous migrations in brown trout, and males to adopt a residency tactic (Ferguson *et al.*, 2017; García-Vega *et al.*, 2017; Huusko *et al.* 2018; Birnie-Gauvin *et al.*, 2019, see also Chapter four of this thesis). Females typically stand to gain more, in fitness terms, from migration to better growth opportunity habitats, as the resulting larger size facilitates the production of more and larger ova. Larger size can also be advantageous to the spawning success of males, but male fitness is generally less limited by body size than is female fitness (Fleming, 1996; Einum and Fleming, 1999; Nielsen *et al.*, 2003; Fleming and Reynolds, 2004). At a genetic level, sex differences in migration rates require either some type of sex linkage, or else differential gene regulation of autosomal genes in males and females. The *vgll3* locus that plays a major role in maturity timing in Atlantic salmon, for example, exhibits sex-dependent dominance patterns (Barson *et al.*, 2015). Particularly relevant in the current context, Pearse *et al.* (2019) found similar sex-dependent dominance at a “supergene” (double chromosomal inversion region) that plays a major role in mediating sex-dependent migration tendencies in rainbow/steelhead trout, *Oncorhynchus mykiss*. Similar major effect loci influencing migration have not been found in *Salmo trutta*, but a number of small-effect outlier genomic regions are evident (Lemopoulos *et al.*, 2018, 2019;

Moran et al. *Unpublished*), suggesting a more polygenic basis to alternative migratory tactics in this species compared to *O. mykiss*.

An additional key finding in this chapter was that movers, defined as those trout captured moving downstream through the RRDT, were consistently longer than stayers, defined as those trout captured by electrofishing within the Rough River. One important caveat here is that some of these so-called stayers might have eventually moved/migrated downstream, but the fact that size differences were maintained across the course of the experiment between trap-captured and electrofished fish is nonetheless striking. This result is unlikely to reflect a bias in catchability towards smaller fish with electrofishing equipment, as if anything the bias goes in the other direction (P. McGinnity, *pers. comm*.). Under the environmentally-cued threshold model of migration (Tomkins and Hazel, 2007), growth rate or some other aspect of energetic/physiological status is hypothesised to be the actual cue for migration, with faster growers becoming energy constrained sooner in life and hence forced to migrate (see Ferguson et al. 2019 and Nevoux et al. 2019 and references therein). Body size is often used as a proxy for individual growth, but this can be problematic as the relationship between growth rate and body size can change with age and hence body size at migration can be an unreliable surrogate for the actual cue triggering migration during critical ontogenetic windows (Ferguson et al. 2019). Moreover, body size can influence survival and capture probabilities, but this is often not taken into account in analyses. Acolas *et al.* (2012) used a capture–mark–recapture approach to show that growth was a better integrative parameter influencing migration probability in partially migratory brown trout, whilst body size was more important for survival. The fact that movers were consistently larger than stayers in our CGE suggests that they were not simply fish that were out-competed and displaced downstream, as larger juveniles tend to win out in territorial contests over smaller juveniles (Lahti *et al.*, 2002; Cutts *et al.*, 1998; Moreau *et al.*, 2014). Whilst initially faster growers might be more likely to adopt a migratory tactic, they can actually be larger than residents at the time of migration, as resources can be reallocated in migrants towards growth spurts prior to migration itself, as larger size may increase survival chances along the migration journey or in the new downstream habitats (Jonsson *et al.*, 2001; Reid *et al.*, 2005;

Hecht *et al.*, 2012; Nevoux *et al.*, 2019). For example, seaward-migrating smolts may need to reach a critical minimum size to survive the transition to marine habitats (Jonsson *et al.*, 2001; Jonsson and Jonsson, 2009; Bond *et al.*, 2015). The latter explanation would not explain why age 0+ and 1+ movers were larger than stayers of the same age, as they seemed to be migrating to downstream freshwater habitats (e.g. Lough Feeagh) rather than marine or brackish habitats at that stage. Still, early-life movers may allocate resources differentially towards growth versus maturation, for example, relative to putative residents (Ohms *et al.*, 2013; Archer *et al.*, 2019, 2020). Crucially, the movers were also found to be in lower condition, on average, than the stayers, which is consistent with recent studies that linked energetic phenotypes of salmonids to their migratory phenotype (Olsson *et al.*, 2006; Wysujack *et al.*, 2009; Boel *et al.*, 2014; Archer *et al.*, 2019, 2021). Genetic correlations have also been documented between growth-related phenotypes and alternative migratory tactics in salmonids (Thériault *et al.*, 2007; Páez *et al.*, 2011), suggesting these traits are linked at a functional level (Dodson *et al.*, 2013), but further molecular and physiological studies are required to characterise these complex linkages, as well as manipulative experiments to disentangle cause from effect.

The Burrishoole anadromous trout run, or “sea trout run”, collapsed in the late 1980s. The documented collapse was linked to sea lice infestations which coincided with the establishment of salmon aquaculture farms in the downstream estuary (Gargan *et al.*, 2003; Poole *et al.*, 2007). Although the exact spawning locations of the anadromous component of the Burrishoole catchment remain unclear, it has long been thought that the Rough River had been a major contributor to past sea trout runs, but other tributaries likely also contributed (J. Magee, unpublished PhD thesis). The results of the current study, which demonstrated a heritable basis to anadromy in brown trout, would suggest that at least some of this rapid life history shift away from anadromy may have been due to an evolutionary response. Rapid evolution in migratory tactics has been observed in other salmonid species such as *O.mykiss* (Pearse *et al.*, 2014). Furthermore, using a common garden experiment, Phillis *et al.*, (2016) was able to show how rapid and opposing changes in size-at-age and threshold size contribute to the contemporary evolution of migration tactics in *O.mykiss*. Together these results

indicate that individuals expressing anadromous migratory tactics that are impeded by migratory barriers, or biological barriers such as the sea lice, may be selected against and thus polymorphic populations may experience rapid evolutionary shifts towards a non-migratory or freshwater life history.

The Erriff catchment also witnessed changes in life history composition shortly after the establishment of commercial Atlantic salmon farms in the local estuary. Both the size and number of sea trout kelts, as well as the estimated numbers of eggs deposited by returning sea trout, significantly decreased after the farms were established (Gargan *et al.*, 2003, 2007, 2012, 2016), though not to the same extent as was observed in the Burrishoole population. Furthermore, Gargan et al., (2016) found a significant positive relationship between the number of sea lice present at the salmon farms and the number of sea lice present on anadromous brown trout caught in the Erriff catchment. These recent environmental changes within both the Burrishoole and Erriff catchments emphasise that phenotypic and demographic responses can occur rapidly in the face of environmental changes and anthropogenic activities via microevolution and/or phenotypic plasticity.

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Chapter Three: Alternative migratory tactics in brown trout (*Salmo trutta*) are underpinned by divergent regulation of metabolic but not neurological genes

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Running title: RNA-seq of alternative brown trout morphs

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Abstract

The occurrence of alternative morphs within populations is common but the underlying molecular mechanisms remain poorly understood. Many animals, for example, exhibit facultative migration, where two or more alternative migratory tactics (AMTs) coexist within populations. In certain salmonid species, some individuals remain in natal rivers all their lives, whilst others (in particular, females) migrate to sea for a period of marine growth. Here we performed transcriptional profiling ("RNA-seq") of the brain and liver of male and female brown trout to understand the genes and processes that differentiate migratory and residency morphs (AMT-associated genes) and how they may differ in expression between the sexes. We found tissue-specific differences with a greater number of genes expressed differentially in the liver (n = 867 genes) compared to the brain (n = 10) between the morphs. Genes with increased expression in resident livers were enriched for Gene Ontology terms associated with metabolic processes, highlighting key molecular-genetic pathways underlying the energetic requirements associated with divergent migratory tactics. In contrast, smolt-biased genes were enriched for biological processes such as response to cytokines, suggestive of possible immune function differences between smolts and residents. Finally, we identified evidence of sex-biased gene expression for AMT-associated genes in the liver (n = 12) but not the brain. Collectively, our results provide insights into tissue-specific gene expression underlying the production of alternative life-histories within and between the sexes, and point towards a key role for metabolic processes in the liver in mediating divergent physiological trajectories of migrants versus residents.

Keywords: alternative life histories, phenotypic plasticity, salmonids, smoltification, sex bias

Introduction

Discrete phenotypic variation, where individuals from the same population develop into two or more morphs, is common in nature (Roff, 1996; Chevin and Lande, 2013). Developmental switches between alternative phenotypes, such as males versus females (Van Dooren and Leimar, 2003), insect castes (Schwander *et al.*, 2010), trophic polymorphisms (Skulason and Smith, 1995), colour polymorphisms (Roulin, 2004), horn polyphenisms (Moczek and Nijhout, 2002) or mating types (Brockmann and Taborsky, 2008; Schunter et al. 2014) can reflect genetic or environmental determination, or some interaction between the two (Roff, 1996; Tomkins and Hazel, 2007; Chevin and Lande, 2013). Recent technical advances in genomics and bioinformatics (Alvarez, Schrey and Richards, 2015; Todd, Black and Gemmell, 2016) allow for novel insights into the molecular mechanisms and developmental plasticity underpinning the production of alternative phenotypes (Smith-Gill, 1983; West-Eberhard, 2003).

Alternative migratory tactics (AMTs), where both migratory and resident individuals coexist within a population (also known as partial, or facultative, migration), represent a particularly interesting type of discrete phenotypic variation that occurs in all major vertebrate groups and many invertebrates (Chapman *et al.*, 2011). The adoption of AMTs is triggered by environmental cues in interaction with genetically-inherited thresholds, with various selective mechanisms involved in the maintenance of AMTs and associated phenotypic variation across time and space (Tomkins and Hazel, 2007; Pulido, 2011; Buoro, Gimenez and Prévost, 2012). Anthropogenic influences can also drive rapid shifts in AMT frequencies via microevolution or phenotypic plasticity (Pulido, 2007; Thériault *et al.*, 2008; McCleave and Edeline, 2009; Phillis *et al.*, 2016), hence understanding the proximate and ultimate drivers of migration versus residency is a topic of increasing conservation and management relevance (Railsback, Harvey and White, 2014).

Diadromous migrations between fresh and saltwater habitats occur in many fishes (McDowall, 1997), which, on top of the rigours of migration itself, involve the added challenge of coping with transitions between different osmotic environments. For

example, salmonid fishes (salmon, trout, charr and relatives) often undertake anadromous migrations between freshwater spawning and marine feeding habitats. Species, populations and individuals vary markedly in rates of anadromy, as well as in the spatial extent and duration of marine migrations (Klemetsen *et al.*, 2003; Quinn and Myers, 2004). In populations where both anadromous and resident individuals occur, they freely interbreed and offspring inherit a propensity for one or other tactic as a polygenic threshold trait (Dodson *et al.*, 2013; Sloat *et al.*, 2014; Kendall *et al.*, 2015; Ferguson *et al.*, 2019). The benefits of anadromy, which include enhanced growth in the marine environment and avoidance of seasonally harsh freshwater conditions, are finely balanced against the costs, which include increased energetic expenditure, potentially greater mortality risks and less scope for early maturation (Hendry and Stearns, 2004; Pavlov and Savvaitova, 2008; Curry *et al.*, 2010; Dodson *et al.*, 2013; Sloat and Reeves, 2014; Kendall *et al.*, 2015; Ferguson, 2017; Ferguson *et al.*, 2019; Nevoux *et al.*, 2019). The life-history trade-offs involved also vary between the sexes, with females typically gaining more in reproductive success terms from the larger potential body sizes afforded by marine feeding. Females are thus more likely to adopt an anadromy tactic and males tend to retain a residency tactic, with males also exhibiting a broader range of ages and sizes at first reproduction (Jonsson and Jonsson, 1993).

Many genes are likely involved in the initial decision to adopt a migratory instead of a residency tactic (Hecht *et al.*, 2013, 2015), potentially including some major effect loci (reviewed by Ferguson *et al.*, 2019). Studies on rainbow/steelhead trout (*Oncorhynchus mykiss*), for example, have identified a migration-associated region (MAR) on chromosome *Omy5* that might harbour a “master control switch” for AMTs (Nichols *et al.*, 2008; Leitwein, Garza and Pearse, 2016; Pearse and Campbell, 2018; Arostegui *et al.*, 2019; Kelson *et al.*, 2019). This MAR involves a 55-Mb double-inversion that acts as a “supergene” influencing sex-specific migratory tendencies via sex-dependent dominance (Pearse *et al.*, 2019). Once an individual has “committed” to a particular AMT – a decision which might occur months or even years in advance of actual migration, depending on the species – a series of molecular changes are set in motion that channel residents and (future) migrants along divergent developmental

trajectories. Migrants and residents can only be differentiated externally at the smoltification stage, when migrants undergo morphological and physiological transformations in preparation for transition to the marine environment. However, internal differences in patterns of energy acquisition and allocation to competing functions, such as metabolism, growth and lipid storage, are apparent much earlier in the life history (reviewed in Dodson *et al.*, 2013; Ferguson *et al.*, 2019).

Complex gene regulatory mechanisms, perhaps involving one or more master regulators (Aubin-Horth, Letcher and Hofmann, 2009) and a series of epigenetic modifications (Baerwald *et al.*, 2016), likely control this phenotypic divergence. Theory on the evolution of developmental plasticity indeed suggests that morph-specific gene expression can decouple developmental pathways and reduce pleiotropic correlations among traits expressed in each morph (Snell-Rood *et al.*, 2010). For example, in *O. mykiss,* differential gene expression between migrant and resident offspring is evident in the brain a full year before smoltification (McKinney *et al.*, 2015). As smoltification approaches, migrants undergo phenotypic "remodelling", including changes in body shape and colour, behavioural changes and hormonal plus enzyme alterations (McCormick, 2012) that are underpinned by transcriptomic differences (Aykanat, Thrower and Heath, 2011; Sutherland *et al.*, 2014; Houde *et al.*, 2019). Complex genetic networks are involved that might include clusters of genes on particular chromosomes, e.g., *Omy12* in *O. mykiss* (Hecht *et al.*, 2012, 2013). Environmental-dependent methylation changes might also play a regulatory role in seawater acclimation of smolts (Morán *et al.*, 2013). Residents, in contrast, increasingly allocate more energy and resources to maturation processes, with males in particular often maturing earlier than (resident or anadromous) females (Jonsson and Jonsson, 1993; McMillan *et al.*, 2012; Archer *et al.*, 2019, 2020).

Here we use RNA sequencing ("RNA-seq") to explore differential gene expression between migrant and resident brown trout (*Salmo trutta*), a species exhibiting facultative anadromy as well as flexible freshwater migration strategies (Ferguson, 2017; Ferguson *et al.*, 2019; Nevoux *et al.*, 2019). Previous studies of AMTs in salmonids have focussed on the brain as a key organ that might regulate divergent phenotypic trajectories by integrating internal and external signals, and found

differential expression in genes associated with metabolism, morphology, olfactory imprinting, osmoregulation and sexual maturation (McKinney *et al.*, 2015; Hale *et al.*, 2016, 2018). In brown trout, genomic studies have found loci associated with variation in the distance of migration (Lemopoulos *et al.*, 2018, 2019), while previous transcriptomic work using cDNA microarrays using a restricted number of genes identified gene expression differences in the liver associated with migratory versus sedentary lifestyles (Giger *et al.*, 2006; 2008). We therefore focussed on the liver and brain as candidate organs mediating AMTs and smoltification processes in brown trout. Our first objective was to identify genes that are differentially expressed between individuals classified as immature smolts (anadromous migratory tactic) or mature non-smolts (residents, i.e., freshwater maturation tactic), here defined as putative "AMT-associated genes". To distinguish these from genes that are differentially expressed as a result of the general stress of a transition from fresh to salt water, we compared putative smolts against both fresh and salt water exposed mature individuals (i.e., residents). Second, we examined whether putative AMT-associated genes demonstrated conserved or tissue-specific expression profiles. Lastly, we asked whether genes differentially expressed between smolts and residents were also differentially expressed between males and females, given their divergent phenotypic trajectories with respect to migration.

2. Materials and Methods

2.1 Laboratory rearing of fish

Fish in our study were reared from the egg stage in a controlled laboratory environment as part of a broader study on genetic and environmental drivers of facultative anadromy (Archer *et al.*, 2019, 2020). Here, we sampled fish from a single population background and environment-of-rearing (i.e., single rearing tank, see Archer *et al.,* 2019, 2020 for full details on fish husbandry) to avoid confounding effects in the gene expression data. The sampled fish represent the offspring of four wild-caught females and five wild-caught males, with each female mated to two males; thus, we had eight full-sibling families nested within four maternal half-sibling families.

The wild-born parents were caught in November 2015 in Tawnyard Lough, a small upland lake in the western reaches of the Erriff catchment in the west of Ireland (53° 37′ 0.00″ N: 09° 40′ 17.10″ W). The lake is fed primarily by the Glendavoch river, within which there is extensive suitable spawning habitat for *Salmo trutta*, along with a series of smaller spawning tributaries. Tawnyard Lough produces a large run of anadromous smolts annually (Gargan *et al.*, 2016) but some juveniles remain within the lake and never go to sea, instead maturing in freshwater. It was not possible to distinguish clearly whether the wild-born parents in our study had been to sea or not, but the Tawnyard population, in general, shows high rates of anadromy (Gargan *et al.*, 2016; Archer *et al.*, 2019, 2020). Thus, we expected a genetic predisposition towards high rates of smolting in our tank-reared fish, but also for some individuals to adopt a freshwater maturation (residency) tactic.

2.2 Determination of smolt status

Over 22 months of tank rearing, 74 fish were routinely assessed for morphological indicators of smoltification, following the criteria of Tanguy *et al.*, (1994). Smolts undergo a change in colouration from dark to silvered flanks, lose colour in the fins and lose their parr marks (the dark traverse bands on the side of young salmonids). They also have pronounced lateral lines and a more fusiform body shape than parr (the juvenile freshwater stage prior to smoltificatoin of migrants). Sixteen putative

smolts were thus identified in early summer 2018 (at age 2+). Such morphological indicators are not always a reliable guide to saltwater tolerance capabilities in this highly phenotypically variable species (Klemetsen, 2013). We, therefore, subjected our putative smolts to a saltwater tolerance test to assess their hypo-osmoregulation capacities. Fifteen putative residents (fish showing no outward signs of smolting) were also subjected to the same saltwater tolerance test, to confirm their non-smolt status.

The trials involved transferring fish from their freshwater tank into another tank at a salinity of 30 parts per thousand (ppt) for 24-hours, a period sufficient to induce hypo-osmoregulation in euryhaline species (Schultz and McCormick, 2012), and then measuring their plasma chloride levels. Low plasma chloride concentrations indicate physiological tolerance to salt water (full details provided in Archer *et al*. 2019, 2020). A salinity of 30 ppt was used rather than full oceanic salinity (35-40 ppt) to minimise unnecessary stress on the fish, and also because wild brown trout often spend large amounts of time in estuaries during seaward migration (Ferguson *et al.*, 2019; Nevoux *et al.*, 2019). Immediately after 24-hr saltwater exposure, the fish were euthanized with an overdose of Tricaine mesylate (MS-222) and a blood sample was collected from the caudal vein using a 21 G needle and a 2.6 ml heparinized syringe. Blood samples were transferred to 2 ml Eppendorf tubes and centrifuged at 8,000 rpm for 3 min to separate the plasma from the erythrocytes and other cellular components. The plasma supernatant was then siphoned off via pipette into a new 1ml Eppendorf tube and stored at −80°C before being measured for plasma chloride concentration (mmol/L) by colometric titration using a Jenway PCLM3 chloride meter (FishVet Group, Oranmore, Ireland).

Given their expected poorer hypo-osmoregulatory abilities, residents were expected to experience higher stress levels than smolts as a result of this acute exposure to saltwater. Thus, comparing gene expression differences between smolts and residents after saltwater testing might confound life history (AMT) differences with stress differences (Fig. 1). To explore this, we also sampled (see below) an additional group of residents (n = 9) who were not subjected to the saltwater challenge but remained within their original freshwater tank and hence experienced similar, or

slightly lower, stress levels than the smolts (who might have experienced mild osmotic stress during the saltwater trial).

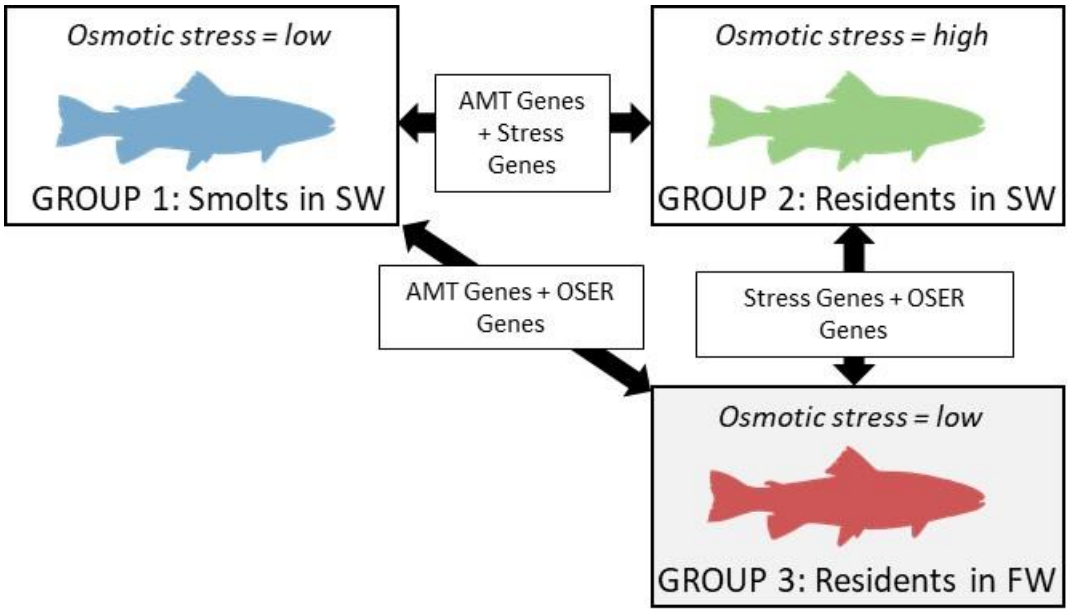


Fig. 1. Identification of putative AMT genes in brown trout. Panel schematic representation of experimental design and associated logic. Livers and brains of trout deemed to have adopted an anadromous tactic (smolts) were RNA-sampled immediately following a 24-hr saltwater (SW) challenge (group 1 = smolts-SW). Livers and brains of a second group of trout deemed to have adopted a resident tactic were sampled following the same SW challenge (group 2 = residents-SW), while a third group were sampled at the same time in fresh water (FW) without having undergone a saltwater challenge (group 3 = residents-FW). Note that smolts could not be tested in FW prior to being subjected to a SW challenge (which is required to define a fish as a smolt or not) because liver and brain sampling is terminal for the fish. Because smolts are by definition physiologically tolerant of SW, group 1 experienced mild osmotic stress. Residents are physiologically intolerant of SW but well suited to life in FW, hence group 2 experienced high osmotic stress while group 3 experience low osmotic stress. Black arrows represent genes that are differentially expressed between each pairwise comparison. AMT genes refer to those that are differentially expressed between the alternative life history tactics and potentially encompass genes involved in morphology, behaviour, physiology (including saltwater tolerance) and reproduction. Stress genes here refer to those for which differential expression between osmotic environments reflects the general stress of transitioning from FW to SW. Here OSER genes (osmotic environmental response genes) refers to genes which would be differentially expressed in response to different environmental salinity.

Fish from all three groups (putative smolts exposed to salt water, putative residents exposed to salt water, putative residents not exposed to salt water) were terminally sampled in early summer 2018 and dissected to determine maturation status and extract liver and brain tissue for transcriptional profiling. Before tissue extraction, every

fish was measured for fork length (to the nearest mm) and weight (to the nearest g) and a small section of tail fin tissue was removed using a sterilized scalpel and stored in 100% ethanol for subsequent DNA analysis (to determine genotypic sex, see below). Each dissection session took place at the same time each morning in an attempt to minimise among-individual variation in gene expression related to circadian rhythms. Visual gonad inspections were performed first and then whole brains and livers were removed and transferred into individual sterile 2ml Eppendorf tubes containing 1ml of RNALater solution. Samples were incubated at 4°C for 24 hours to allow RNALater to permeate through tissues, and then transferred to -80°C storage.

Females were classified as sexually mature if the body cavity was filled with identifiable eggs, whereas males were classed as sexually mature if they had enlarged, white testes, or were running milt. A fish was classified as immature if gonads were undeveloped (see Archer *et al*. 2019 for full details). This maturation status information, coupled with the morphological and physiological evidence for smolting, then allowed us to define three final groups of individuals for whom smolt status (morphological signs of smolting present, low plasma chloride, reproductively immature) or resident status (morphological signs of smolting absent, high plasma chloride if saltwater tested, reproductively mature) could be confidently assigned. From this, we concluded that seven of the salt water tested individuals could be classified as smolts. Which resulted in a final sample set consisting of seven saltwater tested smolts (“smolt-SW”; total n=7: 3 males, 4 females), nine saltwater-tested residents (“resident-SW”; n=9: 5 males, 4 females) and nine freshwater (non-saltwater-tested) residents (“resident-FW”; n=9: 5 males, 4 females). Fish in our smolt-SW group had lower average plasma chloride concentrations (mean = 129.6 mmol/L, standard deviation, sd = 12.6) compared to fish in our resident-SW group (mean = 159.2 mmol/L, sd = 13.9; unpaired t-test: t = 4.4, $P < 0.001$, df = 13.49), confirming the former had adopted a migratory tactic.

2.3 RNA extractions

Total RNA was extracted from each individual brain and liver sample using TRI Reagent (Sigma, Aldrich) and subsequently purified with the GenElute™ Mammalian

Total RNA MiniPrep kits (Sigma Aldrich), following manufacturer's instructions. We DNase-treated each sample using the Turbo DNA-free kit (Qiagen, UK) to remove residual DNA. We initially assessed the quality and quantity of purified RNA using the Nanodrop 2000 (Thermo Fisher, USA) and the Qubit RNA Broad Range assay kit with a Qubit 4 Fluorometer (Thermo Fisher, USA), respectively. Finally, we calculated a relative integrity number (RIN) per sample using an Agilent TapeStation 4200 (Agilent, USA) to ensure purified RNA was not degraded and we only sent samples with a RIN of seven or higher (Sigurgeirsson, Emanuelsson and Lundeberg, 2014) for subsequent library preparation and sequencing.

2.4 Library preparation and sequencing

We shipped samples (n = 49; 24 livers and 25 brains) to Novogene Europe (Cambridge, UK) for mRNA-enriched library preparation and sequencing. In brief, DNase-treated RNA quality was secondarily assessed by TapeStation at Novogene to ensure quality was high and no degradation occurred during transport. For each sample, an individual mRNA-enriched library was generated using the NEBNext Ultra II RNA library preparation kit (NEB, UK). Individual libraries were barcoded with specific identifiers, multiplexed and sequencing (150bp paired-end (PE)) was performed on an Illumina Novaseq6000 generating a total of ~1.29 billion PE reads. For liver samples, the total number of PE reads were ~590 million (mean: ~24.6 million, min: ~20.3 million, max: ~35.4 million) while for brain samples ~699 million reads were generated (mean: ~27.9, min: ~20.4 million, max: ~42.4 million). Sample information, including the total number of paired-end reads generated per sample, is provided in Supporting information Table S1.

2.5 Sequence data quality assessment

To assess the quality of our raw reads, we used FastQC (version 0.11.3; Andrews, 2010) to identify the presence of sequences with low base quality and/or adapter contamination. Overall, FastQC indicated high sequence quality. Following this, we estimated transcript abundance for each sample through quasi-aligning reads with Salmon (version 1.2.0; Patro *et al.*, 2017) against predicted transcripts (cDNA sequences) from the publicly available *Salmo trutta* reference genome assembly

(fSalTru1.1, INSC Assembly GCA_9010011651.1) obtained from Ensembl. Using these estimates, we generated summarised gene-level counts per individual sample using tximport (version 3.10; Soneson *et al* 2015), which were then imported into DESeq2 (version 3.10; Love, Huber and Anders, 2014) for differential expression analysis. For the purpose of reanalysis, gene-level counts are provided in Supporting information Table S2. For differential expression and Gene Ontology enrichment analyses, we used modified scripts initially developed by Colgan *et al.* (2019).

2.6 Differential expression analyses

To first get a broad sense of global differences in transcriptional profiles across our three groups, we ran a principal component analysis (PCA) on variance stabilising transformed gene-level counts using DESeq2. Given expected differences in transcriptional profiles between tissues, we performed independent PCAs for liver and brain samples using the plotPCA function in DESeq2.

For each tissue, we next performed gene-level tests for differential expression between comparisons of interest using DESeq2. We first defined a global model with main effects of “phenotype” – a two level factor indicating whether the individual fish was a smolt or resident – and “testing environment” – a two level factor indicating whether the fish was exposed to the 24-hour saltwater test just prior to terminal sampling (salt), or not (fresh), as explanatory variables. Note that phenotype and testing-environment are not perfectly crossed, as all smolts were salt-water tested (which was necessary to confirm their smolt status in physiological terms), whereas the residents fell under either salt or fresh for testing environment (Fig.1). This global model was then compared against two reduced models, one which contained only a main effect of environment, and one which contained only a main effect of phenotype. This enabled us to generate *P* values, on a gene-by-gene basis, for the effects of phenotype and environment. We then defined differentially expressed genes as those where the absolute log2 fold change was greater than one, and the Benjamini-Hochberg adjusted *P* values were less than 0.05. This, therefore, yielded two lists of genes: those differentially expressed between smolts and residents, and those differentially expressed between salt- versus freshwater testing-environment.

To isolate the effect of phenotype *per se*, we identified the unique subset of genes that were differentially expressed between smolts and residents only and not also between osmotic testing environments. Hereafter we refer to these genes as "AMT-associated genes". Similarly, genes that were differentially expressed between salt- versus freshwater testing-environments, but not also between smolts and residents, were classified as "osmotic environment response genes". Genes that were differentially expressed between both comparisons were labelled as "stress genes". For each set of genes, we also used heatmaps and scatter plots (using the ggplot2 package, version 3.3.2 in R) to explore and illustrate the patterns of (z-score normalised) gene expression with respect to our three groups, i.e., smolt-SW, resident-SW and resident-FW, for livers and brains separately.

Finally, we tested for each tissue whether any of our AMT-associated genes or osmotic environment response genes were also differentially expressed between males and females. To achieve this, a DESeq2 model with a main effect of sex was compared against a null model, with no effects. We defined significantly differentially expressed genes as before (i.e., absolute logFC > 1 between males and females and BH-adjusted $P < 0.05$). We then identified which of these sex-biased genes had also been classified as AMT-associated genes or osmotic environment response genes in the above analyses.

2.7 Gene Ontology term enrichment analyses

For each gene in the *S. trutta* reference genome, we used the functional annotations assigned to the corresponding orthologs in the *Danio rerio* (zebrafish) genome, obtained from Ensembl BioMart (Kinsella *et al.*, 2011), as very little functional information exists for brown trout genes. For both the smolt-biased and resident-biased AMT-associated genes, we tested for enrichment of Gene Ontology (GO) terms using Fisher's exact tests in topGO (version 3.10, Alexa *et al.* 2006), applying the "weight01" algorithm and a node size of 50. We corrected for multiple testing using the Benjamini-Hochberg method to generate adjusted *P* values. As a means of understanding more general processes enriched with differentially expressed genes, we also subset higher level GO terms of significantly enriched GO terms (e.g., Level

Two GO terms that are child terms of each of the three main GO subcategories of ‘Biological Process’, ‘Molecular Function’ and ‘Cellular Component’).

Results

3.1 Distinct AMT-associated transcriptional profile in liver but not brain

For the livers, PCA demonstrated a clear separation of samples based on gene expression with respect to our three groups, i.e., combinations of phenotype and testing-environment (Fig. 2A). The first principal component (PC1) accounted for 21% of the total variance and primarily separated resident-SW individuals, who likely experienced high osmotic stress, from resident-FW, who presumably experienced low osmotic stress, with smolt-SW individuals intermediate. In contrast, PC2, which accounted for 15% of the total variance, primarily separated smolt-SW individuals from residents (Fig. 2A). The combination of PC1 and PC2 separates smolts from residents, with smolts having high values for both PC1 and PC2, but residents having lower PC2 and a range of PC1 values. In contrast, the PCA on the brain samples did not produce clear separation among the three groups based on the first two principal components (Fig. 2B), nor PC3-6 (result not shown).

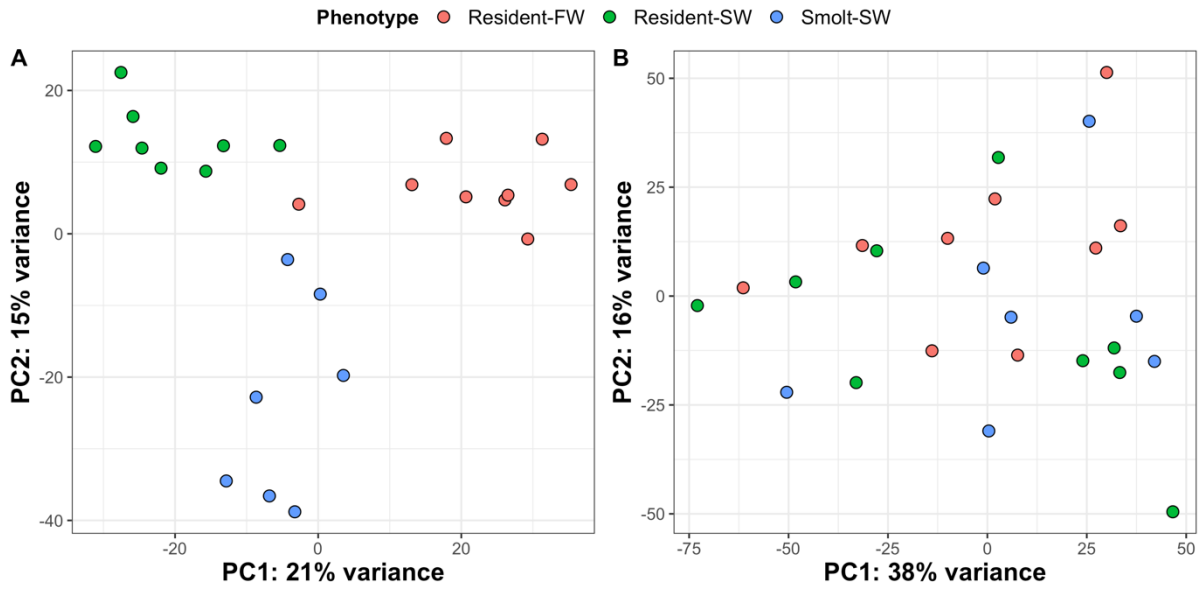


Fig. 2. Distinct transcriptional profiles between resident and smolt livers but not brains. Scatterplots displaying the results of a principal component analysis (PCA) for normalised gene expression values for **(A)** livers and **(B)** brains collected from three

groups differing in phenotype and/or environment. The proportion of variance within gene expression explained by principal component 1 (PC1) and 2 (PC2) in displayed on the x- and y-axes, respectively. Here each group is a categorical variable corresponding to the combination of the individuals phenotype (life history tactic) and osmotic environment the individual experienced for 24-hrs just prior to terminal sampling (salt water or fresh water).

3.2 Liver AMT-associated genes are enriched for metabolic process-related GO terms

We identified 1,670 genes that were significantly differentially expressed (LRT, BH-adjusted $P < 0.05$) between the livers of smolts and residents. A total of 3,426 genes were differentially expressed between fish sampled in salt- and freshwater testing-environments (global model versus reduced model with phenotype effect only). We found 867 genes that were uniquely differentially expressed between the phenotypes but not osmotic testing-environments, which we designate as potential AMT-associated genes. The output of both models, as well as the different subcategories of genes, are provided in Supporting information Table S3.

Of the 867 putative AMT-associated genes (Fig. 3), we identified 430 genes with increased expression in residents relative to smolts, while 437 genes showed increased expression in smolts relative to residents (Supporting information Table S3). The resident-biased genes were enriched (BH-adjusted $P < 0.05$) for 21 GO terms, including 13 terms related to “biological process” (Fig. 4) with the majority categorized under “metabolic processes” (Supporting information Table S4). The three most significant terms were steroid biosynthesis process (GO:0006694), oxidation-reduction process (GO:0055114) and cholesterol metabolic process (GO:0008610) (Supporting information Table S4). In contrast, the 437 genes with smolt-biased expression were significantly enriched for 14 GO terms. The majority of these enriched terms (n = 12) were “biological process”-related GO terms with the three most significant GO terms being response to cytokine (GO:0034097), positive regulation of cell differentiation (GO:0045597) and endothelial cell migration (GO:0043542)(Fig. 4). These terms form part of GO term hierarchies with higher level terms, such as cellular

processes, response to stimulus and metabolic processes (Supporting information Table S4).

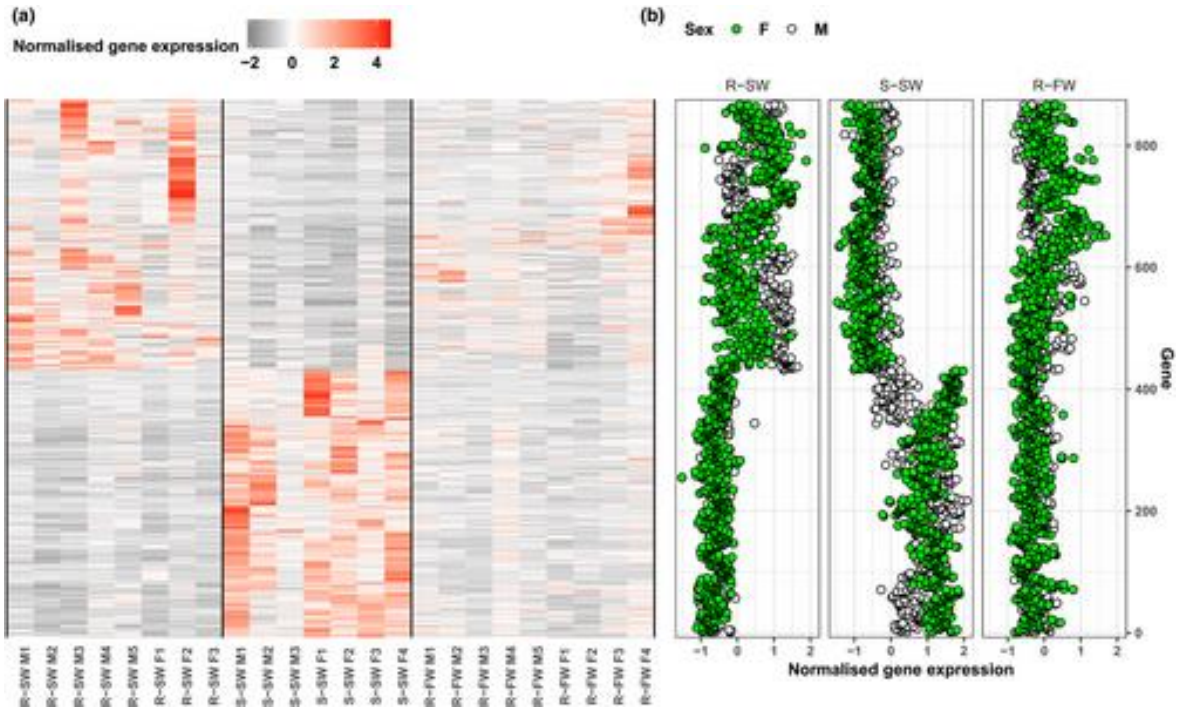


Fig. 3. Putative AMT genes expressed in the brown trout liver. (A) Heatmap displaying the normalised gene-level expression estimates for 867 genes that were uniquely differentially expressed between the livers of resident and migrant brown trout. Each row represents a single gene (y-axis) while for each sample (x-axis), the sample name provides information on phenotype (R = “resident”, S = “smolt”), environment of sampling (SW = “saltwater”, FW = “freshwater”) and sex (M = “male”, F = “female”). **(B)** Scatterplots showing the mean normalised gene expression for each sex within each of three groups (R-SW = “resident saltwater”, S-SW = “smolt saltwater”, R-FW = “resident freshwater”). Each point represents an individual gene and is coloured by sex (green = “female”, white = “male”).

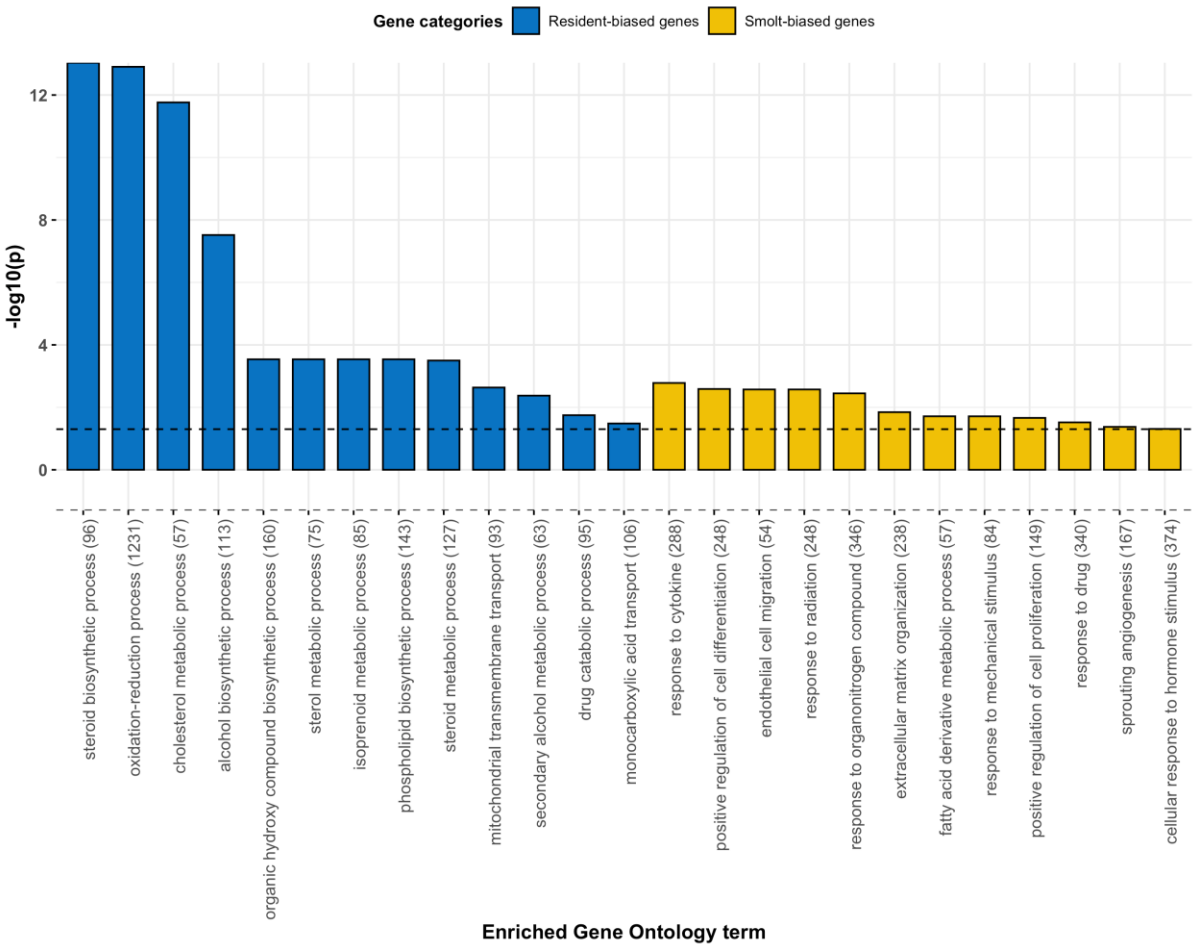


Fig. 4. Gene Ontology enrichment analysis of putative AMT genes in brown trout liver. Enriched Gene Ontology associated with biological process-related terms for genes differentially expressed between alternative life histories. Each bar represents the -log10 of *p* value (Fisher's Exact test) for each GO term with description of GO term, as well as the total number of annotated genes per term provided. The black vertical dashed line represents a -log10(*p*) value equivalent to a *P* value = 0.05 threshold of significance, with bars crossing this threshold being significant.

3.3 Fewer transcriptional changes in brain underlying AMTs

We identified 53 genes that were significantly differentially expressed between the brains of smolts and residents (Supporting information Table S3C). We found 716 genes that were differentially expressed between salt- and freshwater testing-environments (Supporting information Table S3D), and 43 genes that overlapped

between both comparisons. Thus, ten genes were uniquely differentially expressed between the phenotypes but not testing-environments (i.e., brain AMT-associated genes).

Seven of these genes exhibited elevated expression in smolts compared to residents; Sox18 (ENSSTUG00000006705), protein phosphatase PTC7 (ENSSTUG00000008660), E3 ubiquitin-protein ligase RNF38-like (ENSSTUG00000023791), N-acetyltransferase 8-like gene (ENSSTUG00000014735), BUB3 mitotic checkpoint protein (ENSSTUG00000036930), a zinc finger protein 239-like (ENSSTUG00000002250) and an uncharacterised gene (ENSSTUG00000047265). There is an annotated ortholog for this latter gene in *Salmo salar* where it has been described as an ankyrin-3-like (ENSSSAG00000039215). The three genes with increased expression in the brains of residents compared to smolts were a zinc finger and BTB domain-containing protein (ENSSTUG00000030799), sperm tail PG-rich repeat containing protein (ENSSTUG00000039823) and plexin-A1(ENSSTUG00000046884). These genes were not significantly enriched (BH-adjusted $P > 0.05$) for any specific GO term. Furthermore, no overlap was identified between brain and liver AMT-associated genes.

3.4 Sex-biased gene expression in certain AMT-associated genes

We identified evidence of sex-biased gene expression in the liver with 103 genes significantly differentially expressed between males and females (Supporting information Table S5). Of these genes, 12 were also differentially expressed between smolts and residents. We found no significant enrichment (BH-adjusted $P > 0.05$) for GO terms for these 12 genes. The analysed transcriptome as a whole comprised 44,366 transcribed genes, of which 0.23% (103/44,366) exhibited sex-biased expression, whereas just over 1% of our AMT-associated liver genes (12/867) exhibited sex-biased expression. Thus, there was a statistically significant excess of sex-biased AMT-associated genes in comparison to the overall proportion of sex-biased genes genome-wide (chi-squared = 45.7, df = 1, $P < 0.001$). Amongst these 12 sex-biased AMT-associated liver genes, six exhibited elevated expression in residents

relative to smolts and six demonstrated smolt-biased expression. The majority of these genes (n = 9) were also elevated in females relative to males, with the sex differences being typically larger in smolts than residents (Fig. 5). The remaining three sex-biased AMT-associated liver genes had higher expression in male residents compared to female residents.

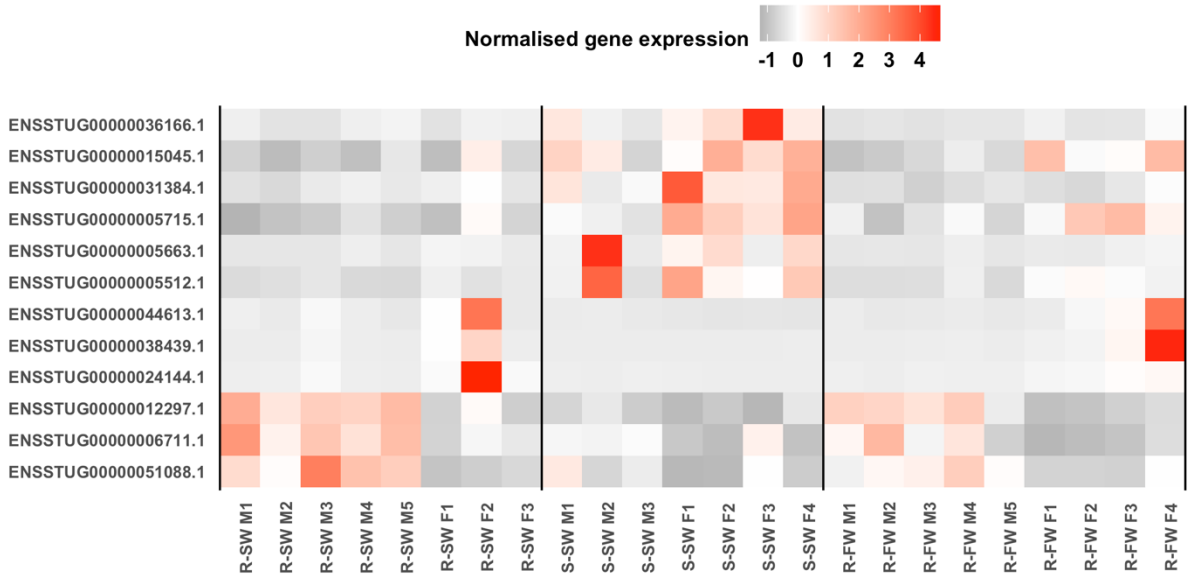


Fig. 5. Sex-biased expression of putative AMT genes. Heatmap displaying the normalised gene-level expression estimates for 12 putative AMT genes that also demonstrated sex-biased gene expression within the liver. For each gene, the Ensembl gene ID is provided (y-axis) while for each sample (x-axis), the sample name provides information on phenotype (R = “resident”, S = “smolt”), environment of sampling (SW = “saltwater”, FW = “freshwater”) and sex (M = “male”, F = “female).

We identified eight genes in the brain that exhibited sex-biased gene expression. However, we found no overlap between these genes and the ten AMT-associated genes that differed between the brains of smolts and residents.

Discussion

Here we compared whole-genome transcriptomic profiles of sea-migratory versus resident brown trout during the presumed migration period, to investigate molecular processes underpinning the generation of well-integrated, ecologically-important, alternative phenotypes. Our study revealed three key findings: 1) extensive transcriptional differences between migratory and resident brown trout for genes underlying metabolic, cellular and immune processes (particularly genes enriched for GO terms associated with metabolic processes, which had the lowest adjusted *P* values; Fig. 4); 2) tissue differences in expression profiles between migrants and residents with distinct life-stage specific patterns evident in the liver but not brain; and 3) sex-biased expression differences in genes associated with AMTs. The genes we identify may be downstream targets of master regulatory genes proposed to trigger AMTs and allow for the transition between aquatic environments; alternatively, they may represent non-target effects of physiological interactions unrelated to migration.

Animal migration can take place over long distances and last prolonged periods requiring dynamic changes in behavioural, physiological, morphological and biochemical aspects of an organism's phenotype. In metabolic terms, migration utilises substantial energetic resources (Wikelski *et al.*, 2003), which likely require tight regulation during such extended periods of metabolic stress. A key organ involved in such regulation is the liver. Here we identified distinct transcriptional profiles between migrants and residents with genes differentially expressed between these alternative phenotypes enriched for Gene Ontology terms involved in a range of key metabolic processes (Fig. 4), including steroid biosynthesis, cholesterol biosynthesis, phospholipid and fatty acid metabolism, with a general reduction in expression of such genes within migrants compared to residents. These findings are in line with previous molecular and biochemical research performed on other salmonids that identified changes in glycogen stores due to the energetic demands of migration, as well as changes in gene expression underlying lipid remodelling during transition from a freshwater to a saltwater environment (Gillard *et al.*, 2018). On top of changes in genes associated with lipid metabolism, we also identified additional metabolic processes that differ between migrants and residents. Such changes may also be related to

differences in reproductive maturity status, whereby energetic demands vary due to residents being fully mature reproductively, while for migrants (who were the same age but immature), reduced expression of metabolic genes may be associated with suppressed reproductive development.

The above findings tally with previous work on brown trout, and on other salmonids exhibiting AMTs, that has established links between various aspects of an individual's physiological condition and its migratory status (reviewed by Dodson *et al.*, 2013; Sloat *et al.*, 2014; Kendall *et al.*, 2015; Ferguson *et al.*, 2017, 2019). For example, energy limitation owing to poor food availability is associated with higher rates, or earlier ages of, migration (Olsson *et al.*, 2006; Wysujack *et al.*, 2009; O'Neal and Stanford, 2011; Morán *et al.*, 2013; Jones, Bergman and Greenberg, 2015; Archer *et al.*, 2019, 2020; Shry *et al.*, 2019), and the energy value of food may also be important (Kendall *et al.*, 2015). Metabolic rate, and how it links to growth efficiency and energy allocation to competing functions (e.g., lipid storage; (Jonsson and Jonsson, 2005; Boel *et al.*, 2014)), is further believed to underpin these associations (Forseth *et al.*, 1999; McCarthy, 2000; Seppänen, Piironen and Huuskonen, 2010; Norin and Malte, 2011; Sloat and Reeves, 2014; Archer *et al*. *In Press*). Our study found that the most differentially expressed gene between smolts and residents was *serine/threonine-protein kinase* (*SBK1*). This gene has elevated expression levels in an *O. mykiss* line selectively bred for fast growth and has been linked with growth hormone-mediated physiological pathways (Cleveland, Gao and Leeds, 2020). Although faster growing parr have a higher propensity to adopt residency, in many salmonid species, the relationships between growth/body size and AMTs are not always consistent (reviewed by Dodson *et al.*, 2013; Ferguson *et al.*, 2019). Another gene of interest was *desert hedgehog gene* (*DHH*) that showed higher expression in smolts compared to residents. Genes in the hedgehog pathway have been associated with tissue growth/regeneration and developmental changes in teleost fishes (Sims, Eble and Iovine, 2009; Marí-Beffa and Murciano, 2010), including aspects of fin growth (Iovine 2007). This is noteworthy as migrants go through a series of morphological changes (e.g., body streamlining, changes in fin shape and colour) during smoltification.

As physiological influences on, or consequences of, life history decisions, may be mediated by hormones, e.g., glucocorticoids (Peiman *et al.*, 2017; but see Jain-Schlaepfer *et al.*, 2018), or by antioxidant capacity (Birnie-Gauvin *et al.*, 2017), our results implicate the liver as an important organ involved in the genetic regulation of these processes. This bolster and expands upon earlier work on *S. trutta*, which based on cDNA microarrays screens of liver samples, found that almost 21% of 900 screened genes were differentially expressed between sedentary and migratory populations (Giger *et al.*, 2008, but also see Giger *et al*., 2006). These included the candidate genes *endozepine* and *transaldolase 1* that are involved in lipid metabolism in the liver, which were upregulated in residents, and the constitutive heat-shock protein HSC70-1, which was upregulated in smolts (Giger *et al.*, 2008). Our study also found that *transaldolase 1* was upregulated in the livers of residents when compared to the livers of smolts, further supporting these findings.

Aside from differences in genes associated with metabolism, smolt-biased genes were enriched for Gene Ontology terms associated with cell proliferation and regulation, and response to stimulus. Migrants face a number of threats when moving between environments, including exhaustion, predation, as well as novel encounters with marine-based pathogens and disease. While immunity is metabolically costly to constitutively express and actively use, it is hypothesised that migrants will boost their immune potential in preparation for novel pathogen encounter during migration (Møller and Erritzøe, 1998; Buehler, Tieleman and Piersma, 2010). Within our study, the most significantly enriched Gene Ontology term for smolt-biased genes was response to cytokines, which suggests changes in the immune profile of migrants. Cytokines are a broad range of small proteins with immunomodulatory capacities (Kishimoto, Taga and Akira, 1994). While the higher hierarchical GO term for response to cyktokines was ‘response to stimulus’, rather than “immune system process”, cytokines are originally produced in response to antigens and hence a link to immune function can be inferred here. Genes falling under the GO term response to cytokines included a number of immune genes with elevated expression in the liver of migrants including *macrophage mannose receptor 1*, *macrophage capping protein*, *LDL receptor related protein 2*, *interleukin 13-receptor*, *lipopolysaccharide-induced tumor necrosis factor*

alpha, as well as seven genes with putative roles in the complement pathway, a key component of the innate immune system that functions in pathogen removal, opsonization and promotion of inflammation. While the fish in our study were not maintained in sterile conditions and we would expect some form of background immune expression, differences in immune regulation between alternative life-history phenotypes may be associated with preparation for travelling to, and residing, in a marine environment. Alternatively, given the energetic trade-offs between immunity and reproduction (Roff, 1992), increased immune potential in migrants may be indirectly related to reduced reproductive investment.

In comparison to the liver, we identified fewer differentially expressed genes in the brain indicating unique tissue-specific profiles that differ between alternative life-history phenotypes. This was surprising, given the brain's proposed role as a control centre for salmonid AMTs via hormonal cascades (Aubin-Horth, Letcher and Hofmann, 2009; Dodson *et al.*, 2013). Indeed, previous research on rainbow trout (*Oncorhynchus mykiss*) (McKinney *et al*. 2015, Hale *et al*. 2016, 2018) identified larger transcriptional differences in the brain between migrants and residents, associated with a range of physiological processes, including phototransduction and circadian rhythm. Here, although we found no enrichment of specific biological processes, there were candidate genes of interest. For example, of the ten genes differentially expressed between migrants and residents, three were annotated as transcription factors, including two zinc finger domain-containing proteins and transcription Sox18A. Transcription factors, such as *vgll3* and *six6* (Barson *et al.*, 2015; Czorlich *et al.*, 2018), have been linked recently to the age of maturity of *Salmo salar*, demonstrating the importance such genes play in the expression of complex phenotypes. While the exact function of the transcription factors identified in our present study are currently unknown, future functional studies may elucidate their role, if any, in migration within brown trout or other species.

For certain facultative salmonids, such as brown trout and rainbow trout, sexes can differ in their rates of anadromy, with females often gaining more, in fitness terms, from anadromy than males (Gross, Coleman and McDowall, 1988; Ohms *et al.*, 2013; García-Vega, Sanz-Ronda and Fuentes-Pérez, 2017; Huusko *et al.,* 2018). Within a

given life history tactic (e.g., anadromy or residency), the sexes can also differ in behaviour, morphology and physiology; for example, anadromous females may spend longer at sea than anadromous males (Thorstad et al. 2016). Given these expected phenotypic differences between the sexes, we examined genes that were differentially expressed both between males and females but also life-history phenotypes and found 12 such genes that were differentially expressed in the liver (out of a total of 103 sex-biased liver genes). While we identified no enrichment of Gene Ontology terms for these 12 genes, one possibility is that they play indirect roles in sex-specific maturation processes (Rossignol, Dodson and Guderley, 2011), given that our smolts were all immature and our residents mature. In mice (*Mus musculus*), for instance, glucocorticoid receptor genes in hepatocytes regulate the expression of sets of genes involved in growth and sexual maturation (Engblom *et al.*, 2007). Sex-biased gene expression in the liver has been previously shown for other salmonids, such as brook charr, *Salvelinus fontinalis* (Sutherland *et al.*, 2018). The lack of observed sex biases in gene expression in the brains of brown trout in our study is surprising given the noted behavioural and hormonal differences between the sexes. Given the cellular complexity of the brain in comparison to the liver, which is more homogenous, our sequencing of the entire brain may have reduced the ability to detect subtle, sub-region specific, differences in gene expression between the sexes. Future studies will benefit from more targeted profiling of specific sections of the brain to elucidate fine-scale differences between the sexes.

Developmental switches in salmonids (Thorpe *et al.*, 1998; Mangel and Satterthwaite, 2008; Dodson *et al.*, 2013; Arostegui *et al.*, 2019) are thought to be triggered by differential expression of one or more 'master regulator' genes early in ontogeny (at least in *O. mykiss*); these in turn orchestrate divergent physiological cascades, likely hormonally-mediated, that result in AMTs (Aubin-Horth, Letcher and Hofmann, 2009; Dodson *et al.*, 2013). A major challenge in these studies is in distinguishing cause from effect. On the one hand, observed gene expression differences between tactics could be the actual cause of physiological changes that originally triggered alternative phenotypes, with these 'decision genes' potentially remaining switched on (in one tactic) from that point onwards. On the other hand, they may simply reflect

'downstream' physiological consequences of earlier activation of master regulators that are only expressed during an early sensitivity window (Dodson *et al.*, 2013; Ferguson *et al.*, 2019). Studies that examine gene expression at older ages (e.g., our current study; Giger *et al.*, 2006, 2008; Seear *et al.*, 2010; Aykanat, Thrower and Heath, 2011; Norman, Ferguson and Danzmann, 2014; Sutherland *et al.*, 2014) might be more likely to identify genes whose differential regulation follow from, rather than causes, the adoption of AMTs. This may be particularly true for organs such as the liver, kidney or gills, which play diverse roles in physiologically responding to hormonal cascades that likely begin in the brain, for example via the light-brain-pituitary axis (Ebbesson *et al.*, 2003). Identifying the actual master regulators is much more difficult and future studies will benefit from time-series of gene expression and profiling of associated phenotypic changes across ontogeny (Dodson *et al.*, 2013). Interestingly, in a comparison of two-year old *O. mykiss* smolts against same-age residents, Baerwald *et al.* (2016) found 57 differentially methylated regions, over half of which were in transcriptional regulatory regions. This suggests a role for epigenetic regulation of gene expression in mediating AMTs, but it remains unknown when these methylation changes are triggered and by what mechanisms.

Conclusions

Our study represents an important step towards understanding molecular mechanisms underlying alternative life history tactics and the regulatory roles played by different organs. Our list of candidate genes showing differential expression between migratory phenotypes and/or the sexes in brown trout could be considered by future salmonid studies that aim to disentangle molecular processes activating developmental switches from those that follow from their activation. This knowledge should inform efforts to conserve wild or cultured populations of facultatively migratory salmonids and other taxa of ecological or economic importance (e.g., Robinson *et al.*, 2016) and their capacity for evolutionary or plastic responses to anthropogenic change (Mangel and Satterthwaite, 2008; Thériault *et al.*, 2008; Railsback, Harvey and White, 2014).

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Ethical statement

All fish were euthanized humanely at the end of the laboratory rearing phase under license. 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.

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Data availability

Raw sequence data files are deposited in the NCBI short read archive (Accession ID: PRJNA670837). Scripts underpinning the analysis of transcript estimation, differential expression, and Gene Ontology term enrichment are for the purpose of review included in Supporting information. Upon publication, scripts will also be hosted on a public repository on GitHub (https://github.com/Joscolgan/salmo_smolt_study). Raw sequence counts for each sample are provided in the Supporting information.

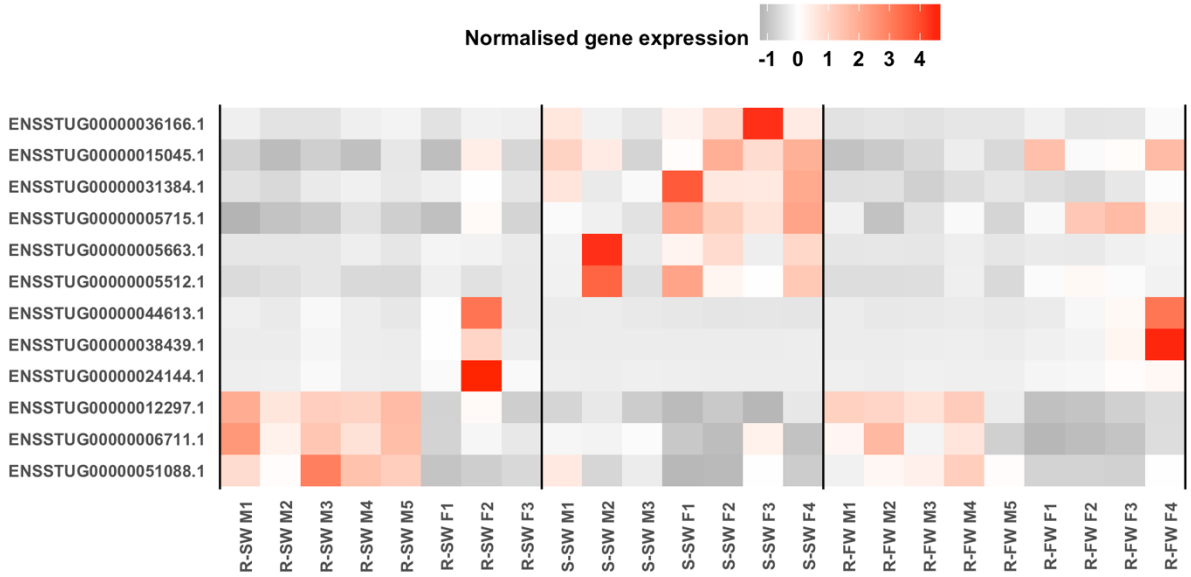
Author Contributions

RW, PAM, PMcG, TJC and TER conceived the study. LA, RW, SH, LH and TER collected data and contributed to experimental design. PG collected and managed broodstock. JC performed microsatellite genotyping. ED managed equipment and laboratory. RW, TJC and TR conducted analyses and wrote the manuscript. All authors contributed to interpretation of results and revisions of the manuscript.

Figures

Fig. 2. Distinct transcriptional profiles between resident and smolt livers but not brains. Scatterplots displaying the results of a principal component analysis (PCA) for normalised gene expression values for **(A)** livers and **(B)** brains collected from three groups differing in phenotype and/or environment. The proportion of variance within gene expression explained by principal component 1 (PC1) and 2 (PC2) in displayed on the x- and y-axes, respectively. Here each group is a categorical variable corresponding to the combination of the individuals phenotype (life history tactic) and osmotic environment the individual experienced for 24-hrs just prior to terminal sampling (salt water or fresh water).

Fig. 3. Putative AMT genes expressed in the brown trout liver. (A) Heatmap displaying the normalised gene-level expression estimates for 867 genes that were uniquely differentially expressed between the livers of resident and migrant brown trout. Each row represents a single gene (y-axis) while for each sample (x-axis), the sample name provides information on phenotype (R = “resident”, S = “smolt”), environment of sampling (SW = “saltwater”, FW = “freshwater”) and sex (M = “male”, F = “female”). **(B)** Scatterplots showing the mean normalised gene expression for each sex within each of three groups (R-SW = “resident saltwater”, S-SW = “smolt saltwater”, R-FW = “resident freshwater”). Each point represents an individual gene and is coloured by sex (green = “female”, white = “male”).



Chapter Four: Autumn outmigrants in brown trout (*Salmo trutta*) are not a demographic dead-end

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Abstract

Genetic identity analysis and physical tagging were used to examine the freshwater return rates and phenotypic characteristics of n=1791 downstream migrating juvenile *Salmo trutta* in the Burrishoole catchment (northwest Ireland) across the period September 2017 to December 2020. In this system, juveniles out-migrate (move from freshwater into brackish or marine habitats) in every month of the year, with distinct seasonal peaks in spring (March through June; mostly silvered smolts) and autumn (September through December; mostly younger, unsilvered fry or parr). Both types exhibited a sex-bias towards females, which was stronger in spring (78% females) than in autumn outmigrants (67%). Sixty-nine returning fish were matched back to previous juvenile outmigrants and similar return rates were found for spring outmigrants (5.0%), autumn outmigrants (3.3%) and fish that outmigrated outside of spring or autumn (2.8%). Spring and autumn outmigrants returned at similar dates (typically mid to late July) but autumn fish were away for longer (median = 612 days; spring outmigrants = 104 days). Autumn outmigrants were 25% smaller than spring outmigrants on the way out and 6% smaller on their return, and within both groups smaller/younger outmigrants spent longer away than larger/older outmigrants. Autumn outmigrants were more likely to return unsilvered as 'slob' trout (84%) than spring outmigrants (31%), suggesting they make greater use of brackish habitats that might be safer, but less productive, than fully marine habitats. However, both types also produced silvered 'sea trout' (≥1+ sea-age), implying neither is locked into a single life-history strategy. Our findings emphasise that autumn outmigrants and the transitional habitats that support their persistence should not be overlooked in salmonid management and conservation.

Keywords: life-history, tactics, salmonid, biocomplexity, intraspecific, migration, telemetry

Introduction

Salmonid fishes are characterised by diverse migration and life-history strategies (Klemetsen et al., 2003; Quinn, 2005; Quinn et al., 2015). Considerable recent attention has been given to the patterns and processes involved in anadromy versus residency (Dodson *et al.*, 2013; Kendall *et al.*, 2014; Sloat *et al.*, 2014; Ferguson *et al.*, 2017, 2019; Nevoux *et al.*, 2019), but less attention has been given to the question of why bimodal patterns of juvenile seaward migration often occur within populations exhibiting anadromy or potamodromy (Birnie-Gauvin *et al.*, 2019; Bourret *et al.*, 2016). Juveniles out-migrating in springtime are typically silvered and primed for hypo-osmoregulation; in standard terminology (which we adhere to in this study) these are referred to as 'smolts', although some authors (e.g., Ferguson *et al.*, 2019; Huusko *et al.*, 2017; Jones *et al.*, 2015) have used this term more broadly to encompass any type of downstream migrating juvenile, irrespective of whether the ultimate destination involves hypo-osmoregulation. In many cases, there is also an earlier pulse of usually younger, smaller, outmigrants that move in the autumn, winter, or early spring (depending on species/region/population) before the 'true' smolt run, which may also be of poorer or more variable hypo-osmoregulatory abilities. This phenomenon occurs in Chinook salmon (*Oncorhynchus tshawytscha* Walbaum) (Anderson & Topping, 2018; Beckman *et al.*, 2003; Bourret *et al.*, 2016; Copeland *et al.*, 2014; Miller *et al.*, 2010), coho salmon (*Oncoryhnchus kisutch* Walbaum) (Quinn *et al.*, 2013; Roni *et al.*, 2012), sockeye salmon (*Oncoryhnchus nerka* Walbaum) (Walsworth *et al.*, 2015); masu salmon (*Oncorhynchus masou* Brevoort) (Kuroki *et al.*, 2020); Atlantic salmon (*Salmo salar* Linnaeus.) (Cunjak *et al.*, 1989; Jonsson & Jonsson, 2014) and brown trout (*Salmo trutta* L.) (Aarestrup *et al.*, 2018; Birnie-Gauvin *et al.*, 2021; Jonsson & Jonsson, 2009; Poole *et al.*, 1996, 2007; Winter *et al.*, 2016). Interestingly, so-called 'autumn parr' migrations are not restricted to anadromous stocks, as they also occur in Baltic Sea populations of Atlantic salmon and brown trout that migrate into brackish water (surface salinity 4.5–6.5 ‰) (Taal *et al.*, 2014), as well as in potamodromous brown trout populations (Kennedy *et al.*, 2022b; Leathe *et al.*, 2014).

The evolutionary reasons for the origins and maintenance of these two distinct migratory phenotypes remain obscure, but the associations with size and age suggest

state-dependent benefits and/or costs. One hypothesis is that the risks and rewards of early versus late outmigration are different for juveniles in different body condition or energetic status. Birnie-Gauvin *et al.*, (2021) found that brown trout that outmigrated in autumn in the Gudsø system, Denmark, were in poorer condition prior to the presumed first migration decision window than spring migrants, which in turn were in lower condition than river-residents. Low physiological condition might be related to high intrinsic metabolic demands, whereby fish with higher standard or maximum metabolic rate are forced to migrate to more productive marine environments, rather than remain resident in their natal river, in order to sustain the associated higher energetic costs (Nevoux *et al.*, 2019; Sloat & Reeves, 2014; Sogard *et al.*, 2012). Those in worst condition during some sensitive period (decision window) might be forced to outmigrate earlier (e.g., autumn rather than spring) to replenish their (e.g., lipid) reserves, as otherwise they could suffer higher freshwater mortality, especially if winter conditions are harsher in freshwater than in saltwater. The benefits of migration at earlier ages/smaller sizes might be countered, however, by increased mortality risk in brackish or marine environments (Jensen *et al.*, 2019). Whilst predators in transitional or ultimate destination habitats might be less abundant in colder winter months or constrained by lower light levels, smaller migrants might still be vulnerable to gape-limited predators. Indeed, autumn outmigrants are typically smaller at the time of migration than spring outmigrants (Birnie-Gauvin *et al.*, 2021; Poole *et al.*, 2007; but see Kennedy *et al.*, 2022b), and hence may suffer higher date-independent mortality post-outmigration for purely size-or growth-related reasons (Dermond *et al.*, 2019; Jonsson & Jonsson, 2009).

Data on how timing and size at outmigration relate to characteristics of returning fish remains sparse for brown trout, owing to challenges in tracking the life-histories of individual fish. The current study builds on previous work on seaward migration timing of brown trout in the Burrishoole catchment, mid-west Ireland (Byrne *et al.*, 2004; de Eyto *et al.*, 2022; Mills *et al.*, 1990; Poole *et al.*, 1996, 2007), using new individual-level information. Our aims were to (1) further characterise the bimodal timing patterns in terms of age, size, and sex of juvenile outmigrants, and (2) use a novel combination of genetic tagging with physical tagging (passive integrated transponder (PIT) tags) to

test for differences between spring outmigrants and autumn outmigrants in their return rates, size and phenotype at return, time spent away and specific growth rates. While previous studies in Norway (Jonsson & Jonsson, 2009) and Denmark (Birnie-Gauvin *et al.*, 2021) have examined similar issues, the Burrishoole differs from these other systems in having a brackish, tidal lagoon at its mouth, which might provide increased habitat options for autumn migrants. We hypothesised that autumn outmigrants make greater use of this lagoon than spring outmigrants, given that the smaller size of the former group might limit their initial survival chances in fully marine habitats. While some of them may eventually leave the lagoon once large enough to transition to full seawater, many may never do so. Thus, we predicted that a higher fraction of autumn outmigrants return as brownish 'slob trout' (a term used in Ireland to refer to semi-anadromous *S. trutta* that utilise brackish coastal habitats such as estuaries; Kennedy and Fitzmaurice 1971) rather than silvered finnock (anadromous individuals returning to freshwater in the same year as outmigration) or sea trout (anadromous individuals spending at least one winter at sea). We further expected growth rates of autumn outmigrants to be lower than those of spring outmigrants, both because they leave freshwater at a time of year when growth is generally lower but also due to their hypothesised greater use of less productive brackish habitats. These habitats may, however, be safer in terms of predation risk (Thorstad *et al.*, 2016) and hence growth costs might be countered by survival benefits. We also test the possibility that autumn outmigrants spend longer away than spring outmigrants to gain as much growth as possible. If they still return at smaller sizes than spring outmigrants, this would limit the fecundity benefits to females and thus sex ratios in autumn outmigrants should be less strongly skewed towards females than in spring outmigrants.

Methods

2.1 Study area and data collection

The Burrishoole system (53°57 N 09°35 W), located in the mid-west region of Ireland within the Nephin Beg Mountain range, consists of over 45 km of shallow streams which are oligotrophic and poorly buffered. The streams/rivers of the system discharge into three main lakes (Lough Bunaveela, freshwater, 46ha; Lough Feeagh, freshwater, 410ha; and Lough Furnace, brackish, 172 ha) before discharging into the north-eastern corner of Clew Bay (Figure 1). Brown trout in the system can be stream residents, potamodromous (migration to larger rivers or lakes), semi-anadromous (migration to brackish areas) or anadromous (migration to full saltwater). The ratios of the different types are not known exactly, but likely fluctuate over time at the catchment level. The system historically produced a healthy run of sea trout, but a collapse occurred in the late 1980s/early 1990s and sea trout numbers have recovered slightly but remained low since (de Eyto *et al.*, 2016; Poole *et al.*, 2007).

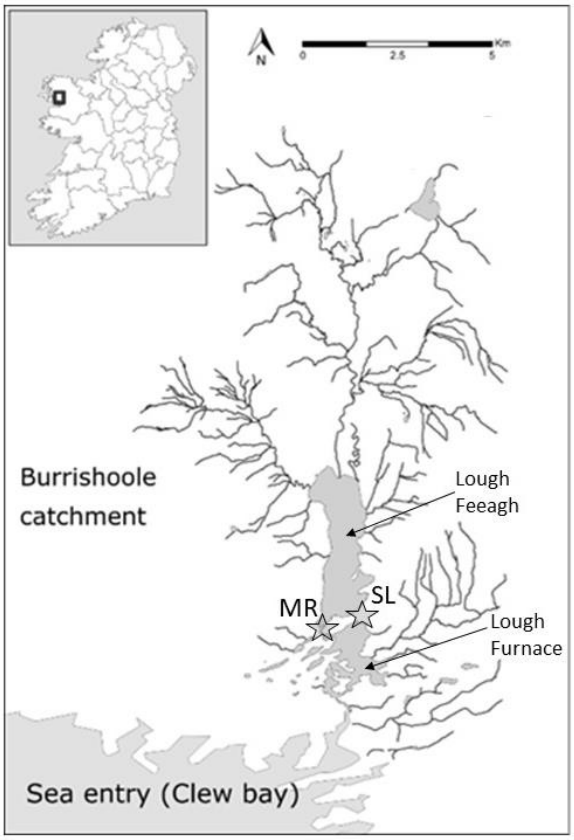


Fig.1: Map of Burrishoole catchment, mid-west Ireland, where the study took place. Migrating *Salmo trutta* were monitored and sampled at sea-entry trapping facilities (marked with star symbols) at the interface between the freshwater (Lough Feeagh) and marine-influenced (Lough Furnace) portions of the system. MR = Mill Race sea-entry traps. SL = Salmon Leap sea-entry traps.

The primary data used in this study derived from a focussed sampling campaign (September 2017 to December 2020), during which genetics samples were taken on both downstream and upstream migrating fish captured at trapping facilities situated on two short rivers that join L. Feeagh to L. Furnace (Mill Race and Salmon Leap sea-entry traps; Figure 1). An individual's multi-locus genotype was then used as a 'genetic tag' to match returning (upstream) individuals to the same previously sampled out-migrating (downstream) individuals (augmented with PIT tag matches), thus allowing for comparisons of the relative performance of autumn versus spring outmigrants in the intervening period in transitional or destination coastal habitats.

2.2 Sampling and phenotypic categorisation of downstream migrants

The recent genetic sampling (2017 to 2020) was augmented with an additional five years of historical monitoring data from 2012 to 2016, which was used purely just to help define autumn versus spring outmigration periods and describe their respective age distributions using a longer time series. In all years, the downstream sea-entry traps were monitored daily during periods of low migration, and twice daily during periods of peak migration. Downstream outmigrants comprise a mix of various categories: smolts (silvered juveniles on first migration to sea, a mix of 2+ and 3+ ages, with occasional 4+), unsilvered juveniles (on presumed first migration, a mix of 0+ and 1+ and older immature fish), silvered kelts (previously spawned adults returning to sea with silvered colouration), unsilvered kelts (previously spawned adults returning to sea unsilvered) and unspawned finnock (that overwintered in freshwater without spawning). During the study period, approximately 80-90% of the smolt run in each year was comprised of age 2+ fish, the rest comprising age 3+ fish (Marine Institute Annual Reports: https://oar.marine.ie/handle/10793/3). Unsilvered outmigrants comprised a mix of predominantly 0+ and 1+ fish, with 0+ fish representing typically

30-50% of these (Mills et al., 1990; Poole et al., 1996; 2007; Marine Institute Annual Reports).

Between Sep 2017 and Dec 2020, 2,095 downstream migrating *S. trutta* (all categories combined; 94% of the total run) were sampled for genetics by taking a small tissue sample (using a tissue sample unit, TSU, kit; Allflex, South Africa) from the caudal fin, which were stored at room temperature in the preservation liquid provided with the TSU kit for later DNA extraction. Of these, 566 (27%) either had new PIT tags (RFID HDX, Biomark) inserted at the traps (449 fish) or already had PIT tags (117 fish) from previous telemetry work in the catchment (R. Finlay, unpublished).

All tissue-sampled fish, in both migration directions, were measured for total length (mm). As the focus was on juveniles on their first seaward migration, kelts were excluded from the downstream migrants. To further exclude possible smaller kelts that might have been mislabelled as smolts or unsilvered juveniles, outmigrants larger than 250mm (the 33rd percentile size of fish labelled as kelts) were also removed. The final sample size of tissue-sampled downstream migrants was 2008 juveniles (putative first-time outmigrants), of which 543 had PIT tags (Table 4.1).

Table 4.1. Summary of number of *S. trutta* sampled in the downstream (DS; juveniles only) and upstream (US) directions at the "sea-entry" traps delimiting the transition between the freshwater and marine-influenced portions of the Burrishoole catchment. All DS fish with PIT tags were also tissue-sampled for genetics (as all captured by hand), whereas only a subset of PIT-tagged fish detected in the US direction were tissue-sampled (those captured by hand – numbers in parentheses in row f) as the remainder were detected automatically via fixed antennae (i.e., not captured by hand).

Variable	2017	2018	2019	2020	Total
a. Number genetic samples in DS direction	195	802	751	260	2008
b. Number DS samples successfully genotyped at 14+ loci	175	744	682	190	1791
c. Number DS fish with PIT tags	193	289	35	26	543
d. Number genetic samples in US direction	20	67	77	78	242
e. Number US samples successfully genotyped at 14+ loci	20	65	75	62	222
f. Number US PIT tag detections (number also genetic sampled)	2 (0)	18 (3)	10 (5)	4 (2)	34 (10)

Across the full time-series (2012-2020), the majority (97%) of silvered smolts outmigrated between March and June, whilst the majority (76%) of unsilvered juveniles outmigrated between September and December (Figure 2). Thus, the year was split into three four-month blocks: March to June (spring outmigrants; 39% of the total), September to December (autumn outmigrants; 50%) and an 'other outmigrants' category (January, February, July, August; 11% of the total). 87% of the spring outmigrants, <1% of the autumn outmigrants, and 10% of the other category were silvered, respectively, the rest being unsilvered. Autumn outmigrating juveniles in year *t* are typically younger by one year on average than spring outmigrants in year *t*+1; thus, there were effectively three pairs of autumn-spring outmigration cohorts: autumn 2017 – spring 2018; autumn 2018 – spring 2019, and autumn 2019 – spring 2020.

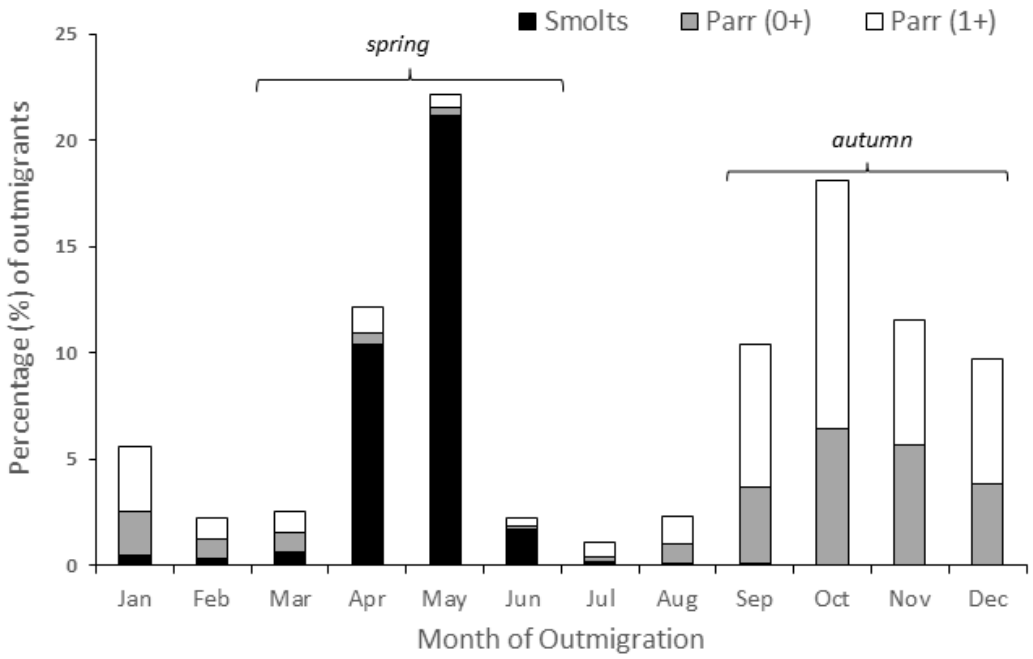


Fig.2 Bimodal pattern of outmigration timing of *Salmo trutta* in the Burrishoole system, based on data from 2012 to 2020 on downstream (seaward) migrating juveniles classified as smolts (based on silver colouration and other morphometric characteristics; n=3324), aged 0+ parr (n=2408) or aged 1+ and older parr (n=3735). Spring outmigrants were defined in this study as those migrating in March through June, whilst autumn outmigrants as those outmigrating from September through

December. Fish outmigrating in January, February, July and August were grouped under an “other” downstream migration category.

2.3 Sampling and phenotypic categorisation of upstream migrants

A total of 242 returning *S. trutta* were tissue sampled in the upstream direction across the focal study period (Sep 2017 to Dec 2020). PIT-tagged fish were detected by fixed antenna (Biomark, with IS1001 Readers) and hand scanners (HPRPlus, Biomark) at the Salmon Leap upstream trap and hand scanners (HRPlus and HPRLite, Biomark) at the Mill Race upstream trap. 34 (14%) of the tissue-sampled fish had associated PIT tag detections, comprising 32 unique individuals (two individuals were detected twice in separate years). Sampling of returning upstream migrants ceased at the end of Dec 2020, so return rates are likely underestimated in absolute terms, especially for the autumn 2019 – spring 2020 cohort pair (for which fish returning after > 1 year away would not have been sampled).

Upstream migrants, which typically return between April and September but sometimes through until December, were categorised as either unsilvered or fully/partially silvered. The unsilvered category corresponds to slob trout: fish that leave freshwater and spend anywhere from a few months to >1 year in transitional waters. The fully/partially silvered returns were further split into finnock (fish that spent less than one year at sea) and sea trout (>1 year at sea).

2.4 DNA extraction

Genomic DNA was extracted using the Promega Wizard® SV 96 Genomic DNA Purification System. DNA quality and quantity was assessed on agarose gels by comparison with a Quick-Load® Purple 100 bp DNA Ladder (New England Biolabs). Concentration of DNA for PCR was adjusted to 2-10ng/µl.

2.5 PCR and Genotyping

Multiplex PCR (polymerase chain reaction) was used to amplify 18 loci (17 microsatellite loci and the SalmoY sex identity locus) in two separate reactions (panels). Forward primers were labelled with fluorescent labels from the Applied

Biosystems (ABI) standard dye sets to enable visualisation on ABI genetic analysers and reverse primers included a GTTT 'pig tail' to minimise stuttering. In panel 1, a single set of primers (One102) amplified two distinct and apparently unlinked loci which could be scored effectively. Primer sequences were taken from relevant publications as follows; *Ssa*197 & *Ssa*85 (O'Reilly *et al.*, 1996); *Ssa*D71 (King *et al.*, 2005); *Ssa*410, *Ssa*416 (Cairney *et al.*, 2000); CAO48828, CAO53293 & CAO60177 (Vasemägi *et al.*, 2005); One102, One103 (primer sequences for this locus amplify two unlinked microsatellite loci) & One108 (Olsen *et al.*, 2000); pp*Str*2 & pp*Str*3 (Keenan *et al.*, 2013); *Cocl*-lav-4 (Rogers *et al.*, 2004); *Sasa*TAP2 (Grimholt *et al.*, 2002); One9Asc (Scribner *et al.*, 1996). All PCRs were performed in a total volume of 3.5µl, including 2-10ng of genomic DNA and 1.75µl Plain Combi PPP Master Mix (TopBio). Primer concentrations (same for forward and reverse primers for each locus) and fluorescent label employed in each panel were as follows:

Panel 1; SalmoYF(VIC) – 0.03µM, Ssa85(NED) – 0.04µM, Oneu9ASC (VIC) – 0.05µM, Ssa416UOS (FAM) – 0.06µM, One102 (NED) – 0.08µM, CAO48828 (VIC) – 0.08µM, One103 (FAM) – 0.1µM, ppStr2 (PET) – 0.12µM, CAO53293 (PET) – 0.15µM, CoclLav4 (VIC) – 0.2µM, One108 (VIC) – 0.25µM

Panel 2; Ssa197 (VIC) – 0.04µM, SsaD71 (NED) – 0.05µM, ppStr3 (FAM) – 0.06µM, SasaTAP2 (NED) – 0.08µM, CAO60177 (FAM) – 0.2µM, Ssa410UOS (PET) – 0.25µM.

Cycling conditions were as follows: Initial denaturation at 95°C for 15 minutes, followed by 25 cycles of 94°C for 30 seconds, 55°C for 60 seconds and 72°C for 90 seconds. A final extension step of 30 minutes at 60°C was performed. Electrophoresis was performed on an ABI3500xl DNA analyser (Applied Biosystems) using POP-7™ Polymer. Each sample was prepared in Hi-Di™ Formamide with GeneScan™ 600 LIZ™ Dye Size Standard (ThermoFisher Scientific) as a size ladder with which to compare allele size. Allele calling was conducted in GeneMarker (SoftGenetics).

2.6 Genetic Identity Analysis

Identity analysis in CERVUS 3.0.7 (Kalinowski *et al.*, 2007) was used to match pairs of samples coming from the same individual, based on having the same multi-locus genotype allowing for one mis-matching locus (e.g., due to genotyping errors) to include 'fuzzy matches'. Only samples scored for at least 14 loci were included, such that a minimum number of 11 loci were common among all pairwise comparisons. The combined non-exclusion probability for identity analysis (i.e., the probability that genotypes do not differ between two randomly chosen individuals) was estimated to be very close to zero ($<5 \times 10^{-10}$) with 11 or more loci. All genetic samples were included in this identity analysis, regardless of direction of migration or fish type (juveniles, finnock, sea trout, slob trout, kelts, etc.).

2.7 Data Analysis: Outmigration

A general linear model (GLM) with Gaussian errors was used to test for differences in length-at-outmigration among the downstream phenotypes (autumn, spring, other). A chi-squared test was used to determine whether the downstream phenotypes differed in their sex ratio (proportion females, as determined by the SalmoY sex identity locus) at outmigration.

2.8 Data Analysis: Return migration

Chi-squared tests were used to determine whether return rates differed among the downstream phenotypes. Return rates were calculated as the number of individuals that returned (as inferred by genetic identity and/or PIT tags) for each downstream phenotype divided by the corresponding number of juveniles that outmigrated and were successfully genotyped at 14 or more loci (a subset of which also had PIT tags). Chi-squared tests were similarly used to test for a non-random association between downstream phenotype and upstream phenotype (slob trout, finnock, sea trout).

To test the hypothesis that post-outmigration growth rates differ among the downstream phenotypes, instantaneous growth rate was calculated as $g = [\log_e(L_2) - \log_e(L_1)]/\Delta t$, where L_2 was length-at-return (upstream recapture) and L_1 was length at

outmigration (first downstream capture), and *t* was the number of intervening days away. This was done for length only, as no information on weight of returning fish was available. Specific growth rate was then calculated as $G = 100(e^g - 1)$, which is interpretable as the percentage increase in length per day (Crane *et al.*, 2020). A GLM with Gaussian errors was then fitted where *G* was the response variable and downstream phenotype was the sole explanatory variable.

Finally, GLMs with Gaussian errors were used to test whether the downstream phenotypes differed in terms of length-at-return and total time spent away (number of days between first downstream capture and next upstream capture). The latter was first natural-log transformed as its raw distribution exhibited right-skew.

All analyses were carried out in R version 4.2.0 (R Core Team, 2022) using base R and the tidyverse (Wickham *et al.*, 2019), lubridate (Grolemund & Wickham, 2011) and gridExtra (Auguie & Antonov, 2017) packages.

2.9 Ethical statement

The data were collected by the Marine Institute under licence (Fisheries Acts 1959–2003) from the Irish Department of Agriculture, Food and Marine and by permission of the Minister of Agriculture, Food and Marine. The Marine Institute complies with EU Directive on Animal Welfare 2010/63/EU and the corresponding Irish Directive SI No 543 of 2012.

Results

3.1 Composition of juvenile outmigrants

Of the 2008 juvenile (first-time) outmigrants sampled for genetics in the downstream direction across the Sep 2017 to Dec 2020 study period, 1,791 (89%) were successfully genotyped at 14 or more microsatellite loci and 543 (27%) also had PIT tags (Table 4.1). The downstream phenotypes differed significantly in length-at-outmigration ($F_{2,1782}$ = 525.33, P < 0.001; Figure 3a). Autumn outmigrants were smaller (mean length-at-outmigration = 140.8mm ± 30.5 (SD), range 86 - 147mm) than spring outmigrants (188.4mm ± 25.8, range 101 - 249mm), whilst the other (non-autumn/spring) downstream phenotype category was intermediate in size (161.5mm ± 39.2, range = 102 – 249mm). Genetic sex information was available for 1,782 of the juvenile outmigrants, of which 1,281 (72%) were scored as females and 501 (28%) as males. The sex ratio was female-biased for all downstream phenotype categories and also differed significantly among them (χ^2 = 23.40, df = 2, p < 0.001), with 67% of the autumn outmigrants, 78% of the spring outmigrants, and 69% of the other category, respectively, being female (Figure 3b).

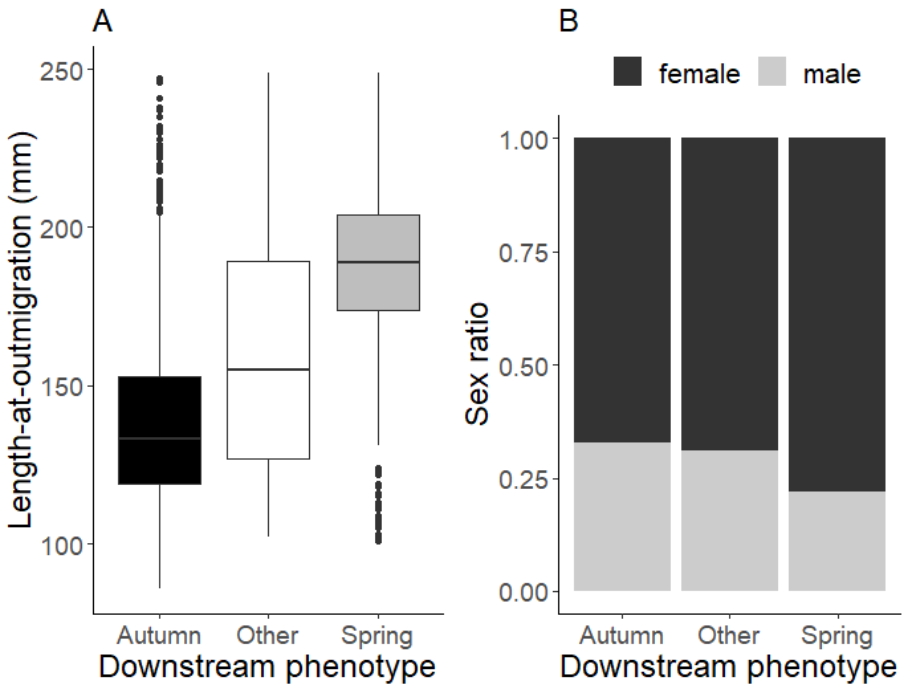


Fig.3: Length (A) and sex ratio (B) differences between the three downstream phenotypes of *Salmo trutta* in the Burrishoole system across the period Sep 2017 to Dec 2020. For the boxplot in A, the dark horizontal lines correspond to medians, the boxes to interquartile ranges (IQR), the whiskers to 1.5 x IQR, and the dots to outliers. Plot A is based on n=1785 outmigrants for which length information was available. Sex ratios in B are for the subset of n=1782 outmigrants with reliable genetic sex information.

3.2 Genetic identity matches

The genetic identity analysis in CERVUS identified 159 matches, i.e., pairs of tissue-samples with identical genotypes across 11 or more loci. All matches were exact, i.e., no mismatching loci in any case. Sixteen genetic matches had PIT tag readings for both samples in the pair, and in 13 (81%) of these cases the PIT tag codes matched exactly, confirming the samples came from the same individual fish. The three cases where the PIT tag codes did not match may have represented instances where fish shed their original tag and were retagged; these records were excluded from further analyses as we could not be sure they were the same individuals. After additionally excluding cases where the same individual was sampled twice on the same day, this left 111 matches.

Seventy-seven of these matched cases where the two samples in the pair were only involved in that single match ('twins'). The remaining matches could be grouped into 17 'triplets' (three samples that all matched to each other), three 'quadruplets' (four samples all matching) and one 'quintuplet' (five samples all matching). Thus the 111 retained matches were inferred to group into 98 genetically distinct individuals, i.e., the same fish tissue-sampled on two or more occasions.

After filtering to retain only those fish deemed to be first-time outmigrants (1st capture in downstream direction; kelts removed), this yielded n=50 genetically identified individuals. Only the first and second capture occasions were retained for each of

these fish for the analyses of return rates. An additional 19 putative first-time outmigrants were identified as having returned via PIT tag matching, i.e., upstream detected individuals matched via their unique PIT tag to previous downstream detections (but not also matched via genetics, as no tissue sample was taken on the second capture occasion). Nine fish were identified via both PIT tag matching and genetic matching. In total, 69 juvenile outmigrants were identified as having returned via genetic and/or PIT-tag matching (Table 4.2), of which 62 comprised downstream (1st capture) to upstream (2nd capture) matches, and seven comprised downstream to downstream matches (where the intervening upstream return went undetected). The distribution of days away had a large peak of 14 fish that spent ≤14 days away (Figure 4). These fish might have simply drifted passively through the downstream sea-entry traps, rather than migrated actively. Hereafter, for operational purposes, they are referred to as ‘wanderers’, whilst fish that spent more than two weeks away are referred to as ‘true migrants’.

Table 4.2: Summary of numbers of *S. trutta* outmigrating by month across the study period (Sep 2017 to Dec 2020) in the Burrishoole catchment and corresponding numbers and characteristics of returns identified by genetic and/or PIT tag matching.

Variable	MONTH OF OUTMIGRATION:												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	**Total**
a. Number outmigrants sampled for genetics	80	46	72	154	526	76	23	38	241	389	225	138	**2008**
b. Number outmigrants successfully genotyped (14+ loci)	66	44	61	142	475	67	15	31	227	356	193	114	**1791**
c. Total number returns (matched by genetics and/or PIT tags)	3	0	4	11	15	1	2	1	3	17	9	3	**69**
d. Number of wanderer returns (away for ≤ 2 weeks)	0	0	0	1	4	1	2	0	1	6	1	0	**16**
e. Number of true migrant returns (away for > 2 weeks)	3	0	4	10	11	0	0	1	2	11	8	3	**53**
f. Total return rate (c/b)	4.5%	0.0%	6.6%	7.7%	3.2%	1.5%	13.3 %	3.2%	1.3%	4.8%	4.7%	2.6%	**3.9%**
g. Return rate of true migrants (e/b)	4.5%	0.0%	6.6%	7.0%	2.3%	0.0%	0.0%	3.2%	0.9%	3.1%	4.1%	2.6%	**3.0%**
h. Percentage slob trout among true migrant returns	100 %	-	100 %	0%	50%	-	-	100 %	50%	100 %	80%	100 %	**69%**
i. Percentage sea trout among true migrant returns	0%	-	0%	20%	25%	-	-	0%	50%	0%	20%	0%	**14%**
j. Percentage finnock among true migrant returns	0%	-	0%	80%	25%	-	-	0%	0%	0%	0%	0%	**17%**

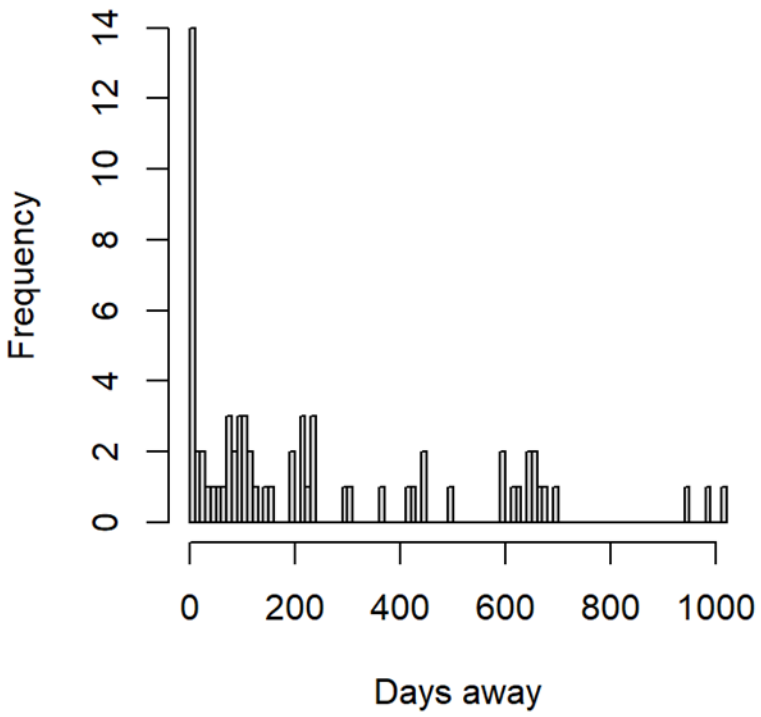


Fig 4: The distribution of days away (number of days between first downstream capture and next upstream or downstream capture at the sea-entry traps) for *Salmo trutta* in the Burrishoole catchment, based on n=69 individuals that outmigrated during the period Sep 2017 to Dec 2020 and were identified via genetic and/or PIT-tag matching as having returned at some point during this same period. Each bar represents a 14-day bin width. The initial spike of n=14 fish spending <=14 days away were categorised as “wanderers” and those spending >14 days away as “true migrants”.

3.3 Return rates

In total, 32 autumn outmigrants were detected as having returned (eight wanderers and 24 true migrants), i.e., subsequently recaptured or detected via antenna in the upstream or downstream directions. The corresponding number of returns for spring outmigrants was 31 (six wanderers and 25 true migrants), and for fish outmigrating in other months was six (two wanderers and four true migrants). The percentage of wanderers did not differ among the autumn (25%), spring (19%) and other (33%) categories ($\chi^2 = 0.66$, df = 2, p = 0.718).

The total return rates for autumn, spring and other outmigrants were 3.3%, 5.0% and 2.8%, respectively. These return rates were not statistically significantly different (χ^2 = 3.66, df = 2, p = 0.161). When limiting the analysis to only true migrants, the corresponding return rates were 2.5%, 4.1% and 1.8%, for autumn, spring and other outmigrants, respectively (χ^2 = 4.20, df = 2, p = 0.123).

3.4 Relationship between downstream phenotype and upstream phenotype

Considering the wanderers only, all returned the same colour as they left, bar one fish (an autumn outmigrant) which was brown on downstream migration and partially silvered on upstream migration. True migrants differed in their phenotype upon return (slob trout, finnock or sea trout) depending on their phenotype at outmigration (χ^2 = 33.39, df = 6, p < 0.001), with more autumn migrants returning as slob trout (87%) than their spring counterparts (47%; Figure 5). The two autumn outmigrants that returned fully/partially silvered were classified as sea trout, whilst three of the nine spring outmigrants that returned fully/partially silvered were sea trout and six were finnock. All four of the true migrant returns from the other downstream phenotype category were slob trout.

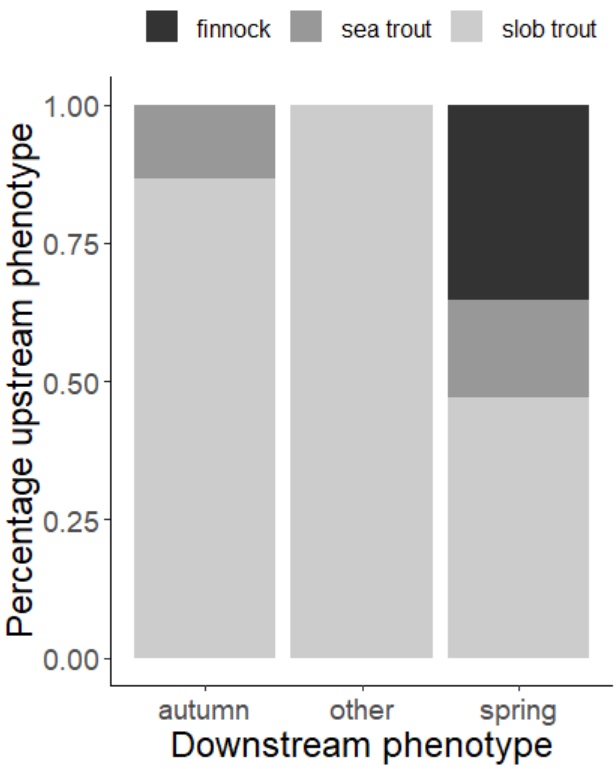


Fig. 5: The relationship between downstream phenotype and upstream phenotype for *Salmo trutta* in the Burrishoole catchment, based on fish that outmigrated during the period Sep 2017 to Dec 2020 and were identified via genetic matching as having returned at some point during this period. Data for the subset of n=36 true migrants for which return colour information was available: unsilvered (brown) returns were classed as slob trout, and fully/partially silvered returns as either finnock (< 1 year away) or sea trout (> 1 year away).

3.5 Specific growth rates

The specific growth rate of true migrants that outmigrated in autumn was lower (mean = 0.12% day^{-1}) than that of spring outmigrants (mean = 0.21% day^{-1}), with that of the other downstream phenotype category being lower again (mean = 0.07% day^{-1}). Overall, the downstream phenotypes differed in their specific growth rates (Figure 6a; $F_{2,27}$ =4.80; P = 0.016). Considering upstream phenotype only, finnock had the highest specific growth rates (0.27% day^{-1}), followed by sea trout (0.16% day^{-1}) and then slob trout (0.12% day^{-1}) (model with an effect of upstream phenotype only: $F_{2,27}$ = 7.79; P = 0.002).

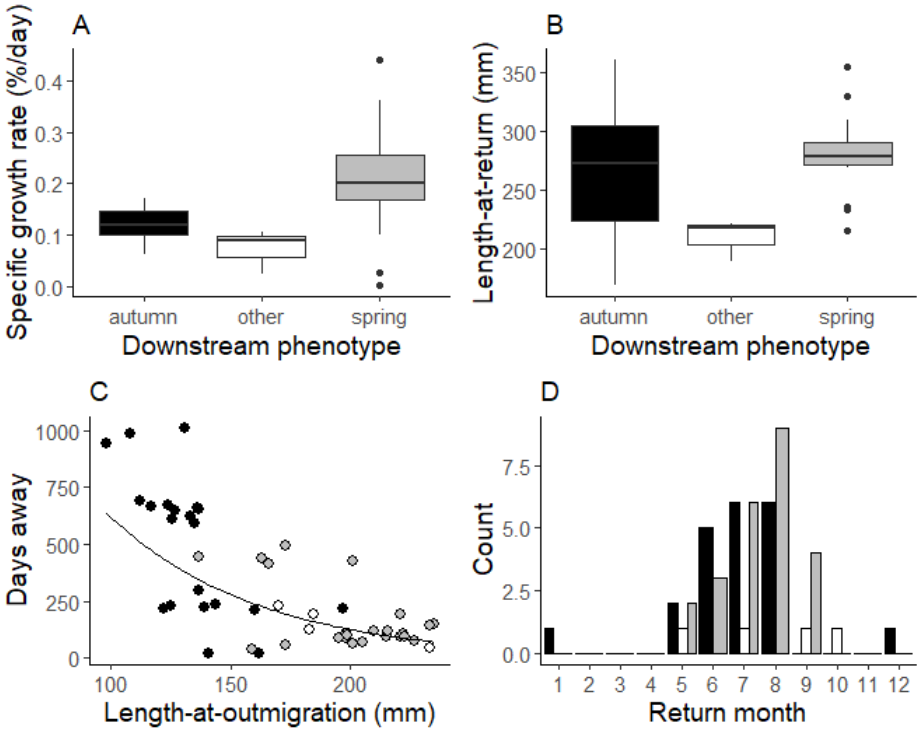


Fig.6: Characteristics of returning (upstream migrating) *Salmo trutta* in the Burrishoole catchment, based on fish that outmigrated during the period Sep 2017 to Dec 2020 and were identified via genetic matching as having returned at some point during this same period. In all panels, autumn outmigrants are black, spring outmigrants are grey and the other (non-autumn/spring) outmigration category is white. (A) Specific growth rate as a function of downstream phenotype. (B) Length-at-return as a function downstream phenotype. (C) Days away as a function of length-at-outmigration (fitted curve corresponds to back-transformed fit from linear regression of log(days away) versus length-at-outmigration). (D) Distribution of return months (1=Jan, 12=Dec).

3.6 Length at return and time spent away

Length at return did not differ significantly among the downstream phenotypes when the latter was the only explanatory variable in the model ($F_{2,28}$ = 3.19; P = 0.06), with autumn outmigrants being 6% smaller on average than spring outmigrants on their return upstream (mean length-at-return: autumn outmigrants = 264.5mm; spring outmigrants = 279.4mm; Figure 6b). When days away was included in the model, it had a significant positive effect on length at return ($F_{1,27}$ = 6.75; P = 0.015) and the

residual effect of downstream phenotype then became significant ($F_{2,27}$ = 10.24; P < 0.001). The interaction between days away and downstream phenotype was not significant ($F_{2,25}$ = 0.12; P = 0.891). Length-at-return was uncorrelated (r = -0.16, df = 29, P = 0.395) with length-at-outmigration.

Considering only the n=49 true migrants whose second capture was in the upstream direction, 32 (65%) spent less than a full year away, 14 (29%) spent more than a year but less two full years away, and three (6%) spent over two full years away. The downstream phenotypes differed in the number of days spent away ($F_{2,46}$ = 7.77, P = 0.001), with the median number for each type being 612 days, 104 days, and 160 days, respectively, for autumn, spring and other. When length-at-outmigration was included in the model, it had a statistically significant negative effect on (the log of) days away (slope = – 0.02; $F_{1,44}$ = 29.52, P = 0.001) and there was no residual effect of downstream phenotype ($F_{2,44}$ = 0.09, P = 0.916). This indicates that initial size differences among the downstream phenotypes accounts for the differences in days away between them (Figure 6c). Return dates did not differ significantly among the downstream phenotypes (Figure 6d; Kruskall-Wallace χ^2 = 2.50, P = 0.287), with median upstream dates of July 18th, Aug 2nd and Aug 18th for the autumn, spring and other categories, respectively.

Discussion

This study used a novel methodology that combined genetic and physical tagging to show that autumn migrants make an important contribution to the total return of upstream *S. trutta* in the Burrishoole system and are thus not a demographic or evolutionary 'dead-end', bolstering recent claims that they should not be overlooked in salmonid research and management (Birnie-Gauvin *et al.*, 2019). Whilst autumn migrants were more likely than spring outmigrants to return as brownish slob trout, both types also produced sea trout, a pattern hinted at, but not rigorously addressed, by older tagging work in the catchment (Mills *et al.*, 1990; Poole *et al.*, 1996). Thus, although the propensity to adopt different migratory behaviours/utilise different coastal habitats may vary between autumn and spring outmigrants, neither appears locked into a single strategy. The presence of a tidal lagoon at the interface between

freshwater and the sea differentiates the Burrishoole from other systems where autumn migrating trout have been documented (River Imsa, Norway: Jonsson & Jonsson, 2009; River Gudsø, Denmark: Birnie-Gauvin *et al.*, 2021), although autumn and spring migrants might make different use of estuarine habitats in these Scandinavian systems. Whilst we could not demonstrate differences in relative utilisation of Lough Furnace with behavioural data (as we did not track movements once fish left freshwater), the fact that daily specific growth rate of autumn outmigrants was almost half that of spring outmigrants (Figure 6a) is consistent with the former group making greater use of less productive brackish habitat in the lagoon. Autumn outmigrants also spent an additional winter away compared to spring outmigrants, which could have contributed to their lower growth rate averaged across the entire sojourn compared to spring migrants, regardless of any differences in habitat use. Daily apparent mortality (calculated as $1 - R^{1/t}$, where R = return rate and t = median days spent away) across this period was lower for autumn (0.6%) compared to spring (3.0%) outmigrants because R was similar for each group, yet autumn migrants were away for considerably longer (Figure 6c). Collectively, these results suggest that autumn fish utilise safer habitats post-outmigration than spring fish and that growth-survival trade-offs could play a role in maintaining both tactics (Stearns 1989).

Sex ratios were skewed towards females for all downstream phenotype categories, with the bias being stronger for spring outmigrants (Figure 3b). An overall female bias in migratory individuals was not unexpected and has been well-documented before in this species and other salmonids (Ferguson *et al.*, 2019; Nevoux et al. 2019). Birnie-Gauvin *et al.* (2021) similarly found that spring outmigrating *S. trutta* in the Gudsø River (Denmark) were 75% females (33/44 fish sampled), whilst autumn outmigrants were 63% female (26/41 fish sampled). Typically, female reproductive success in salmonids is more limited by body size than is male reproductive success (O'Sullivan *et al.*, 2019; Seamons *et al.*, 2004), making migration to more productive (brackish or fully marine) habitats more attractive to females. Whilst specific growth rate during the 'marine' sojourn was lower for autumn outmigrants, they in fact returned at similar (albeit a broader range of) sizes to spring outmigrants (Figure 6b) because they spent longer away (Figure 6c). Low sample sizes precluded meaningful analysis of sex

differences in length-at-return, hence it remains unknown whether the fecundity of female autumn outmigrants is generally lower than that of female spring migrants. Another possibility is that some autumn fish might not be active migrants seeking habitats with higher growth potential, but rather juveniles displaced downstream under density-dependent competition for limited freshwater rearing sites (consistent with their smaller average size than spring outmigrants; Figure 3a) or 'wandering nomads' that just happen to drift downstream. Less-extreme sex ratio skew would then be expected compared to spring migrants that are actively migrating to marine habitats. The wandering rate – here defined as the proportion of all returns that spent <2 weeks away – was somewhat higher in autumn (8/32 fish = 25%) than spring outmigrants (6/31 = 19%), but the difference was not statistically significant and low sample size may have precluded detection of a subtle effect. In complex systems, male and female brown trout may exploit different habitats in both freshwater and coastal areas (Jonsson 1989) and the connections between sex, habitat utilisation and life-history strategies warrant further study.

The lack of obvious differences in return rates or return sizes among the downstream phenotypes implies that the overall fitness of each tactic might be similar, with tactic variation being maintained by some sort of balancing selection mechanism (Dodson *et al.,* 2013). Autumn migration could persist as a condition-dependent tactic, for example, that 'makes the best of a bad job'. That is, individuals in poor physiological/energetic condition at the fry/parr stage might be under selection to migrate early (in autumn/winter months) to productive coastal/marine habitats in order to recuperate energy, rather than spending an additional winter in freshwater where conditions are potentially harsher, especially for energy-depleted fish (Birnie-Gauvin *et al.*, 2021). Autumn outmigrants may also get a head start on the best downstream and/or marine feeding opportunities come springtime (Jonsson & Jonsson, 2009). Such benefits can be countered, however, by higher mortality in saltwater habitats compared to freshwater, which in turn can depend on body size (e.g., due to gape-limited predators). If brackish habitats are safer than fully marine habitats, small autumn migrants might ameliorate to some extent these predation risks. Physiological stresses need not be lower, however; e.g., salinity levels in Lough Furnace can

fluctuate on various timescales (Kelly *et al.*, 2020), whilst anoxic conditions occur at depths below 6m (Kelly *et al.*, 2017). Migrants remaining within Lough Furnace might behaviourally respond by remaining closer to the surface and/or utilising shallow areas, lake fringes and stream inflow habitat (R. Poole, *pers. observation*).

Survival rates were probably underestimated in absolute terms for both autumn and spring outmigrants in the current study for two reasons. First, some fraction of Burrishoole outmigrants likely stray to other catchments. Straying rates in anadromous *S. trutta* could be as much as 15-20% (Thorstad *et al.*, 2016), with immature finnock in particular known to often overwinter in non-natal catchments (Kennedy *et al.*, 2022a). It remains unknown whether straying rates differ between autumn and spring outmigrants in the Burrishoole system; the fact that autumn outmigrants spent longer somewhere downstream of the sea-entry traps suggests more scope for straying, but this may be counteracted by a greater tendency to stay in Lough Furnace. Second, some fish that outmigrated later in the study period might not have returned prior to the cessation of sampling in Dec 2020. Again, given the longer time spent away by autumn outmigrants, their survival rates might have been underestimated more than that of spring outmigrants.

Somewhat unexpectedly, only ~25% of upstream returns could be matched genetically (as being the same individuals) to previous downstream migrants in this study. Genetic sampling began in Sep 2017, so any juveniles outmigrating prior to this could not have been matched to fish returning during the study period. Indeed, matching rates were lower for fish returning in 2017 (0%) compared to 2018 and 2019 (both 23%) and 2020 (27%), whilst the median number of days spent away by matched fish increased from 105 days for 2018 returns, to 153 days for 2019 returns, to 621 for 2020 returns. This indicates that total return rates were especially underestimated for the earlier return years. Straying rates are very unlikely to have been as high as 70+%, so many downstream migrants must have gone unsampled. Trapping efficiency is lower for outmigrants under 100mm, which can pass through the screens/grids. Moreover, any trapped outmigrants under this size were purposefully not sampled for genetics, or tagged with PIT tags, as the sampling protocols were not designed originally with the specific questions of this study in mind. Such very small fish were likely all 0+ autumn

fry migrants; if these fish survived worse than outmigrants >100mm, overall return rates for autumn migrants would then be overestimated relative to spring outmigrants. Outmigrants under 100mm would be too small to smoltify and go to sea the following spring, but many of them surviving the initial transition into brackish Lough Furnace might rear there for a further year or more before becoming smolts in the following spring and then migrate out into fully marine habitats. Given the observed negative relationship between size-at-outmigration and time spent away (Figure 6c), unsampled autumn fry outmigrants <100mm might spend long periods away and hence reach a similar eventual size-at-return as larger outmigrants. Thus, fry outmigrants on the lower end of the size spectrum could still be demographically important.

Recent reductions in the numbers and size of silvered trout returning to Burrishoole and other catchments associated with sea-lice infestations and other anthropogenic stressors (de Eyto *et al.*, 2016; Gargan *et al.*, 2017; Poole *et al.*, 2007) suggest that the balance of costs and benefits has now shifted, such that autumn outmigration may nowadays be a tactic on par, in fitness terms, with spring outmigration. Future sampling of *S. trutta* inhabiting brackish Lough Furnace plus acoustic tagging work could shed light on their post-outmigration movements and habitat associations, which might be profitably coupled with physiological work to test for osmoregulatory differences at outmigration. Failure to consider autumn outmigrants in stock assessments could lead to overestimation of return rates and underestimation of freshwater production (Birnie-Gauvin *et al.*, 2019). Maintaining diverse and healthy transitional coastal habitats should promote the continued maintenance of multiple migration tactics and foster increased population resilience in the face of anthropogenic threats.

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Figures and Tables

Chapter Five: General Discussion

Understanding how, and why, intraspecific phenotypic diversity arises and persists in nature is fundamental to both evolutionary biology and effective biodiversity conservation in an ever-changing environment (Roff, 1992; Woodruff, 2001). Phenotypes vary greatly not only across species, but also among populations of the same species and even among individuals within the same population (Roff, 1996). This phenotypic diversity encompasses a range of physiological, behavioural and life-history traits which are collectively influenced by genetic and environmental factors (Fusco and Minelli, 2010). The mechanisms underpinning phenotypic diversity can be understood from ultimate and proximate perspectives (Tinbergen 1963), where ultimate mechanisms concern evolutionary function and phylogenetic history of phenotypes (West-Eberhard, 2003) and proximate mechanisms relate to the environmental or ontogenetic factors influencing phenotypic expression (Ehrenreich and Pfennig, 2016). Genetic considerations are relevant to both, as evolution requires a heritable basis to phenotypes under selection (ultimate factors), whilst gene expression and its regulation underly organismal development and phenotypic plasticity (proximate factors). With this in mind, the overarching objective of this thesis was to advance the understanding of the underlying genetic mechanisms shaping migratory phenotypes at both the population and individual levels in brown trout, an economically important species that can express an enormous range of life history tactics.

Overview of each data chapter

In Chapter Two, I specifically aimed to test for heritable differences in migratory life histories among neighbouring, yet genetically partially isolated, brown trout populations in the setting of a common garden. Additionally, I explored the phenotypic correlates of early movement behaviours and patterns in the timing of movements between progeny of experimental crosses and for naturally produced fish. Anadromy rates differed among the different genetic backgrounds, largely following the basic predictions where progeny of parents from populations with a naturally higher rate of anadromy in the wild were more likely to undertake seaward migrations in this common

garden setting. Additionally, I was able to document group-specific and individual variation in movement rates out of the nursery stream (Rough River) during certain key migratory periods. Collectively, these results point toward heritable differences in migration strategies between populations, as well as size/age-related variation in migration patterns within populations, with implications for conservation and fisheries management.

In Chapter Three, I performed transcriptional profiling of the brain and liver of male and female brown trout to understand the genes and processes that differentiate migratory and residency morphs and associated sex-differences in gene expression. I then examined whether putative genes associated with migratory or residency morphs demonstrated generalised or tissue-specific expression profiles. Lastly, I investigated whether genes differentially expressed between migrants and residents were also differentially expressed between males and females, given their divergent phenotypic trajectories with respect to migration. Tissue-specific differences were found, with a greater number of genes expressed differentially in the liver compared to the brain between the migratory and resident morphs. Genes with increased expression in the livers of resident fish were enriched for Gene Ontology terms associated with metabolic processes. In contrast, smolt-biased genes were enriched for a range of biological processes, many of which were suggestive of possible immune function differences between smolts and residents. Finally, a subset of the genes that were differentially expressed between migrants and residents (AMT genes) were also found to be differentially expressed between males and females in the liver, but no sex-biased gene expression was overserved for AMT genes in the brain. Collectively, these results identify a set of candidate genes involved in the production of alternative life-histories and provide insights into tissue-and sex-specific expression of these genes, in particular underlining a key role for metabolic processes in mediating divergent physiological trajectories of migrants and residents.

In Chapter Four, I focussed on understanding variable migration timing tactics within a single population; namely, younger autumn migrating parr versus spring-migrating smolts in brown trout. Building on previous work in the Burrishoole catchment, this chapter further characterised bimodal downstream migration timing patterns in terms

of age, size, and sex of juvenile outmigrants. I then used genetic identity analysis together with information from passive integrated transponder tags to test for differences between spring outmigrants and autumn outmigrants with reference to their return rates, size and colour at return, time spent away and specific growth rates with a view to understanding proximate and ultimate factors influencing these alternative migratory morphs. A key finding here was that autumn outmigrants returned to the catchment at a similar rate to spring outmigrants, despite the former being smaller at outmigration and spending longer away than the latter. Furthermore, I recorded that autumn outmigrants had lower specific growth rates post-outmigration and were also more likely to return unsilvered when compared to spring outmigrants, suggesting that autumn outmigrants make greater use of brackish habitats that might be safer, but less productive, than fully marine habitats. Nevertheless, both types were recorded to produce silvered returns (finnock or sea trout), albeit in different proportions, implying neither is locked into a single life-history strategy. These findings emphasise that autumn outmigrants and the transitional habitats that support their persistence should not be overlooked in salmonid management and conservation.

In what follows of this Chapter Five, I synthesise the results of the data chapters and discuss their contribution in the context of the wider literature towards developing an integrated understanding of the links between genetics, life history and ecology of brown trout and other salmonids. Finally, I address limitations of the experimental design employed in this thesis and identify interesting avenues for future research.

Proximate and ultimate drivers of migration

Over half a century has elapsed since Tinbergen first suggested that one can ask four fundamental questions about any observed animal behaviour (Bergman and Beehner, 2022). The first two questions operate at the ultimate level of explanation, and the second two at the proximate level. Question one (*Why is the animal performing the behaviour?*) concerns functional relevance and asks how the behaviour increases the animal's fitness; question two (*How did the behaviour evolve?*) then considers how natural selection and phylogenetic constraints have modified the behaviour over evolutionary time (Tinbergen, 1963; Burkhardt, 2014; Bergman and Beehner, 2022).

Question three (*What causes the behaviour to be performed?*) asks which stimuli elicit and/or what physiological mechanisms are responsible for the onset of the behaviour? Finally, question four (*How has the behaviour developed during the lifetime of the individual?*) considers how the behaviour has been influenced by experience and learning (Tinbergen, 1963; Burkhardt, 2014; Bergman and Beehner, 2022). The latter two proximate questions are thus more concerned with the environmental and developmental factors influencing trait expression within individuals over the course of their lives, whereas the first two ultimate questions focus on how and why the trait evolves at the population level, or diverges among populations/species, over micro/macro-evolutionary timescales. It is important to realise that the proximate mechanisms determining a given trait themselves reflect the action of past natural selection or evolutionary constraints and indeed can respond evolutionarily to ongoing environmental change. Thus, considering both ultimate and proximate mechanisms and how they interact is essential to a holistic, integrated, understanding of phenotypic variability and trends in natural populations (Stamps, 2003; Bergman and Beehner, 2022). Moreover, it is not as simple as the proximate questions falling more under "ecology" and the ultimate questions more under "genetics": ecology and genetics are each relevant to both sides of the coin. The abiotic and biotic environment, for example, influence the developmental trajectories and traits of individuals via plasticity (proximate), but also influence evolutionary dynamics when they act as selective pressures (ultimate). Likewise, gene expression and its regulation play a key role in guiding development in a particular environment or mediating plastic responses to environmental variation (proximate), but likewise genetic variation provides the raw material for evolution and adaptive divergence (ultimate).

Tinbergen's framework was originally developed specifically for behavioural traits, but the approach can be applied to any phenotype, including migration. Indeed, migration is particularly interesting but also complex in this regard in that it encompasses a suite of inter-linked physiological, behavioural and life-history traits, referred to collectively as "migratory syndromes" (Dingle and Drake, 2007). While this thesis did not attempt to exhaustively address all aspects of Tinbergen's four questions, it did implicitly consider both ultimate and proximate aspects of migration-related phenotypes in

brown trout. Chapter 2 was particularly focused on testing whether phenotypic divergence among geographically proximate brown trout populations in migration/anadromy propensities had a heritable basis that would be consistent with local adaptation. The results were consistent with such a scenario, although a full reciprocal transplant experiment in which phenotypes as well as fitness outcomes are measured would be required to fully test the adaptive divergence aspects. While total lifetime fitness could not be measured in the wild common garden experiment, components of fitness such as early survival and freshwater growth rates were considered.

Chapter 3 was more focussed on the proximate issues of what genes and molecular processes play a role in determining the migratory phenotypes of individual fish, this time in a laboratory-based common garden experiment where environmental factors were tightly controlled. Despite experiencing the same environmental conditions, some of the fish in this experiment developed into smolts and others into residents, suggesting that heritable factors shaped by past (adaptive or neutral) evolution determined their migratory phenotypes. These findings complement previous work, based on the same laboratory experiments, that focussed more on the environmental drivers of migration in brown trout, i.e., phenotypic plasticity in response to food (Archer et al. 2019, 2020) and temperature (Archer et al. 2020), although heritable differences among populations was also considered by Archer et al. (2019).

Chapter 4 then considered natural phenotypic variation in a wild setting, specifically whether brown trout migrated as either autumn parr or spring smolts. The relative roles of genes versus environment in determining this phenotypic variation was not assessed in this study, but insights were gleaned into fitness consequences of these alternative tactics by measuring their survival and growth post-outmigration. Crucially, the autumn migrant phenotype was shown not to be an evolutionary or demographic dead-end, although fitness data across the full life-cycle would be required to quantitatively assess the selection pressures at play.

Many interesting and important questions regarding the ultimate and proximate drivers of migration in brown trout remain (Ferguson et al. 2019; Nevoux et al. 2019) and this

thesis has just scratched the surface in that regard. For example, future studies could consider phenotypic plasticity and genotype by environment interactions in wild settings and link migration decisions to fitness outcomes at the individual level. Data spanning multiple years or locations would then be required to measure selection parameters and relate them to putative ecological drivers. This thesis also did not consider broader phylogenetic patterns (Tinbergen's second question), but comparative studies of salmonids or other fishes that vary substantially in their migration tactics would make great avenues for further research.

Between-population differentiation in migration tactics

There is an enormous range of life history and migratory tactics within salmonid fishes (species belonging to the family Salmonidae) (Forseth *et al.*, 1999; Klemetsen *et al.*, 2003; Quinn and Myers, 2004; Dodson *et al.*, 2013; Ferguson *et al.*, 2017, 2019; Hutchings *et al.*, 2019). Salmonids typically spawn in freshwater and juveniles rear in their natal streams/rivers for varying lengths of time, depending on the species or population. In many salmonid species, various forms of migration occur, including anadromy and potamodromy, with spatial and temporal aspects of migration varying hugely across species but also among populations of the same species (Klemetsen *et al.*, 2003; Quinn, 2018; Ferguson *et al.*, 2019; Nevoux *et al.*, 2019). Phenotypic divergence among populations in migratory tactics can be explained by genetic divergence (which in turn reflects the results of past natural selection, i.e. adaptive divergence, or genetic drift, neutral divergence) or phenotypic plasticity, i.e. direct effects of the different environments each population is exposed on individual phenotypes (proximate factors). Common garden experiments (CGEs) are one way to tease apart the relative roles of genetic versus environmental factors in explaining geographic variation in phenotypes among populations (de Villemereuil *et al.*, 2016). The results of the CGE reported in Chapter Two showed that there are heritable differences in migration strategies between geographically proximate brown trout populations. The main prediction was that trout deriving from populations that naturally exhibit divergent migratory tactics should maintain native migratory life history frequencies when reared under shared environmental conditions. The shared environment in this case was the home environment of the Rough below-falls

population. Consistent with expectations, an excess of seaward-migrating progeny from Erriff (high anadromy background population) mothers and a deficit of seaward-migrating progeny from Rough Below (low anadromy background) mothers was observed at the sea-entry traps. Although not significant statistically, the effects were in the expected direction for the fathers too, with fewer smolts assigning to sires from the Rough Above (resident) population compared to Rough Below or Erriff sires.

CGEs have been a popular method of studying the genetic bases of migratory and other traits in salmonid models (McGinnity *et al.*, 2004; Piché *et al.*, 2008; Doctor *et al.*, 2014; Phillis *et al.*, 2016; Archer *et al.*, 2019; Lemopoulos *et al.*, 2019). They can be set up as laboratory experiments or conducted in semi or fully wild environments, with each having advantages and limitations. CGEs in laboratory settings have the advantage of being able to completely control for environmental conditions as well as other parameters such as fish density and composition. However, laboratory based common garden experiments, although useful are not always able to reproduce comparable results to common garden experiments set in wild environments, especially if traits of interest are subject to strong genotype by environment (G x E) interactions (Hutchings, 2011). In contrast, CGEs undertaken in natural, or semi natural, conditions can provide a more realistic representation of trait expression in ecologically complex settings. Wild CGEs are not without their own issues and problems, however. For instance, the experimental fish are subject to intraspecific competition from wild populations of the same species (when not stocked into empty habitat) and interspecific interactions with predators, parasites, competitors, etc. Whilst these ecological interactions are part and parcel of wild environments, they may be amplified to unnatural levels if experimental fish are stocked in on top of pre-existing, naturally produced, conspecifics. Furthermore, these wild environments are subject to unpredictable and sometimes extreme natural events, which could potentially introduce noise to findings and results.

While potentially very powerful, a downside of CGEs is that they only allow one to test for genetic differentiation in a given trait (or set of traits) in a single environment. Reciprocal transplant experiments represent an even more powerful approach (Johnson *et al.*, 2022), in that different genetic populations are compared both in home

and away environments, allowing for tests of local adaptation (Westley *et al.*, 2013; O'Toole *et al.*, 2015) as well as explorations of phenotypic plasticity and genotype-by-environment interactions (Doctor *et al.*, 2014; Phillis *et al.*, 2016). However, reciprocal transplants can be difficult to accomplish for logistic, economic and biological reasons. Furthermore, these experiments often have to run for a long enough timeframe to allow for lifetime measures of fitness outcomes, ideally, and in the case of brown trout could be as long as five to six years.

Overall, the results in Chapter Two support the findings of many contemporary studies which call for a major revision in conservation and management strategies for facultatively anadromous salmonid species (Middlemas *et al.*, 2009; Newson *et al.*, 2009; Quinn *et al.*, 2015; Birnie-Gauvin and Aarestrup, 2019; Birnie-Gauvin *et al.*, 2019). Migratory life history varies greatly among populations and therefore a blanket conservation program developed for a fully non-anadromous population may not be the most appropriate strategy for a partially or fully anadromous population where individuals are exposed to a different and potentially broader array of natural stressors (e.g. marine predation) and anthropogenic threats (e.g. industrial fishing, pollution or aquacultural developments).

Within-population variation in migration tactics

As well as showing strong among-population differentiation in life histories, brown trout can show tremendous variation in life history tactics within a single population (Birnie-Gauvin *et al.*, 2019; Ferguson *et al.*, 2019; Nevoux *et al.*, 2019). Some of this within-population variation has been shown to have a heritable genetic basis (Thériault *et al.*, 2007; Páez *et al.*, 2011), but environmentally-induced differences among individuals (phenotypic plasticity) also plays a role (Klemetsen, 2013; Ferguson *et al.*, 2017, 2019; Birnie-Gauvin *et al.*, 2019; Nevoux *et al.*, 2019). This thesis was able to showcase a variety of life history tactics within the Burrishoole catchment, as well as within the Erriff (non-local) group reared in the Burrishoole environment (chapter two). In Chapter Two, I found that trout that were captured moving downstream through the RRDT (putative migrants) were consistently longer than trout that remained within the Rough River (putative residents). This result could be interpreted as lending further support

to the environmentally-cued threshold model of migration (Tomkins and Hazel, 2007), as growth rate has been hypothesised to be a cue for migration in brown trout and other salmonids which exhibit facultative migration (Doctor *et al.*, 2014; Ferguson *et al.*, 2019; Archer *et al.*, 2021). It is theorized that faster growing individuals will have a higher energic demand and will therefore become energetically constrained by the natal environment sooner and forced to migrate (Ferguson *et al.*, 2019; Nevoux *et al.*, 2019b; Robertsen *et al.*, 2019; Shry *et al.*, 2019; Archer, *et al.*, 2020). The faster growing movers were also found to be in lower condition than stayers on average, which again lends support to the environmentally-cued threshold model and is consistent with studies that link energetic phenotype of salmonids to migratory propensity (Olsson *et al.*, 2006; Wysujack *et al.*, 2009; Boel *et al.*, 2014; Archer *et al.*, 2019, 2020, 2021). Differential growth within the natal environment, in turn, could be explained by a mix of genetic and environmental influences (e.g. some fry might end up in better quality microhabitats than others), or indeed genotype-environment associations could be at play, where e.g. genotypes predisposed to faster initial growth might outcompete other genotypes for access to the best feeding territories, such that growth differences get further amplified.

In Chapter Four, I was able to showcase variable migratory tactics within a single population; namely, younger autumn migrating parr versus spring-migrating smolts in the Burrishoole system. Brown trout exhibit variation in migratory tactics beyond the borders of the decision to migrate or to remain in the natal river. Many populations of brown trout show variable life histories with reference to age of migration (Forseth *et al.*, 1999; Jonsson *et al.*, 2001), timing of migration (Birnie-Gauvin and Aarestrup, 2019; Harvey *et al.*, 2020), destination of migration (Ferguson *et al.*, 2019) and distance travelled in a potamodromous, anadromous or semi-anadromous (migration to brackish or estuarine environment) setting. Furthermore, sex can also play a huge role in influencing migratory life history (Ferguson *et al.*, 2017, 2019; Nevoux *et al.*, 2019). I found that sex ratios were skewed towards females for most downstream migratory phenotype categories (Chapter Two and Chapter Four). Female bias in migratory individuals was not unexpected as the fitness advantages migration gives to females have long been hypothesised or documented within this species and other

salmonids (Hutchings and Gerber, 2002; Ferguson *et al.*, 2017, 2019; Birnie-Gauvin *et al.*, 2019). As female reproductive success in salmonids is limited by body size (Johnsson *et al.*, 2001; Seamons *et al.*, 2004; Ferguson *et al.*, 2019; O'Sullivan *et al.*, 2020), undertaking anadromous migration, or migration into brackish or estuarine environments, is a much more attractive option to females than it would be to males. In complex systems, like the Burrishoole which is thought to be comprised of many discrete and genetically distinct populations (Cross *et al.*, 1992; Coughlan *et al.*, 2006; Poole, Dillane, DeEyto, *et al.*, 2007; Keenan *et al.*, 2013; de Eyto *et al.*, 2016), male and female brown trout may exploit different habitats in both freshwater and coastal areas (Jonsson, 1985) and the connections between sex, habitat utilisation and life-history strategies warrant further study.

Recent studies have emphasised the importance of understanding variation in the timing of migration, as well as many other 'overlooked' aspects of salmonid life histories (Finstad and Hein, 2012; Birnie-Gauvin and Aarestrup, 2019; Birnie-Gauvin *et al.*, 2019; Harringmeyer *et al.*, 2021). These traits and variations need to be fully understood in order to advise and inform contemporary management and conservation strategies. In Chapter Four I found that autumn outmigrants produced more slob trout, when compared to spring outmigrants, although it must be noted that both autumn and spring outmigrants produced sea trout. Poole *et al.*, (1996), Mills *et al*., (1990), and Cross and Rogan.,(1992) reported similar patterns, although these studies did not report on these findings in great detail. Furthermore, Chapter Four reports that there could be a degree of plasticity in the migratory life history of these outmigrants, as the results suggest that neither autumn nor spring outmigrants appear to be locked into a single migratory strategy. As this study did not track the movements of the trout once the fish had left freshwater, we can only speculate upon how each migratory phenotype uses estuarine and marine habitats. However, it would not be a huge logical jump to use the studies conducted on salmonids in Scandinavian and North American systems to make informed predictions on Burrishoole outmigrant habitat use. Many studies have suggested that autumn and spring migrants might make different use of estuarine habitats (Klemetsen *et al.*, 2003; Varpe, 2017; Birnie-Gauvin *et al.*, 2019), and consistent with this I was able to show that daily specific

growth rate of autumn outmigrants was almost half that of spring outmigrants which would be suggestive of autumn outmigrants making greater use of less productive brackish habitat in the lagoon. Furthermore, autumn outmigrants spent an additional winter away compared to spring outmigrants, which could have contributed to their lower growth rate averaged across the entire sojourn compared to spring migrants.

Within the Burrishoole we can see the evolutionary maintenance of multiple phenotypes within a single population. Some sort of balancing selection mechanism is required for this, such as sexual conflict (see above section regarding sex bias in migratory life history), fluctuating selection, heterozygote advantage, or frequency dependent selection. Sudden or sustained changes in marine, estuarine or freshwater environments, however, can tip the balance towards one particular phenotype and lead to plastic or evolutionary changes in life histories. As previously stated in this thesis's introduction and again in Chapter Two, the Burrishoole population historically hosted a healthy anadromous sea trout run which collapsed in the late 1980s (Poole *et al.*, 1996). The collapse coincided with the establishment of Atlantic salmon aquaculture facilities situated in Clew Bay, the body of water the Burrishoole River meets at its terminus. There is substantial evidence that sea lice infections contracted from these salmon farms were responsible for the decline in sea trout populations in the West of Ireland (Poole *et al.*, 1996; Gargan *et al.*, 2003; Coughlan *et al.*, 2006). If the parasites are responsible for the decline, anadromous phenotypes would be heavily selected against and selection pressures would shift from balancing to more consistently directional over a relatively brief period of time. During periods where the lice may be more abundant in number, possibly in the warmer seasons of the year (Rikardsen, 2004; Rittenhouse *et al.*, 2016; James *et al.*, 2021), the selection against the anadromous phenotypes could be much greater than in the cooler seasons. In addition to influencing the relative numbers of anadromous versus resident fish in the catchment, such changes in the marine environment could also affect the selective pressures on autumn versus spring migrants. The results of Chapter Four suggested that autumn and spring outmigrants might utilise the estuarine and marine environments differently, and hence may have different levels of exposure to the parasites.

Growth rates, timing of migration and energetic phenotypes all appear to be linked at a functional level (Dodson *et al.*, 2013; Nevoux *et al.*, 2019). Further progress on fully understanding these functional linkages will require molecular and physiological studies that capitalise on ongoing technical advances in genomics and animal instrumentation (Hagen-Larsen *et al.*, 2005; Carruthers *et al.*, 2018), which might also be profitably coupled with common garden or reciprocal transplant experiments to probe fine-scale adaptive divergence within single catchments.

Molecular basis of migration tactics

Over the last few decades, modern genetic and genomic techniques have been key tools that have allowed researchers to gain a deeper understanding of the genetic architecture of quantitative traits. In salmonids, these technologies have opened new avenues of research into the molecular basis and genetic architecture of migration and anadromy. A popular model for genetic, genomic, and transcriptomic investigations in recent years has been rainbow/steelhead trout (*Oncorhynchus mykiss*), another facultatively anadromous salmonid (Hale *et al.*, 2013, 2016, 2018; Pearse *et al.*, 2014; Arostegui *et al.*, 2019). Possibly, the most exciting result found from these studies has been the discovery of the Omy05 region, a major locus on Chromosome 5 in *O. mykiss* determined to have a strong influence on migratory propensity (Hecht *et al.*, 2013; Pearse *et al.*, 2014; Leitwein *et al.*, 2016; Arostegui *et al.*, 2019). After the discovery of the Omy05 region many more investigations have been conducted across different salmonid populations and species to find a master locus for migratory life histories, (Narum *et al.*, 2011; Barson *et al.*, 2015; Nichols *et al.*, 2016; Lemopoulos *et al.*, 2019; Kurko *et al.*, 2020) with varying levels of success.

In some respects, *O. mykiss* and *Salmo trutta* share many life-history characteristics: both species can express multiple migratory life histories, have similar life stages and reproductive strategies, share similar freshwater habitats, and both are ecologically and economically important species. Therefore, one could hypothesise that the molecular mechanisms controlling migratory life history in these related species may be similar. Yet, studies have found little in the way of overlap in genomic regions of interest between the two species (Sutherland *et al.*, 2014; Lemopoulos *et al.*, 2018,

2019; Moran *et al.,* in prep). This does not imply that such loci do not exist, but rather suggests that the molecular mechanisms behind the migratory decision may vary between species, possibly even between populations. Another possibility is that while different genomic regions might correlate with migration propensity in different salmonid species or populations, similar genes or biological pathways (e.g. gene ontology terms) might be involved. However, it could also be the case that relatively few of the causative genes which influence the migratory decision have been discovered thus far.

A key related issue is to understand the gene regulation mechanisms that give rise to alternative migratory phenotypes within a single population. One way in which I attempted to do this was to use RNA sequencing (RNA-Seq) to try to detect transcriptomic level differences between smolts and residents. In recent years RNA-Seq has emerged as a powerful approach for the detection of differential gene expression with both high-throughput and high-resolution capabilities (Robles *et al.*, 2012). Furthermore, RNA-Seq experiments have become a popular tool to investigate the molecular basis of phenotypic plasticity (Gibbons *et al.*, 2017). Therefore, they have been a frequently used tool when looking at the molecular mechanism involved in the migratory life history of salmonid fishes (Norman *et al.*, 2014; Schunter *et al.*, 2014; Alvarez *et al.*, 2015; McKinney *et al.*, 2015). In Chapter Three, the experimental subjects all shared the same genetic background (Erriff) and were reared under the same environmental conditions within a single tank, yet they expressed multiple different phenotypes (smolt versus resident). Therefore, this experiment points more towards heritable differences in gene expression underpinning these divergent life-histories, rather than environmental effects, but in natural environments that vary spatially and temporally both genetic variation and plastic changes in gene expression will likely affect migratory decisions and associated phenotypic differences among individuals and populations. Thus gene expression itself can be considered a “molecular phenotype” for which genetic versus environmental contributions can be dissected out, e.g. using common garden or reciprocal transplant experiments, or pedigree information (Oomen and Hutchings, 2017, 2022).

Yet, these more traditional approaches, although powerful, do not seek to identify specific genes or loci of interest and do not shed further light on the genomic basis of phenotypic variation (de Villemereuil *et al.*, 2016). Resultingly, there is a lack of understanding to this day regarding the mechanisms linking genotype to phenotype, which in turn influence how a species responds to environmental change (Fraser *et al.*, 2014; Oomen and Hutchings, 2022). As more studies are conducted, it is becoming increasingly apparent that the complex molecular and gene regulation mechanisms that control phenotypic divergence in salmonid migratory decisions are likely to involve one or more master regulatory genes, as well as epigenetic modifications (Aubin-Horth *et al.*, 2009; Baerwald *et al.*, 2016; Oomen and Hutchings, 2017).

The notion of gene regulation via differential methylation and histone modifications playing a role in salmonid migratory life history decisions may indicate that gene expression differences between one or two distinct migratory phenotypes may actually begin much earlier in the developmental process. These molecular-level differences are likely to occur even before individuals on different life-history trajectories start to look physically distinct from one another. These gene expression differences could be influenced by events occurring very early in life, where methylation and acetylation may occur due to environmental conditions (Morán *et al.*, 2013; Baerwald *et al.*, 2016). Furthermore, environmental effects experienced by parents, often very early in their own lives, can sometimes be transmitted via methylation marks to their offspring, but the prevalence and importance of such putative epigenetic inheritance remains unclear (Burton and Metcalfe, 2014). From the few epigenetic studies to date on salmonids, there are suggestions that some epigenetic changes in the parental genomes might be transmitted to their offspring (Wellband *et al.*, 2021). Furthermore, epigenetic inheritance mediated via effects of temperature on gene regulation could potentially influence the life-history traits of offspring and perhaps grand-offspring, irrespective of energy status (Oomen and Hutchings, 2017), although further studies are needed to verify and gain further insight into these mechanisms.

In Chapter Three I was able to highlight extensive transcriptional differences between migratory and resident brown trout for genes underlying metabolic, cellular and immune processes. Furthermore, this body of work was able to document tissue-

specific differences in expression profiles between the migratory phenotypes, with differential expression evident in the liver but not brain. This result lends support to many studies and the notion that migratory phenotype is heavily linked with energetic phenotype and metabolic processes (Forseth *et al.*, 1999; McCarthy, 2000; Lahti *et al.*, 2002; Archer, *et al.*, 2020). Finally, in Chapter Three I was able to detect sex-biased expression differences in genes associated with alternative migratory tactics. These differences were found in the liver again, but not the brain, which is surprising given the documented hormonal and behavioural differences between male and female salmonids that might be under the control of physiological feedback loops that start in the brain (Hale *et al.*, 2018; Cleveland *et al.*, 2020). Future studies should focus on targeted profiling of specific regions of the brain, as this method could highlight fine-scale within-organ differences in gene expression between the sexes.

Conservation and management

Brown trout hold biological, cultural, ecological and economic importance in almost all environments they are found (Ferguson *et al.*, 2017; Nevoux *et al.*, 2019). Yet, the tremendous variation that the species shows in life history can present challenges when it comes to developing comprehensive conservation and management plans (Antunes *et al.*, 1999; Ferguson and Prodöhl, 2022). The results of this thesis would further support that different migratory phenotypes may need independent management strategies, or that brown trout may need a multi-layered management approach that considers the numerous habitats that an anadromous or potamodromous individual may encounter on its migratory journey. Direct and indirect human stressors such as habitat loss and degradation, introduction of invasive species, overexploitation, climate change and habitat fragmentation, among others, threaten aquatic communities and their inhabitants (Muhlfeld *et al.*, 2019; Quratulann *et al.*, 2021; Ferguson and Prodöhl, 2022). Natal homing in brown trout can produce fine-scale, often quite complex, population structuring (Ferguson and Prodöhl, 2022), and within any given local population, multiple discrete migratory phenotypes such as anadromous, potamodromous and resident individuals can coexist (Birnie-Gauvin and Aarestrup, 2019; Birnie-Gauvin *et al.*, 2019; Ferguson *et al.*, 2019). This life-history complexity argues for conservation and management strategies that are established

on a population-by-population basis and tailored to local conditions and constraints, but this of course comes with logistical complexities.

In recent decades, aquaculture activities have had detrimental effects on wild salmonid populations (McGinnity *et al.*, 2004, 2009; Solem *et al.*, 2014; Vollset *et al.*, 2018; O'Sullivan *et al.*, 2020). Aquaculture facilities are becoming increasingly problematic with the frequent unintentional release and escape of farmed fish (Antunes *et al.*, 1999; McGinnity *et al.*, 2009). These farmed fish present a particular threat to native fish populations in surrounding areas, and many studies have suggested that these facilities should be strategically placed in order to minimise the potential damage these farms can inflict on natural populations (Rikardsen, 2004; Middlemas *et al.*, 2010). A major issue that aquaculture farms present to the anadromous components of brown trout populations is the transmission of parasites and pathogens. Sea lice infestations have been continuously associated with Atlantic salmon farming, which have led to reduced marine survival and changes associated with migration, growth, physiology and reproduction (Gargan *et al.*, 2003; Ferguson *et al.*, 2017; Vollset *et al.*, 2018; James *et al.*, 2021). These infections can be detrimental to anadromous individuals, as the results of Taranger *et al.* (2015) suggest, as this study found a mortality rate of between 50% and 100% in sea trout within 15km of salmon farms. In the Burrishoole system, the return rates or anadromous trout were diminished by two thirds between 1988 and 1998 (Poole *et al.*, 1996; Poole *et al.*, 2007), with the beginning of these declines coinciding with the establishment of the aquaculture facilities in Clew Bay.

Limitations of this work and future research suggestions

This thesis sets out to explore the underlying genetic mechanisms by which alternative life history tactics in facultatively migratory brown trout are determined. While the results have shed important new light on these genetic aspects, many important questions remain and my findings point the way to various new avenues for future research.

In Chapter Two, I showed that anadromy rates differed among the different genetic backgrounds and I was able to observe and document group-specific differences in

movement rates out of the nursery stream (Rough River) via the RRDT data during certain key migratory periods. Collectively, these results point toward heritable differences in migration strategies between populations, as well as size/age-related variation in migration patterns within population. However, one major limitation in the common garden experiment of Chapter Two was the lack of a pure Rough Above Falls group in the experiment. Unfortunately, it was not possible to include such a group in the experiment, as the fecundity of Rough Above females is so low (given their diminutive size) that an unfeasibly large number of dams would have been required to yield sufficient surviving progeny. If a pure Rough Above group could have been included in this experiment, I would have predicted this group to produce even fewer, if any, smolts, compared to hybrid groups involving Rough Above sires. Documenting this would have increased confidence in the conclusion that above-falls populations have evolved genetically towards residency, and comparisons with both types of hybrid groups (where the sire or the dam is from the Rough Above population, and the other parent is from the Rough Below or Erriff populations) would further allow insights into the effects of maternal versus paternal genotype. Even if resident populations retain the ability to produce smolts under the right environmental circumstances, the physiological capacity to cope with the freshwater to marine transition might have degraded over the generations under relaxed selection in such populations. Future work could test this by exposing different genetic groups to saltwater challenges in captivity or by measuring osmoregulatory changes under natural conditions.

The common garden experiment of Chapter Two found no evidence for survival differences in the Rough River between offspring from different population genetic backgrounds, based on their relative representation in the electrofishing samples. This result could be due to the selective environments in the Burrishoole and Erriff systems being similar in nature at the freshwater stage. Therefore, there is not enough of an environmental difference between these two freshwater habitats to illicit a substantial adaptive divergence among Erriff and Rough River trout. Numerous studies of brown trout have documented molecular or quantitative genetic evidence for local adaptation at regional (Meier *et al.*, 2011) or more local scales (Westley *et al.*, 2013). It must also be noted that “gold standard” evidence for local adaptation comes from reciprocal

transplant experiments, preferably with replication at the population level (Kawecki and Ebert, 2004; Fraser *et al.*, 2011). Historically, the complexity of such experiments performed with brown trout has led to mixed, or inconsistent results been performed with brown trout (e.g. Stelkens *et al.*, 2012; Westley *et al.*, 2013; Labonne *et al.*, 2020). However, these difficulties highlight the complexity of factors determining the extent and scale of local adaptation. Therefore, a large-scale reciprocal transplant could be established between these populations or even including a population from a different geographic region where environmental differences would be more dramatic. This type of experiment could help disentangle not only the effects of genetics and environmental variation on the migratory phenotypes of brown trout, but the data generated could also help gain deeper understanding of plastic responses in phenotype as well as looking at the effects of local adaptation within brown trout populations.

In Chapter Three I was able to explore the differences between migratory phenotypes at a transcriptomic level and this chapter revealed that there are indeed differences in levels of gene expression between smolts and residents. To do this I looked at the gene expression profiles in the brain and liver of smolts and resident brown trout. This research showed that the genes that were differentially expressed were mainly involved in metabolic pathways rather than neurological processes. However, this study only considered a single tank of trout which were all from the same genetic background, i.e. a single population which all experienced the same environmental conditions. Thus, while this study does provide a good platform to assess heritable differences between these trout, it is limited when considering phenotypic plasticity. Furthermore, although this study highlights some interesting developmental and metabolic genes it does not unravel the regulatory mechanisms of these genes. Further studies could build on this by considering histone modifications as well as methylation and acetylation changes between these phenotypes to gain further insight into the epigenetic regulation of phenotypic variation in brown trout.

Chapter Four used a novel methodology that combined genetic and physical tagging to show that autumn migrants make an important contribution to the total return of upstream *S. trutta* in the Burrishoole system, bolstering recent claims that they should

not be overlooked in salmonid research and management (Birnie-Gauvin and Aarestrup, 2019; Birnie-Gauvin *et al.*, 2019). However, one limitation of this study is that no data were collected on movements or habitat use of the outmigrants once they had passed out of the sea entry traps. Therefore, there is a need for future sampling of *S. trutta* that are inhabiting brackish Lough Furnace, plus acoustic tagging to track their movements and habitat use within the lagoon and beyond into fully marine environments. This sort of study would be profitably coupled with physiological work to test for osmoregulatory differences at outmigration.

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